IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

COLD SPRING HARBOR LABORATORY, Plaintiff, v. GUARDANT HEALTH, INC., Defendant.	Civil Action No. 25-263-JCG

PLAINTIFF'S DISCLOSURE OF ASSERTED CLAIMS AND INFRINGEMENT CONTENTIONS FOR U.S. PATENT NOS. 10,947,589 AND 12,234,510

Pursuant to Paragraph 2 of the Third Amended Scheduling Order (D.I. 37), Plaintiff Cold Spring Harbor Laboratory ("Plaintiff" or "CSHL") hereby provides its infringement contentions to Defendant Guardant Health, Inc. ("Defendant" or "Guardant"), which includes charts attached as Exhibits A and B for U.S. Patent Nos. 10,947,589 (the "'589 Patent") and 12,234,510 (the "'510 Patent"), respectively.

As set out in CSHL's May 1, 2025, First Amended Complaint, incorporated in full by reference herein, Plaintiff contends that Defendant, either alone or in conjunction with others, has infringed and continues to infringe, literally and/or under the doctrine of equivalents, one or more claims of the '589 Patent and the '510 Patent (collectively the "Patents-in-Suit") under 35 U.S.C. § 271 since at least March 16, 2021, and February 25, 2025, respectively, by making, using, selling, and/or offering for sale in the United States and/or importing into the United States the "Accused Products" (collectively, the "Guardant360 tests"), including all liquid biopsy or tissue biopsy tests marketed under the tradename "Guardant360", including at least the following tests:

a. Guardant360® Liquid Test ("Guardant360 Liquid");

b. Guardant360® Tissue Test ("Guardant 360 Tissue"); and

c. Guardant360® CDx Test ("Guardant360 CDx").

Plaintiff's Infringement Contentions comprise the following charts, attached as Exhibits A and B, setting forth where in the Accused Products each element of each asserted claim is found:

Patent in Suit	Priority Date	Asserted Claims	Defendant's Accused Products
10,947,589	Oct. 22, 2010	1-3, 6-15, and 17-19	Guardant360 Liquid, Guardant360 Tissue, and Guardant360 CDx
12,234,510	Oct. 22, 2010	1-12, 15-23, 26-29 and 31-34	Guardant360 Liquid, Guardant360 Tissue, and Guardant360 CDx

Plaintiff's Infringement Contentions identify where each limitation of the asserted claims of the Patents-in-Suit is met with respect to each Accused Product. Where Plaintiff cites documents or other evidence in support of its infringement contentions, Plaintiff has identified exemplary evidence for each claim limitation and reserves its right to rely upon additional or alternative evidence as the case proceeds. Plaintiff has cited representative portions of identified references for the Accused Products even where a particular reference may contain additional examples of infringement for a claim element. Where a claim element is implemented in the same or substantially the same way for each product of an Accused Product family, Plaintiff provides an exemplary illustration or description setting forth specifically where the limitation is found in the Accused Products, without necessarily repeating the same illustration or description for each version of each Accused Product in the family. To the extent Plaintiff's infringement contentions do not cite support for each member of an Accused Product family, such an omission of evidence shall not be construed as an admission of non-infringement or a waiver of the right to rely on such evidence at a later date.

Plaintiff contends that each element of each asserted claim is literally met with respect to each Accused Product unless otherwise indicated. But to the extent that any claim element is found not to be literally met with respect to the Accused Products, Plaintiff contends that the

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element is met under the doctrine of equivalents because there are no substantial differences between the element and the Accused Products, and the element and Accused Products perform substantially the same function, in substantially the same way, to achieve substantially the same result.

Plaintiff further contends that Defendant has willfully infringed and continues to willfully infringe the patents-in-suit. Defendant has continued its infringement despite knowledge of the patents-in-suit and its infringement. (See, e.g., D.I. 15 ¶¶59-78.)

Plaintiff's Infringement Contentions are based upon information reasonably and presently available to Plaintiff through publicly available information. Plaintiff reserves the right, consistent with the Federal Rules of Civil Procedure, the Local Rules and Standing Orders, and the Court's Scheduling Order (D.I. 37), to amend its infringement contentions to assert additional patents and/or claims, accuse additional products, and update its infringement theories as additional evidence and information becomes available or as otherwise appropriate.

Pursuant to Paragraph 3 of the Third Amended Scheduling Order (D.I. 37), Plaintiff has produced and made available the documents in the table below to accompany its Disclosure of Asserted Claims and Infringement Contentions. Plaintiff is in the process of gathering and preparing additional documents described in Paragraph 3 of the Third Amended Scheduling Order (D.I. 37) and will produce or make them available for inspection and copying under the Protective Order once entered.

Patent/Publication Number or Reference Title	Bates Range
U.S. Patent No. 9,834,822	CSHL-GUARDANT00001926 through CSHL-GUARDANT00001981

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Patent/Publication Number or Reference Title	Bates Range
U.S. Patent No. 12,241,128	CSHL-GUARDANT00001861 through CSHL-GUARDANT00001925
Guardant360 Tissue Webpage (2025) https://www.guardantcomplete.com/hcp/solutions/guardant360-tissue ("Guardant360 Tissue Webpage")	CSHL-GUARDANT00001987 through CSHL-GUARDANT00001991
Guardant360 Liquid Webpage (2025) https://www.guardantcomplete.com/hcp/solutions/guardant360-liquid ("Guardant360 Liquid Webpage")	CSHL-GUARDANT00001999 through CSHL-GUARDANT00002004
Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023.	CSHL-GUARDANT00001411 through CSHL-GUARDANT00001443
Guardant360 CDx Technical Information, Guardant Health (July 2021) ("Guardant360 CDx Technical Information")	CSHL-GUARDANT00001309 through CSHL-GUARDANT00001410
Guardant360 Liquid Assay Specifications (2025) ("Guardant360 Liquid Assay Specifications")	CSHL-GUARDANT00002028 through CSHL-GUARDANT00002029
Guardant360 Tissue Assay Specifications (2025) ("Guardant360 Tissue Assay Specifications")	CSHL-GUARDANT00002030 through CSHL-GUARDANT00002031
Guardant360 CDx Webpage (2025) https://www.guardantcomplete.com/hcp/solutions/guardant360-cdx ("Guardant360 CDx Webpage")	CSHL-GUARDANT00001992 through CSHL-GUARDANT00001998
Guardant360 Educational Booklet, Guardant Health EU (Nov. 23, 2020) ("Guardant360 Educational Booklet")	CSHL-GUARDANT00001826 through CSHL-GUARDANT00001831
Guardant360 TissueNext – Tissue-Based Test for Complete Genomic Testing Across all Advanced Solid Cancers, Codex Genetics (2024) https://www.codexgenetics.com/guardant-health/guardant360-tissuenext.html ("Guardant360 TissueNext – Codex Webpage")	CSHL-GUARDANT00001982 through CSHL-GUARDANT00001986
Guardant360 Instructions for Use – Blood Collection Kit, Guardant Health (June 2021) ("Guardant Blood Collection Kit Instructions")	CSHL-GUARDANT00001859 through CSHL-GUARDANT00001860

Patent/Publication Number or Reference Title	Bates Range
Lanman et al., *Analytical and Clinical Validation of a Digital Sequencing Panel for Qualitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA*, PLOS One (Oct. 16, 2015) ("Lanman et al.")	CSHL-GUARDANT00001455 through CSHL-GUARDANT00001481
Nakamura et al., *Targeted Therapy Guided by Circulating Tumor DNA Analysis in Advanced Gastrointestinal Tumors*, Nature Medicine (Sept. 16, 2024) ("Nakamura et al.")	CSHL-GUARDANT00001832 through CSHL-GUARDANT00001858
Odegaard et al., *Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies*, American Association for Cancer Research (Aug. 1, 2018) ("Odegaard et al.")	CSHL-GUARDANT00001482 through CSHL-GUARDANT00001493
Integrated DNA Technologies, Inc. Custom Adapter Configurator Webpage (2025) https://www.idtdna.com/site/order/adapter ("IDT Custom Adapter Webpage")	CSHL-GUARDANT00002005 through CSHL-GUARDANT00002007
Integrated DNA Technologies, Inc., Illumina xGen NGS Adapters & Indexing Primers for Illumina sequencing (2025) https://www.idtdna.com/pages/products/next-generation-sequencing/workflow/xgen-ngs-library-preparation/ngs-adapters-indexing-primers/adapters-indexing-primers-for-illumina ("IDT Illumina Adapters and Primers Webpage")	CSHL-GUARDANT00002008 through CSHL-GUARDANT00002029

OF COUNSEL:

John M. Desmarais
Brian D. Matty
Kevin Goon
Ashley DaBiere
Desmarais LLP
230 Park Avenue
New York, NY 10169

Dated: July 11, 2025

/s/ Kelly E. Farnan
Kelly E. Farnan (#4395)
Sara M. Metzler (#6509)
Richards, Layton & Finger, P.A.
One Rodney Square
920 N. King Street
Wilmington, DE 19801
(302) 651-7700
farnan@rlf.com
metzler@rlf.com

Attorneys for Plaintiff
Cold Spring Harbor Laboratory

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EXHIBIT A

EXHIBIT A

Cold Spring Harbor Laboratory's Infringement Contentions

Cold Spring Harbor Laboratory v. Guardant Health, Inc., C.A. No. 25-263-JCG

Defendant's Guardant360 Tests Infringe The '589 Patent

Defendant Guardant Health, Inc. ("Defendant" or "Guardant"), either alone or in conjunction with others, have infringed, and continue to infringe, claims 1-3, 6-15, and 17-19 (the "Asserted Claims") of U.S. Patent No. 10,947,589 ("the '589 Patent") under 35 U.S.C. §§ 271(a), (b), (c), and/or (f), either literally and/or under the doctrine of equivalents, by making, using, selling, and/or offering for sale in the United States and/or importing into the United States tests marketed under the tradenames "Guardant360®," "Guardant360® Liquid," "Guardant360® Tissue," and "Guardant360® CDx" (collectively, the "Guardant360 tests"). As described in the chart below, use (performance) of the Guardant360 tests practices the method of the Asserted Claims of the '589 Patent through the normal and intended operation of the products under Defendant's protocols.

Guardant, either alone or in conjunction with others, directly infringes each Asserted Claim of the '589 Patent under 35 U.S.C. § 271(a), either literally and/or under the doctrine of equivalents, at least by selling, and/or offering for sale the performance of the Guardant360 tests and by using the Guardant360 tests according to their normal and intended operation under Guardant's protocols. For example, Guardant makes the Guardant360 tests and markets, offers for sale, sells, and distributes the Guardant360 tests, including through its website, www.guardanthealth.com. (*See, e.g.*, D.I. 15 ¶10.) For example, Guardant uses the Guardant360 tests according to their normal and intended operation under Guardant's protocols. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 79-84.) To the extent that Guardant itself does not perform all steps of the Guardant360 tests, Guardant directly infringes by directing and controlling the performance of the Guardant360 tests in an infringing manner according to Guardant's protocols. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 79-84.) For example, Guardant establishes the manner and timing of the performance of the Guardant360 tests in an infringing manner and conditions the receipt of a benefit upon that performance. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 79-84.)

Additionally, Guardant, either alone or in conjunction with others, indirectly infringes the Asserted Claims under 35 U.S.C. § 271(b) by making, importing, selling, and/or offering for sale the Guardant360 tests. For example, Guardant actively, knowingly, and intentionally induces third parties, including without limitation third-party laboratories, to use the Guardant360 tests, in a way that Guardant knew or should have known infringed the Asserted Claims. For example, Guardant has induced and continues to induce infringement by selling or otherwise supplying the Guardant360 tests with the knowledge and intent that those third parties will use the Guardant360 tests in the manner designed and instructed by Guardant to infringe the '589 Patent and with the knowledge and intent to encourage and facilitate third party infringement through the dissemination of the Guardant360 tests and/or the creation and dissemination of promotional and marketing materials, supporting materials, instructions, product manuals, and/or technical information related to the Guardant360 tests. Guardant knows and intends for their third-party laboratories in the United States to use the

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Guardant360 tests according to the instructions and requirements provided by Guardant. Guardant contributed to the design of the Guardant360 tests to be used according to the instructions and requirements provided by Guardant, which results in infringement of the Asserted Claims. If third-party laboratories in the U.S. were to use the Guardant360 tests in a manner other than that instructed by Guardant, the kits would not work for their intended purpose, *i.e.*, to test for copy number variations. On information and belief, third-party laboratories and other third parties have directly infringed the Asserted Claims of the '589 Patent, through the normal and customary use of the Guardant360 tests. Furthermore, Guardant has had knowledge of the '589 Patent and its infringement since at least the issue date of the '589 Patent. (*See, e.g.*, D.I. 15 ¶¶ 59-78.)

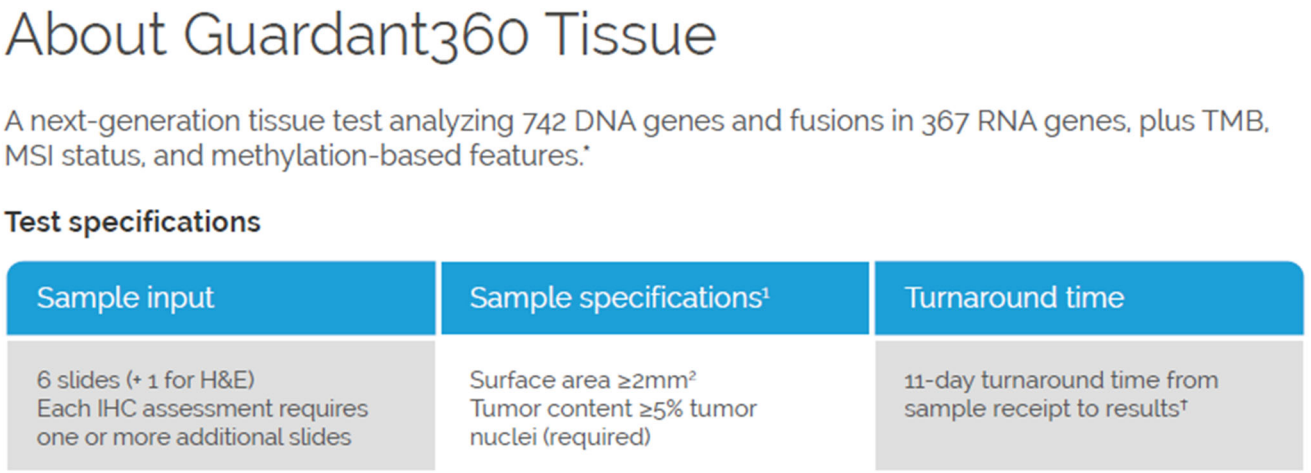
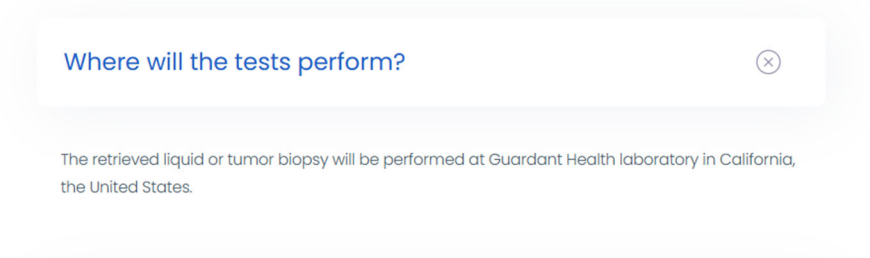
Additionally, Guardant is liable for infringement under 35 U.S.C. § 271(f). For example, Guardant has supplied or caused to be supplied—and continue to supply or cause to be supplied—in or from the United States components of the invention of one or more claims of the '589 Patent that are especially made or especially adapted for use in the invention and not a staple article or commodity of commerce suitable for substantial noninfringing use, where such components are uncombined in whole or in part, knowing that such components are so made or adapted and intending that such components will be combined outside of the United States in a manner that would infringe the patent if such combination occurred within the United States.

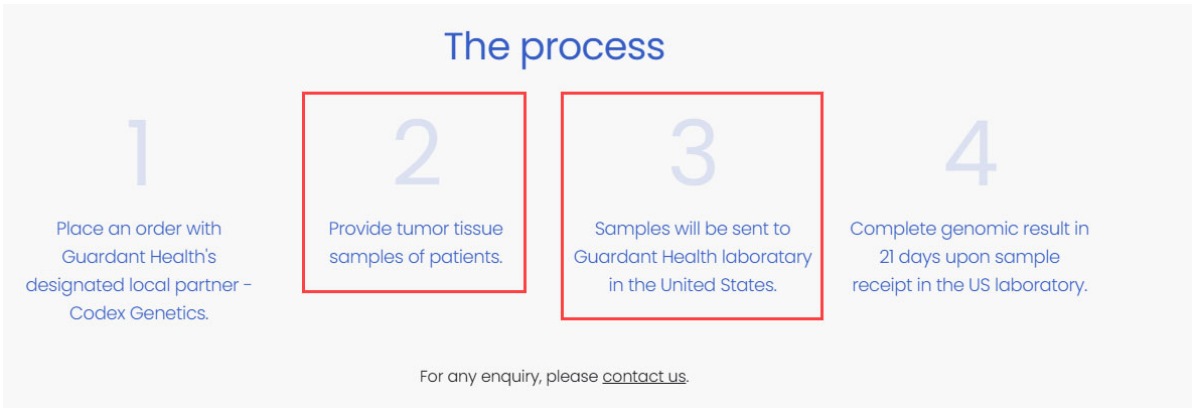
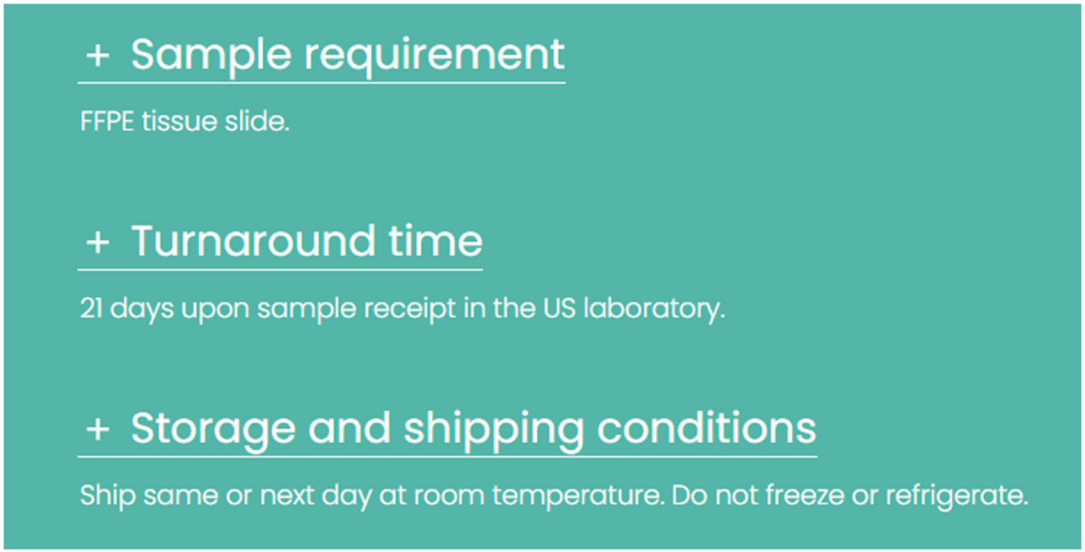
The claim charts below identify specifically where each element of each asserted claim is found in the performance of the Guardant360 tests, based upon the information available to date. CSHL contends that each element of each asserted claim is literally met unless otherwise indicated. But to the extent that any claim element is found not to be literally met with respect to the Guardant360 tests, CSHL contends that the element is met under the doctrine of equivalents because there are no substantial differences between the element and the Guardant360 tests, and the element and the Guardant360 tests perform substantially the same function, in substantially the same way, to achieve substantially the same result. *See, e.g.*, *Graver Tank & Mfg. Co. v. Linde Air Prod. Co.*, 339 U.S. 605 (1950); *Warner-Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17 (1997).

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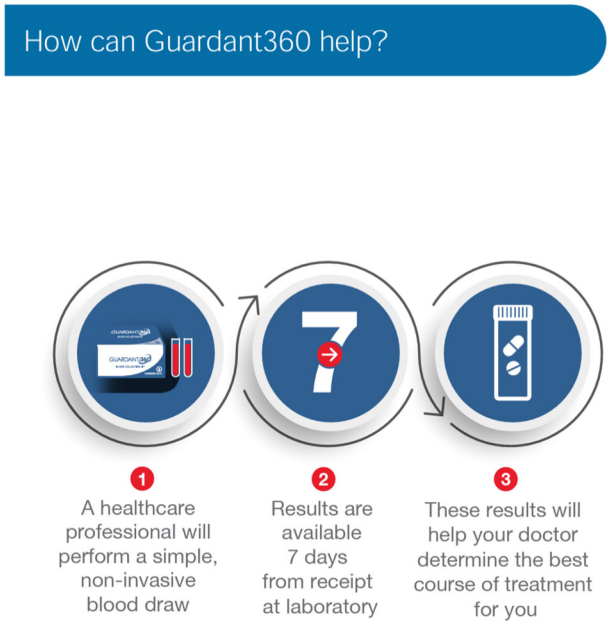
ASSERTED CLAIM 1

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
[Preamble] A method comprising:	To the extent the preamble is limiting, Guardant provides a method for analyzing DNA using next-generation sequencing (NGS). For example, the Guardant360 tests are NGS panels, the performance of which utilizes sequencing of DNA obtained from a biological sample, such as blood or tumor tissue. 5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Assay Specifications at 1.  Guardant360 TissueNext – Codex Webpage.

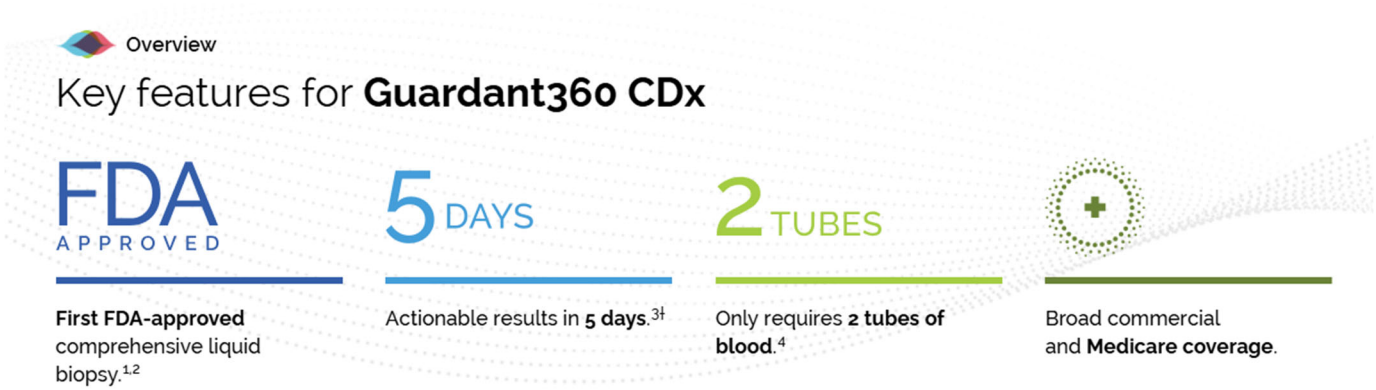
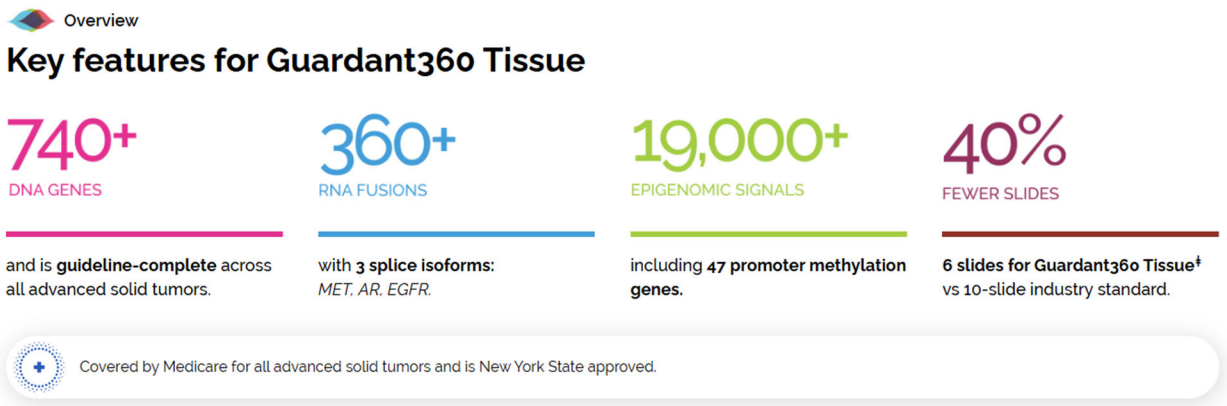
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

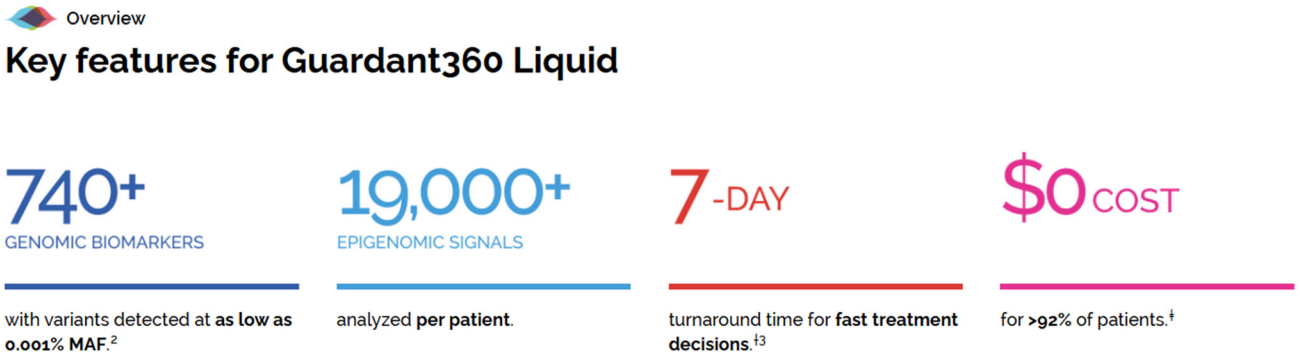
GUARDANT HEALTH EX.1020.011

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Educational Booklet at 5. Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. This test is indicated to prevent repeat invasive biopsy when an initial biopsy is QNS or otherwise unavailable/unobtainable as well as when cancer has progressed or recurred despite treatment. Actionable genomic alterations are those for which there is an FDA-approved treatment or that serve as eligibility criteria for later phase clinical trials.")

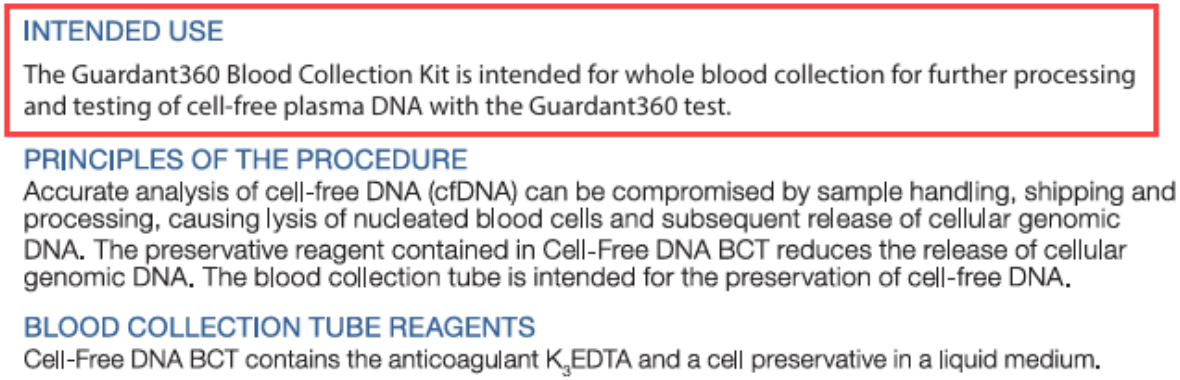
GUARDANT HEALTH EX.1020.012

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Odegaard et al. at 3539 ("To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.") Odegaard et al. at 3539 ("Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical sample. Blood-to-tissue clinical to those documented in the medical record of 543 consecutive lung cancer patients.") Odegaard et al. at 3540 ("Here, we describe the analytic (technical characterization using controlled and often contrived samples) and clinical (characterization using real-world patient samples, contexts, and comparators) validation of a comprehensive ctDNA sequencing assay for clinical genotyping of advanced solid tumors, including large-scale comparisons with orthogonal clinical plasma-and tissue-genotyping methods.") Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.") '822 patent, claim 1 ("A method, comprising:…")

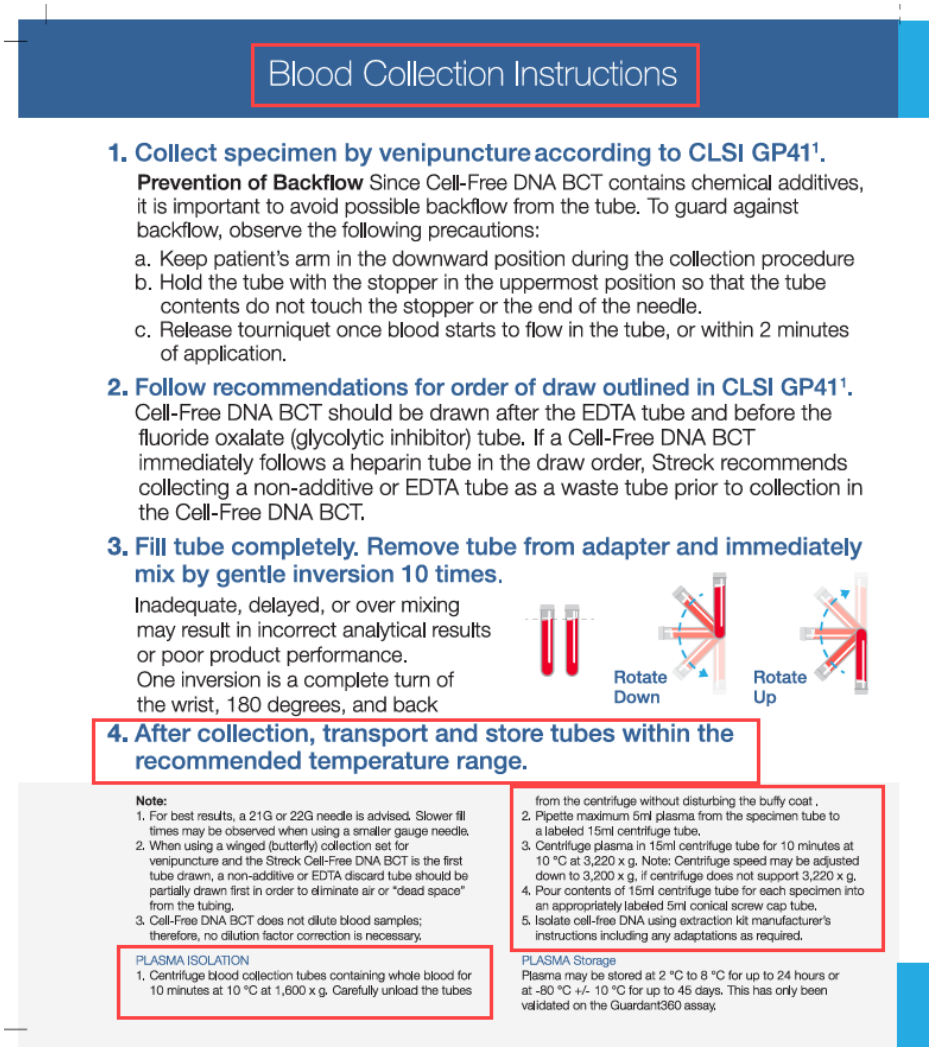
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 CDx Webpage.  Guardant360 Tissue Webpage.

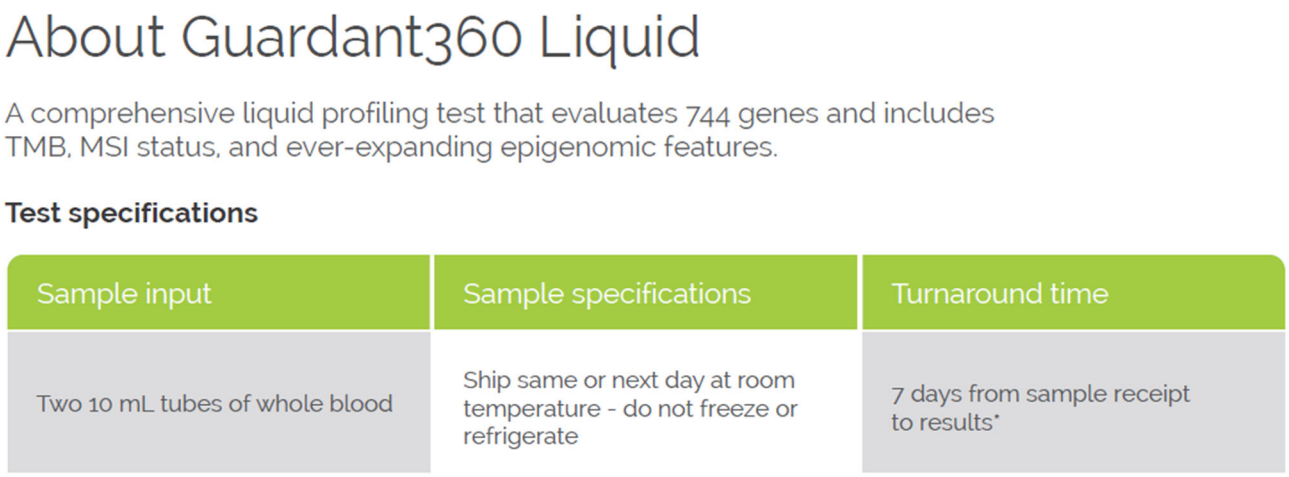
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Webpage.
[1a.] obtaining segments of genomic nucleic acids from a sample;	Guardant obtains segments of genomic nucleic acids from a sample. For example, the Guardant360 tests require obtaining cell-free DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples.

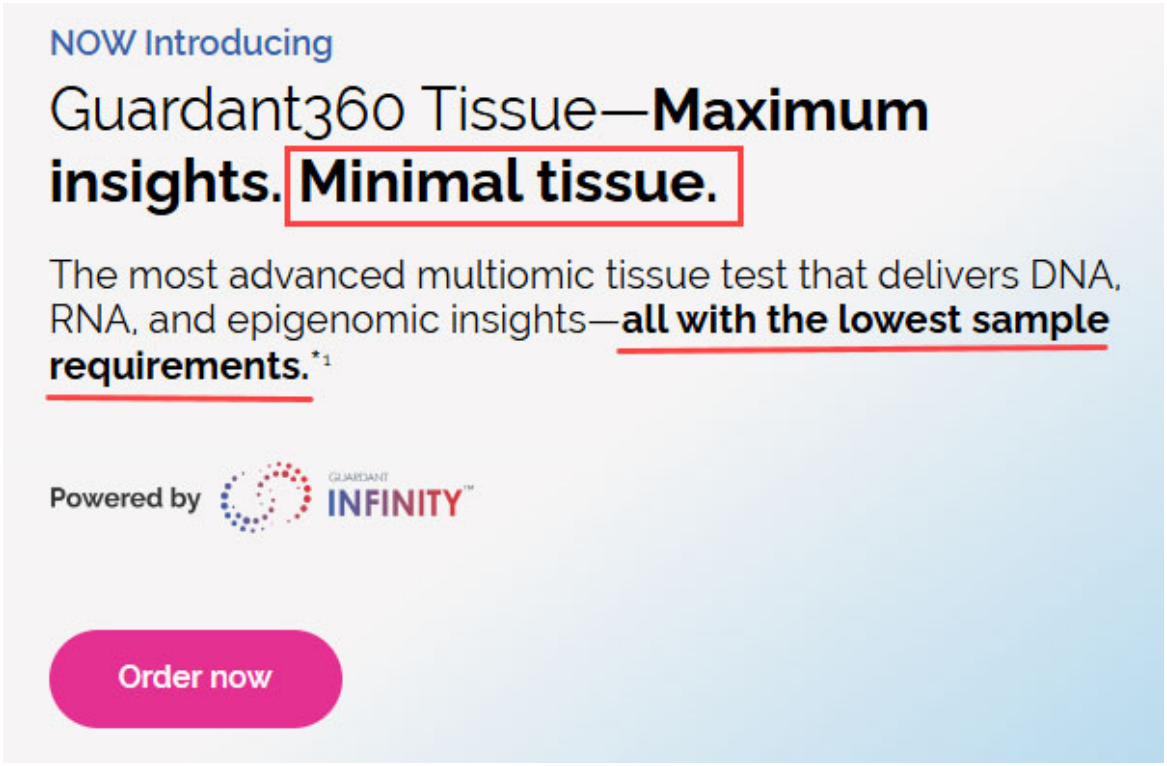
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.  Guardant Blood Collection Kit Instructions at 2.

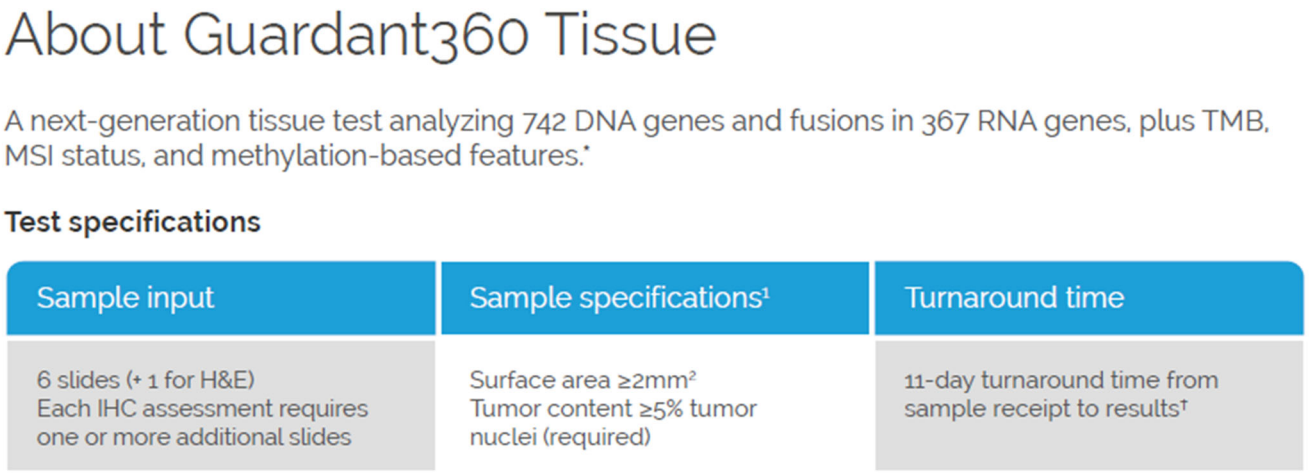
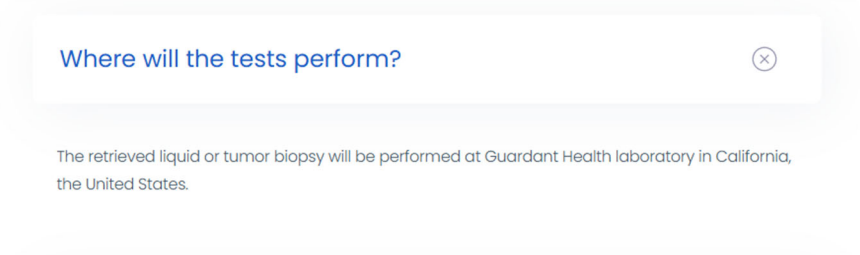
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

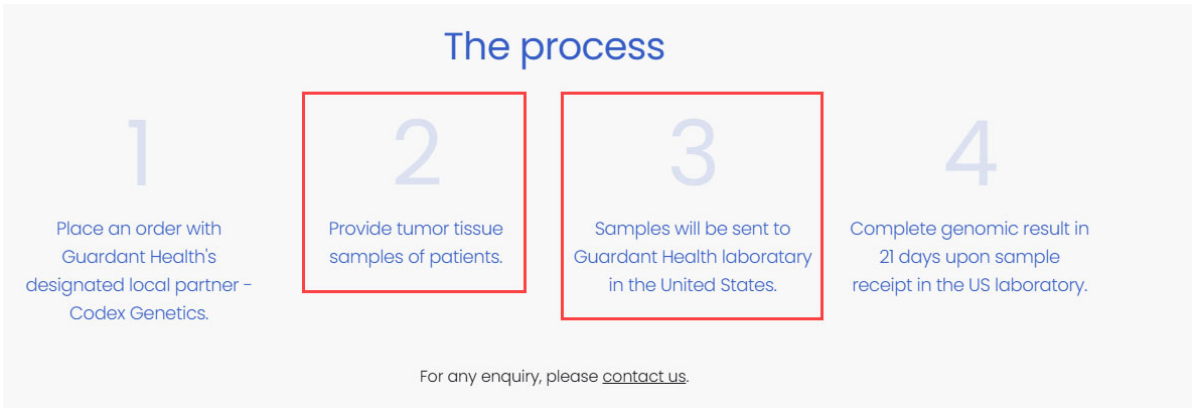
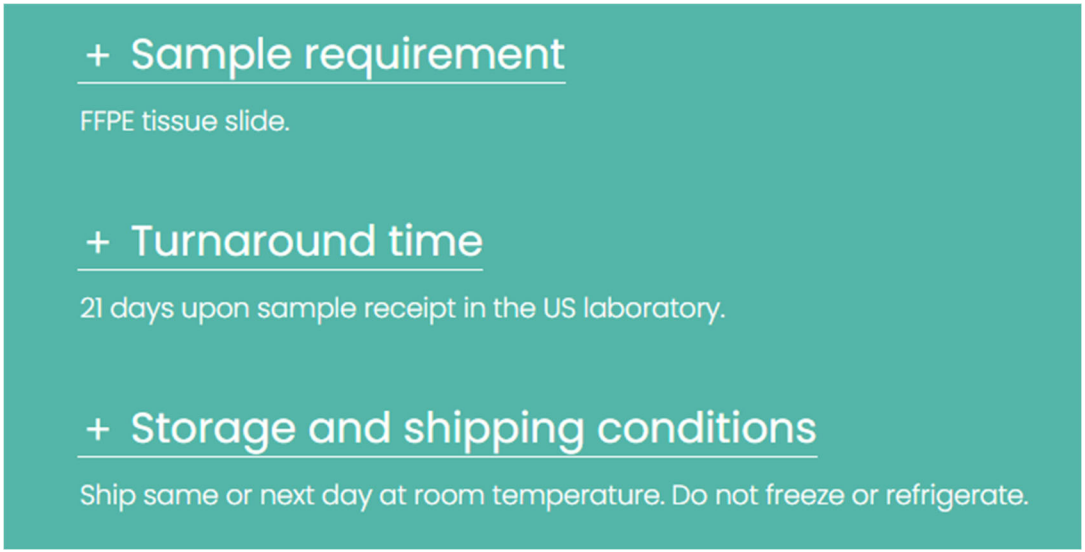
GUARDANT HEALTH EX.1020.017

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 1.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1

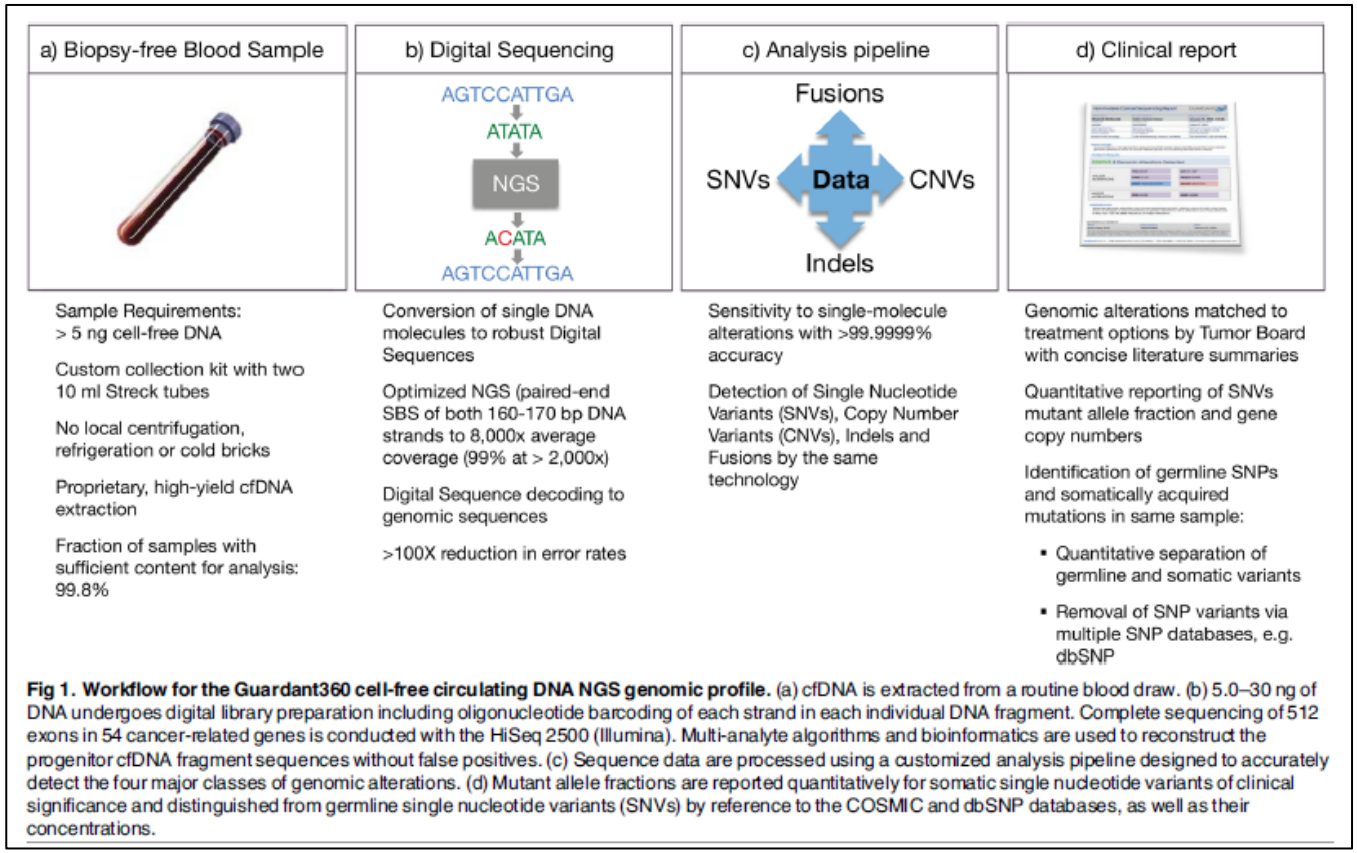
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Assay Specifications at 1.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 3 ("[D]igital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Guardant360 CDx Technical Information at 5 ("Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. A minimum of 5 mL of blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. . . . Plasma is isolated via centrifugation and cfDNA is extracted.").) Lanman et al. at 1–2 ("Digital sequencing of plasma-derived DNA is indicated int advanced cancer patients to prevent repeated invasive biopsies when the initial biopsy is inadequate, unobtainable for genomic testing or uninformative, or when the patient's cancer has progressed despite its treatment.") Lanman et al. at 2 ("Next-generation sequencing (NGS) of solid tumor tissue has advanced our ability to perform multiplex testing for an ever-growing number of targetable genomic alterations utilizing small needle biopsies or archival pathology slides. As the number of genomic targets with matched therapies grows, tissue biopsy material can be quickly exhausted before multiple "companion diagnostics" tests can be completed.) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 3–4 ("Here we report the analytical and clinical validation of the 54-gene (512 exons) Guardant360 panel of mutations and gene amplifications in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), NCCN Task Force recommendations and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines.") Lanman et al. at 4 ("With high sensitivity and the ultra-high specificity required to comprehensively sequence cfDNA, the Guardant360 test has the potential to evaluate the multiple genomic alterations cited in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with tumor heterogeneity [34], to interrogate all actionable genomic alterations via comprehensive sequencing of a multigene panel, and to prevent the time delays, cost and complications inherent in invasive biopsies.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Lanman et al. at 5 (Fig. 1 above)

Lanman et al. at 5 ("cfDNA is extracted from a routine blood draw.")

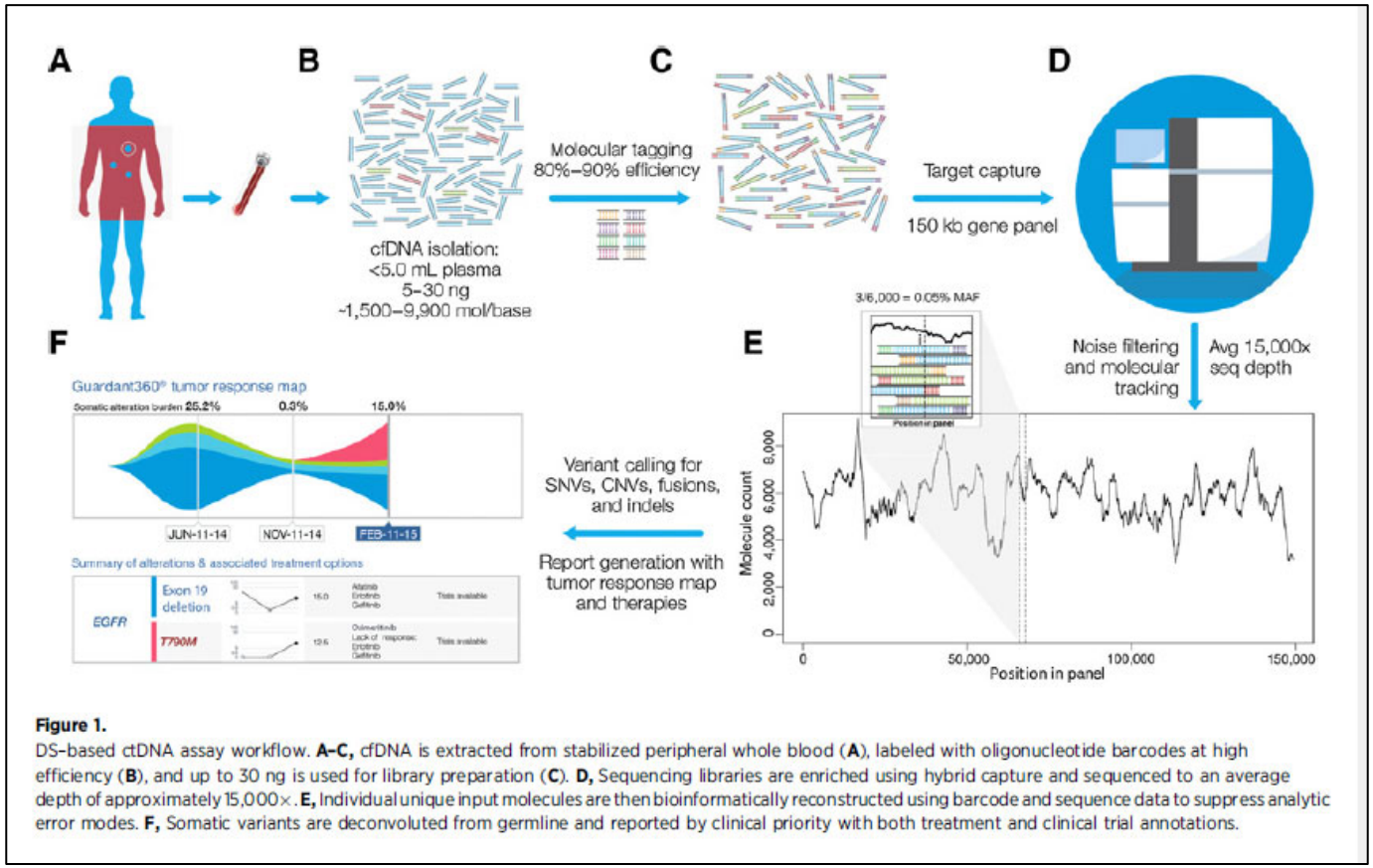
Lanman et al. at 12 ("The analytic validation studies of the Guardant360 cfDNA panel highlight its differences from both solid tumor and other cell-free circulating tumor DNA genomic testing methods.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	The digital sequencing platform enables single molecular sensitivity, an analytic sensitivity that is two orders of magnitude greater than an Illumina HiSeq with standard prep while maintaining near-perfect specificity (Fig 1A and 1B).") Lanman et al. at 12 ("The Guardant360 panel at the time of this study sequences ~78,000 base pairs (78 kbp), providing complete coverage for all exons in 18 genes and the critical exons in the remainder of the 54 total genes.") Lanman et al. at 14 ("The clinical sensitivity for plasma-based DNA NGS via digital sequencing was equivalent to the clinical sensitivity of tissue-based DNA NGS sequencing.") Lanman et al., 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.") Lanman et al. at 14 ("The clinical utility of the Guardant360 diagnostic test in advanced cancer is supported by results in clinical practice. Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guide-line-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 17 ("Guardant360 could be used in lieu of a repeat invasive biopsy for NGS, particularly for patients with visceral primary disease or metastases to lung, liver or bone. Secondly, in patients with visceral primary or metastatic disease, when a tumor has progressed or recurred based on clinical symptomatology, imaging or serological protein biomarkers, e.g. CEA, CA-125, CA 15–3, PSA, etc., the Guardant360 panel could be used to re-evaluate for new targeted therapies, enabling real-time adaptive management of a patient's continuously evolving cancer while obviating further invasive tissue biopsies.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 17 ("At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a minimum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual in Streck Cell-Free DNA Blood Collection (Streck) tubes, which contain a proprietary formaldehyde-free preservative that stabilizes white blood cells, preventing the release of genomic DNA and allowing isolation of high-quality cfDNA.") Lanman et al. at 18 ("The cell-free DNA from these cell lines is extracellular DNA released by the culture cells and the fragmentation pattern is essentially identical to circulating DNA except the size distribution peaks tend to be slightly broader. Similar biological mechanisms to those in play in circulating peripheral blood in vivo occur with the cell culture media, i.e. DNA digestion of histone-protected genomic DNA.") Lanman et al. at 18 ("Plasma samples and supernatant from cultured cell lines were processed using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. Cell-free DNA is extracted from 1.5 mL to 5 mL plasma and subsequently concentrated and size selected using Agencourt Ampure XP beads (Beckman Coulter) and subsequently quantified using Qubit1 2.0 fluorometer. The DNA is extracted using standard extraction procedures, including proteinase K digestion of histone and other DNA-binding proteins and isoproponal precipitation. The DNA is then end-repaired to repair ends of DNA with a 5' phosphate prior to ligation of adapters necessary for downstream sequencing.") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries.")

GUARDANT HEALTH EX.1020.026

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3539 ("To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.") Odegaard et al. at 3539 ("Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical sample. Blood-to-tissue clinical to those documented in the medical record of 543 consecutive lung cancer patients.") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.).") Odegaard et al. at 3540 ("Plasma-blood comprehensive tumor genotyping expands access to standard-of-care precision oncology to patients previously ineligible due to barriers associated with tumor sampling.") Odegaard et al. at 3540 ("Here, we describe the analytic (technical characterization using controlled and often contrived samples) and clinical (characterization using real-world patient samples, contexts, and comparators) validation of a comprehensive ctDNA sequencing assay for clinical genotyping of advanced solid tumors, including large-scale comparisons with orthogonal clinical plasma-and tissue-genotyping methods.") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.). Briefly, 2 x 10 mL of peripheral venous blood was collected in Streck Cell-Free DNA BCT (Streck, Inc.), packaged into a provided padded thermal insulation block within a secondary biohazard containment barrier, and shipped overnight to Guardant Health at ambient temperature. …To isolate plasma, whole blood was centrifuged (1,600 x *g* for 10 minutes at 10°C), and the resulting supernatant was clarified by additional centrifugation (3,220 x *g* for 10 minutes at 10°C).") Odegaard et al. at 3540 ("All cell-free DNA (cfDNA) extraction, processing, and sequencing was performed in a CLIA-certified, CAP-accredited laboratory (Guardant Heath, Inc.) as described previously (24). Briefly, cfDNA was extracted from the entire plasma aliquot prepared from a single 10-mL tube

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	described above (QIAamp Circulating Nucleic Acid Kit, Qiagen, Inc.), which yielded a median of 48.5 ng of cfDNA (range, 2–1,050 ng). Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3542 (Figure 1). Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials , and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).") Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.") Odegaard et al. at 3547 ("These data demonstrate applicability of ctDNA-based genotyping to treatment paradigms based on tissue genotyping; however, ctDNA has the potential to not only match but to surpass tissue genotyping in for important ways.") '822 patent, claim 1 ("[P]roviding a population of cell free DNA ('cfDNA') molecules obtained from a bodily sample from a subject.")
[1b.] tagging the segments with nucleic acid tags, thereby generating a plurality of unique tagged nucleic acid molecules,	Guardant tags the segments with nucleic acid tags, thereby generating a plurality of unique tagged nucleic acid molecules, such that each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
such that each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags;	For example, during library preparation, Guardant attaches library adapters containing barcodes to the ends of the DNA fragments, creating a set of unique tagged nucleic acid molecules. Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").) Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

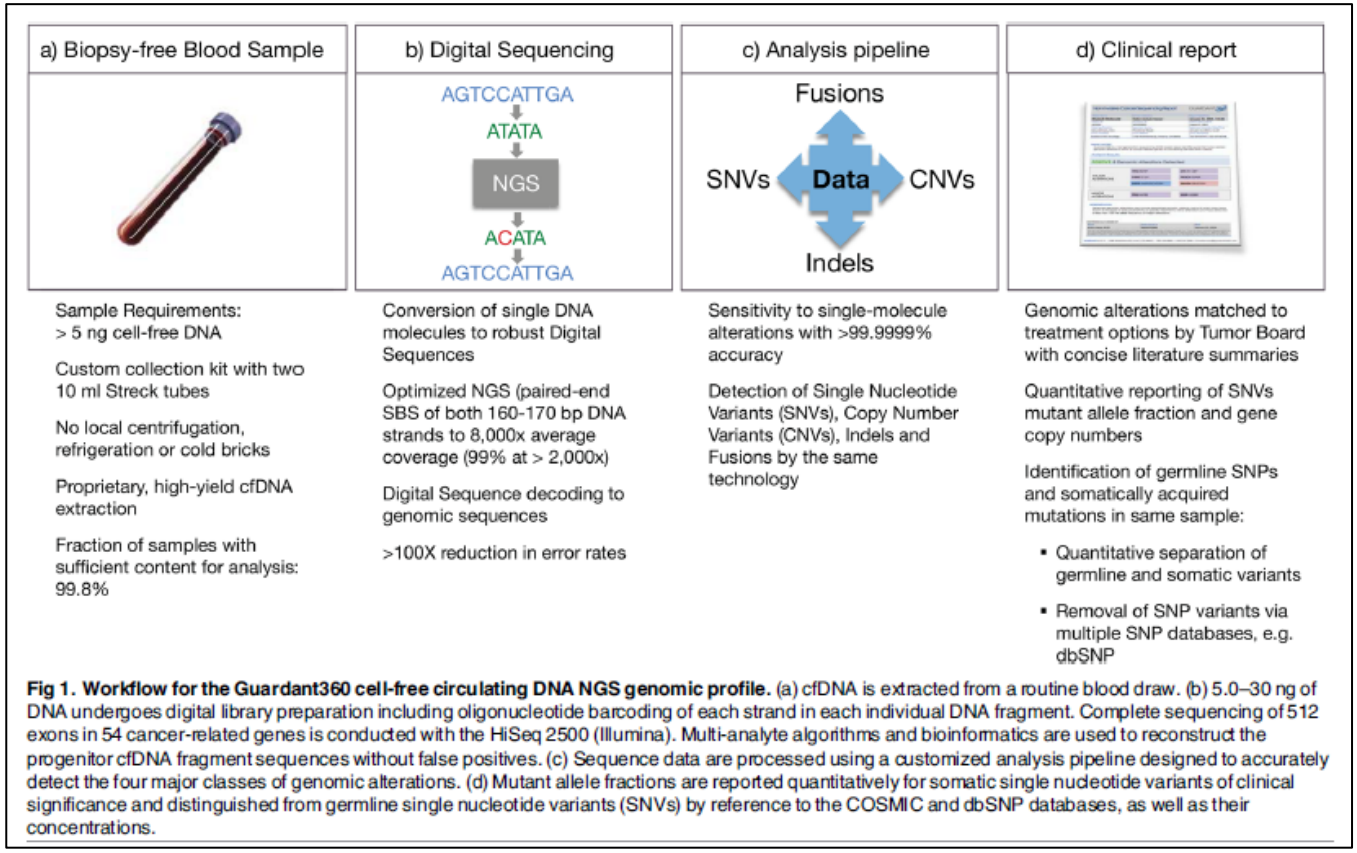


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the double stranded input cfDNA samples is separately encoded with said oligonucleotides. This digital conversion process occurs with high efficiency with greater than 80% of the original plasma of cfDNA molecules converted.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.033

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

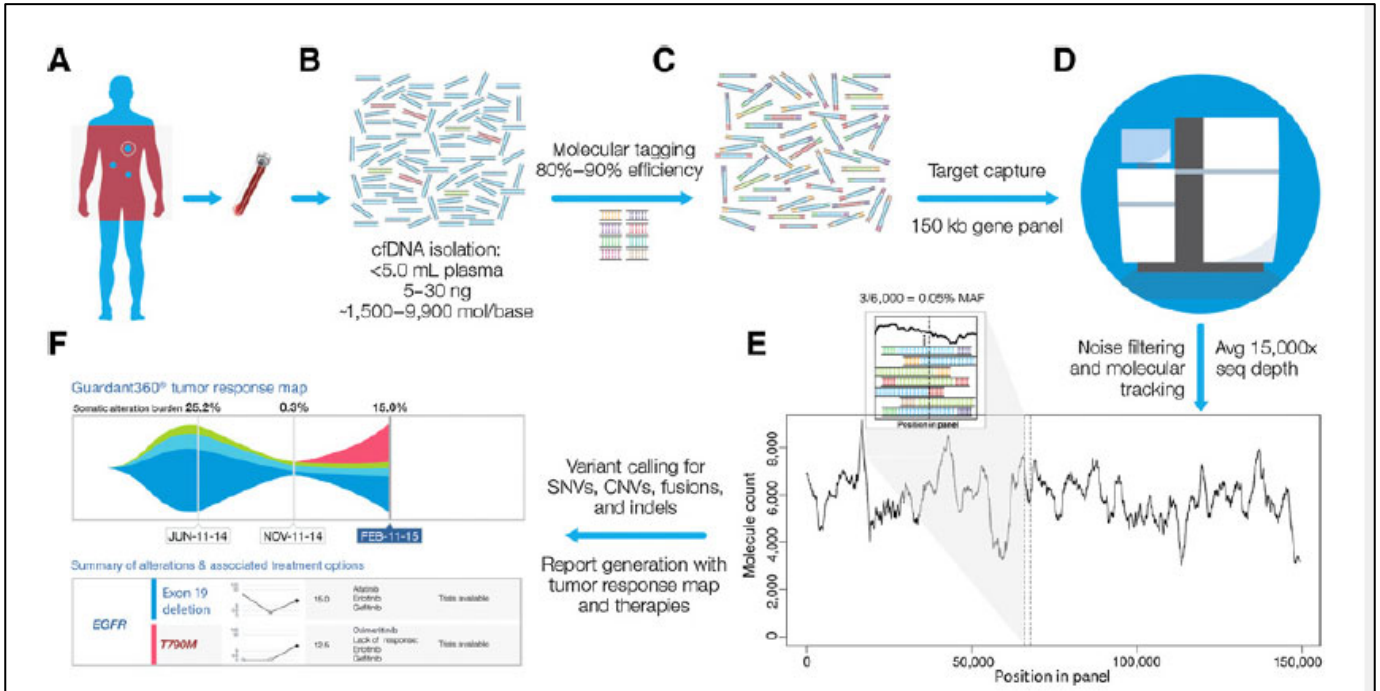


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1) ("Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data.").

'822 patent, 3:25-35 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step. In some embodiments, the barcode is a polynucleotide, which may further comprise random sequence or a

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	fixed or semi-random set of oligonucleotides that in combination with the diversity of molecules sequenced from a select region enables identification of unique molecules and be at least a 3, 5, 10, 15, 20, 25, 30, 40, 45, or 50mer base pairs in length.") '822 patent, 1:49-63 ("The disclosure provides for a method for detecting copy number variation comprising: a) sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide are optionally attached to unique barcodes; b) filtering out reads that fail to meet a set threshold; c) mapping sequence reads obtained from step (a) to a reference sequence; d) quantifying/counting mapped reads in two or more predefined regions of the reference sequence; e) determining a copy number variation in one or more of the predefined regions by (i) normalizing the number of reads in the predefined regions to each other and/or the number of unique barcodes in the predefined regions to each other; and (ii) comparing the normalized numbers obtained in step (i) to normalized numbers obtained from a control sample.") '822 patent, 6:26-27 ("In some embodiments each tagged parent polynucleotide in the set is uniquely tagged.")
[1c.] subjecting the plurality of the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating amplified tagged nucleic acid molecules;	Guardant subjects the plurality of the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating amplified tagged nucleic acid molecules. For example, Guardant uses PCR amplification to amplify the library of unique tagged nucleic acid molecules to create an enriched library that can be sequenced. Laman at 18 ("These digital sequence libraries are amplified and then subsequently enriched for target genes using biotinylated custom baits of RNA probes"), 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.").) Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The resulting DNA is amplified by PCR to create libraries suitable for enrichment….Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.")

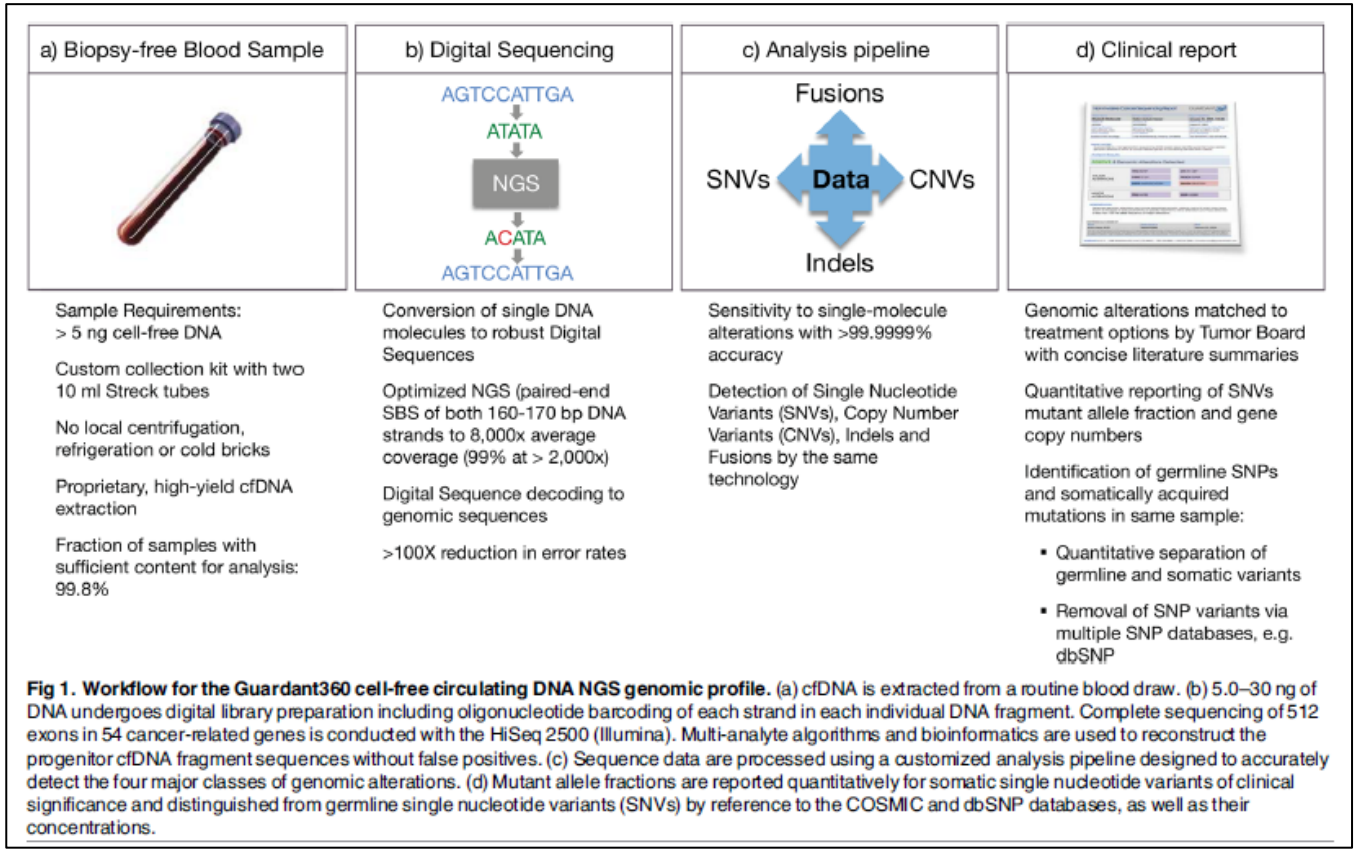
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Technical Information at 5 ("The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform."); *id.* at 6 (listing "Illumina NextSeq 550 Sequencing System" as an instrument for use with the Guardant360 CDx Assay).) Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.") Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides [76].") Lanman et al. at 17 ("For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs.") Lanman et al. at 18 ("These digital sequence libraries are amplified and then subsequently enriched for target genes using biotinylated custom baits of RNA probes.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3540 ("Here, we describe definitive analytic and clinical validation studies of a comprehensive tumor-derived cell-free DNA sequencing assay for clinical genotyping of advanced solid tumors and report clinical experience applying this technology in the clinical care of 10,593 consecutive patients.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.") '822 patent, claim 1 ("[A]mplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The resulting DNA is amplified by PCR to create libraries suitable for enrichment….Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1d.] generating tag associated sequence reads by sequencing the amplified tagged nucleic acid molecules of step (c);	Guardant generates tag associated sequence reads by sequencing the amplified tagged nucleic acid molecules of step (c). For example, Guardant sequences the enriched libraries of unique tagged nucleic acid molecules. Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Paired-end sequencing by synthesis is performed with the Illumina NextSeq 550 Sequencing system. The amplified cfDNA is analyzed by parallel sequencing of amplified target genes to an average depth of coverage of greater than 2,700 unique molecules.") Guardant 360CDx Technical Information at 5 ("The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 Platform.").) Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.") Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

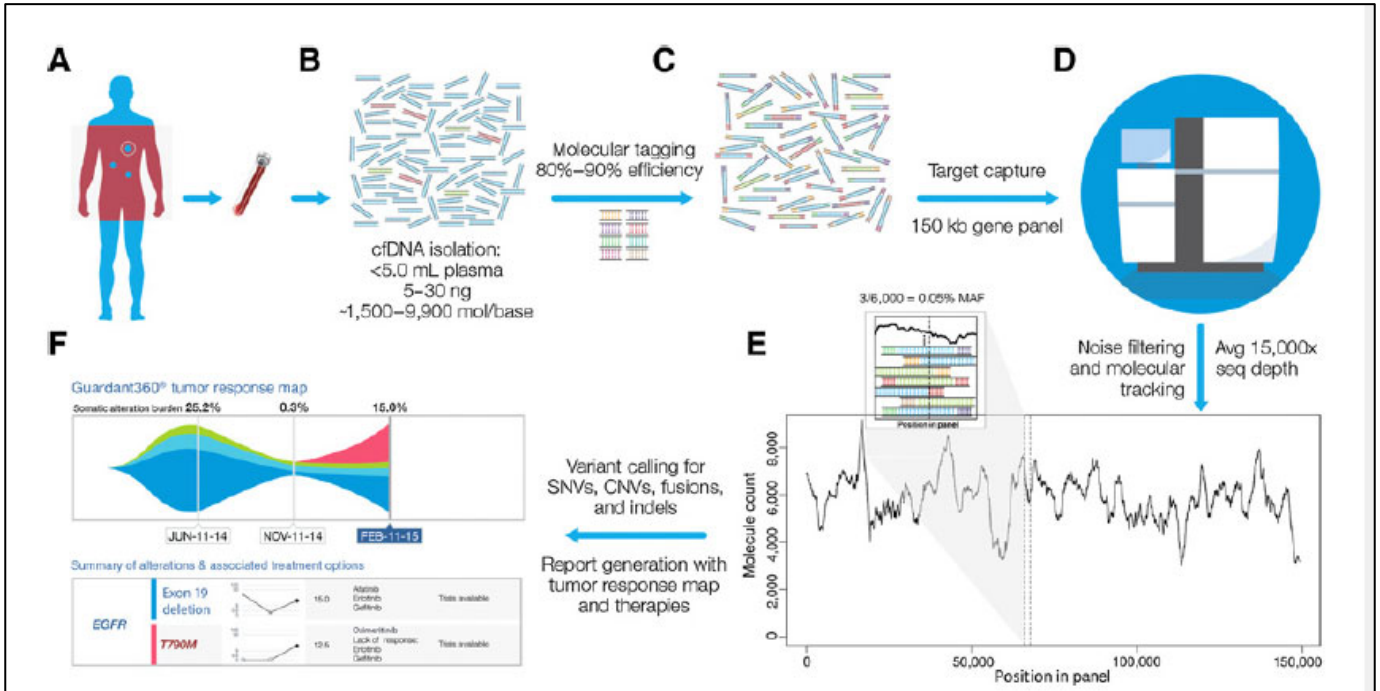


Lanman et al. at 5. (Fig. 1 above)

Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call. This process is akin to building a separate noise model for each and every one of the 78,000 bases in the sequencing panel. Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3539 ("High-concordance with orthogonal clinical plasma- and tissue-based genotyping methods supports the clinical accuracy of digital sequencing across all four types of targetable genomic

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	alterations. Digital sequencing's clinical applicability is further supported by high rates of technical success and biomarker target discovery.") Odegaard et al. at 3539 ("All cell-free DNA (cfDNA) extraction, processing, and sequencing was performed in a CLIA-certified, CAP accredited laboratory (Guardant Health, Inc.) as described previously (24).") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).").) Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1). Sequencing reads are then used to reconstruct each individual cfDNA molecule present in the original patient sample with high-fidelity using proprietary double-stranded consensus representation.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[S]equencing the population of amplified progeny polynucleotides to produce a set of sequence reads.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1e.] assigning each of the amplified tagged nucleic acid molecules to a location on a reference genome from the same species from which the sample has been obtained by mapping each of the tag associated sequence reads of step (d) to a location of the reference genome; and	Guardant assigns each of the amplified tagged nucleic acid molecules to a location on a reference genome from the same species from which the sample has been obtained by mapping each of the tag associated sequence reads of step (d) to a location of the reference genome. For example, Guardant uses software, such as CASAVA, to map the amplified unique tagged molecules to a reference genome, which can be used to reconstruct the genome attributed to the sample. Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations.") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv: 1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("Fusion events were detected by merging overlapping paired-end reads to form a representation of the sequenced cfDNA molecule which was then, aligned, mapped to initial unique cfDNA molecules based on molecular barcoding alignment information, including soft clipping.") '822 patent, claim 1 ("[M]apping sequence reads of the set of sequence reads to one or more reference sequences from a human genome.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1f.] at a plurality of locations on the	At a plurality of locations on the reference genome, Guardant counts the number of unique tagged nucleic acid molecules that have been assigned to the same location on the reference genome based on the

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
reference genome, counting the number of unique tagged nucleic acid molecules that have been assigned to the same location on the reference genome based on the mapping step to obtain a count for each location of the plurality of locations on the reference genome, thereby obtaining copy number information from genomic nucleic acids in the sample.	mapping step to obtain a count for each location of the plurality of locations on the reference genome, thereby obtaining copy number information from genomic nucleic acids in the sample. For example, Guardant determines the copy number variation (CNV) or amplification of a given gene or locus by measuring the total number of unique copies of the sequence mapped to a location on the reference genome. Prospectus at 121 ("Copy number variants manifest in higher than expected coverage, or number of unique molecules mapping to a given amplified region of the genome, compared to that expected for a typical diploid genome. Since observed coverage is highly variable from region to region depending on sequence composition and efficiency of amplification for each individual sample, it is highly non-uniform even when no copy number variants are present. As such, copy number variation is significantly harder to identify than small sequence variants, such as SNVs, insertions, and deletions considered in the previous figure.").) Guardant360 CDx Technical Information at 1 ("Guardant360® CDx is a qualitative next generation sequencing-based *in vitro* diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of . . . copy number amplifications (CNAs) in two (2) genes . . . .") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 3-4 (Table 2—reporting copy number amplifications of genes *ERBB2* and *MET*).

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

Table 2. Genes Containing Alterations Detected by the Guardant360® CDx

Alteration Type	Genes
Single Nucleotide Variants (SNVs)	*AKT1, ALK, APC, AR, ARAF, ATM*, BRAF, BRCA1**, BRCA2**, CCND1, CDH1, CDK4, CDK6, CDK12*, CDKN2A, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MTOR, MYC, NF1, NFE2L2, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, RHEB, ROS1, SMAD4, SMO, STK11, TERT, TSC1, VHL*
Indels	*AKT1, ALK, APC, ATM*, BRAF, BRCA1**, BRCA2**, CDH1, CDK12*, CDKN2A, EGFR, ERBB2, ESR1, FGFR2, GATA3, HNF1A, HRAS, KIT, KRAS, MET, MLH1, NF1, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL*

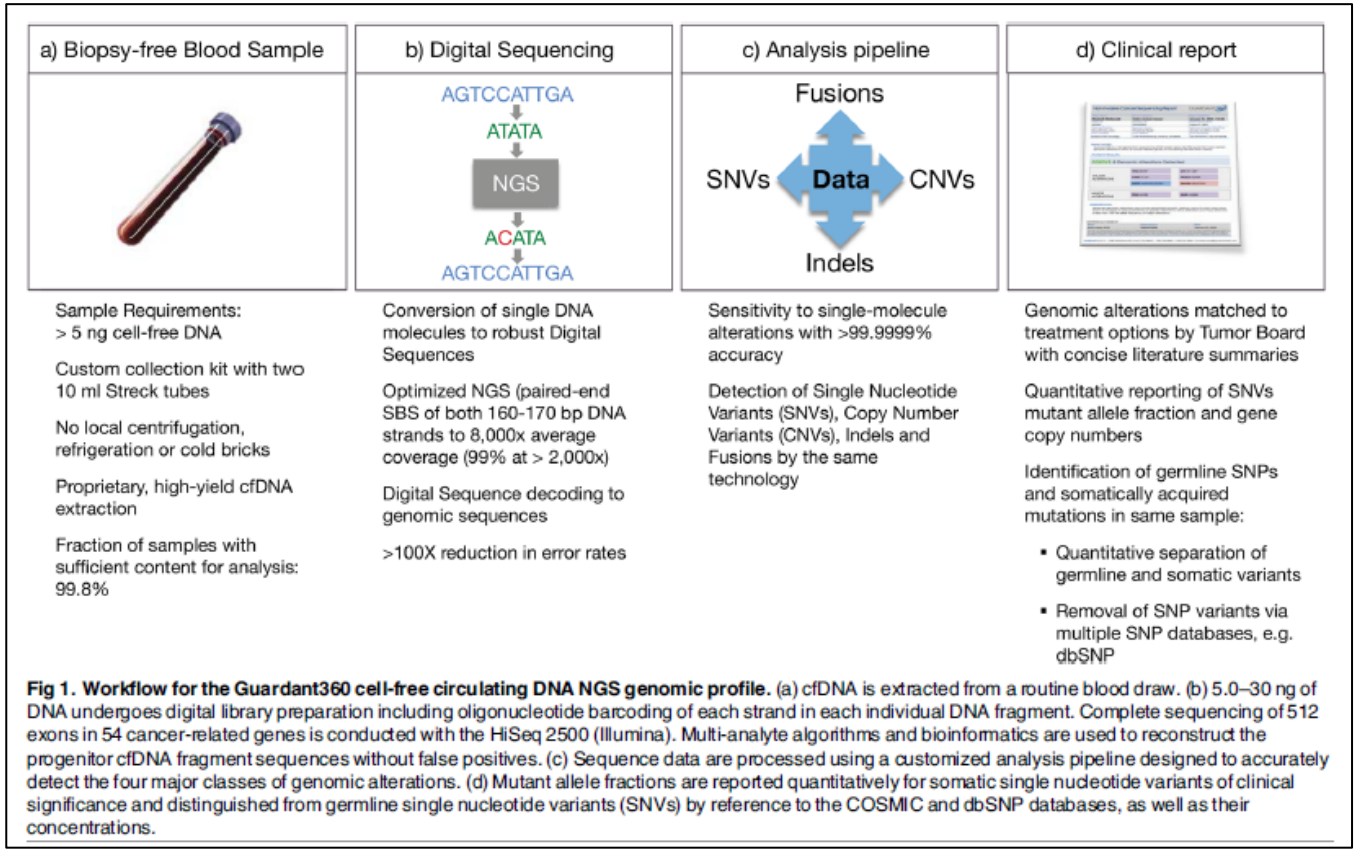
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Alteration Type	Genes
Copy Number Amplifications (CNAs)	*ERBB2, MET*
Fusions / Rearrangements	*ALK, NTRK1, RET, ROS1*

*Reporting is enabled for pathogenic germline alterations only. Somatic alterations will not be reported.
**Reporting is enabled for both germline and somatic alterations.

Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detectes (sic) single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23]."

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Lanman et al. at 5. (Fig. 1 above).

Lanman et al. at 14 ("Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 17 ("Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call. This process is akin to building a separate noise model for each and every one of the 78,000 bases in the sequencing panel. Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlap-ping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 20 ("[T]he Guardant360 panel expresses the absolute copy number or focal amplification of a gene in the plasma, and when 2.2 or higher, this may represent a clinically significant increase in the copy number of the gene in cancer cells in tumor samples.") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv: 1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3540 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at gene level. CAN determinations were based on training set-established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.") '822 patent, title ("Systems and methods to detect rare mutations and copy number variation.") '822 patent, claim 1 ("[G]rouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide; g) at each genetic locus of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus; and h) determining a frequency of one or more bases called at the locus from among the families." '822 patent, claim 2 ("The method of claim 1, further comprising detecting, at one or more loci, at least one single nucleotide variant, at least one gene fusion and at least one copy number variant.") '128 patent, 67:9-14 ("Guardant360™ is a targeted cfDNA NGS panel using hybrid capture and complete exon sequencing for single nucleotide variant detection in 70 genes, copy number amplifications (CNA) in

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	16 genes, fusions in six genes, and small indels in EGFR, ERBB2 and MET exon 14 skipping (see FIG. 20).") Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows−Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 2

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, further comprising estimating a copy number at multiple locations within a region of the reference genome.	Guardant estimates a copy number at multiple locations within a region of the reference genome. For example, Guardant determines the copy number of different genes, which are located at different locations in a given region of the reference genome. Guardant Technical Information at 1 ("Guardant360® CDx is a qualitative next generation sequencing-based *in vitro* diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in two (2) genes, and fusions in four (4) genes.")

<table>
<tr><th>Claim Term</th><th>Evidence Showing Defendant's Practice of Each Claim Term</th></tr>
<tr><td></td><td>Guardant Technical Information Table 3 (page 6):

Table 3. Genes Containing Alterations Reported by Guardant360 CDx
<table><tr><th>Alteration Type</th><th>Genes</th></tr><tr><td>Single Nucleotide Variants (SNVs)</td><td>AKT1, ALK, APC, AR, ARAF, ATM*, BRAF, BRCA1**, BRCA2**, CCND1, CDH1, CDK4, CDK6, CDK12*, CDKN2A, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MTOR, MYC, NF1, NFE2L2, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, RHEB, ROS1, SMAD4, SMO, STK11, TERT, TSC1, VHL</td></tr><tr><td>Indels</td><td>AKT1, ALK, APC, ATM*, BRAF, BRCA1**, BRCA2**, CDH1, CDK12*, CDKN2A, EGFR, ERBB2, ESR1, FGFR2, GATA3, HNF1A, HRAS, KIT, KRAS, MET, MLH1, NF1, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL</td></tr><tr><td>Copy Number Amplifications (CNAs)</td><td>ERBB2, MET</td></tr><tr><td>Fusions</td><td>ALK, NTRK1, RET, ROS1</td></tr></table>*Reporting is enabled for pathogenic germline alterations only. Somatic alterations will not be reported.
** Reporting is enabled for both germline and somatic alterations.

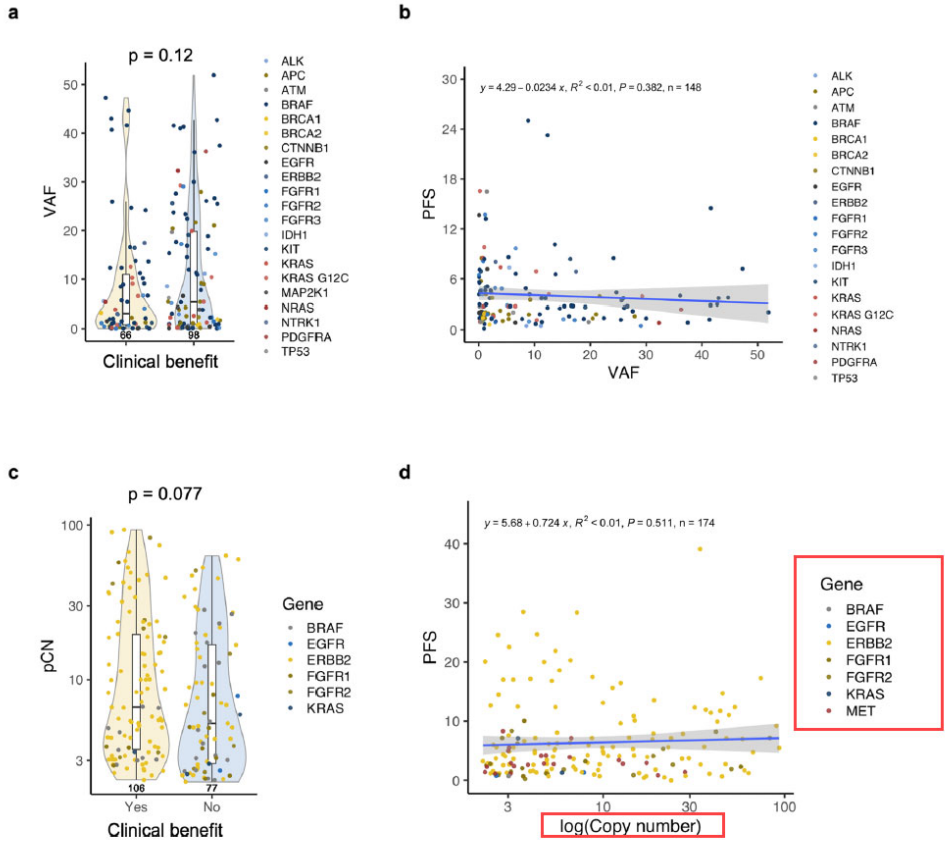
Odegaard et al. at 3543 ("To validate CNA detection performance, cell line cfDNA harboring known amplifications of ERBB2, MET, EGFR, MYC, CCND1, and CCNE1 was used to bracket the expected LOD95 in 120 near-LOD observations. LOD95 estimates constructed for each gene independently ranged from 2.24 copies (ERBB2) to 2.76 (CCND2, the smallest gene on the panel), median 2.44 (Fig. 3A; Supplementary Fig. S2D). Using multiplex-baselined ddPCR, we validated CNA accuracy in 14 cell lines comprising 43 amplifications (3–74 copies, median 4) in 10 genes."); 3545 ("We similarly assessed the accuracy of copy number determination relative to ddPCR in 70 samples with detectable CNAs in 8 genes (copy numbers 2.33–48.0) and 49 samples without.")

'128 patent, 67:9-14 ("Guardant360™ is a targeted cfDNA NGS panel using hybrid capture and complete exon sequencing for single nucleotide variant detection in 70 genes, copy number amplifications (CNA) in</td></tr>
</table>

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	16 genes, fusions in six genes, and small indels in EGFR, ERBB2 and MET exon 14 skipping (see FIG. 20).") Lanman et al. at 3 ("The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23]."); 9 ("CNVs for three genes, EGFR, ERBB2 and MET, were determined by utilizing nine cell lines well-characterized by fluorescence in situ hybridization (FISH) [35–39]. The cfDNA from these cell lines was extracted from cell culture media and sequenced from libraries prepared using 15 ng input amounts over two separate flow cells. Results showed an excellent correlation with high diagnostic accuracy (Table 3)."); 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 9 ("CNVs for three genes, EGFR, ERBB2 and MET, were determined by utilizing nine cell lines well-characterized by fluorescence in situ hybridization (FISH) [35–39]. The cfDNA from these cell lines was extracted from cell culture media and sequenced from libraries prepared using 15 ng input amounts over two separate flow cells. Results showed an excellent correlation with high diagnostic accuracy (Table 3).") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Extended Data Fig. 8 | Association of variant allelic fraction (VAF) or plasma copy number (pCN) with therapeutic efficacy. a. VAF of variants targeted in patients with clinical benefit and no benefit. The clonality was significantly higher in patients with clinical benefit (two-sided p = 0.001, Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within 1.5× of the interquartile range; and dots indicate outliers. **b.** Correlation of progression-free survival (PFS) with VAF. Shaded area represents 95% confidence interval. P values were calculated using the two-sided Wald test. **c.** pCN of gene amplification targeted in patients with clinical benefit and no benefit. The pCN was significantly higher in patients with clinical benefit (two-sided p = 0.077, Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within 1.5× of the interquartile range; and dots indicate outliers. **d.** Correlation of PFS with pCN. Shaded area represents 95% confidence interval. P values were calculated using the two-sided Wald test.

Nakamura et al. at Extended Data Fig. 8.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Nakamura et al. at "Methods" ("Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described43. Guardant360 interrogates 74 genes and detects single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study, 2 × 10 ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 3

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, further comprising comparing a count from counts obtained in step (f) for a location on the reference genome to a count for the same location on the reference genome	Guardant compares a count from counts obtained in step(f) for a location on the reference genome to a count for the same location on the reference genome obtained from a reference sample, thereby estimating a relative copy number at the location. For example, Guardant uses software to align sequence reads of a given location that were obtained from a sample to a location on a reference genome (e.g., the genome of a healthy patient), and compares the number of sequence reads associated with that locus obtained from the sample to the number of counts of sequence reads associated with that locus on the reference genome.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
obtained from a reference sample, thereby estimating a relative copy number at the location.	Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Guardant Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ $\geq$ 60).") Odegaard et al. at 3541 ("Copy number determination was assessed using a compendium of multiplexed PCR assays against reference genes in combination with target-specific PCR assays. Copy number was determined from the average of the reference gene signal relative to the total target signal.") Odegaard et al. at 3541 ("CNAs, in contrast, are not detectable at the level of individual molecules and are instead defined by statistical overrepresentation of reference sequences in the cfDNA. As such, we utilized the CLSI "Classical Approach" (CLSI EP17-A2) to statistically determine copy number LOD based on variation observed in titration series replicates and normal samples by calculating the limit where estimated copy number and Z-score values jointly have a 95% probability of exceeding the decision threshold.") Odegaard et al. at 3541 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. CNA determinations were based on training set–established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.") Nakamura et al. at "Methods." ("Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described43. Guardant360 interrogates 74 genes and detects single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study, 2 × 10 ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") '822 patent at 9:8-31 ("In another embodiment wherein the sets derive from a common sample, the method further comprising: a. inferring copy number variation for the plurality of sets based on a comparison of the inferred number of tagged parent polynucleotides in each set mapping to each of a plurality of reference sequences. In another embodiment the original number of polynucleotides in each set is further inferred. This disclosure also provides a system comprising a computer readable medium for performing the aforesaid methods. This disclosure also provides a method of determining copy number variation in a sample that includes polynucleotides, the method comprising: a. providing at least two sets of first polynucleotides, wherein each set maps to a different reference sequence in a genome, and, for each set of first polynucleotides; i. amplifying the polynucleotides to produce a set of amplified polynucleotides; ii. sequencing a subset of the set of amplified polynucleotides, to produce a set of sequencing reads; iii. group sequences reads sequence from amplified polynucleotides into families, each family amplified from the

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	same first polynucleotide in the set; iv. inferring a quantitative measure of families in the set; b. determining copy number variation by comparing the quantitative measure of families in each set.") '822 patent at 10:52-11:3 ("This disclosure also provides a computer readable medium in non-transitory, tangible form comprising executable code configured to perform the following steps: a. accessing a data file comprising a plurality of sequence reads; b. filtering out reads that fail to meet a set threshold; c. mapping sequence reads derived from the sequence onto a reference sequence; d. identifying a subset of mapped sequence reads that align with a variant of the reference sequences at each mappable base position; e. for each mappable base position, calculating a ratio of (a) a number of mapped sequence reads that include a variant as compared to the reference sequence, to (b) a number of total sequence reads for each mappable base position; f. normal- izing the ratios or frequency of variance for each mappable base position and determining potential rare variant(s) or other genetic alteration(s); and g. comparing the resulting number for each of the regions with potential rare variant(s) or mutation(s) to similarly derived numbers from a reference sample.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library. The deviation from ideal distribution is calculated and modeled through a non-parametric regression to remove sample-preparation- and sequencing-associated biases. Subsequently, the mode of the normalized number of fragments covering each gene is calculated to estimate the fragment number corresponding to two copies to derive a baseline diploid value. All values of unique fragments for each gene are then normalized by this baseline value. The baseline derivation is informed by molecule counts data from a large set of normal samples from healthy donors' plasma (Normal Set) provided by AllCells (Alameda, CA) with donor consent. The age range of donors is 20 to 40 years and input plasma is screened to provide a minimum input of 15 ng of cfDNA. A statistical z-score for each gene is calculated by comparing the normalized gene fragment number to that derived from the Normal Set to estimate the likelihood of the gene exhibiting amplification and remove any remaining region specific bias.")

ASSERTED CLAIM 6

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, further comprising (l) summing the counts from the counts obtained in step (f) for locations at a region of the reference genome; (m) summing the counts obtained from a reference sample for the same region of the reference genome; and comparing the sums of the counts obtained in step (l) and step (m), thereby estimating a relative copy number at the region of the reference genome.	Guardant (l) sums the counts from the counts obtained in step (f) for locations at a region of the reference genome; (m) sums the counts obtained from a reference sample for the same region of the reference genome; and compares the sums of the counts obtained in step (l) and step (m), thereby estimating a relative copy number at the region of the reference genome. For example, Guardant uses an algorithm to process, map, and compare the number of times a sequence appears in a sample to the number of times a sequence appears in a reference genome. Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("CNAs, in contrast, are not detectable at the level of individual molecules and are instead defined by statistical overrepresentation of reference sequences in the cfDNA. As such, we utilized the CLSI "Classical Approach" (CLSI EP17-A2) to statistically determine copy number LOD based on variation observed in titration series replicates and normal samples by calculating the limit where estimated copy number and Z-score values jointly have a 95% probability of exceeding the decision threshold.") Odegaard et al. at 3541 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. CNA determinations were based on training set–established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Guardant Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60).") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library. The deviation from ideal distribution is calculated and modeled through a non-parametric regression to remove sample-preparation- and sequencing-associated biases. Subsequently, the mode of the normalized number of fragments covering each gene is calculated to estimate the fragment number corresponding to two copies to derive a baseline diploid value. All values of unique fragments for each gene are then normalized by this baseline value. The baseline derivation is informed by molecule counts data from a large set of normal samples from healthy donors' plasma (Normal Set) provided by AllCells (Alameda, CA) with donor consent. The age range of donors is 20 to 40 years and input plasma is screened to provide a minimum input of 15 ng of cfDNA. A statistical z-score for each gene is calculated by comparing the normalized gene fragment number to that derived from the Normal Set to estimate the likelihood of the gene exhibiting amplification and remove any remaining region specific bias.") '822 patent at 9:8-31 ("In another embodiment wherein the sets derive from a common sample, the method further comprising: a. inferring copy number variation for the plurality of sets based on a comparison of the inferred number of tagged parent polynucleotides in each set mapping to each of a plurality of reference sequences. In another embodiment the original number of polynucleotides in each set is further inferred. This disclosure also provides a system comprising a computer readable medium for performing the aforesaid methods. This disclosure also provides a method of determining copy number variation in a sample that includes polynucleotides, the method comprising: a. providing at least two sets of first

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	polynucleotides, wherein each set maps to a different reference sequence in a genome, and, for each set of first polynucleotides; i. amplifying the polynucleotides to produce a set of amplified polynucleotides; ii. sequencing a subset of the set of amplified polynucleotides, to produce a set of sequencing reads; iii. group sequences reads sequence from amplified polynucleotides into families, each family amplified from the same first polynucleotide in the set; iv. inferring a quantitative measure of families in the set; b. determining copy number variation by comparing the quantitative measure of families in each set.") '822 patent at 10:52-11:3 ("This disclosure also provides a computer readable medium in non-transitory, tangible form comprising executable code configured to perform the following steps: a. accessing a data file comprising a plurality of sequence reads; b. filtering out reads that fail to meet a set threshold; c. mapping sequence reads derived from the sequence onto a reference sequence; d. identifying a subset of mapped sequence reads that align with a variant of the reference sequences at each mappable base position; e. for each mappable base position, calculating a ratio of (a) a number of mapped sequence reads that include a variant as compared to the reference sequence, to (b) a number of total sequence reads for each mappable base position; f. normal- izing the ratios or frequency of variance for each mappable base position and determining potential rare variant(s) or other genetic alteration(s); and g. comparing the resulting number for each of the regions with potential rare variant(s) or mutation(s) to similarly derived numbers from a reference sample.") Nakamura et al. at "Methods" ("Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described43. Guardant360 interrogates 74 genes and detects single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study, 2 × 10 ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming.

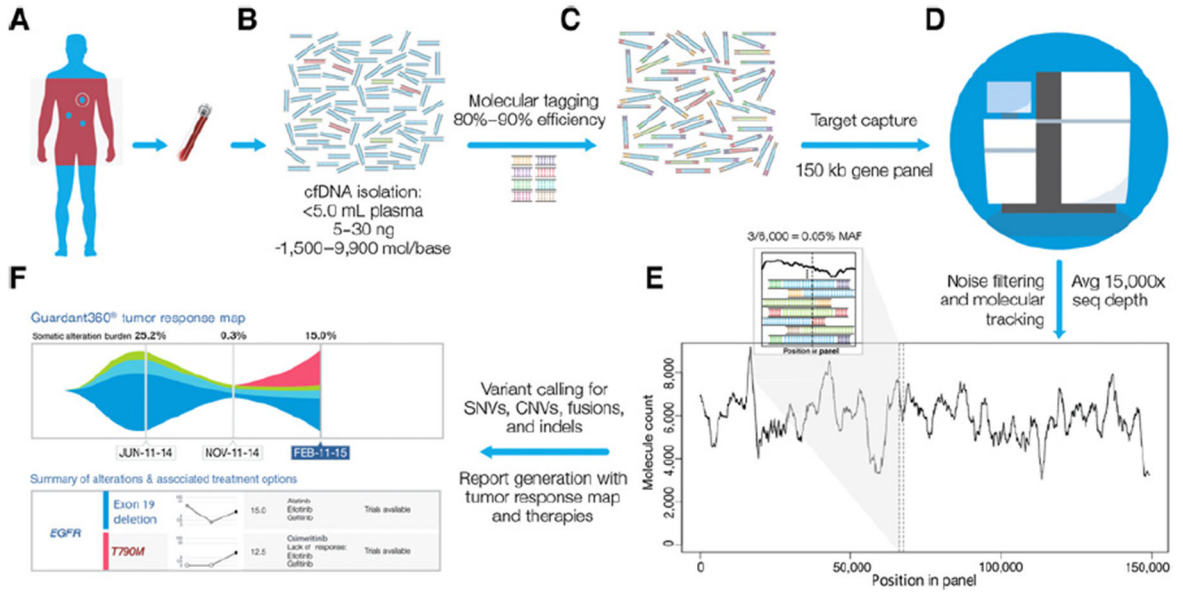
GUARDANT HEALTH EX.1020.060

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 7

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the unique tagged nucleic acid molecules are subject to a hybrid capture method prior to PCR in step (c) or the amplified tagged nucleic acid molecules produced in step (c) are subject to a hybrid capture method prior to step (d).	Guardant subjects the unique tagged nucleic acid molecules to a hybrid capture method prior to PCR in step (c) or to a hybrid capture method prior to step (d). For example, Guardant amplifies DNA to create a library using hybrid capture, tags the DNA, and also amplifies the unique tagged nucleic acid molecules using hybrid capture to create an enriched library that can be sequenced. Lanman et al. at 18 ("These digital sequence libraries are amplified and then subsequently enriched for target genes using biotinylated custom baits of RNA probes") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The resulting DNA is amplified by PCR to create libraries suitable for enrichment….Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Guardant360 CDx Technical Information at 5 ("The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform."); *id.* at 6 (listing "Illumina NextSeq 550 Sequencing System" as an instrument for use with the Guardant360 CDx Assay).) Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides [76].") Lanman et al. at 17("For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3540 ("Here, we describe definitive analytic and clinical validation studies of a comprehensive tumor-derived cell-free DNA sequencing assay for clinical genotyping of advanced solid tumors and report clinical experience applying this technology in the clinical care of 10,593 consecutive patients.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries,

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

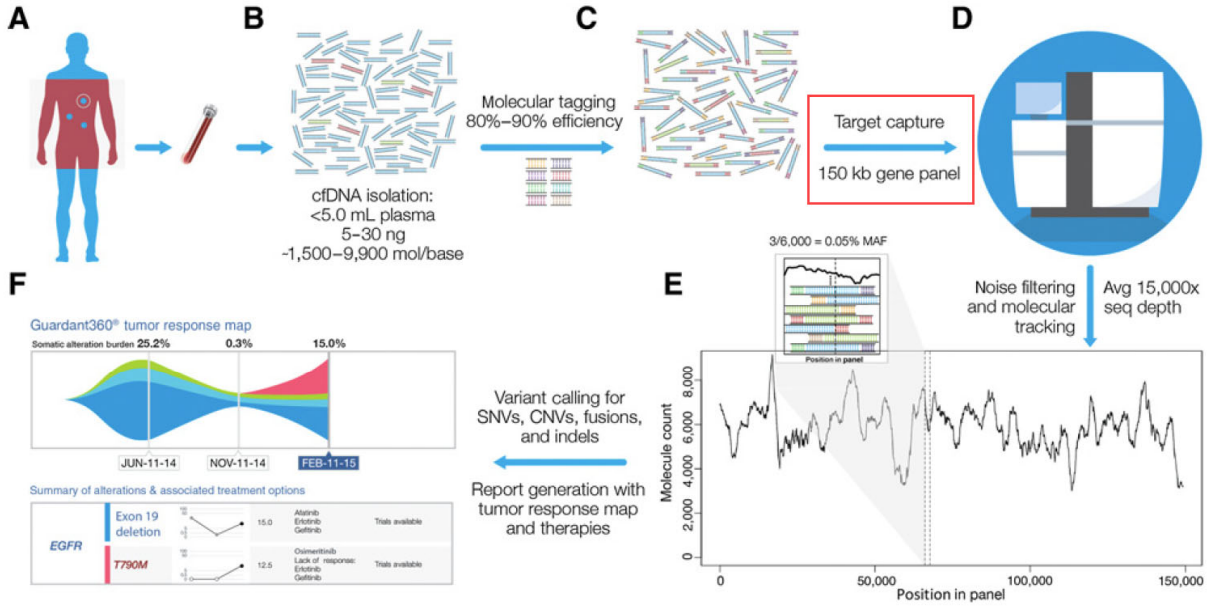
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3542 ("cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced"). '822 patent, claim 1 ("[A]mplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The resulting DNA is amplified by PCR to create libraries suitable for enrichment….Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence."). Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 1 ("Guardant360 CDx is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in two (2) genes, and fusions in four (4) genes."). Guardant 360CDx Technical information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform."). '128 patent, 67:9-14 ("Guardant360™ is a targeted cfDNA NGS panel using hybrid capture and complete exon sequencing for single nucleotide variant detection in 70 genes, copy number amplifications (CNA) in 16 genes, fusions in six genes, and small indels in EGFR, ERBB2 and MET exon 14 skipping (see FIG. 20).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina).")

ASSERTED CLAIM 8

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 7, wherein each of the unique tagged nucleic acid molecules subjected to the hybrid capture method has a nucleic acid sequence which is complementary to a capture probe on an array or on a microarray.	Each of the unique tagged nucleic acid molecules subjected by Guardant to a hybrid capture has a nucleic acid sequence which is complementary to a capture probe on an array or on a microarray. For example, Guardant uses arrays containing capture probes that target the unique tagged nucleic acid molecules. Guardant CDx Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60). None of the primers or probes mapped to the representative microbial contaminant genomes.") Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina)."). Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).") Odegaard et al. at 3542 ("The combination of high-efficiency (80%–90%) input molecule labeling, optimized assay conditions, and hybridization probe design allows DS to recover 60%–85% of all input molecules postalignment (Fig. 2A), depending on cfDNA input, across the entire range of G:C content (Fig. 2B) with highly uniform coverage (13% quartile-based coefficient of variation with >96% of exonic panel positions covered above 0.2 x median panel coverage; Fig. 2C).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3542 (Fig. 1) Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET. The gene list was selected to focus on those genomic alterations that are currently actionable, which is defined as being targets of sensitivity or resistance to an FDA-approved matched therapy and/or a targeted therapy in later stage clinical trials. The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides [76].") '822 patent at 38:6-9("Often, the identifier is a bar-code oligonucleotide that is used to tag the polynucleotide; but, in some cases, different unique identifiers are used. For example, in some cases, the unique identifier is a hybridization probe.")

ASSERTED CLAIM 9

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein each of the tagged nucleic acid molecules differs at more than one nucleotide.	Each of the nucleic acid molecules tagged by Guardant differs at more than one nucleotide. For example, Guardant attaches tags to nucleic acid molecules such that each copy of a given sequence contains a tag, creating nucleic acid molecules where each has a unique sequence. Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the double stranded input cfDNA samples is separately encoded with said oligonucleotides.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	This digital conversion process occurs with high efficiency with greater than 80% of the original plasma of cfDNA molecules converted.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.)."), 3542 ("Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

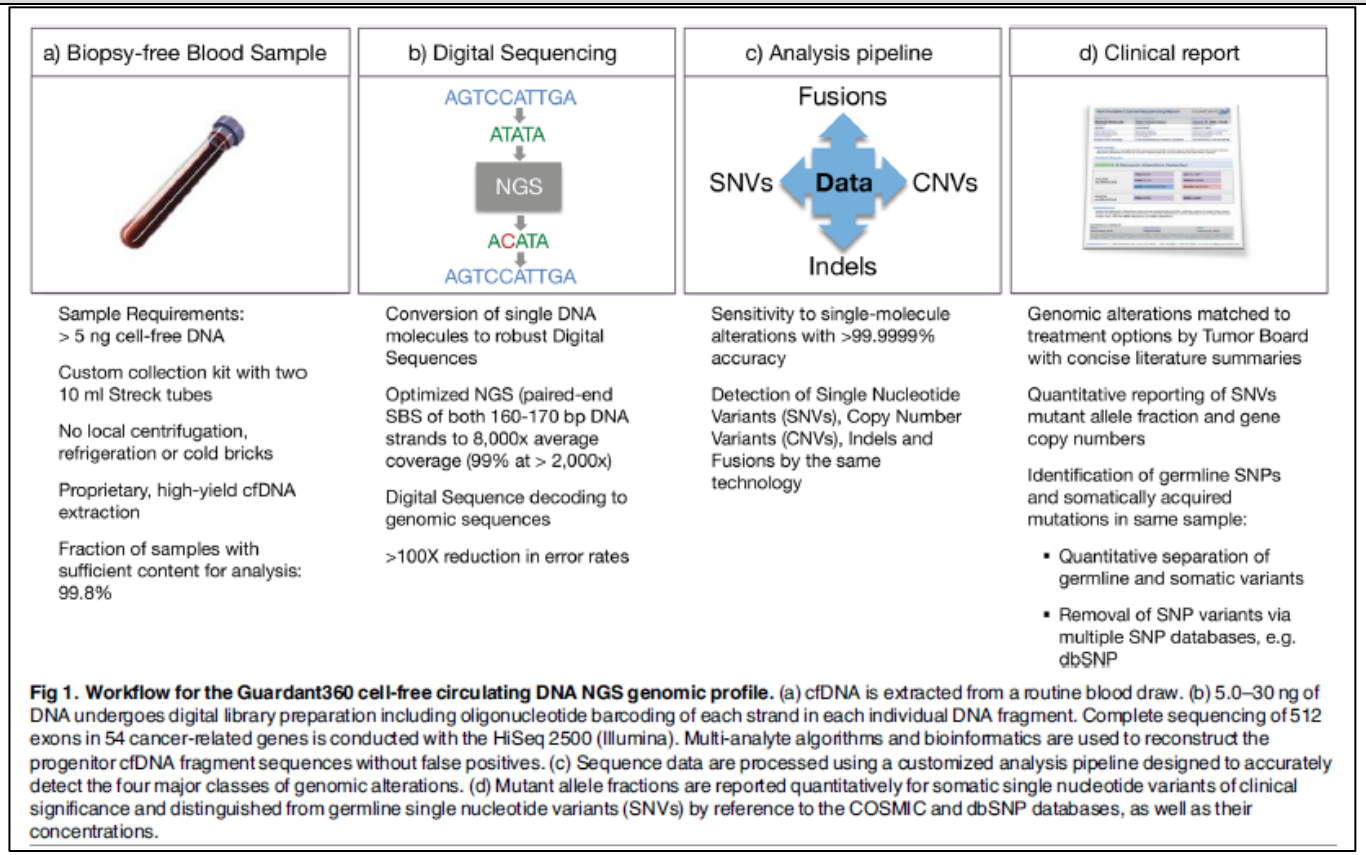
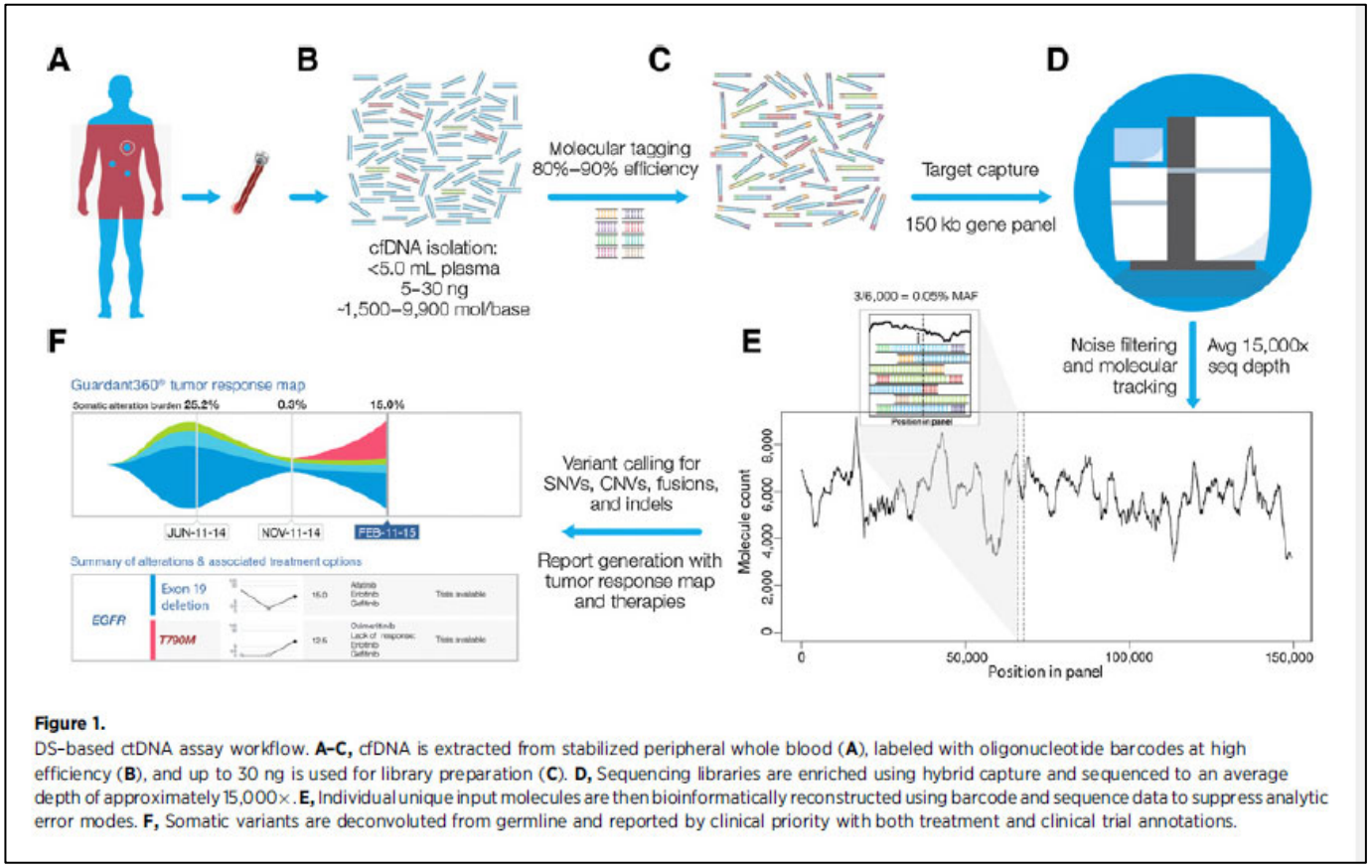


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1).

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	'822 patent, 3:25-35 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step. In some embodiments, the barcode is a polynucleotide, which may further comprise random sequence or a fixed or semi-random set of oligonucleotides that in combination with the diversity of molecules sequenced from a select region enables identification of unique molecules and be at least a 3, 5, 10, 15, 20, 25, 30, 40, 45, or 50mer base pairs in length.") '822 patent, 1:49-63 ("The disclosure provides for a method for detecting copy number variation comprising: a) sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide are optionally attached to unique barcodes; b) filtering out reads that fail to meet a set threshold; c) mapping sequence reads obtained from step (a) to a reference sequence; d) quantifying/counting mapped reads in two or more predefined regions of the reference sequence; e) determining a copy number variation in one or more of the predefined regions by (i) normalizing the number of reads in the predefined regions to each other and/or the number of unique barcodes in the predefined regions to each other; and (ii) comparing the normalized numbers obtained in step (i) to normalized numbers obtained from a control sample.") '822 patent, 6:26-27 ("In some embodiments each tagged parent polynucleotide in the set is uniquely tagged.")

ASSERTED CLAIM 10

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein said tagging the segments with nucleic acid tags comprises ligation of adaptors comprising	Guardant's method of tagging the segments with nucleic acid tags comprises ligation of adaptors comprising the tags to at least one end of the segments of the genomic nucleic acids. For example, Guardant incorporates barcodes into adaptors that it attaches to the ends of nucleic acid molecules obtained from samples.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
the tags to at least one end of the segments of the genomic nucleic acids.	Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1). Sequencing reads are then used to reconstruct each individual cfDNA molecule present in the original patient sample with high-fidelity using proprietary double-stranded consensus sequence representation.") Lanman et al. at 18 ("The DNA is extracted using standard extraction procedures, including proteinase K digestion of histone and other DNA-binding proteins and isoproponal precipitation. The DNA is then end-repaired to repair ends of DNA with a 5' phosphate prior to ligation of adapters necessary for downstream sequencing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations. Custom scripts are then used to (a) remove spurious variants ("noise") created by sequencing errors, (b) identify all germline single nucleotide polymorphisms (SNPs) and somatic single nucleotide variants (SNVs), and (c) call somatic SNVs, while removing erroneous variants resulting from sequencing errors, DNA damage, strand bias, etc. These steps complete the digital sequencing process and enable post-sequencing removal of the false positives associated with detecting somatic variants present in cfDNA at low concentrations.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

using blunt-end ligation. The resulting DNA is amplified by PCR to create libraries suitable for enrichment.")

6.7 Guardbanding/ Robustness

The purpose of the guardbanding study was to evaluate cfDNA input at the minimum input amount (5 ng) and the maximum amount (30 ng), adapter volume tolerances for ligation steps, hybridization time tolerances in the enrichment process and wash buffer 2 temperature tolerances in the enrichment process (**Table 20**).

Table 20. Guardbanding Study Overview

Guardbanding Condition	Reference condition	Condition 1	Condition 2
cfDNA Input amount	5 ng	2.5 ng	4 ng
cfDNA Input amount	30 ng	36 ng	45 ng
Adapter volume	18.0 µL	16.2 µL	19.8 µL
Hybridization Time	12 hours	24 hours	N/A
Wash Buffer Temperature	71°C	70°C	72°C

Guardant360 CDx Technical Information at 27.

ASSERTED CLAIM 11

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein each of the tagged nucleic acid molecules comprise more than one tag.	Guardant tags each of the nucleic acid molecules with more than one tag. For example, Guardant attaches one or more barcodes to the nucleic acid molecules. '822 patent at 3:25-28 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step.") '822 patent at 15:20-25 ("In some embodiments , the methods further comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to sequencing . In some embodiments, each barcode attached to extracellular polynucleotides or fragments thereof prior to sequencing is unique.") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call. This process is akin to building a separate noise model for each and every one of the 78,000 bases in the sequencing panel. Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.")

ASSERTED CLAIM 12

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the tags further comprise a sample tag.	Guardant tags each of the nucleic acid molecules with a sample tag. For example, Guardant multiplexes by pooling nucleic acid molecules obtained from different biological samples, and attaches sample tags to nucleic acid molecules so that it can trace sequencing reads back to the correct sample. Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guideline-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 1("Specifically, theoretical conversion efficiency of amplicon-based methods on cell-free DNA can be approximated by (1-x/166)*100%, where x is the length of the amplicon. For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs. High efficiency conversion is critical since it translates directly to sensitivity, i.e., one cannot achieve single-molecule sensitivity if only a fraction of a few input DNA molecules are convertible to analyzable material.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guideline-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 21 ("The 165 matched plasma and tissue samples were both analyzed on the Illumina Hi-Seq 2500 platform, the cfDNA analyzed using the digital sequencing method described herein and the DNA isolated from tissue samples analyzed using standard prep as per the manufacturer's instructions [76].") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("All variant detection analyses were performed using the locked clinical Guardant360 bioinformatics pipeline and reported unaltered by post hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads).") Odegaard et al. at 3541 ("Copy number determination was assessed using a compendium of multiplexed PCR assays against reference genes in combination with target-specific PCR assays. Copy number was determined from the average of the reference gene signal relative to the total target signal.") Odegaard et al. at 3541 ("Frequency of false positive calls was defined using a set of 408 validation runs (324 LOD pools and 84 healthy cfDNA donor runs across both NextSeq and HiSeq instruments) for which truth was orthogonally defined. Analytic specificity was determined by analysis of pooled samples at high dilution that had been orthogonally defined at high VAF. Precision was determined utilizing in-process positive controls from the clinical sequencing workflow and pooled clinical samples.") Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Guardant CDx Technical Information at 16 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform.") Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows−Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 13

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the tagged nucleic acid molecules generated from step (b) and that differ by a	During or after the counting step, Guardant ignores tagged nucleic acid molecules generated from step (b) that differ by a single nucleotide. For example, Guardant discards sequence reads that contain a sequencing error at a single position (i.e., those sequence reads that do not comprise the same sequence as other amplicons attributable to the same

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
single nucleotide are ignored during or after the counting step.	original starting molecule) to avoid considering such sequence reads in the analysis of the total number of counts of a given sequence. Odegaard et al. at 3540 ("As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads). Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("Specific reporting thresholds were determined by retrospective and training set analyses but were generally unique post realignment molecules meeting quality requirements, which in an average sample corresponds to a detection limit of approximately 0.04% VAF. To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. CNA determinations were based on training set–established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline. Per-sample relative tumor burden was determined by normalization to the mutational burden expected for tumor type and ctDNA fraction and reported as a Z-score.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations. Custom scripts are then used to (a) remove spurious variants ("noise") created by sequencing errors, (b) identify all germline single nucleotide polymorphisms (SNPs) and somatic single nucleotide variants (SNVs), and (c) call somatic SNVs, while removing erroneous variants resulting from sequencing errors, DNA damage, strand bias, etc. These steps complete the digital sequencing process and

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	enable post-sequencing removal of the false positives associated with detecting somatic variants present in cfDNA at low concentrations.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel."). Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence."); ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") '822 patent, 1:49-63 ("The disclosure provides for a method for detecting copy number variation comprising: a) sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide are optionally attached to unique barcodes; b) filtering out reads that fail to meet a set threshold; c) mapping sequence reads obtained from step (a) to a reference sequence; d) quantifying/counting mapped reads in two or more predefined regions of the reference sequence; e) determining a copy number variation in one or more of the predefined regions by (i) normalizing the number of reads in the predefined regions to each other and/or the number of unique barcodes in the

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	predefined regions to each other; and (ii) comparing the normalized numbers obtained in step (i) to normalized numbers obtained from a control sample.")

ASSERTED CLAIM 14

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the unique tagged nucleic acid molecules are pooled with another plurality of unique tagged nucleic acid molecules having a different sample tag prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules of step (c).	Prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules of step (c), Guardant pools unique tagged nucleic acid molecules with another plurality of unique tagged nucleic acid molecules having a different sample tag. For example, Guardant multiplexes by pooling nucleic acid molecules obtained from different biological samples, and attaches different sample tags to nucleic acid molecules from different samples so that it can trace sequence reads back to the correct sample. Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guideline-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 1 ("Specifically, theoretical conversion efficiency of amplicon-based methods on cell-free DNA can be approximated by (1-x/166)*100%, where x is the length of the amplicon. For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs. High efficiency conversion is critical since it translates directly to sensitivity, i.e., one cannot achieve single-molecule sensitivity if only a fraction of a few input DNA molecules are convertible to analyzable material.") Lanman et al. at 21 ("The 165 matched plasma and tissue samples were both analyzed on the Illumina Hi-Seq 2500 platform, the cfDNA analyzed using the digital sequencing method described herein and the DNA isolated from tissue samples analyzed using standard prep as per the manufacturer's instructions [76].") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries,

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	which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All variant detection analyses were performed using the locked clinical Guardant360 bioinformatics pipeline and reported unaltered by post hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads).") Odegaard et al. at 3541 ("Copy number determination was assessed using a compendium of multiplexed PCR assays against reference genes in combination with target-specific PCR assays. Copy number was determined from the average of the reference gene signal relative to the total target signal.") Odegaard et al. at 3541 ("Frequency of false positive calls was defined using a set of 408 validation runs (324 LOD pools and 84 healthy cfDNA donor runs across both NextSeq and HiSeq instruments) for which truth was orthogonally defined. Analytic specificity was determined by analysis of pooled samples at high dilution that had been orthogonally defined at high VAF. Precision was determined utilizing in-process positive controls from the clinical sequencing workflow and pooled clinical samples.") Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.").

ASSERTED CLAIM 15

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 14, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.	Guardant deconvolutes the tag associated sequence reads by grouping the tag associated sequence reads. For example, Guardant attributes amplified molecules containing the same unique sample tag to the original sample, and groups those sequence reads to the same sample. As another example, Guardant attributes amplified molecules containing the same unique molecular tag to the original molecule, and groups those sequence reads to the same original starting molecule. Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The Guardant360 CDx Software uses a custom-developed analysis bioinformatics pipeline (BIP) software module. The BIP

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Odegaard et al. at 3540 ("All variant detection analyses were performed using the locked clinical Guardant360 bioinformatics pipeline and reported unaltered by post hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("CNAs, in contrast, are not detectable at the level of individual molecules and are instead defined by statistical overrepresentation of reference sequences in the cfDNA. As such, we utilized the CLSI "Classical Approach" (CLSI EP17-A2) to statistically determine copy number LOD based on variation observed in titration series replicates and normal samples by calculating the limit where estimated copy number and Z-score values jointly have a 95% probability of exceeding the decision threshold.") Odegaard et al. at 3541 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. CNA determinations were based on training set–established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60).") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library. The deviation from ideal distribution is calculated and modeled through a non-parametric regression to remove sample-preparation- and sequencing-associated biases. Subsequently, the mode of the normalized number of fragments covering each gene is calculated to estimate the fragment number corresponding to two copies to derive a baseline diploid value. All values of unique fragments for each gene are then normalized by this baseline value. The baseline derivation is informed by molecule counts data from a large set of normal samples from healthy donors' plasma (Normal Set) provided by AllCells (Alameda, CA) with donor consent. The age range of donors is 20 to 40 years and input plasma is screened to provide a minimum input of 15 ng of cfDNA. A statistical z-score for each gene is calculated by comparing the normalized gene fragment number to that derived from the Normal Set to estimate the likelihood of the gene exhibiting amplification and remove any remaining region specific bias.") '822 patent at 9:8-31 ("In another embodiment wherein the sets derive from a common sample, the method further comprising: a. inferring copy number variation for the plurality of sets based on a comparison of the inferred number of tagged parent polynucleotides in each set mapping to each of a plurality of reference sequences. In another embodiment the original number of polynucleotides in each set is further inferred. This disclosure also provides a system comprising a computer readable medium for performing the aforesaid methods. This disclosure also provides a method of determining copy number variation in a sample that includes polynucleotides, the method comprising: a. providing at least two sets of first polynucleotides, wherein each set maps to a different reference sequence in a genome, and, for each set of first polynucleotides; i. amplifying the polynucleotides to produce a set of amplified polynucleotides; ii. sequencing a subset of the set of amplified polynucleotides, to produce a set of sequencing reads; iii. group

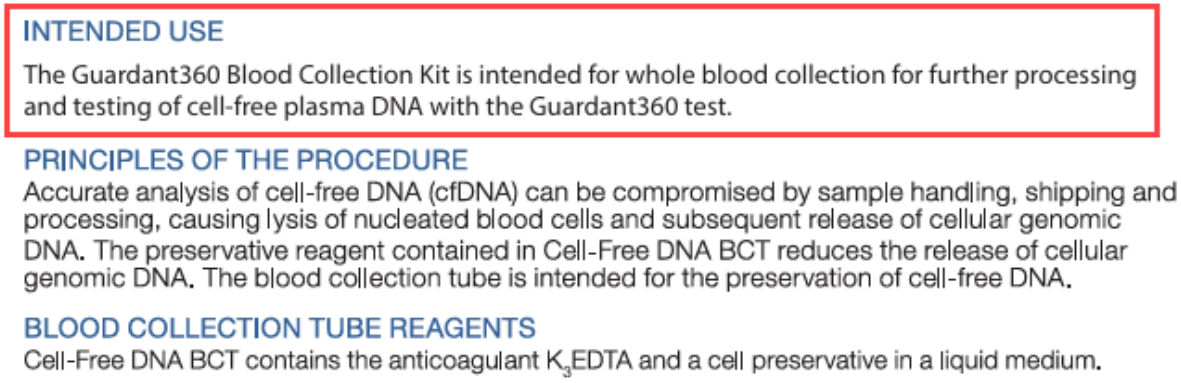
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	sequences reads sequence from amplified polynucleotides into families, each family amplified from the same first polynucleotide in the set; iv. inferring a quantitative measure of families in the set; b. determining copy number variation by comparing the quantitative measure of families in each set.") '822 patent at 10:52-11:3 ("This disclosure also provides a computer readable medium in non-transitory, tangible form comprising executable code configured to perform the following steps: a. accessing a data file comprising a plurality of sequence reads; b. filtering out reads that fail to meet a set threshold; c. mapping sequence reads derived from the sequence onto a reference sequence; d. identifying a subset of mapped sequence reads that align with a variant of the reference sequences at each mappable base position; e. for each mappable base position, calculating a ratio of (a) a number of mapped sequence reads that include a variant as compared to the reference sequence, to (b) a number of total sequence reads for each mappable base position; f. normal- izing the ratios or frequency of variance for each mappable base position and determining potential rare variant(s) or other genetic alteration(s); and g. comparing the resulting number for each of the regions with potential rare variant(s) or mutation(s) to similarly derived numbers from a reference sample.") Nakamura et al. at "Methods" ("Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described43. Guardant360 interrogates 74 genes and detects single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study, 2 × 10 ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing

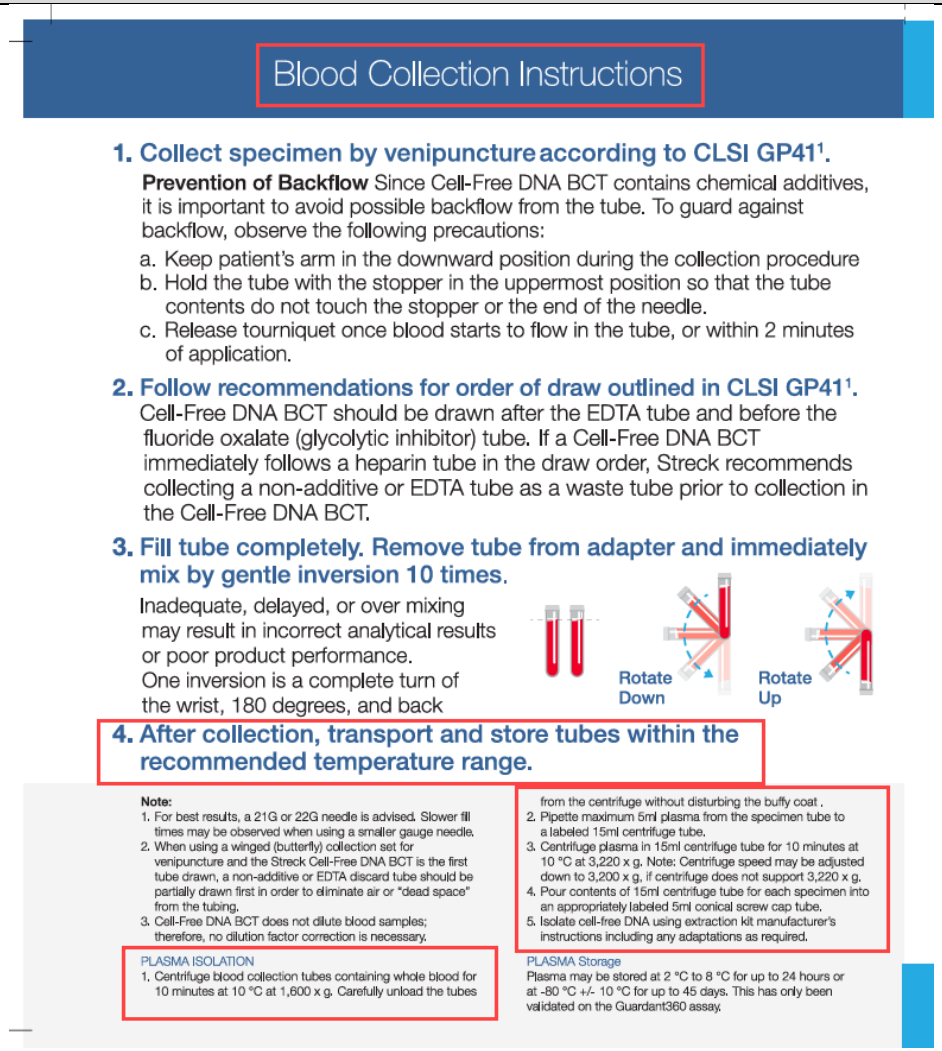
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

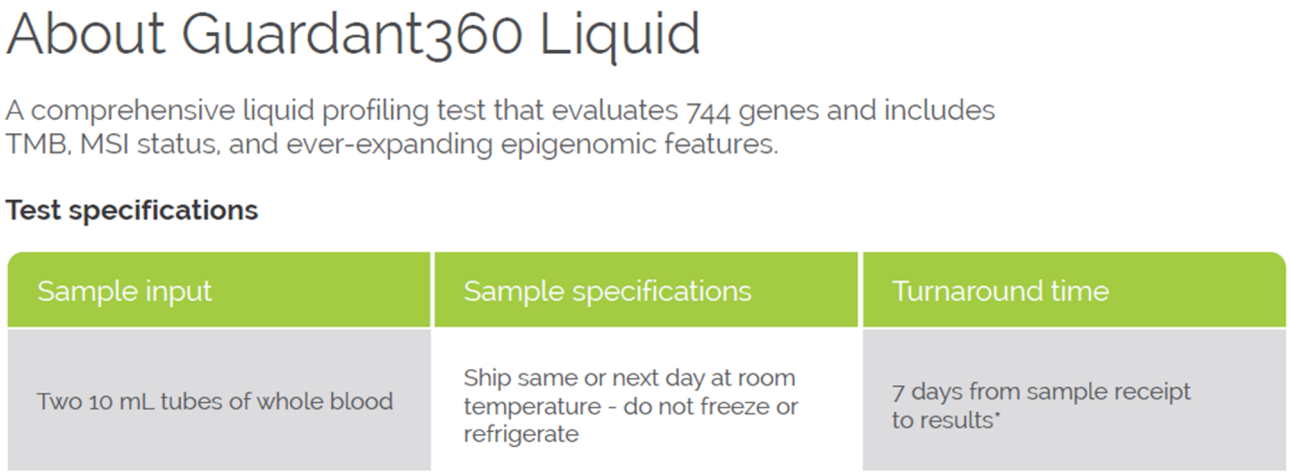
ASSERTED CLAIM 17

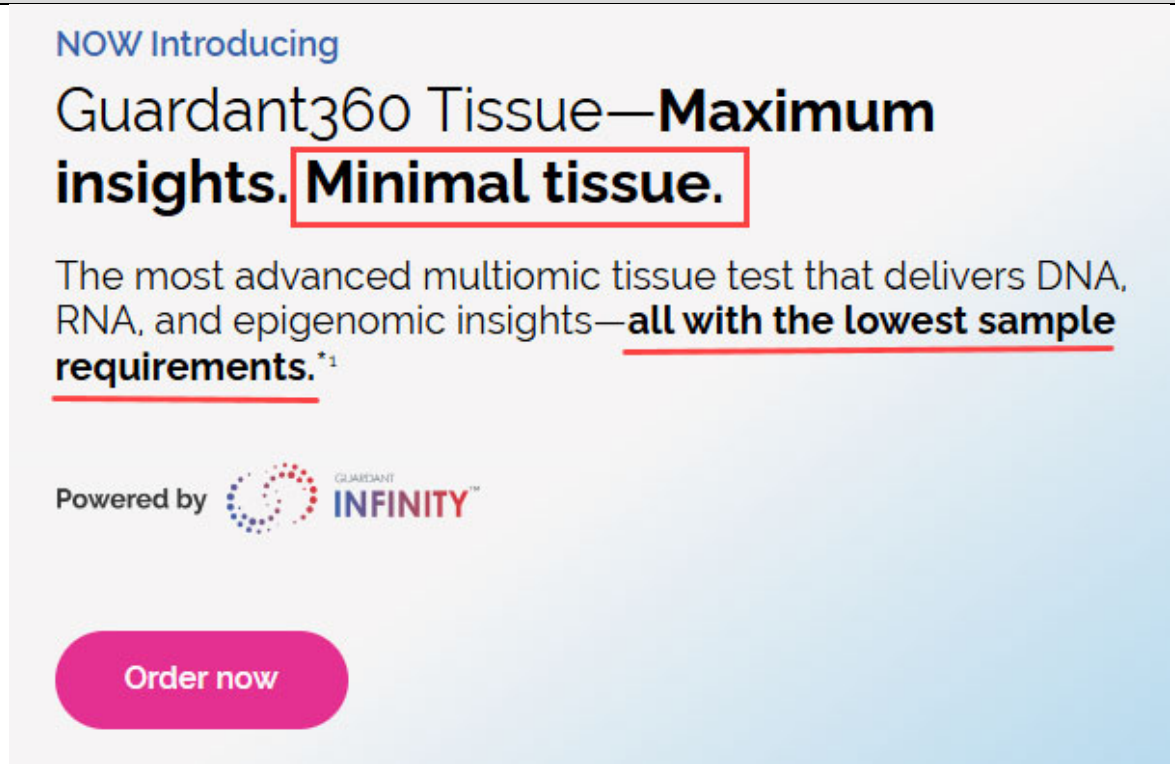
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the genomic nucleic acids are obtained from blood or other bodily fluids.	Guardant obtains the genomic nucleic acids from blood or other bodily fluids. For example, the Guardant360 tests require obtaining cell-free DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.  Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 1.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Lanman et al. at 3 ("digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Guardant360 CDx Technical Information at 5 ("Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. A minimum of 5 mL of blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. . . . Plasma is isolated via centrifugation and cfDNA is extracted.").)

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 1–2 ("Digital sequencing of plasma-derived DNA is indicated int advanced cancer patients to prevent repeated invasive biopsies when the initial biopsy is inadequate, unobtainable for genomic testing or uninformative, or when the patient's cancer has progressed despite its treatment.") Lanman et al. at 2 ("Next-generation sequencing (NGS) of solid tumor tissue has advanced our ability to perform multiplex testing for an ever-growing number of targetable genomic alterations utilizing small needle biopsies or archival pathology slides. As the number of genomic targets with matched therapies grows, tissue biopsy material can be quickly exhausted before multiple "companion diagnostics" tests can be completed.) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 3–4 ("Here we report the analytical and clinical validation of the 54-gene (512 exons) Guardant360 panel of mutations and gene amplifications in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), NCCN Task Force recommendations and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines.") Lanman et al. at 4 ("With high sensitivity and the ultra-high specificity required to comprehensively sequence cfDNA, the Guardant360 test has the potential to evaluate the multiple genomic alterations cited in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with tumor heterogeneity [34], to interrogate all actionable genomic alterations via comprehensive sequencing of a multigene panel, and to prevent the time delays, cost and complications inherent in invasive biopsies.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

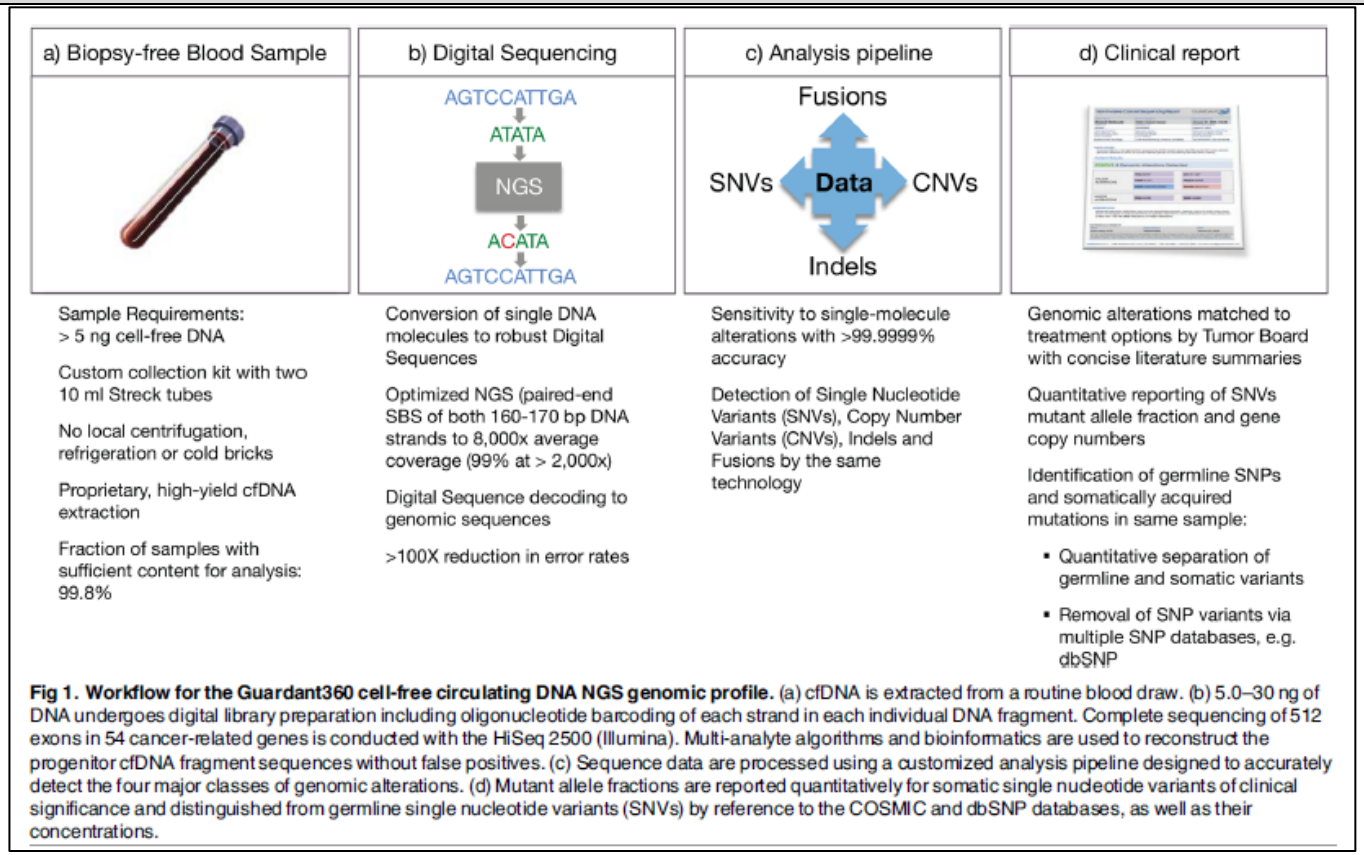


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Fig. 1 above)

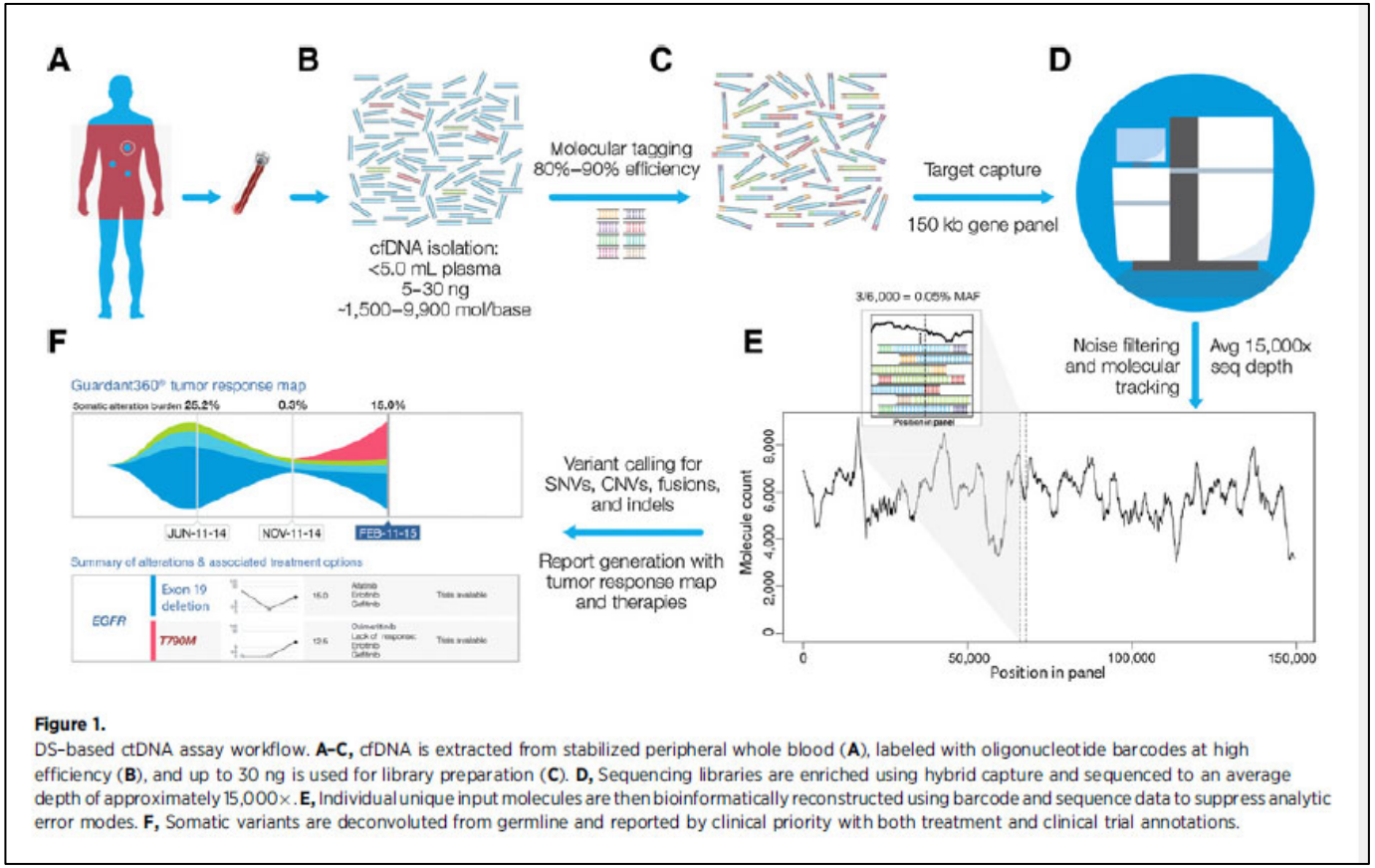
Lanman et al. at 5 ("cfDNA is extracted from a routine blood draw.")

Lanman et al. at 12 ("The analytic validation studies of the Guardant360 cfDNA panel highlight its differences from both solid tumor and other cell-free circulating tumor DNA genomic testing methods. The digital sequencing platform enables single molecular sensitivity, an analytic sensitivity that is two orders of

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	magnitude greater than an Illumina HiSeq with standard prep while maintaining near-perfect specificity (Fig 1A and 1B).") Lanman et al. at 12 ("The Guardant360 panel at the time of this study sequences ~78,000 base pairs (78 kbp), providing complete coverage for all exons in 18 genes and the critical exons in the remainder of the 54 total genes.") Lanman et al. at 14 ("The clinical sensitivity for plasma-based DNA NGS via digital sequencing was equivalent to the clinical sensitivity of tissue-based DNA NGS sequencing.") Lanman et al., 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.") Lanman et al. at 14 ("The clinical utility of the Guardant360 diagnostic test in advanced cancer is supported by results in clinical practice. Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guide-line-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 17 ("Guardant360 could be used in lieu of a repeat invasive biopsy for NGS, particularly for patients with visceral primary disease or metastases to lung, liver or bone. Secondly, in patients with visceral primary or metastatic disease, when a tumor has progressed or recurred based on clinical symptomatology, imaging or serological protein biomarkers, e.g. CEA, CA-125, CA 15–3, PSA, etc., the Guardant360 panel could be used to re-evaluate for new targeted therapies, enabling real-time adaptive management of a patient's continuously evolving cancer while obviating further invasive tissue biopsies.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that

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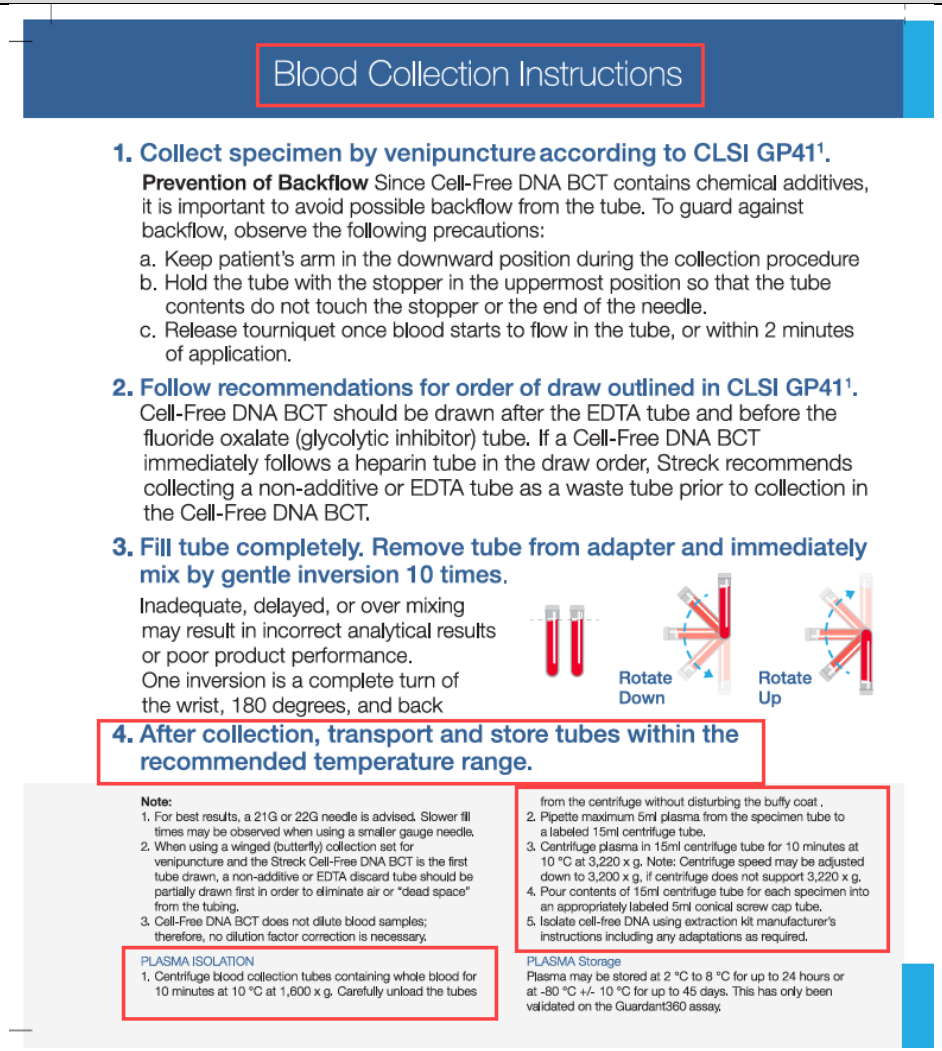
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically

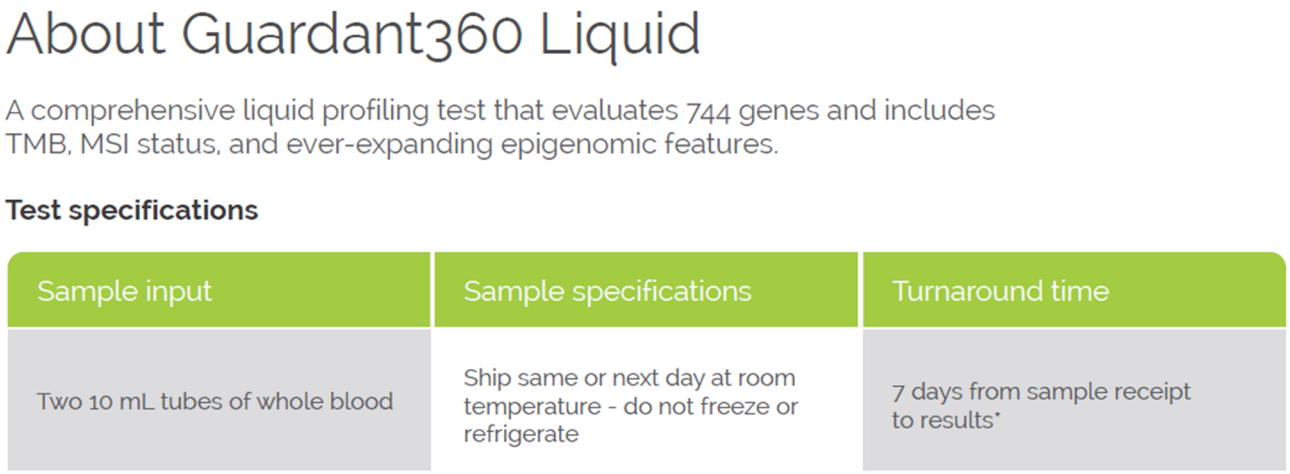
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	relevant biomarkers, including those for all approved and many investigational drugs and clinical trials , and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).") Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.") Odegaard et al. at 3547 ("These data demonstrate applicability of ctDNA-based genotyping to treatment paradigms based on tissue genotyping; however, ctDNA has the potential to not only match but to surpass tissue genotyping in for important ways.") '822 patent, claim 1 ("[P]roviding a population of cell free DNA ('cfDNA') molecules obtained from a bodily sample from a subject.") Odegaard et al. at 3542 ("cfDNA is extracted from stabilized whole blood") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.).") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.")

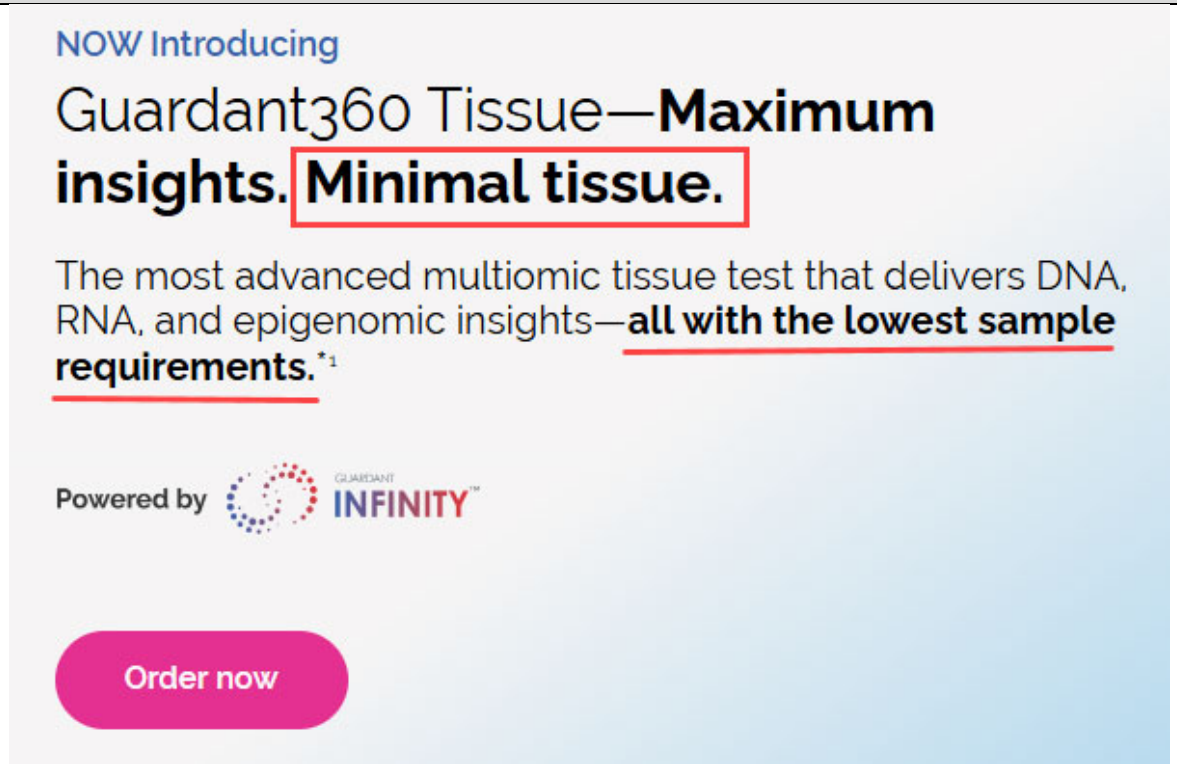
ASSERTED CLAIM 18

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the genomic nucleic acids are obtained from tumor cell DNA in a bodily fluid.	Guardant obtains the genomic nucleic acids from tumor cell DNA in a bodily fluid. For example, the Guardant360 tests require obtaining cell-free tumor DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples. 5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.

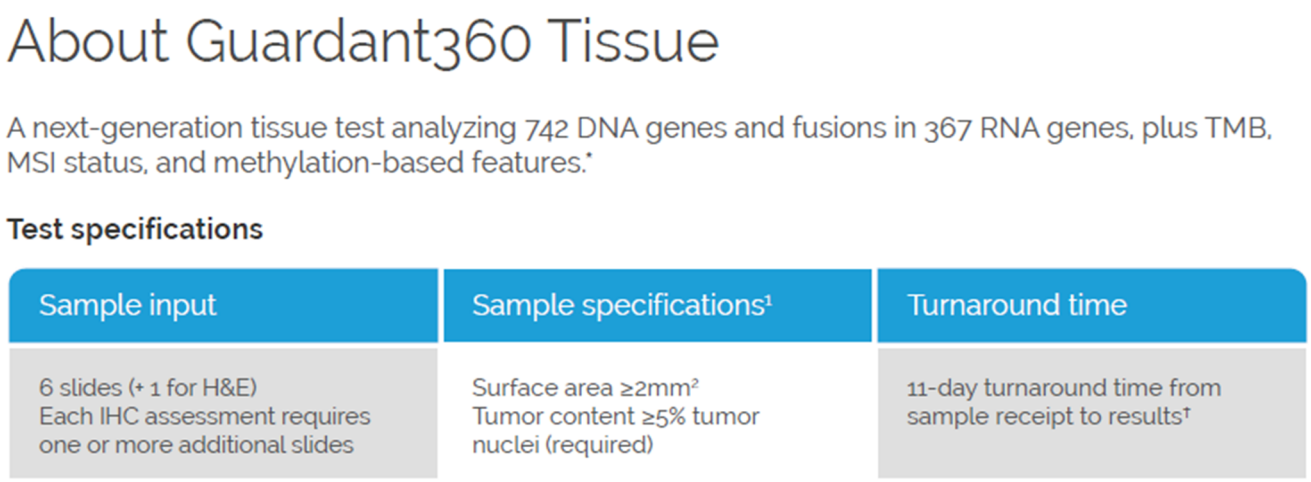
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2. **INTENDED USE** The Guardant360 Blood Collection Kit is intended for whole blood collection for further processing and testing of cell-free plasma DNA with the Guardant360 test. **PRINCIPLES OF THE PROCEDURE** Accurate analysis of cell-free DNA (cfDNA) can be compromised by sample handling, shipping and processing, causing lysis of nucleated blood cells and subsequent release of cellular genomic DNA. The preservative reagent contained in Cell-Free DNA BCT reduces the release of cellular genomic DNA. The blood collection tube is intended for the preservation of cell-free DNA. **BLOOD COLLECTION TUBE REAGENTS** Cell-Free DNA BCT contains the anticoagulant K_3EDTA and a cell preservative in a liquid medium. Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 1.

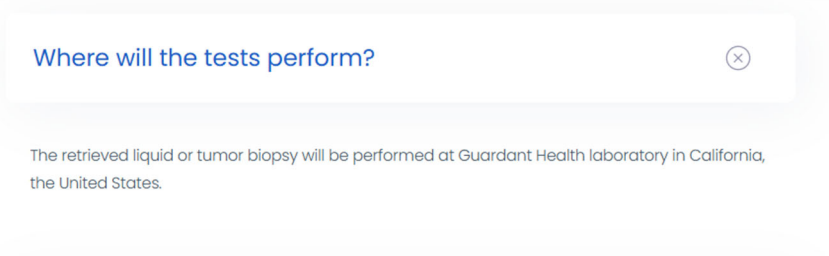
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Webpage.

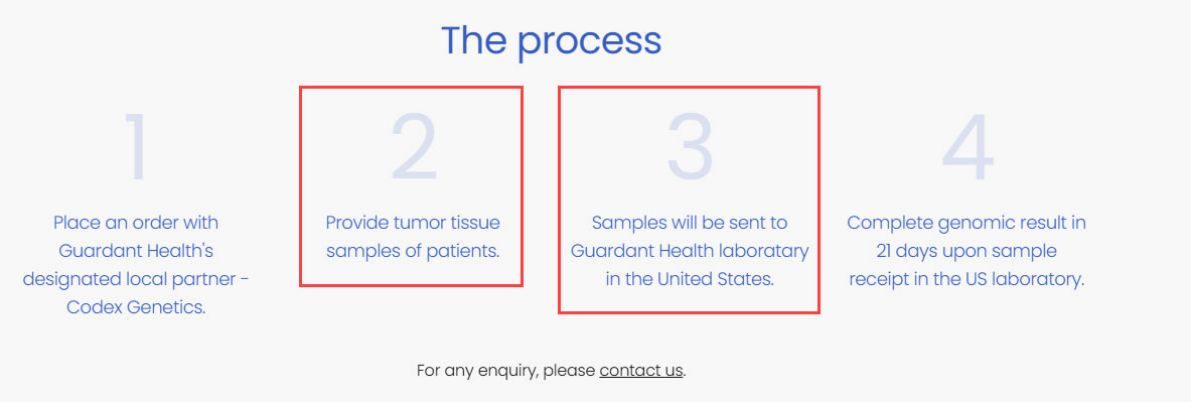
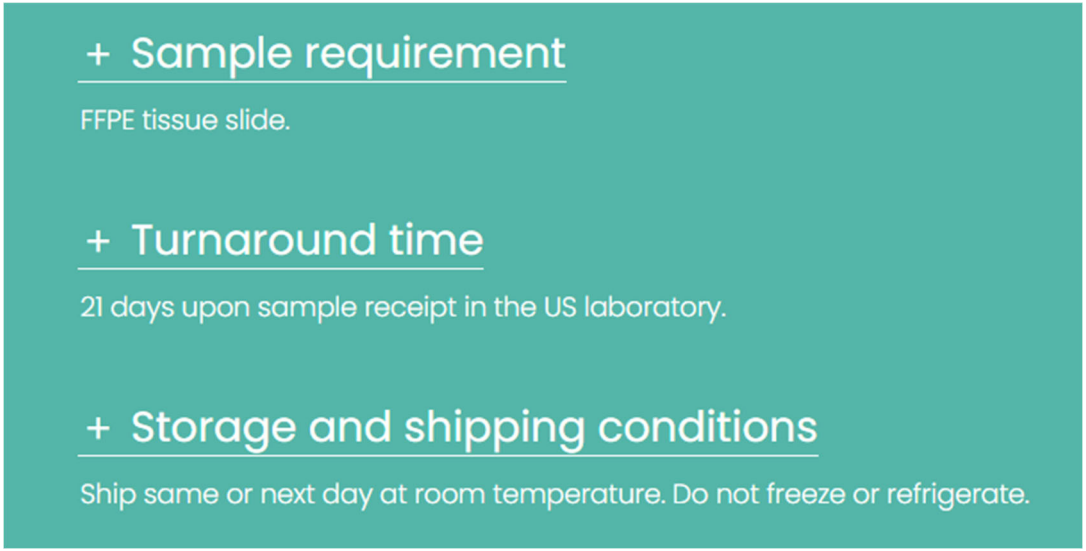
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Guardant360 Tissue Assay Specifications at 1.



Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

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	Lanman et al. at 3 ("digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Guardant360 CDx Technical Information at 5 ("Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. A minimum of 5 mL of blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. . . . Plasma is isolated via centrifugation and cfDNA is extracted.").) Lanman et al. at 1–2 ("Digital sequencing of plasma-derived DNA is indicated int advanced cancer patients to prevent repeated invasive biopsies when the initial biopsy is inadequate, unobtainable for genomic testing or uninformative, or when the patient's cancer has progressed despite its treatment.") Lanman et al. at 2 ("Next-generation sequencing (NGS) of solid tumor tissue has advanced our ability to perform multiplex testing for an ever-growing number of targetable genomic alterations utilizing small needle biopsies or archival pathology slides. As the number of genomic targets with matched therapies grows, tissue biopsy material can be quickly exhausted before multiple "companion diagnostics" tests can be completed.) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 3–4 ("Here we report the analytical and clinical validation of the 54-gene (512 exons) Guardant360 panel of mutations and gene amplifications in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), NCCN Task Force recommendations and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines.") Lanman et al. at 4 ("With high sensitivity and the ultra-high specificity required to comprehensively sequence cfDNA, the Guardant360 test has the potential to evaluate the multiple genomic alterations cited in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with tumor heterogeneity [34], to interrogate all actionable genomic alterations via comprehensive sequencing of a multigene panel, and to prevent the time delays, cost and complications inherent in invasive biopsies.")

GUARDANT HEALTH EX.1020.107

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

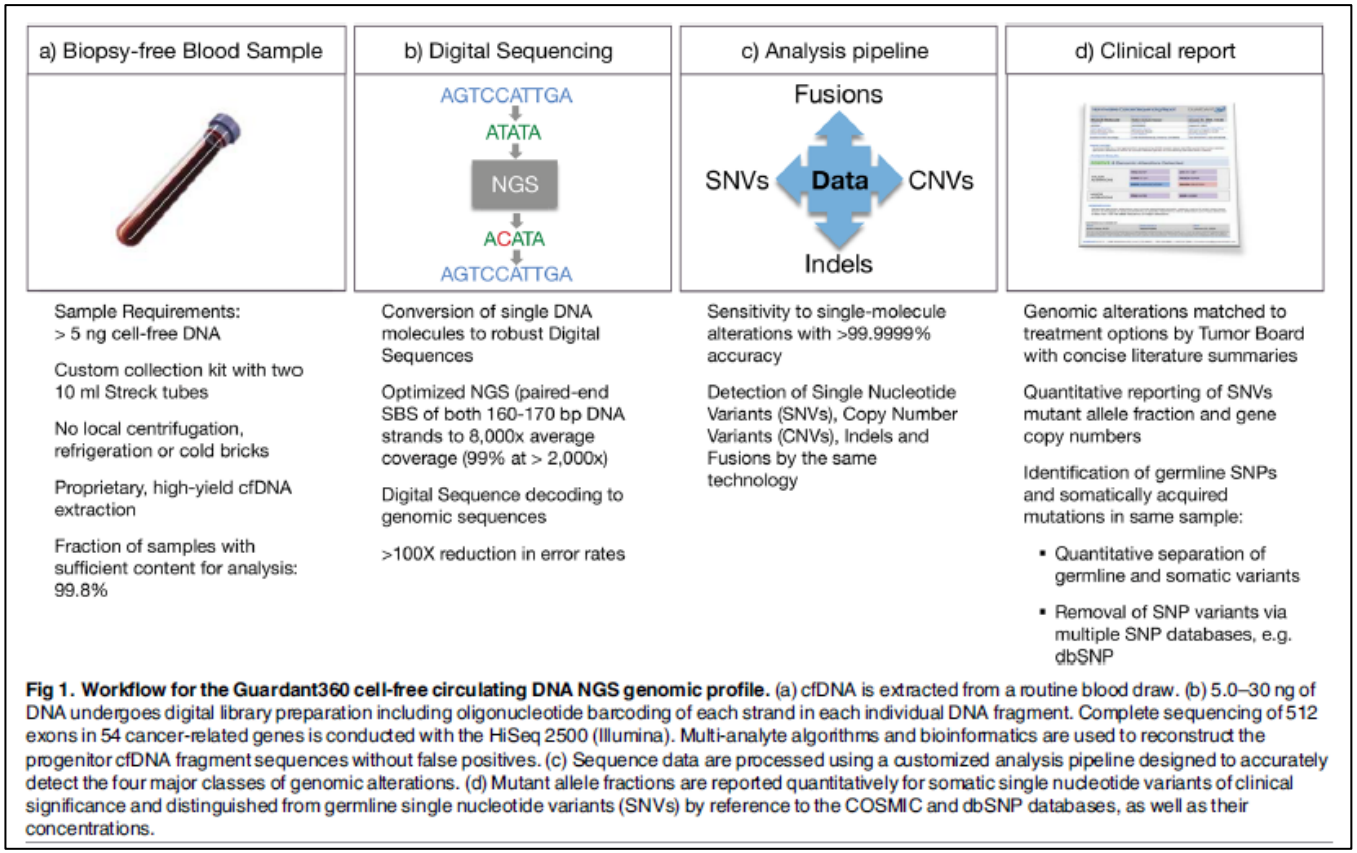


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Fig. 1 above)

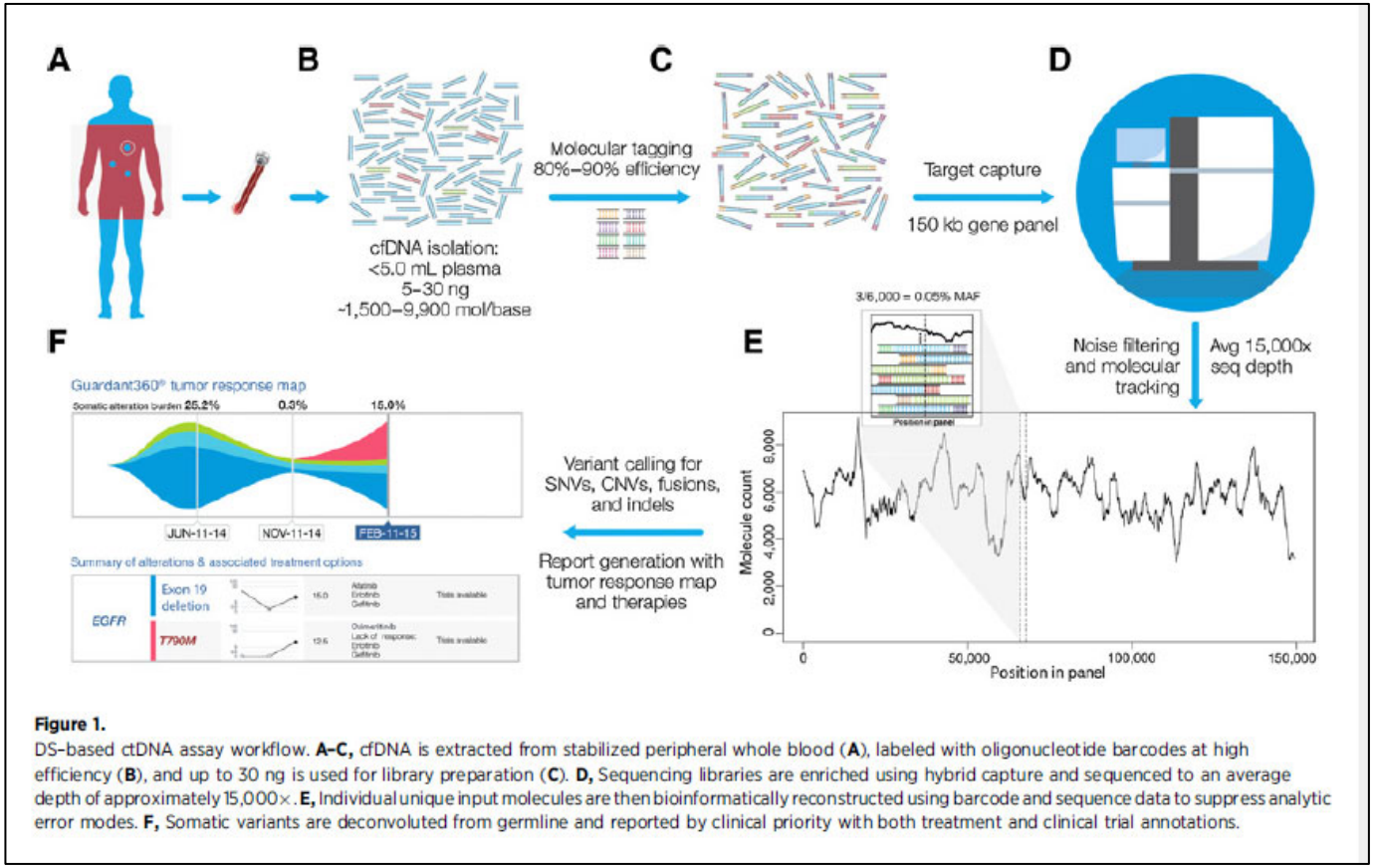
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EXHIBIT A

Cold Spring Harbor Laboratory's Infringement Contentions

Cold Spring Harbor Laboratory v. Guardant Health, Inc., C.A. No. 25-263-JCG

GUARDANT HEALTH EX.1020.115

EXHIBIT B

EXHIBIT B

Cold Spring Harbor Laboratory's Infringement Contentions

Cold Spring Harbor Laboratory v. Guardant Health, Inc., C.A. No. 25-263-JCG

Defendant's Guardant360 Tests Infringe The '510 Patent

Defendant Guardant Health, Inc. ("Defendant" or "Guardant"), either alone or in conjunction with others, have infringed, and continue to infringe, claims 1-12, 15-23, 26-29 and 31-34 (the "Asserted Claims") of U.S. Patent No. 12,234,510 (the "'510 Patent") under 35 U.S.C. §§ 271(a), (b), (c), and/or (f), either literally and/or under the doctrine of equivalents, by making, using, selling, and/or offering for sale in the United States and/or importing into the United States tests marketed under the tradenames "Guardant360®," "Guardant360® Liquid," "Guardant360® Tissue," and "Guardant360® CDx" (collectively, the "Guardant360 tests"). As described in the chart below, use (performance) of the Guardant360 tests practices the method of the Asserted Claims of the '510 Patent through the normal and intended operation of the products under Defendant's protocols.

Guardant, either alone or in conjunction with others, directly infringes each Asserted Claim of the '510 Patent under 35 U.S.C. § 271(a), either literally and/or under the doctrine of equivalents, at least by selling, and/or offering for sale the performance of the Guardant360 tests and by using the Guardant360 tests according to their normal and intended operation under Guardant's protocols. For example, Guardant makes the Guardant360 tests and markets, offers for sale, sells, and distributes the Guardant360 tests, including through its website, www.guardanthealth.com. (*See, e.g.*, D.I. 15 ¶ 10.) For example, Guardant uses the Guardant360 tests according to their normal and intended operation under Guardant's protocols. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 88-93.) To the extent that Guardant itself does not perform all steps of the Guardant360 tests, Guardant directly infringes by directing and controlling the performance of the Guardant360 tests in an infringing manner according to Guardant's protocols. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 88-93.) For example, Guardant establishes the manner and timing of the performance of the Guardant360 tests in an infringing manner and conditions the receipt of a benefit upon that performance. (*See, e.g.*, D.I. 15 ¶¶ 43-58, 88-93.)

Additionally, Guardant, either alone or in conjunction with others, indirectly infringes the Asserted Claims under 35 U.S.C. § 271(b) by making, importing, selling, and/or offering for sale the Guardant360 tests. For example, Guardant actively, knowingly, and intentionally induces third parties, including without limitation third-party laboratories, to use the Guardant360 tests, in a way that Guardant knew or should have known infringed the Asserted Claims. For example, Guardant has induced and continues to induce infringement by selling or otherwise supplying the Guardant360 tests with the knowledge and intent that those third parties will use the Guardant360 tests in the manner designed and instructed by Guardant to infringe the '510 Patent and with the knowledge and intent to encourage and facilitate third party infringement through the dissemination of the Guardant360 tests and/or the creation and dissemination of promotional and marketing materials, supporting materials, instructions, product manuals, and/or technical information related to the Guardant360 tests. Guardant knows and intends for their third-party laboratories in the United States to use the

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Guardant360 tests according to the instructions and requirements provided by Guardant. Guardant contributed to the design of the Guardant360 tests to be used according to the instructions and requirements provided by Guardant, which results in infringement of the Asserted Claims. If third-party laboratories in the U.S. were to use the Guardant360 tests in a manner other than that instructed by Guardant, the kits would not work for their intended purpose, *i.e.*, to test for copy number variations. On information and belief, third-party laboratories and other third parties have directly infringed the Asserted Claims of the '510 Patent, through the normal and customary use of the Guardant360 tests. Furthermore, Guardant has had knowledge of the '510 Patent and its infringement since at least the issue date of the '510 Patent. (*See, e.g.*, D.I. 15 ¶¶ 59-78.)

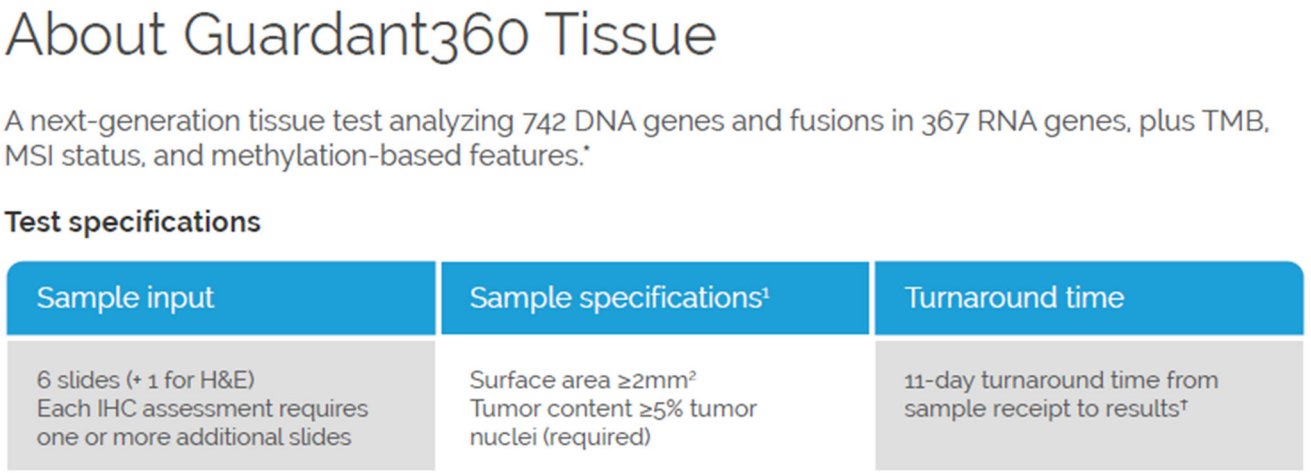
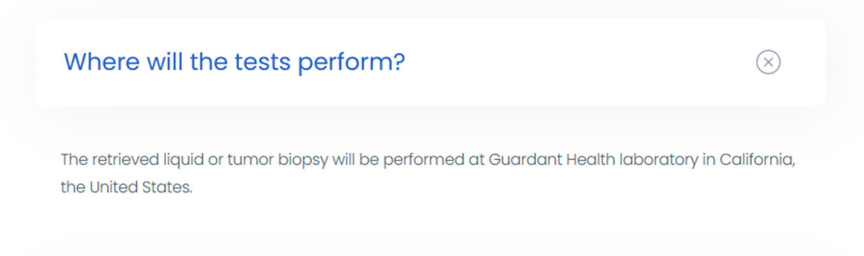
Additionally, Guardant is liable for infringement under 35 U.S.C. § 271(f). For example, Guardant has supplied or caused to be supplied—and continue to supply or cause to be supplied—in or from the United States components of the invention of one or more claims of the '510 Patent that are especially made or especially adapted for use in the invention and not a staple article or commodity of commerce suitable for substantial noninfringing use, where such components are uncombined in whole or in part, knowing that such components are so made or adapted and intending that such components will be combined outside of the United States in a manner that would infringe the patent if such combination occurred within the United States.

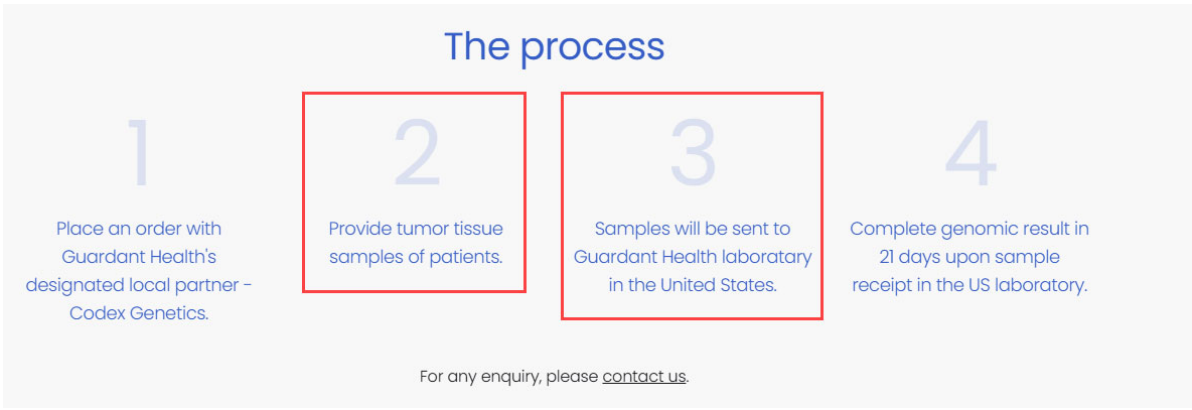
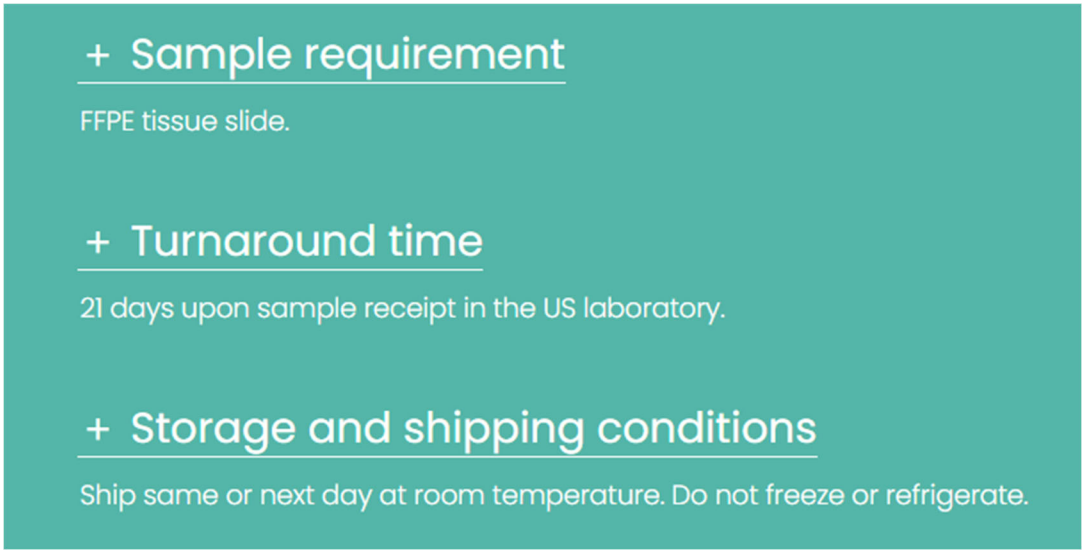
The claim charts below identify specifically where each element of each asserted claim is found in the performance of the Guardant360 tests, based upon the information available to date. CSHL contends that each element of each asserted claim is literally met unless otherwise indicated. But to the extent that any claim element is found not to be literally met with respect to the Guardant360 tests, CSHL contends that the element is met under the doctrine of equivalents because there are no substantial differences between the element and the Guardant360 tests, and the element and the Guardant360 tests perform substantially the same function, in substantially the same way, to achieve substantially the same result. *See, e.g.*, *Graver Tank & Mfg. Co. v. Linde Air Prod. Co.*, 339 U.S. 605 (1950); *Warner-Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17 (1997).

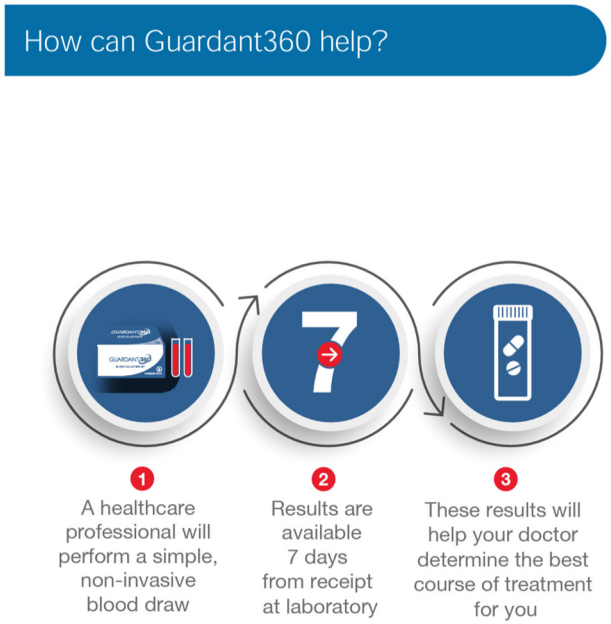
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ASSERTED CLAIM 1

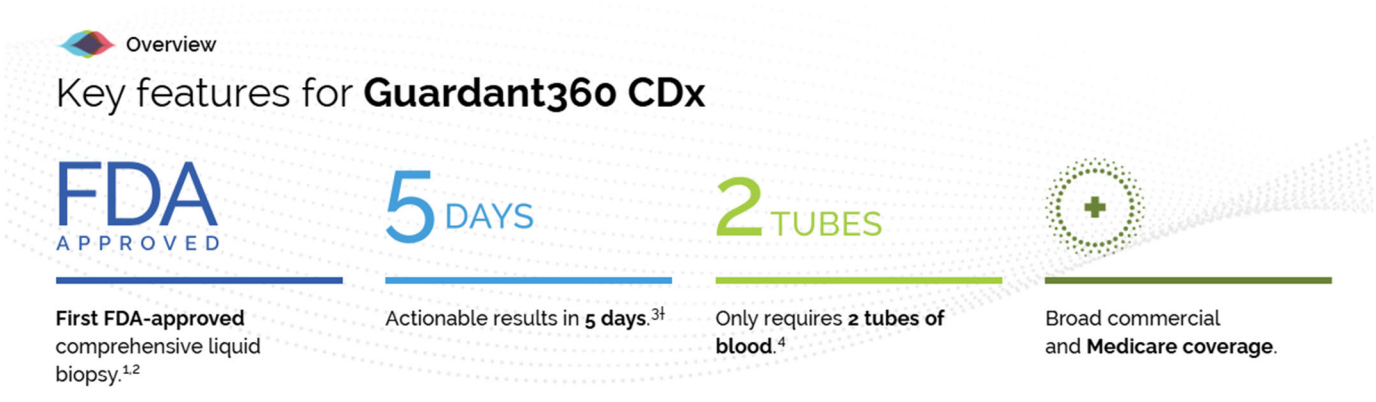
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
[Preamble] A method comprising:	To the extent the preamble is limiting, Guardant provides a method for analyzing DNA using next-generation sequencing (NGS). For example, the Guardant360 tests are NGS panels, the performance of which utilizes sequencing of DNA obtained from a biological sample, such as blood or tumor tissue. 5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4

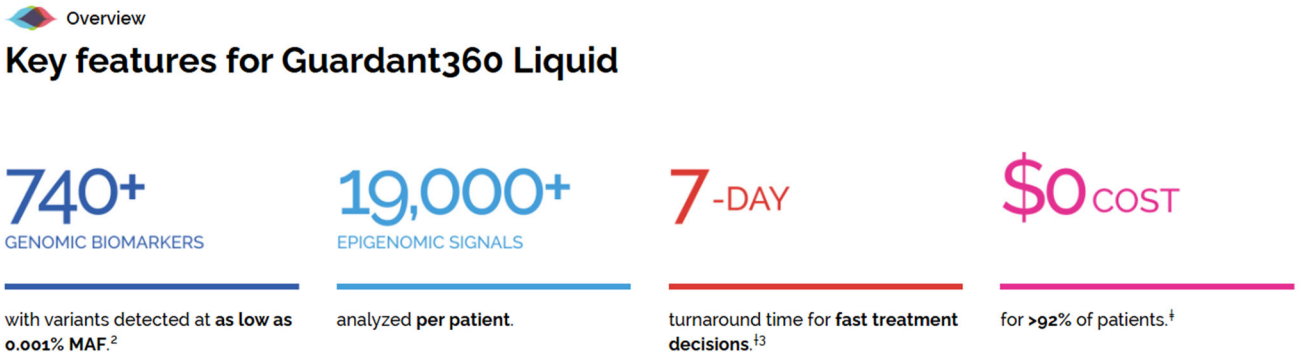
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Assay Specifications at 1.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

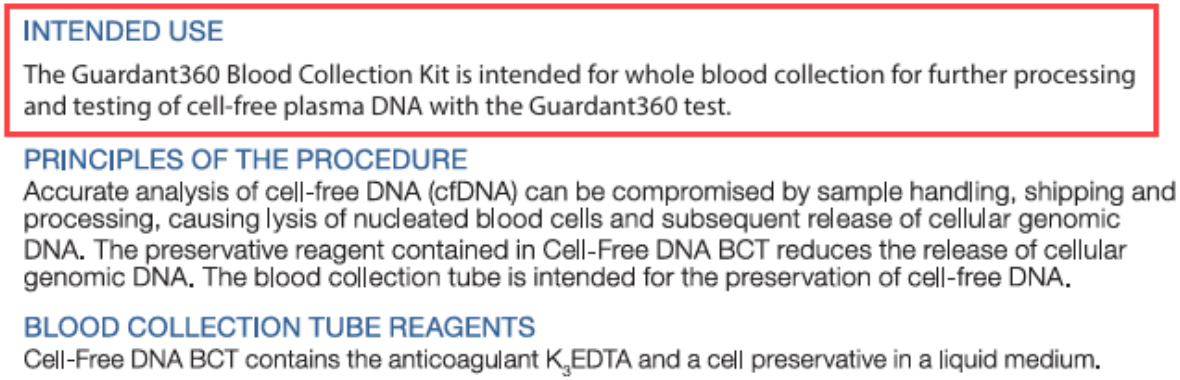
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Educational Booklet at 5. Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. This test is indicated to prevent repeat invasive biopsy when an initial biopsy is QNS or otherwise unavailable/unobtainable as well as when cancer has progressed or recurred despite treatment. Actionable genomic alterations are those for which there is an FDA-approved treatment or that serve as eligibility criteria for later phase clinical trials.")

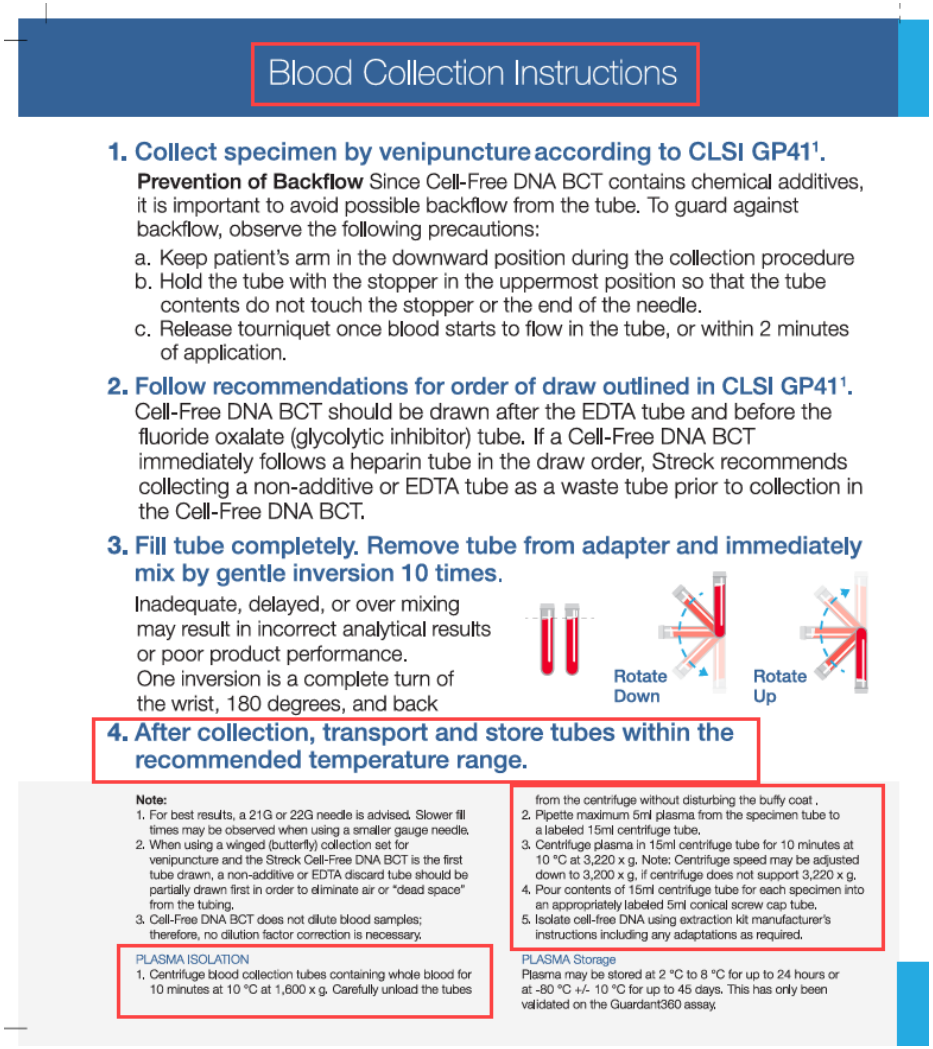
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.")
	Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.")
	Odegaard et al. at 3539 ("To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.")
	Odegaard et al. at 3539 ("Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical sample. Blood-to-tissue clinical to those documented in the medical record of 543 consecutive lung cancer patients.")
	Odegaard et al. at 3540 ("Here, we describe the analytic (technical characterization using controlled and often contrived samples) and clinical (characterization using real-world patient samples, contexts, and comparators) validation of a comprehensive ctDNA sequencing assay for clinical genotyping of advanced solid tumors, including large-scale comparisons with orthogonal clinical plasma-and tissue-genotyping methods.")
	Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.")
	'822 patent, claim 1 ("A method, comprising:…")

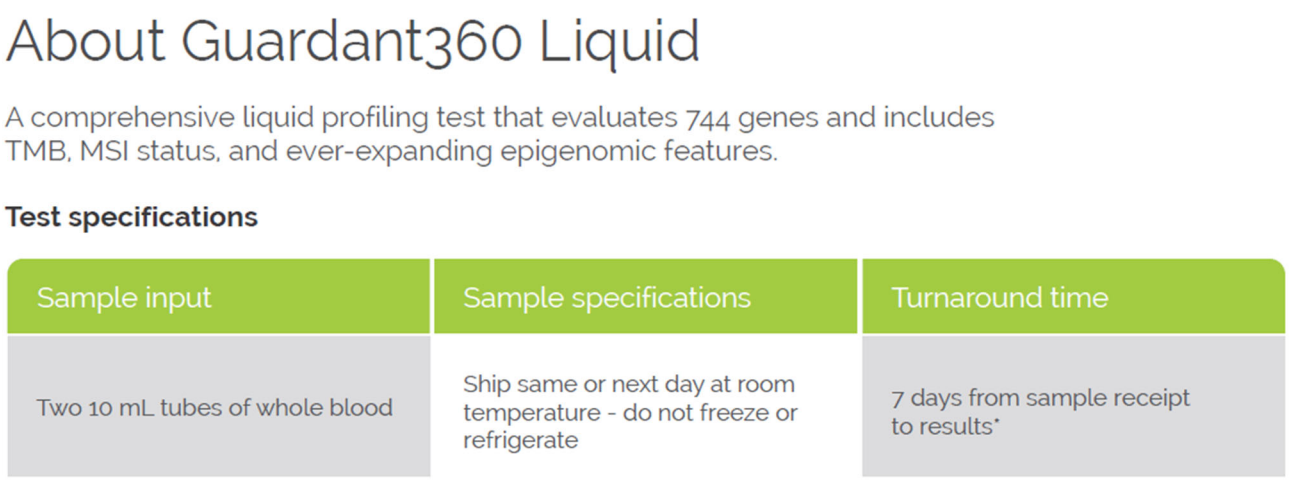
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 CDx Webpage  Guardant360 Tissue Webpage.

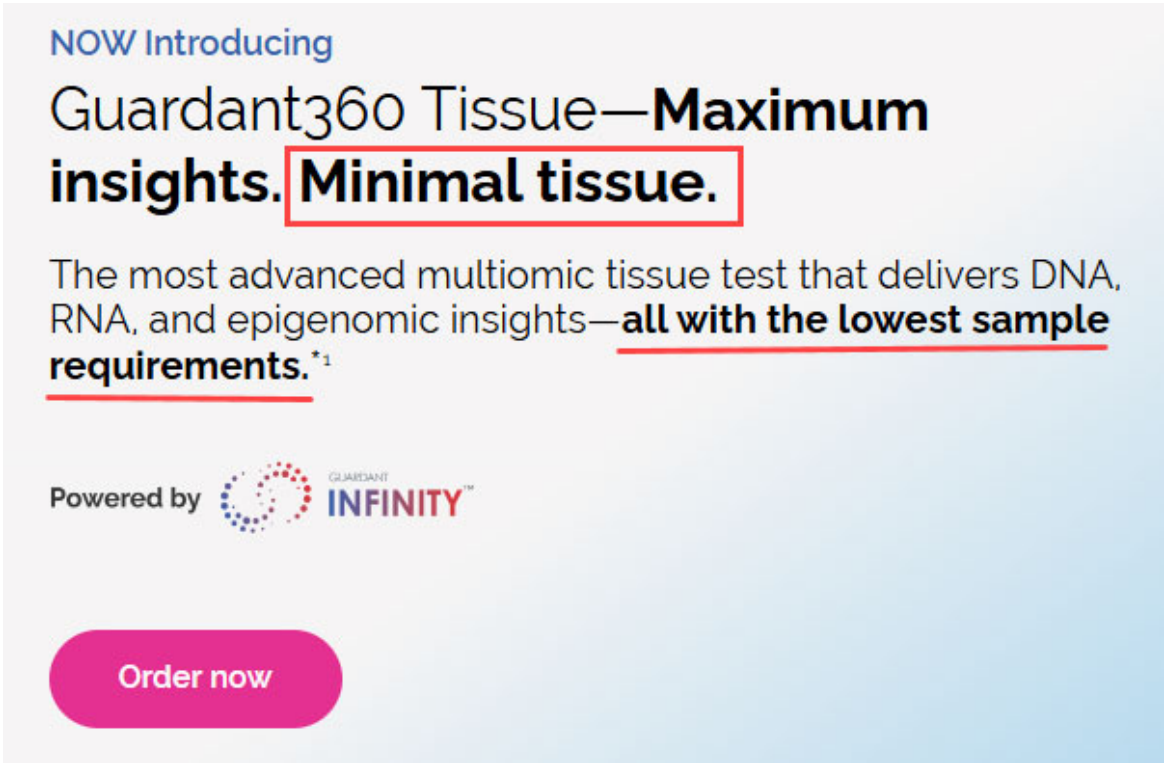
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Webpage.
[1a.] obtaining segments of genomic nucleic acids from a sample containing the genomic nucleic acids;	Guardant obtains segments of genomic nucleic acids from a sample containing the genomic nucleic acids. For example, the Guardant360 tests require obtaining cell-free DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples.

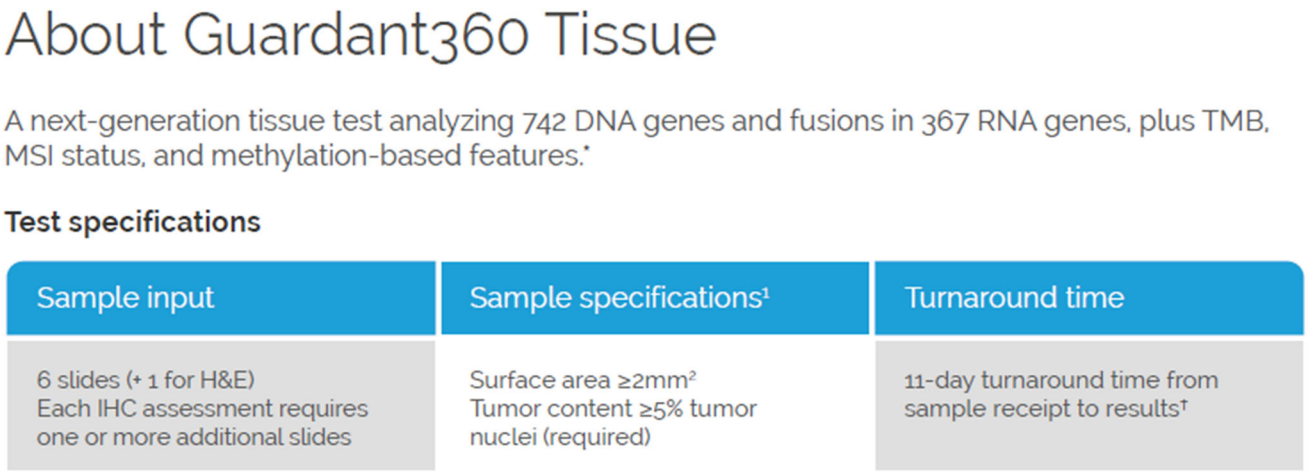
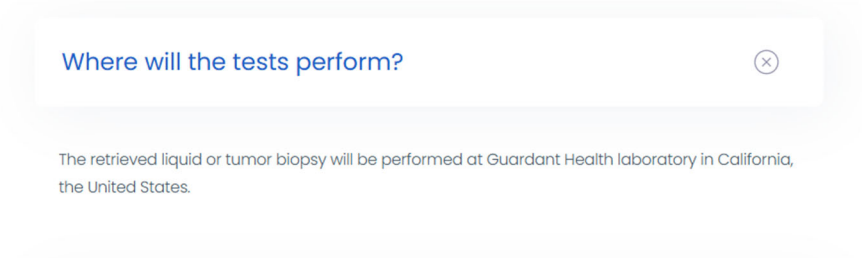
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.  Guardant Blood Collection Kit Instructions at 2.

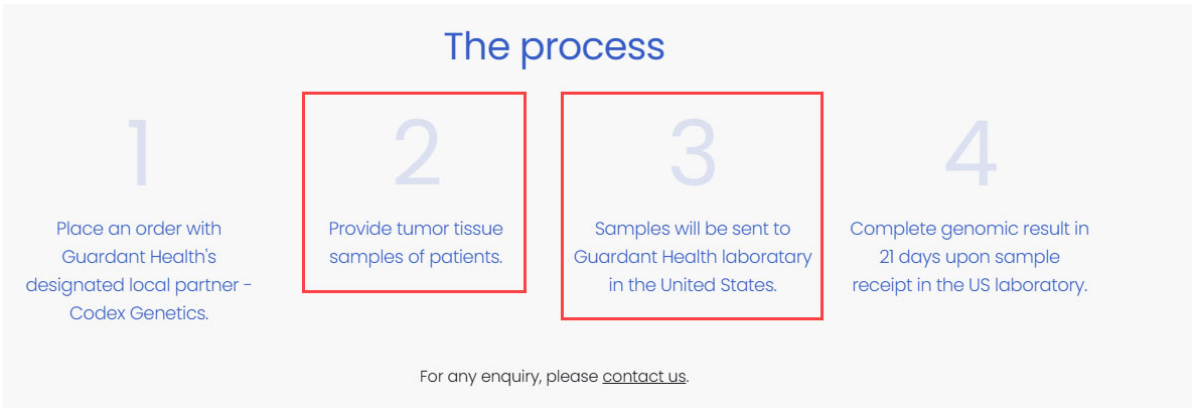
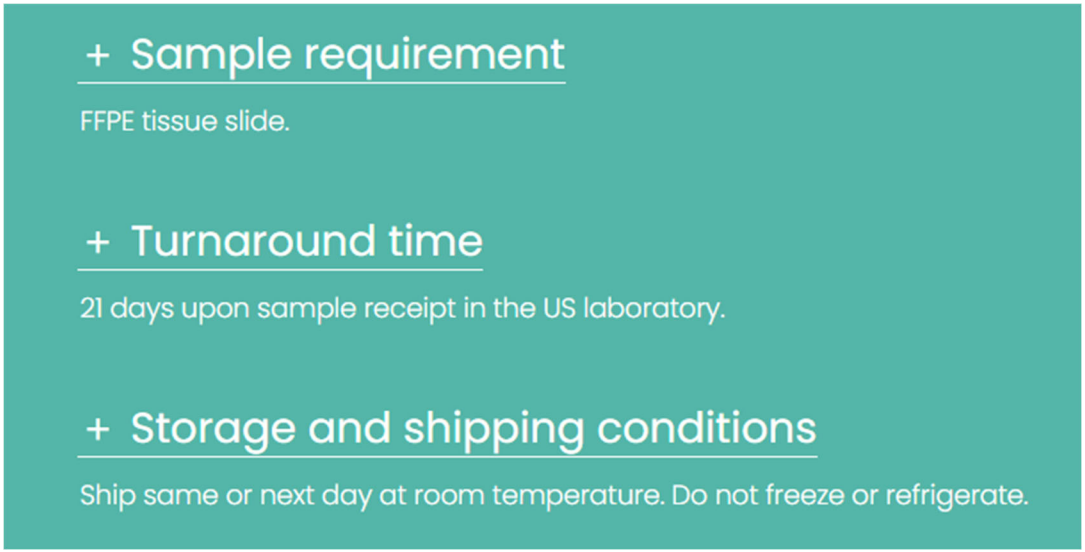
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 1.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1

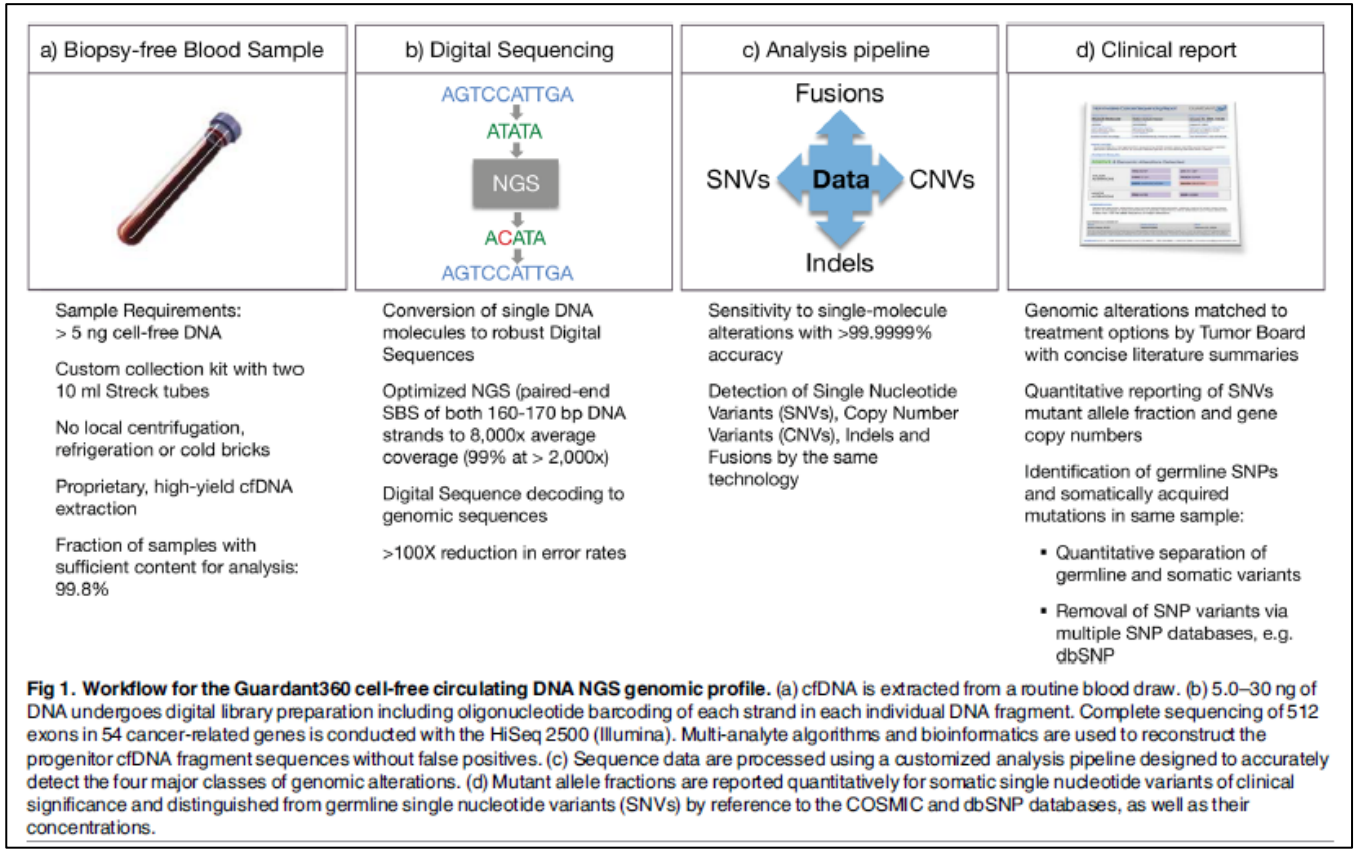
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Assay Specifications at 1.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 3 ("[D]igital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 1–2 ("Digital sequencing of plasma-derived DNA is indicated int advanced cancer patients to prevent repeated invasive biopsies when the initial biopsy is inadequate, unobtainable for genomic testing or uninformative, or when the patient's cancer has progressed despite its treatment.") Lanman et al. at 2 ("Next-generation sequencing (NGS) of solid tumor tissue has advanced our ability to perform multiplex testing for an ever-growing number of targetable genomic alterations utilizing small needle biopsies or archival pathology slides. As the number of genomic targets with matched therapies grows, tissue biopsy material can be quickly exhausted before multiple "companion diagnostics" tests can be completed.) Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 3–4 ("Here we report the analytical and clinical validation of the 54-gene (512 exons) Guardant360 panel of mutations and gene amplifications in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), NCCN Task Force recommendations and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines.") Lanman et al. at 4 ("With high sensitivity and the ultra-high specificity required to comprehensively sequence cfDNA, the Guardant360 test has the potential to evaluate the multiple genomic alterations cited in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with tumor heterogeneity [34], to interrogate all actionable genomic alterations via comprehensive sequencing of a multigene panel, and to prevent the time delays, cost and complications inherent in invasive biopsies.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Lanman et al. at 5. (Fig. 1 above)

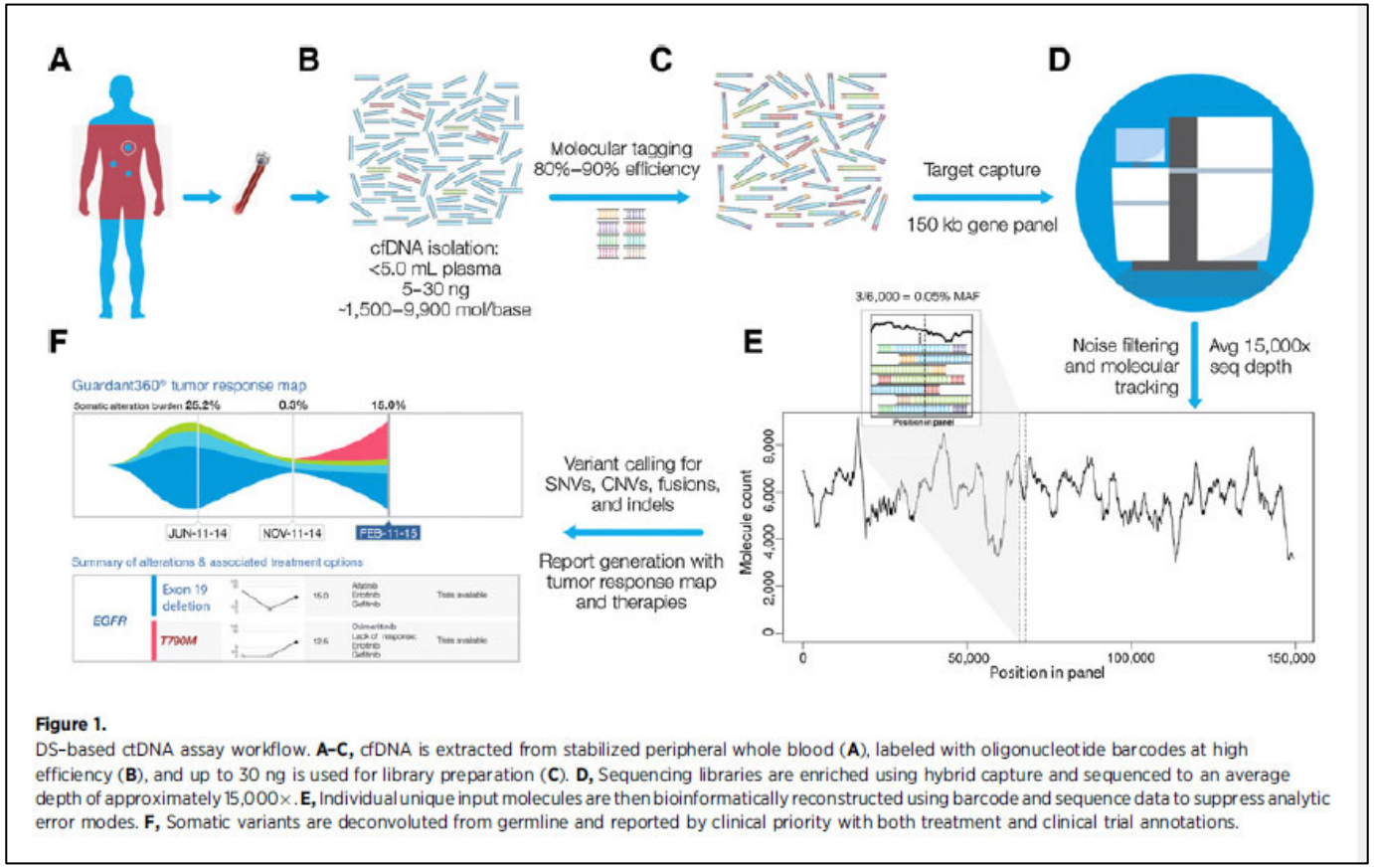
Lanman et al. at 5 ("cfDNA is extracted from a routine blood draw.")

Lanman et al. at 12 ("The analytic validation studies of the Guardant360 cfDNA panel highlight its differences from both solid tumor and other cell-free circulating tumor DNA genomic testing methods. The digital sequencing platform enables single molecular sensitivity, an analytic sensitivity that is two

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	orders of magnitude greater than an Illumina HiSeq with standard prep while maintaining near-perfect specificity (Fig 1A and 1B).") Lanman et al. at 12 ("The Guardant360 panel at the time of this study sequences ~78,000 base pairs (78 kbp), providing complete coverage for all exons in 18 genes and the critical exons in the remainder of the 54 total genes.") Lanman et al. at 14 ("The clinical sensitivity for plasma-based DNA NGS via digital sequencing was equivalent to the clinical sensitivity of tissue-based DNA NGS sequencing.") Lanman et al., 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.") Lanman et al. at 14 ("The clinical utility of the Guardant360 diagnostic test in advanced cancer is supported by results in clinical practice. Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guide-line-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 17 ("Guardant360 could be used in lieu of a repeat invasive biopsy for NGS, particularly for patients with visceral primary disease or metastases to lung, liver or bone. Secondly, in patients with visceral primary or metastatic disease, when a tumor has progressed or recurred based on clinical symptomatology, imaging or serological protein biomarkers, e.g. CEA, CA-125, CA 15–3, PSA, etc., the Guardant360 panel could be used to re-evaluate for new targeted therapies, enabling real-time adaptive management of a patient's continuously evolving cancer while obviating further invasive tissue biopsies.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with

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	algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 17 ("At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a minimum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual in Streck Cell-Free DNA Blood Collection (Streck) tubes, which contain a proprietary formaldehyde-free preservative that stabilizes white blood cells, preventing the release of genomic DNA and allowing isolation of high-quality cfDNA.") Lanman et al. at 18 ("The cell-free DNA from these cell lines is extracellular DNA released by the culture cells and the fragmentation pattern is essentially identical to circulating DNA except the size distribution peaks tend to be slightly broader. Similar biological mechanisms to those in play in circulating peripheral blood in vivo occur with the cell culture media, i.e. DNA digestion of histone-protected genomic DNA.") Lanman et al. at 18 ("Plasma samples and supernatant from cultured cell lines were processed using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. Cell-free DNA is extracted from 1.5 mL to 5 mL plasma and subsequently concentrated and size selected using Agencourt Ampure XP beads (Beckman Coulter) and subsequently quantified using Qubit1 2.0 fluorometer. The DNA is extracted using standard extraction procedures, including proteinase K digestion of histone and other DNA-binding proteins and isoproponal precipitation. The DNA is then end-repaired to repair ends of DNA with a 5' phosphate prior to ligation of adapters necessary for downstream sequencing.") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries.")

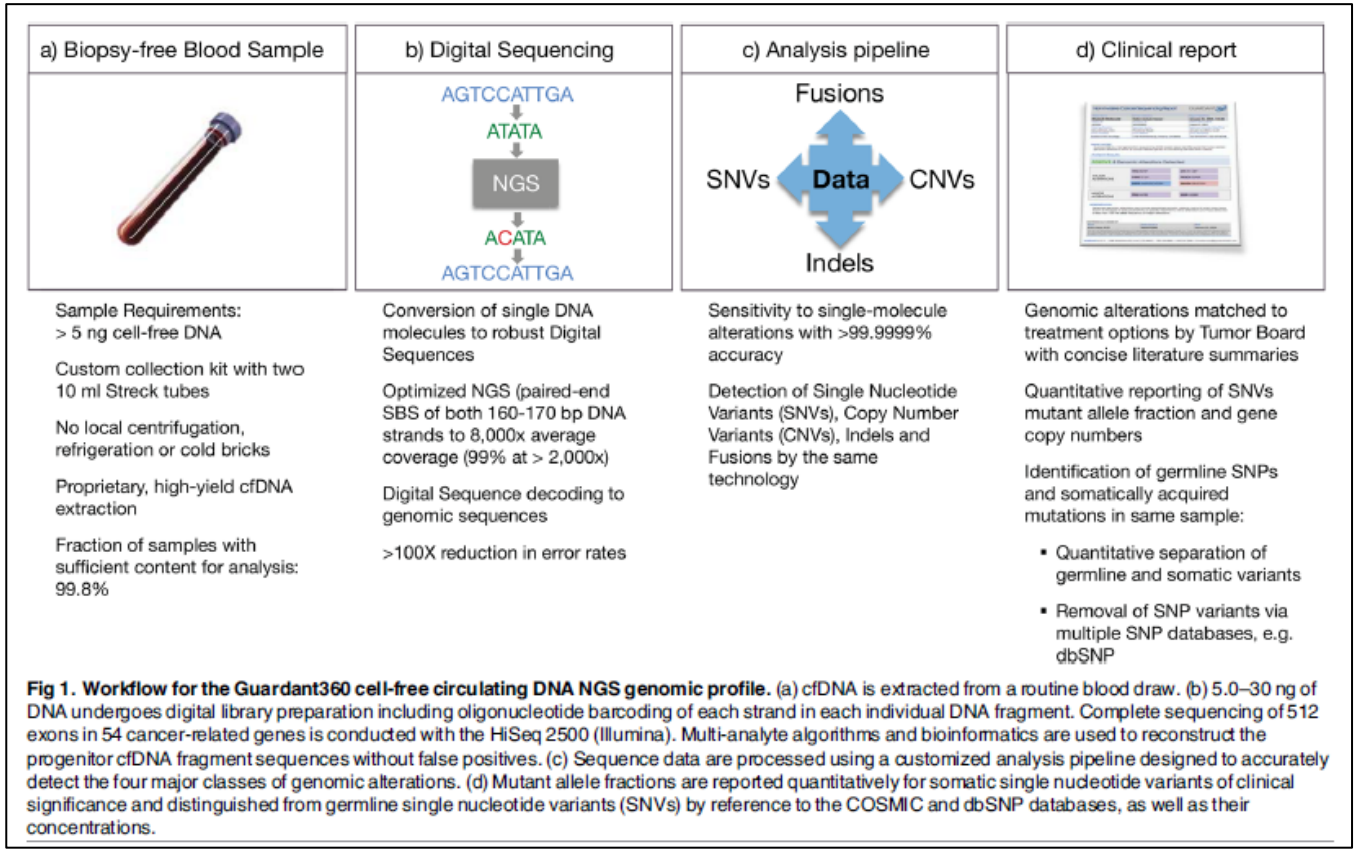
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3539 ("To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.") Odegaard et al. at 3539 ("Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical sample. Blood-to-tissue clinical to those documented in the medical record of 543 consecutive lung cancer patients.") Odegaard et al. at 3540 ("Plasma-blood comprehensive tumor genotyping expands access to standard-of-care precision oncology to patients previously ineligible due to barriers associated with tumor sampling.") Odegaard et al. at 3540 ("Here, we describe the analytic (technical characterization using controlled and often contrived samples) and clinical (characterization using real-world patient samples, contexts, and comparators) validation of a comprehensive ctDNA sequencing assay for clinical genotyping of advanced solid tumors, including large-scale comparisons with orthogonal clinical plasma-and tissue-genotyping methods.") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.). Briefly, 2 x 10 mL of peripheral venous blood was collected in Streck Cell-Free DNA BCT (Streck, Inc.), packaged into a provided padded thermal insulation block within a secondary biohazard containment barrier, and shipped overnight to Guardant Health at ambient temperature. …To isolate plasma, whole blood was centrifuged (1,600 x *g* for 10 minutes at 10°C), and the resulting supernatant was clarified by additional centrifugation (3,220 x *g* for 10 minutes at 10°C).") Odegaard et al. at 3540 ("All cell-free DNA (cfDNA) extraction, processing, and sequencing was performed in a CLIA-certified, CAP-accredited laboratory (Guardant Heath, Inc.) as described previously (24). Briefly, cfDNA was extracted from the entire plasma aliquot prepared from a single 10-mL tube described above (QIAamp Circulating Nucleic Acid Kit, Qiagen, Inc.), which yielded a median of 48.5 ng of cfDNA (range, 2–1,050 ng). Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng

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	input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1).

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	Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials , and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).") Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.") Odegaard et al. at 3547 ("These data demonstrate applicability of ctDNA-based genotyping to treatment paradigms based on tissue genotyping; however, ctDNA has the potential to not only match but to surpass tissue genotyping in for important ways.") '822 patent, claim 1 ("[P]roviding a population of cell free DNA ('cfDNA') molecules obtained from a bodily sample from a subject.") Guardant360 CDx Technical Information at 5 ("Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. A minimum of 5 mL whole blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. . . . Plasma is isolated via centrifugation and cfDNA is extracted.")
[1b.] randomly tagging the segments of genomic nucleic acids	Guardant randomly tags the segments of genomic nucleic acids with different nucleic acid tags, thereby generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids, such that

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
with different nucleic acid tags, thereby generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids, such that each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags;	each of the unique tagged nucleic acid molecules comprises a segment of the segments of the genomic nucleic acids from step (a) and a nucleic acid tag of the nucleic acid tags. For example, during library preparation, Guardant attaches library adapters containing barcodes to the ends of the isolated DNA fragments, creating a set of unique tagged nucleic acid molecules. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

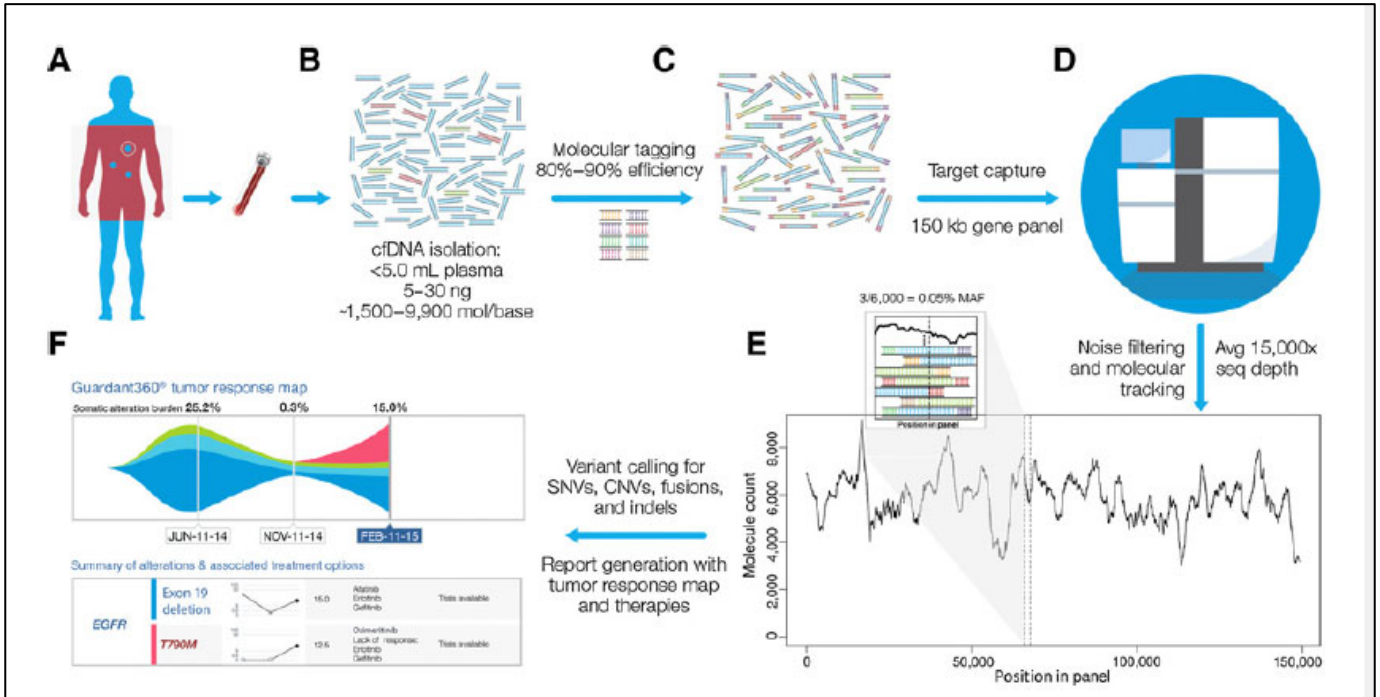
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	

Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1) ("Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data.")

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	(i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.") '822 patent, 3:25-35 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step. In some embodiments, the barcode is a polynucleotide, which may further comprise random sequence or a fixed or semi-random set of oligonucleotides that in combination with the diversity of molecules sequenced from a select region enables identification of unique molecules and be at least a 3, 5, 10, 15, 20, 25, 30, 40, 45, or 50mer base pairs in length.") '822 patent, 1:49-63 ("The disclosure provides for a method for detecting copy number variation comprising: a) sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide are optionally attached to unique barcodes; b) filtering out reads that fail to meet a set threshold; c) mapping sequence reads obtained from step (a) to a reference sequence; d) quantifying/counting mapped reads in two or more predefined regions of the reference sequence; e) determining a copy number variation in one or more of the predefined regions by (i) normalizing the number of reads in the predefined regions to each other and/or the number of unique barcodes in the predefined regions to each other; and (ii) comparing the normalized numbers obtained in step (i) to normalized numbers obtained from a control sample.") '822 patent, 6:26-27 ("In some embodiments each tagged parent polynucleotide in the set is uniquely tagged.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

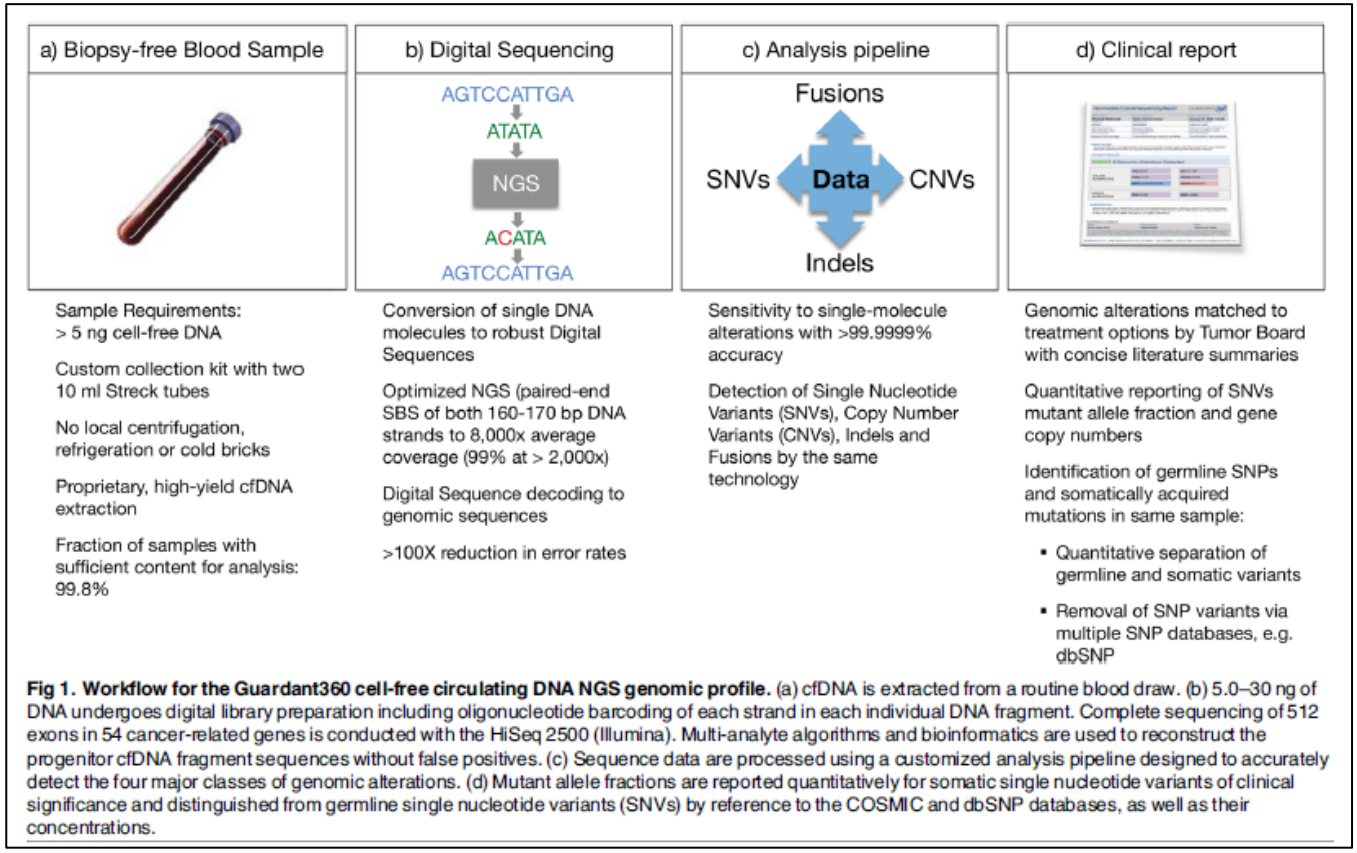
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
[1c.] subjecting the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating copies of the unique tagged nucleic acid molecules;	Guardant subjects the unique tagged nucleic acid molecules to a polymerase chain reaction (PCR), thereby generating copies of the unique tagged nucleic acid molecules. For example, Guardant uses PCR amplification to amplify the library of unique tagged nucleic acid molecules to create an enriched library that can be sequenced. Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.") Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides [76].") Lanman et al. at 17 ("For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs.") Lanman et al. at 18 ("These digital sequence libraries are amplified and then subsequently enriched for target genes using biotinylated custom baits of RNA probes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3540 ("Here, we describe definitive analytic and clinical validation studies of a comprehensive tumor-derived cell-free DNA sequencing assay for clinical genotyping of advanced solid tumors and report clinical experience applying this technology in the clinical care of 10,593 consecutive patients.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.") '822 patent, claim 1 ("[A]mplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The resulting DNA is amplified by PCR to create libraries suitable for enrichment….Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1d.] generating tag associated sequence reads by sequencing the copies of the unique tagged nucleic acid molecules of step (c);	Guardant generates tag associated sequence reads by sequencing the copies of the unique tagged nucleic acid molecules of step (c). For example, Guardant sequences the enriched libraries of unique tagged nucleic acid molecules. Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.") Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



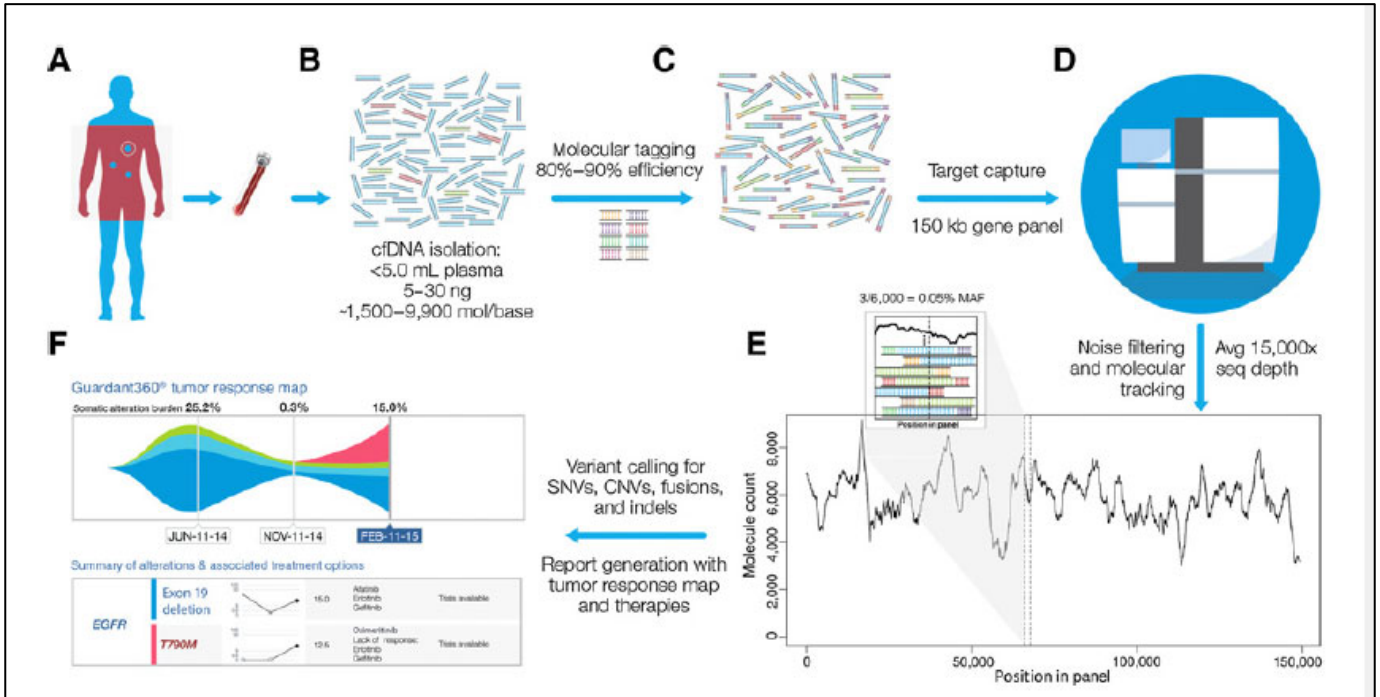
Lanman et al. at 5. (Fig. 1 above)

Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call. This process is akin to building a separate noise model for each and every one of the 78,000 bases in the sequencing panel. Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3539 ("High-concordance with orthogonal clinical plasma- and tissue-based genotyping methods supports the clinical accuracy of digital sequencing across all four types of targetable genomic

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	alterations. Digital sequencing's clinical applicability is further supported by high rates of technical success and biomarker target discovery.") Odegaard et al. at 3539 ("All cell-free DNA (cfDNA) extraction, processing, and sequencing was performed in a CLIA-certified, CAP accredited laboratory (Guardant Health, Inc.) as described previously (24).") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1). Sequencing reads are then used to reconstruct each individual cfDNA molecule present in the original patient sample with high-fidelity using proprietary double-stranded consensus representation.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). Guardant360 CDx Technical Information at 5 ("The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Paired-end sequencing by synthesis is performed with the Illumina NextSeq 550 Sequencing system. The amplified cfDNA is analyzed by parallel sequencing of amplified target genes to an average depth of coverage of greater than 2,700 unique molecules.") '822 patent, claim 1 ("[S]equencing the population of amplified progeny polynucleotides to produce a set of sequence reads.") Nakamura et al. at "Methods" ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1e.] assigning each of the unique tagged nucleic acid molecules to a location on a genome by mapping each of the tag associated sequence reads of step (d) to a location in the genome; and	Guardant assigns each of the unique tagged nucleic acid molecules to a location on a genome by mapping each of the tag associated sequence reads of step (d) to a location in the genome. For example, Guardant uses software, such as CASAVA, to map the amplified unique tagged molecules to a reference genome, which can be used to reconstruct the genome attributed to the sample. Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules. First, 150bp paired-end reads are aligned to the reference genome. Trim_galore, a wrapper script for cutadapt, is used to remove lower quality 3' adapter sequences, and custom scripts are used to remove unaligned sequences and trim low quality tails and adapter contaminations.") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv: 1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("Fusion events were detected by merging overlapping paired-end reads to form a representation of the sequenced cfDNA molecule which was then, aligned, mapped to initial unique cfDNA molecules based on molecular barcoding alignment information, including soft clipping.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") '822 patent, claim 1 ("[M]aping sequence reads of the set of sequence reads to one or more reference sequences from a human genome.") Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")
[1f.] in a plurality of locations in the genome, obtaining relative copy number information of the genomic nucleic acids from the sample based on the number of unique tagged nucleic acid molecules that have been assigned to each of the plurality of locations in the genome.	In a plurality of locations in the genome, Guardant obtains relative copy number information of the genomic nucleic acids from the sample based on the number of unique tagged nucleic acid molecules that have been assigned to each of the plurality of locations in the genome. For example, Guardant determines the copy number variation (CNV) or amplification of a given gene or locus by measuring the total number of unique copies of the sequence mapped to a location on the reference genome. Prospectus at 121 ("Copy number variants manifest in higher than expected coverage, or number of unique molecules mapping to a given amplified region of the genome, compared to that expected for a typical diploid genome. Since observed coverage is highly variable from region to region depending on sequence composition and efficiency of amplification for each individual sample, it is highly non-uniform even when no copy number variants are present. As such, copy number variation is significantly harder to identify than small sequence variants, such as SNVs, insertions, and deletions considered in the previous figure.").) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detectes (sic) single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23]."

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

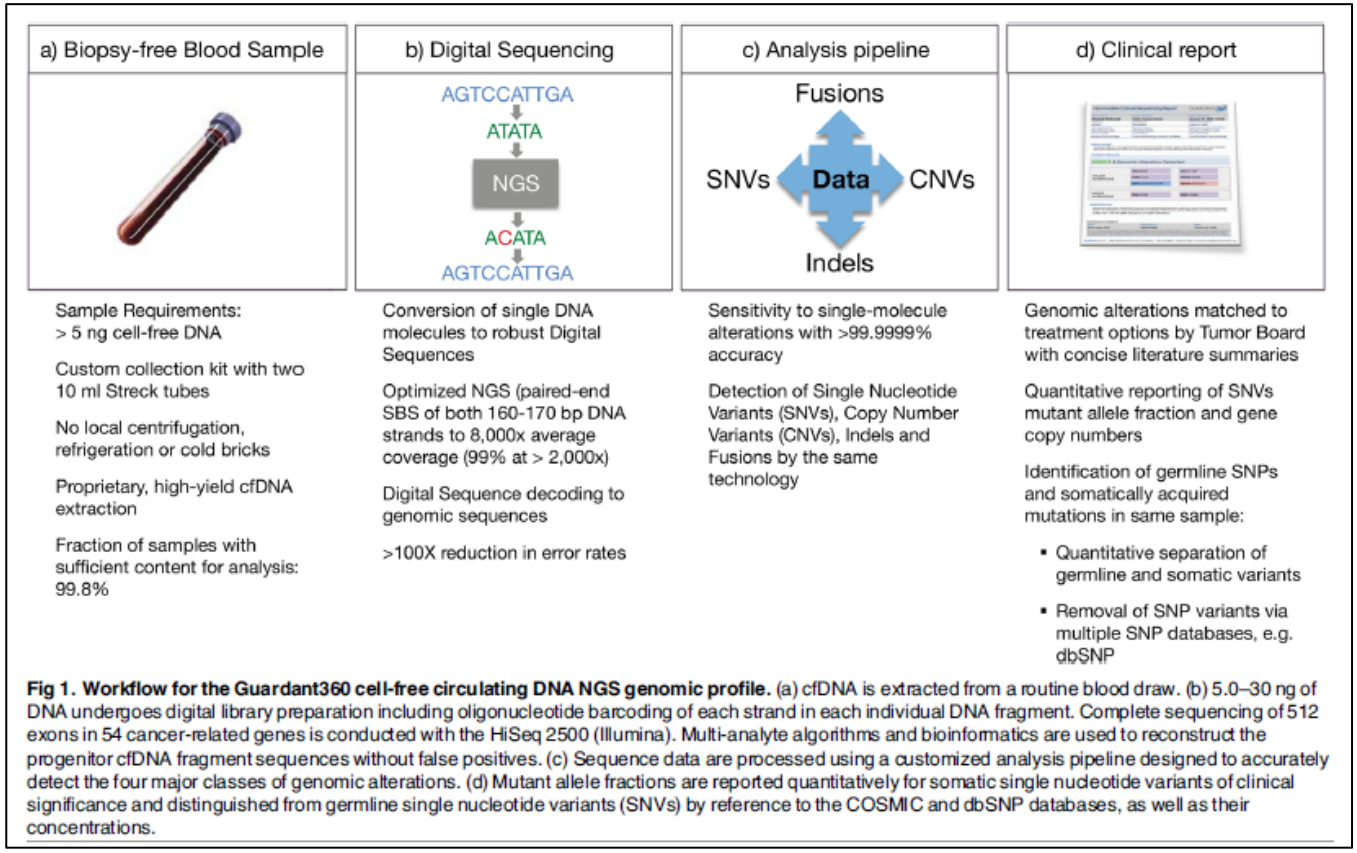


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5. (Fig. 1 above).

Lanman et al. at 14 ("Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 17 ("Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call. This process is akin to building a separate noise model for each and every one of the 78,000 bases in the sequencing panel. Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlap-ping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 20 ("[T]he Guardant360 panel expresses the absolute copy number or focal amplification of a gene in the plasma, and when 2.2 or higher, this may represent a clinically significant increase in the copy number of the gene in cancer cells in tumor samples.") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv: 1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3540 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at gene level. CAN determinations were based on training set-established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.") '822 patent, title ("Systems and methods to detect rare mutations and copy number variation.") '822 patent, claim 1 ("[G]rouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide; g) at each genetic locus of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus; and h) determining a frequency of one or more bases called at the locus from among the families." '822 patent, claim 2 ("The method of claim 1, further comprising detecting, at one or more loci, at least one single nucleotide variant, at least one gene fusion and at least one copy number variant.") '128 patent, 67:9-14 ("Guardant360™ is a targeted cfDNA NGS panel using hybrid capture and complete exon sequencing for single nucleotide variant detection in 70 genes, copy number amplifications (CNA) in

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	16 genes, fusions in six genes, and small indels in EGFR, ERBB2 and MET exon 14 skipping (see FIG. 20).") Guardant360 CDx Technical Information at 1 ("Guardant360® CDx is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of . . . copy number amplifications (CNAs) in two (2) genes . . . .") **Table 2. Genes Containing Alterations Detected by the Guardant360® CDx** **Alteration Type** / **Genes** Single Nucleotide Variants (SNVs): *AKT1, ALK, APC, AR, ARAF, ATM*, BRAF, BRCA1**, BRCA2**, CCND1, CDH1, CDK4, CDK6, CDK12*, CDKN2A, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MTOR, MYC, NF1, NFE2L2, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, RHEB, ROS1, SMAD4, SMO, STK11, TERT, TSC1, VHL* Indels: *AKT1, ALK, APC, ATM*, BRAF, BRCA1**, BRCA2**, CDH1, CDK12*, CDKN2A, EGFR, ERBB2, ESR1, FGFR2, GATA3, HNF1A, HRAS, KIT, KRAS, MET, MLH1, NF1, PDGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TSC1, VHL* PMA P200010/S010: FDA Summary of Safety and Effectiveness Data 3 of 33 **Alteration Type** / **Genes** Copy Number Amplifications (CNAs): *ERBB2, MET* Fusions / Rearrangements: *ALK, NTRK1, RET, ROS1* *Reporting is enabled for pathogenic germline alterations only. Somatic alterations will not be reported. **Reporting is enabled for both germline and somatic alterations.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 1; *id.* at 3-4 (Table 2—reporting copy number amplifications of genes ERBB2 and MET). Nakamura et al. at "Methods" ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 2

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the polymerase chain reaction is performed in the presence of region specific PCR primers.	Guardant performs the polymerase chain reaction in the presence of region specific PCR primers. For example, Guardant uses specific PCR primer pairs associated with particular regions of the tagged nucleic acid molecule, which can be used to selectively amplify molecules. Lanman et al. at 18 ("For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence. Amplified enriched libraries are pooled in equimolar amounts, denatured, and diluted to appropriate concentration for sequencing.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant CDx Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60). None of the primers or probes mapped to the representative microbial contaminant genomes.")

ASSERTED CLAIM 3

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the unique tagged nucleic acid molecules differ at more than one nucleotide or wherein the sequences of the nucleic acid tags are known prior to said generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids.	Guardant tags nucleic acid molecules that differ at more than one nucleotide, or tags nucleic acid molecules with tags that have sequences known prior to generating unique tagged nucleic acid molecules from the segments of genomic nucleic acids. For example, Guardant uses non-redundant oligonucleotides to tag nucleic acid molecules. Guardant also uses oligonucleotide barcodes known to be non-random at the time they are ligated to the nucleic acid molecules. Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

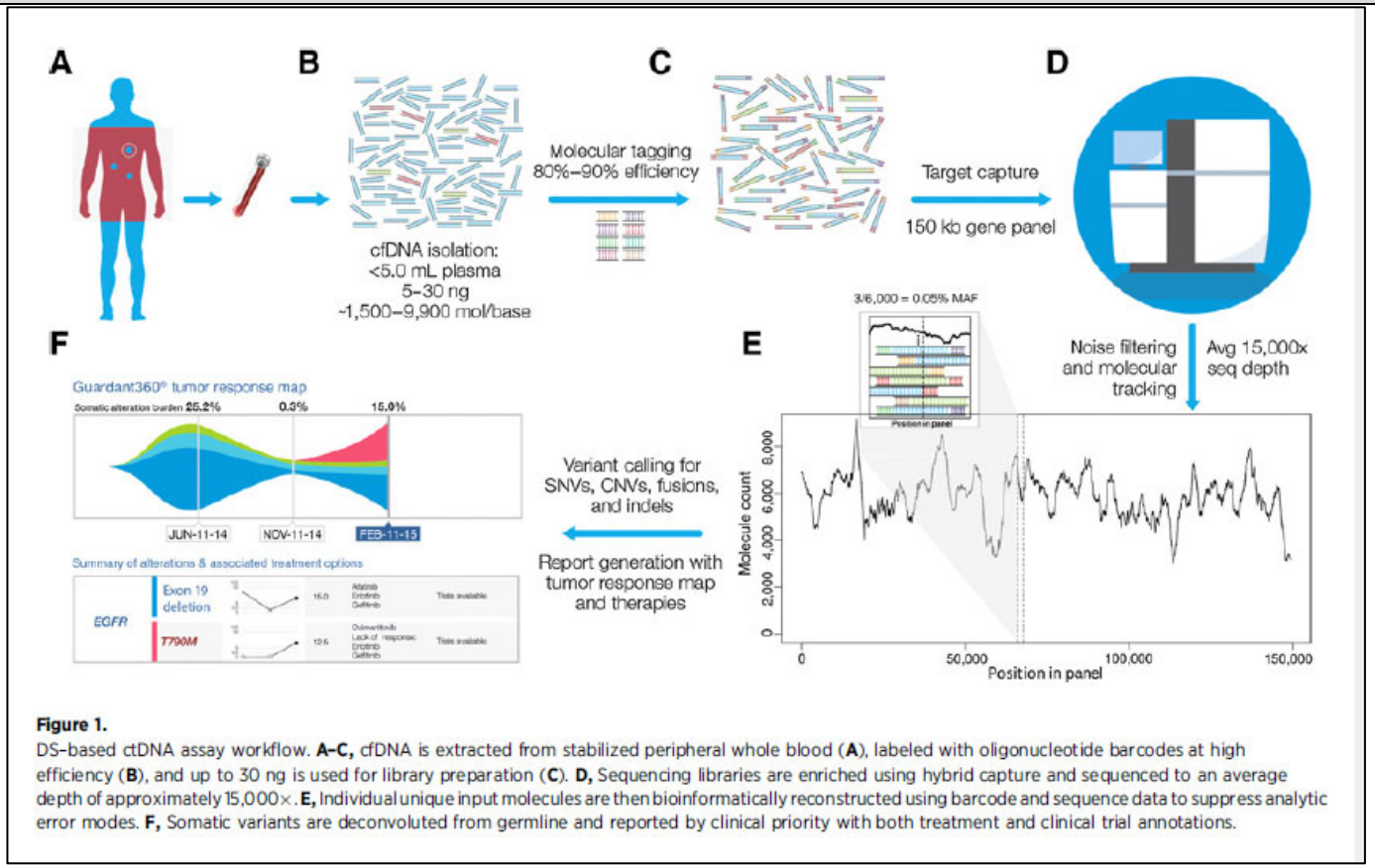


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina).")

ASSERTED CLAIM 5

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the nucleic acid tags comprise a sample tag or a sample tag set.	Guardant tags nucleic acid molecules with a sample tag or a sample tag set. For example, Guardant multiplexes by pooling nucleic acid molecules obtained from different biological samples, and attaches sample tags to nucleic acid molecules so that it can trace sequencing reads back to the correct sample. Lanman et al. at 1("Specifically, theoretical conversion efficiency of amplicon-based methods on cell-free DNA can be approximated by (1-x/166)*100%, where x is the length of the amplicon. For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs. High efficiency conversion is critical since it translates directly to sensitivity, i.e., one cannot achieve single-molecule sensitivity if only a fraction of a few input DNA molecules are convertible to analyzable material.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guideline-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 21 ("The 165 matched plasma and tissue samples were both analyzed on the Illumina Hi-Seq 2500 platform, the cfDNA analyzed using the digital sequencing method described herein and the DNA isolated from tissue samples analyzed using standard prep as per the manufacturer's instructions [76].") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("As described previously, base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fast1 (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads)." Odegaard et al. at 3541 ("Copy number determination was assessed using a compendium of multiplexed PCR assays against reference genes in combination with target-specific PCR assays. Copy number was determined from the average of the reference gene signal relative to the total target signal.") Odegaard et al. at 3541 ("Frequency of false positive calls was defined using a set of 408 validation runs (324 LOD pools and 84 healthy cfDNA donor runs across both NextSeq and HiSeq instruments) for which truth was orthogonally defined. Analytic specificity was determined by analysis of pooled samples at high dilution that had been orthogonally defined at high VAF. Precision was determined utilizing in-process positive controls from the clinical sequencing workflow and pooled clinical samples.") Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).") Nakamura et al. at "Methods" ("Base call files generated using RTA software v 2.12 (Illumina) were demultiplexed using bcl2fastq (v2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming.") Guardant CDx Technical Information at 16 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The Guardant360 CDx Software uses a custom-developed analysis bioinformatics pipeline (BIP) software module. The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream.")

ASSERTED CLAIM 6

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 5, wherein the unique tagged nucleic acid molecules are pooled with a plurality of unique tagged nucleic acid molecules having a different sample tag prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules product of step (c).	Guardant pools unique tagged nucleic acid molecules with a plurality of unique tagged nucleic acid molecules having a different sample tag prior to the PCR or prior to said sequencing of the amplified tagged nucleic acid molecules product of step (c). For example, Guardant multiplexes by pooling nucleic acid molecules obtained from different biological samples, and attaches different sample tags to nucleic acid molecules from different samples so that it can trace sequence reads back to the correct sample. Lanman et al. at 1 ("Specifically, theoretical conversion efficiency of amplicon-based methods on cell-free DNA can be approximated by (1-x/166)*100%, where x is the length of the amplicon. For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs. High efficiency conversion is critical since it translates directly to sensitivity, i.e., one cannot achieve single-molecule sensitivity if only a fraction of a few input DNA molecules are convertible to analyzable material.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guideline-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 21 ("The 165 matched plasma and tissue samples were both analyzed on the Illumina Hi-Seq 2500 platform, the cfDNA analyzed using the digital sequencing method described herein and the DNA isolated from tissue samples analyzed using standard prep as per the manufacturer's instructions [76].") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All variant detection analyses were performed using the locked clinical Guardant360 bioinformatics pipeline and reported unaltered by post hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads).") Odegaard et al. at 3541 ("Copy number determination was assessed using a compendium of multiplexed PCR assays against reference genes in combination with target-specific PCR assays. Copy number was determined from the average of the reference gene signal relative to the total target signal.") Odegaard et al. at 3541 ("Frequency of false positive calls was defined using a set of 408 validation runs (324 LOD pools and 84 healthy cfDNA donor runs across both NextSeq and HiSeq instruments) for which truth was orthogonally defined. Analytic specificity was determined by analysis of pooled samples at high dilution that had been orthogonally defined at high VAF. Precision was determined utilizing in-process positive controls from the clinical sequencing workflow and pooled clinical samples.") Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically relevant biomarkers, including those for all approved and many investigational drugs and clinical trials, and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced (Fig. 1).") Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence.") Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.")

ASSERTED CLAIM 7

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 6, further comprising deconvoluting the tag associated sequence reads by grouping the tag associated sequence reads.	Guardant deconvolutes the tag associated sequence reads by grouping the tag associated sequence reads. For example, Guardant attributes amplified molecules containing the same unique sample tag to the original sample, and groups those sequence reads to the same sample. As another example, Guardant attributes amplified molecules containing the same unique molecular tag to the original molecule, and groups those sequence reads to the same original starting molecule. Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("The Guardant360 CDx Software uses a custom-developed analysis bioinformatics pipeline (BIP) software module. The BIP software module uses the raw data (output) from the targeted sequencing, partitions the data based on the sample index sequence (barcode) of each read to separate reads originating from individual samples, and executes a proprietary algorithmic reconstruction of the digitized sequencing signals based on molecular barcodes for high-fidelity molecule-based alteration calling downstream. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence alteration data is performed.") Odegaard et al. at 3540 ("All variant detection analyses were performed using the locked clinical Guardant360 bioinformatics pipeline and reported unaltered by post hoc analyses. All decision thresholds were determined using independent training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3541 ("CNAs, in contrast, are not detectable at the level of individual molecules and are instead defined by statistical overrepresentation of reference sequences in the cfDNA. As such, we utilized the CLSI "Classical Approach" (CLSI EP17-A2) to statistically determine copy number LOD based on

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	variation observed in titration series replicates and normal samples by calculating the limit where estimated copy number and Z-score values jointly have a 95% probability of exceeding the decision threshold.") Odegaard et al. at 3541 ("To detect CNAs, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. CNA determinations were based on training set–established decision thresholds for both absolute copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample's own diploid baseline.") Guardant Technical Information at 16 ("Primer and probe specificity were addressed by mapping panel probes to the human genome. When mapped to the human genome (hg19) with decoy sequences, unplaced contigs, and representative microbial contaminants genomes, 97.6% of probes uniquely map to the genome (MAPQ ≥ 60).") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained. To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library. The deviation from ideal distribution is calculated and modeled through a non-parametric regression to remove sample-preparation- and sequencing-associated biases. Subsequently, the mode of the normalized number of fragments covering each gene is calculated to estimate the fragment number corresponding to two copies to derive a baseline diploid value. All values of unique fragments for each gene are then normalized by this baseline value. The baseline derivation is informed by molecule counts data from a large set of normal samples from healthy donors' plasma (Normal Set) provided by AllCells (Alameda, CA) with donor consent. The age range of donors is 20 to 40 years and input plasma is screened to provide a minimum input of 15 ng of cfDNA. A statistical z-score for each gene is calculated by comparing the normalized gene fragment number to that derived from the Normal Set to estimate the likelihood of the gene exhibiting amplification and remove any remaining region specific bias.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	'822 patent at 9:8-31 ("In another embodiment wherein the sets derive from a common sample, the method further comprising: a. inferring copy number variation for the plurality of sets based on a comparison of the inferred number of tagged parent polynucleotides in each set mapping to each of a plurality of reference sequences. In another embodiment the original number of polynucleotides in each set is further inferred. This disclosure also provides a system comprising a computer readable medium for performing the aforesaid methods. This disclosure also provides a method of determining copy number variation in a sample that includes polynucleotides, the method comprising: a. providing at least two sets of first polynucleotides, wherein each set maps to a different reference sequence in a genome, and, for each set of first polynucleotides; i. amplifying the polynucleotides to produce a set of amplified polynucleotides; ii. sequencing a subset of the set of amplified polynucleotides, to produce a set of sequencing reads; iii. group sequences reads sequence from amplified polynucleotides into families, each family amplified from the same first polynucleotide in the set; iv. inferring a quantitative measure of families in the set; b. determining copy number variation by comparing the quantitative measure of families in each set.") '822 patent at 10:52-11:3 ("This disclosure also provides a computer readable medium in non-transitory, tangible form comprising executable code configured to perform the following steps: a. accessing a data file comprising a plurality of sequence reads; b. filtering out reads that fail to meet a set threshold; c. mapping sequence reads derived from the sequence onto a reference sequence; d. identifying a subset of mapped sequence reads that align with a variant of the reference sequences at each mappable base position; e. for each mappable base position, calculating a ratio of (a) a number of mapped sequence reads that include a variant as compared to the reference sequence, to (b) a number of total sequence reads for each mappable base position; f. normal- izing the ratios or frequency of variance for each mappable base position and determining potential rare variant(s) or other genetic alteration(s); and g. comparing the resulting number for each of the regions with potential rare variant(s) or mutation(s) to similarly derived numbers from a reference sample.") Nakamura et al. at "Methods" ("Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described43. Guardant360 interrogates 74 genes and detects

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study, 2 × 10 ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

ASSERTED CLAIM 8

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the nucleic acid tags are double-stranded.	Guardant tags nucleic acid molecules with nucleic acid tags that are double-stranded. For example, Guardant ligates oligonucleotide heptamer barcodes to each half of individual double-stranded cell-free DNA, creating a library where each single-stranded half the cell-free DNA is separately encoded with an oligonucleotide. Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the double stranded input cfDNA samples is separately encoded with said oligonucleotides. This digital conversion process occurs with high efficiency with greater than 80% of the original plasma of cfDNA molecules converted.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("To determine the CNV or amplification of a given gene, we first measure the total number of unique fragments covering each gene comprised of both halves of the original parent molecules. Regions with copy-number alteration bias will have uneven representation of each half of the original double-stranded library.") Lanman et al. at 22 ("After isolation from plasma, each single-stranded half of the original double-stranded 5–30 ng input cfDNA sample is separately encoded with oligonucleotide heptamers to create a self-referenced digital sequence duplex library with properties similar to differential signaling in digital communications."0

ASSERTED CLAIM 9

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the unique tagged nucleic acid molecules are subjected to hybrid capture prior to the PCR or the amplified tagged nucleic acid molecules produced in step (c) are subjected to hybrid	Guardant subjects the unique tagged nucleic acid molecules to hybrid capture prior to the PCR, or subjects the amplified tagged nucleic acid molecules produced in step (c) to hybrid capture prior to step (d). For example, Guardant amplifies DNA to create a library using hybrid capture, tags the DNA, and also amplifies the unique tagged nucleic acid molecules using hybrid capture to create an enriched library that can be sequenced. Lanman et al. at 1 ("Next-generation sequencing of cell-free circulating solid tumor DNA addresses two challenges in contemporary cancer care.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
capture prior to step (d).	Lanman et al. at 1 ("Digital Sequencing is a novel method for high-quality sequencing of circulating tumor DNA simultaneously across a comprehensive panel of over 50 cancer-related genes with a simple blood test.") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma. At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a mini-mum average base coverage of 3,000X and a minimum Qscore of 20."sing Lanman et al. at 17 ("The exons in each gene targeted by the capture probes are listed in S4 Table. The enriched digital sequence libraries are then analyzed using HiSeq 2500 Sequencing System (Illumina) paired-end sequencing with fluorescent reversible terminator deoxyribonucleotides.") Lanman et al. at 17("For x = 100bp, theoretical efficiency is 39% and drops significantly with higher levels of multiplexing due to inversely lower individual concentrations of specific PCR primer pairs.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).).

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina).") '128 patent, 67:9-14 ("Guardant360™ is a targeted cfDNA NGS panel using hybrid capture and complete exon sequencing for single nucleotide variant detection in 70 genes, copy number amplifications (CNA) in 16 genes, fusions in six genes, and small indels in EGFR, ERBB2 and MET exon 14 skipping (see FIG. 20).") '822 patent, claim 1 ("[A]mplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides.") Odegaard et al. at 3540 ("Here, we describe definitive analytic and clinical validation studies of a comprehensive tumor-derived cell-free DNA sequencing assay for clinical genotyping of advanced solid tumors and report clinical experience applying this technology in the clinical care of 10,593 consecutive patients.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3541 ("For short (<50-70 bp) indels, a generative background noise model was constructed to account for PCR artifacts arising frequently in homopolymeric or repetitive contexts, allowing for strand-specific and late PCR errors.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

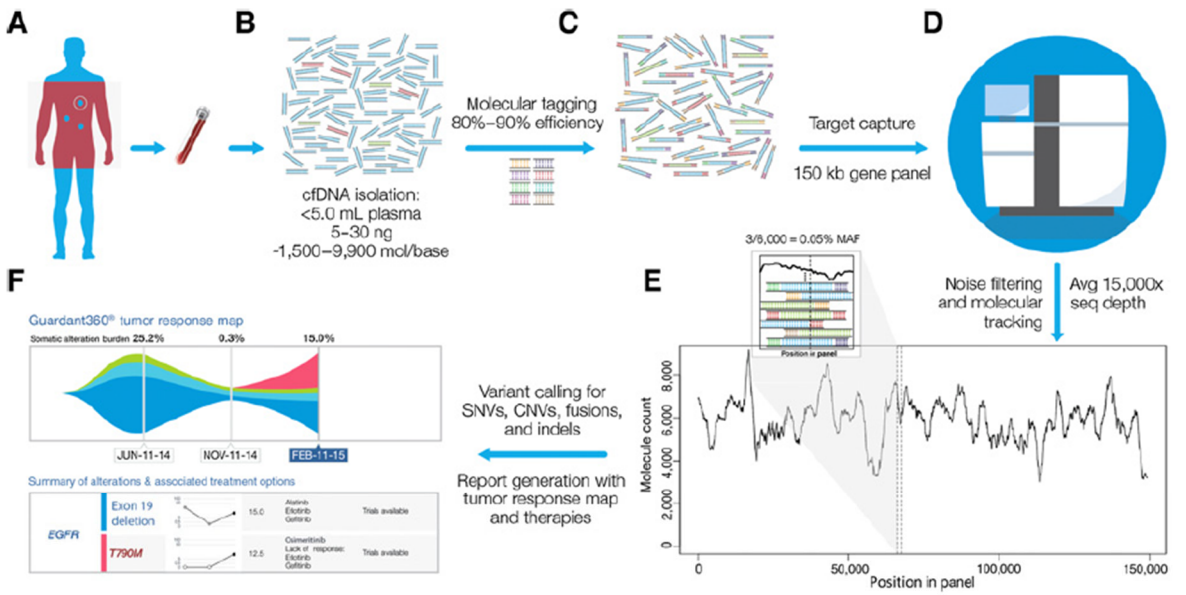


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 ("cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ("molecular barcodes"), and used to prepare sequencing libraries, which are then enriched using hybrid capture and sequenced").

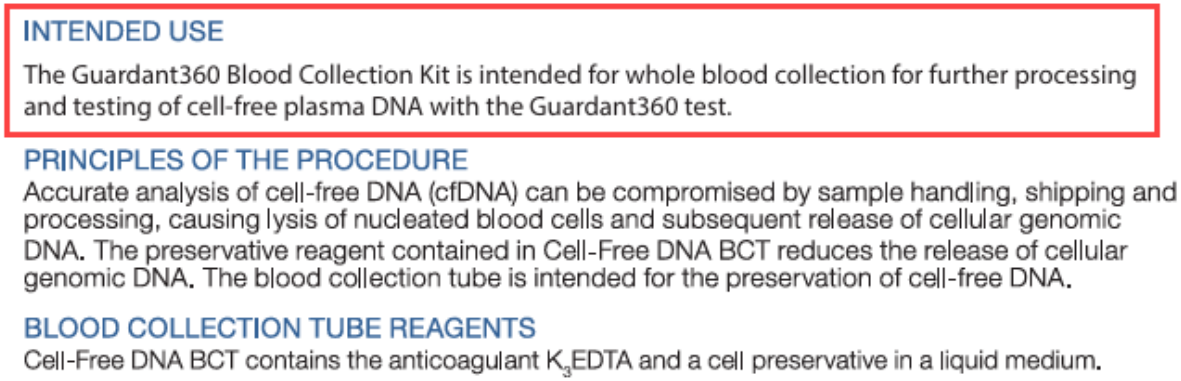
Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("Amplified libraries are enriched for genes of interest using hybrid target capture with custom biotinylated RNA probes. Each

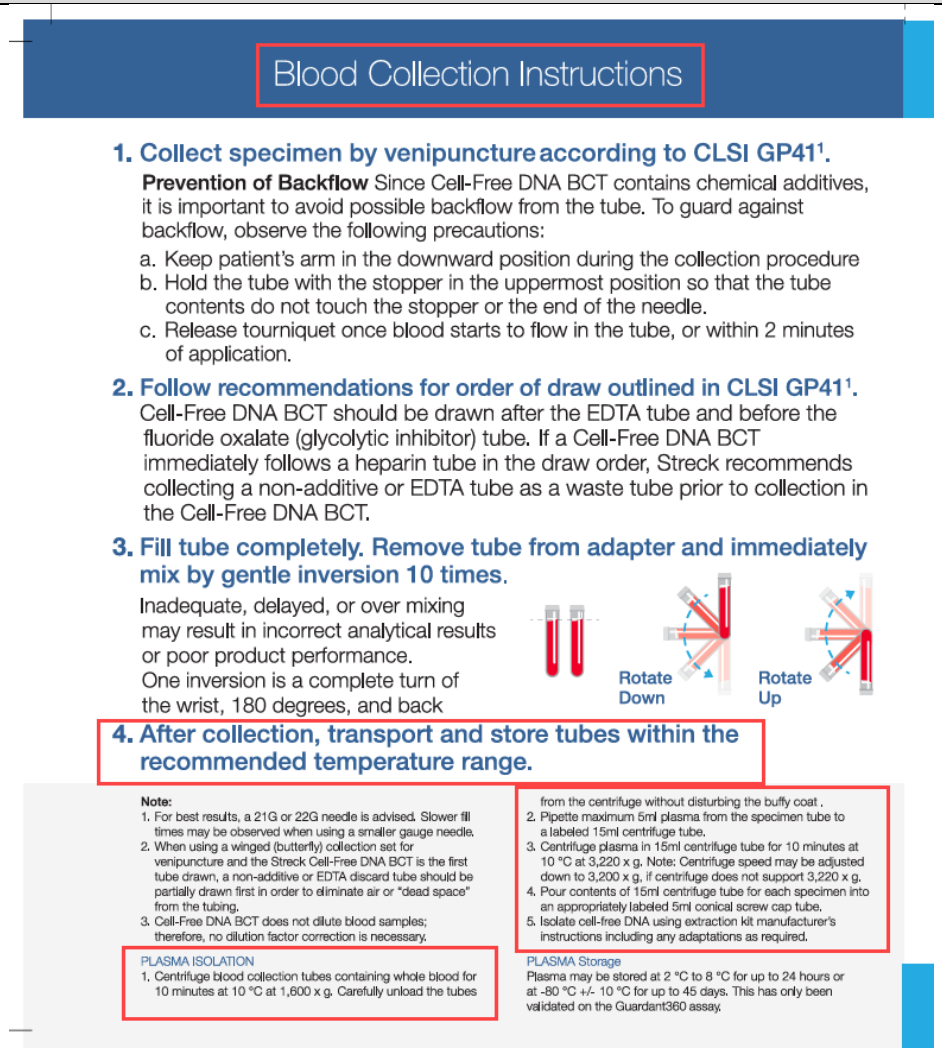
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	enriched library is amplified by PCR using a unique index primer that also contains a sequencing flow cell attachment sequence."). Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 1 ("Guardant360 CDx is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in two (2) genes, and fusions in four (4) genes."). Guardant 360CDx Technical information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture. The enriched libraries are then sequenced using next generation sequencing on the Illumina NextSeq 550 platform.").

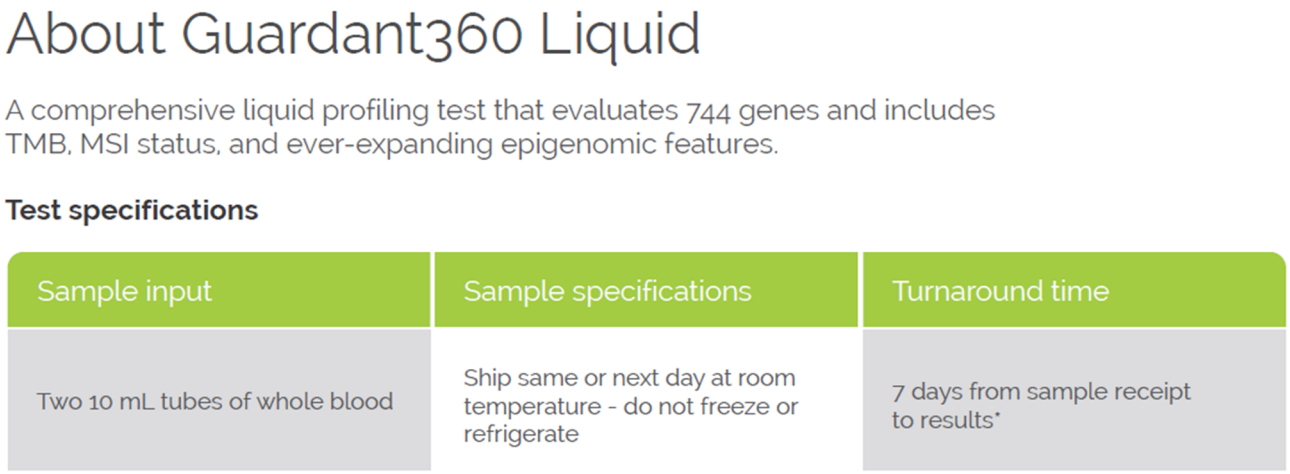
ASSERTED CLAIM 10

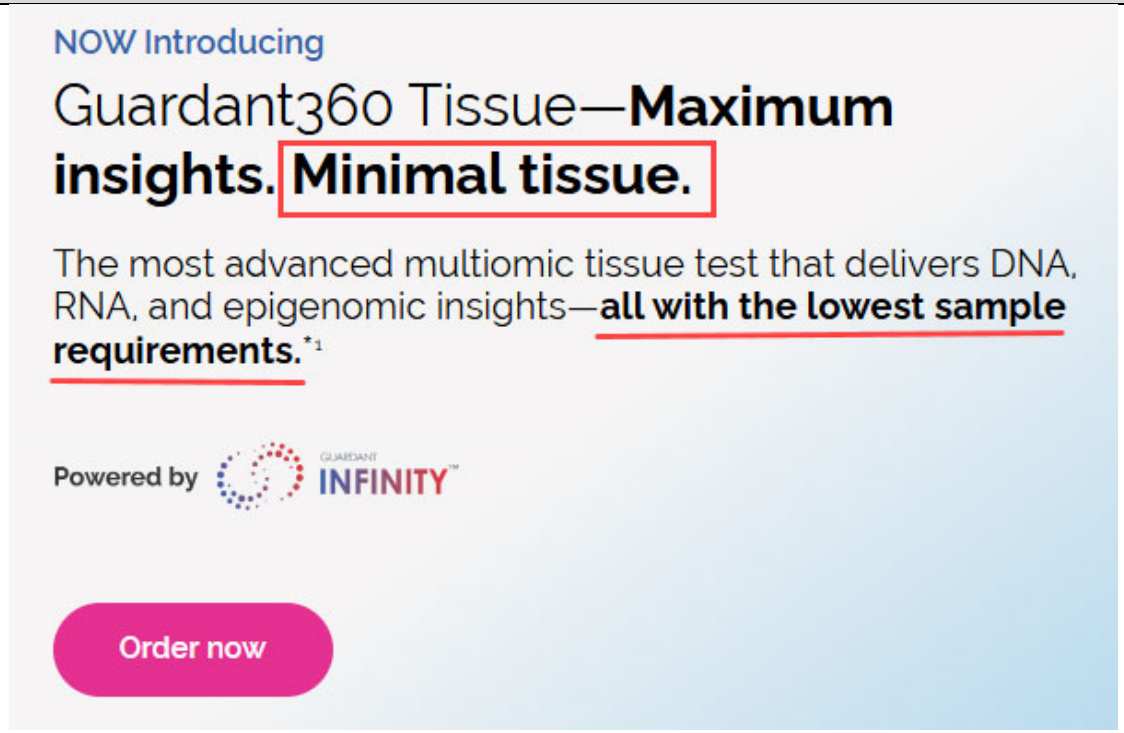
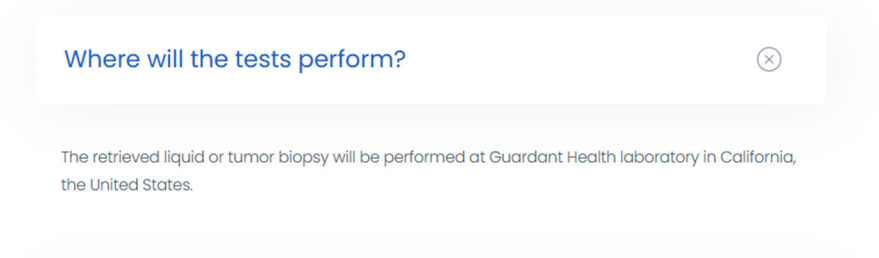
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the genomic nucleic acids are from a single species, a single cell, two or more organisms, two or more species, or from a population of microbes.	Guardant obtains nucleic acids from a single species, a single cell, two or more organisms, two or more species, or from a population of microbes. For example, the Guardant360 tests require obtaining cell-free DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples.

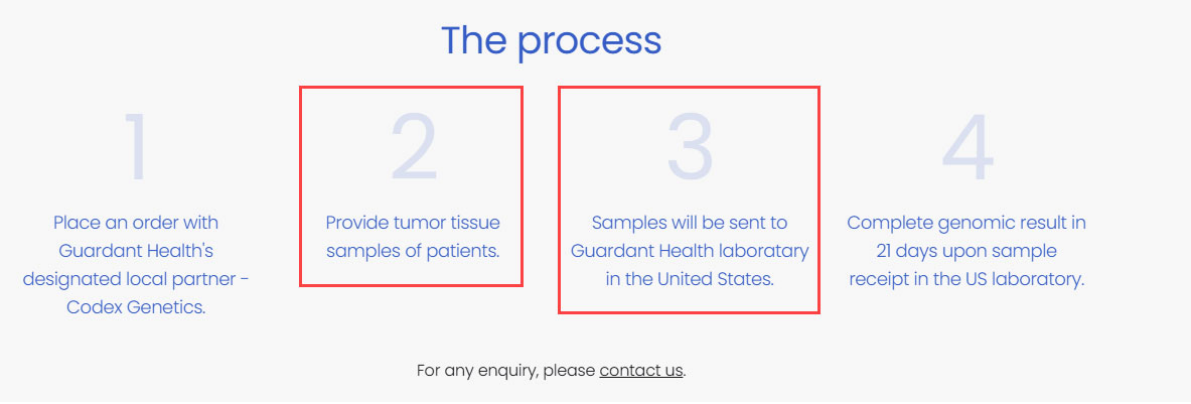
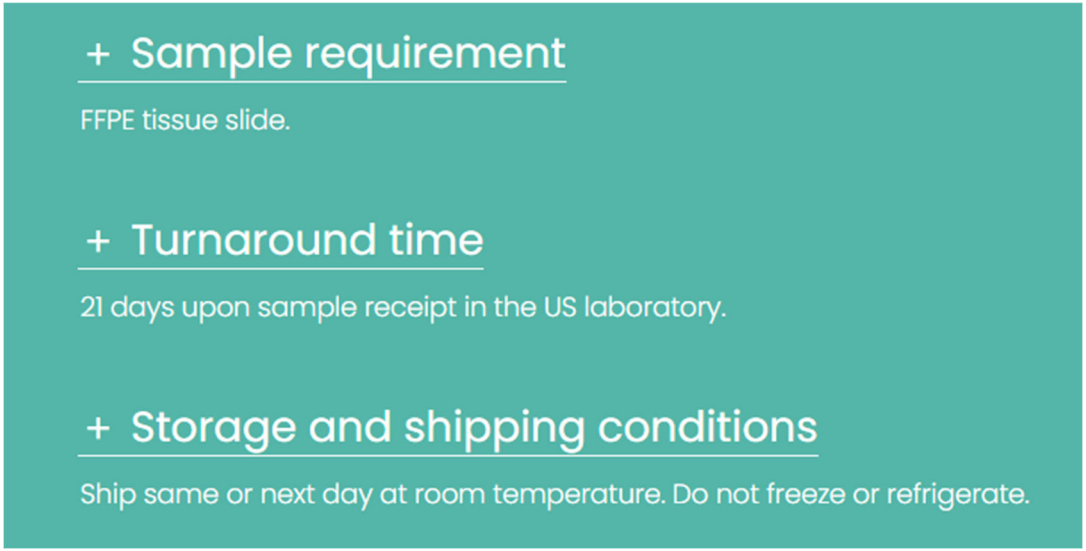
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.  Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Webpage.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

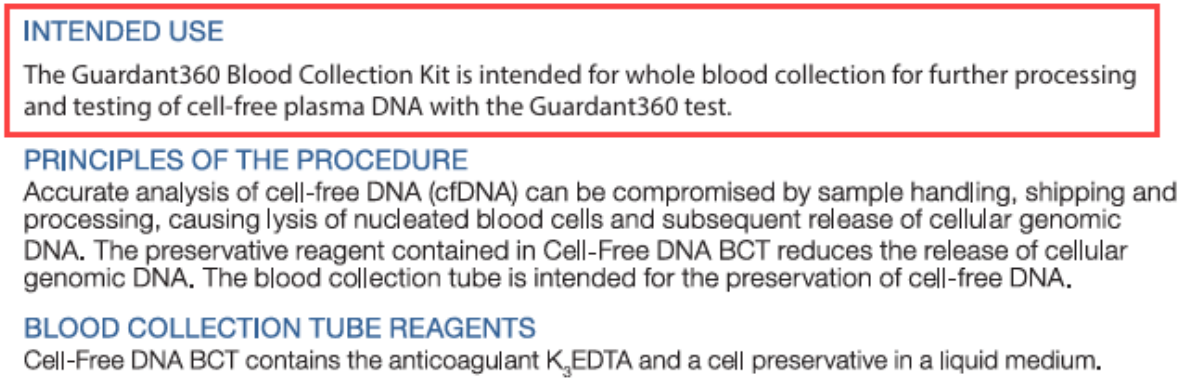
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3542 ("cfDNA is extracted from stabilized whole blood") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.).") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Nakamura et al. at 176 ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina).") Guardant 360CDx Technical information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma.")

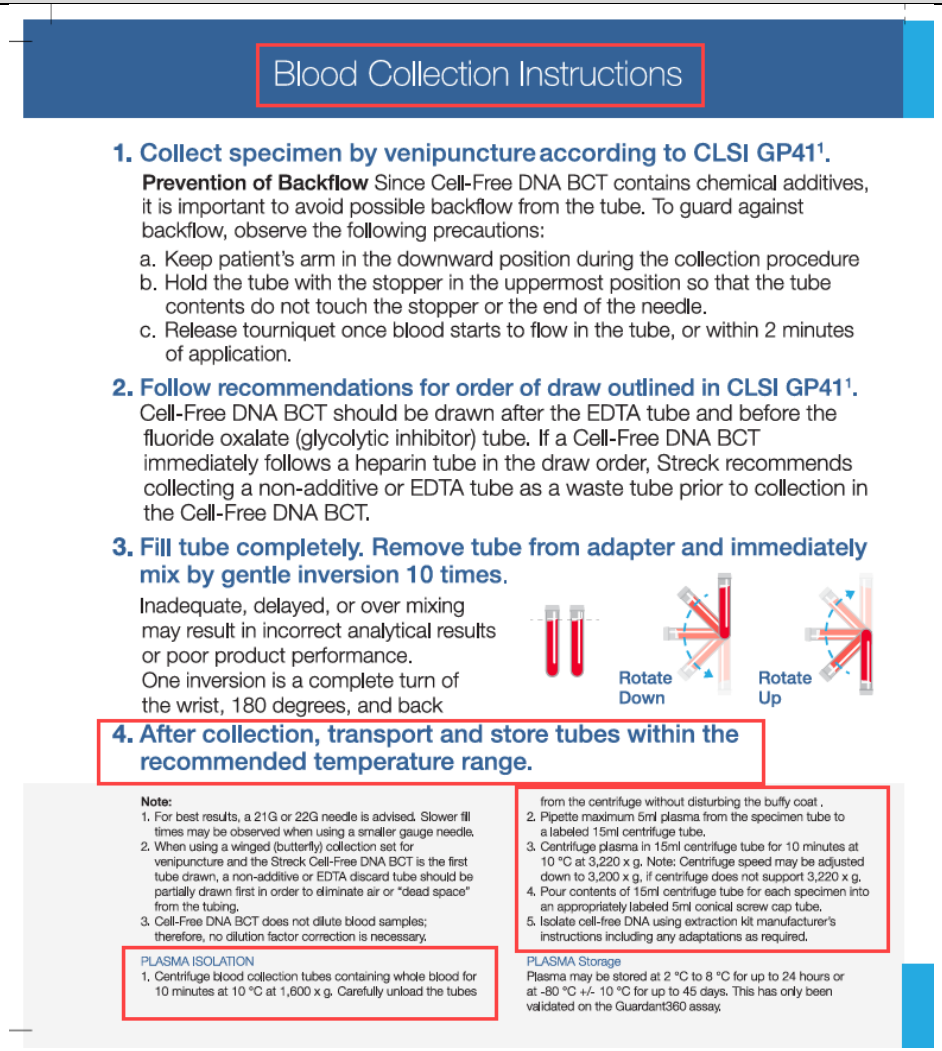
ASSERTED CLAIM 11

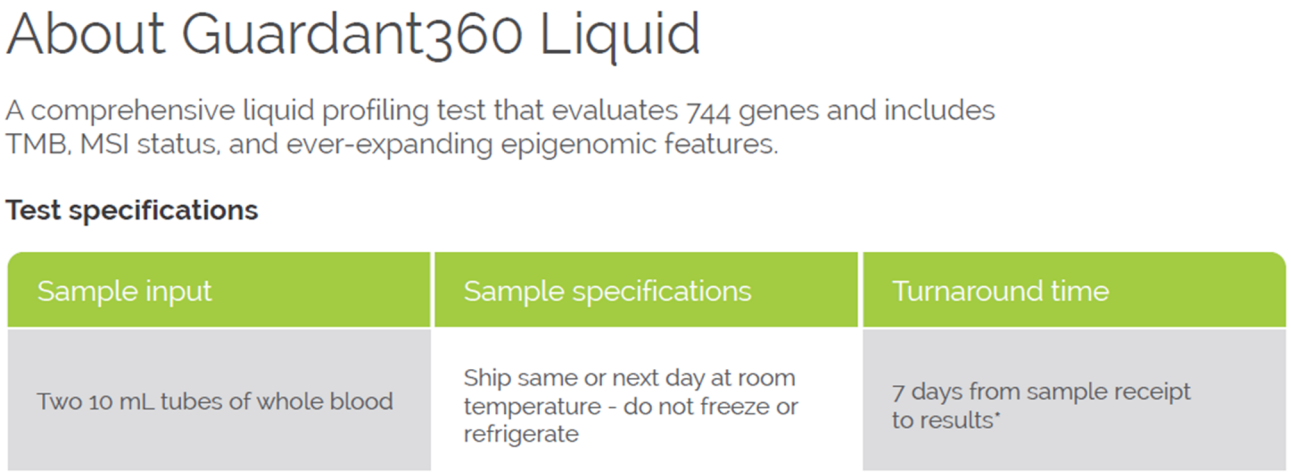
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the sample is a blood sample or bone marrow sample.	Guardant obtains the sample from blood or bone marrow. For example, the Guardant360 tests require obtaining cell-free DNA collected from a blood sample or tumor DNA collected from a tissue sample. Guardant also provides its customers with instructions for collecting DNA from blood or tissue samples, which are returned to Guardant for sample processing, and conditions receipt of the benefits of attaining results from the Guardant360 tests on the customer collecting DNA from blood or tissue samples.

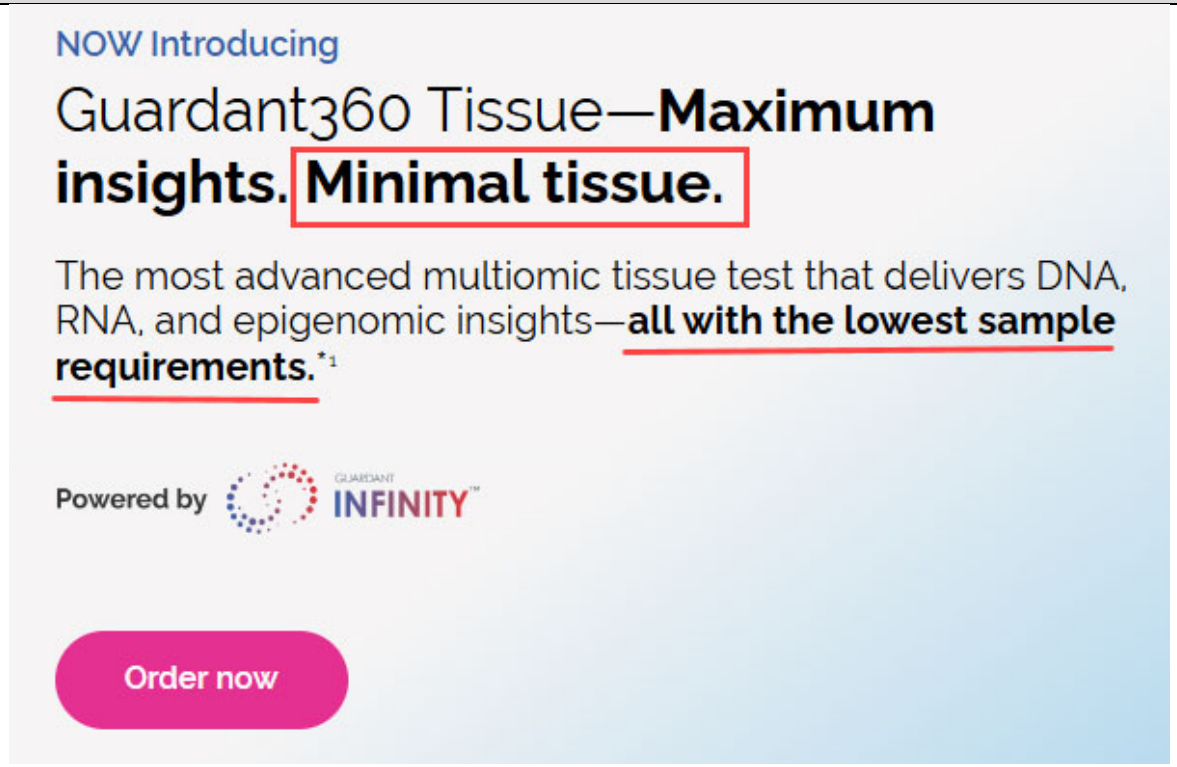
GUARDANT HEALTH EX.1020.183

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	5.3. Principles of the Procedure Guardant360 CDx is performed by a single laboratory, the Guardant Health Clinical Laboratory, located in Redwood City, CA, USA. Guardant360 CDx is composed of the following major processes: • Whole Blood Collection and Shipping • Plasma Isolation and cfDNA Extraction • Library Preparation and Enrichment • DNA Sequencing • Data Analysis and Reporting Guardant360 CDx Technical Information at 4.  Guardant Blood Collection Kit Instructions at 2.

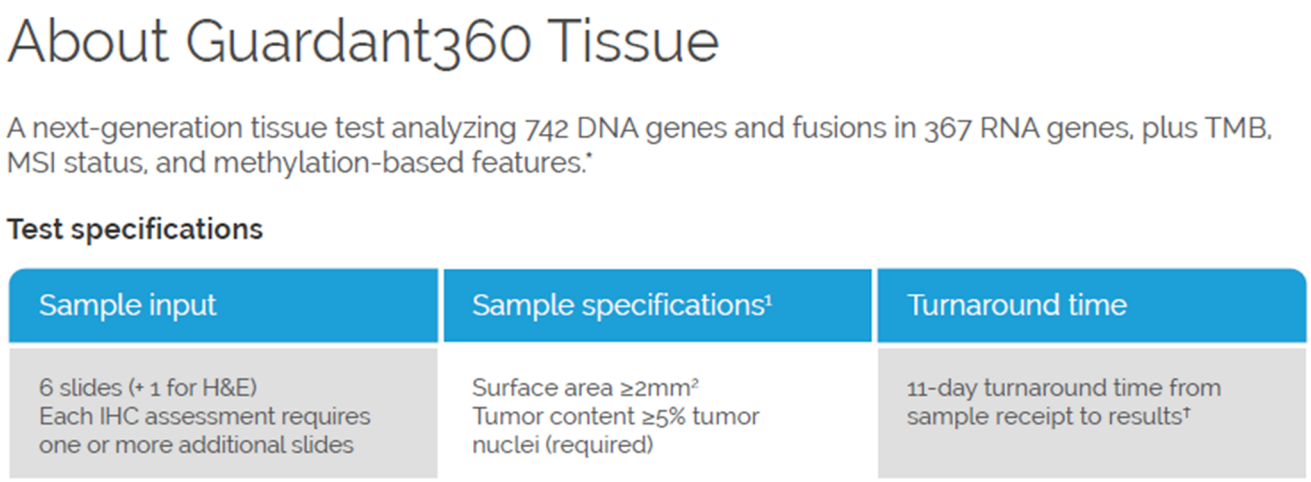
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 2.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant Blood Collection Kit Instructions at 1.

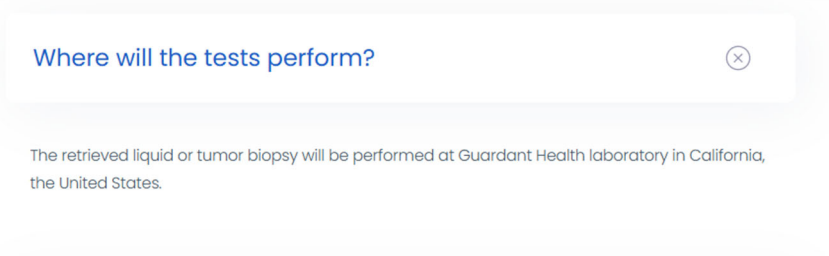
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Liquid Assay Specifications at 1.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 Tissue Webpage.

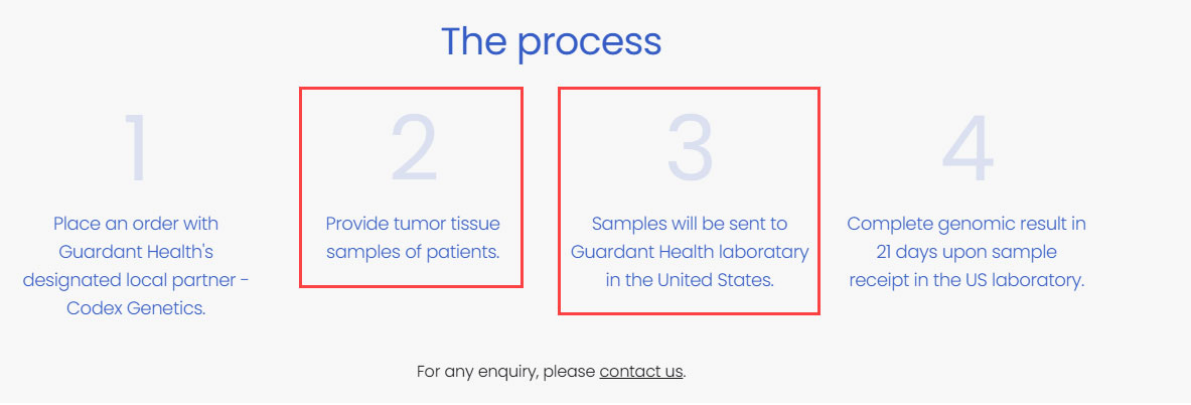
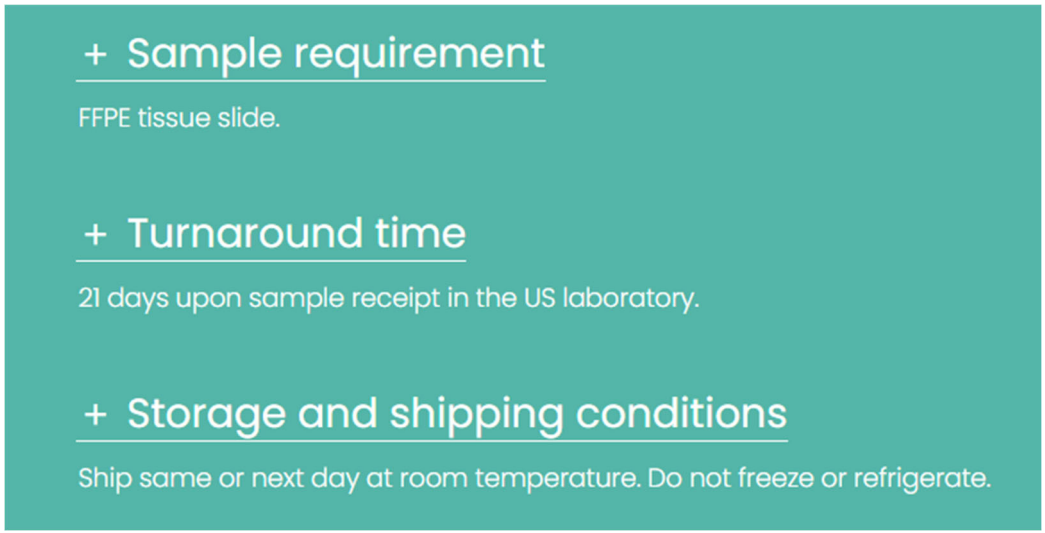
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



Guardant360 Tissue Assay Specifications at 1.



Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 Guardant360 TissueNext – Codex Webpage.  Guardant360 TissueNext – Codex Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 3 ("digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.") Guardant360 CDx Technical Information at 5 ("Whole blood specimens are processed in the Guardant Health Clinical Laboratory within 7 days of blood collection. A minimum of 5 mL of blood must be received in order to achieve optimal performance for the Guardant360 CDx assay. . . . Plasma is isolated via centrifugation and cfDNA is extracted.").) Lanman et al. at 1–2 ("Digital sequencing of plasma-derived DNA is indicated int advanced cancer patients to prevent repeated invasive biopsies when the initial biopsy is inadequate, unobtainable for genomic testing or uninformative, or when the patient's cancer has progressed despite its treatment.") Lanman et al. at 2 ("Next-generation sequencing (NGS) of solid tumor tissue has advanced our ability to perform multiplex testing for an ever-growing number of targetable genomic alterations utilizing small needle biopsies or archival pathology slides. As the number of genomic targets with matched therapies grows, tissue biopsy material can be quickly exhausted before multiple "companion diagnostics" tests can be completed.) Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives.") Lanman et al. at 3–4 ("Here we report the analytical and clinical validation of the 54-gene (512 exons) Guardant360 panel of mutations and gene amplifications in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), NCCN Task Force recommendations and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines.") Lanman et al. at 4 ("With high sensitivity and the ultra-high specificity required to comprehensively sequence cfDNA, the Guardant360 test has the potential to evaluate the multiple genomic alterations cited in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with tumor heterogeneity [34], to interrogate all actionable genomic alterations via comprehensive sequencing of a multigene panel, and to prevent the time delays, cost and complications inherent in invasive biopsies.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

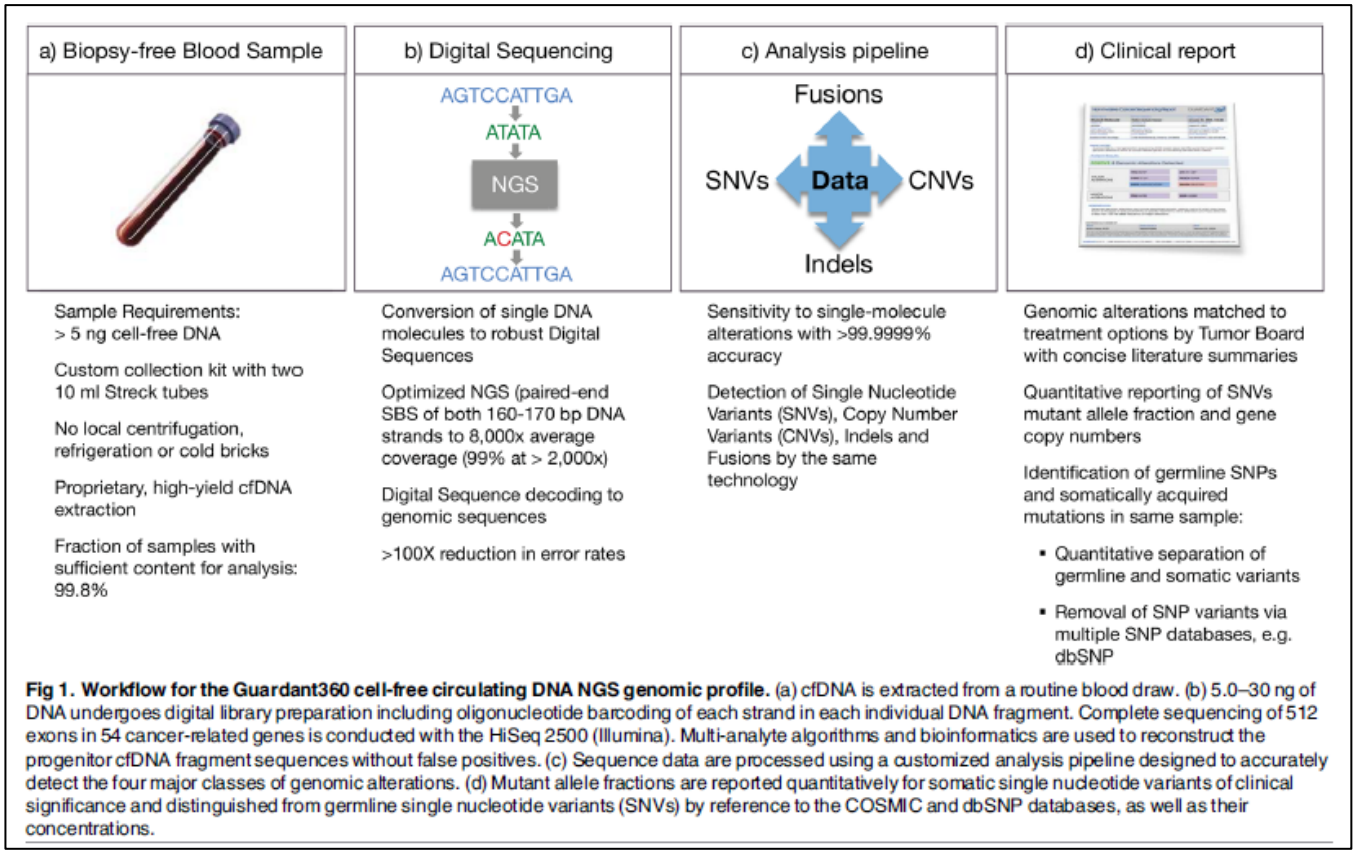


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Fig. 1 above)

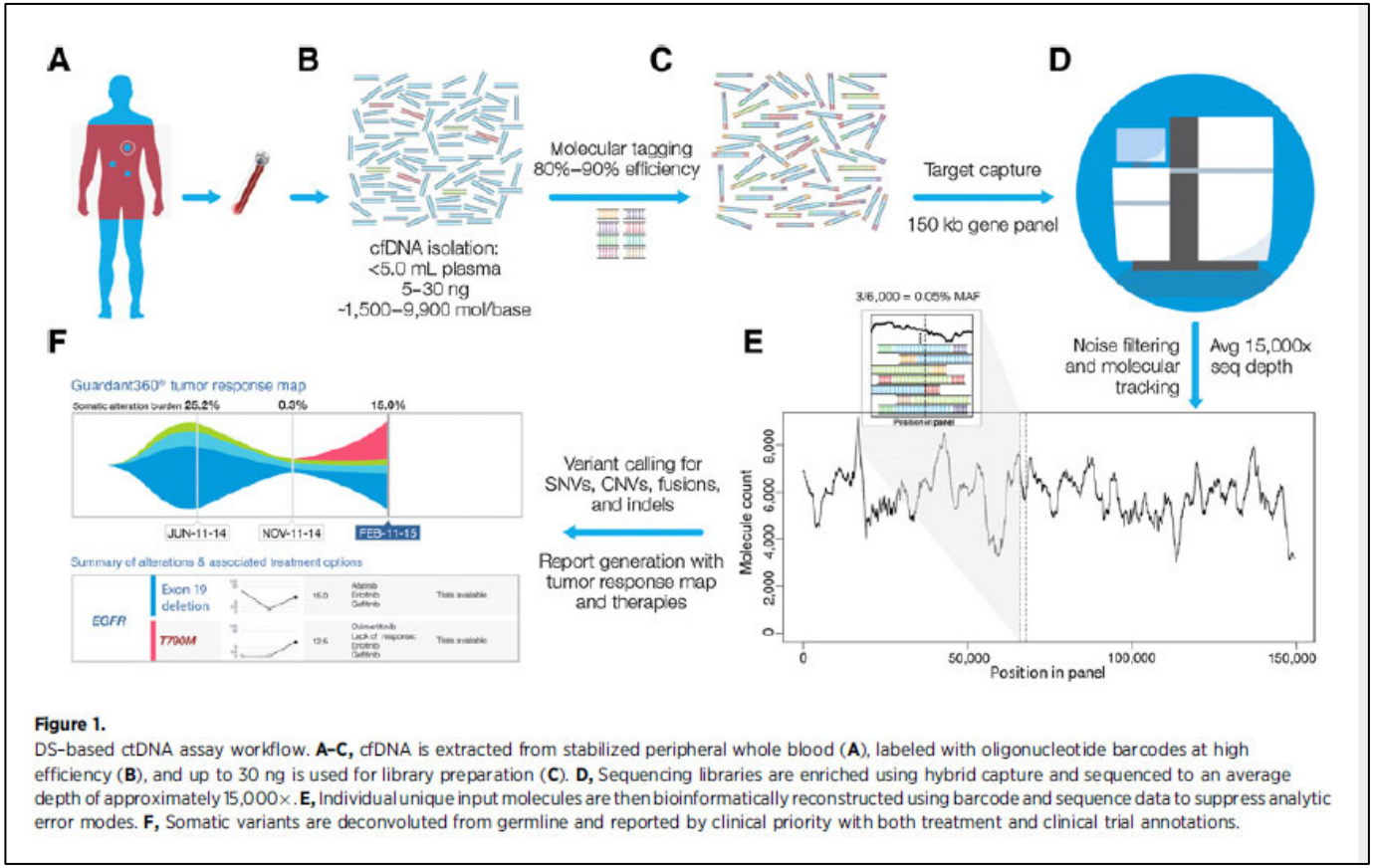
Lanman et al. at 5 ("cfDNA is extracted from a routine blood draw.")

Lanman et al. at 12 ("The analytic validation studies of the Guardant360 cfDNA panel highlight its differences from both solid tumor and other cell-free circulating tumor DNA genomic testing methods. The

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	digital sequencing platform enables single molecular sensitivity, an analytic sensitivity that is two orders of magnitude greater than an Illumina HiSeq with standard prep while maintaining near-perfect specificity (Fig 1A and 1B).") Lanman et al. at 12 ("The Guardant360 panel at the time of this study sequences ~78,000 base pairs (78 kbp), providing complete coverage for all exons in 18 genes and the critical exons in the remainder of the 54 total genes.") Lanman et al. at 14 ("The clinical sensitivity for plasma-based DNA NGS via digital sequencing was equivalent to the clinical sensitivity of tissue-based DNA NGS sequencing.") Lanman et al., 14 ("This study validates the Guardant360 assay across many cancer types, but only in stage III/IV cancers.") Lanman et al. at 14 ("The clinical utility of the Guardant360 diagnostic test in advanced cancer is supported by results in clinical practice. Seventy-six percent of 1,000 consecutively tested patients had a reported somatic single nucleotide variant amongst 54 genes or a copy number amplification in three genes. This was accomplished without an invasive tissue biopsy.") Lanman et al. at 16 ("By obviating the need for an invasive biopsy when the patient's initial biopsy is QNS for molecular testing, the Guardant360 panel enables biopsy-free multiplex testing of all current guide-line-recommended genomic targets without the costs and complications involved in invasive tissue biopsies for NGS.") Lanman et al. at 17 ("Guardant360 could be used in lieu of a repeat invasive biopsy for NGS, particularly for patients with visceral primary disease or metastases to lung, liver or bone. Secondly, in patients with visceral primary or metastatic disease, when a tumor has progressed or recurred based on clinical symptomatology, imaging or serological protein biomarkers, e.g. CEA, CA-125, CA 15–3, PSA, etc., the Guardant360 panel could be used to re-evaluate for new targeted therapies, enabling real-time adaptive management of a patient's continuously evolving cancer while obviating further invasive tissue biopsies.") Lanman et al. at 17 ("The studies presented here are the first to provide analytic and clinical validation of the accuracy of a comprehensive tumor profiling test that utilizes NGS of circulating tumor DNA to identify genomic alterations in hundreds of exons in over 50 genes with unparalleled specificity. Digital sequencing

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	relies on massively parallel sequencing of circulating tumor DNA fragments combined with algorithms that utilize multiple analytic inputs to accurately compute quantitative SNV concentrations and absolute gene copy numbers in plasma.") Lanman et al. at 17 ("The Guardant360 cfDNA NGS panel identifies SNVs via sequencing of 54 genes spanning approximately 78,000 base pairs, including complete exon coverage in all exons in 18 genes and the critical exons in 36 genes (gene list in S1 Table) as well as copy number variations (CNVs) in ERBB2, EGFR, and MET.") Lanman et al. at 17 ("At the time of this study, the Guardant360 panel targeted region was 78,000 base pairs (78 kbp) per sample and each base was sequenced at average raw coverage depth of 8,000X with a minimum average base coverage of 3,000X and a minimum Qscore of 20.") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual in Streck Cell-Free DNA Blood Collection (Streck) tubes, which contain a proprietary formaldehyde-free preservative that stabilizes white blood cells, preventing the release of genomic DNA and allowing isolation of high-quality cfDNA.") Lanman et al. at 18 ("The cell-free DNA from these cell lines is extracellular DNA released by the culture cells and the fragmentation pattern is essentially identical to circulating DNA except the size distribution peaks tend to be slightly broader. Similar biological mechanisms to those in play in circulating peripheral blood in vivo occur with the cell culture media, i.e. DNA digestion of histone-protected genomic DNA.") Lanman et al. at 18 ("Plasma samples and supernatant from cultured cell lines were processed using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. Cell-free DNA is extracted from 1.5 mL to 5 mL plasma and subsequently concentrated and size selected using Agencourt Ampure XP beads (Beckman Coulter) and subsequently quantified using Qubit1 2.0 fluorometer. The DNA is extracted using standard extraction procedures, including proteinase K digestion of histone and other DNA-binding proteins and isoproponal precipitation. The DNA is then end-repaired to repair ends of DNA with a 5' phosphate prior to ligation of adapters necessary for downstream sequencing.") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3539 ("To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.") Odegaard et al. at 3539 ("Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical sample. Blood-to-tissue clinical to those documented in the medical record of 543 consecutive lung cancer patients.") Odegaard et al. at 3540 ("Plasma-blood comprehensive tumor genotyping expands access to standard-of-care precision oncology to patients previously ineligible due to barriers associated with tumor sampling.") Odegaard et al. at 3540 ("Here, we describe the analytic (technical characterization using controlled and often contrived samples) and clinical (characterization using real-world patient samples, contexts, and comparators) validation of a comprehensive ctDNA sequencing assay for clinical genotyping of advanced solid tumors, including large-scale comparisons with orthogonal clinical plasma-and tissue-genotyping methods.") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.). Briefly, 2 x 10 mL of peripheral venous blood was collected in Streck Cell-Free DNA BCT (Streck, Inc.), packaged into a provided padded thermal insulation block within a secondary biohazard containment barrier, and shipped overnight to Guardant Health at ambient temperature. …To isolate plasma, whole blood was centrifuged (1,600 x *g* for 10 minutes at 10°C), and the resulting supernatant was clarified by additional centrifugation (3,220 x *g* for 10 minutes at 10°C).") Odegaard et al. at 3540 ("All cell-free DNA (cfDNA) extraction, processing, and sequencing was performed in a CLIA-certified, CAP-accredited laboratory (Guardant Heath, Inc.) as described previously (24). Briefly, cfDNA was extracted from the entire plasma aliquot prepared from a single 10-mL tube described above (QIAamp Circulating Nucleic Acid Kit, Qiagen, Inc.), which yielded a median of 48.5 ng of cfDNA (range, 2–1,050 ng). Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")  **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). Odegaard et al. at 3542 ("The assay, Guardant360, utilizes NGS-based digital sequencing (DS; ref. 24) to comprehensively profile 73 cancer-related genes (Supplementary Fig. S1) comprising both therapeutically

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	relevant biomarkers, including those for all approved and many investigational drugs and clinical trials , and cancer-specific biomarkers that may be used to establish both ctDNA presence and quantity. Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).") Odegaard et al. at 3546 ("In contrast, ctDNA-based liquid biopsies decouple biomarker assessment from tissue, enabling tumor-specific genotyping using safe, inexpensive, and near-painless peripheral blood sampling Although promising, successful implementation of liquid biopsy is fraught with challenges including ctDNA's short fragment length (~165 bp), scant material (median 48ng/10 mL) whole blood vs. micrograms in typical tissue sections), low tumor representation (VAFs typically to- to 100-fold lower than tissue), and absent reference materials and methods. We have developed an NGS-based ctDNA diagnostic that overcomes these challenges to provide accurate tumor-specific genotyping for all guideline-recommended advanced solid tumor somatic biomarkers from a single peripheral blood draw.") Odegaard et al. at 3547 ("These data demonstrate applicability of ctDNA-based genotyping to treatment paradigms based on tissue genotyping; however, ctDNA has the potential to not only match but to surpass tissue genotyping in for important ways.") '822 patent, claim 1 ("[P]roviding a population of cell free DNA ('cfDNA') molecules obtained from a bodily sample from a subject.") Odegaard et al. at 3542 ("cfDNA is extracted from stabilized whole blood") Odegaard et al. at 3540 ("All patient samples were collected and processed in accordance with the Guardant360 Clinical Blood Collection Kit instructions (Guardant Health, Inc.).") Lanman et al. at 18 ("Two 10mL tubes of whole blood were collected per individual") Lanman et al. at 3 ("Guardant360 is an NGS panel of 54 clinically actionable genes (S1 Table) utilizing digital sequencing of cell-free circulating tumor DNA isolated from a simple, non-invasive blood draw.")

ASSERTED CLAIM 12

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids. For example, Guardant uses oligonucleotide heptamer barcodes to create a library where original cell-free DNA molecules are separately encoded. Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Nakamura et al. at 176 ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina).")

ASSERTED CLAIM 15

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 12, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions. For example, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of the same length. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

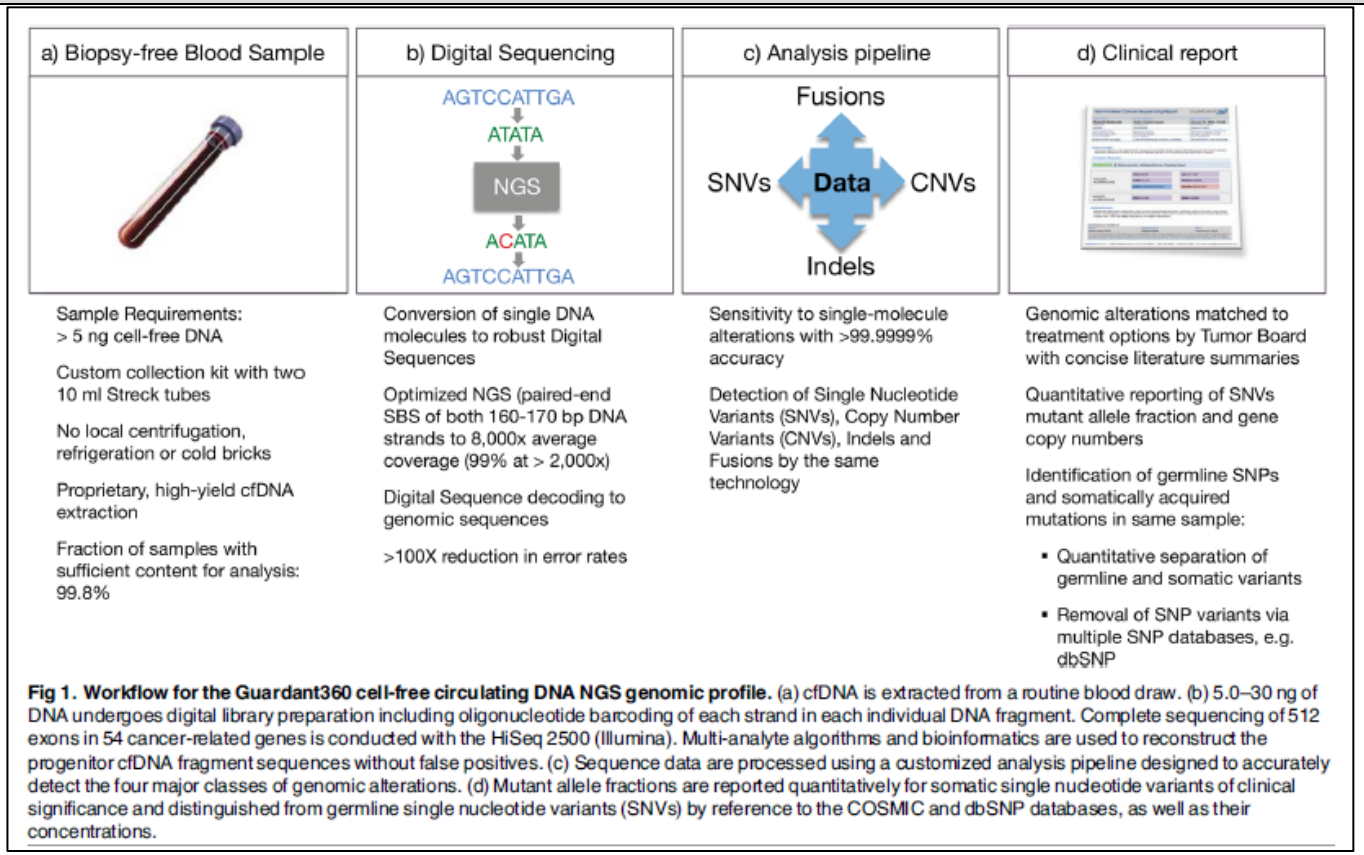
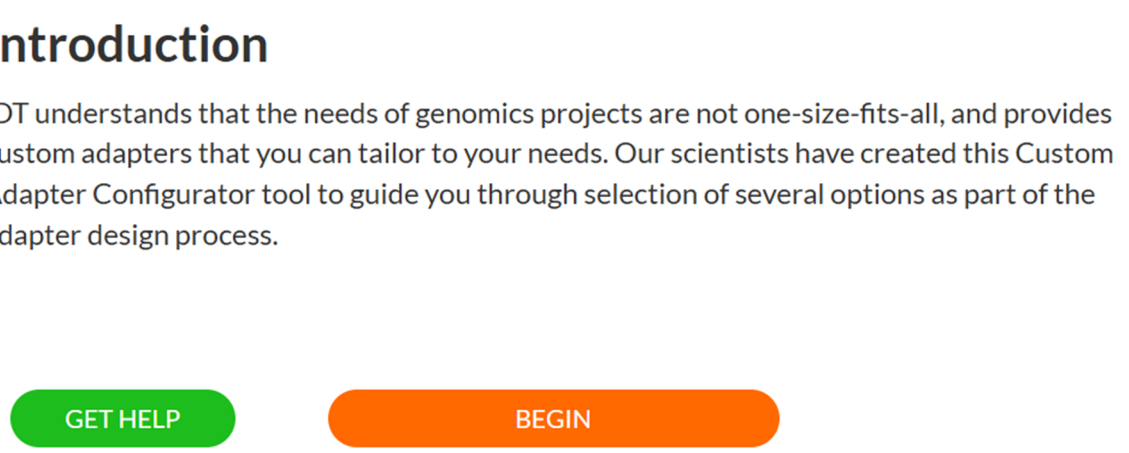


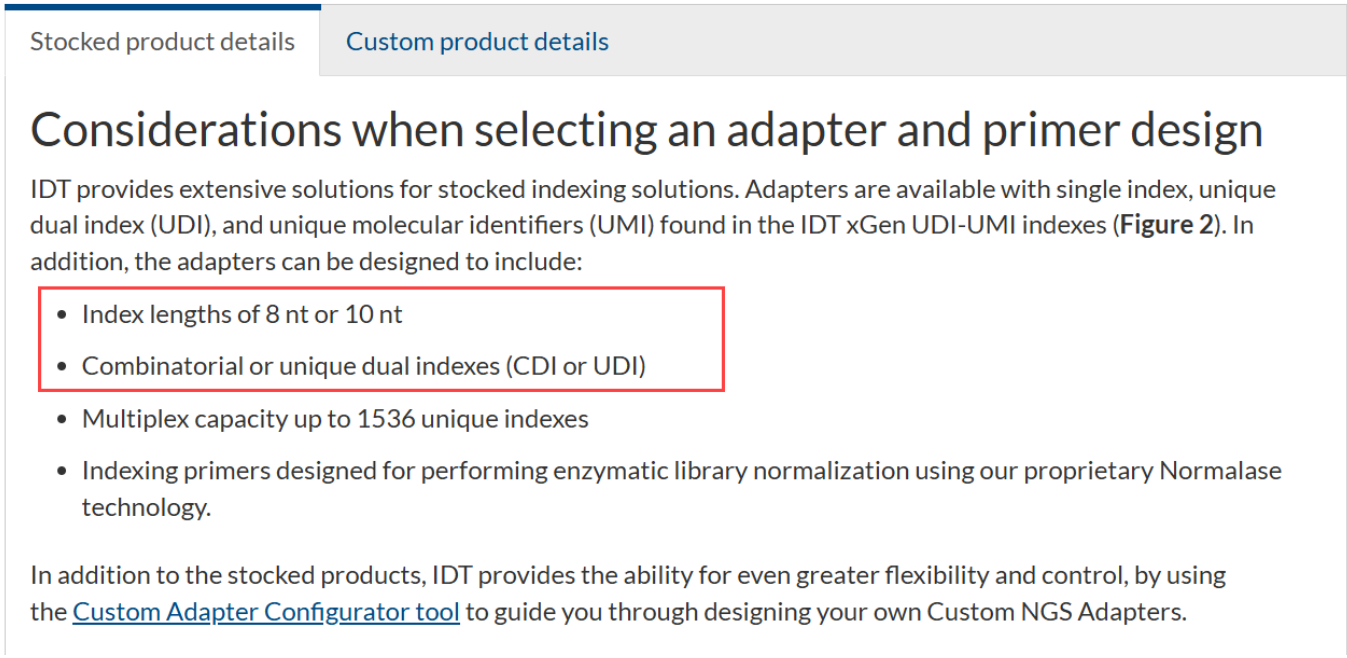
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

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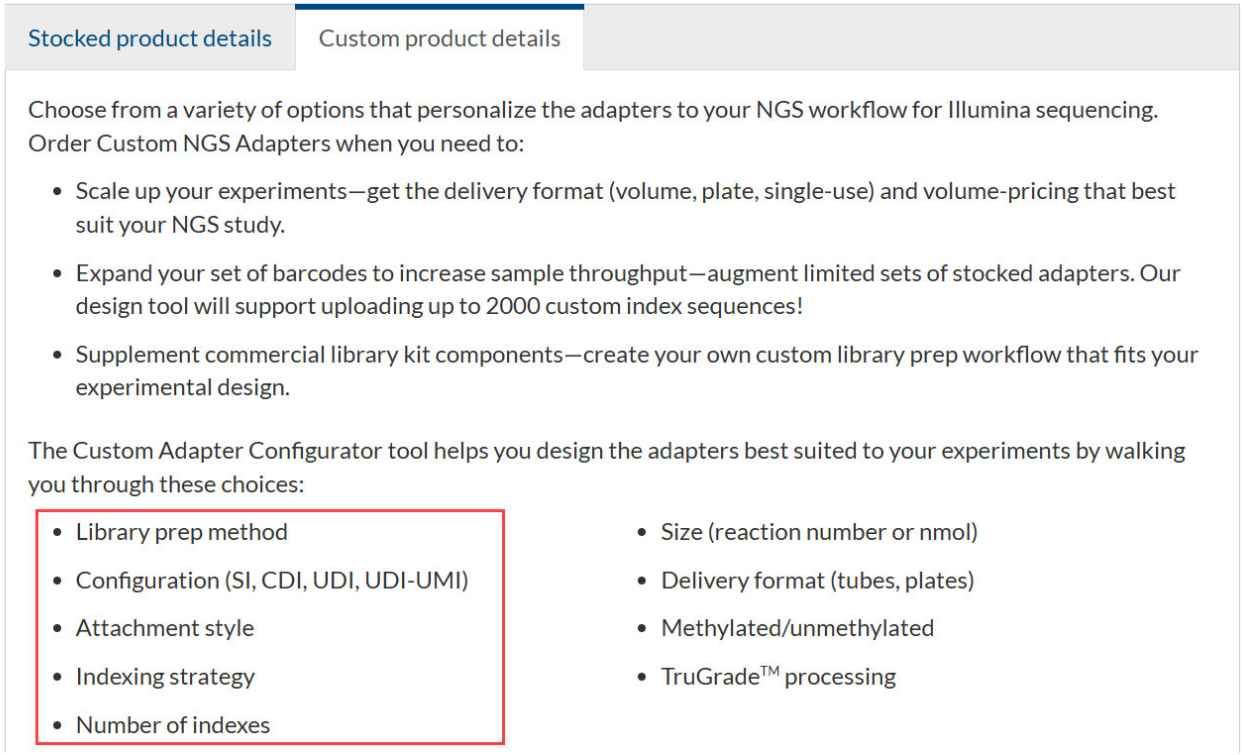
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

GUARDANT HEALTH EX.1020.203

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



IDT Illumina Adapters and Primers Webpage.

Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

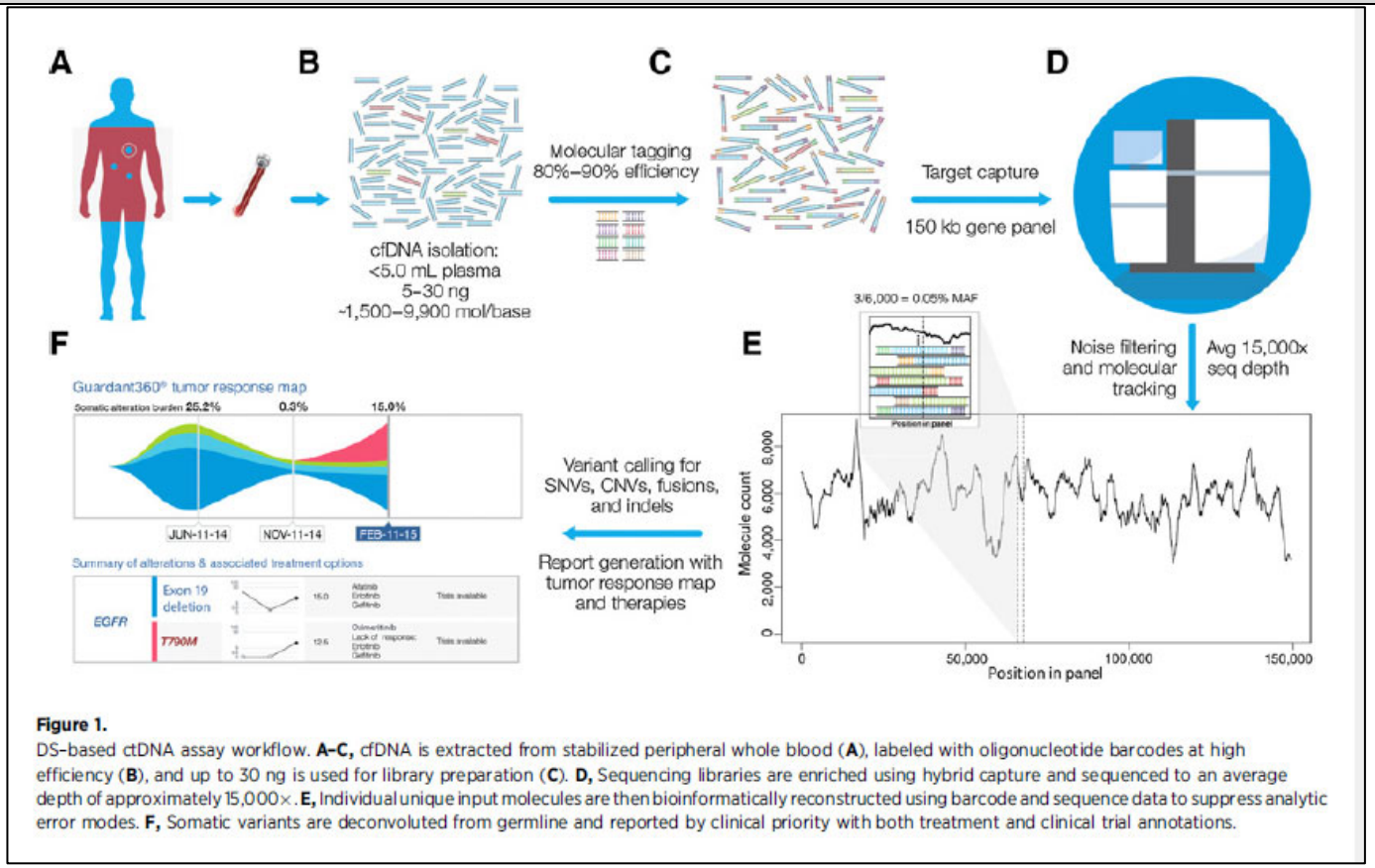


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 16

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 15, wherein the length of the constant portions and the length of the variable portions is the same.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the length of the constant portions and the length of the variable portions is the same. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of the same length. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

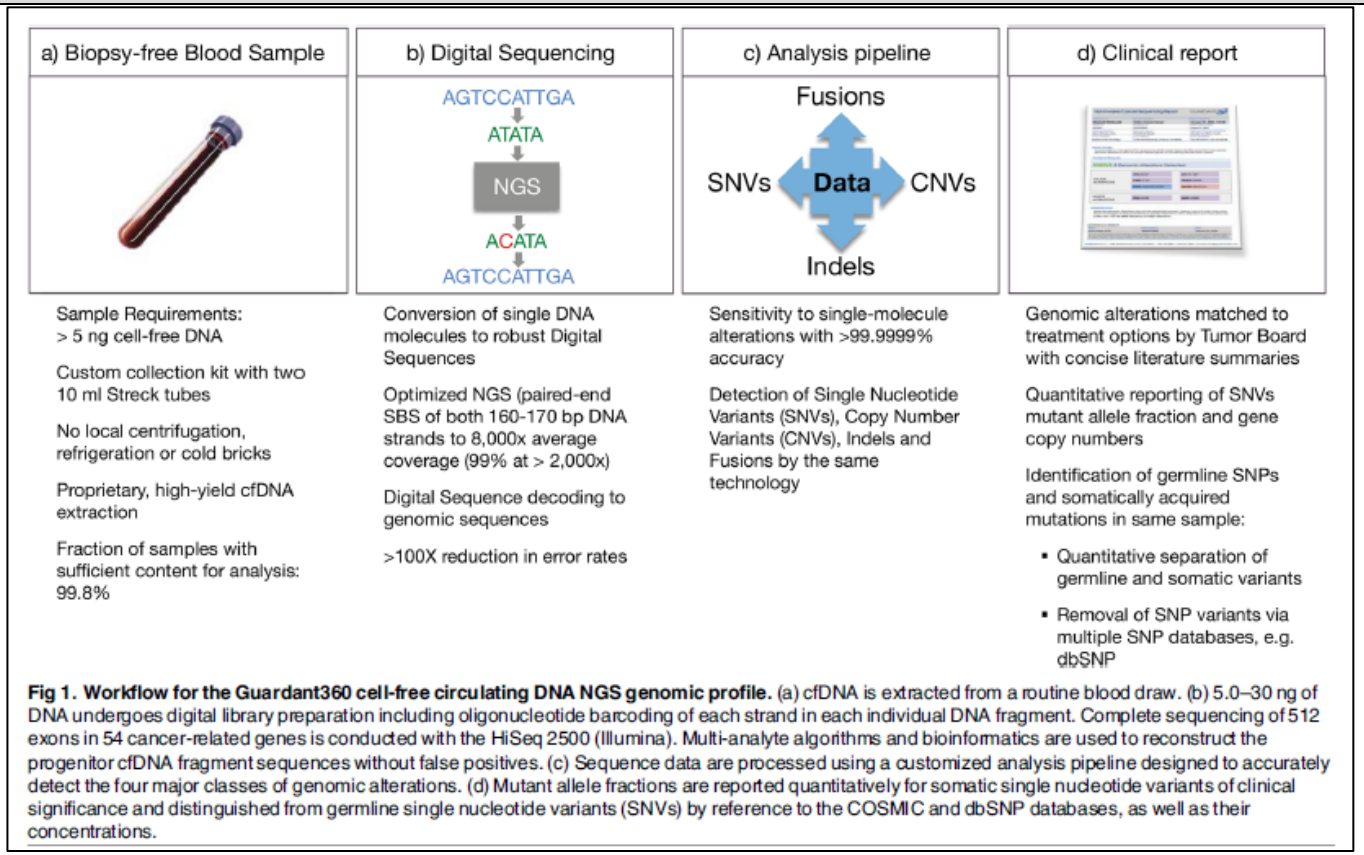
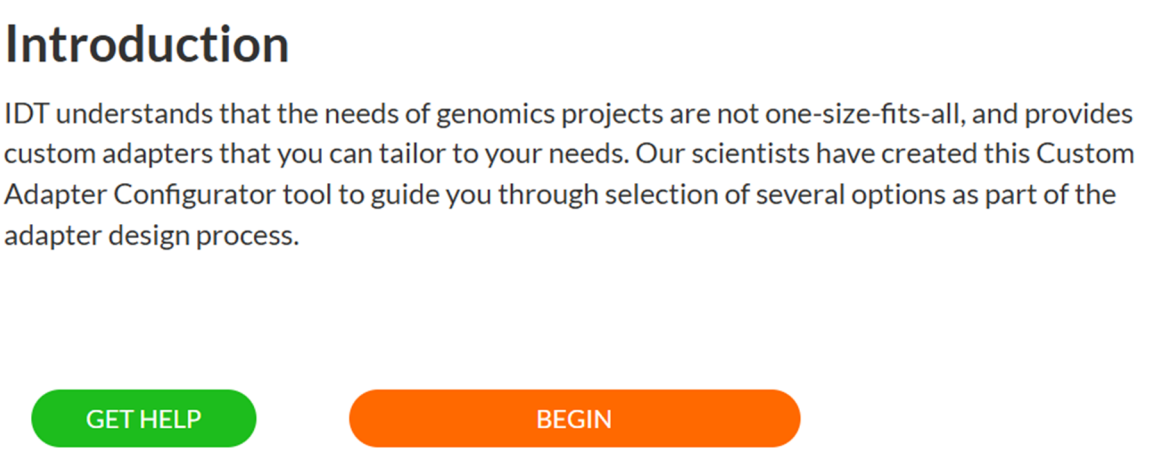


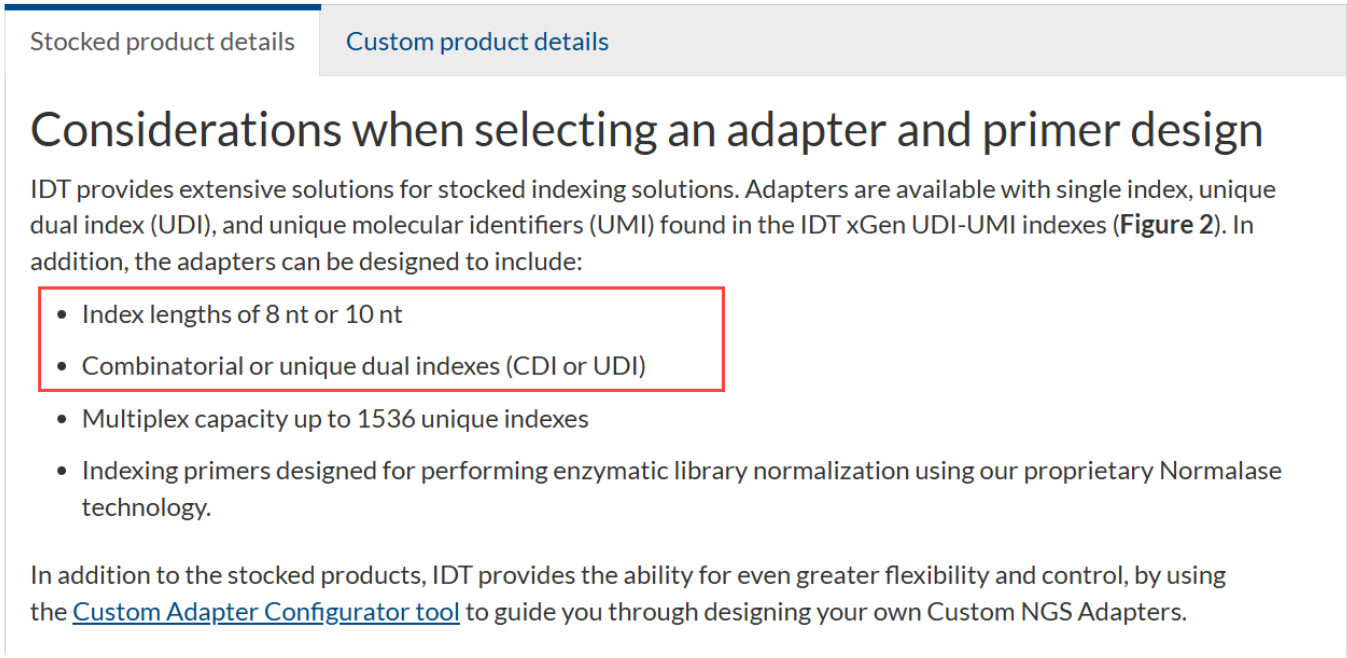
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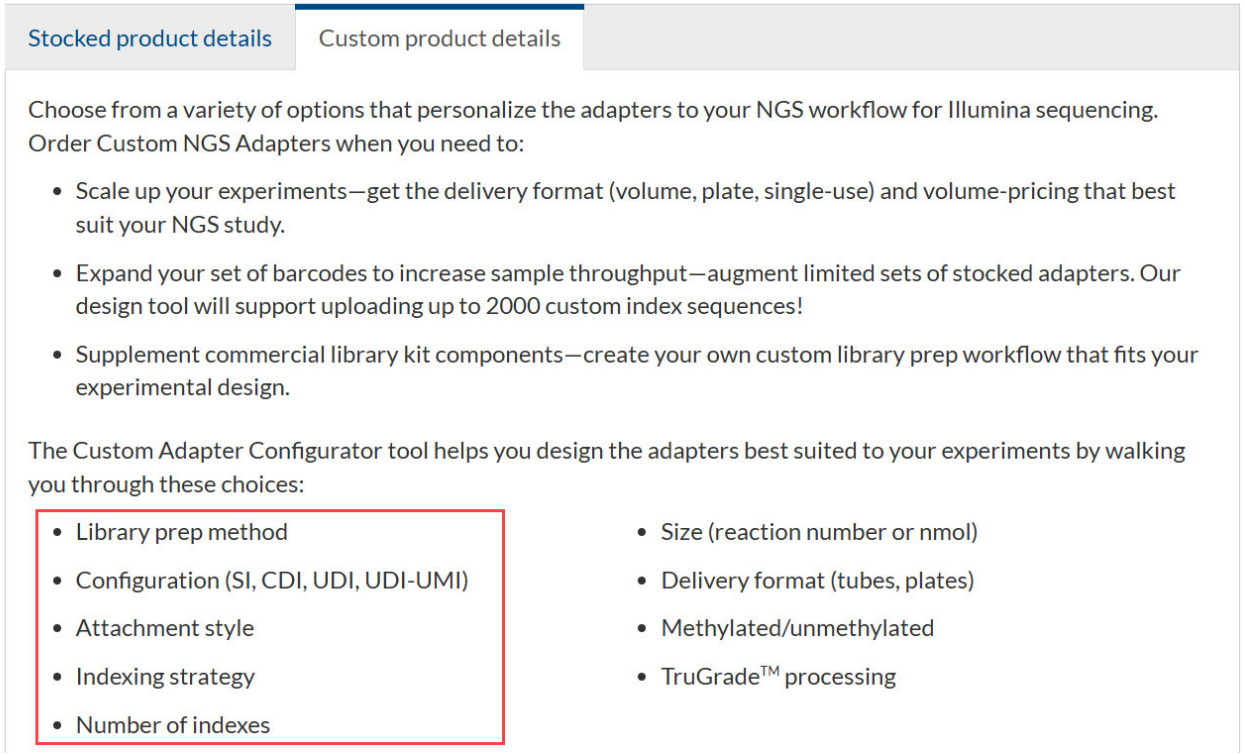
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

GUARDANT HEALTH EX.1020.212

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



IDT Illumina Adapters and Primers Webpage.

Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

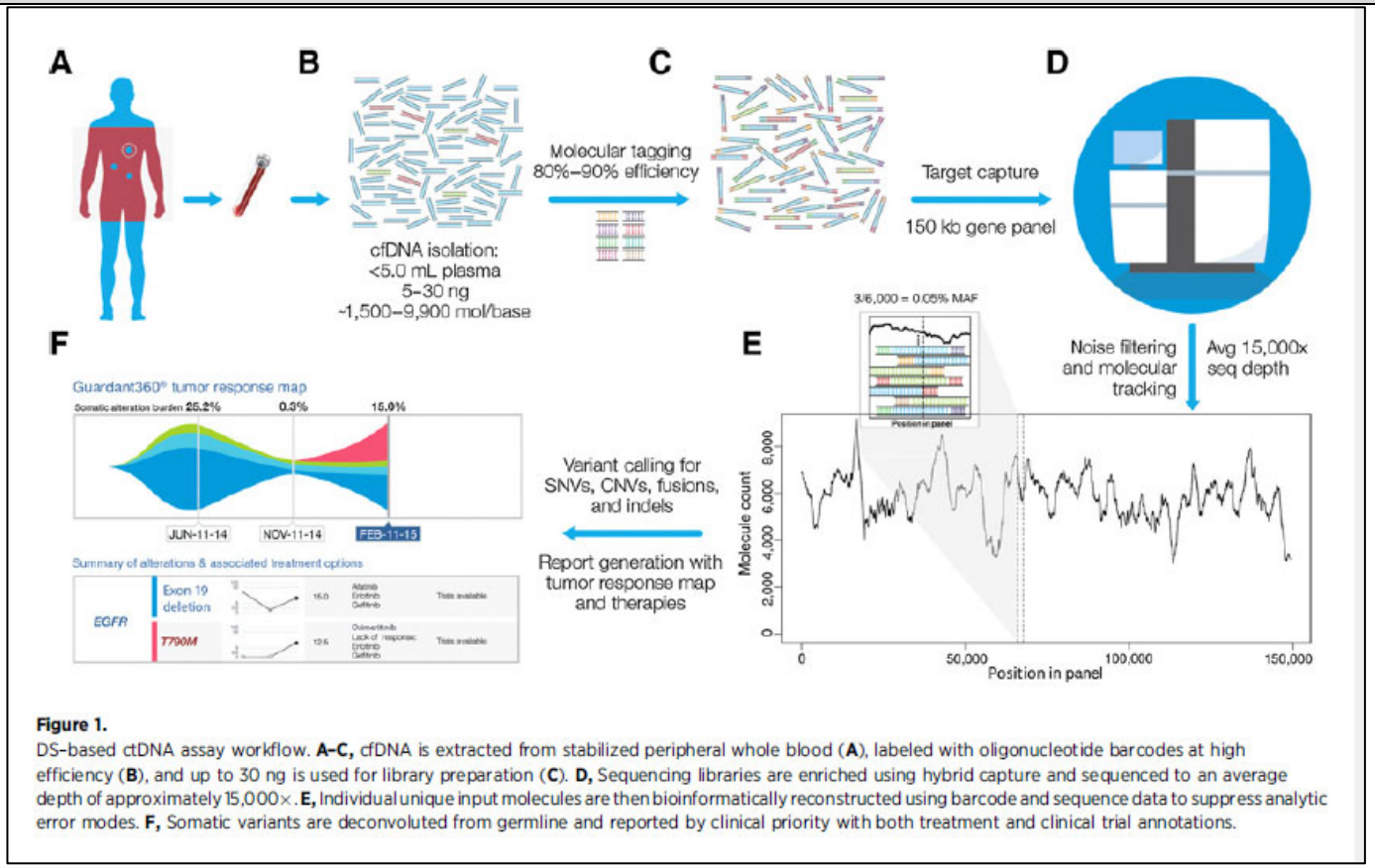


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 17

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 15, wherein the length of the constant portions and the length of the variable portions are different.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the length of the constant portions and the length of the variable portions are different. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of different lengths. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.217

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

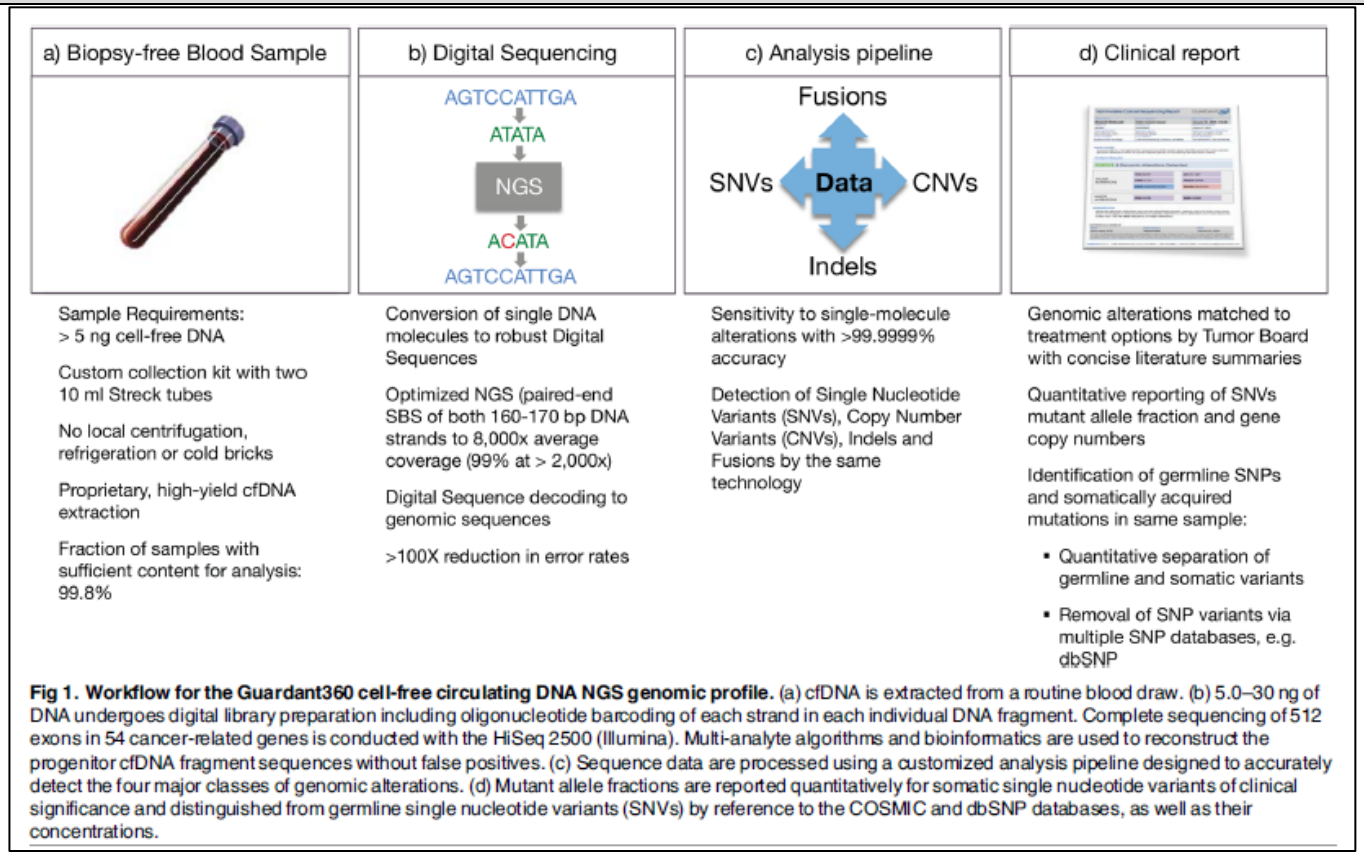
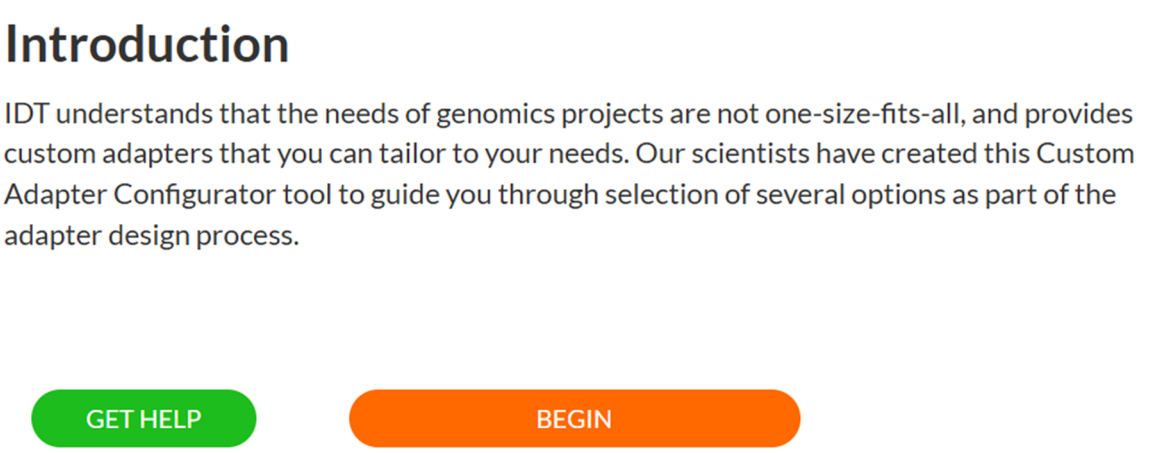


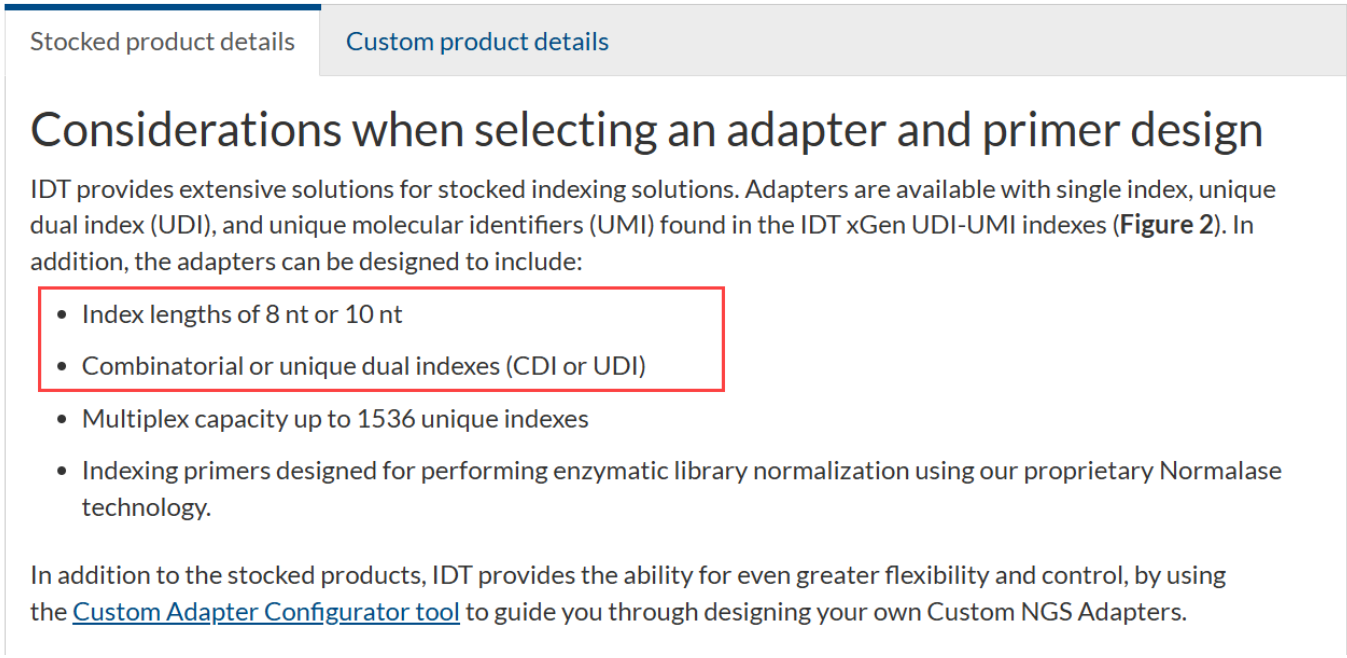
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

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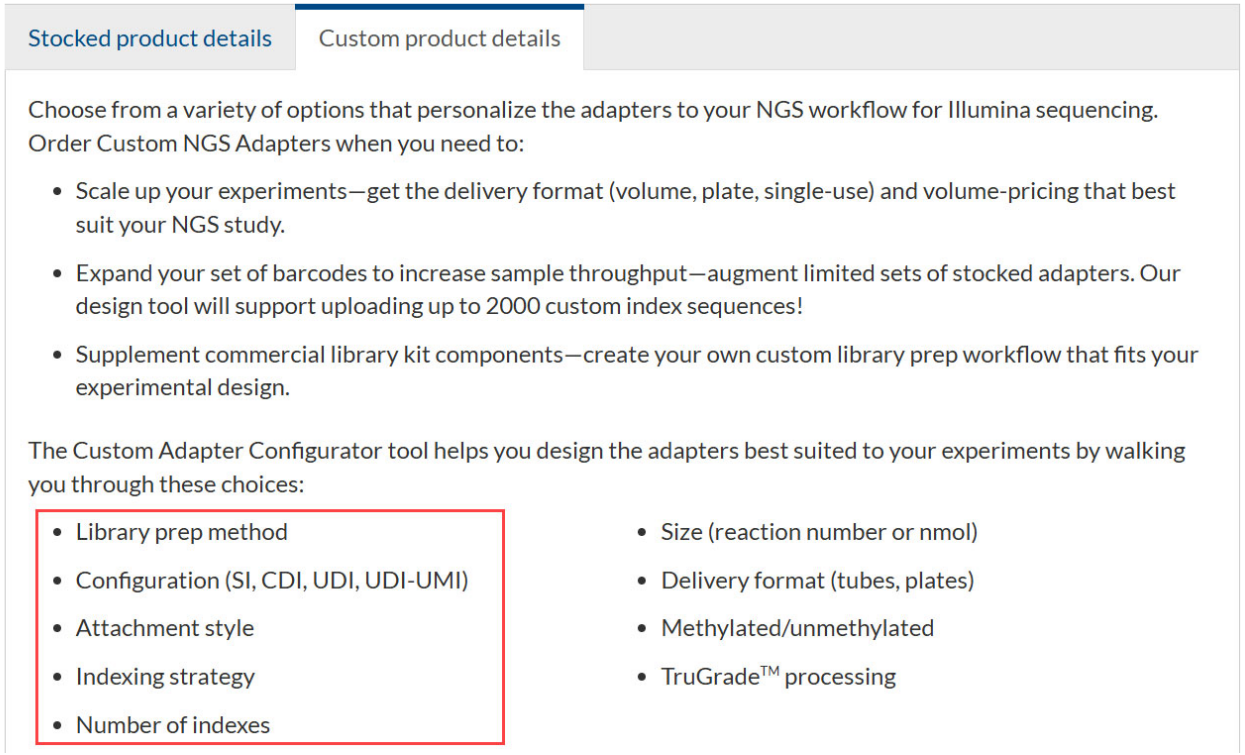
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



IDT Illumina Adapters and Primers Webpage.

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GUARDANT HEALTH EX.1020.223

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

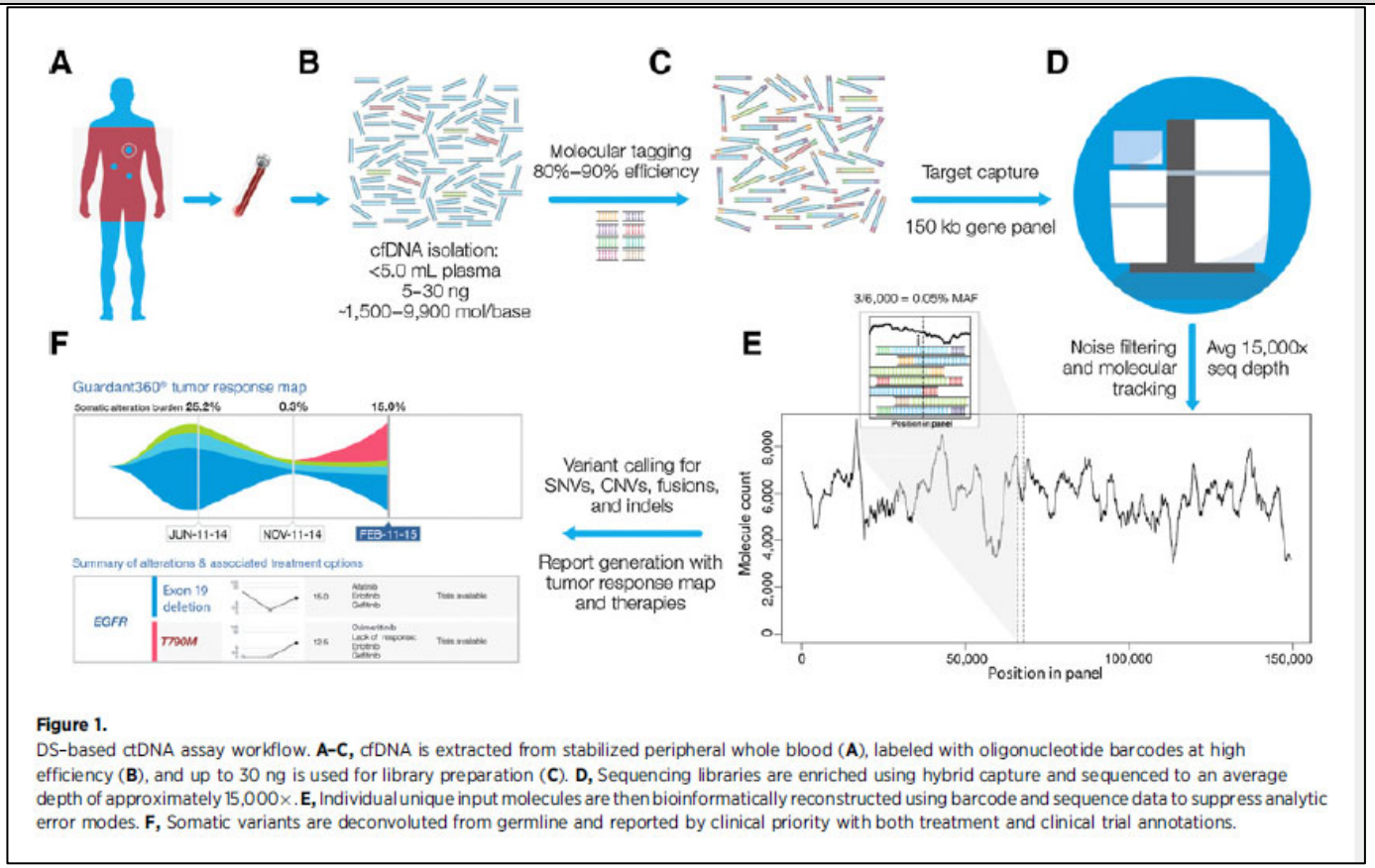


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ASSERTED CLAIM 18

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.226

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

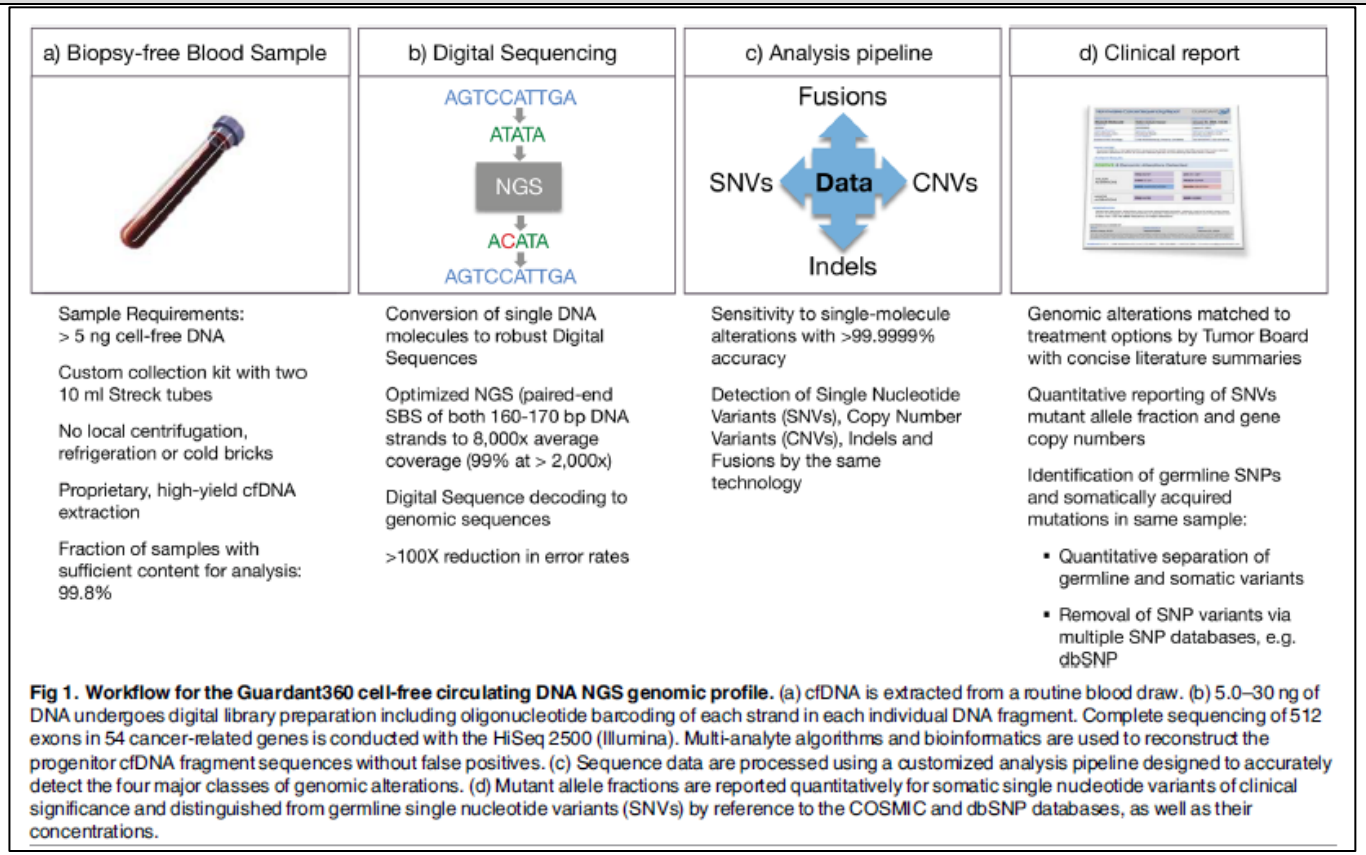
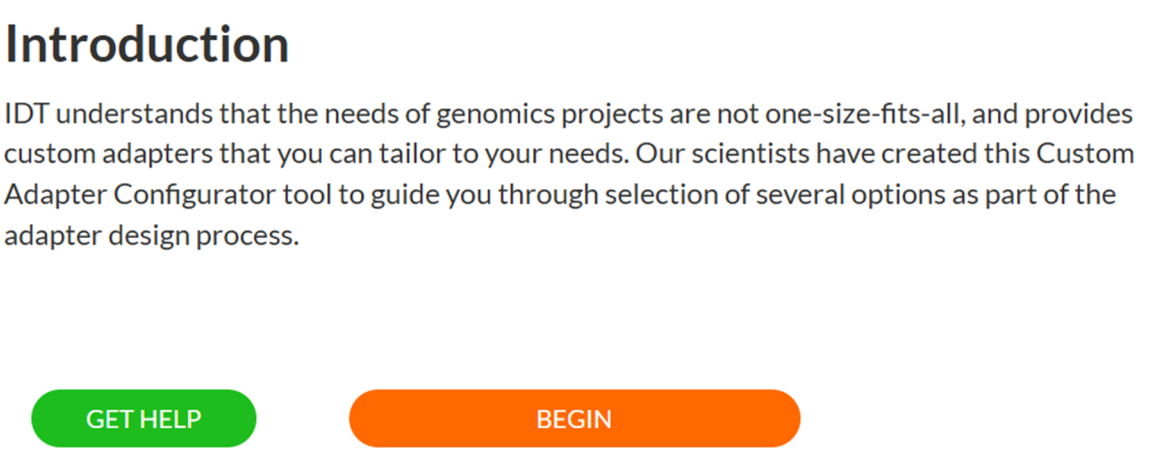


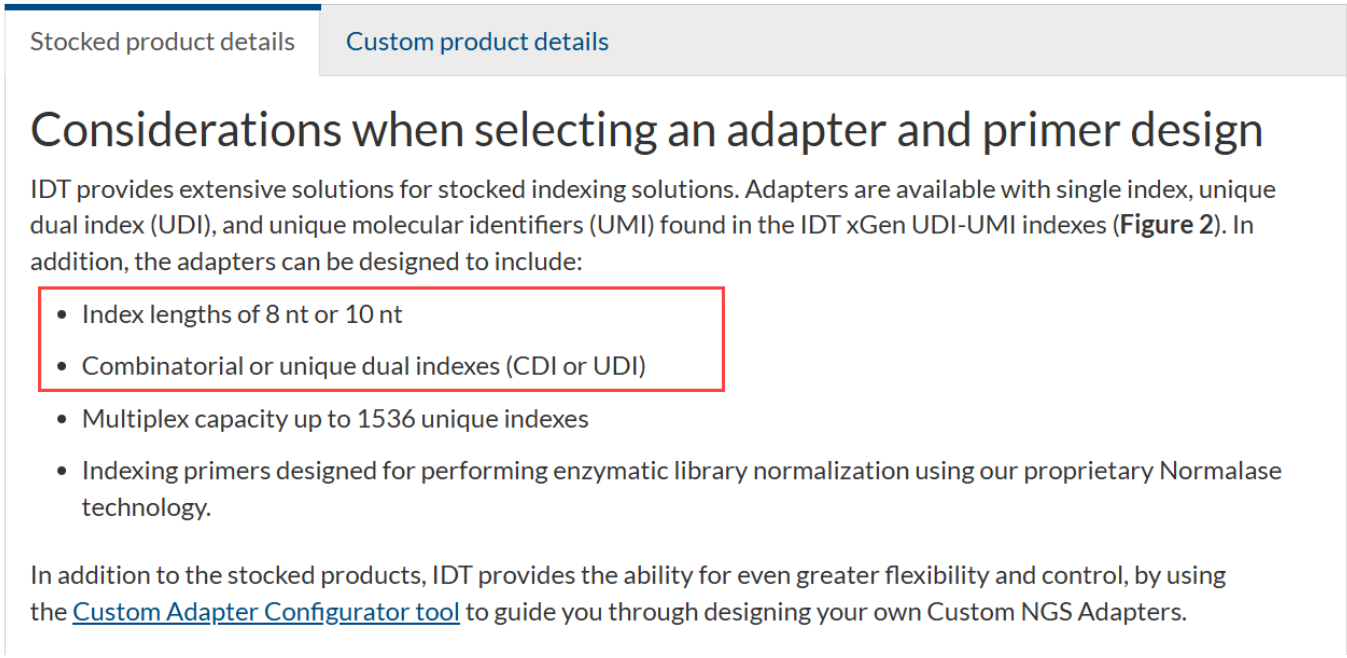
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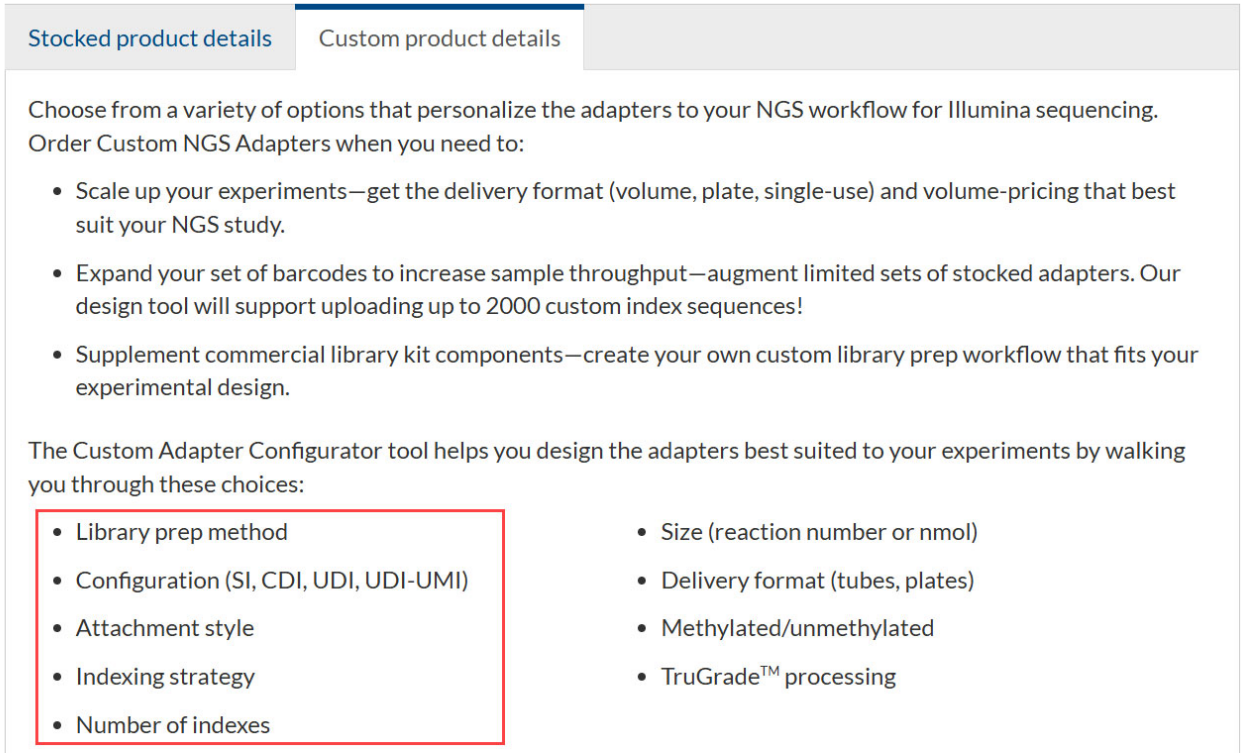
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

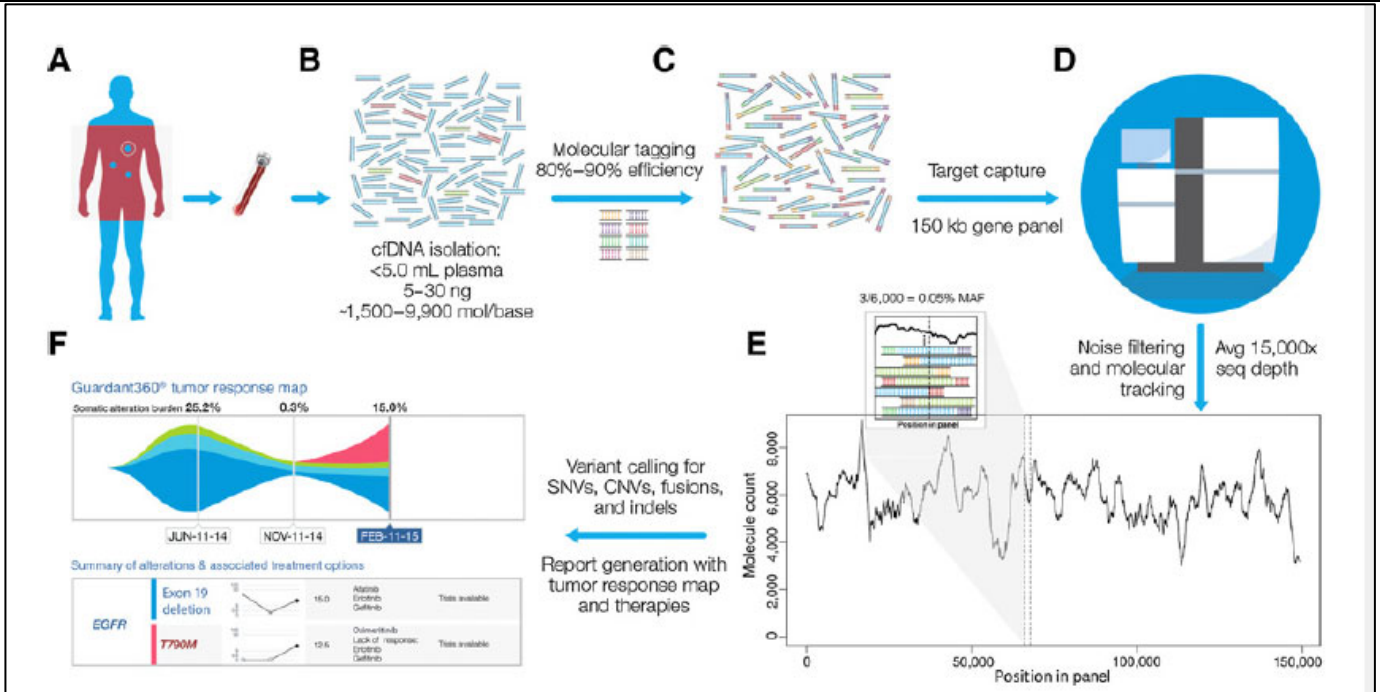


IDT Illumina Adapters and Primers Webpage.

Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.232

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 19

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 15, wherein the variable portions consist of only three species of nucleotides.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the variable portions consist of only three species of nucleotides. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions consisting of only three species of nucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.235

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

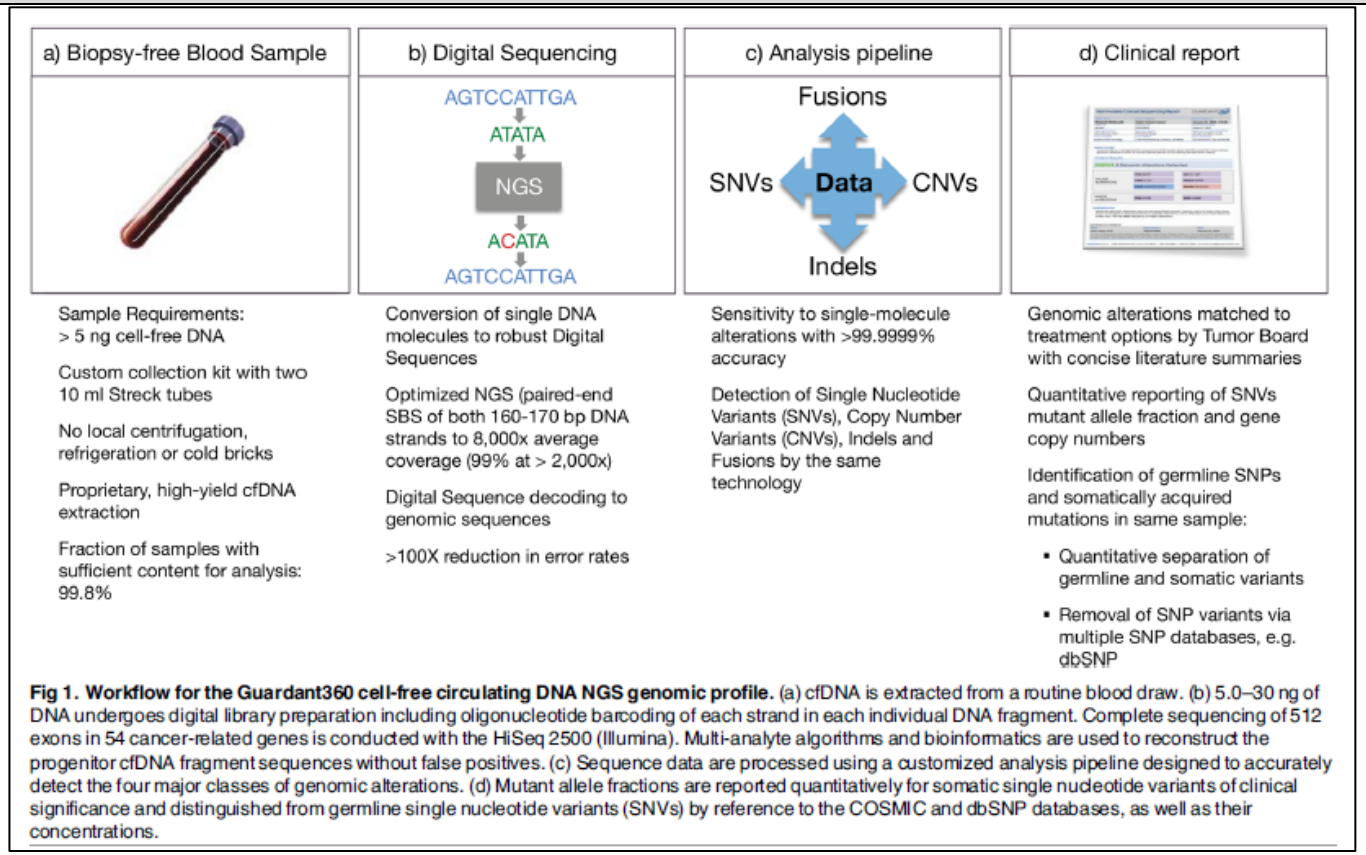
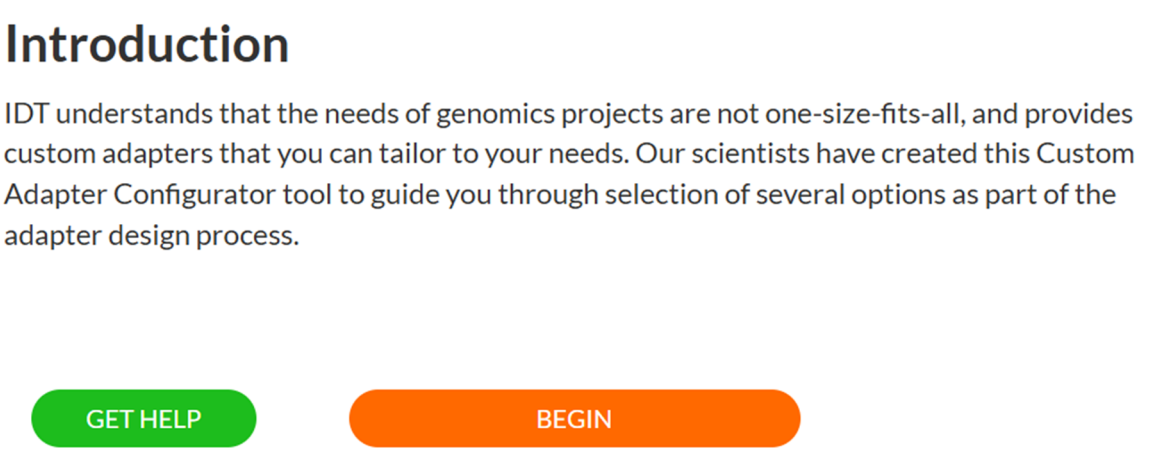


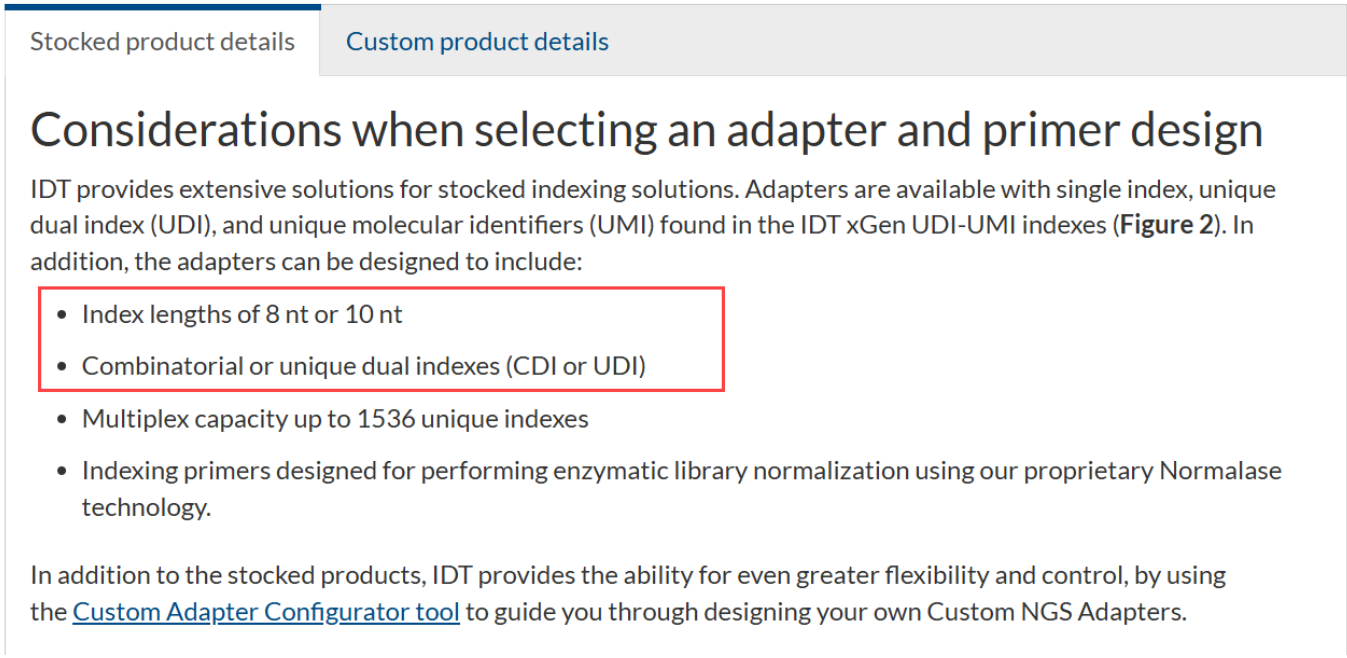
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

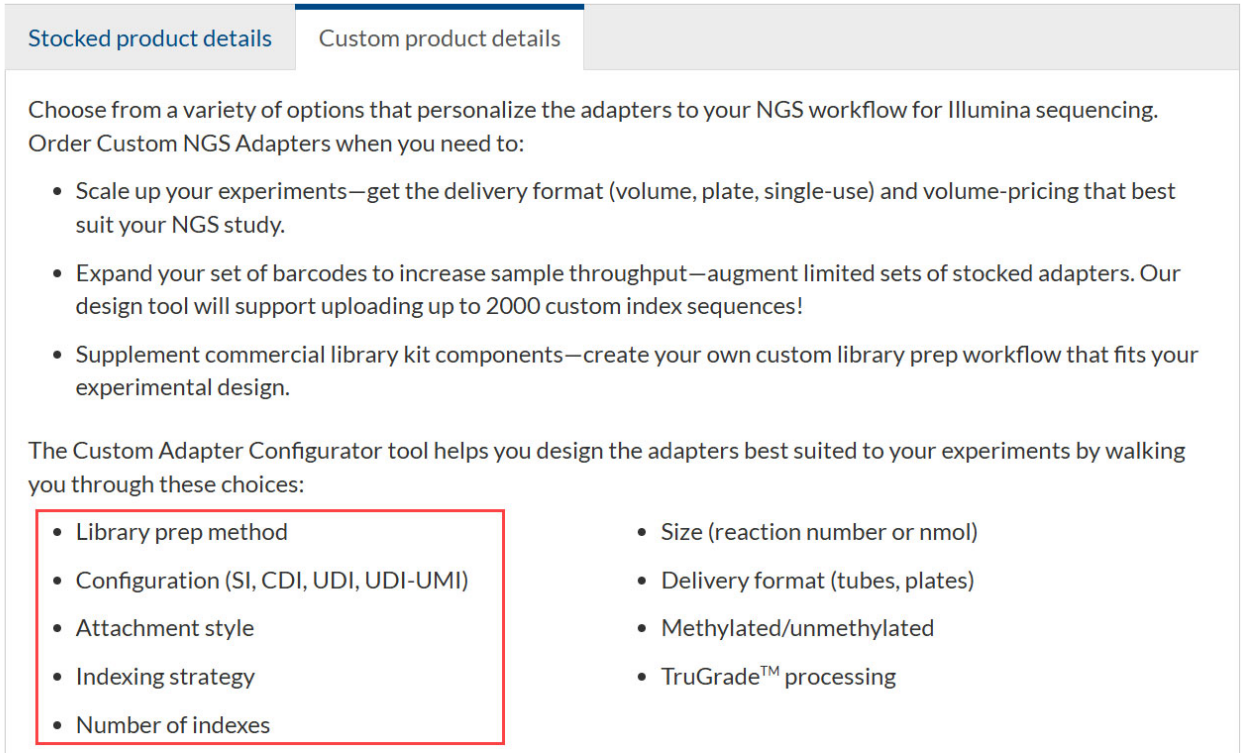
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

GUARDANT HEALTH EX.1020.239

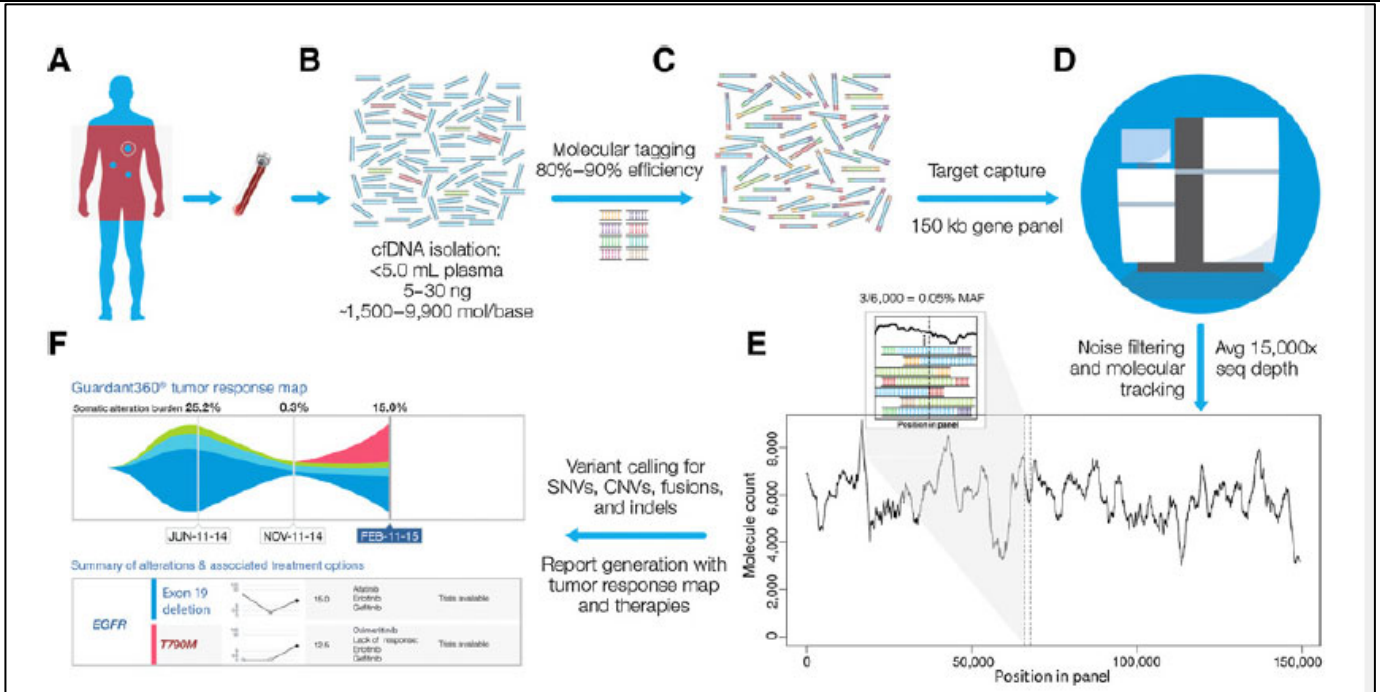
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term



IDT Illumina Adapters and Primers Webpage.

Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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ASSERTED CLAIM 20

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 12, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides. For example, on information and belief, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions constructed by a set of dinucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.244

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

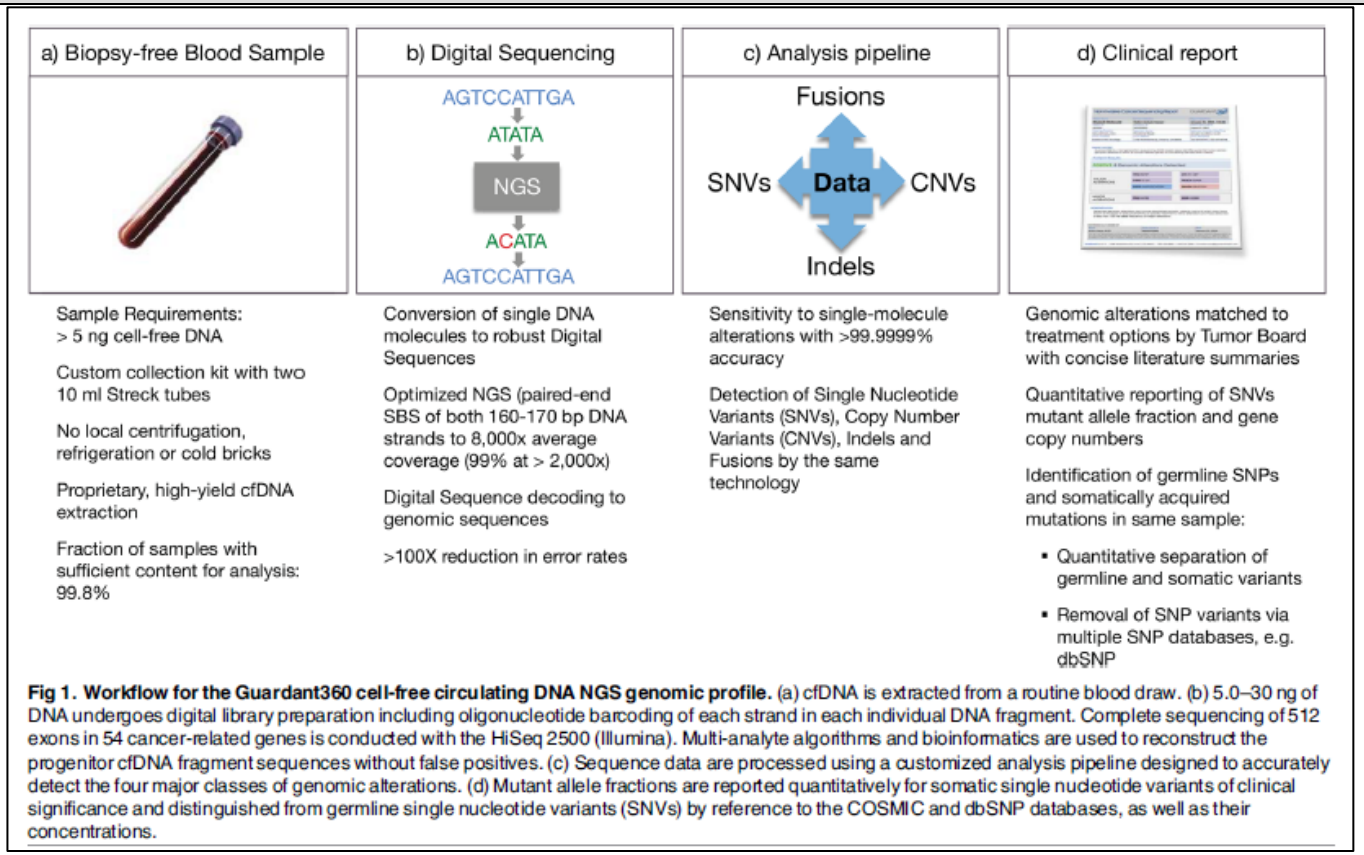
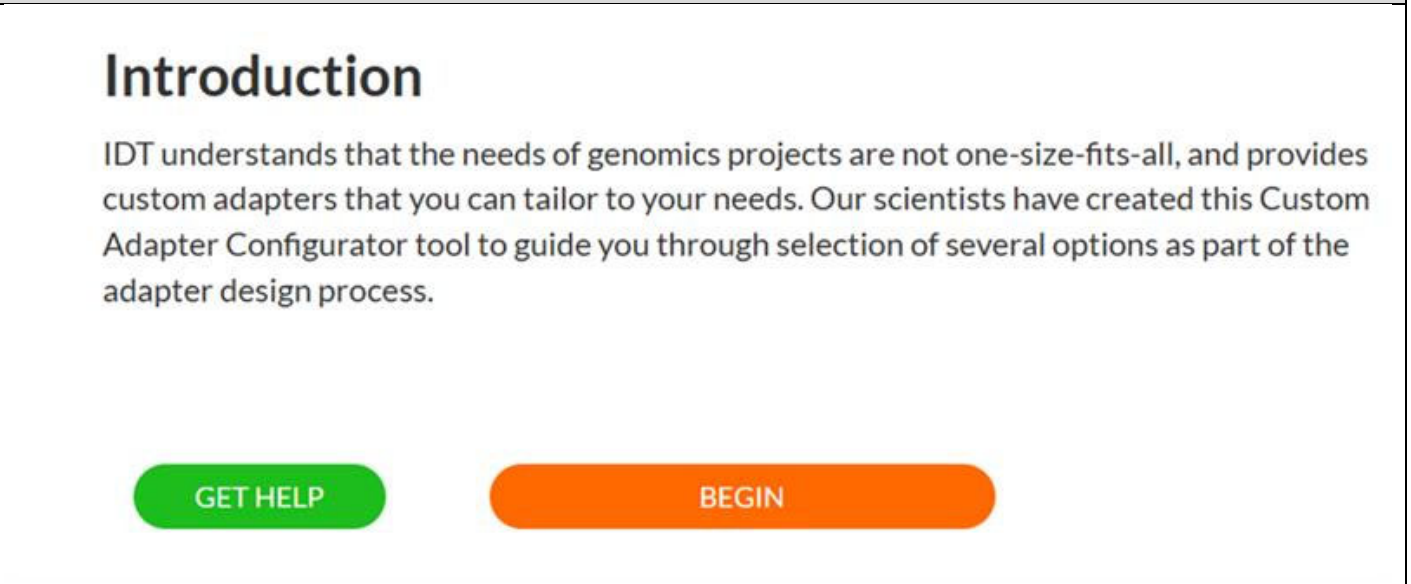


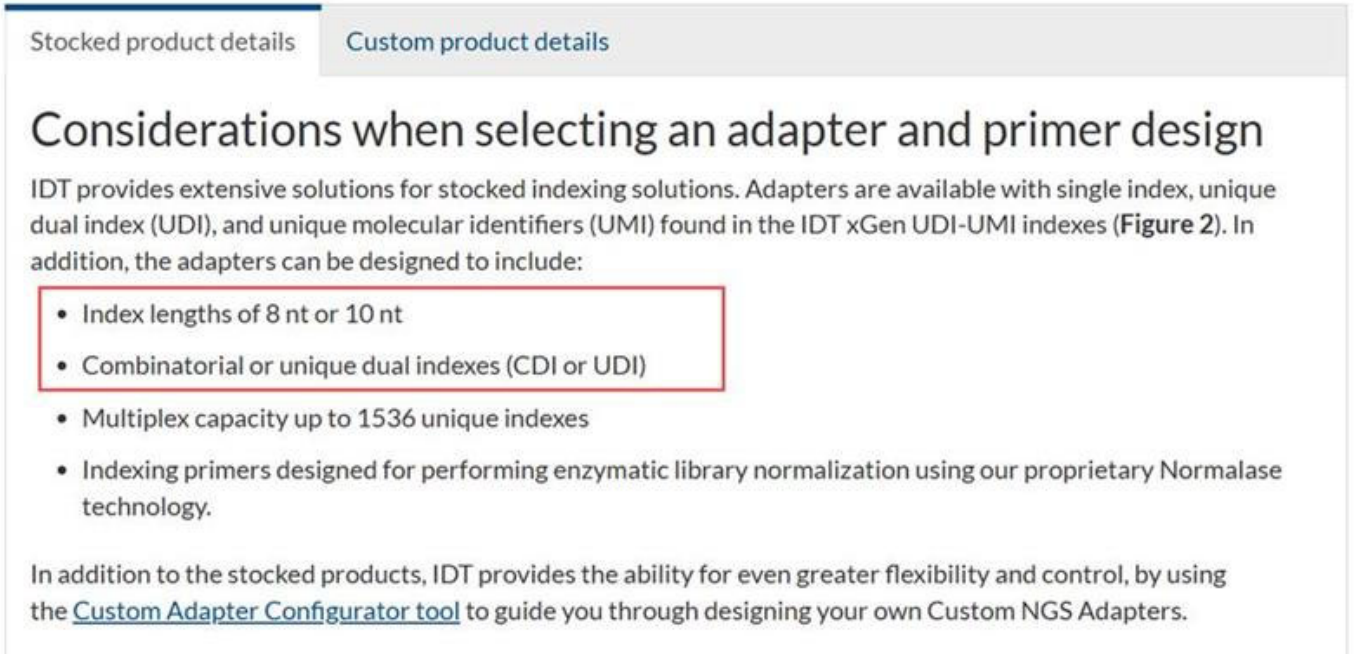
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

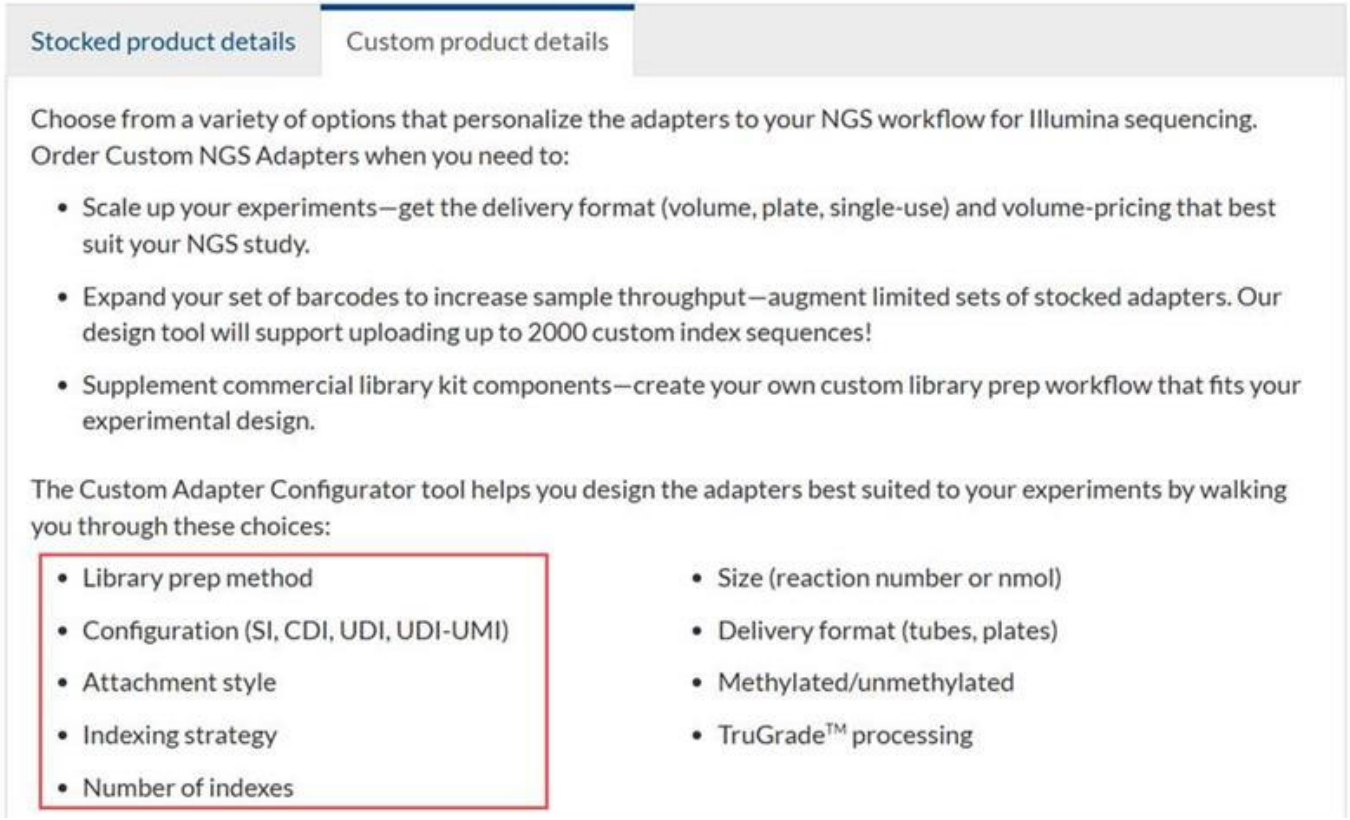
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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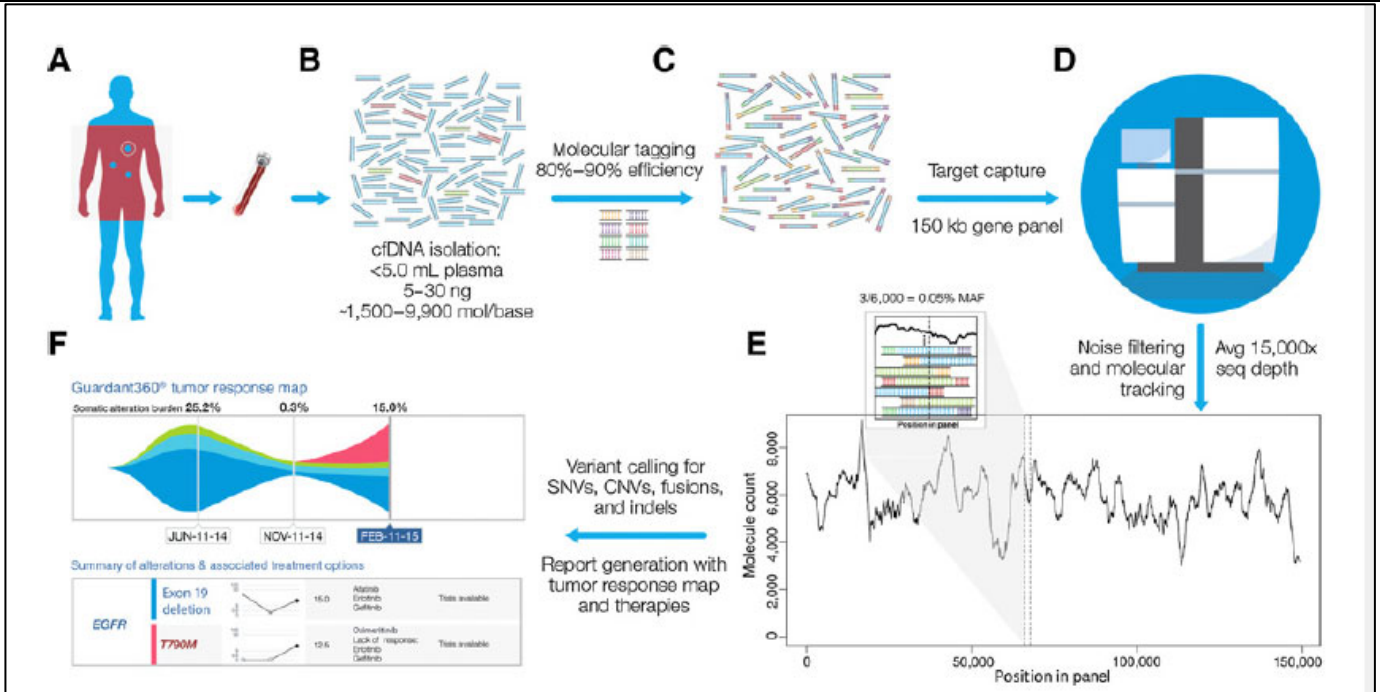
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.250

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

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ASSERTED CLAIM 21

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 12, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides. For example, on information and belief, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions constructed by a set of trinucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.253

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

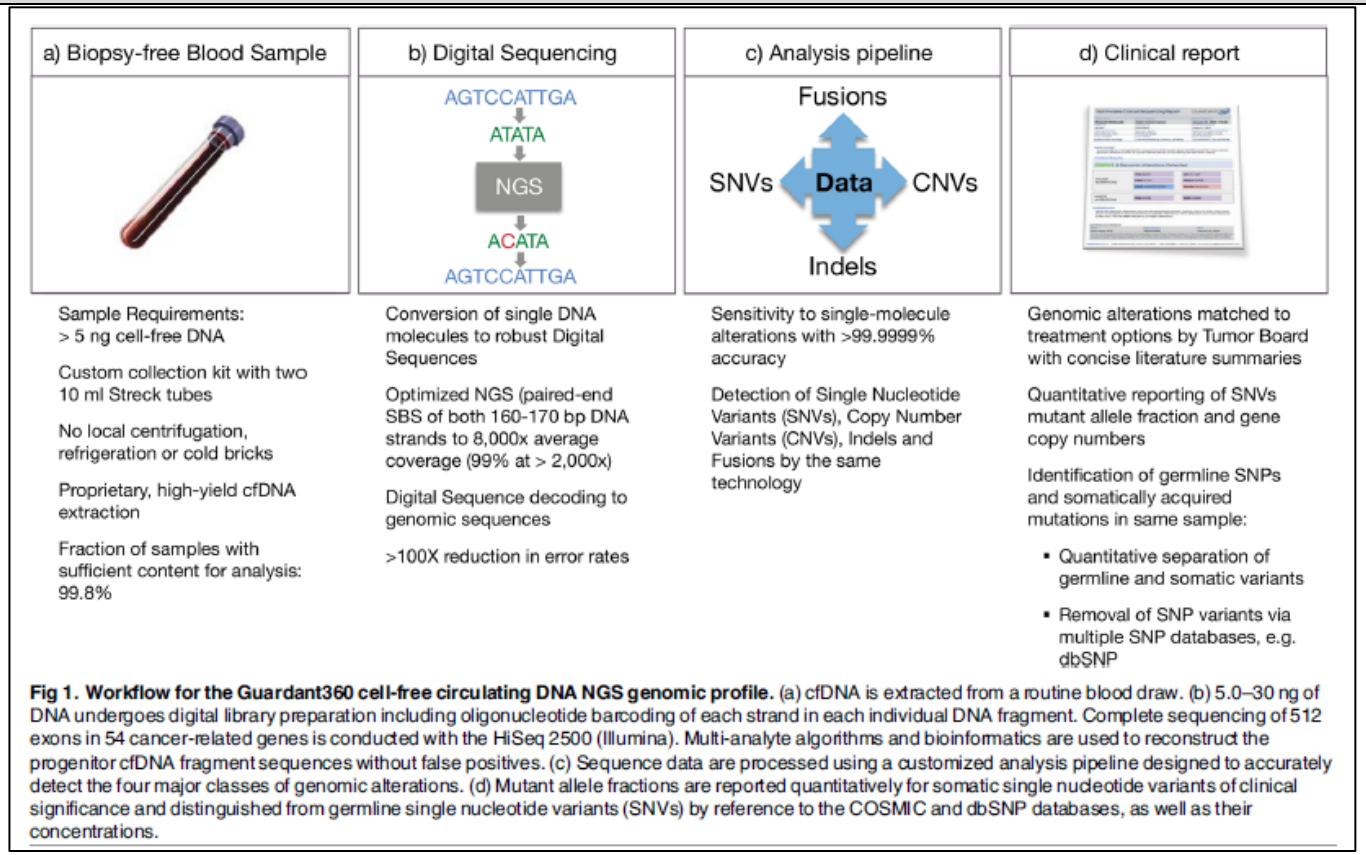
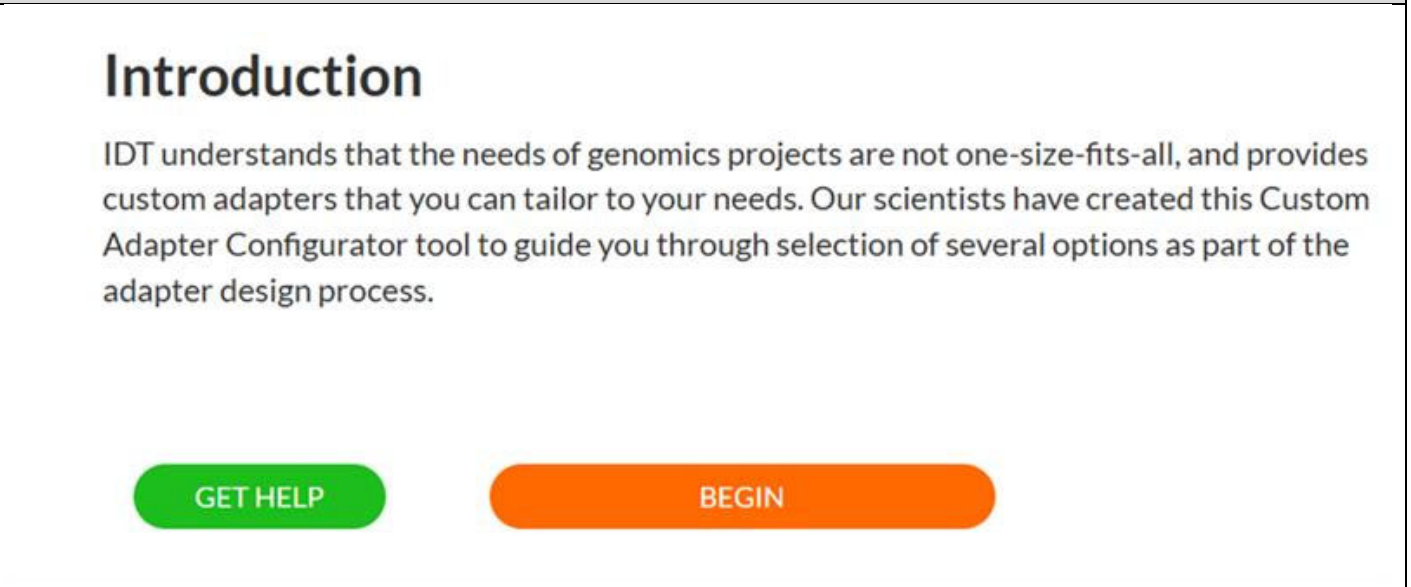


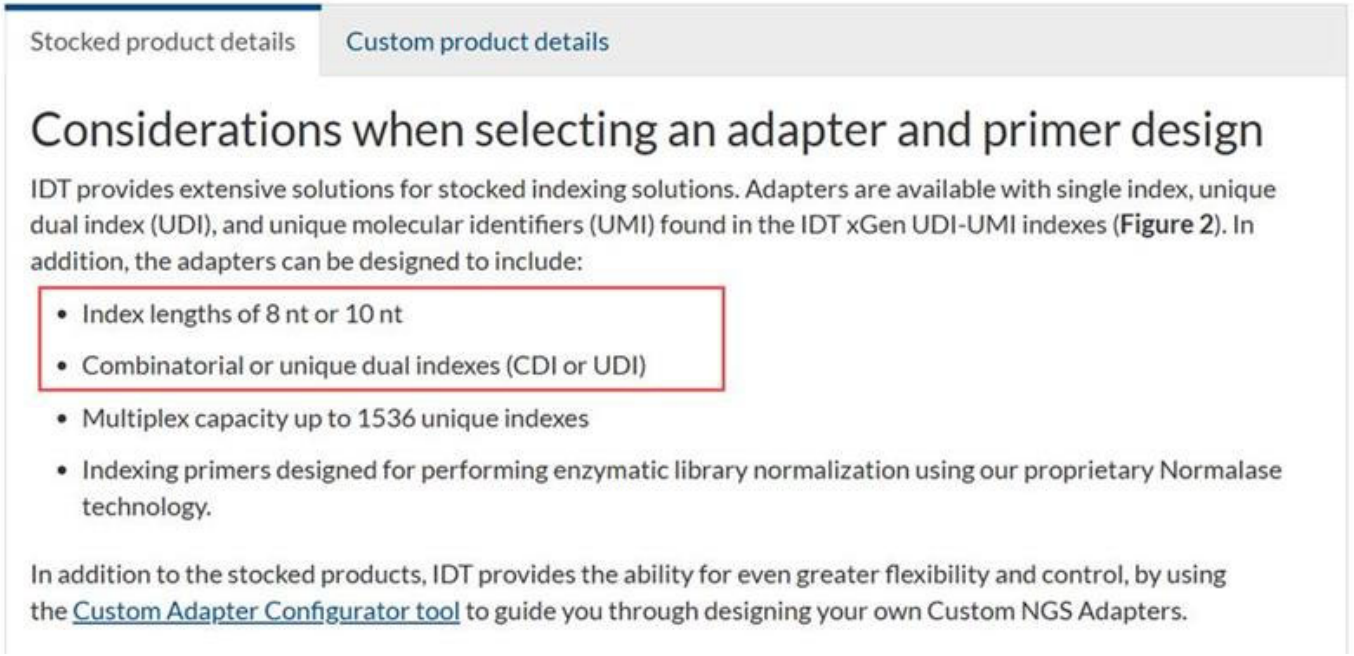
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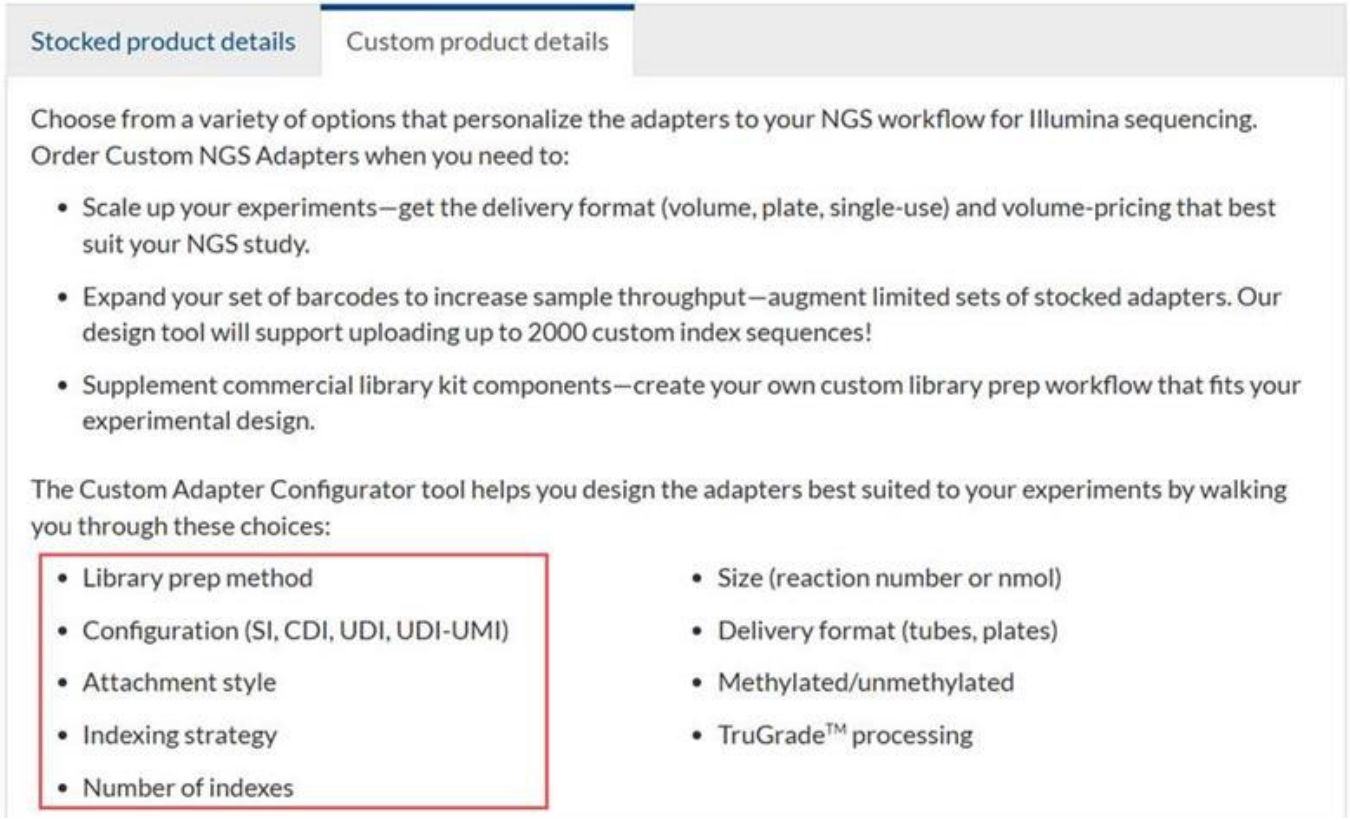
Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

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	 IDT Illumina Adapters and Primers Webpage. Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.259

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

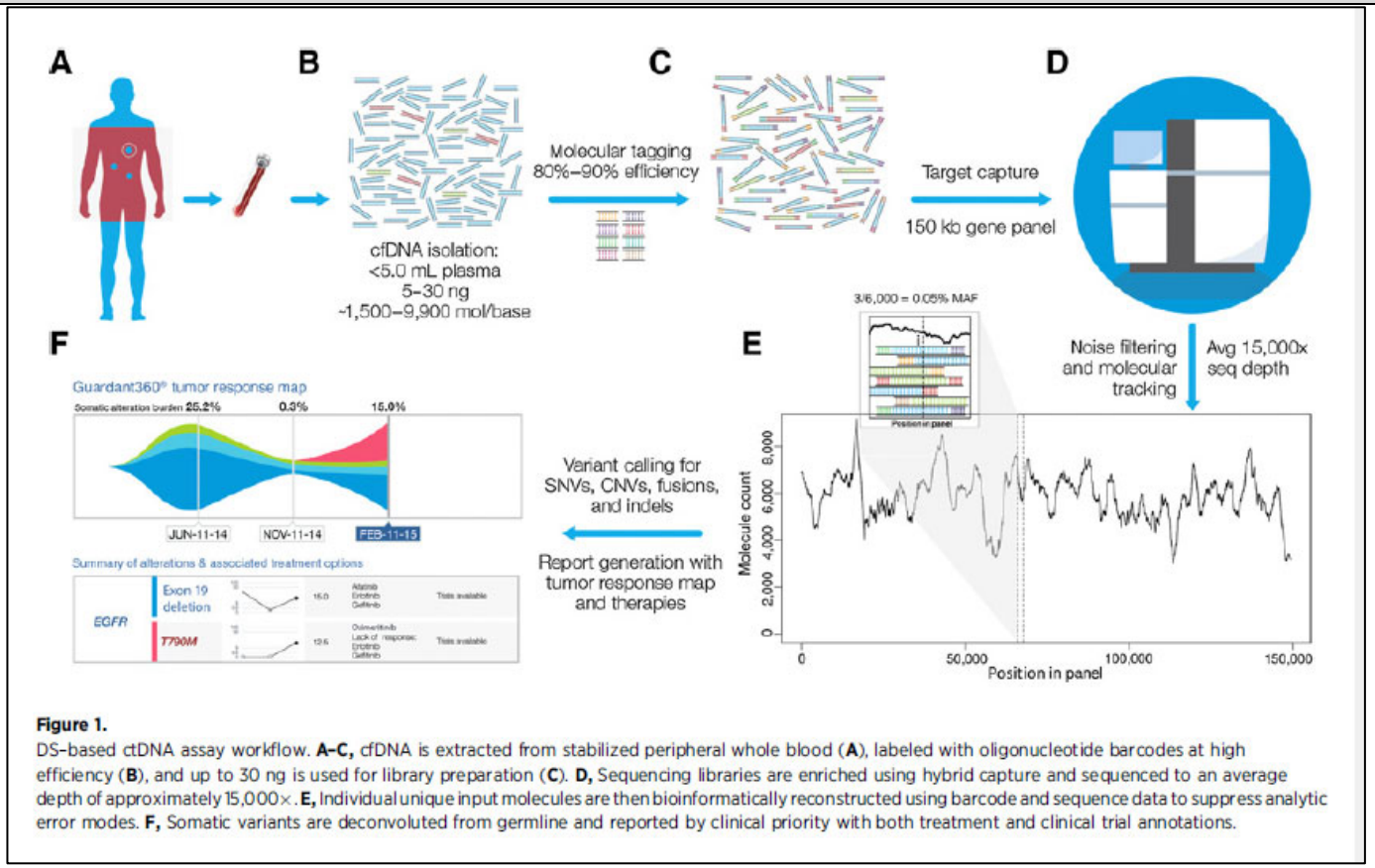


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

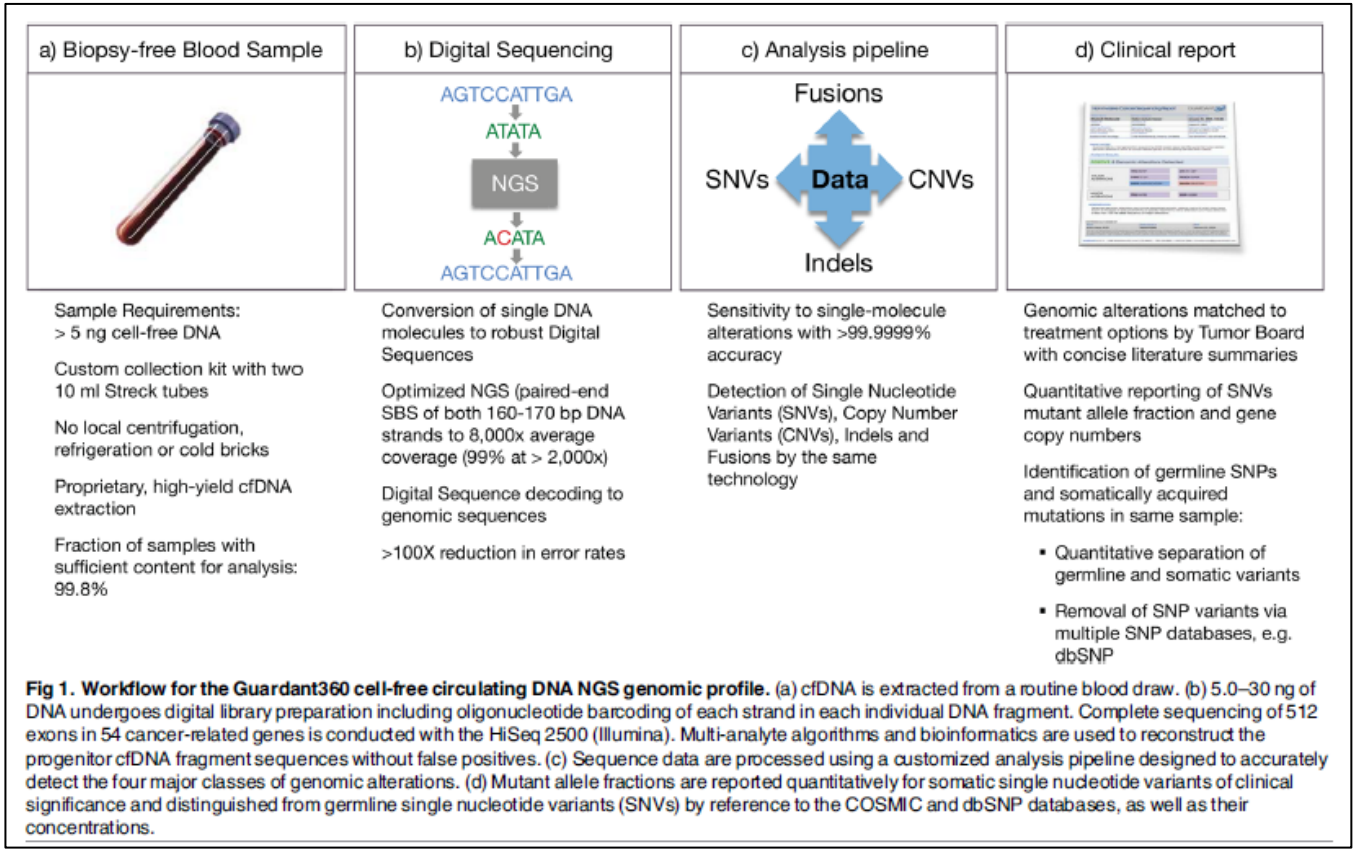
Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 22

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags.	Guardant generates unique tagged nucleic acid molecules, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags. For example, Guardant uses enough distinct nucleic acid tags such that multiple copies containing the same unique tag-target sequence combination can be traced back to the same original starting molecule. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")  **Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile.** (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations. Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries,

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

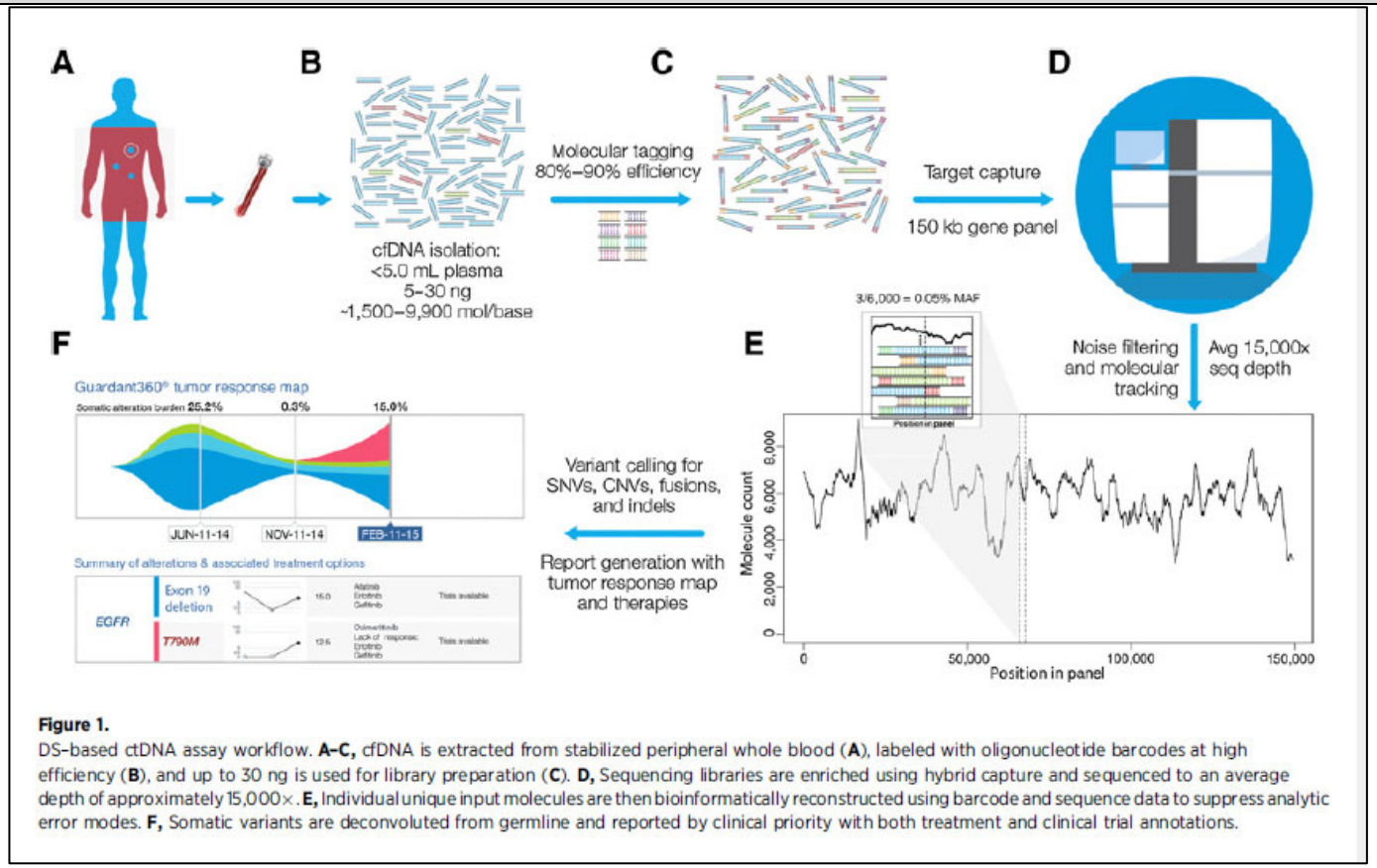


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

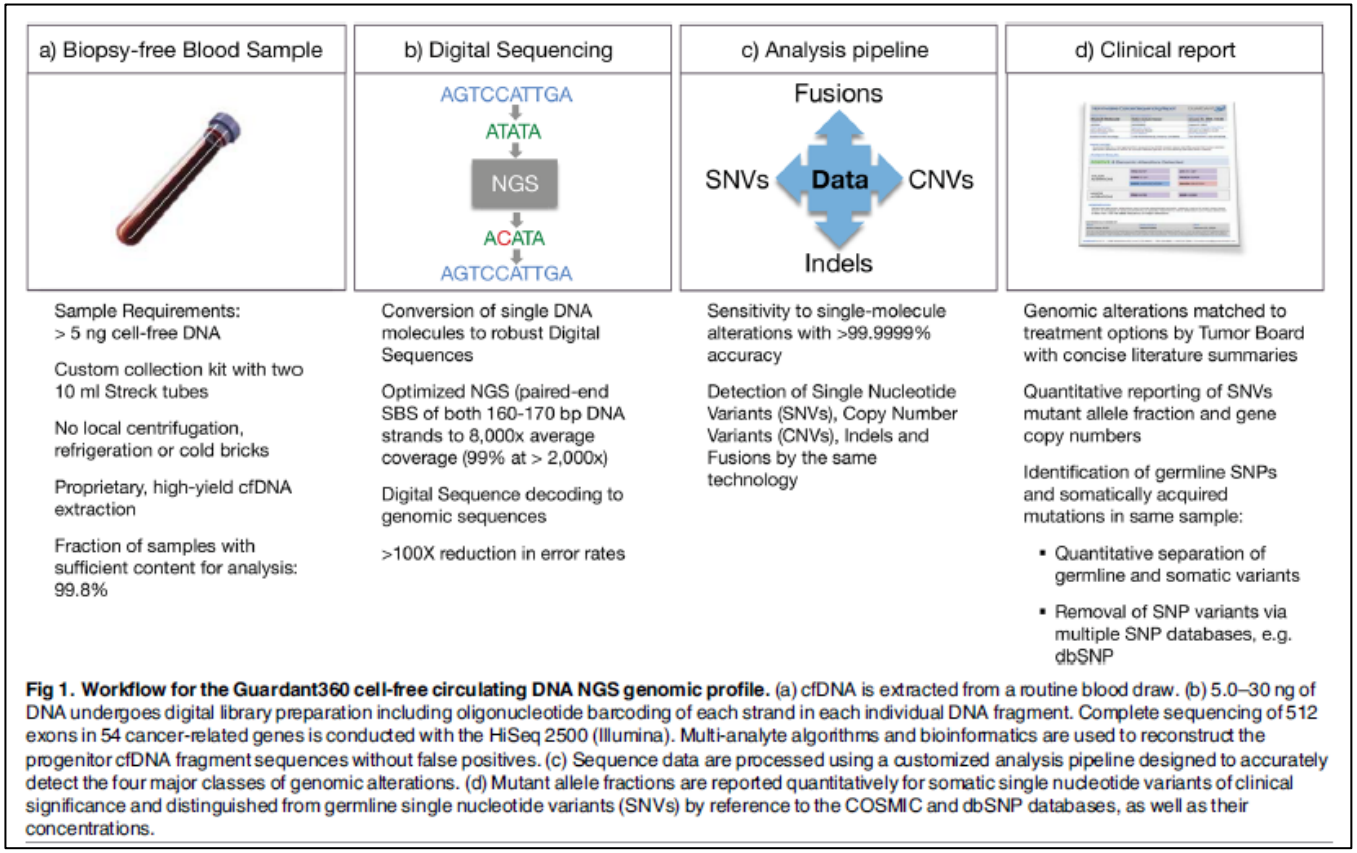
Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 23

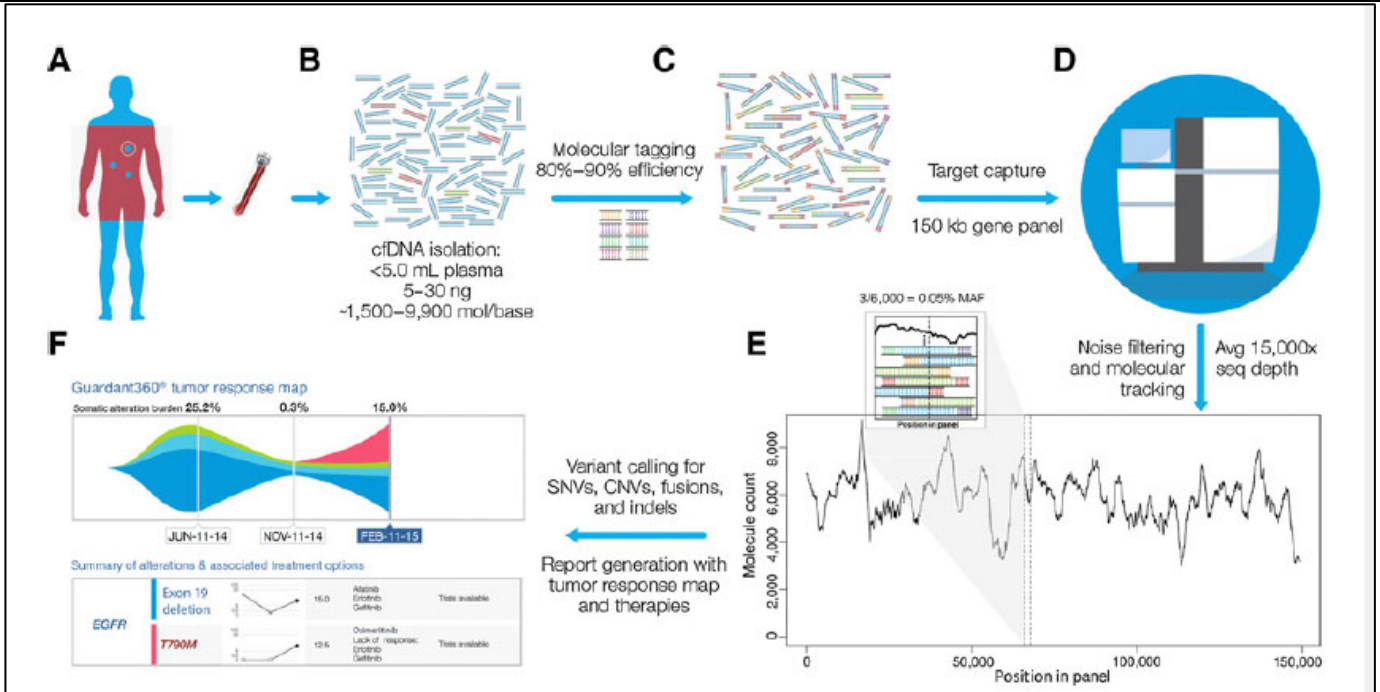
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the unique tagged nucleic acid molecules are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids. For example, Guardant uses oligonucleotide heptamer barcodes to create a library where original cell-free DNA molecules are separately encoded, such that multiple copies containing the same unique tag-target sequence combination can be traced back to the same original starting molecule. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")  **Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile.** (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.") Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.")

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 26

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags comprise nucleotide sequences with constant portions and variable portions.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions. For example, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of the same length. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.272

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

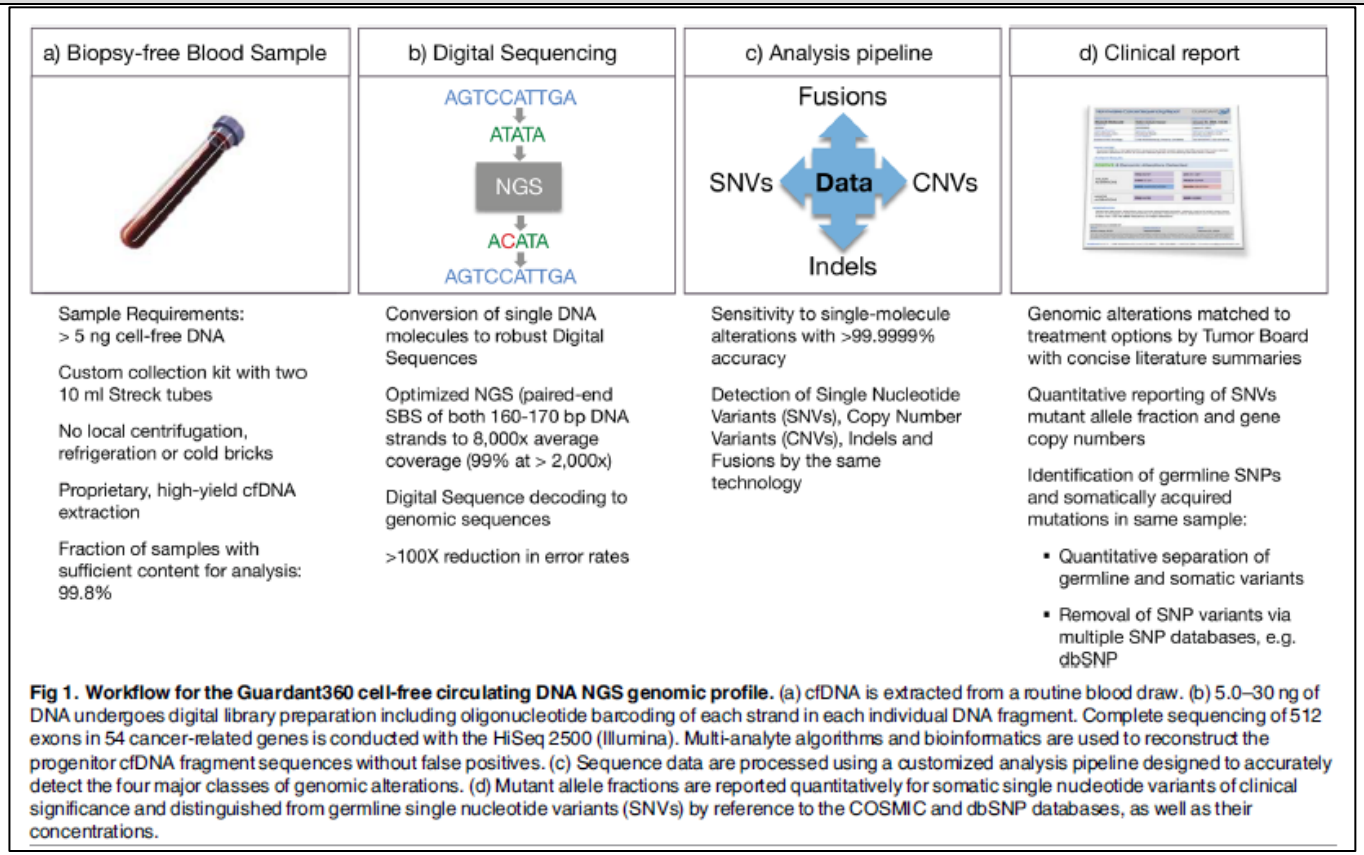
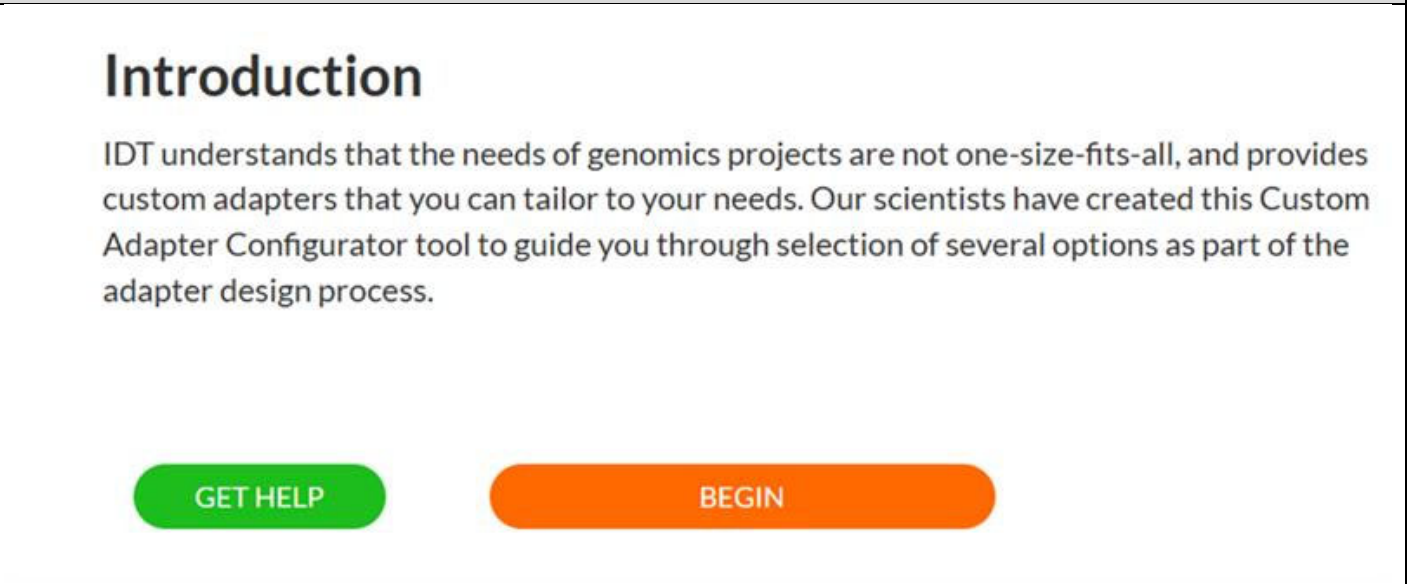


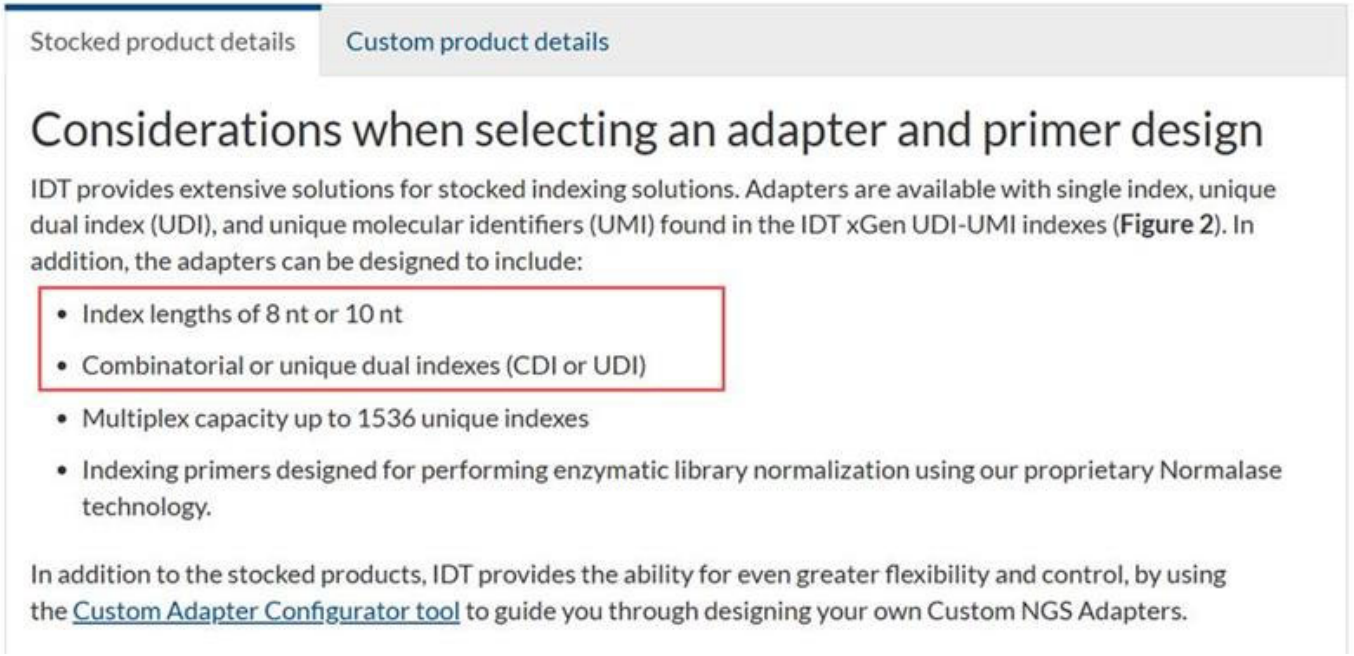
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

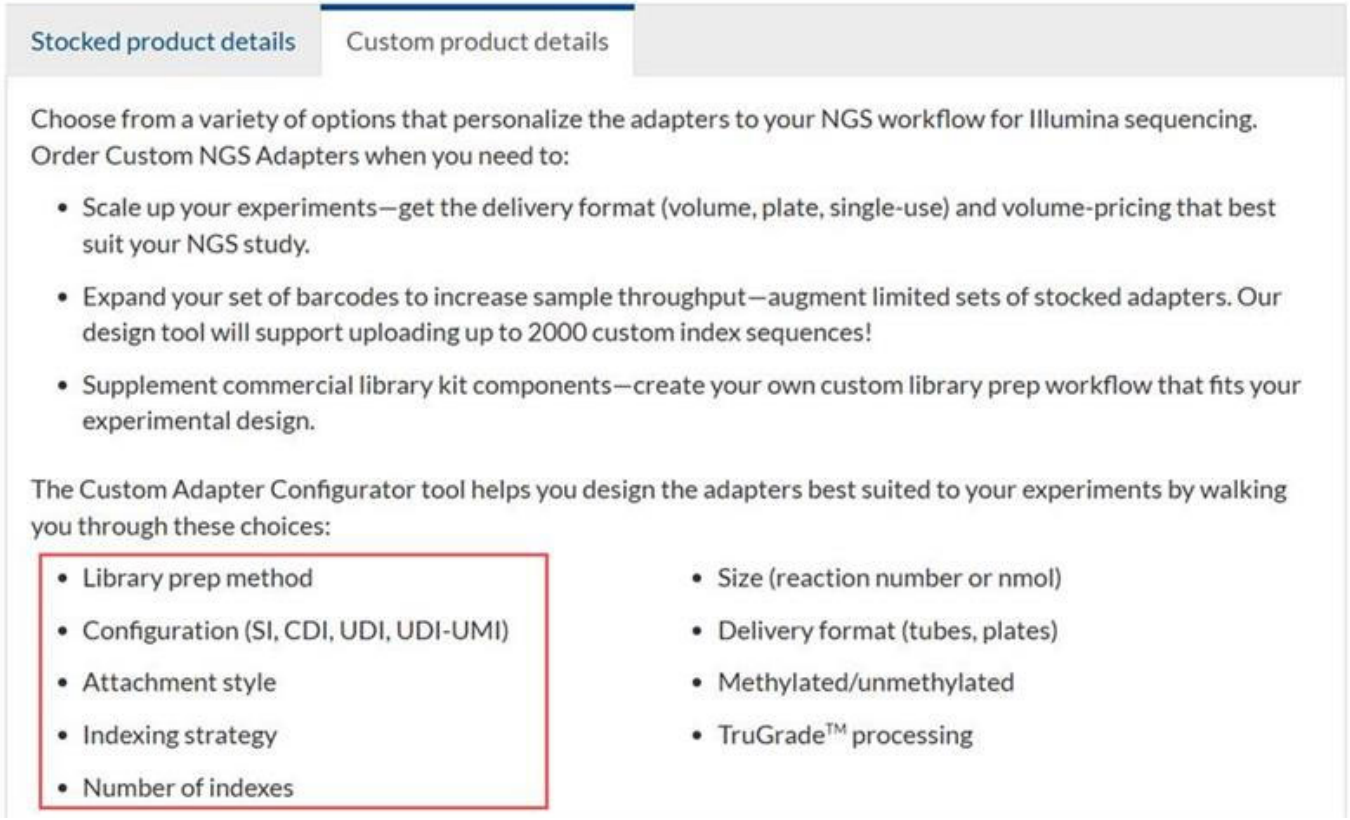
Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

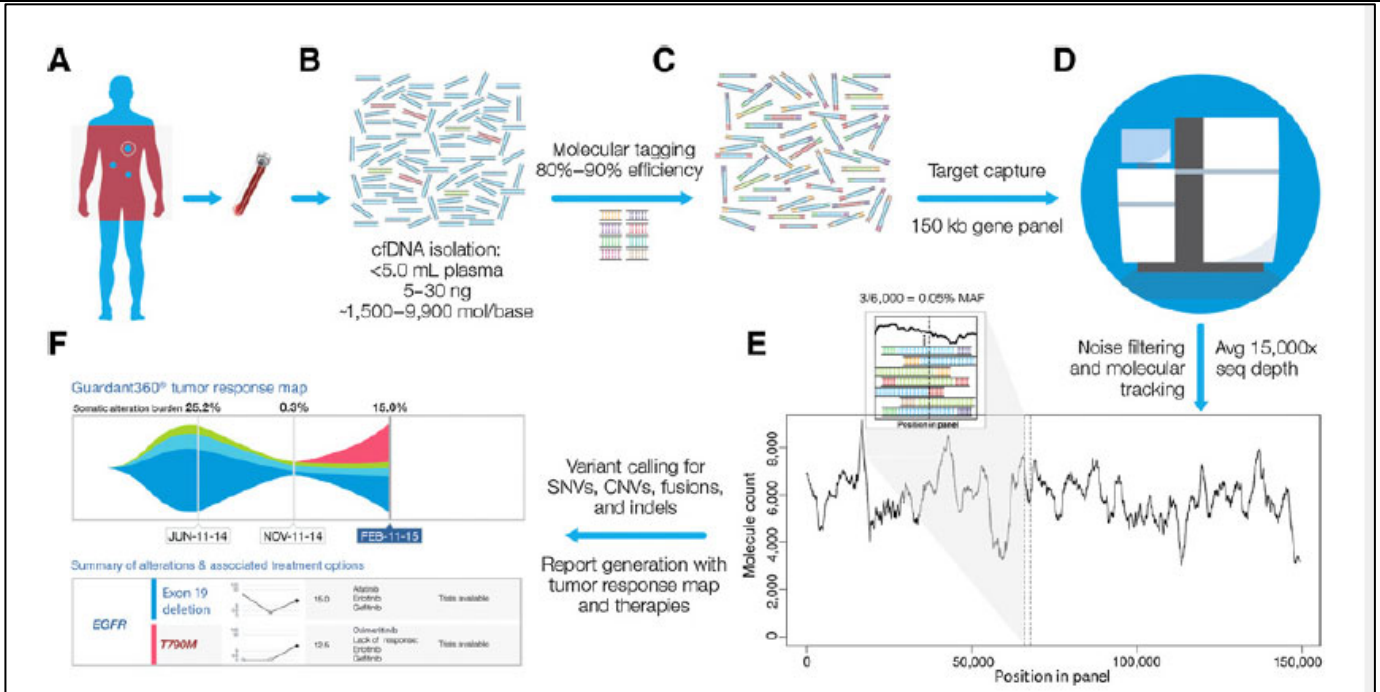
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage. Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.278

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

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	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 27

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 26, wherein the length of the constant portions and the length of the variable portions is the same.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the length of the constant portions and the length of the variable portions is the same. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of the same length. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

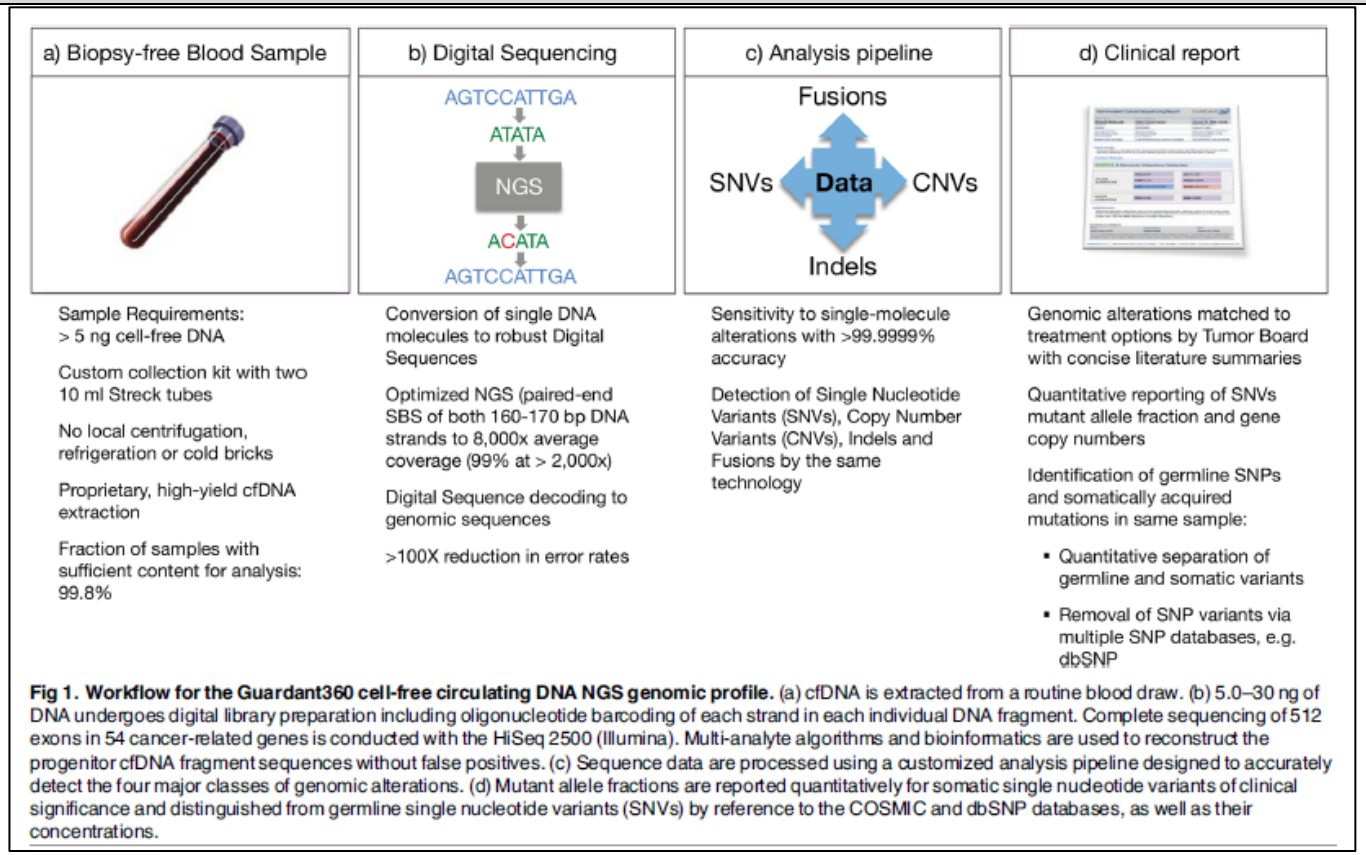
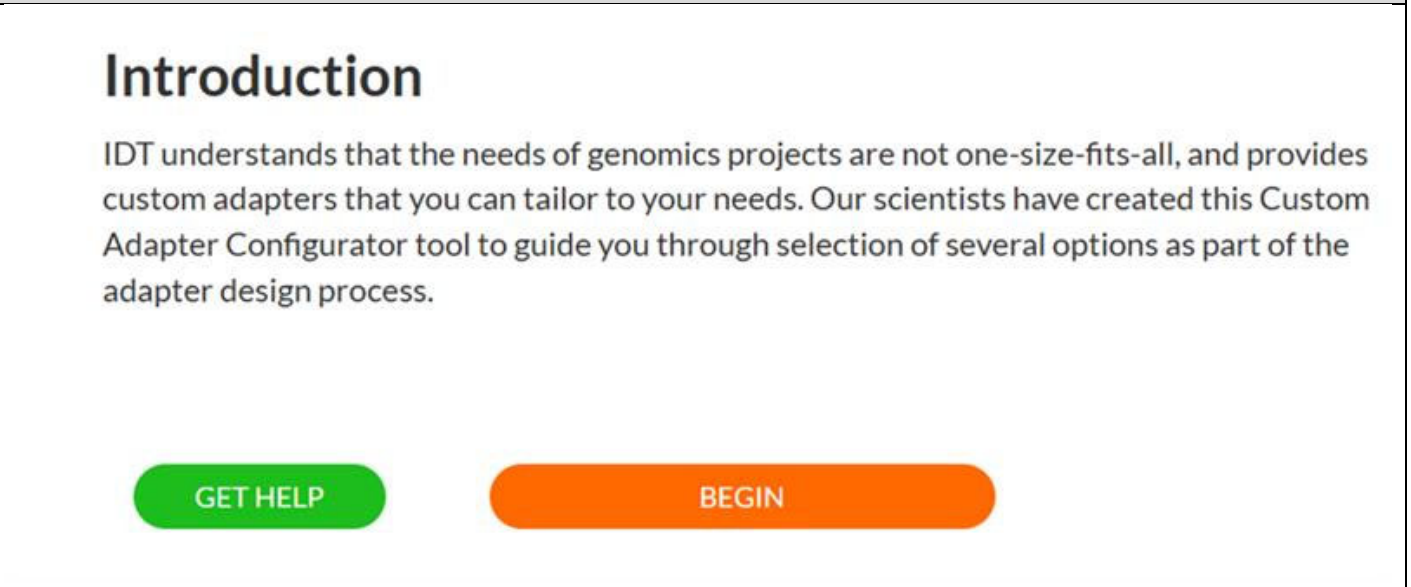


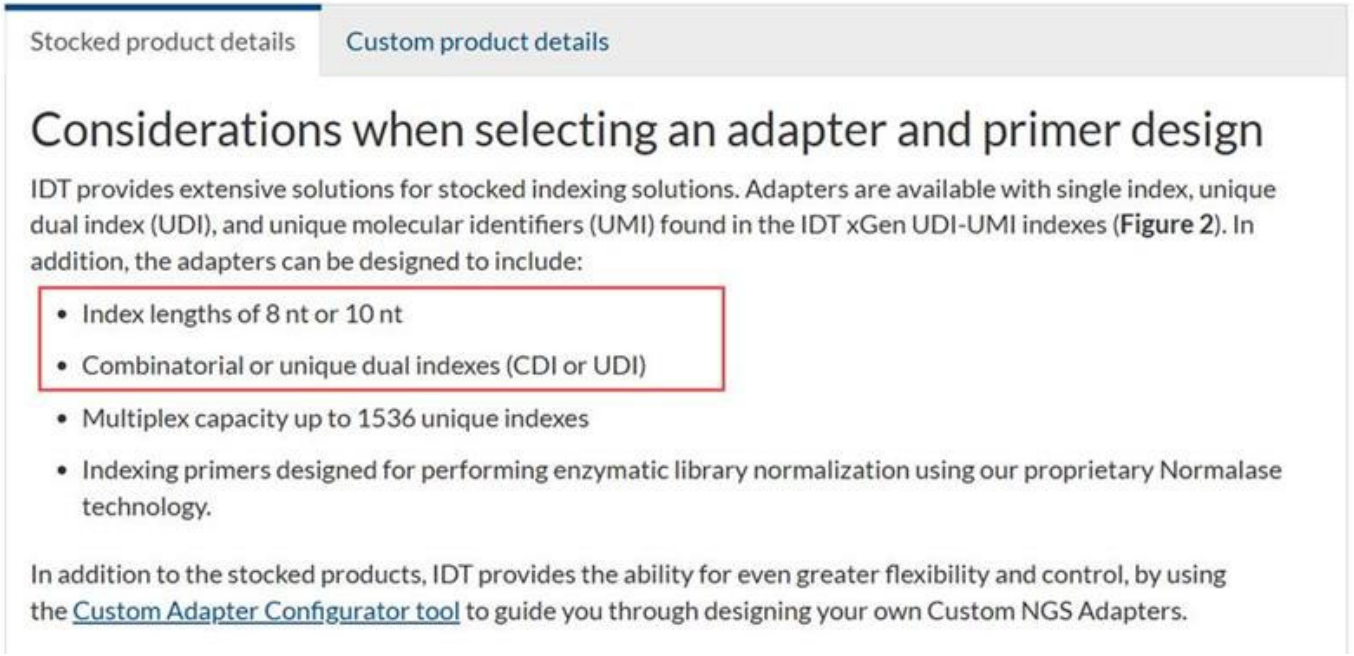
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

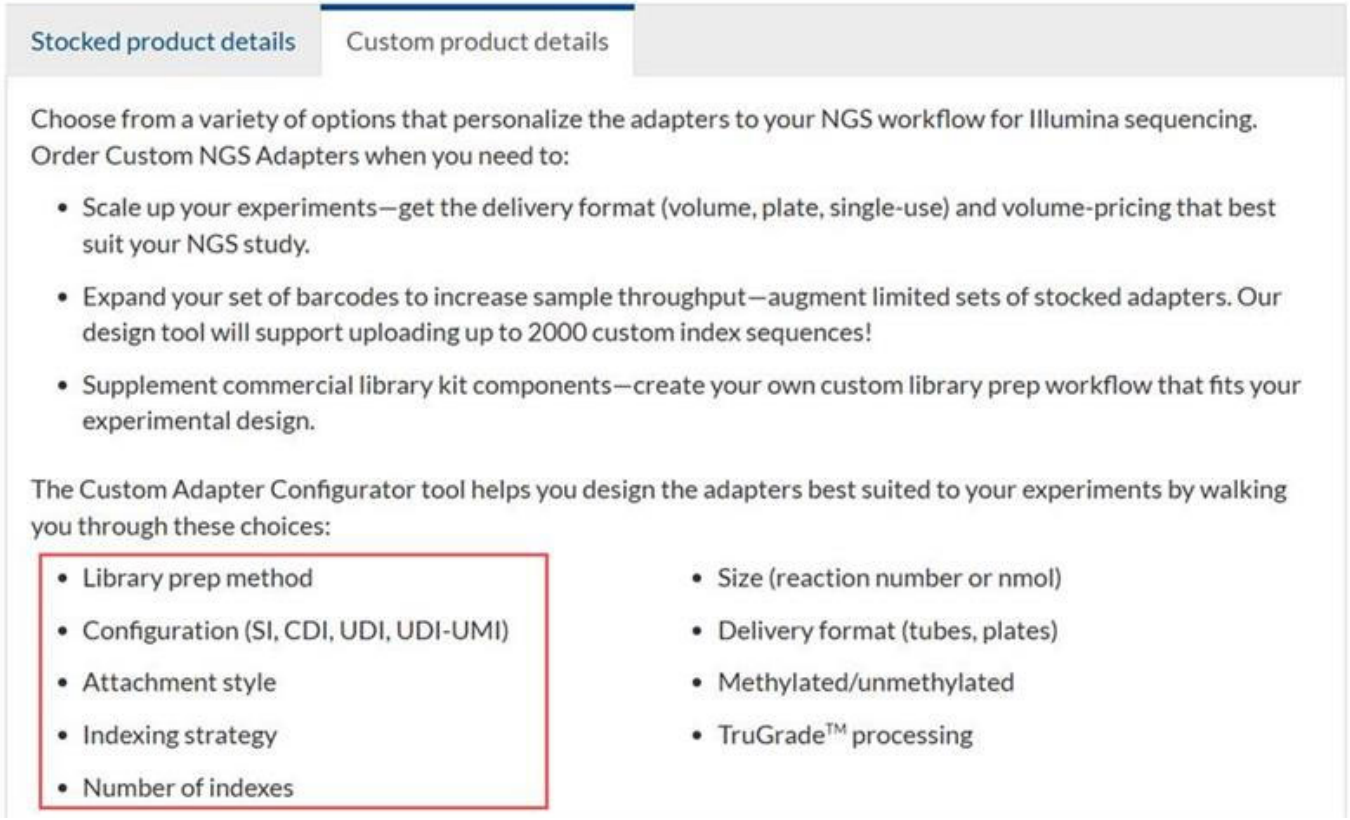
Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.287

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

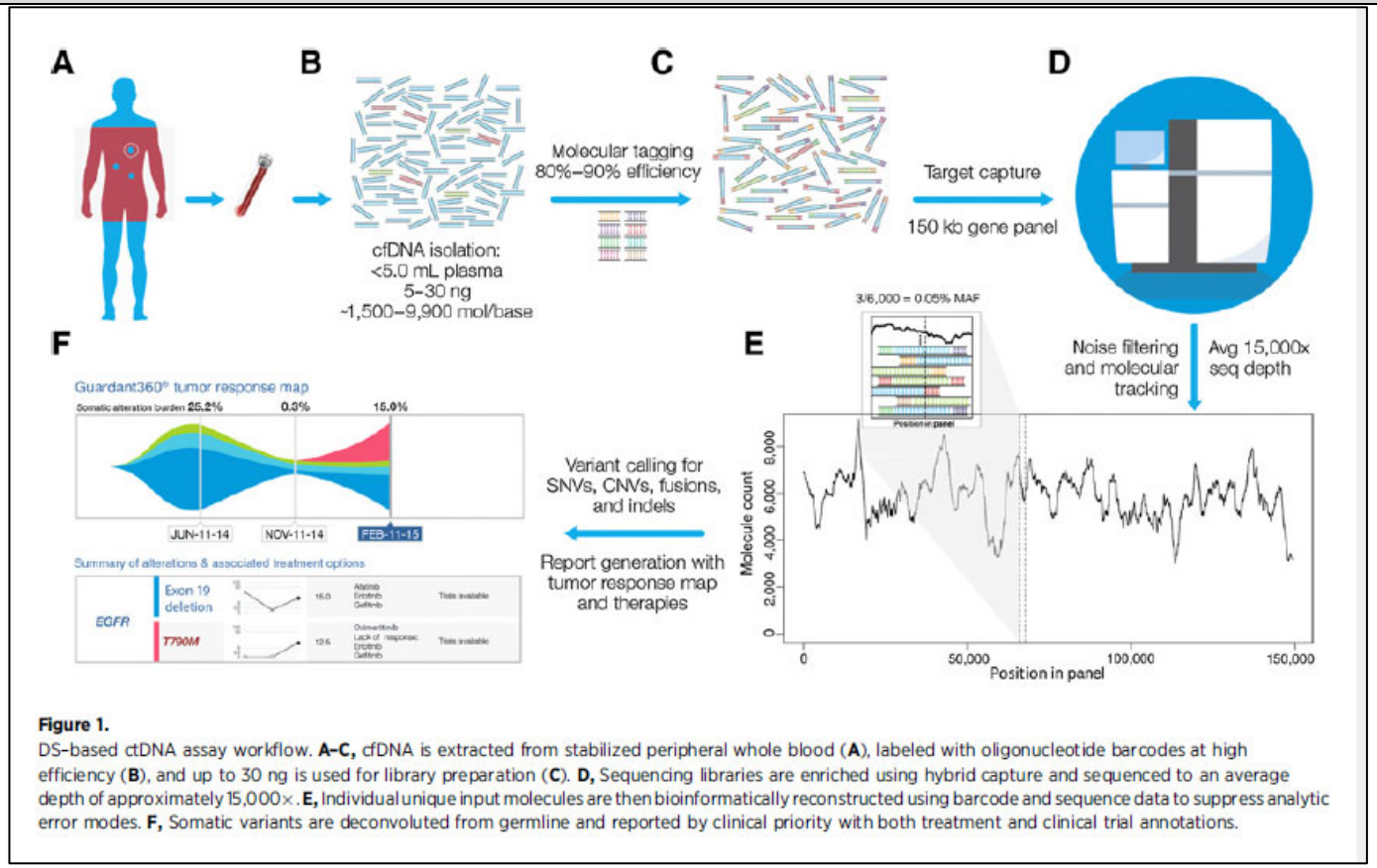


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 28

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 26, wherein the length of the constant portions and the length of the variable portions are different.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the length of the constant portions and the length of the variable portions are different. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers that may contain constant and variable portions of different lengths. Nakamura et al. at "Methods" ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.290

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

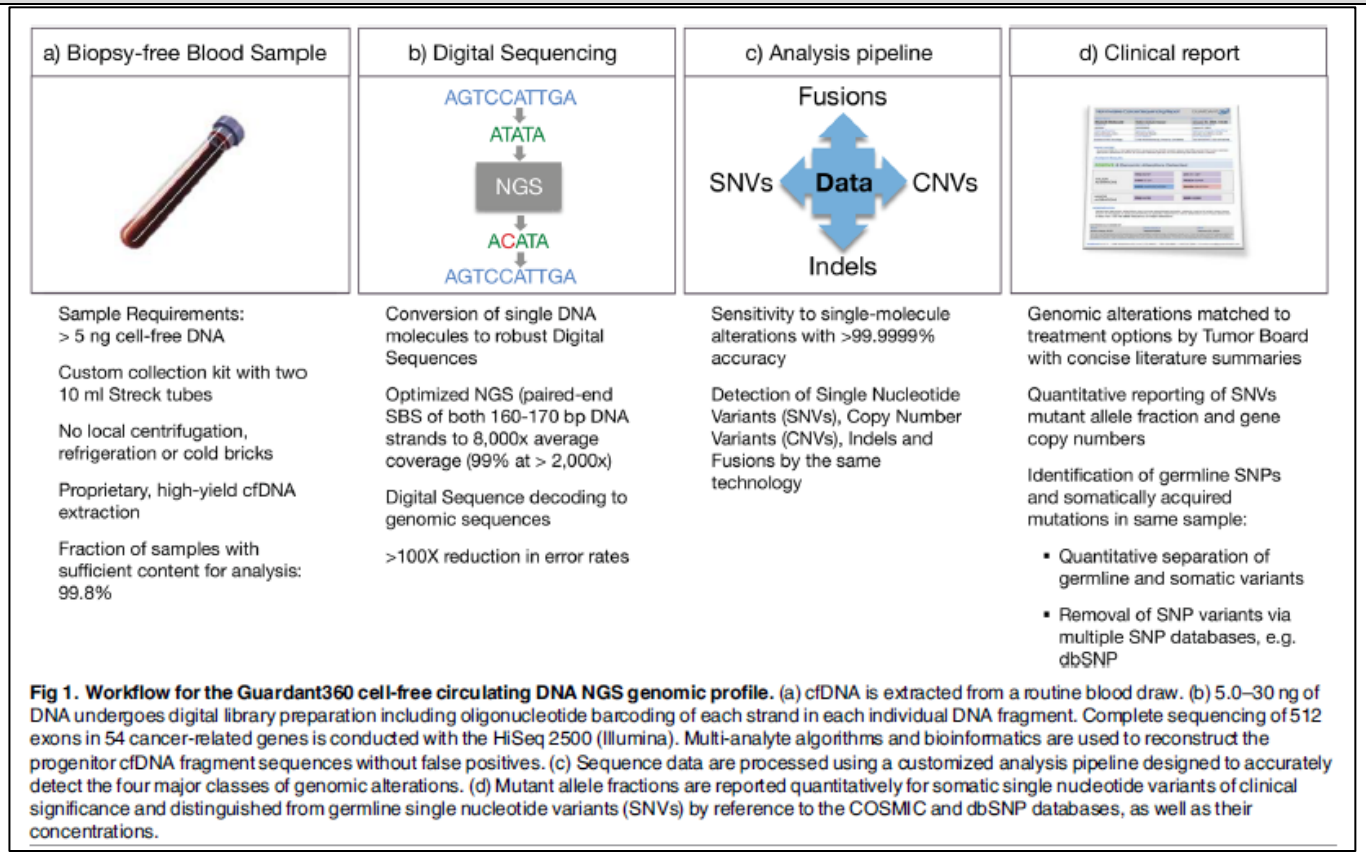
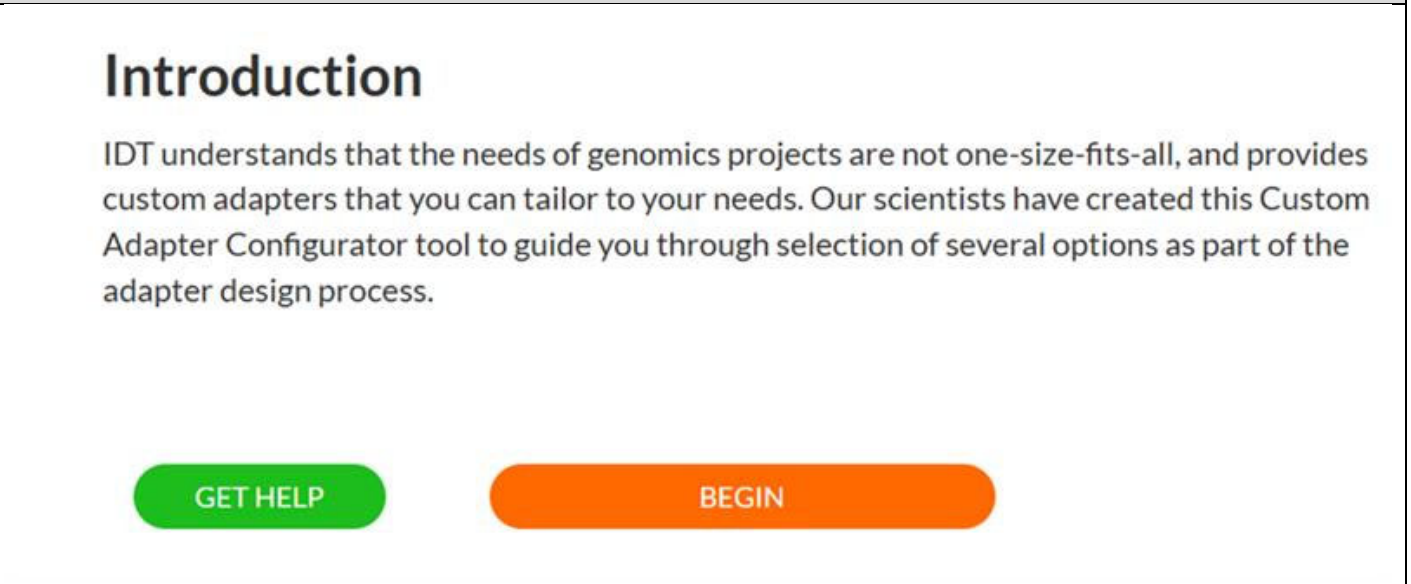


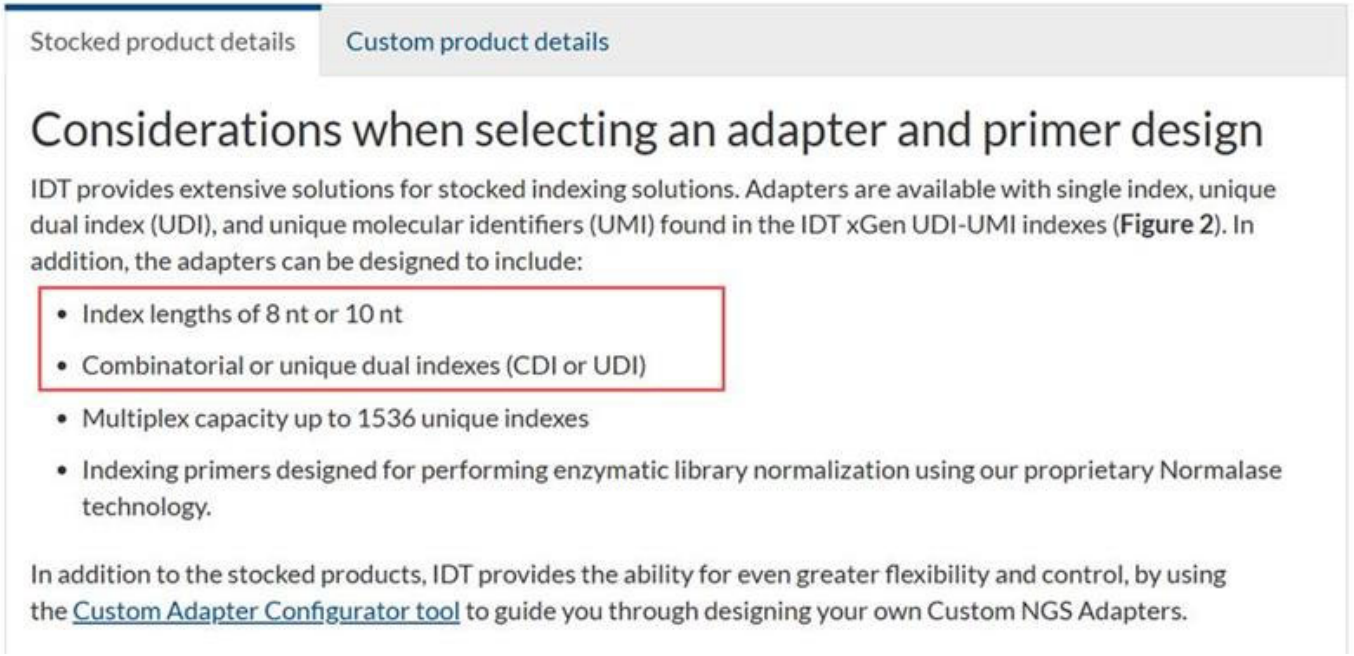
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

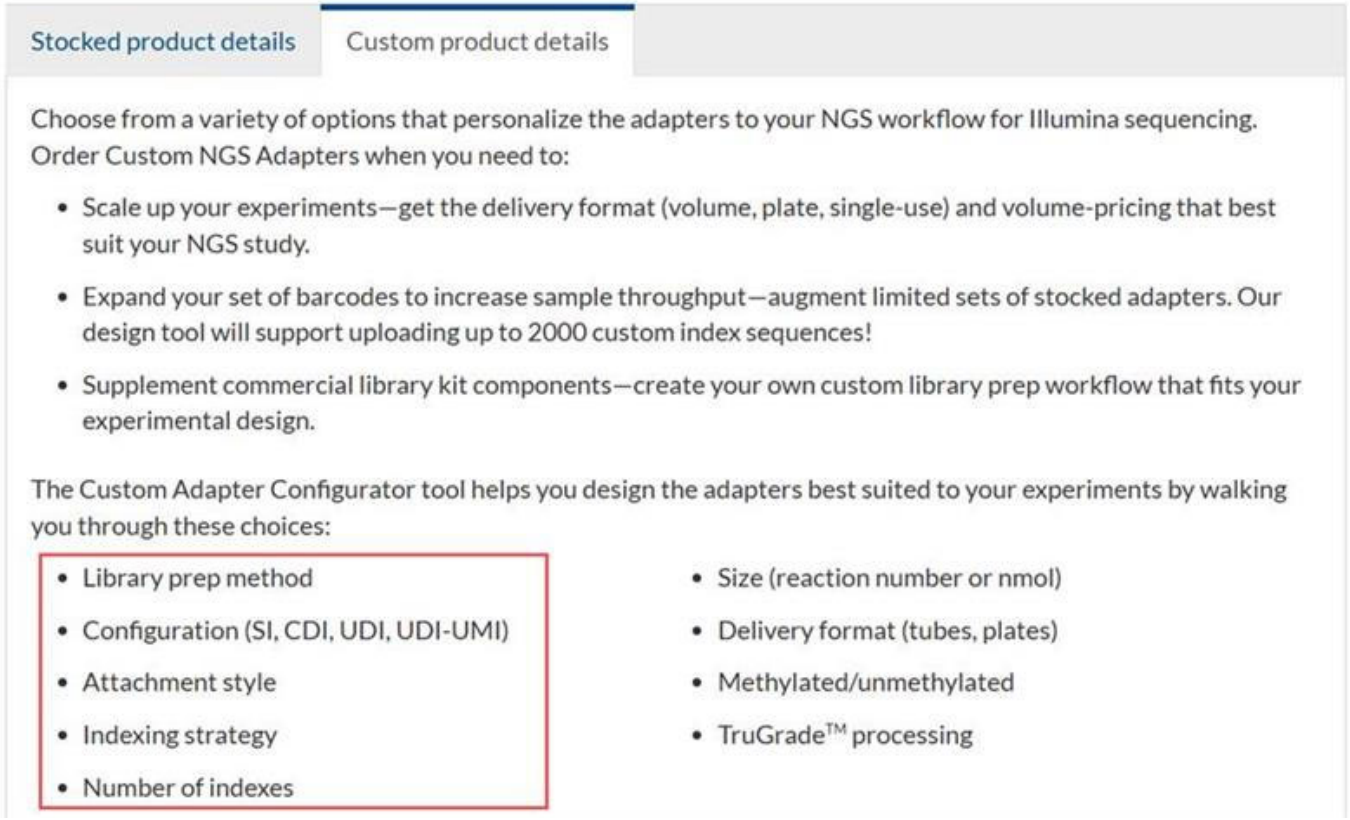
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

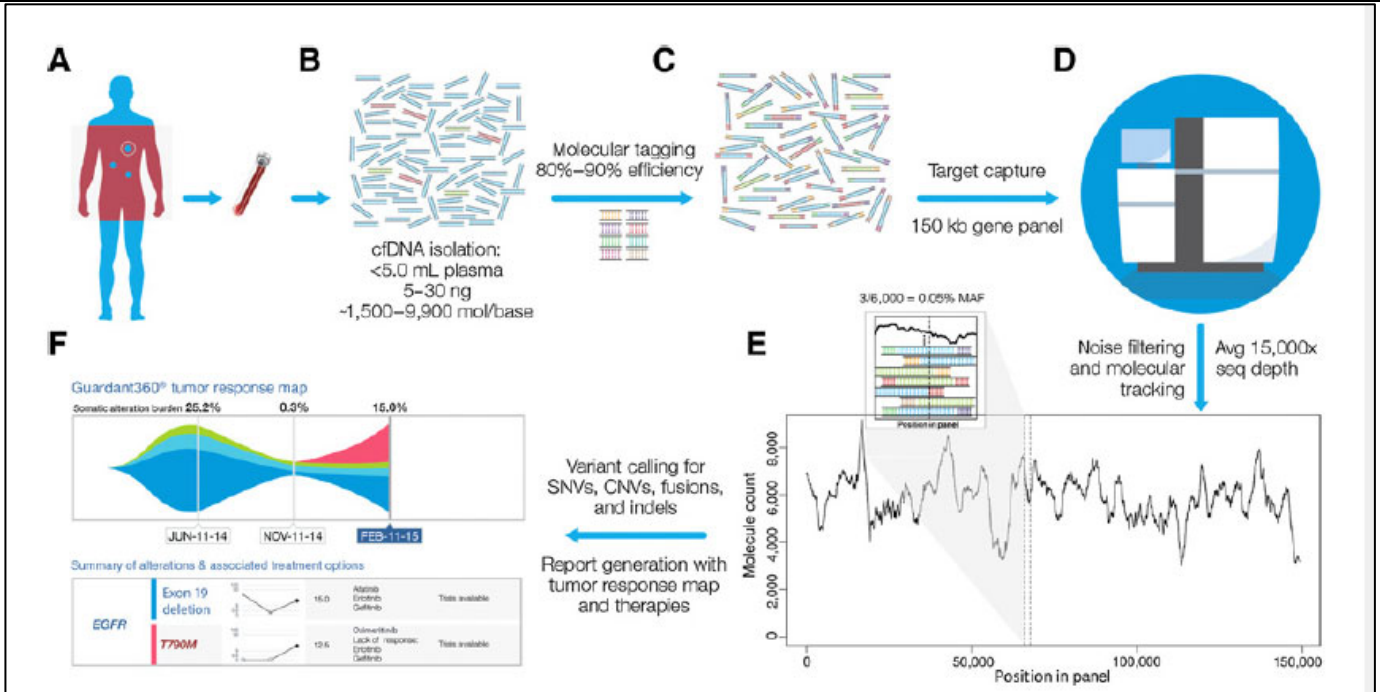
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.296

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 29

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 26, wherein the variable portions consist of only two species of nucleotides.	Guardant generates unique tagged nucleic acid molecules using nucleic acid tags that comprise nucleotide sequences with constant portions and variable portions, wherein the variable portions consist of only two species of nucleotides. For example, on information and belief, Guardant ligates library adapters containing inline barcodes to nucleic acid molecules, where the adapters comprise constant portions and the barcodes comprise variable portions or vice versa. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions consisting of only two species of nucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.299

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

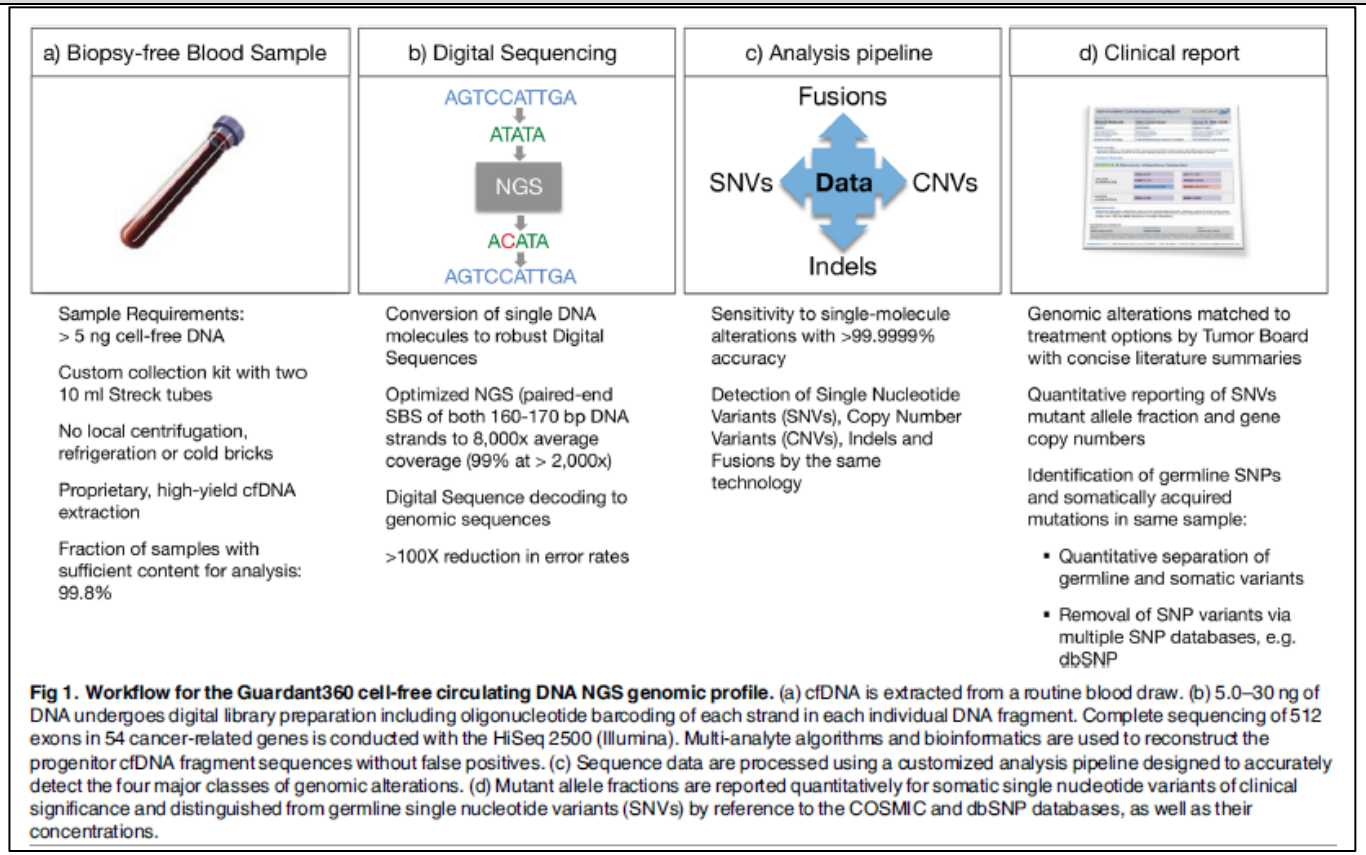
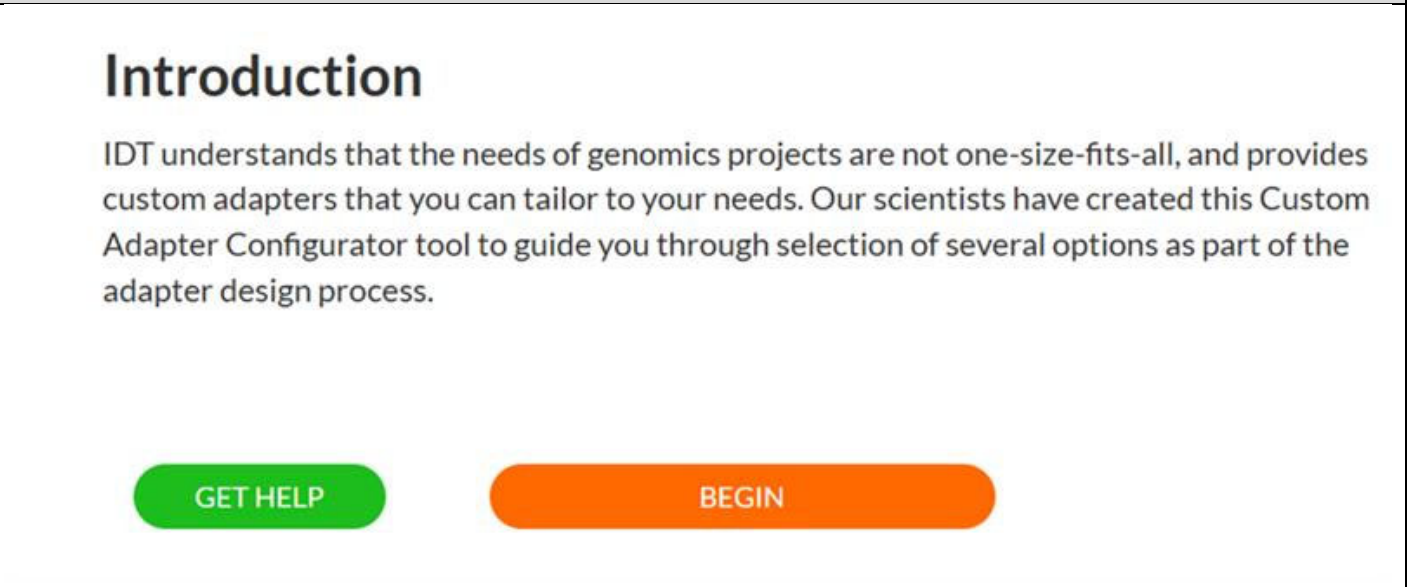


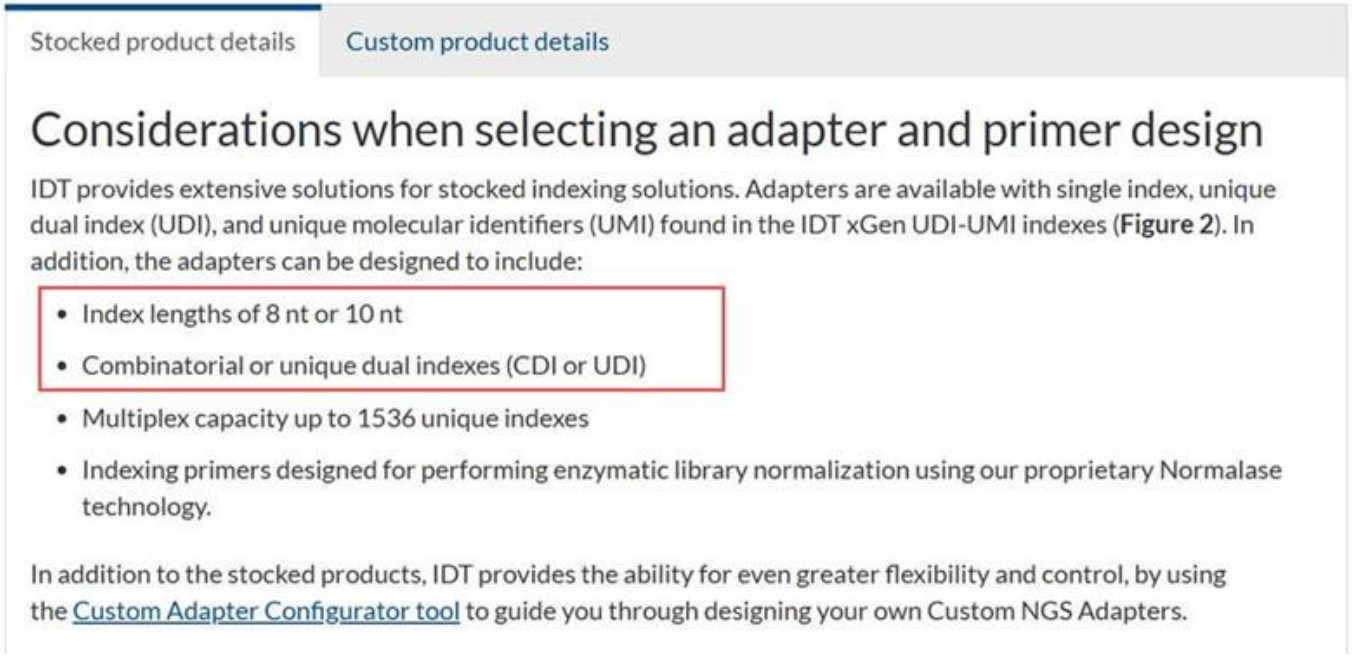
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

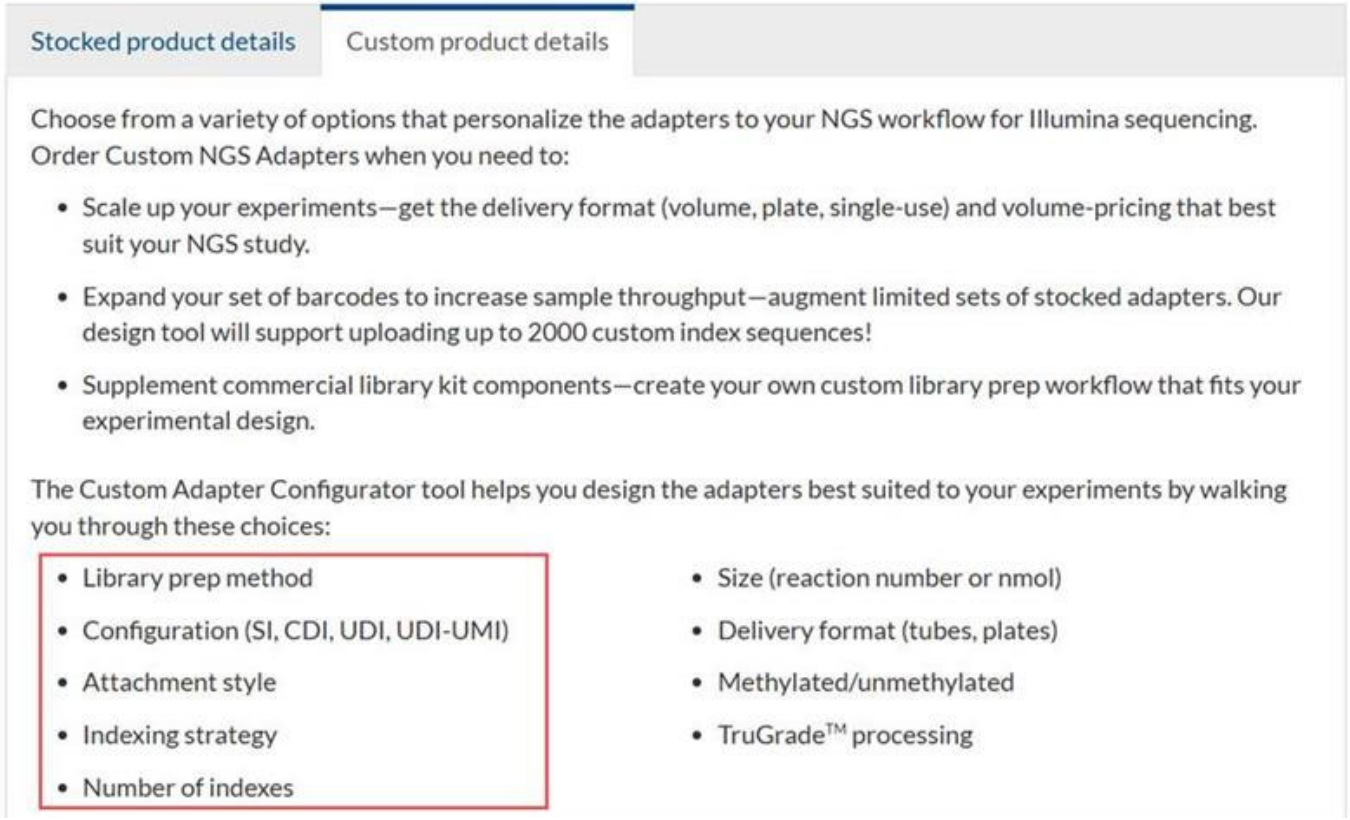
Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

GUARDANT HEALTH EX.1020.303

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage. Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.305

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

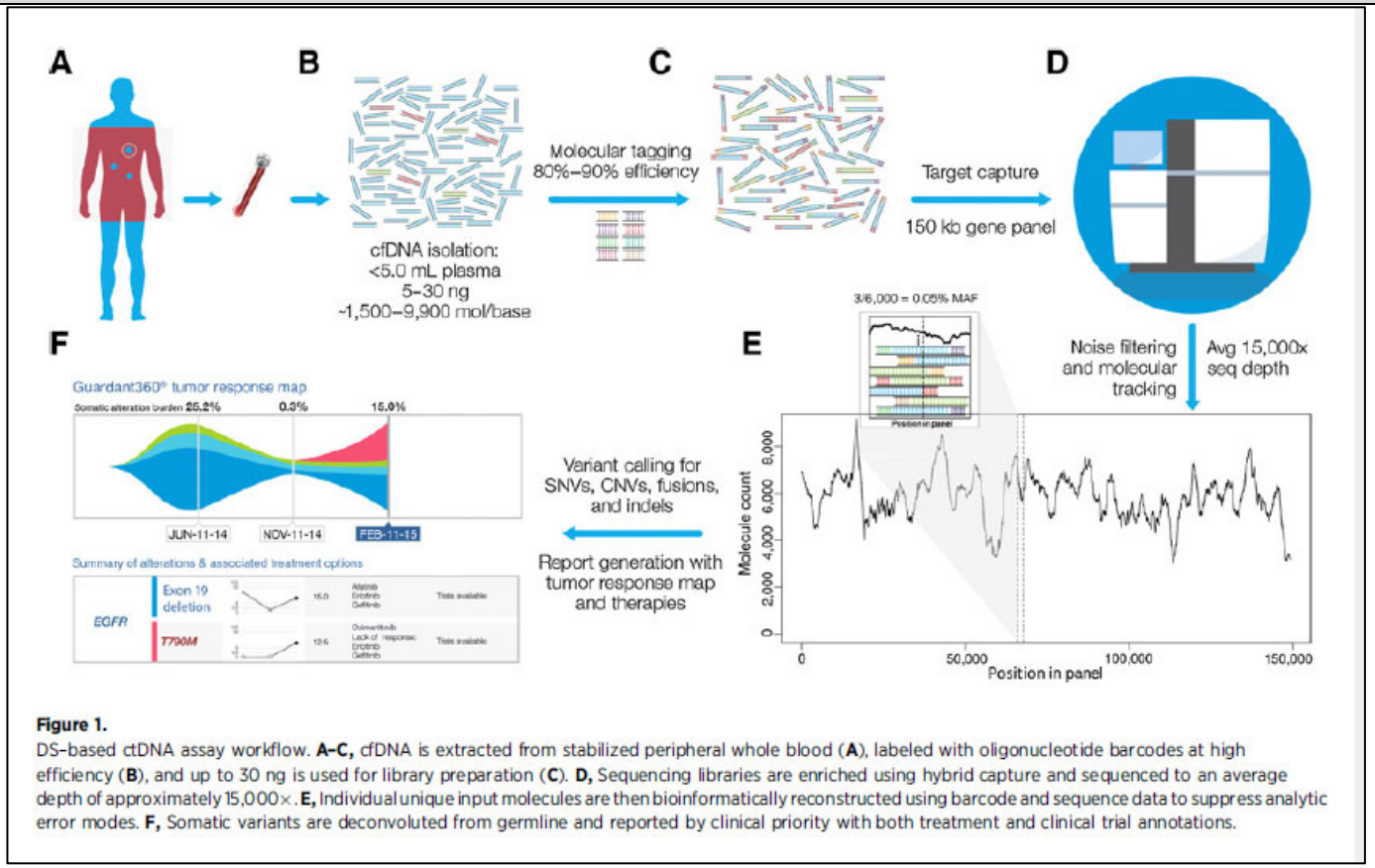


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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ASSERTED CLAIM 31

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids, wherein the nucleic acid tags comprise variable portions constructed by a set of dinucleotides. For example, on information and belief, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions constructed by a set of dinucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.308

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

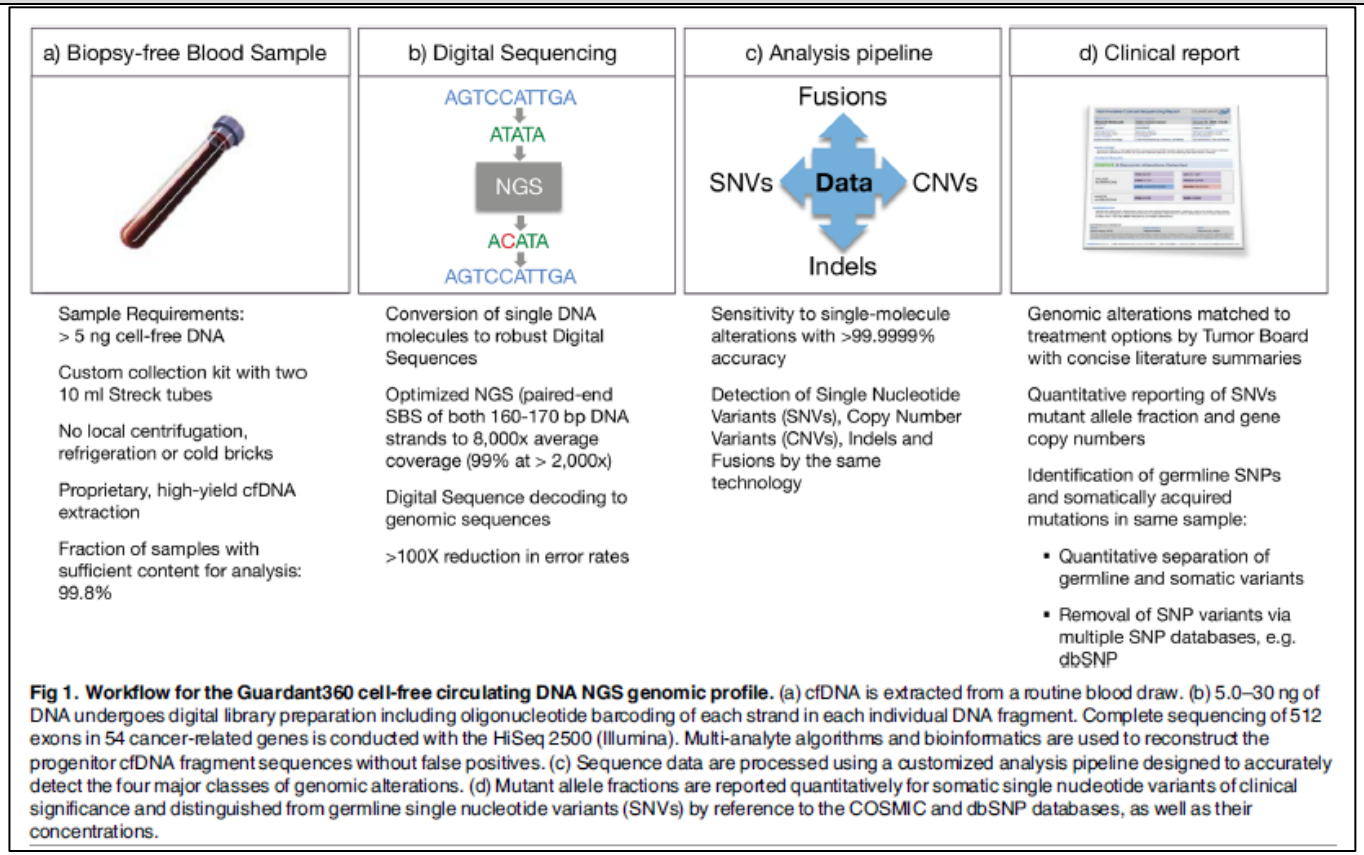
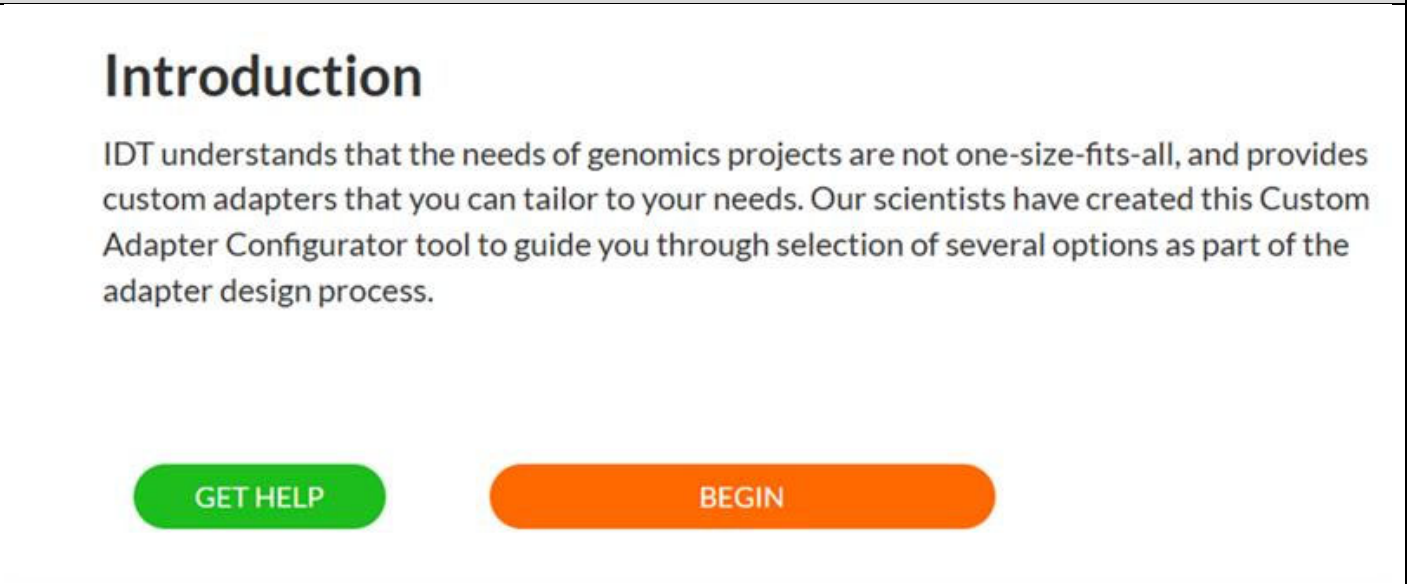


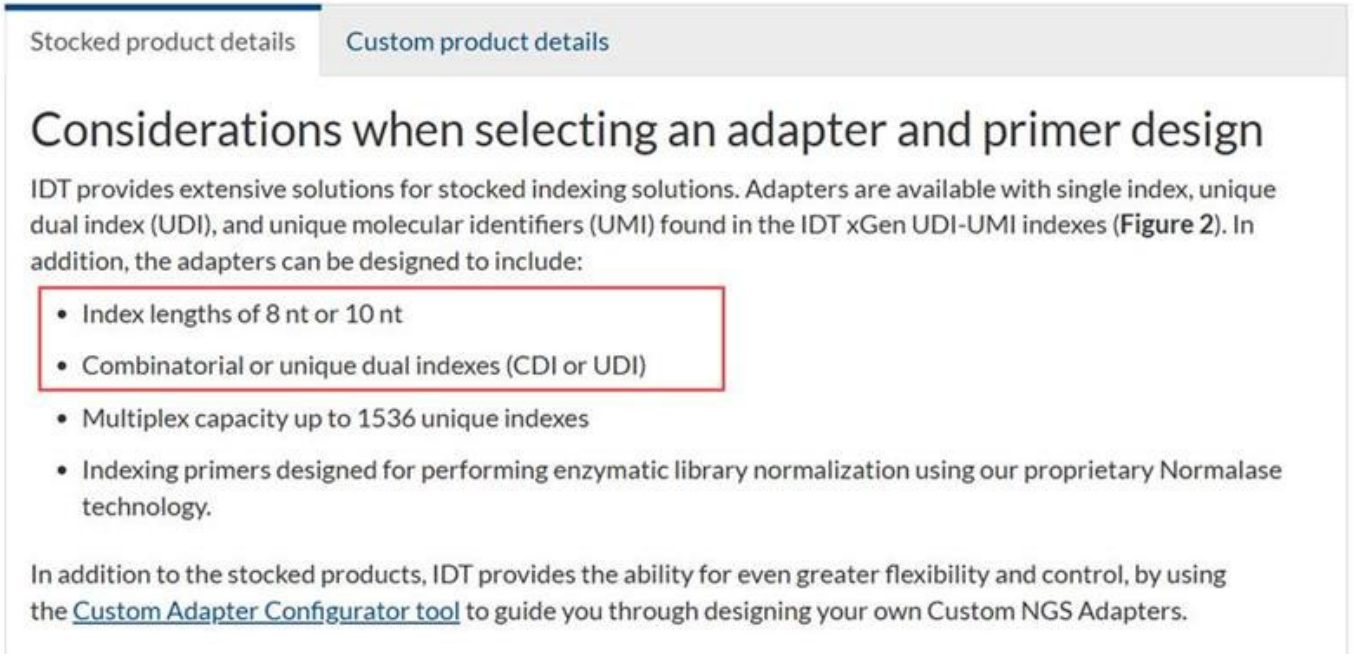
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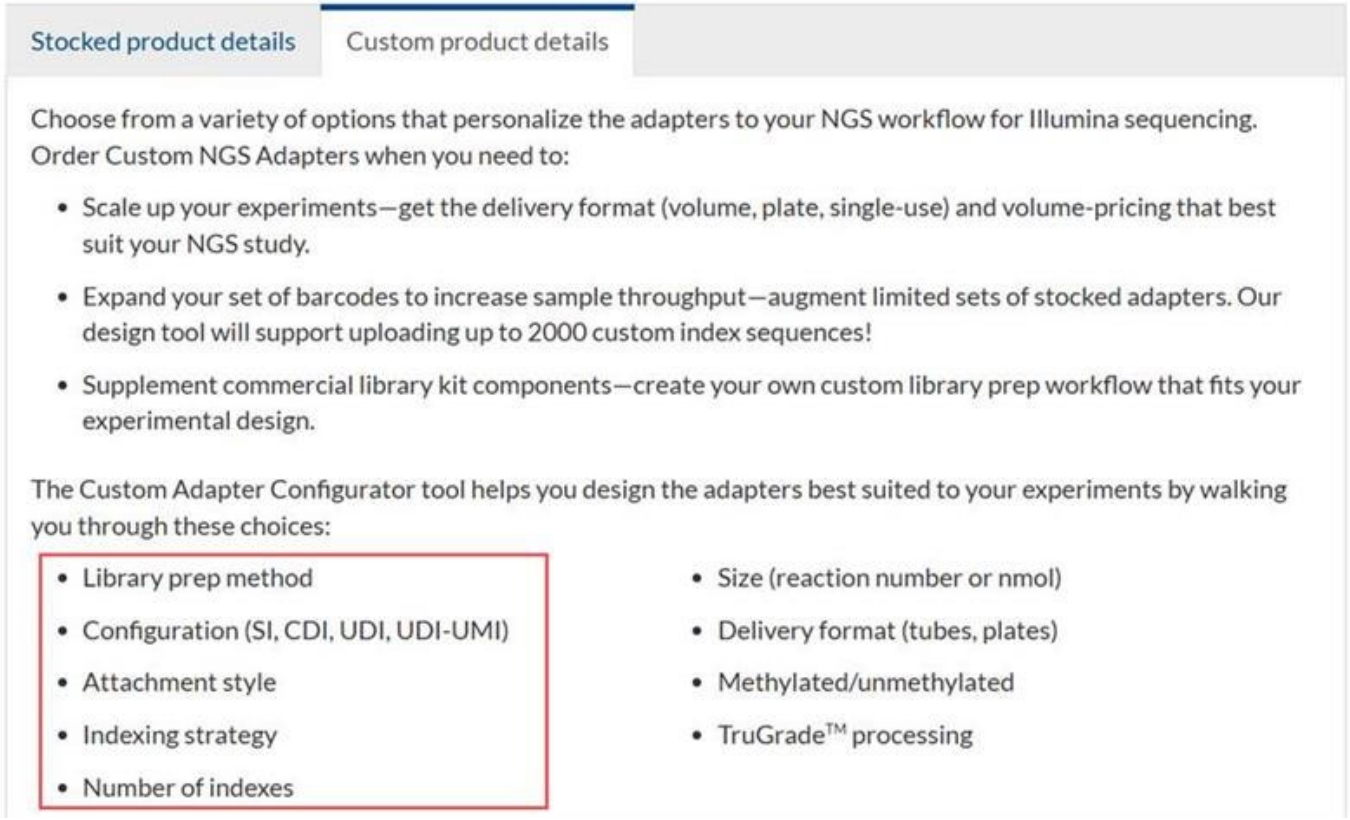
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

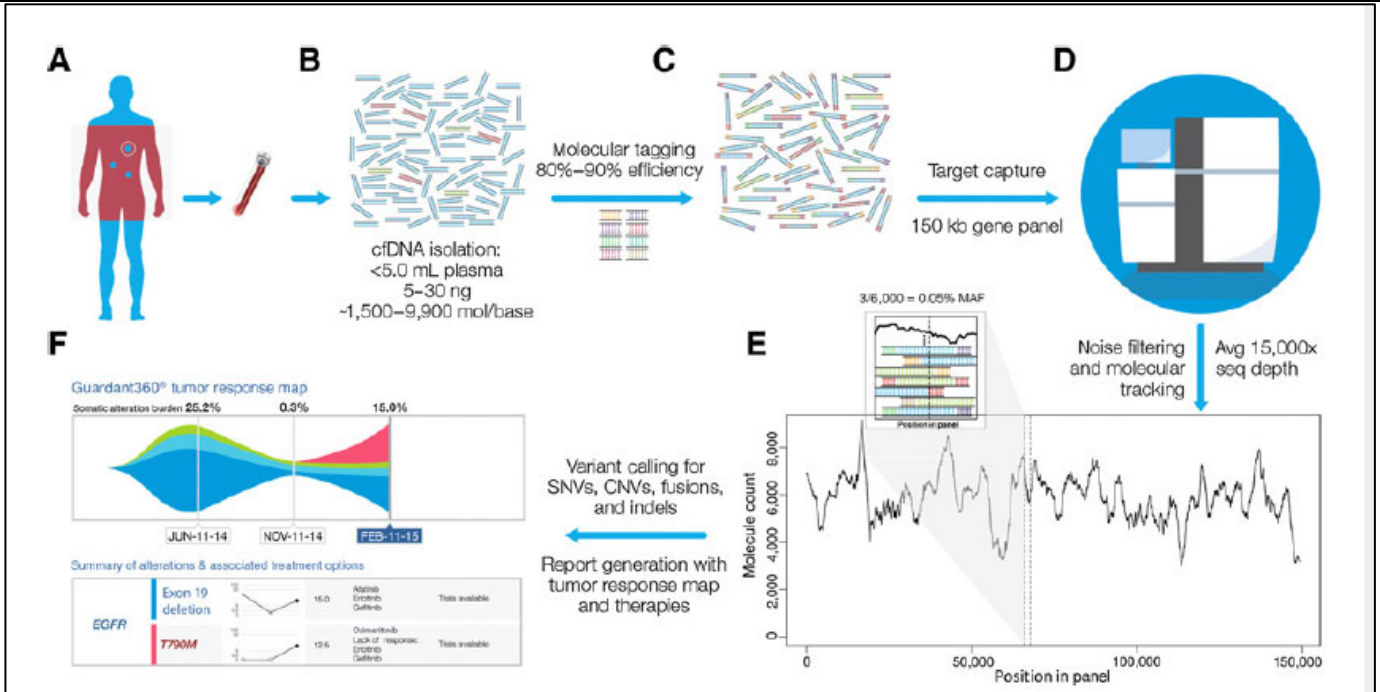
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage. Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.314

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 32

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides.	Guardant generates unique tagged nucleic acid molecules that are unique based on a combination of sequence information in the nucleic acid tags and sequence information in the segments of the genomic nucleic acids, wherein the nucleic acid tags comprise variable portions constructed by a set of trinucleotides. For example, on information and belief, Guardant tags cell-free DNA with oligonucleotide heptamer barcodes. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom adapters and primers with variable portions constructed by a set of trinucleotides. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.317

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

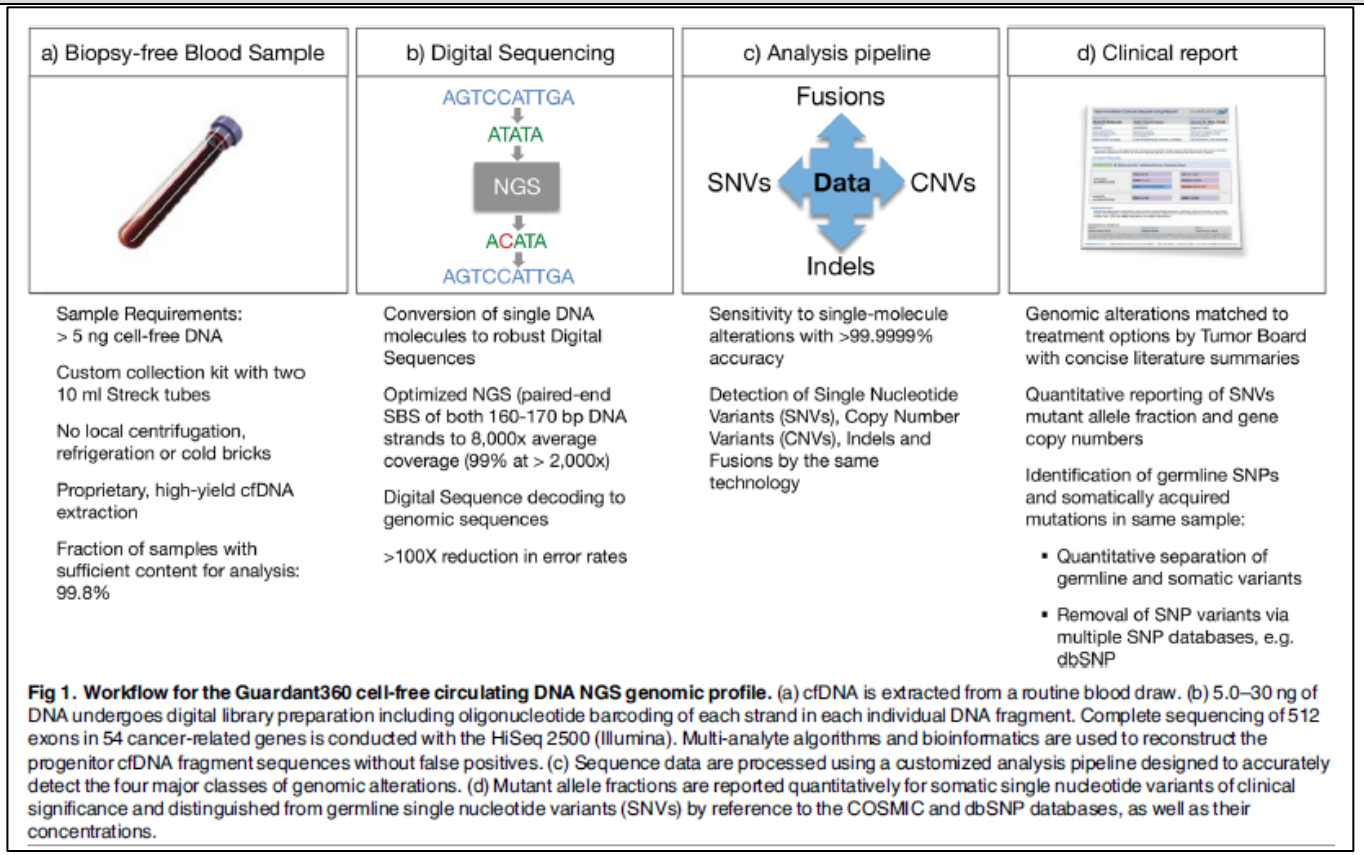
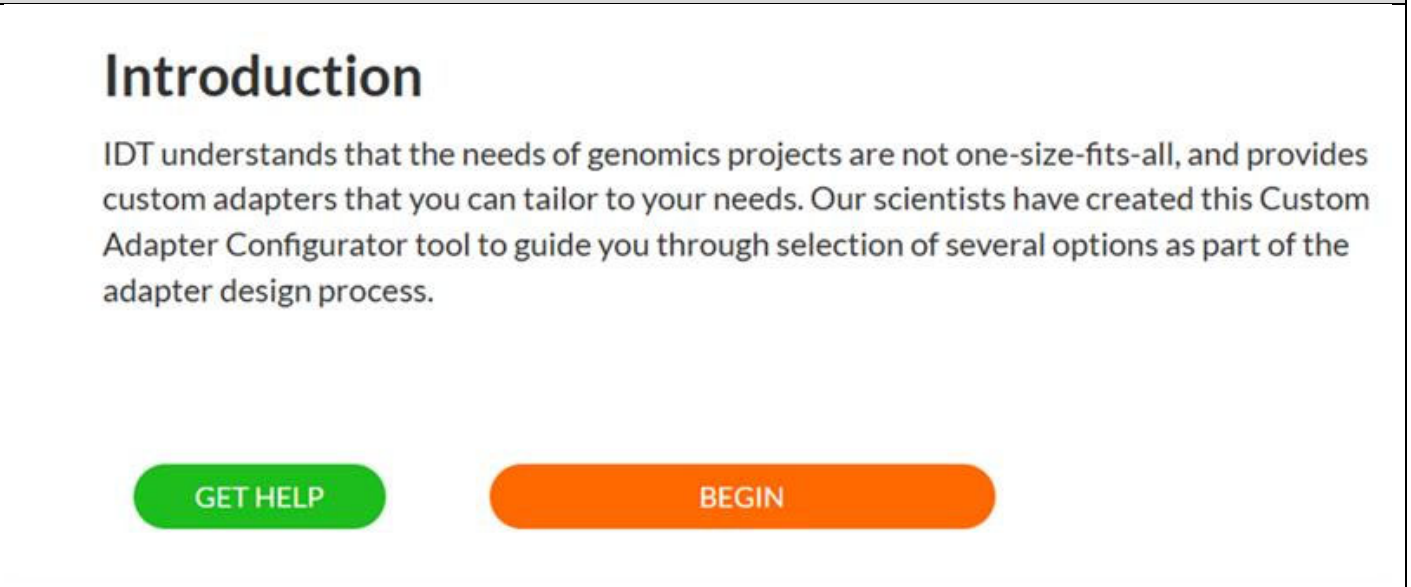


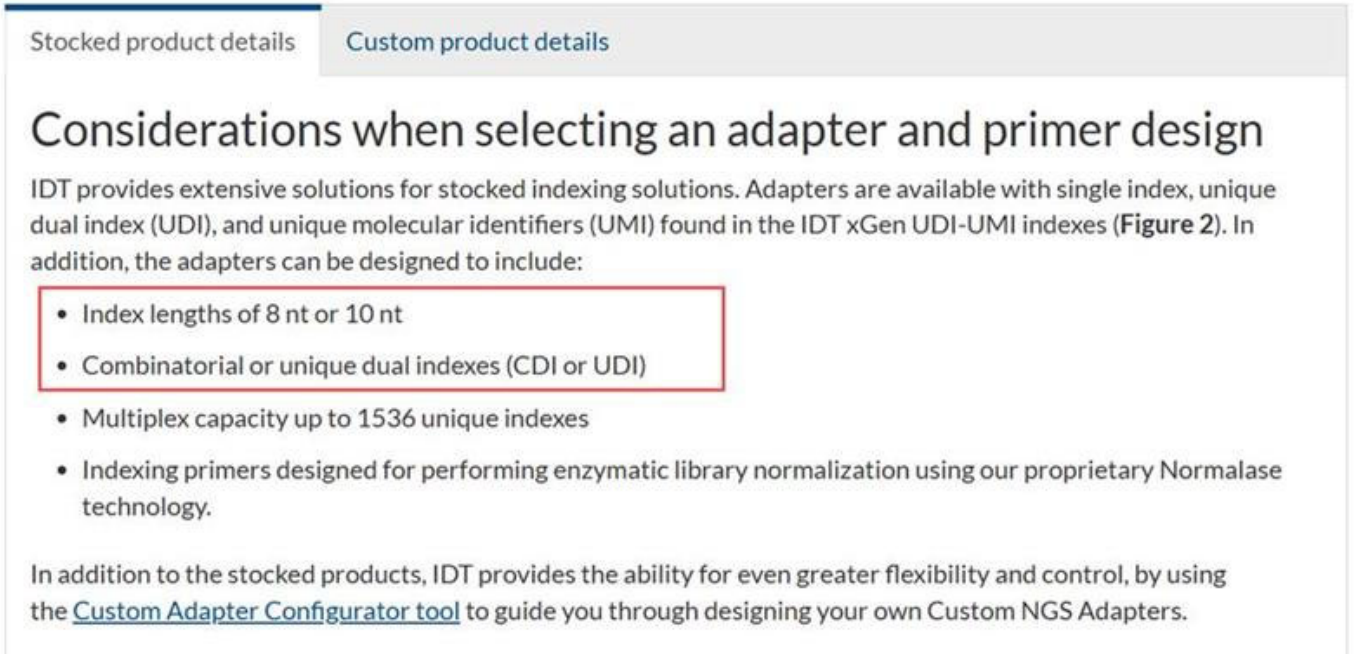
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

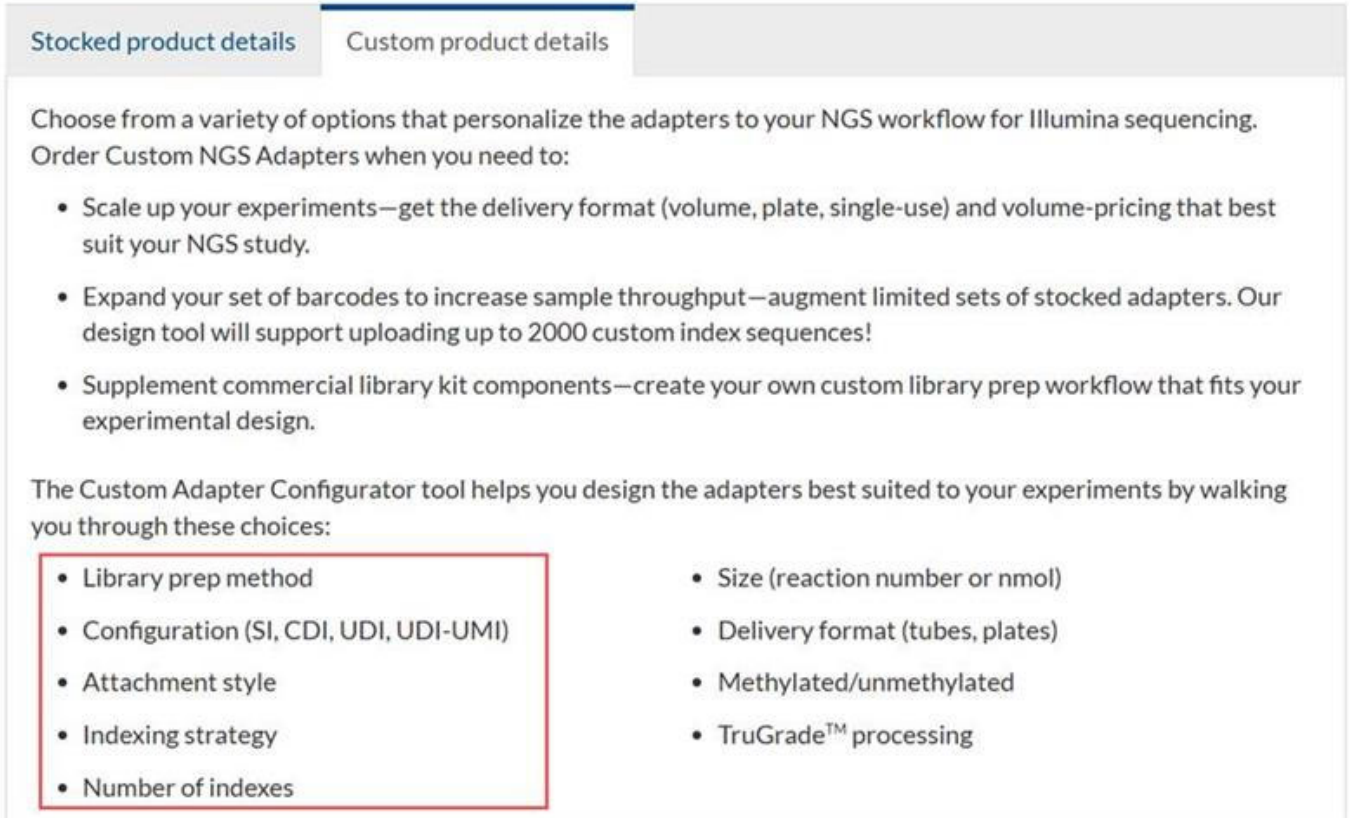
Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).")

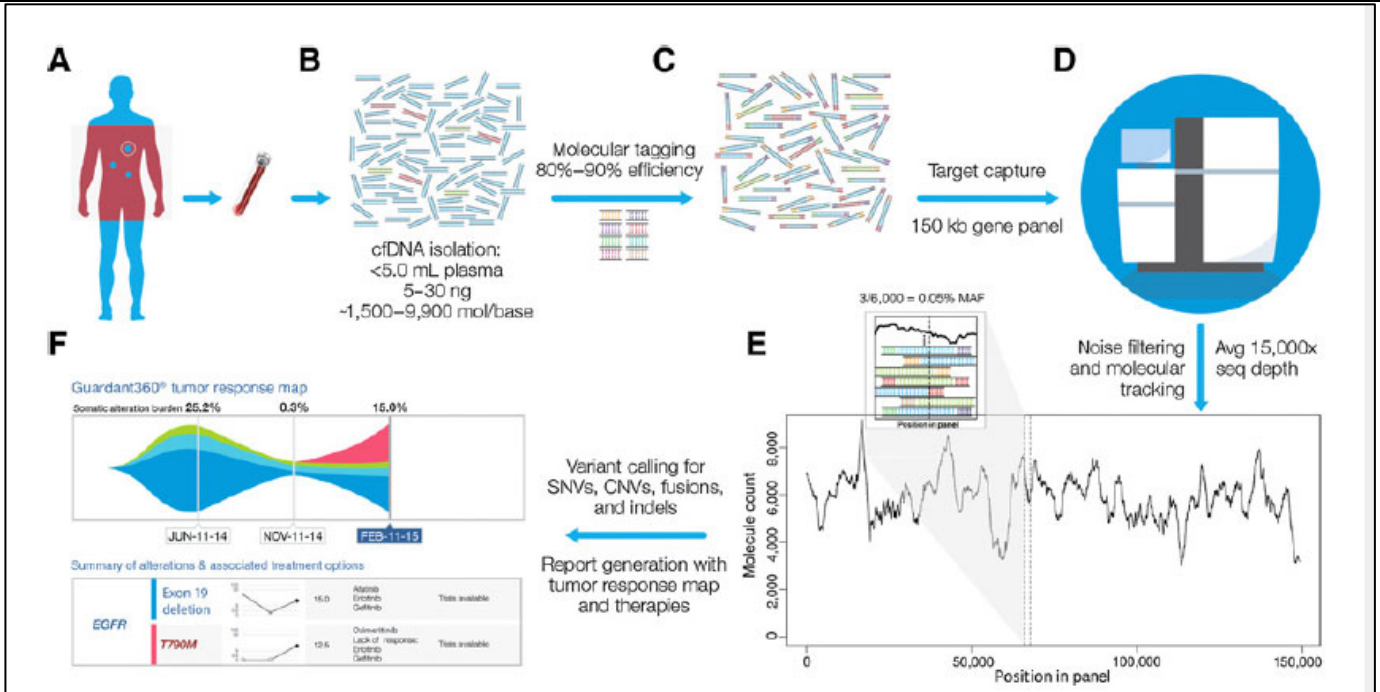
Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage.

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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

GUARDANT HEALTH EX.1020.323

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 **Figure 1.** DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations. Odegaard et al. at 3542 (Figure 1). '822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 33

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags comprise nucleotide sequences that are not known before the tagged nucleic acid molecules are generated.	Guardant generates unique tagged nucleic acid molecules, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags and the nucleic acid tags comprise nucleotide sequences that are not known before the tagged nucleic acid molecules are generated. For example, Guardant aligns sequencing reads to a reference genome and uses consensus representations consensus representations of the sequence of the original unique DNA molecule by using inferring the molecular barcode sequence. As another example, Guardant uses random barcode sequences. '822 patent, 3:25-35 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step. In some embodiments, the barcode is a polynucleotide, which may further comprise random sequence or a fixed or semi-random set of oligonucleotides that in combination with the diversity of molecules sequenced from a select region enables identification of unique molecules and be at least a 3, 5, 10, 15, 20, 25, 30, 40, 45, or 50mer base pairs in length.") Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	(Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.326

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

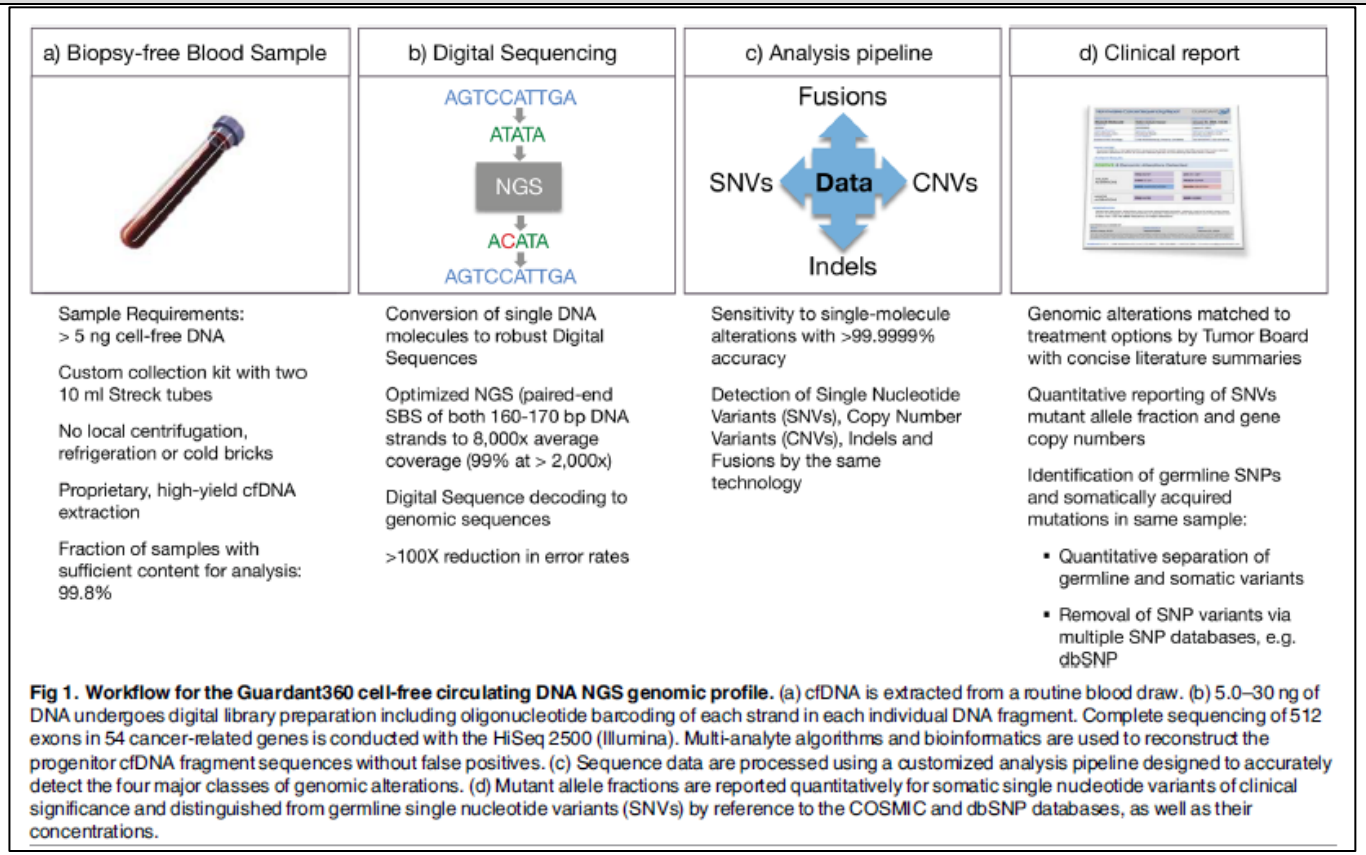


Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

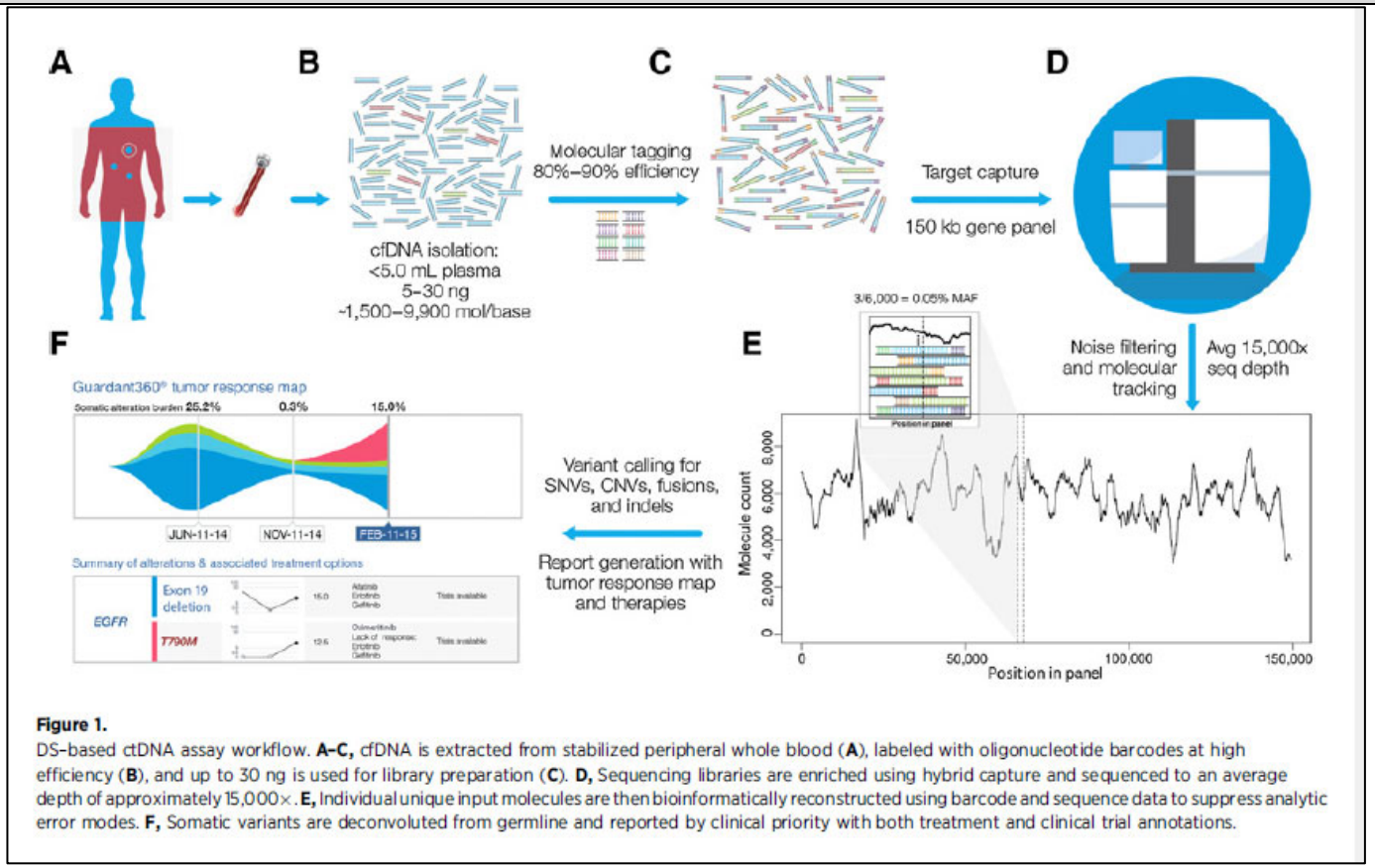


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 34

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags comprise random nucleotide sequences.	Guardant generates unique tagged nucleic acid molecules, wherein the nucleic acid tags comprise a sufficiently large number of distinct nucleic acid tags and the nucleic acid tags comprise random nucleotide sequences. For example, Guardant uses a random set of oligonucleotides as barcode sequences. On information and belief, in performing its Guardant360 tests, Guardant also uses tags provided by Integrated DNA Technologies, which provides customers with indexes with a variety of characteristics, including an option to create custom tags with random nucleotide sequences. '822 patent, 3:25-35 ("Further, the methods of the disclosure comprise attaching one or more barcodes to the extracellular polynucleotides or fragments thereof prior to any amplification or enrichment step. In some embodiments, the barcode is a polynucleotide, which may further comprise random sequence or a fixed or semi-random set of oligonucleotides that in combination with the diversity of molecules sequenced from a select region enables identification of unique molecules and be at least a 3, 5, 10, 15, 20, 25, 30, 40, 45, or 50mer base pairs in length.") Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")

GUARDANT HEALTH EX.1020.332

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

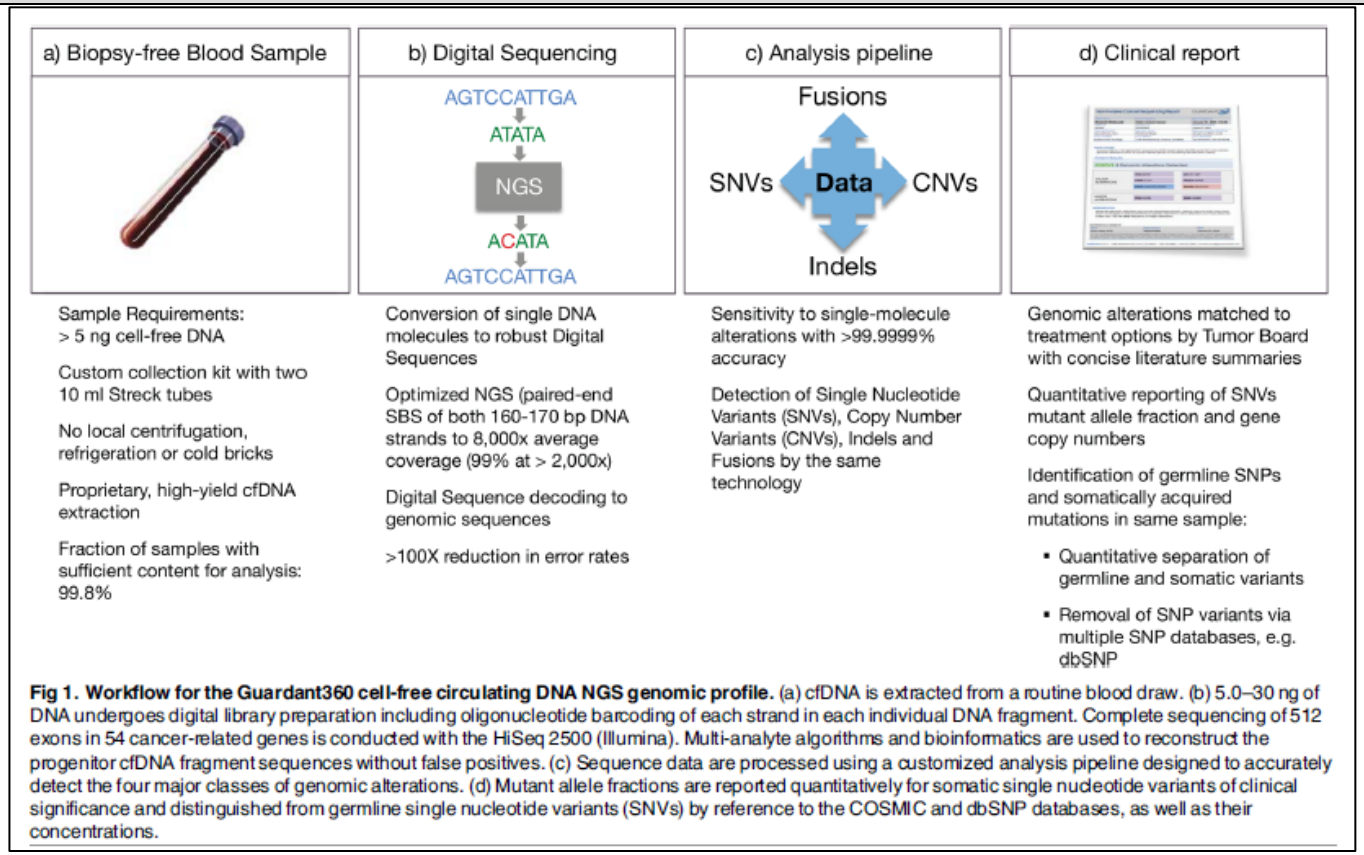
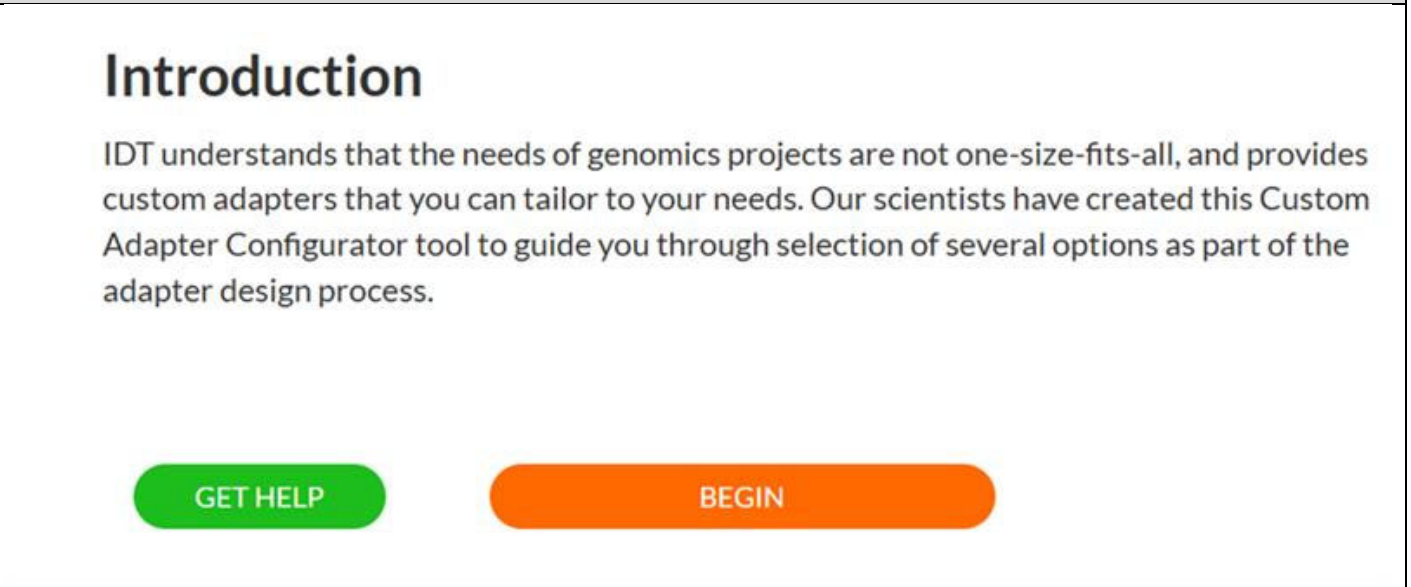


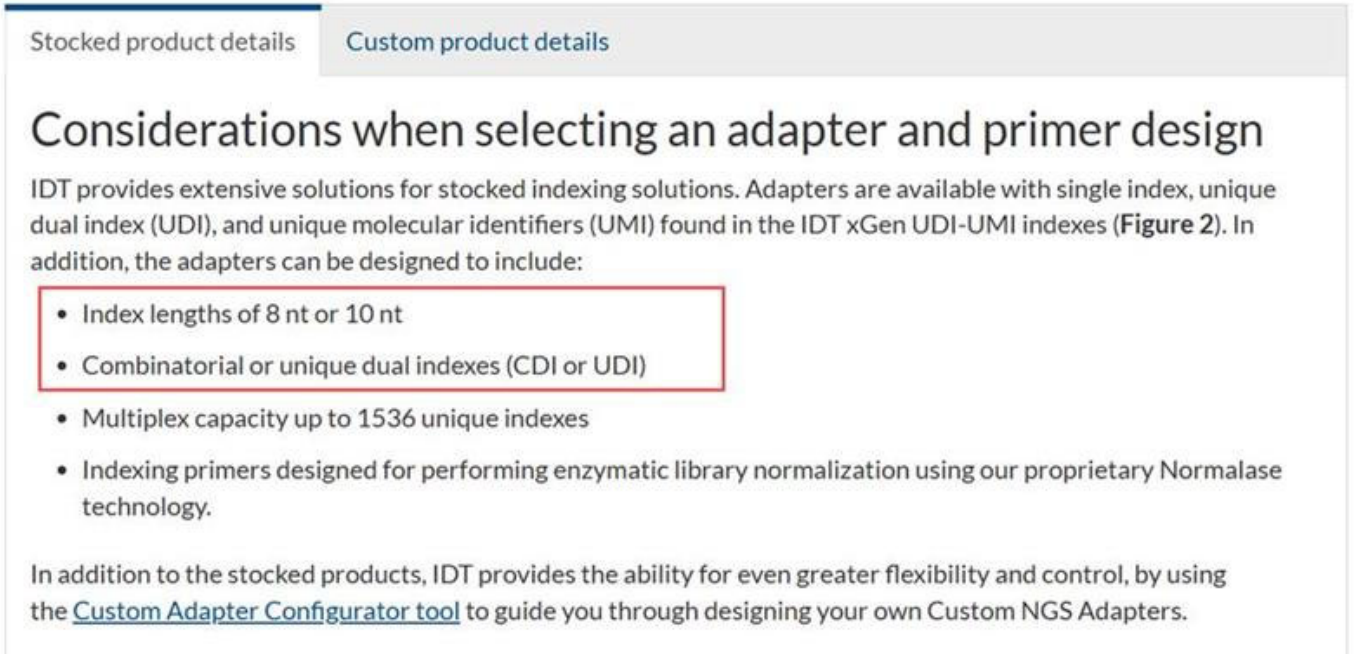
Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile. (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations.

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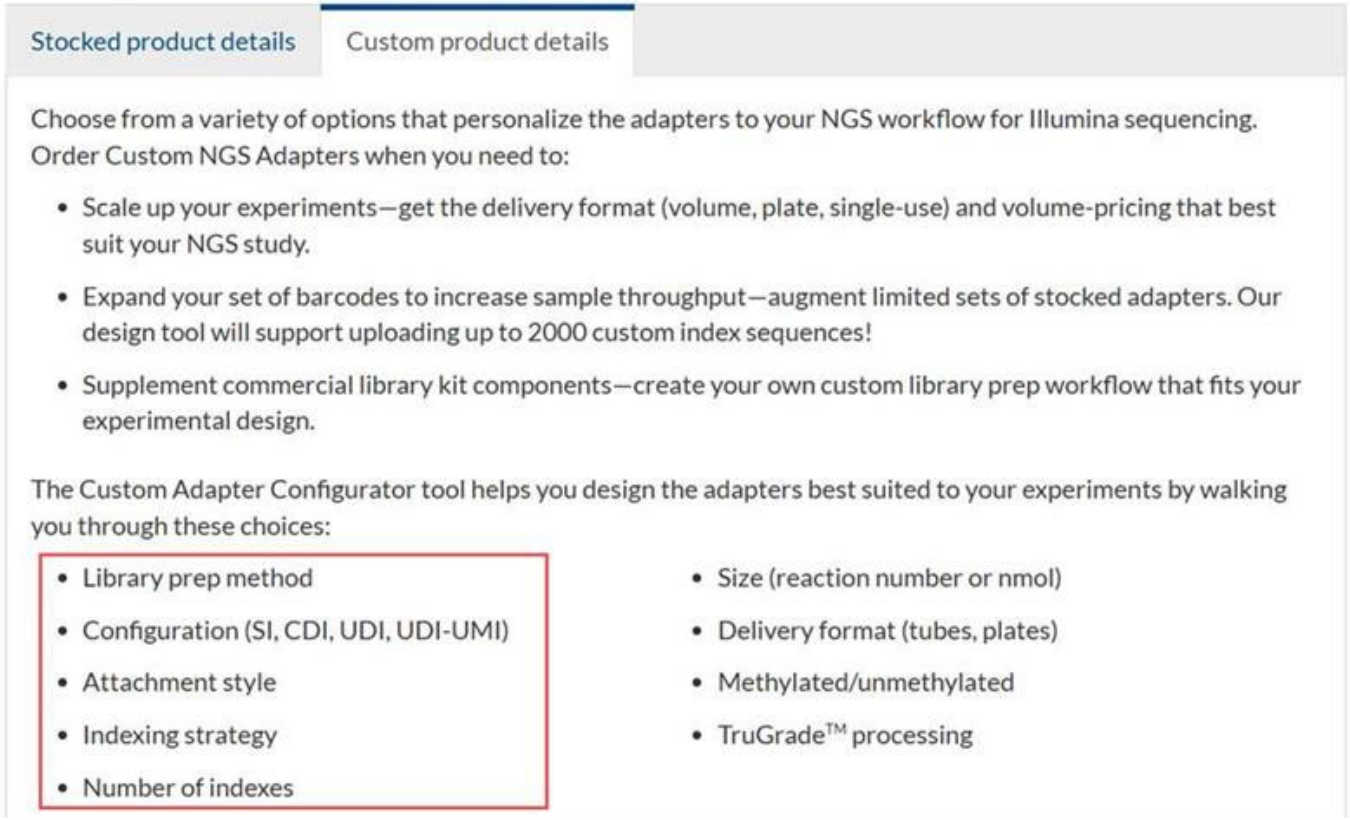
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Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Custom Adapter Webpage.

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
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GUARDANT HEALTH EX.1020.336

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	 IDT Illumina Adapters and Primers Webpage. Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed

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GUARDANT HEALTH EX.1020.338

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

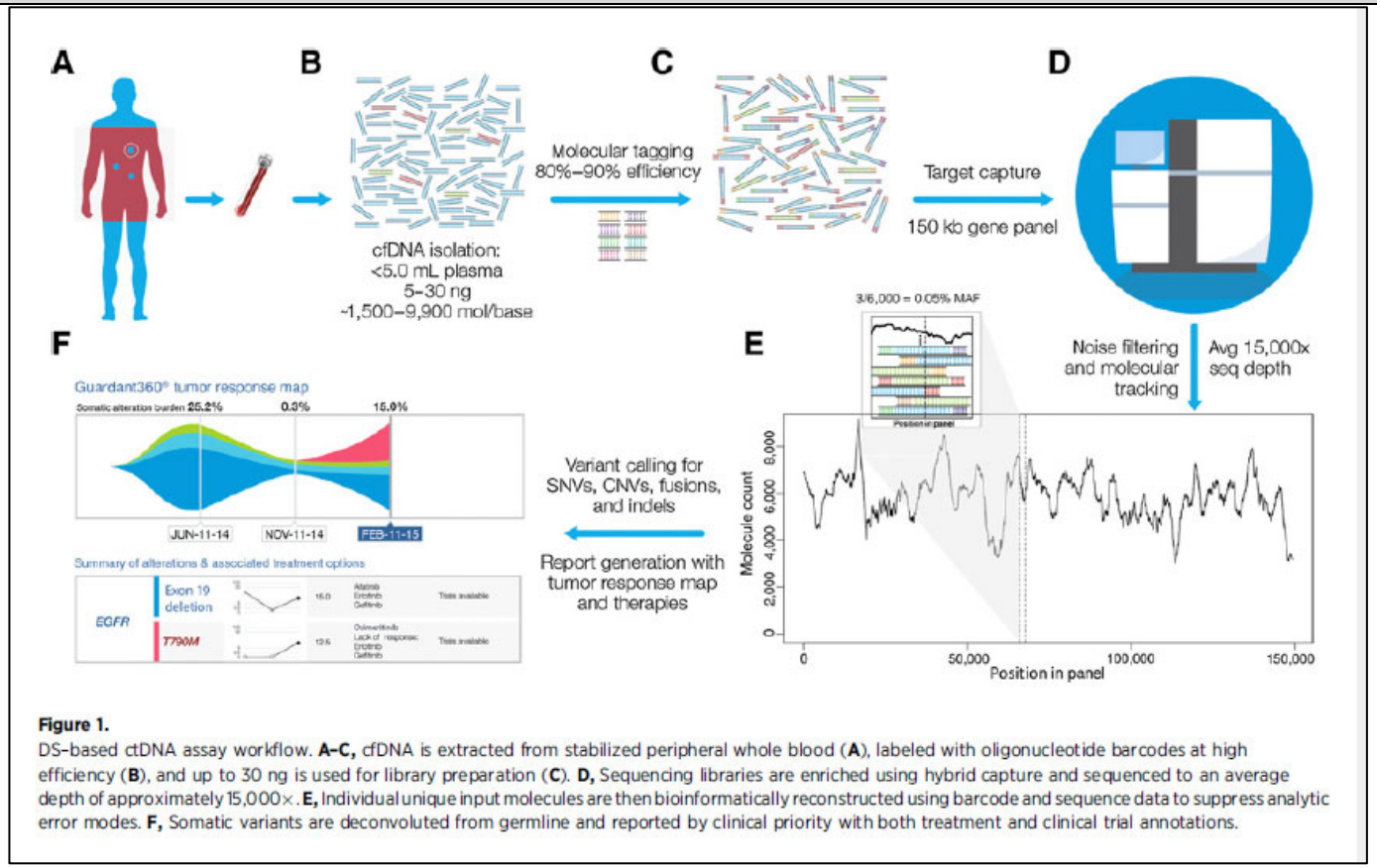


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

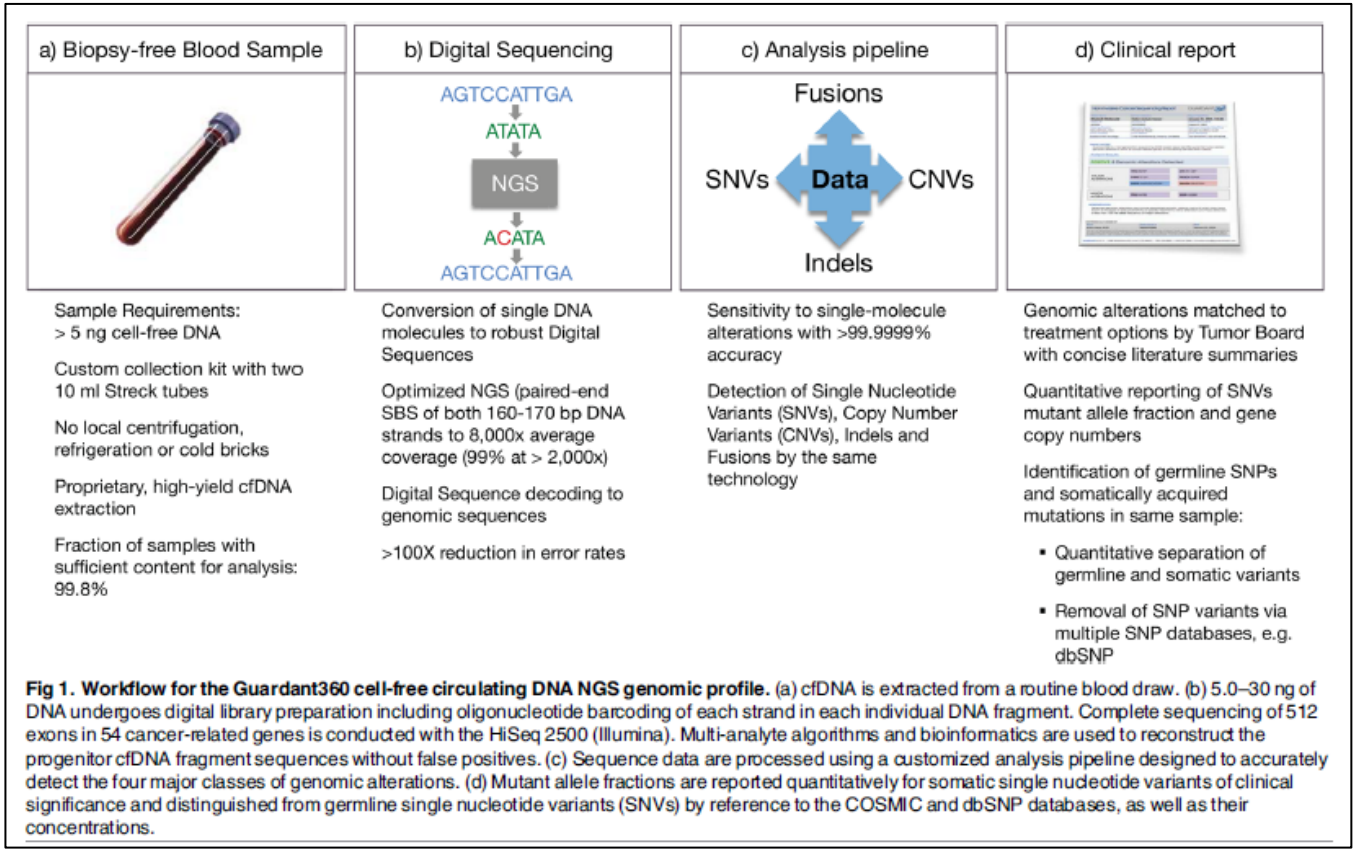
Odegaard et al. at 3542 (Figure 1).

'822 patent, claim 1 ("[C]onverting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Guardant360 CDx Summary of Safety and Effectiveness Data – January 27, 2023 at 7 ("During library preparation, cfDNA fragment ends are repaired and library adapters containing inline barcodes are attached using blunt-end ligation.") Guardant360 CDx Technical Information at 5 ("Plasma is isolated via centrifugation and cfDNA is extracted from plasma. cfDNA, 5 to 30 ng, is then used to prepare sequencing libraries which are enriched by hybridization capture.").)

ASSERTED CLAIM 35

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 22, wherein the nucleic acid tags are substantially unique nucleic acid tags.	Guardant uses nucleic acid tags that are substantially unique nucleic acid tags. For example, Guardant uses nonredundant molecular barcodes to individually tag each unique cfDNA molecule. Nakamura et al. at "Methods" ("5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	reconstruction to eliminate nearly all false positives. The test detects single nucleotide variants in all 54 genes and copy number amplifications in EGFR, ERBB2 (codes for HER2) and MET [23].")  **Fig 1. Workflow for the Guardant360 cell-free circulating DNA NGS genomic profile.** (a) cfDNA is extracted from a routine blood draw. (b) 5.0–30 ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment. Complete sequencing of 512 exons in 54 cancer-related genes is conducted with the HiSeq 2500 (Illumina). Multi-analyte algorithms and bioinformatics are used to reconstruct the progenitor cfDNA fragment sequences without false positives. (c) Sequence data are processed using a customized analysis pipeline designed to accurately detect the four major classes of genomic alterations. (d) Mutant allele fractions are reported quantitatively for somatic single nucleotide variants of clinical significance and distinguished from germline single nucleotide variants (SNVs) by reference to the COSMIC and dbSNP databases, as well as their concentrations. Lanman et al. at 5 (Figure 1 explaining "5.0–30ng of DNA undergoes digital library preparation including oligonucleotide barcoding of each strand in each individual DNA fragment.")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	Lanman et al. at 18 ("Isolated cfDNA fragments are subsequently converted to digital sequence libraries. Briefly, in the digital sequencing workflow, non-unique oligonucleotide heptamer barcodes are ligated to each half of individual double-stranded cfDNA. This generates a duplex library whereby each single-stranded half of the original double-stranded input cfDNA sample is separately encoded with said oligonucleotides.") Lanman et al. at 19 ("Post-sequencing, the digital decoding process comprises analysis of both strands of each unique cfDNA molecule to greatly increase the accuracy ("transmission characteristics") of each individual base call.") Lanman et al. at 19 ("Each strand of a double-stranded cfDNA molecule is individually tagged, allowing custom software to compare the two complementary strands to ascertain whether either has acquired an erroneous variant due to a sequencing error, library preparation error, or DNA damage during sample processing.") Lanman et al. at 19 ("Software utilized includes CASAVA (version 1.8.4), the open source BWA-MEM aligner, and a custom read pile-up process that utilizes information encoded by digital-sequencing oligonucleotides to reconstruct the set of unique cfDNA molecules.") Lanman et al. at 19 ("Because digital sequencing enables tracking and quantification of all unique cfDNA fragments overlapping a given genomic site, PCR duplicates and artifacts of amplification during library preparation can easily be removed.") Lanman et al. at 19 ("Leveraging the ability of digital sequencing technology (DST) to absolutely quantify the number of unique DNA fragments in a sequenced sample, the copy number of a given gene in plasma may also be ascertained.") Odegaard et al. at 3540 ("Five to 30 ng of extracted cfDNA (66% of samples processed with 30 ng input) was labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries,

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
	which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.).") Odegaard et al. at 3540 ("All decision thresholds were determined using training cohorts, locked, and applied prospectively to all validation and clinical samples. As described previously (24), base call files generated by Illumina's RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of reads).") Odegaard et al. at 3540 ("Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions.") Odegaard et al. at 3542 ("Briefly, cfDNA is extracted from stabilized whole blood, labeled at high efficiency with nonrandom oligonucleotide adapters ('molecular barcodes'), and used to prepare sequencing libraires, which are then enriched using hybrid capture and sequenced (Fig. 1).")

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term

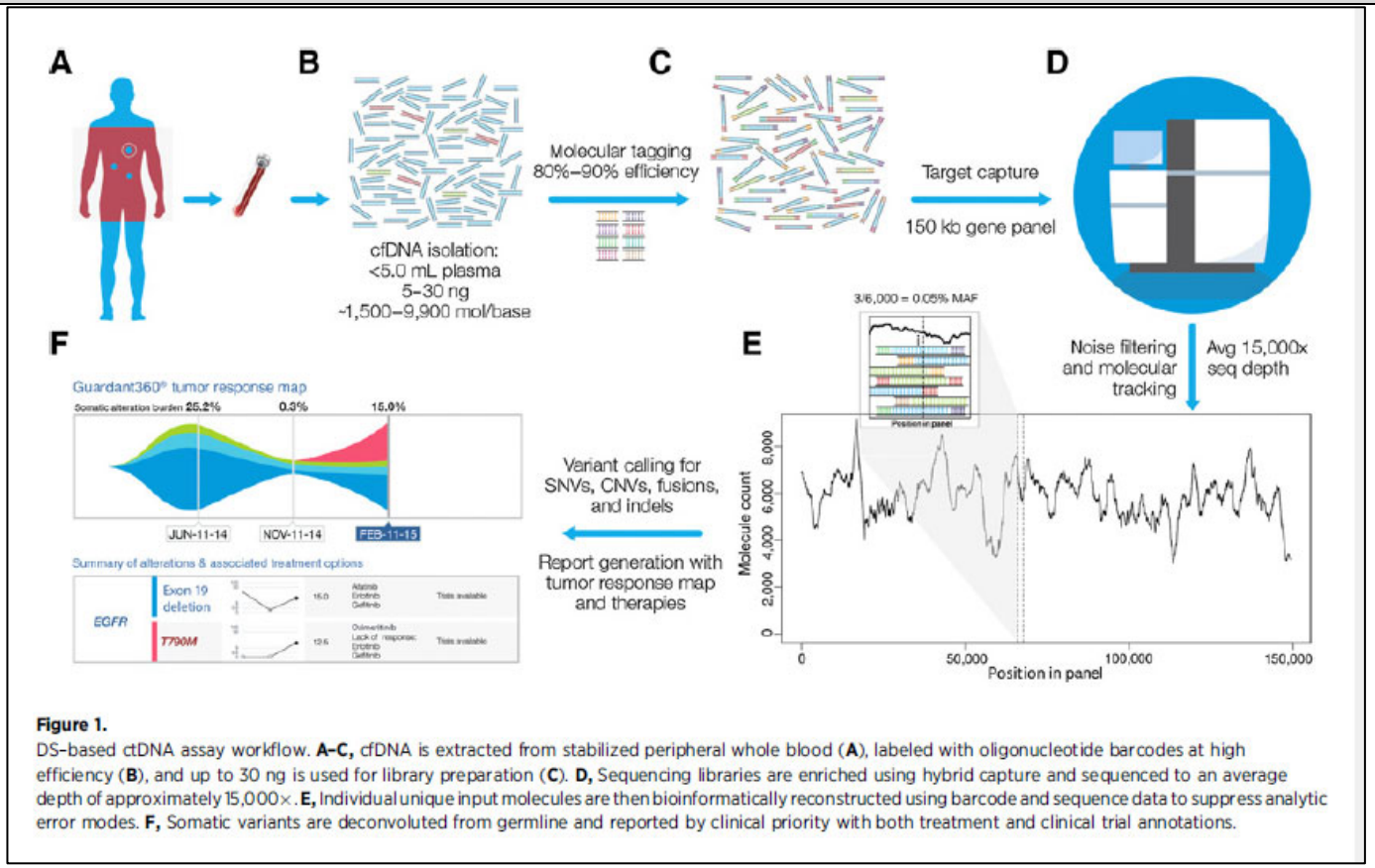


Figure 1.
DS-based ctDNA assay workflow. **A–C,** cfDNA is extracted from stabilized peripheral whole blood (**A**), labeled with oligonucleotide barcodes at high efficiency (**B**), and up to 30 ng is used for library preparation (**C**). **D,** Sequencing libraries are enriched using hybrid capture and sequenced to an average depth of approximately 15,000×. **E,** Individual unique input molecules are then bioinformatically reconstructed using barcode and sequence data to suppress analytic error modes. **F,** Somatic variants are deconvoluted from germline and reported by clinical priority with both treatment and clinical trial annotations.

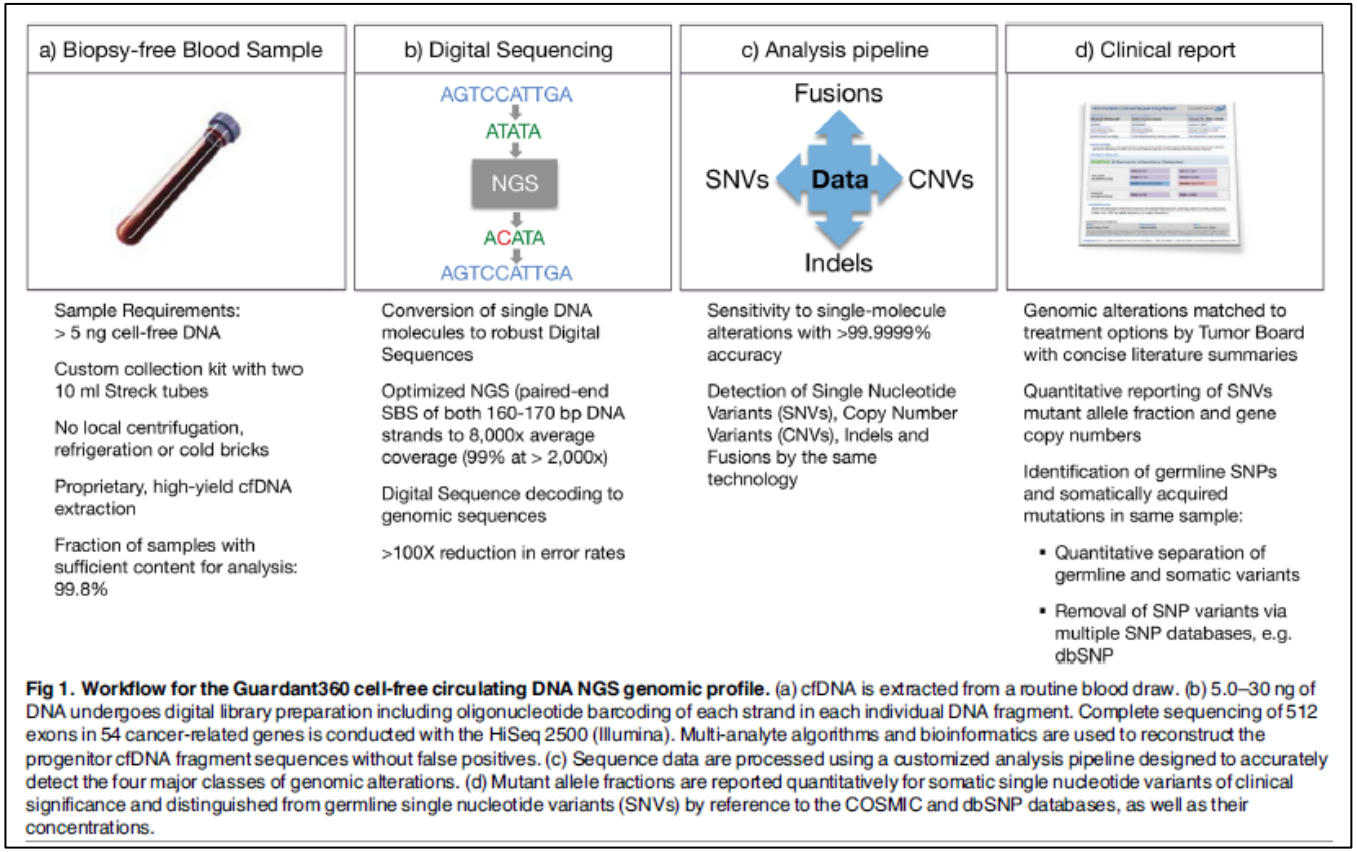
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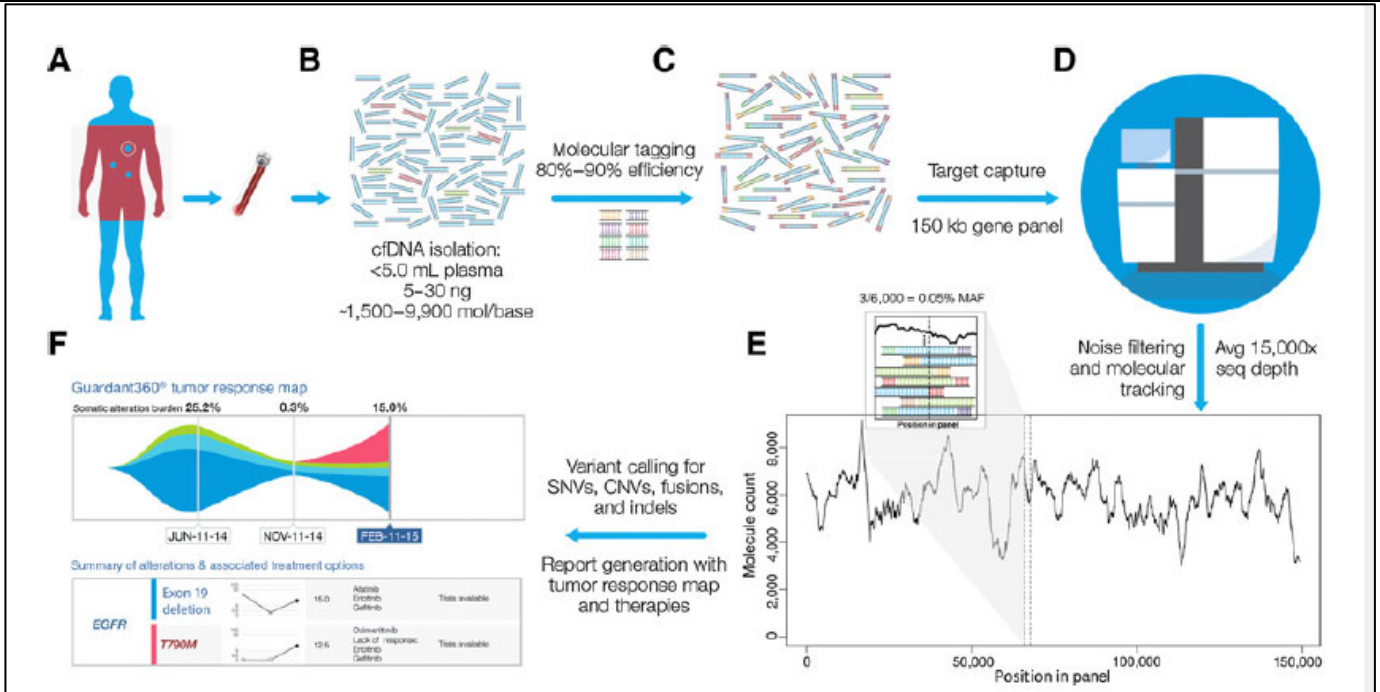
ASSERTED CLAIM 36

Claim Term	Evidence Showing Defendant's Practice of Each Claim Term
The method of claim 1, wherein the nucleic acid tags are substantially unique nucleic acid tags.	Guardant uses nucleic acid tags that are substantially unique nucleic acid tags. For example, Guardant uses nonredundant molecular barcodes to individually tag each unique cfDNA molecule. Nakamura et al. at "Methods" ("5−30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows−Wheeler aligner-MEM algorithm. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel.") Lanman et al. at 3 ("Digital sequencing (see Materials and Methods) employs pre-sequencing preparation of a digital library of individually tagged cfDNA molecules combined with post-sequencing bioinformatic

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CERTIFICATE OF SERVICE

I hereby certify that on July 11, 2025, true and correct copies of the foregoing document were caused to be served on the following counsel of record as indicated:

BY ELECTRONIC MAIL
Brian E. Farnan
Michael J. Farnan
Farnan LLP
919 N. Market Street, 12th Floor
Wilmington, DE 19801

BY ELECTRONIC MAIL
Edward R. Reines
Jones Day
1755 Embarcadero Road
Palo Alto, CA 94303

BY ELECTRONIC MAIL
Derek C. Walter
Jones Day
555 California Street, 26th Floor
San Francisco, CA 94104

/s/ Kelly E. Farnan
Kelly E. Farnan (#4395)