



Search Biocompare

Products

Articles

Multimedia

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)

Write a Review

Resources

Register

Sign In

[Life Science Articles](#) > [Editorial Articles](#) > Maximize Your NGS with Sequence Capture

Maximize Your NGS with Sequence Capture

November 06, 2012

Post

Share 0

Share

Email

For all that genome-sequencing costs have fallen, they're still relatively high – for many researchers, at least those with large sample sizes, prohibitively so.



This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)

2% of the genome that actually codes for protein is specifically read out.

And it isn't just small labs doing this work either. Even the 1000 Genomes Project Consortium, including sequencing facilities at BGI-Shenzhen, the Broad Institute, and the Wellcome Trust Sanger Institute, among others, takes advantage of sequence capture technologies. As they detail in a November 1 *Nature* paper on nearly 1,100 human genomes, "Primary data generated for each sample consist of low-coverage (average 5x) whole-genome and high-coverage (average 80x across a consensus target of 24Mb spanning more than 15,000 genes) exome sequence data, and high density SNP array information." [1]

There are two basic approaches to sequence capture: PCR-based and hybridization-based, the latter of which can be done either in solution or on the surface of an oligonucleotide microarray. In practice, says Baiju Parikh, marketing manager for sequencing solutions at Roche Applied Science, few researchers use the array-based method any more, and none of the major manufacturers still offers such tools off-the-shelf

Hybridization-based Tools

Solution-based capture tools are available from [Agilent Technologies](#), [Roche NimbleGen](#), [Illumina](#), and [Life Technologies](#), the former two of which were used in the 1000 Genomes Project Consortium paper. All are based on solution hybridization selection (SHS), in which a pool of biotinylated oligonucleotides is used to capture some subset of a sequencing library of interest. Following pull-down with streptavidin beads and washing, the hybridized fragments are released and ready to sequence.

Agilent's SureSelect technology is based on 120-mer RNA oligonucleotides. The company's SureSelect Human All Exon V4 exome-capture kits come in two varieties, an exon-only version capturing 51 Mb of genomic DNA, and an exon plus untranslated regions kit covering 71 Mb. The protocol is simple enough: shear DNA, ligate sequencing adaptors, PCR amplify, capture, elute, and amplify again.

Agilent also offers a SureSelect Methyl-Seq kit for specific capture of potentially methylated regions, such as promoters and CpG islands totally 80 Mb. Unlike the All Exon SureSelect kit, the Methyl-Seq kit skips the pre-capture PCR amplification step to preserve methylation of the genomic DNA. That way, following capture, the DNA can be bisulfite treated to identify methylated bases.

"We spent a lot of time optimizing the library preparation to work without PCR," says Olle Ericsson, Marketing Director for DNA sequencing applications, who adds that the company is unique in offering such a product. "The only other way to get single-base-pair methylation analysis is whole-genome sequencing, and that's very inefficient," he says.

Roche NimbleGen's solution-based SeqCap EZ products use 2.1 million biotinylated DNA oligonucleotides ranging from 55 to 105 bases in length to effect sequence capture.

"That's the big differentiator for us," says Parikh, of the product's high probe density. That density allows the company to design multiple redundant probes over each region, reducing the likelihood that mutations or SNPs will negatively impact capture efficiency. At the same time, Parikh adds, the probe density also gives the company design maneuverability to optimize and increase capture uniformity.

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)

According to Parikh, NimbleGen will send a proposed design within 24 hours, which the user can either accept or modify. Agilent's answer to NimbleDesign is SureDesign (which replaces eArray for SureSelect products); Life Technologies' TargetSeq Custom Enrichment Kits must be ordered by submitting a form and genome coordinates to the company directly.

PCR-based capture tools

Agilent and Life Technologies also offer PCR-based enrichment solutions. Life Technologies' version is called AmpliSeq, while Agilent's is HaloPlex. HaloPlex, says Ericsson, is intended mostly for clinical labs or small research labs using benchtop sequencers like Illumina's MiSeq or Life Technologies' Ion Torrent PGM.

In HaloPlex, a DNA sample is fragmented with restriction enzymes. A double-stranded universal probe flanked by single-stranded sequence-specific capture ends captures and circularizes desired targets, which are then amplified using universal primers. This design, says Ericsson, gives HaloPlex effectively infinite multiplexing capability. "You can do up to 250,000 PCRs in one single tube," he says.

Automating the process

For those doing more traditional PCR-based capture, [RainDance Technologies](#) and [Fluidigm](#) have devised automated solutions to simplify the process.

Fluidigm's Access Array System uses a microfluidic plate to set up and run up to 2,304 individual PCR reactions (48 x 48). The plate and accompanying hardware automate the process of mixing sample and primer for each of 48 samples and 48 primer sets.

According to Julian Walker, Senior Product Manager at Fluidigm, the system can set up and run those reactions in 4 hours, including just 20 minutes of hands-on time.

Each 48.48 microfluidic plate costs a few hundred dollars, Walker says, on top of the Fluidigm instrumentation itself. But, he says, the result is reproducibility and data uniformity, including greater than 85% of coverage within 5x of the mean, greater than 90% of reads that map to the genome (meaning "you're not producing a lot of garbage"), and more than 95% of reads mapping to the target (specificity).

RainDance Technologies' RDT 1000 system simplifies the process of PCR multiplexing by mixing template, buffer, reagents, and primer pairs in individual picoliter reaction droplets within an oil emulsion. As a result, researchers can perform thousands of individual reactions in a single tube. In late 2011, the company launched a high-throughput version of the platform, the ThunderStorm™ system, for up to 96 samples per run.

Intuitively, as sequencing prices continue to fall, it may seem as if the need for sequence capture tools will wane. For his part, Parikh doubts that will happen; instead, more and more researchers could end up turning to these tools to squeeze ever more data from every sequencer run, and to ease the load on computational pipelines.

"As the cost of sequencing drops and bioinformatics pipelines get more savvy, the need for sequence capture will actually grow," he says.

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)

References

[1] 1000 Genomes Project Consortium, "An integrated map of genetic variation from 1,092 human genomes," Nature, 491:56–65, 2012.

[2] M.J. Clark et al., "Performance comparison of exome DNA sequencing technologies," Nat Biotechnol, 29:908–12, 2011.

The image at the top of the page is from [Roche NimbleGen](#).

Related Products

KAPA HyperPrep Kit, PCR-free

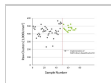
The KAPA Hyper Prep Kit is a versatile, streamlined solution for DNA library preparation

The novel one-tube chemistry, optimally formulated and ...

Library Quantification Kit - Illumina/Universal

KAPA Library Quantification Kits contain all the reagents needed for the accurate, reliable and reproducible qPCR-based quantification of NGS

...



KAPA Library Quantification Kits

KAPA Library Quantification Kits contain all the reagents needed for the accurate, reliable and reproducible qPCR-based quantification of NGS ...

Join the Discussion

Comments (0)

≡ best



Sign In

Subscribe to Newsletter

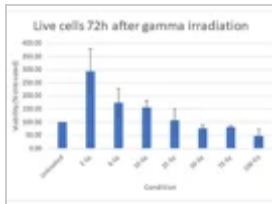
This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)[Sign up](#)

Product Review

Simple and Effective Tool for Quick Cell Counting



Lana Jagannathan

★★★★★ (4)

We use this cell counter for basic cell culture for a variety of cells and cell lines, including cancer cell lines and human donor-derived PBMCs. [Read More](#)

[More Product Reviews »](#)

Connect With Us



Biocompare is the leading resource for up-to-date product information, product reviews, and new technologies for life scientists.

About Biocompare

[About Biocompare](#)[Contact Us](#)[Meet the Editors](#)

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)[Disclaimer & Terms of Use](#)

Advertise with Us

[Frequently Asked Questions](#)[Request Advertising Information](#)[Editorial Calendar](#)[View Online Media Kit](#)[View Ad Specifications](#)

Specialized Search Tools

[Antibodies](#)[ELISA Kits](#)[Cellular Assays](#)[Enzymes](#)[Chemicals & Reagents](#)[Chromatography](#)[Cell Isolation](#)[Nucleic Acid Purification](#)[Pipettors](#)[Transfection Reagents](#)[Microplate Readers](#)[Next Gen Sequencers](#)[PCR Machines](#)[Laboratory Incubators](#)[Services](#)

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

Get Quote	Compare
-----------	---------

- Cells & Microorganisms
- Recombinant Proteins
- Inhibitors
- Protein Purification Kits
- Cell Culture Media
- NGS Library Prep
- Microscopes & Imagers
- Blood & Tissue
- Liquid Handling
- Flow Cytometers
- Spectrophotometers
- Laboratory Shakers
- Antibody Production Services

Site Map

- Products
- Articles
- Videos
- Promotions
- News
- Events
- Submit A Review
- Subscribe eNewsletters

A publication of

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)

Stay up to date on news and special offers related to these products. Free subscription!

[Get Quote](#)[Compare](#)

This website utilizes technologies such as cookies to enable essential site functionality, as well as for analytics, personalization, and targeted advertising purposes. To learn more, view the following link: [Privacy Policy](#)