IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

PARSE BIOSCIENCES, INC.

Petitioner,

v.

10X GENOMICS, INC,

Patent Owner

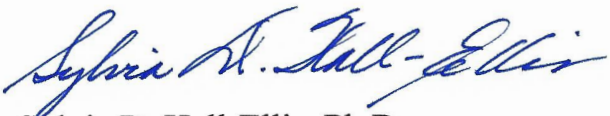
IPR2023-XXXXX

U.S. PATENT NO. 10,150,995

DECLARATION OF SYLVIA D. HALL-ELLIS, Ph.D.

Dated: April 19, 2023

By:



Sylvia D. Hall-Ellis, Ph.D.

I, Sylvia D. Hall-Ellis, declare as follows:

I. INTRODUCTION

1. My name is Sylvia D. Hall-Ellis. I have been retained as an expert by Parse Biosciences, Inc., the Petitioner, in the above-captioned *inter parte* review.

2. I have written this Declaration at the request of the Petitioner to provide my expert opinion regarding the authenticity and public availability of three publications. My Declaration sets forth my opinions in detail and provides the basis for my opinions regarding the authenticity and public availability of these publications. If called to testify in the above-captioned proceeding, I will testify regarding the opinions and bases set forth below.

3. I reserve the right to supplement or amend my opinions, and bases for them, in response to any additional evidence, testimony, discovery, argument, and/or other additional information that may be provided to me after the date of this Declaration.

4. I am being compensated for my time spent working on this matter at my normal consulting rate of $350 per hour, plus reimbursement for any additional reasonable expenses. My compensation is not in any way tied to the content of this Declaration, the substance of my opinions, or the outcome of this proceeding. I have no other interests in this proceeding or with any of the parties.

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5. All the materials that I considered and relied upon are discussed explicitly in this Declaration.

II. QUALIFICATIONS

6. I am currently an Adjunct Professor in the School of Information at San José State University in San José, California. I obtained a Master of Library Science from the University of North Texas in 1972 and a Ph.D. in Library and Information Science from the University of Pittsburgh in 1985. Over the last fifty years, I have held various positions in the field of library and information resources. I was first employed as a librarian in 1966 and have been involved in the field of library sciences since, holding numerous positions.

7. I am a member of the American Library Association (ALA) and its Association for Library Collections & Technical Services (ALCTS) Division, and I served on the Committee on Cataloging: Resource and Description (which wrote the new cataloging rules) and as the chair of the Committee for Education and Training of Catalogers and the Competencies and Education for a Career in Cataloging Interest Group. I also served as the founding Chair of the ALCTS Division's Task Force on Competencies and Education for a Career in Cataloging. Additionally, I have served as the Chair for the ALA Office of Diversity's Committee on Diversity, as a member of the REFORMA National Board of Directors, as a member of the Editorial Board for the ALCTS premier cataloging

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journal, *Library Resources and Technical Services*, and as a Co-Chair of the Membership Committee for the Library Research Round Table (LRRT) of the American Library Association. Currently I serve as a member of the LRRT Nominating Committee.

8. I have also given over one hundred presentations in the field, including several on library cataloging systems and Machine-Readable Cataloging ("MARC") standards. My current research interests include library cataloging systems, metadata, and organization of electronic resources.

9. I have been deposed twenty-seven times: (1) *Symantec Corp. v. Finjan, Inc.*, Petition for *Inter Partes Review* of U.S. Patent No. 7,613,926, May 26, 2016, on behalf of Symantec Corp.; (2) *Symantec Corp. v. Finjan, Inc.*, 14-cv-299-HSG (N.D. Cal.), on behalf of Symantec Corp., September 14, 2017; (3) one deposition for ten matters: *Intellectual Ventures I LLC v. AT&T Mobility LLC*; AT&T Mobility II LLC, New Cingular Wireless Services, Inc., SBC Internet Services, Inc., Wayport, Inc., and Cricket Wireless LLC, C.A. No. 12-193 (LPS); *Intellectual Ventures II LLC v. AT&T Mobility LLC*; AT&T Mobility II LLC, New Cingular Wireless Services, Inc., SBC Internet Services, Inc., Wayport, Inc., and Cricket Wireless LLC, C.A. No. 13-1631 (LPS); *Intellectual Ventures I LLC v. T-Mobile USA, Inc. and T-Mobile US, Inc.*, C.A. No. 13-1632 (LPS); *Intellectual Ventures II LLC v. T-Mobile USA, Inc. and T-Mobile US, Inc.*, C.A.

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No. 13-1633 (LPS); *Intellectual Ventures I LLC, v. Nextel Operations, Inc., Sprint Spectrum L.P., Boost Mobile, LLC and Virgin Mobile USA, L.P.*, C.A. No. 13-1634 (LPS); *Intellectual Ventures II LLC v. Nextel Operations, Inc., Sprint Spectrum L.P., Boost Mobile, LLC and Virgin Mobile USA, L.P.*, C.A. No. 13-1635 (LPS); *Intellectual Ventures I LLC, v. United States Cellular Corporation*, C.A. No. 13-1636 (LPS); *Intellectual Ventures I LLC v. United States Cellular Corporation*, C.A. No. 13-1637 (LPS); *Intellectual Ventures II LLC v. AT&T Mobility LLC, AT&T Mobility II LLC, New Cingular Wireless Services, Inc.*, C.A. No. 15-799 (LPS); *Intellectual Ventures I LLC v. T-Mobile USA, Inc. and T-Mobile US, Inc.*, C.A. No. 15-800 (LPS), on behalf of AT&T Mobility LLC; AT&T Mobility II LLC, Boost Mobile, LLC Cricket Wireless LLC, Nextel Operations, Inc., New Cingular Wireless Services, Inc., SBC Internet Services, Inc., Sprint Spectrum L.P., T-Mobile USA, Inc., T-Mobile US, Inc., United States Cellular Corporation Virgin Mobile USA, L.P., and Wayport, Inc., November 15, 2016; (4) *Hitachi Maxell, LTD., v. Top Victory Electronics (Taiwan) Co. Ltd., et al.*, 2:14-cv-1121 JRG-RSP (E.D. Texas), on behalf of Top Victory Electronics (Taiwan) Co. LTD, *et. al.*, January 20, 2016; (5) *Sprint Spectrum, L.P. v. General Access Solutions, Ltd*., Petition for *Inter Partes Review* of U.S. Patent No. 7,173,916, on behalf of Sprint Spectrum L.P., July 13, 2018; (6) *Nichia Corporation v. Vizio, Inc*., 8:16-cv-00545; on behalf of Vizio, Inc., October 12, 2018; (7) *Intellectual Ventures I*

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LLC, v. T-Mobile USA, Inc., T-Mobile US, Inc., Ericsson Inc., and Telefonaktiebolaget LM Ericsson, 2:17-cv-00557 (JRG), on behalf of T-Mobile USA, Inc., T-Mobile US, Inc., Ericsson Inc., and Telefonaktiebolaget LM Ericsson, October 19, 2018; (8) Pfizer, Inc. v. Biogen, Inc., Petition for *Inter Partes Review* of U.S. Patent No. 8,821,873, on behalf of Pfizer, November 3, 2018; (9) Finjan, Inc. v. ESET, LLC and ESET SPOL. S.R.O., 3:17-cv-00183-CAB-BGS, on behalf of ESET, January 15, 2019; (10) *Finjan, Inc. v. Cisco Systems, Inc.*, 5:17-cv-00072-BLF-SVK, on behalf of Cisco Systems, Inc., September 6, 2019; (11) *Facebook, Inc., Instagram, LLC and Whatsapp Inc. v. Blackberry Limited*, Petition for *Inter Partes Review* of U.S. Patent No. 9,349,120 B2, on behalf of Facebook, Inc., Instagram, LLC and Whatsapp Inc. December 20, 2019; (12) *3Shape A/S and 3Shape Inc. v. Align Technology, Inc.*, Petition for *Inter Partes Review* of U.S. Patent No. 7,156,661, IPR 2020-00222 and IPR 2020-00223, August 10, 2020, on behalf of 3Shape A/S and 3Shape Inc.; (13) *Finjan Inc. v. Rapid7, Inc. and Rapid7 LLC*, Northern District of Delaware; 1:18-cv-01519-MN, September 15, 2020; (14) *VLSI Technology LLC v. Intel Corporation*, Western District of Texas, 6:19-cv-00254, 6:19-cv-00255, 6:19-cv-00256, on behalf of Intel Corporation, September 23, 2020; (15) *Finjan Inc. v. Sonicwall, Inc.*, Northern District of California, 5:17-cv-04467-BLF-HRL, on behalf of Sonicwall, Inc., October 27, 2020; (16) *VLSI Technology, LLC v. Intel*

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Corporation, District of Delaware, 1:18-cv-00966-CFC-CJB, February 5, 2021, on behalf of the Intel Corporation; (17) *Unified Patents, LLC v. Good Kaisha IP Bridge 1*, Petition for *Inter Partes Review* of U. S. Patent 7,817,868, February 11, 2021, on behalf of Unified Patents; (18) *Finjan, Inc. v. Qualsys, Inc.*, Northern District of California, 4:18-cv-07229-YGR, March 1, 2021, on behalf of Qualsys, Inc.; (19) *Qualcomm, Inc. v. Monterey Research LLC*, Petition for *Inter Partes Review* of U. S. Patent 6,534,805, May 6, 2021, on behalf of Qualcomm, Inc.; (20) *Hulu, LLC v. Sound View Innovations, LLC*, Petition for *Inter Partes Review* of U. S. Patent 5,806,062, May 14, 2021, on behalf of Hulu, LLC; (21) *VLSI Technology LLC v. Intel Corporation*, Western District of Texas, 6:19-cv-00254, 6:19-cv-00255 and 6:19-cv-00256, August 3, 2021, on behalf of Intel Corporation; (22) *Liquidia Technologies, Inc. v. United Therapeutics Corporation*, Petition for *Inter Partes Review* of U. S. Patent 10,716,793 B2, October 20, 2021, on behalf of Liquidia Technologies, Inc.; (23) *EcoFactor, Inc. v. Google, Inc.*, Western District of Texas, 6:20-cv-00075 (ADA), 6:20-0078-ADA, and 6:20-cv-00080 ADA, October 27, 2021, on behalf of Google, Inc.; (24) *Liquidia Technologies, Inc. v. United Therapeutics Corporation*, Petition for *Inter Partes Review* of U. S. Patent 10,716,793 B2, March 11, 2022, on behalf of Liquidia Technologies, Inc.; (25) *Juniper Networks, Inc. v. Swarm Technology LLC*, Petition for *Inter*

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Partes Review of U. S. Patent 9,852,004, May 5, 2022, on behalf of Juniper Networks, Inc.; (26) *ParkerVision, Inc. v. Intel Corporation*, Western District of Texas, 6:20-cv-108 (ADA), October 26, 2022, on behalf of the Intel Corporation; and, (27) *CommScope v. TQ Delta*, Petition for *Inter Partes Review* of U. S. Patents 9,094,348 and 10,833,809, February 16, 2023, on behalf of CommScope.

10. My full curriculum vitae is attached as Appendix 1.

III. PRELIMINARIES

A. Scope of Declaration and Legal Standards

11. I am not an attorney and will not offer opinions on the law. I am, however, rendering my expert opinion on the authenticity of the documents referenced herein and on when and how each of these documents was disseminated or otherwise made available to the extent that persons interested and ordinarily skilled in the subject matter or art, exercising ordinary diligence, could have located the documents on or after May 23, 2013.

12. I am informed by counsel that a printed publication qualifies as publicly accessible as of the date it was disseminated or otherwise made available such that a person interested in and ordinarily skilled in the relevant subject matter could locate it through the exercise of ordinary diligence.

13. While I understand that the determination of public accessibility under

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the foregoing standard rests on a case-by-case analysis of the facts particular to an individual publication, I also understand that a printed publication is rendered "publicly accessible" if it is cataloged and indexed by a library such that a person interested in the relevant subject matter could locate it (*i.e.*, I understand that cataloging and indexing by a library is sufficient, though there are other ways that a printed publication may qualify as publicly accessible). One manner of sufficient indexing is indexing according to subject matter category. I understand that the cataloging and indexing by a single library of a single instance of a particular printed publication is sufficient, even if the single library is in a foreign country. I understand that, even if access to a library is restricted, a printed publication that has been cataloged and indexed therein is publicly accessible so long as a presumption is raised that the portion of the public concerned with the relevant subject matter would know of the printed publication. I also understand that the cataloging and indexing of information that would guide a person interested in the relevant subject matter to the printed publication, such as the cataloging and indexing of an abstract for the printed publication, is sufficient to render the printed publication publicly accessible.

14. I understand that routine business practices, such as general library cataloging and indexing practices, can be used to establish an approximate date on which a printed publication became publicly accessible.

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B. Persons of Ordinary Skill in the Art

15. I am told by counsel that the subject matter of this proceeding relates to methods of analyzing nucleic acid sequences.

16. I have been informed by counsel that a "person of ordinary skill in the art at the time of the invention" ("POSITA") is a hypothetical person who is presumed to be familiar with the relevant field and its literature at the time of the inventions. This hypothetical person is also a person of ordinary creativity, capable of understanding the scientific principles applicable to the pertinent field.

I was told by counsel a POSITA would have had (1) a Masters and/or Ph.D. in molecular biology, genetics, chemistry, engineering, or a related discipline and at least two years of post-Masters, postdoctoral, or industry experience or (2) a Bachelor of Science in such disciplines and at least five years of academic or industry experience (including any experimental work toward a graduate degree), relating to DNA sample preparation techniques (including electroporation and permeabilization), tagmentation, amplification methods, and DNA sequencing technologies (including next generation sequencing). I was also told that the ordinary skilled artisan in 2013 would have been familiar with and would have fully understood the following concepts and techniques:

- The format of DNA packaging in living cells.
- The dynamic nature of DNA packaging in living cells.

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- Next generation sequencing techniques, and methods of preparing nucleic acid for sequencing.
- The tagmentation process, and commercially available products for carrying out tagmentation.
- Methods of permeabilizing cells for nucleic acid analysis.
- Epigenetic analysis and techniques therefore.

17. I have been further informed by counsel that a person of ordinary skill in the art would have been familiar with and able to understand the information known in the art relating to these fields, including the information disclosed in the publications discussed in this Declaration.

18. It is my opinion that such a person would have been engaged in research, learning, study, and practice in the field, and formal instruction so that bibliographic resources relevant to his or her research would be familiar. After May 23, 2013, such a person would have had access to a vast array of long-established print resources in the field as well as to a rich set of online resources providing indexing information, abstracts, and full text services.

IV. LIBRARY PROFESSIONAL PRACTICES

19. In preparing this Declaration, I used authoritative databases, such as the OCLC bibliographic database, the Library of Congress Online Catalog, and the Library of Congress Subject Authorities, to confirm citation details of the various publications discussed. Unless I note otherwise below in reference to a specific

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serial publication, it is my expert opinion that this standard protocol was followed for the serial publications discussed below.

20. *Indexing*. A researcher may discover material relevant to his or her topic in a variety of ways. One common means of discovery is to search for relevant information in an index of periodicals and other publications. Having found relevant material, the researcher will then normally obtain it online, look for it in libraries, or purchase it from the publisher, a bookstore, a document delivery service, or other provider. Sometimes, the date of a document's public accessibility will involve both indexing and library date information. However, date information for indexing entries is often unavailable. This is especially true for online indices.

21. Indexing services use a wide variety of controlled vocabularies to provide subject access and other means of discovering the content of documents. The Library of Congress Subject Authorities includes standard forms of terms and cross references that are included in bibliographic records. Subject headings are terms that an individual seeking a document regardless of format can enter in the search bar of the online catalog. Subjects also connect an authenticated term (one included in the Library of Congress subject headings list) with related, broader, and narrower terms. The formats in which these access terms are presented vary from service to service.

22. Online indexing services commonly provide bibliographic

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information, abstracts, and full-text copies of the indexed publications, along with a list of the documents cited in the indexed publication. These services also often provide lists of publications that cite a given document. A citation of a document is evidence that the document was publicly available and in use by researchers no later than the publication date of the citing document. Prominent indexing services include *Scopus*, the *IEEE Xplore* database, the *ACM Digital Library*, *Google Scholar*, and the *Internet Archive*.

V. ONLINE LIBRARY CATALOGS

23. Learning to use an online library catalog begins in elementary school and continues through high school and post-secondary studies. Undergraduate students further develop their skills to use the online catalog at the academic institution where they are studying through information literacy instruction which is provided by professional reference and user experience librarians. The skills required to search, identify, and obtain the resources identified in this declaration are fundamental to using the online catalogs in libraries and information centers. Therefore, a POSITA who has attained the undergraduate and/or graduate educational levels described above (see paragraphs 17-18) possesses the searching and retrieval skills needed to search library catalogs.

24. Professional catalog and metadata librarians prepare and enhance MARC records to provide points of access for each item in the library collection.

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Using a set of internationally adopted guidelines, rules for the assignment of classification numbers, and the assignment of subject headings from standardized lists, taxonomies, folksonomies, locally developed lists. An information seeker (including POSITAs) can search the online library catalog in a number of ways to identify a specific item or resources on a defined topic.

25. The continued development of the software that supports integrated online library catalogs resulted in the potential to search by an individual creator or author, title proper or variant title, a series that contains the desired item, subjects, corporate bodies responsible in part for the creation of an item, or standard identification numbers associated with the item (e.g., International Standard Book Numbers (ISBN), International Standard Serial Numbers (ISSN), and Publisher's Numbers). Each of these data elements appears in a prescribed location within the MARC framework so that the software can parse, index, and display data elements resulting from a user-initiated search.

VI. LIBRARY CATALOGING PRACTICES AND MARC RECORDS

26. I am fully familiar with the library cataloging standard known as the MARC standard, which is an industry-wide standard method of storing and organizing library catalog information.[1] MARC was first developed in the 1960s

[1] The full text of the standard is available from the Library of Congress at http://www.loc.gov/marc/bibliographic/ (last visited March 2, 2023).

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by the Library of Congress. A MARC-compatible library is one that has a catalog consisting of individual MARC records for each of its items. Today, MARC is the primary communications protocol for the transfer and storage of bibliographic metadata in libraries.[2]

27. MARC is a framework into which descriptive bibliographic data are transcribed to interact with the software in online library catalogs to provide access to books, journals, and other resources in the collection. The bibliographic data provide points of access and can be searched by a POSITA to identify and obtain resources in the library collection. An information seeker (or POSITA) can search a local online library catalog or the holdings of a group of libraries in a state or region or in the global catalog WorldCat.

[2] Almost every major library in the world is MARC-compatible. *See, e.g.*, *MARC Frequently Asked Questions (FAQ)*, Library of Congress, https://www.loc.gov/marc/faq.html (last visited March 2, 2023) ("MARC is the acronym for Machine-Readable Cataloging. It defines a data format that emerged from a Library of Congress-led initiative that began nearly fifty years ago. It provides the mechanism by which computers exchange, use, and interpret bibliographic information, and its data elements make up the foundation of most library catalogs used today."). MARC is the ANSI/NISO Z39.2-1994 (reaffirmed 2016) standard for Information Interchange Format.

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28. MARC records are not designed for public viewing. Although a significant number of libraries provide access to the MARC version of a bibliographic record, the public display is designed to show information in a succinct manner that is quickly understood and useful to the information seeker. Libraries determine the default search for the online catalog to make the entry of search terms efficient and result in a successful search. Information seekers can enter a keyword, title, author, or standard number for the item. Libraries may also provide a search capability called "Summon" that allows the information seeker to enter known information about the item to conduct a search.

29. Since at least the early 1970s and continuing to the present day, MARC has been the primary communications protocol for the transfer and storage of bibliographic metadata in libraries.[3] As explained by the Library of Congress:

> You could devise your own method of organizing the bibliographic information, but you would be isolating your library, limiting its options, and creating much more

[3] A complete history of the development of MARC can be found in *MARC: Its History and Implications* by Henrietta D. Avram (Washington, DC: Library of Congress, 1975) and available online from the Hathi Trust (https://babel.hathitrust.org/cgi/pt?id=mdp.39015034388556;view=1up;seq=1; last visited March 2, 2023).

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> work for yourself. Using the MARC standard prevents duplication of work and allows libraries to better share bibliographic resources. Choosing to use MARC enables libraries to acquire cataloging data that is predictable and reliable. If a library were to develop a "home-grown" system that did not use MARC records, it would not be taking advantage of an industry-wide standard whose primary purpose is to foster communication of information.
>
> Using the MARC standard also enables libraries to make use of commercially available library automation systems to manage library operations. Many systems are available for libraries of all sizes and are designed to work with the MARC format. Systems are maintained and improved by the vendor so that libraries can benefit from the latest advances in computer technology. The MARC standard also allows libraries to replace one system with another with the assurance that their data will still be compatible.

Why Is a MARC Record Necessary? LIBRARY OF CONGRESS.[4]

30. Thus, almost every major library in the world is MARC-compatible. *See, e.g.*, *MARC Frequently Asked Questions (FAQ)*, LIBRARY OF CONGRESS.[5] ("MARC is the acronym for MAchine-Readable Cataloging. It defines a data

[4] http://www.loc.gov/marc/umb/um01to06.html#part2

[5] https://www.loc.gov/marc/faq.html

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format that emerged from a Library of Congress-led initiative that began fifty years ago. It provides the mechanism by which computers exchange, use, and interpret bibliographic information, and its data elements make up the foundation of most library catalogs used today."). MARC is the ANSI/NISO Z39.2-1994 standard (reaffirmed in 2016) for Information Interchange Format. The full text of the standard is available from the Library of Congress.[6]

31. Examining the MARC records for a specific item reveals the comprehensive data transcribed about a particular item at the time that cataloging and classification occurred. In addition to the creator, title, subjects, and standard numbers, additional information may provide additional and relevant data depending on the type of resource. Understanding the full extent of bibliographic data for an item and the points of access associated with it provide essential information that can be used to determine the indexing and public availability for documents described in this declaration.

32. A MARC record comprises several fields, each of which contains specific data about the work. Each field is identified by a standardized, unique, three-digit code corresponding to the type of data that follow. For example, a work's title is recorded in Field 245; the primary author of the work is transcribed in Field 100; an item's International Standard Book Number ("ISBN") consisting

[6] http://www.loc.gov/marc/bibliographic/

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of ten or thirteen digits is transcribed in Field 020; an item's International Standard Serial Number ("ISSN") is transcribed in Field 022; the Library of Congress classification notation is recorded in Field 050; and the publication date is recorded in Field 260 under the subfield "c." If a work is a periodical, then its publication frequency is recorded in Field 310, and the publication dates (e.g., the first and last publication) are recorded in Field 362, which is also referred to as the enumeration/chronology field.[7]

33. The library that created the record is recorded in Field 040 in subfield "a" with a unique library code. When viewing the MARC record online via Online Computer Library Center's ("OCLC") bibliographic database, hovering over this code with the mouse reveals the full name of the library. I used this method of "mousing over" the library codes in the OCLC database to identify the originating library for the MARC records discussed in this Declaration. Where this "mouse over" option was not available, I consulted the Directory of OCLC Libraries to identify the institution that created the MARC record.[8]

34. MARC records also include several fields that include subject matter classification information. An overview of MARC record fields is available

[7] http://www.loc.gov/marc/bibliographic/bd3xx.html

[8] https://www.oclc.org/en/contacts/libraries.html

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through the Library of Congress.[9] For example, 6XX fields are termed "Subject Access Fields."[10] Among these, for example, is the 650 field; this is the "Subject Added Entry – Topical Term" field.[11] The 650 field is a "[s]ubject added entry in which the entry element is a topical term." These entries "are assigned to a bibliographic record to provide access according to generally accepted thesaurus-building rules (e.g., *Library of Congress Subject Headings* (LCSH), *Medical Subject Headings* (MeSH))." *Id.* Further, MARC records include call numbers, which themselves include a classification number. For example, the 050 field is the "Library of Congress Call Number."[12] A defined portion of the Library of Congress Call (LCC) Number is the classification number, and "source of the classification number is *Library of Congress Classification* and the *LC Classification-Additions and Changes*." Thus, included in the 050 field is a subject matter classification. Further, the 082 field is the "Dewey Decimal Call Number."[13] A defined portion of the Dewey Decimal Call (DDC) Number is the classification number, and "source of the classification number is the *Dewey Decimal*

[9] http://www.loc.gov/marc/bibliographic/

[10] http://www.loc.gov/marc/bibliographic/bd6xx.html

[11] http://www.loc.gov/marc/bibliographic/bd650.html

[12] http://www.loc.gov/marc/bibliographic/bd050.html

[13] http://www.loc.gov/marc/bibliographic/bd082.html

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Classification and Relative Index." Thus, included in the 082 field is a subject matter classification. Each item in a library has a single classification number. A library selects a classification scheme (*e.g.*, the Library of Congress classification scheme just described or a similar scheme such as the Dewey Decimal classification scheme) and uses it consistently. When the Library of Congress assigns the LCC classification number, it appears as part of the 050 field. When the Library of Congress assigns the DDC classification number, it appears as part of the 082 field. If a local library assigns the classification number, it appears in a 090 field. In either scenario, the MARC record includes a classification number that represents a subject matter classification.

35. The OCLC was created "to establish, maintain and operate a computerized library network and to promote the evolution of library use, of libraries themselves, and of librarianship, and to provide processes and products for the benefit of library users and libraries, including such objectives as increasing availability of library resources to individual library patrons and reducing the rate of rise of library per-unit costs, all for the fundamental public purpose of furthering ease of access to and use of the ever-expanding body of worldwide scientific,

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literary and educational knowledge and information."[14] Among other services, OCLC and its members are responsible for maintaining the WorldCat database (http://www.worldcat.org/), used by independent and institutional libraries throughout the world.

36. OCLC also provides its members online access to MARC records through its OCLC bibliographic database. When an OCLC member institution acquires a work, it creates a MARC record for this work in its computer catalog system in the ordinary course of its business. MARC records created at the Library of Congress are tape-loaded into the OCLC database through a subscription to MARC Distribution Services daily or weekly. Once the MARC record is created by a cataloger at an OCLC member institution or is tape-loaded from the Library of Congress, the MARC record is then made available to any other OCLC members online, and therefore made available to the public. Accordingly, once the MARC record is created by a cataloger at an OCLC member institution or is tape-loaded from the Library of Congress or another library anywhere in the world, any publication corresponding to the MARC record has been cataloged and indexed according to its subject matter such that a person interested in that subject matter

[14] Third Article, Amended Articles of Incorporation of OCLC Online Computer Library Center, Incorporated (available at https://www.oclc.org/content/dam/oclc/membership/articles-of-incorporation.pdf).

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could, with reasonable diligence, locate and access the publication through any library with access to the OCLC bibliographic database or through the Library of Congress.

37. When an OCLC member institution creates a new MARC record, OCLC automatically supplies the date of creation for that record. The date of creation for the MARC record appears in the fixed Field (008), characters 00 through 05. The MARC record creation date reflects the date on which, or shortly after which, the item was first acquired or cataloged. Initially, Field 005 of the MARC record is automatically populated with the date the MARC record was created in year, month, day format (YYYYMMDD) (some of the newer library catalog systems also include hour, minute, second (HHMMSS)). Thereafter, the library's computer system may automatically update the date in Field 005 every time the library updates the MARC record (*e.g.*, to reflect that an item has been moved to a different shelving location within the library). Field 005 is visible when viewing a MARC record via an appropriate computerized interface, but when a MARC record is printed to hardcopy, no "005" label appears. The initial Field 005 date (*i.e.*, the date the MARC record was created) does appear, however, next to the label "Entered."[15] The date upon which the most recent update to Field 005

[15] Field 005 is visible when viewing a MARC record via an appropriate computerized interface. But when a MARC record is printed directly to hardcopy

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occurred also appears, next to the label "Replaced." Thus, when an item's MARC record has been printed to hardcopy—as is the case with the exhibit to this Declaration—the date reflected next to the label "Entered" is necessarily on or after the date the library first cataloged and indexed the underlying item.

38. Once one library has cataloged and indexed a publication by creating a MARC record for that publication, other libraries that receive the publication do not create additional MARC records—the other libraries instead rely on the original MARC record. They may update or revise the MARC record to ensure accuracy, but they do not replace or duplicate it. This practice does more than save libraries from duplicating labor. It also enhances the accuracy of MARC records. Further, it allows librarians around the world to know that a particular MARC record is authoritative (in contrast, a hypothetical system wherein duplicative records were created would result in confusion as to which record is authoritative).

39. Catalogers can create MARC records for all types of print, online, and digital resources. The date of creation of the MARC record by a cataloger at an OCLC member institution reflects when the underlying item is accessible to the public. Upwards of two-thirds to three-quarters of book sales to libraries come from a jobber or wholesaler for online and print resources. These resellers make it

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from the OCLC database, the "005" label is not shown. The date in the 005 field instead appears next to the label "Replaced."

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their business to provide books to their customers as fast as possible, often providing turnaround times of only a single day after publication. Libraries purchase a significant portion of the balance of their books directly from publishers themselves, which provide delivery on a similarly expedited schedule. In general, libraries make these purchases throughout the year as the books are published and shelve the books as soon thereafter as possible to make the books available to their patrons. Thus, books are generally available at libraries across the country within just a few days of publication.

40. *Journals.* MARC records that cover serial publications include both serially-published monographs and journals. OCLC hosts MARC records for more than 320 million serial publications. Serial publications are those publications that have the same collective title but are intended to be continued indefinitely with enumeration such as a volume or issue number (*e.g.*, magazines, journals, etc.). In the OCLC bibliographic database, the first issue of the serial publication is typically cataloged (*i.e.*, a corresponding MARC record is created), but the date is left open-ended with the use of a punctuation mark such as a dash. OCLC serial publication MARC records represent the entire run of the serial title. With knowledge of the first issue published, future issues can be predicted based on the information provided in the MARC record, for example in field 362. In my extensive professional experience, is it highly unusual for a library to stop

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collecting and shelving a serial publication prior to the end of its publication run. If a subscription to a serial publication ends its run or is cancelled before the end of its run, the library will denote that it has stopped receiving new volumes by filling in the end date in the MARC record.

41. The handling of printed journal subscriptions is shown on the cover or the first few pages of individual issues. As was the best practice among libraries, issues arrived at a central facility and were immediately received, verified as part of a subscription, checked in, and stamped or labeled with the institution's name and date. Determining that the issue was part of the library subscription ensured that the entire set of publications for the year had been received so that they could be professionally bound and retained. This process also verified that all of the published issues arrived so that the library staff did not have to request or claim an issue that did not arrive as expected. In large public libraries with branches and multi-campus libraries within academic institutions, the journals were sorted and delivered to the subscribing unit. The new issue was placed in the public area; the older issue was stored so that it remained available.

42. The foregoing process has been standard library practice longer than I have been working in the profession. I first learned the steps in the process in the late 1970s and later supervised it. Although the checking in process has become automated and now links electronically to holdings records for the MARC record

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for each serial title, the manual placement of a stamp or label and placing the issue in a public area has not changed for 50 years. Unless I note otherwise below in reference to a specific serial publication, it is my expert opinion that this standard protocol was followed for the serial publication discussed below.

VII. PUBLICATION 1: "*Using Chip-Seq Technology to Generate High-Resolution Profiles of Histone Modifications*" by Henriette O'Geen, Lorigail Echipare, and Peggy J. Farnham. EXHIBIT A ("O'Geen")

43. Exhibit A is a copy of the title page, copyright page, table of contents, and extract from the book titled *Epigenetics Protocols*, 2nd edition, edited by Trygve O. Tollefsbol and issued by Humana Press, part of Springer-Science + Business Media issued with a 2011 copyright date. The book titled *Epigenetics Protocols*, 2nd edition, is Volume 791 of the publisher's series Methods in Molecular Biology. The extract, Chapter 20, is titled "Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications" by Henriette O'Geen, Lorigail Echipare, and Peggy J. Farnham (hereafter "O'Geen"), which appears on pages 265-286. Exhibit A is a true and correct copy of the O'Geen chapter from the book titled *Epigenetics Protocols*, 2nd edition, held by the Steenbock Library at the University of Wisconsin -- Madison. Exhibit A is complete; no pages are missing, and the text on each page appears to flow seamlessly from one page to the next; further, there are no visible alterations to the document. Exhibit A is a true and correct copy in a condition that creates no

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suspicion about its authenticity.

44. Attachment 1A is a true and correct copy of the MARC record for this monograph from the online catalog in the Steenbock Library at the University of Wisconsin – Madison. The library ownership is indicated by the presence of the library's code (GZM) in field 049. I personally identified and retrieved the library catalog record which is Attachment 1A.

45. Based on finding a print copy of the book titled *Epigenetics Protocols*, 2nd edition, in the Steenbock Library at the University of Wisconsin – Madison and MARC record in its online library catalog attached as Attachment 1A, it is my opinion that the O'Geen book chapter was publicly available on September 21, 2011, as indicated in field 008 ("110921"). The International Standard Book Number (ISBN) on Exhibit A (978-1-61779-315-8) matches the ISBN on page 3 of Exhibit A. Therefore, Exhibit A is the same book as the one that the cataloger in the Steenbock Library at the University of Wisconsin – Madison used to create the MARC record that is Attachment 1A.

46. Attached hereto as Attachment 1B is a true and correct copy of the MARC record for the book titled *Epigenetics Protocols*, 2nd edition, from the OCLC bibliographic database. I personally identified and retrieved the MARC record that is Attachment 1B. As previously noted, the library that created the record is recorded in field 040 with a unique library code. For Attachment 1B that

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library code is "DLC," which means that the MARC record for this book was created in the Library of Congress. The library continues to update and enhance this MARC record to meet current cataloging rules. The most recent enhancement to Attachment 1B occurred on June 27, 2022, as shown in the "Replaced" field ("20220627"). I personally identified and retrieved the MARC record that is Attachment 1B.

47. Attachment 1B further includes an entry in field 050 ("QH599 $b .E65 2011") as described above, this includes a subject matter classification number consistent with the Library of Congress classification system (analogous to the Dewey Decimal classification system), an entry in field 060 ("W1 $b ME9616J v.791 2011") as described above, this includes a subject matter classification number consistent with the National Library of Medicine classification system (analogous to the Dewey Decimal classification system), and an entry in field 082 ("572.8/7") as described above, this includes a subject matter classification number consistent with the Dewey Decimal classification system. Attachment 1B further includes four descriptor terms reading "Chromatin $v Laboratory manuals" (see Attachment 1C, Library of Congress subject heading sh85025333 and Attachment 1D, Library of Congress subject heading sh8700908), "Telomere $v Laboratory manuals" (see Attachment 1E, Library of Congress subject heading sh94005630 and Attachment 1D, Library of Congress subject heading sh8700908), "ADP-

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ribosylation $v Laboratory manuals" (see Attachment 1F, Library of Congress subject heading sh89006797 and Attachment 1D, Library of Congress subject heading sh8700908), and "Epigenesis $v Laboratory manuals" (see Attachment 1G, Library of Congress subject heading sh85044382 and Attachment 1D, Library of Congress subject heading sh8700908) in the 650 fields. Thus, as of its cataloging, the publication corresponding to the MARC record attached hereto as Attachment 1B was indexed according to its subject matter by virtue of at least four independently sufficient classifications: the field 050 entry, the field 060entry, the field 082 entry, and the field 650 entries. Further, as of July 25, 2011, the MARC record attached hereto as Attachment 1B was accessible through any library with access to the OCLC bibliographic database or the online catalog at a library that added this book to its collection, which means that the corresponding publication was publicly available on or before that same date through any library with access to the OCLC bibliographic database or through an individual library.

48. Further supporting my opinion that Exhibit A was publicly accessible to ordinarily skilled and interested researchers in the field is the fact that the book titled *Epigenetics Protocols,* 2nd edition, was registered with the U.S. Copyright Office as item TX0007451979 on November 23, 2011, and indicated a publication date of September 15, 2011 (see Attachment 1H, U. S. Copyright Office Public Catalog). As noted above, this would have provided a POSITA with keyword

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searching capabilities and other tools to locate this book.

49. Attachment 1B indicates that the book titled *Epigenetics Protocols*, 2nd edition, as cataloged at the Library of Congress is currently available from 41 libraries. In view of above, the book titled *Epigenetics Protocols*, 2nd edition, was publicly available on or shortly September 21, 2011, because by that date it had been received, cataloged, and indexed in the Steenbock Library at the University of Wisconsin – Madison and made part of the OCLC bibliographic database. For these reasons, it is my opinion that O'Geen was published and accessible to the public on or shortly after September 21, 2011.

VIII. PUBLICATION 2: "*Rapid, Low-Input, Low-Bias Construction of Shotgun Fragment Libraries by High-Density in Vitro Transposition*" by Andrew Adey, Hilary G. Morrison, Asan, Xu Xun, Jacob O. Kitzman, Emily H. Turner, Bethany Stackhouse, Alexandra P. MacKenzie, Nicholas C. Caruccio, Xiuqing Zhang, and Jay Shendure. EXHIBIT B ("Adey")

50. Attached hereto as Exhibit B is a copy of the journal article "Rapid, Low-Input, Low-Bias Construction of Shotgun Fragment Libraries By High-Density in Vitro Transposition" by Andrew Adey, Hilary G. Morrison, Asan, Xu Xun, Jacob O. Kitzman, Emily H. Turner, Bethany Stackhouse, Alexandra P. MacKenzie, Nicholas C. Caruccio, Xiuqing Zhang, and Jay Shendure (hereafter "Adey"). The Adey article was published in Volume 11 of the open access journal *Genome Biology* and was found in the National Library of Medicine (Bethesda, Maryland). The Adey article appears beginning on page R119 of this issue dated

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December 2010. Exhibit B is a true and correct copy of the Adey article (17 pages). Specifically, the text of the article is complete; no pages are missing, and the text on each page appears to flow seamlessly from one page to the next; further, there are no visible alterations to the document. The Adey article is also available from the publisher, BMC Springer,[16] through the National Library of Medicine,[17] and through the digital repository *ResearchGate*.[18] The publisher indicates that the Adey article was published online on December 8, 2010. Exhibit B was found within the custody of a library – a place where an authentic copy of this journal would likely be. Exhibit B is a true and correct copy in a condition that creates no suspicion about its authenticity.

51. Attached hereto as Attachment 2A is a true and correct copy of the MARC record for the open access journal *Genome Biology* in the online catalog at the National Library of Medicine. The library ownership is indicated by the presence of the library's code (NLM) in field 040. The library continues to update this MARC record and enhanced the MARC record to meet current cataloging

[16] https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-12-r119

[17] https://pubmed.ncbi.nlm.nih.gov/21143862/

[18] https://www.researchgate.net/publication/49669878_Rapid_low-input_low-bias_construction_of_shotgun_fragment_libraries_by_high-density_in_vitro_transposition

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rules. The most recent enhancement to Attachment 2A occurred on April 29, 2022, as shown in field 005 ("20220429"). I personally identified and retrieved the MARC record that is Attachment 2A.

52. Based on finding a print copy of Exhibit B in the National Library of Medicine and MARC record in its online library catalog attached as Attachment 2A, it is my opinion that the Adey article published in the open access journal *Genome Biology* was publicly available on or shortly after December 8, 2010, as shown on the cover. Attachment 2A also shows that Exhibit B was catalogued with a descriptor term reading "Biology" (see Attachment 2B, National Library of Medicine subject heading D001695) in the 650 field.

53. As noted in the holdings information (362 field), the National Library of Medicine has received the open access journal *Genome Biology* since 2000 and continues to receive the publication. In view of the MARC record for Exhibit B, the Adey article was publicly available on or shortly after December 8, 2010, because the serial title had been received, cataloged, and indexed in the National Library of Medicine and made part of its online catalog database.

54. Attached hereto as Attachment 2C is a true and correct copy of the MARC record for the open access journal *Genome Biology* obtained from the OCLC bibliographic database. As previously noted, the library that created the record is recorded in field 040 with a unique library code. For Attachment 2C, that

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library code is "NLM," which means that the MARC record for this open access serial was cataloged at the National Library of Medicine. A cataloger in the National Library of Medicine created OCLC record number 45320094 on July 21, 2000, as shown in the "Entered" field in the MARC record for this exhibit ("20000721"). The library continues to update this MARC record and enhanced the MARC record to meet current cataloging rules. The most recent enhancement to Attachment 2C occurred on August 9, 2022, as shown in the "Replaced" field ("20220809"). I personally identified and retrieved the MARC record that is Attachment 2C. The "BLvl" entry in Attachment 2C is "s," which indicates that the open access journal *Genome Biology* is a serial publication. Accordingly, the MARC record for Exhibit B corresponds to the open access journal *Genome Biology* from the time the serial title began publication and includes the date of the Adey article.

55. Attachment 2C includes an entry in field 050 ("QH447 $b .G465") (as described above, a subject matter classification number consistent with the Library of Congress classification system (analogous to the Dewey Decimal classification system) and an entry in field 060 ("W1 $b GE366FQ") (a subject matter classification number consistent with the National Library of Medicine classification system (analogous to the Dewey Decimal classification system). Attachment 2C further includes three descriptor terms reading "Genomes $v

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Periodicals" (*see* Attachment 2D, Library of Congress subject heading sh2009125753), "Biology $v Periodicals" (*see* Attachment 2E, Library of Congress subject heading sh2007101986), and "Molecular biology $v Periodicals" (*see* Attachment 2F, Library of Congress subject heading sh2008107986) in the 650 fields. Thus, as of its cataloging, the publication corresponding to the MARC record attached hereto as Attachment 2C was indexed according to its subject matter by virtue of at least three independently sufficient classifications: the field 050 entry, the field 060 entry, and the field 650 entries. As of July 21, 2000, the MARC record attached hereto as Attachment 2C was accessible through any library with access to the OCLC bibliographic database or the online catalog at a library that subscribed to the open access serial, which means that the corresponding publication was publicly available on or before that same date through any library with access to the OCLC bibliographic database or through an individual library.

56. Attachment 2C indicates that the open access journal *Genome Biology* as cataloged at the National Library of Medicine is currently available from 53 libraries. In view of the above, this issue of the open access journal *Genome Biology* was publicly available on or shortly after December 8, 2010, because by that date it had been cataloged, indexed, and received in the National Library of Medicine and made part of the OCLC bibliographic database. For these reasons, it

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is my opinion that Adey was published and accessible to the public on or shortly after December 8, 2010.

IX. PUBLICATION 3: "*Ultrasonic Drug Delivery – A General Review*" by William G. Pitt, Ghaleb A. Husseini, and Bryant J. Staples. EXHIBIT C ("PITT")

57. Attached hereto as Exhibit C is a copy of the journal article "Ultrasonic Drug Delivery – A General Review" by William G. Pitt, Ghaleb A. Husseini, and Bryant J. Staples (hereafter "Pitt"). The Pitt article was published in Volume 1, Number 1 of the journal *Expert Opinion on Drug Delivery* and was found in the National Library of Medicine (Bethesda, Maryland). The Pitt article appears beginning on page 37 of this issue dated November 2004. Exhibit C is a true and correct copy of the Pitt article (pages 37-56). Specifically, the text of the article is complete; no pages are missing, and the text on each page appears to flow seamlessly from one page to the next; further, there are no visible alterations to the document. The Pitt article is also available from the publisher, the Taylor & Francis Group,[19] through the National Library of Medicine,[20] and through the digital repository *Semantic Scholar*.[21] The publisher indicates that the Pitt article

[19] https://www.tandfonline.com/doi/abs/10.1517/17425247.1.1.37

[20] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1361256/

[21] https://www.semanticscholar.org/paper/Ultrasonic-drug-delivery-%E2%80%93-a-general-review-Pitt-Husseini/da5598bf14810e0f8cd992d60233fd0c67d8557d

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was published online on February 23, 2005. Exhibit C was found within the custody of a library – a place where an authentic copy of this journal would likely be. Exhibit C is a true and correct copy in a condition that creates no suspicion about its authenticity.

58. Attached hereto as Attachment 3A is a true and correct copy of the MARC record for the journal *Expert Opinion on Drug Delivery* in the online catalog at the National Library of Medicine. The library ownership is indicated by the presence of the library's code (NLM) in field 040. The library continues to update this MARC record and enhanced the MARC record to meet current cataloging rules. The most recent enhancement to Attachment 3A occurred on March 10, 2023, as shown in field 005 ("20230310"). I personally identified and retrieved the MARC record that is Attachment 3A.

59. Based on finding a print copy of Exhibit C in the National Library of Medicine and MARC record in its online library catalog attached as Attachment 3A, it is my opinion that the Pitt article published in the journal *Expert Opinion on Drug Delivery* was publicly available on or shortly after February 23, 2005, as shown on the cover. Attachment 3A also shows that Exhibit C was catalogued with a descriptor term reading "Drug Delivery Systems" (see Attachment 3B, National Library of Medicine subject heading D016503) in the 650 field.

60. As noted in the holdings information (362 field), the National Library

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of Medicine has received the journal *Expert Opinion on Drug Delivery* since November 2004 and continues to receive the publication. In view of the MARC record for Exhibit C, the Pitt article was publicly available on or shortly after February 23, 2005, because the serial title had been received, cataloged, and indexed in the National Library of Medicine and made part of its online catalog database.

61. Attached hereto as Attachment 3C is a true and correct copy of the MARC record for the journal *Expert Opinion on Drug Delivery* obtained from the OCLC bibliographic database. As previously noted, the library that created the record is recorded in field 040 with a unique library code. For Attachment 3C, that library code is "NLM," which means that the MARC record for this open access serial was cataloged at the National Library of Medicine. A cataloger in the National Library of Medicine created OCLC record number 57731789 on August 30, 2004, as shown in the "Entered" field in the MARC record for this exhibit ("20040830"). The library continues to update this MARC record and enhanced the MARC record to meet current cataloging rules. The most recent enhancement to Attachment 3C occurred on April 3, 2020, as shown in the "Replaced" field ("20200403"). I personally identified and retrieved the MARC record that is Attachment 3C. The "BLvl" entry in Attachment 3C is "s," which indicates that the journal *Expert Opinion on Drug Delivery* is a serial publication. Accordingly,

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the MARC record for Exhibit C corresponds to the journal *Expert Opinion on Drug Delivery* from the time the serial title began publication and includes the date of the Pitt article.

62. Attachment 3C includes an entry in field 050 ("RS199.5") (as described above, a subject matter classification number consistent with the Library of Congress classification system (analogous to the Dewey Decimal classification system) and an entry in field 060 ("W1 $b EX52LF") (a subject matter classification number consistent with the National Library of Medicine classification system (analogous to the Dewey Decimal classification system). Attachment 3C further includes two descriptor terms reading "Drug delivery devices $v Periodicals" (*see* Attachment 3D, Library of Congress subject heading sh87007331 and Attachment 1C, Library of Congress subject heading sh85099890) and "Drug delivery systems $v Periodicals" (*see* Attachment 3E, Library of Congress subject heading sh88007108 and Attachment 1C, Library of Congress subject heading sh85099890) in the 650 fields. Thus, as of its cataloging, the publication corresponding to the MARC record attached hereto as Attachment 3C was indexed according to its subject matter by virtue of at least three independently sufficient classifications: the field 050 entry, the field 060 entry, and the field 650 entries. As of August 30, 2004, the MARC record attached hereto as Attachment 3C was accessible through any library with access to the

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OCLC bibliographic database or the online catalog at a library that subscribed to the open access serial, which means that the corresponding publication was publicly available on or before that same date through any library with access to the OCLC bibliographic database or through an individual library.

63. Attachment 3C indicates that the journal *Expert Opinion on Drug Delivery* as cataloged at the National Library of Medicine is currently available from 27 libraries. In view of the above, this issue of the journal *Expert Opinion on Drug Delivery* was publicly available on or shortly after February 23, 2005, because by that date it had been cataloged, indexed, and received in the National Library of Medicine and made part of the OCLC bibliographic database. For these reasons, it is my opinion that Exhibit C was published and accessible to the public on or shortly after February 23, 2005.

X. SUMMARY OF OPINIONS

64. In view of the foregoing, it is my opinion that the publications described above was publicly available no later than the corresponding date listed in the table below:

Publication	Publicly Available No Later Than
O'Geen, Henriette, Lorigail Echipare, and Peggy J. Farnham. "Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications" (Chapter 20, pp. 265-286). *Epigenetics Protocols*, 2nd ed., edited by Trygve O. Tollefsbol. Methods of Molecular Biology, vol. 791. New York: Humana Press, 2011.	September 21, 2011

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Publication	Publicly Available No Later Than
Adey, Andrew, Hilary G. Morrison, Asan, Xu Xun, Jacob O. Kitzman, Emily H. Turner, Bethany Stackhouse, Alexandra P. MacKenzie, Nicholas C. Caruccio, Xiuqing Zhang, and Jay Shendure. "Rapid, Low-Input, Low-Bias Construction of Shotgun Fragment Libraries by High-Density in Vitro Transposition." *Genome Biology*, vol. 11 (December 2010): R119.	December 8, 2010
Pitt, William G., Ghaleb A. Husseini, and Bryant J. Staples. "Ultrasonic Drug Delivery – A General Review." *Expert Opinion on Drug Delivery*, vol. 1, no. 1 (November 2004): 37–56.	February 23, 2005

65. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

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Exhibit A

Methods in Molecular Biology 791

Springer Protocols



Trygve O. Tollefsbol *Editor*

Epigenetics Protocols

Second Edition

Humana Press

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Epigenetics Protocols

Second Edition

Edited by

Trygve O. Tollefsbol

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Chapter 20

Using ChIP-Seq Technology to Generate High-Resolution Profiles of Histone Modifications

Henriette O'Geen, Lorigail Echipare, and Peggy J. Farnham

Abstract

The dynamic modification of DNA and histones plays a key role in transcriptional regulation through altering the packaging of DNA and modifying the nucleosome surface. These chromatin states, also referred to as the epigenome, are distinctive for different tissues, developmental stages, and disease states and can also be altered by environmental influences. New technologies allow the genome-wide visualization of the information encoded in the epigenome. For example, the chromatin immunoprecipitation (ChIP) assay allows investigators to characterize DNA–protein interactions in vivo. ChIP followed by hybridization to microarrays (ChIP-chip) or by high-throughput sequencing (ChIP-seq) are both powerful tools to identify genome-wide profiles of transcription factors, histone modifications, DNA methylation, and nucleosome positioning. ChIP-seq technology, which can now interrogate the entire human genome at high resolution with only one lane of sequencing, has recently surpassed ChIP-chip technology for epigenomic analyses. Importantly, for the study of primary cells and tissues, epigenetic profiles can be generated using as little as 1 μg of chromatin. In this chapter, we describe in detail the steps involved in performing ChIP assays (with a focus on characterizing histone modifications in primary cells) either manually or using the IP-Star ChIP robot, followed by a detailed protocol to prepare successful libraries for Illumina sequencing. Critical quality control checkpoints are discussed. Although not a focus of this chapter, we also point the reader to several methods by which massive ChIP-seq data sets can be analyzed to extract the tremendous information contained within.

Key words: Chromatin immunoprecipitation, ChIP-seq, Next generation sequencing, Epigenomics, Histone modifications, IP-Star, ChIP robot

1. Introduction

Although the genetic information encoded in our DNA plays a major role in specifying our individual phenotypes, it is becoming increasingly clear that epigenetic information is also an important contributor to our mental and physical attributes (1–8). Our epigenome is defined as methylated DNA and modified histone

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proteins (around which both methylated and unmethylated DNA are wrapped). DNA methylation and histone modifications undergo global changes during transitions in developmental states and in diseases such as cancer and therefore are major contributors to the dynamic nature of chromatin. Histone modifications such as acetylation (e.g., acetylation of lysine 9 of histone H3 which is called H3K9Ac) are typically associated with open and accessible chromatin regions, while histone methylation can be associated with either open or compacted (also referred to as heterochromatic) chromatin regions, depending on the specific histone amino acid that is methylated (9–12). For example, mono- or trimethylation of lysine 4 on histone H3 (H3K4me1 or H3K4me3) and trimethylation of histone H3 on lysine 36 (H3K36me3) are associated with open chromatin. However, each of these marks represents a unique category of open chromatin, with H3K4me3 marking gene promoter regions, H3K4me1 marking transcriptional enhancers, and H3K36me3 marking transcribed regions of the genome. In contrast, trimethylation of lysines 9 and 27 on histone H3 (H3K9me3 and H3K27me3, respectively) is associated with compacted chromatin regions resulting in repression of target genes. Although both of these modifications mark repressive chromatin, H3K9me3 and H3K27me3 regulate distinct sets of target genes; H3K27me3 predominantly represses homeobox transcription factors and H3K9me3 predominantly targets the genes of zinc finger transcription factors (13). Knowing the genome-wide pattern of single histone modifications, such as the six marks described above, provides a great deal of information about cell identity and disease state (10, 14–17). Accordingly, these six marks have been selected to provide "roadmaps" of the epigenomic profiles of primary cells by the Roadmap Epigenome Mapping Centers (http://www.roadmapepigenomics.org/). However, it is also becoming increasingly clear that different combinations of histone marks can provide even more detailed information. For example, the presence of both the open chromatin mark H3K4me3 and the compacted chromatin mark H3K9me3 at a promoter can identify imprinted genes (18). We are just beginning to understand the interrelationships between specific histone modifications and transcriptional regulation and more insights will certainly be forthcoming with the analysis of more and more epigenetic profiles.

Currently, the method of choice to study the epigenome is the chromatin immunoprecipitation (ChIP) assay. To perform a ChIP assay, proteins such as histones or transcription factors are covalently crosslinked to their genomic DNA substrates in living cells. This provides an opportunity to take a snapshot of histone or other protein–DNA interactions in a given cell type, in cells taken at different developmental stages, or in cells altered by disease. After isolation and fragmentation of chromatin, the protein–DNA complexes are captured using antibodies specific to the histone or transcription factor of interest. After reversal of crosslinks, the

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ChIP DNA is then purified and analyzed by either hybridization to microarrays (ChIP-chip) or by high-throughput sequencing (ChIP-seq). While multiple DNA microarrays are needed to cover the entire human genome, resulting in high costs for comprehensive studies, ChIP-seq offers the possibility to interrogate the entire genome in one sequencing run. Therefore, ChIP-seq has generally replaced ChIP-chip for comprehensive epigenomic studies. To date there are four high-throughput sequencing platforms that have been used for ChIP-seq (see ref. 19 for review): (1) 454 Genome Sequencer FLX from Roche (20, 21), (2) Genome Analyzer GA2 from Illumina (9, 18, 22), (3) Sequencing by Oligo Ligation/Detection (SOLiD) from Applied Biosystems (23, 24), and (4) true Single Molecular Sequencing (tSMS) by Helicos (19, 25). Unlike the first three methods that use an amplification step, the Helicos platform sequences unamplified DNA. Another difference among the platforms include read length; the Illumina, SOLiD, and Helicos platforms produce short read lengths of on average 35–50 bp whereas the 454 platform produces longer reads of 200–400 bp. Most ChIP-seq studies to date have used the Illumina sequencing platform (sometimes called Solexa sequencing). Therefore, this chapter describes in detail the steps needed to prepare ChIP samples and libraries for high-throughput sequencing using the Illumina GA2 platform and includes descriptions of quality control steps necessary to ensure a successful ChIP-seq experiment (see Fig. 1).

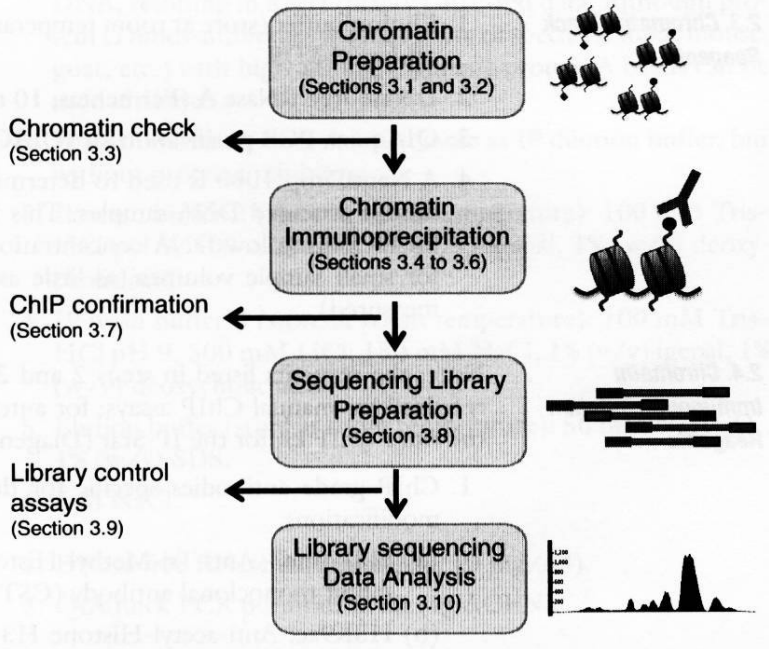


Fig. 1. ChIP-seq diagram summarizing the main experimental steps and quality check points.

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2. Materials

2.1. Crosslinking Reagents

1. Crosslinking reagent: formaldehyde solution (37% w/w).
2. Stopping reagent: glycine (electrophoresis grade).
3. Wash solution: phosphate-buffered saline (PBS).

2.2. Chromatin Preparation Reagents

1. Protease inhibitor stock solutions (store in small aliquots at −20°C): 10 mg/ml aprotinin (in water), 10 mg/ml leupeptin (in water), 100 mM PMSF (in isopropanol).
2. Cell lysis buffer (store at room temperature): 5 mM PIPES pH 8, 85 mM KCl. Add igepal fresh each time to give a final concentration of 1% (10 µl/ml). Warm buffer in 37°C water bath and vortex briefly to help mixing of igepal. After mixing has occurred, place buffer containing igepal on ice to allow solution to cool down and then add protease inhibitors [PMSF (10 µl/ml f.c.), aprotinin (1 µl/ml f.c.), and leupeptin (1 µl/ml f.c.)].
3. Nuclei lysis buffer (store at room temperature): 50 mM Tris–HCl pH 8, 10 mM EDTA, and 1% (w/v) SDS. Place buffer on ice right before use to avoid precipitation of SDS and add protease inhibitors [PMSF (10 µl/ml f.c.), aprotinin (1 µl/ml f.c.), and leupeptin (1 µl/ml f.c.)] just prior to use.
4. Bioruptor UCD-200 (Diagenode) or equivalent is used for sonication.

2.3. Chromatin Check Reagents

1. Elution buffer (store at room temperature): 50 mM $NaHCO_3$, 1% (w/v) SDS.
2. DNase-free RNase A (Fermentas; 10 mg/ml).
3. QIAquick PCR purification kit (QIAGEN).
4. A NanoDrop 1000 is used to determine the concentration of double-stranded DNA samples. This instrument is invaluable for measuring low DNA concentrations (e.g., 10 ng/µl) and for small sample volumes (as little as 1 µl of sample can be measured).

2.4. Chromatin Immunoprecipitation Reagents

Note, the reagents listed in steps 2 and 3 of Subheading 2.4 are required for manual ChIP assays; for automated ChIP assays, use the Auto ChIP kit for the IP-Star (Diagenode).

1. ChIP grade antibodies specific for the following six histone modifications:
   (a) H3K4me3: Anti-Tri-Methyl-Histone H3 (Lys4) (C42D8) rabbit monoclonal antibody (CST #9751S).
   (b) H3K9ac: Anti-acetyl-Histone H3 (Lys9) rabbit antibody (Millipore #07-352).

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(c) H3K27me3: Anti-Tri-Methyl-Histone H3 (Lys27) (C36B11) rabbit monoclonal antibody (CST #9733S).

(d) H3K9me3: Anti-Tri-Methyl-Histone H3 (Lys9) rabbit antibody (CST #9754S).

(e) H3K36me3: Anti-Tri-Methyl-Histone H3 (Lys36) rabbit antibody (CST #9763S).

(f) H3K4me1: Anti-Mono-Methyl-Histone H3 (Lys4) rabbit antibody (Diagenode #pAb-037-050).

2. Protease inhibitor stock solutions (store in small aliquots at −20°C): 10 mg/ml aprotinin (in water), 10 mg/ml leupeptin (in water), 100 mM PMSF (in isopropanol).
3. IP dilution buffer (store at 4°C): 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% (v/v) igepal, 0.25% (w/v) deoxycholic acid, 1 mM EDTA pH 8. Add protease inhibitors [PMSF (10 µl/ml f.c.), aprotinin (1 µl/ml f.c.), and leupeptin (1 µl/ml f.c.)] just prior to use. This buffer is used to adjust the salt and SDS concentrations for the immuno-precipitation step.

2.5. Capture the Antibody/Chromatin Complexes and to Reverse Crosslinks

Note, the reagents listed below are required for manual ChIP assays; for automated ChIP assays, use the Auto ChIP kit for the IP-Star (Diagenode).

1. Magnetic protein G beads (Cell Signaling Technology) and magnetic rack. Do not use magnetic beads that have been blocked with foreign DNA, such as herring sperm or salmon sperm DNA. This may result in sequencing of the blocking DNA, resulting in lower quality ChIP-seq data. Although protein G binds antibodies from a variety of species (rabbit, mouse, goat, etc.) with high affinity, magnetic protein A beads can be used if desired.
2. IP wash buffer 1 (store at 4°C): same as IP dilution buffer, but without protease inhibitors.
3. IP wash buffer 2 (store at room temperature): 100 mM Tris–HCl pH 9, 500 mM LiCl, 1% (v/v) igepal, 1% (w/v) deoxycholic acid.
4. IP wash buffer 3 (store at room temperature): 100 mM Tris–HCl pH 9, 500 mM LiCl, 150 mM NaCl, 1% (v/v) igepal, 1% (w/v) deoxycholic acid.
5. Elution buffer (store at room temperature): 50 mM $NaHCO_3$, 1% (w/v) SDS.
6. 5 M NaCl.

2.6. DNA Purification

1. DNase-free RNase A (Fermentas; 10 mg/ml).
2. QIAquick PCR purification kit (QIAGEN).

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2.7. ChIP Confirmation

1. SYBR-Green qPCR mix, such as SYBR Green JumpStart Taq ReadyMix (SIGMA).
2. Positive and negative control primer sets (see Note 1).

2.8. Sequencing Library Preparation

1. End-It DNA END Repair Kit (Epicentre).
2. Klenow (3′–5′ exo minus) (NEB; 5,000 U/ml).
3. 100 mM dATP.
4. LigaFast DNA ligase (Promega; 3 U/μl).
5. Oligo-only kits for single end or paired end read sequencing are available from Illumina (#FC-102-1003 and PE-102-1003, respectively). Alternatively, adapter oligos and PCR primers compatible with Illumina sequencing can be purchased elsewhere; HPLC purification is recommended. The paired end DNA oligonucleotides are more universal since the resulting library can be sequenced with either single end or paired end sequencing primers. The following stock solutions are prepared: 15 μM Paired End Adapter Oligo mix, 25 μM Paired End PCR primer 1.01, and 25 μM Paired End PCR primer 2.01.

   Paired End DNA oligonucleotide sequences (Oligonucleotide sequences© 2006 and 2008 Illumina, Inc. All rights reserved).

   PE Adapters

   5′ P-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG

   5′ ACACTCTTTCCCTACACGACGCTCTTCCGATCT

   PE PCR Primer 1.01

   5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCC CTACACGACGCTCTTCCGATCT

   PE PCR Primer 2.01

   5′ CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATT CCTGCTGAACCGCTCTTCCGATCT
6. 2% Agarose precast E-Gel® (Invitrogen #G501802), loading dye such as TrackIt™ CyanOrange Loading Buffer (Invitrogen #10482-028), 50 or 100 bp DNA markers (e.g., TrackIt™ 50 bp DNA Ladder, Invitrogen #10488-043).
7. QIAquick Gel Extraction Kit (QIAGEN #28704).
8. Phusion DNA polymerase (NEB #F531).
9. QIAquick PCR purification kit (QIAGEN #28104) and MinElute PCR purification kit (QIAGEN #28004).
10. Agencourt AMPure system (Beckman Coulter Genomics #A29152).

2.9. Library Control Assay

1. DNA High Sensitivity Kit (Agilent Cat# 5067–4626) for use with the Agilent 2100 Bioanalyzer.

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2. SYBR-Green qPCR mix, such as SYBR Green JumpStart Taq ReadyMix (SIGMA #S4438).
3. Positive and negative control primer sets (see Note 1).

2.10. Sequencing Reagents

These reagents will be supplied by the sequencing facility.

3. Methods

3.1. Preparation of Crosslinked Cells

1. Cell cultures should be healthy and not density-arrested prior to crosslinking. For primary cells or tissues, the samples can be snap frozen in liquid nitrogen immediately after collection or snap frozen after crosslinking. The amount of cells needed for ChIP-seq will vary depending on the antibody used and the abundance of the histone mark of interest. In general between 100,000 and 500,000 cells are used per histone antibody. In a chemical hood, prepare 1% formaldehyde solution in PBS and add directly to frozen cell pellet. Resuspend cell pellet by pipetting up and down. Alternatively for cultured cells, add formaldehyde (37% stock) directly to tissue culture media to a final concentration of 1%.
2. Rotate primary cells in a tightly closed tube or rock cultured cells on a shaking platform for 10 min at room temperature. Do not crosslink for longer periods since this may cause cells to form aggregates that do not sonicate efficiently.
3. Stop crosslinking reaction by adding glycine to a final concentration of 0.125 M. We use a 10× (1.25 M) stock solution. Continue to rotate/rock at room temperature for 5 min.
4. For primary cells or other cells crosslinked in suspension, centrifuge cells at 430 rcf for 5 min at 4°C, discard the solution, wash the pellet twice with ice-cold 1× PBS (mix by pipetting, pellet cells by centrifugation at 430 rcf for 5 min at 4°C and discard wash solution). For adherent cells, pour off media and rinse plates twice with ice-cold 1× PBS and pour off wash solution. Using a cell scraper, transfer adherent cells from the culture dish to a 15-ml conical tube on ice. Centrifuge the crosslinked cells at 430 rcf for 5 min at 4°C. It is important to carefully aspirate supernatants so as to not lose cells. Note: media containing formaldehyde should be treated as hazardous waste.
5. Cells may be used immediately for a chromatin preparation or snap frozen in liquid nitrogen and stored at −80°C.

3.2. Preparation of Chromatin

1. If using frozen crosslinked cells thaw them on ice; keep all cells and chromatin samples on ice at all times. Prepare the cell lysis buffer (1 ml cell lysis buffer per 1×10^7 cells): add Igepal

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(10 µl per ml cell lysis buffer, agitate at 37°C to dissolve, cool on ice), then add protease inhibitors [PMSF (10 µl/ml), aprotinin (1 µl/ml), and leupeptin (1 µl/ml)]. Resuspend cell pellet in freshly prepared ice-cold cell lysis buffer by pipetting. The final volume of cell lysis buffer should be sufficient so that there are no clumps of cells. Incubate on ice for 15 min.

2. Homogenize cells using a glass dounce homogenizer (type B) to break open the cells and release nuclei. Homogenize cells on ice with 20 strokes. Omit this step when processing less than one million cells.
3. Centrifuge cells at 430 rcf for 5 min at 4°C.
4. Discard the supernatant and resuspend the nuclear pellet in nuclei lysis (NL) buffer plus protease inhibitors. Be careful not to use too much NL buffer as it may lead to dilute chromatin; we suggest ~20 µl/10^6 cells. Incubate on ice for 30 min.
5. An optional flash-freezing step may help break open nuclei more efficiently. This step is critical if the homogenizing in step 2 is omitted. After incubation of nuclei in NL buffer for 30 min, flash freeze samples in liquid nitrogen, thaw at room temperature (once thawed, immediately transfer to ice; do not allow samples to warm up to room temperature), and proceed to sonication.
6. Sonicate cells in a coldroom and/or on ice to achieve average chromatin length of 200–500 bp (see Note 2). Larger chromatin fragments can negatively influence data quality and can lead to failure of the ChIP-seq experiment. Therefore, before processing large quantities of cells, sonication conditions should be optimized for each cell type (see Note 3).
7. Transfer sonicated samples into an Eppendorf tube and centrifuge using a microcentrifuge at 10,000 rcf for 10 min at 4°C. Carefully transfer the supernatant (sonicated chromatin) to a new tube while avoiding cell debris. Keep sonicated chromatin at 4°C while performing quantification and determining chromatin size; then proceed with the ChIP assays (see Note 4).

3.3. Determination of Chromatin Size and Concentration

1. Take an aliquot of chromatin sample from Subheading 3.2, step 7 prepared above. A typical size determination uses chromatin from 100,000 to 200,000 cells (see Note 5).
2. Add ChIP elution buffer to a total volume of 100 µl and then 12 µl 5 M NaCl to give a final salt concentration of 0.54 M. Boil samples in a water bath for 20 min to reverse crosslinks.
3. Allow sample to cool down, add 1 µl DNase-free RNase (10 mg/ml), and incubate for 20 min at 37°C. This step is important because the presence of RNA results in false estimation of chromatin size.

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4. Purify DNA using a PCR purification kit, elute DNA in 25 µl water. Measure chromatin concentration by NanoDrop and calculate the chromatin yield (see Note 6).
5. Run remaining chromatin on a 1.2% agarose gel to visualize average size. If the chromatin is larger than ~600 bp, adjust the sonication conditions by adding more pulses and repeat steps 1–5.

3.4. Chromatin Immunoprecipitation

ChIP is usually done within the same day as the chromatin preparation to avoid any concern about the quality of chromatin (see Note 4). The steps detailed in Subheadings 3.4 and 3.5 are for manual ChIP assays. However, ChIP reactions can be automated using a ChIP robot (e.g., the IP-Star from Diagenode) (see Note 7).

1. Measure volume of chromatin and divide chromatin as needed. The amount of chromatin needed for each ChIP reaction varies depending on the histone modification. For histone marks covering a small portion of the genome displaying sharp peaks, such as H3K4me3, we typically use 1 µg chromatin. Based on our experience, we prefer to use 5 µg chromatin for spreading histone marks such as H3K9me3 or H3K36me3 that cover large portions of the genome.
2. Optional: An IgG negative control sample can be included along with experimental antibodies. However, oftentimes chromatin cannot be spared for a control ChIP when using small amounts of primary cells. In this case, one can rely on regions bound by the activating histone marks as negative controls for the repressive histone marks and vice versa (Fig. 2a).

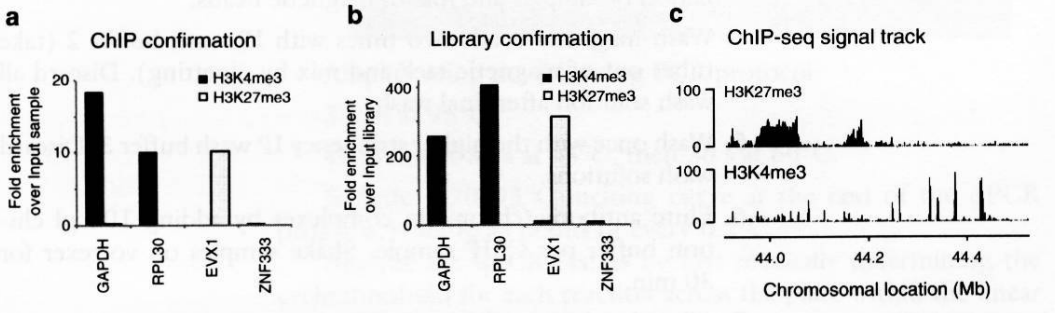


Fig. 2. ChIP-seq experiments using the IP-Star ChIP robot. ChIP assays using antibodies specific for H3K4me3 and H3K27me3 were performed using 1 µg chromatin from Ntera2 cells and the IP-Star ChIP robot (Diagenode). Libraries were prepared as outlined in Subheading 3.8. Quantitative PCR confirms specific enrichment over input in (**a**) the ChIP samples and (**b**) the ChIP-seq libraries. *Black bars* represent H3K4me3 enrichment and *white bars* represent H3K27me3 enrichment. Primer sets used are shown on the *x*-axis. GAPDH and RPL30 are positive control primer sets for H3K4me3 and negative control primer sets for H3K27me3. EVX1 was used as a positive primer set for H3K27me3 and a negative primer set for H3K4me3. The ZNF333 primer set was used as a negative control for both histone marks. (**c**) ChIP-seq binding patterns for H3K4me3 and H3K27me3 obtained from samples prepared using the IP-Star are shown for a region on chromosome 17 encompassing the HOXB gene cluster. The peak height is plotted along the *y*-axis; chromosomal coordinates (hg18 coordinates) are shown on the *x*-axis. Samples were sequenced at the DNA Technologies Core at UC Davis (http://genomecenter.ucdavis.edu/dna_technologies/).

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3. Save volume corresponding to 500 ng of chromatin to prepare an input sample (often also referred to as total DNA). Store the reserved amount at −20°C until the next day and then reverse the crosslinks in the input chromatin at the same time as the crosslinks in the ChIP samples are reversed.
4. Dilute chromatin fivefold with ice-cold IP Dilution buffer (1 volume chromatin and 4 volumes IP Dilution buffer) containing protease inhibitors.
5. Add an antibody specific to the histone mark of interest to capture the protein/chromatin complexes (see Note 8). Although antibody amounts are determined empirically, we typically use between 1 and 5 µg antibody per ChIP assay. Always record catalogue number and lot number of antibodies used.
6. Incubate 8–16 h on a rotating platform at 4°C.

3.5. Capture of Antibody/Chromatin Complexes and Reversal of Crosslinks

Step 1 is carried out at 4°C, whereas steps 2–9 are carried out at room temperature.

1. Add 15 µl magnetic protein G beads to each ChIP sample ranging from 1 to 5 µg chromatin starting material and incubate on a rotating platform for 2 h at 4°C.
2. At room temperature, allow beads to settle for 1 min in a magnetic separation rack. Carefully remove the supernatant without disturbing magnetic beads.
3. Wash magnetic beads two times with IP Dilution buffer (take tubes out of magnetic rack and mix by pipetting). Efficient washing is critical to reduce background. Avoid cross contamination of samples and loss of magnetic beads.
4. Wash magnetic beads two times with IP wash buffer 2 (take tubes out of magnetic rack and mix by pipetting). Discard all wash solution after final wash.
5. Wash once with the higher stringency IP wash buffer 3. Discard wash solutions.
6. Elute antibody/chromatin complexes by adding 100 µl elution buffer per ChIP sample. Shake samples on vortexer for 30 min.
7. Allow beads to settle for 1 min in a magnetic separation rack. Carefully transfer the supernatant containing antibody/chromatin complexes to a siliconized tube.
8. Add 12 µl of 5 M NaCl per 100 µl elution buffer mix to give a final concentration of 0.54 M NaCl.
9. At this point, thaw the input sample from the previous day (Subheading 3.4, step 3). Dilute 1 volume input sample with 4 volumes ChIP elution buffer (e.g., add 80 µl ChIP elution

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buffer to 20 µl input sample). Add 12 µl of 5 M NaCl per 100 µl elution buffer mix.

10. Incubate all samples in a 67°C water bath overnight to reverse formaldehyde crosslinks.

3.6. DNA Purification

1. Allow samples to cool, add 1 µl of RNaseA; incubate at 37°C for 20 min.
2. Purify DNA with a PCR clean up kit, one column per sample. Elute each sample with 40 µl EB buffer.
3. Assess ChIP enrichments by quantitative PCR (qPCR) before proceeding to preparation of Solexa libraries.

3.7. ChIP Confirmation

Enrichment of histone marks in the ChIP samples are determined by quantitative real-time PCR (qPCR). The input sample is diluted with EB to give a final concentration of 2 ng/µl and serves as a reference. Prepare a master reaction mix for each library with triplicate reactions per primer set. Add extra reagents for 10% of the total number of reagents to account for loss of volume. Add 14 µl of reaction mix to each PCR reaction well. Add 2 µl primer mix to each well.

Recipe for one reaction:

1 µl	Undiluted ChIP sample or diluted Input sample (2 ng/µl)
4.5 µl	Nuclease-free H20
7.5 µl	2× SYBR Green mix (containing polymerase)
2 µl	5 µM target primer mix (containing both Forward and Reverse primers)
15 µl	Total reaction volume

Amplify using the following PCR protocol:

3 min at 95°C.

40 cycles of 30 s at 95°C, then 30 s at 60°C.

Include a 70–95°C melting curve at the end of the qPCR program, reading all points or every 0.2°C.

Analyze the qPCR results by first manually determining the cycle threshold for each reaction across the plate within the linear range of the amplification curve. Calculate the average cycle threshold for each triplicate reaction of each sample. The relative DNA amount is then calculated for any given primer set as 2 to the power of the cycle threshold (cT) difference between input chromatin and ChIP samples, where cT is the average value.

$$\text{Relative DNA amount} = 2^{(\text{cT input} - \text{cT sample})}$$

The enrichment is then calculated by comparing relative enrichment for the target and a negative control. This is accomplished by dividing the relative DNA amount of each sample for a target primer

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set by the corresponding value for a negative control primer set. The resulting quotient represents the fold enrichment. The fold enrichment will vary depending on the histone marks as well as the location of the chosen target primer set (see Fig. 2a for an example).

3.8. Preparation of the Sequencing Library

The library protocol is based on the Illumina Sample Preparation Kit for Genomic DNA with some modifications. This protocol describes the preparation of libraries from ChIP DNA that are compatible with the Illumina sequencing platforms. Libraries are prepared from the ChIP sample as well as matching input DNA from the same cell type (see Note 9). Boiled chromatin samples should not be used since single-stranded DNA will lower the library preparation efficiency.

3.8.1. End-Repair

End-repair is performed using the "End-It DNA End Repair Kit" from Epicentre. This step ensures that all DNA fragments are converted to 5′-phosphorylated blunt-ended DNA. The entire ChIP DNA volume from Subheading 3.6 is used. Combine and mix the following components in a siliconized Eppendorf tube:

1–34 µl	ChIP DNA from Subheading 3.6 or 200 ng input DNA
5 µl	10× End-Repair Buffer
5 µl	10 mM ATP
5 µl	2.5 mM dNTP Mix
1 µl	End-Repair Enzyme Mix
50 µl	Total reaction volume

Incubate at room temperature for 45 min and purify DNA using a PCR purification kit (such as QIAquick PCR purification kit), elute in 34 µl EB buffer.

3.8.2. Addition of an "A" Base to the 3′ End of DNA Fragments

Before starting, prepare stocks of 1 mM dATP from 100 mM dATP stock (e.g., add 5 µl of 100 mM dATP to 495 µl sterile RNase DNase free water), and store aliquots of 11 µl at −20°C. Once thawed, 1 mM dATP solution should not be refrozen. Combine and mix the following components in PCR tubes:

34 µl	DNA from Subheading 3.8.1
5 µl	10× Klenow buffer
10 µl	1 mM dATP
1 µl	Klenow fragment (3′–5′ exo minus)
50 µl	Total reaction volume

Incubate for exactly 30 min at 37°C using a PCR machine. Purify DNA using a PCR purification kit (such as MinElute PCR purification kit), elute in 12 µl EB buffer.

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3.8.3. Ligation of Adapters to DNA Fragments

The Paired End Adapter Oligo mix is diluted 1:10 in water before use to adjust for the small quantity of ChIP DNA.

Combine and mix the following components in a siliconized Eppendorf tube:

12 µl	DNA from Subheading 3.8.2
15 µl	2× DNA ligase buffer
1 µl	1:10 dilution of PE Adapter Oligo mix
2 µl	LigaFast DNA ligase
30 µl	Total reaction volume

Incubate for 15 min at room temperature. Purify DNA using a PCR purification kit (such as QIAquick PCR purification kit), elute in 19 µl EB buffer.

3.8.4. Size Selection of DNA Fragments

Size selection of the sample ensures removal of unused adapters and selection of proper fragment size for amplification and sequencing (see Note 10). We use precast agarose gels to minimize risk of contamination.

1. Dilute 10 µl of 6× Cyan/Orange with 50 µl EB buffer to obtain 1× Cyan/Orange buffer dye. Add 1 µl of 1× Cyan/Orange buffer dye to eluted DNA from Subheading 3.8.3.
2. Prerun e-gels according to manufacturer's instructions.
3. Load 20 µl of appropriately diluted 50 or 100 bp DNA ladder (500 ng ladder per well) in one well per gel. Skip at least one well between marker and samples to avoid contamination.
4. Load 20 µl DNA with dye in each well. Skip at least one well between samples to avoid contamination.
5. Load 20 µl of EB buffer in each of the empty wells.
6. Run the gel until desired size separation is achieved (30 min for e-gels).
7. Take a gel picture to visualize sample.
8. Size select samples: using a fresh razor blade, excise two gel pieces of 200–400 bp and 400–600 bp (see Note 11). The DNA concentrations may be too low for the sample to be visible by eye; in this case, use markers as a guide. Keep exposure to UV light to a minimum to reduce DNA damage. Alternatively, a non-UV transilluminator can be used. Gel slizes can be stored at –20°C.
9. Solubilize gels at room temperature using the QIAgen gel extraction buffer by shaking on the vortexer for 30 min.
10. Purify on one QIAquick column using QIAquick PCR Purification Kit. Elute in 25 µl EB buffer.

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3.8.5. Amplification of Adapter-Modified DNA Fragments and Gel Purification

Because we make libraries from both the small (200–400 bp) and the big (400–600 bp) size selected DNA fragments from Subheading 3.8.4, we prepare two amplification reactions (and two libraries) per ChIP sample. We also prepare a 200–400-bp and a 400–600-bp size-selected input library. Therefore, if all six histone modifications are analyzed, there will be 14 amplification reactions and 14 libraries (12 ChIP libraries and 2 input libraries). For these reactions, dilute Paired End primers 1:4 with sterile water.

Combine and mix the following components in PCR tubes:

23 µl	DNA from step 2
25 µl	2× Phusion DNA polymerase
1 µl	Paired End PCR primer 1.01 (1:4 dilution)
1 µl	Paired End PCR primer 2.01 (1:4 dilution)
50 µl	Total reaction volume

Amplify using the following PCR protocol:

30 s at 98°C.

15 cycles: 10 s at 98°C, 30 s at 65°C, 30 s at 72°C.

5 min at 72°C.

Hold at 4°C.

Perform 15 cycles of amplification.

3.8.6. Library Purification

Purify library samples from Subheading 3.8.5 using the Agencourt AMPure system following manufacturer's instructions.

1. Mix Ampure beads thoroughly before addition.
2. Add 90 µl of Ampure beads to each 50 µl DNA sample from step 5. Pipette several times to ensure proper mixing.
3. Use magnetic rack to separate bead–DNA complexes and discard the supernatant. Allow beads to settle, this may take several minutes.
4. Wash bead–DNA complexes using 70% ethanol without disturbing the beads. Leave the tube in the magnetic rack and add 200 µl of 70% ethanol. After 30 s, discard 70% ethanol by pipetting.
5. Repeat wash one more time for a total of two washes.
6. Allow beads to air dry for 10–20 min.
7. Add 30 µl of EB buffer and elute DNA on the vortexer for 30 min.
8. Place tubes back in magnetic rack to collect DNA, transfer liquid to siliconized Eppendorf tubes. Store libraries at –20°C.

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3.9. Library Quality Control Assays

3.9.1. Library Quantification

The constructed libraries are assessed on a Bioanalyzer using the DNA High Sensitivity chip. The High Sensitivity DNA chip allows sizing and quantification of DNA samples in the single-digit pg/μl concentration range; the sequencing flow cell is loaded according to the library concentration (check with your sequencing facility for their requirements). The Bioanalyzer also allows visualization of possible adapter contamination; adapter dimers are visible as a sharp peak at approximately 120 bp. Libraries having large adapter dimer peaks should not be sequenced (contaminating adapter dimers can be removed by an additional gel size selection step as described in 3.8.4). For libraries that are not quantifiable, five additional cycles can be performed as in Subheading 3.8.5. However, over-amplification should be avoided to reduce the risk of PCR artifacts.

3.9.2. Library Enrichment Confirmation

To verify that a ChIP library has maintained a specific enrichment of target sites, perform qPCR on the ChIP-seq library using both positive targets and negative control primer pairs. The input library serves as a control to normalize the qPCR data to determine the relative enrichment of a given target (see Note 9).

A. Real-time quantitative PCR (qPCR)

Analyze the ChIP-seq sample as well as the appropriately sized input library for reference. Prepare a master reaction mix for each library with triplicate reactions per primer set. Add extra reagents for 10% of the total number of reagents to account for loss of volume. Add 15 μl of reaction mix to each PCR reaction well. Add 2 μl of primer mix to each well.

2 μl	2 ng library from Subheading 3.8.6
3.5 μl	Nuclease-free H_2O
7.5 μl	2× SYBR Green mix (containing polymerase)
2 μl	5 μM target primer mix
15 μl	Total reaction volume

Amplify using the following PCR protocol:

3 min at 95°C.

40 cycles of 30 s at 95°C then 30 s at 60°C.

Include a 70–95°C melting curve at the end of the qPCR program, reading all points or every 0.2°C.

B. Determine enrichment

Analyze library enrichments by qPCR as described in Subheading 3.7. The enrichment values vary depending on histone modification and placement of primers (see Fig. 2b, for an example). If enrichment is acceptable, then the sample can be provided to a sequencing facility (e.g., http://genomecenter.ucdavis.edu/dna_technologies/) for high-throughput sequencing; do not proceed with sequencing unless the positive targets are at least 20-fold enriched.

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3.10. Library Sequencing and Data Analysis

After the sequencing is performed, the short tags (~25–50 nts) are mapped to the human genome, the tags that map uniquely to only one location in the genome are selected, the unique tags are extended to the average size of the library fragments (~200 nt), and then the extended fragments are grouped into consecutive bins running the length of each chromosome. The binned data can be visualized using the USCS browser (http://www.genome.ucsc.edu/) or the Affymetrix Integrated Genome Browser (http://www.affymetrix.com/partners_programs/-programs/developer/tools/download_igb.affx; see Fig. 2c). Target sites can be identified using a variety of peak calling methods (26–33). Most peak calling programs account for binding pattern in peak shape, but algorithms need to be adjusted for spreading histone marks covering larger regions of the genome. Sole-search is a peak-calling program that was initially developed to identify transcription factor binding sites, which typically display peaks (34). Applying this type of program to spreading histone marks such as H3K9me3 produces a large number of small peaks rather than identifying a binding region. Sole-search version 2 has been modified to address this issue and offers the choice of using the peak or histone method for peak calling (Blahnik et al., in preparation). We note that the histone method can be used to call peaks for both types of binding; it correctly identifies sharp peaks for H3K4me3, and also calls broad regions occupied by H3K9me3 (Fig. 3).

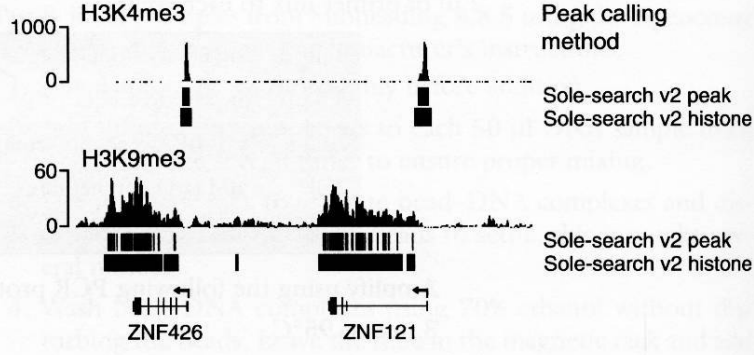


Fig. 3. Comparison of target identification for histone modifications having peak-like vs. spreading binding patterns. Libraries were prepared from ChIP samples using an antibody for H3K4me3 (K562 chromatin) and an antibody for H3K9me3 (Ntera2 chromatin) and were sequenced using the Illumina platform. As expected, sharp H3K4me3 peaks are observed proximal to the transcription start sites, whereas H3K9me3 covers larger chromatin regions; two zinc finger genes on chromosome 19 are shown to illustrate the different binding patterns (the number of tags is plotted along the *y*-axis and the hg19 chromosomal coordinates are shown on the *x*-axis). Targets were identified with Sole-search version 2 using the peak method as well as the histone method of the program; targets identified using both methods are depicted underneath each ChIP-seq signal track. The peak method (alpha value 0.01; FDR 0.001) works well for H3K4me3 but not for H3K9me3, whereas the histone method (alpha value 0.0001; FDR 0.01) can identify the sharp peaks in the H3K4me3 dataset as well as the broad binding regions of the H3K9me3 dataset.

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The number of reads required to identify all sites bound by a particular histone mark depends on the characteristics of this mark. Similar to most site-specific transcription factors, the binding patterns of certain modified histones (H3K9ac and H3K4me3) can be identified as sharp peaks. For analysis of these modifications throughout the human genome, ten million sequenced tags should be sufficient. With the current Illumina GA2 platform, 20–40 million sequenced reads can be obtained routinely in a single sequencing run and 75% or more of the reads can be mapped to the human genome. Thus, one lane is usually sufficient to identify the regions bound by H3K9ac and H3K4me3. However, reads should ideally come from two independent ChIP samples, with the binding sites identified in each replicate having at least a 60% overlap. Therefore, a minimum of two lanes of sequencing (one lane each from two independent ChIP assays) are usually performed for each of the histone modifications. As noted above, certain histone modifications do not have a peak-like binding pattern, but instead spread over large chromatin regions. Spreading histone marks require more reads to identify significant enrichment over background. For spreading marks such as H3K36me3, H3K4me1, or H3K27me3, 20–40 million reads may be required. H3K9me3 is not only a spreading mark but it is also present on repetitive regions of the genome (such as centromeres). For this mark, up to 50% of the reads may map to more than one place in the human genome and are discarded from analysis. Thus, up to 40–80 million sequenced reads may be required to achieve 20–40 million mapped reads (see Fig. 4). For the Illumina GA2 machine this translates to ~2–3 lanes of sequencing. Fortunately, the newest Illumina technology (HiSeq) will greatly increase the number of reads/lane. However, HiSeq will generally provide more reads/lane than is required for many site-specific factors or certain modified histones. Therefore, multiplexing and barcoding of ChIP-seq libraries will become necessary to ensure the most cost-effective sequencing strategy (35).

4. Notes

1. Use the primer design program Primer3 (36) or another suitable program to design the target and control primers, making the product length 90–150 bp. If possible, design primers for at least two positive targets as well as for two negative control regions. Before testing the ChIP sample or library, it is important to determine that the primers work for the SYBR Green-based real-time PCR assay. To do this, use input DNA and run a melting curve following the real-time PCR reaction conditions and view the dissociation curve to ensure that the desired amplicon was detected, as seen by a single peak.

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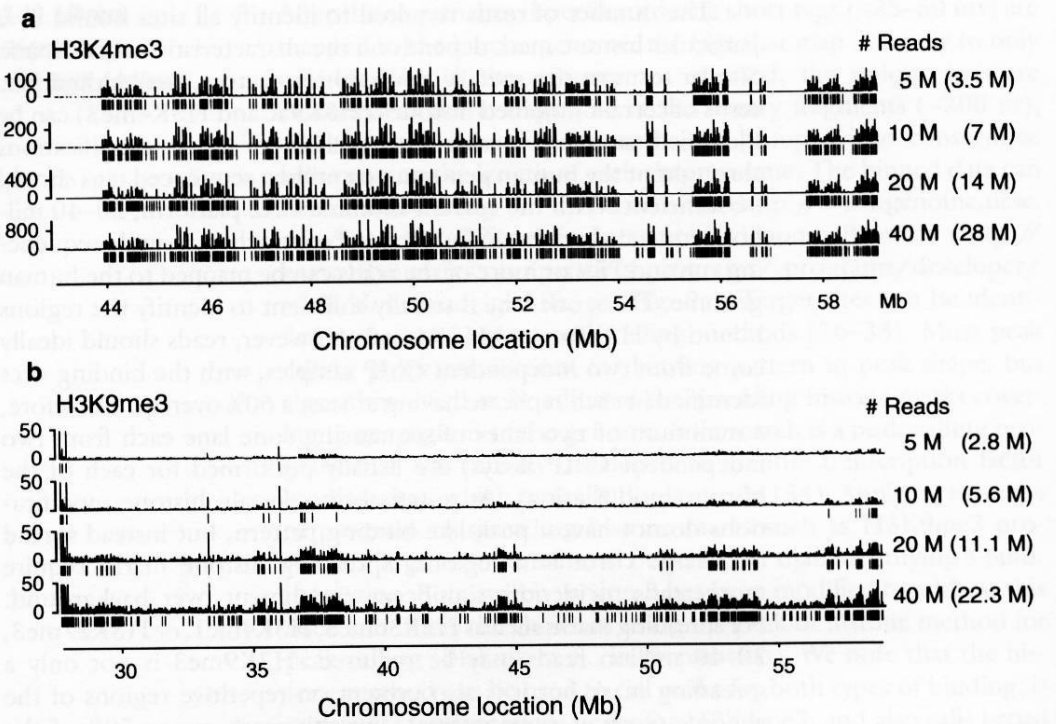


Fig. 4. Required read number varies depending on the histone mark. The ChIP-seq binding patterns from 5, 10, 20, and 40 million (M) sequenced reads for the libraries described in Fig. 3 are shown for a region on chromosome 19 (hg19 coordinates). The number of sequences that uniquely mapped to the genome is given in *brackets* and is representative for a typical ChIP-seq experiment. Peak height is plotted along the *y*-axis and the chromosomal location is shown on the *x*-axis. Called peaks are indicated as *black bars* below each track. While ten million reads are sufficient to identify most H3K4me3 sites in the human genome, more reads are required for the spreading mark H3K9me3. For example, in the region shown, only one peak is called with five million reads and only 20 peaks are identified with ten million reads; appropriate peak calling is achieved when ~40 million reads are analyzed.

2. Chromatin can be sheared by sonication or digested by micrococcal nuclease. Although both methods work well, this protocol is based on sonication. An alternative method using micrococcal nuclease is available from Cell Signaling Technology (http://www.cellsignal.com).
3. Sonication conditions should be optimized for each cell type and chromatin size determined before processing large quantities of cells, especially if the cells are collected from patients. We use the BioRuptor UCD-200 (Diagenode) on high setting for sonication. Wear hearing protection! Volumes between 0.5 and 2 ml are sonicated in 15 ml tubes, volumes between 0.1 and 0.3 ml are sonicated in 1.5 ml Eppendorf tubes, and volumes between 10 and 100 µl are sonicated in 0.5 ml tubes. The pulse duration, intensity, and number will vary depending on the sonicator, the extent of cross-linking, and cell type.

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Ideally, the least amount of input energy that gives satisfactory fragmentation should be used. We commonly sonicate 20–30 min (pulses of 30 s at setting high, with 1.5 min pauses in between pulses).

4. It is recommended to prepare chromatin and set up ChIP assays on the same day for best results. If necessary, unused chromatin can be snap frozen in liquid nitrogen and stored at −80°C for use at a later time.
5. In some cases the cell number is extremely limited. As an alternative to determining chromatin yield before the ChIP assay, chromatin can be isolated after incubation with magnetic protein G beads in Subheading 3.4, step 2. Do not discard the supernatant, but reverse crosslinks and purify DNA instead. This sample can substitute for the Input sample.
6. A typical chromatin yield ranges from 5 to 10 μg chromatin per one million tissue culture cells. In our experience, primary cells often give lower chromatin yields than cultured cells. If the yield drops below 1 μg chromatin per one million cells, the ChIP reaction mixture becomes too dilute and the ChIP quality may be significantly compromised.
7. While manual ChIP assays are very reproducible when performed by experienced investigators, the IP-Star® (Diagenode) offers an alternative by automating Subheadings 3.4 and 3.5. The ChIP robot can perform up to 16 ChIP assays at a time, requiring only manual set-up of the tubes and solutions. Automation of the ChIP assay can enhance consistency of ChIP results from one experiment to the other (especially for novice researchers) or between different users. We use the IP-Star in combination with the Auto ChIP kit (Diagenode) following the manufacturer's instructions. Typically, an automated ChIP assay starts with 1–5 μg chromatin (see Fig. 2 for PCR analysis of ChIPs performed using 1 μg of chromatin and the IP-Star); if higher chromatin amounts are required, it may be best to perform manual ChIP assays. Chromatin concentration is important since the chromatin sample has to be diluted to a total reaction volume of 100 μL with Buffer A (Diagenode) to ensure appropriate dilution of the SDS that is present in the nuclei lysis buffer. Therefore, the chromatin sample used per ChIP should not exceed 20 μL to allow fivefold dilution with buffer A; more dilute chromatin (higher volumes) may decrease ChIP enrichment and are thus not desirable. Volumes of antibody and Protein G magnetic beads used per assay are the same as described in the manual ChIP protocol. The main difference in the manual and the automated protocol is that in the IP-Star, protein G magnetic beads are coated with antibody before proceeding to 8 h of IP reaction.

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8. The biggest limitations to histone ChIP assays are the specificity of the antibody and the variability between different lot numbers of the same antibody. Companies have acknowledged this problem and some are making an effort to provide antibody specificity information. However, the number of ChIP validated antibodies is still very small and it is extremely important to test histone antibodies for cross reactivity with other histone marks. Antibody specificity can be tested using histone dot blots (each dot on the membrane contains a histone peptide carrying a specific modification(s)). The dot blot can be probed with the histone antibody of interest using a Western blotting protocol. Antibodies with high specificity will only recognize one dot corresponding to the histone modification(s) that was used to raise the antibody. Antibody efficiency can vary significantly between different batches, resulting in variation of the quality of the resultant ChIP-seq data. It is therefore important to record antibody details, such as catalog number, lot number, batch of affinity purification, etc. and to test each new antibody batch before performing a ChIP assay.
9. An input library is also critical for determining a baseline genome for the identification of binding sites. It is important that the input library size matches the size of the ChIP-seq library. For each cell type, 20–40 million sequenced tags of an input library are required. The same input library used to determine enrichment can be used for sequencing.
10. Gel size selection is the most variable step among protocols used by different investigators. For example, some protocols incorporate a size selection of the ChIP sample, whereas others size select after adapter ligation or after amplification (some protocols size select at more than one of these steps). We have chosen to perform size selection only once at the step after adapter ligation for three reasons: (1) each size selection results in loss of sample, so we limit the protocol to only one size selection step, (2) selecting after adapter ligation allows the removal of unincorporated adapters from the amplification reaction (which would not be possible if size selection was done after the ChIP assay but before adapter ligation), and (3) by selecting prior to PCR, we only amplify ChIP fragments of the proper length. This is especially important when the sonicated sample contained a significant fraction of fragments bigger than 500 bp (which can sometimes occur with certain cell types).
11. We prepare two libraries for each sample because the efficiency of sonication varies for different chromatin regions. Compacted chromatin sonicates less efficiently than accessible chromatin regions. The smaller portion of sonicated chromatin (200–400 bp) contains more promoter fragments, while fragments from compacted chromatin are overrepresented in the bigger

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fragment (400–600 bp). We proceed with both sized fragments through the library-making process and determine enrichment of positive controls by qPCR in both the large and small libraries for each sample. We typically find that the smaller sized library gives the highest enrichment for histone marks of open chromatin (e.g., H3K9Ac, H3K4me3, H3K4me1, and H3K36me3) whereas the larger sized library gives the highest enrichment for compacted chromatin (e.g., H3K9me3 or H3K27me3).

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Exhibit B

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Genome Biology

METHOD Open Access

Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density *in vitro* transposition

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Abstract

We characterize and extend a highly efficient method for constructing shotgun fragment libraries in which transposase catalyzes *in vitro* DNA fragmentation and adaptor incorporation simultaneously. We apply this method to sequencing a human genome and find that coverage biases are comparable to those of conventional protocols. We also extend its capabilities by developing protocols for sub-nanogram library construction, exome capture from 50 ng of input DNA, PCR-free and colony PCR library construction, and 96-plex sample indexing.

Background

Massively parallel DNA sequencing methods are rapidly achieving broad adoption by the life sciences research community [1,2]. As the productivity of these platforms continues to grow with hardware and software optimizations, the bottleneck experienced by researchers is increasingly at the front end (the construction of sequencing libraries) and at the back end (data analysis and interpretation) rather than in the sequencing itself.

The input material for commonly used platforms, such as the Illumina Genome Analyzer [3], the Roche (454) Genome Sequencer [4], the Life Technologies SOLiD platform [5], as well as for 'real-time' third-generation sequencers such as Pacific Biosciences [6], consists of complex libraries of genome- or transcriptome-derived DNA fragments flanked by platform-specific adaptors. The standard method for constructing such libraries is entirely *in vitro* and typically includes fragmentation of DNA (mechanical or enzymatic), end-polishing, ligation of adaptor sequences, gel-based size-selection, and PCR amplification (Figure 1a). This core protocol may be preceded by additional steps depending on the specific application, such as cDNA synthesis for RNA-seq libraries [7].

Although generally effective, several aspects of the standard method are throughput-limiting or otherwise suboptimal. These include: (1) Labor: there are several labor-intensive enzymatic manipulations with obligate clean-up steps. (2) Time: the protocol requires 6-10 hours from beginning to end, often including an overnight incubation. (3) Automation: although 96-plex, semi-automated processing has been achieved by large-scale genome centers [8], many researchers lack access to the requisite robotic liquid handling systems and/or instruments for parallelized mechanical fragmentation. (4) Sample indexing: incorporation of barcoded adaptors, which enable concurrent analysis of multiple samples and post-sequencing deconvolution, still requires most steps to be carried out on individual samples prior to pooling [9]. (5) High input requirements: standard protocols for shotgun DNA sequencing suggest 1-10 μg DNA as input material per library. This is often not possible, for example in cancer genomics where sample material can be limited. (6) Coverage bias: biases in sequence coverage correlated with G+C content can arise from steps secondary to library construction, including gel purification [10] and PCR amplification [11]. Amplification-free versions of these protocols may reduce G+C biases and eliminate PCR duplicates [11,12], while potentially increasing input requirements.

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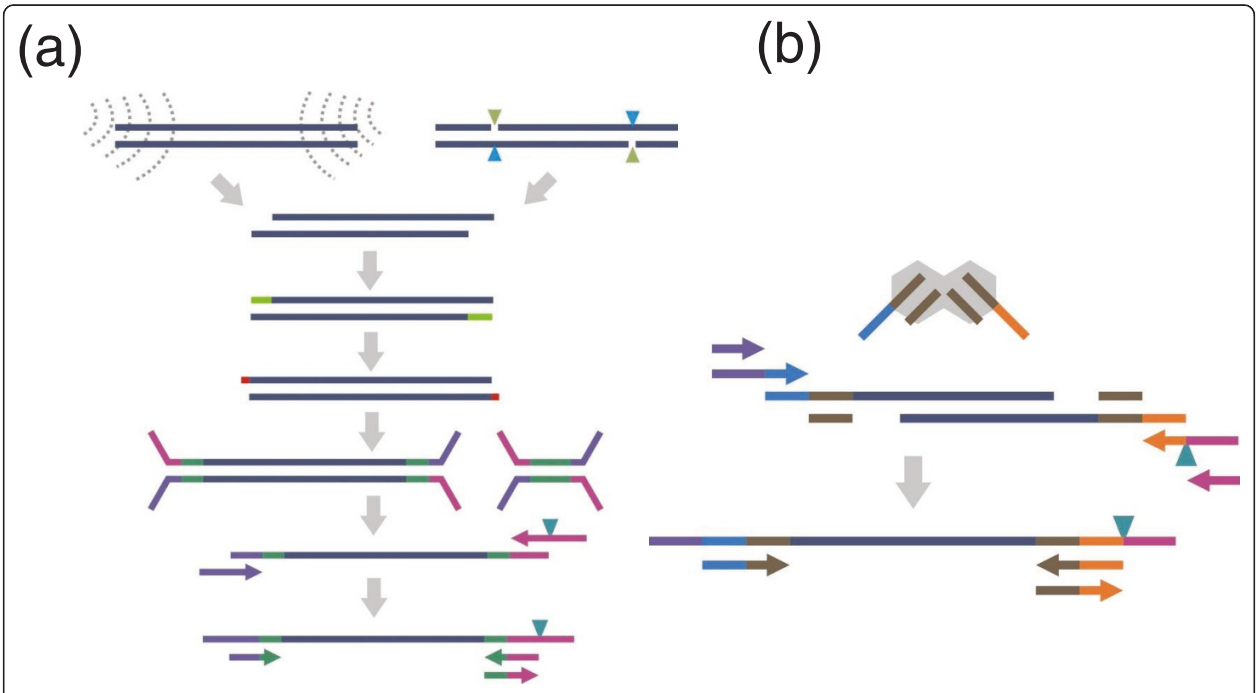


Figure 1 Methods for constructing *in vitro* fragment libraries. **(a)** In the conventional protocol, mechanical or endonuclease fragmentation is followed by end-polishing, A-tailing, adaptor ligation and PCR. **(b)** With transposase-mediated adaptor insertion, fragmentation and adaptor insertion occur in a single 5-min *in vitro* step, followed by PCR. For both methods, a primer-embedded sample-specific barcode can be incorporated during PCR amplification (black triangle). Dark blue: Genomic DNA. Light green: End repaired sequence. Red: A-tail. Magenta/dark green and purple/dark green: Adaptors. Mid blue/brown/orange: Transposase adaptors. Cyan/light green triangles: Endonuclease fragmentation. Grey curved dotted lines: Sonication. Grey hexagon: Transposase.

In the alternative approach that we characterize and extend here, a hyperactive derivative of the Tn5 transposase is used to catalyze *in vitro* integration of synthetic oligonucleotides into target DNA at a high density ('Nextera', Epicentre, Madison, WI, USA). Wild-type Tn5 transposon DNA is flanked by two inverted IS50 elements, each containing two 19 bp sequences required for function (outside end and inside end). A 19 bp hyperactive derivative (mosaic end, ME) is sufficient for transposition provided that the intervening DNA is long enough to allow the two ends to come in close proximity in order to form a complex with a Tn5 transposase homodimer. The relatively low activity of the wild-type Tn5 transposase was cumulatively increased through several classes of mutation [13]. In a classical *in vitro* transposition reaction, hyperactive Tn5 transposomes (hyperactive transposase mutant bound to ME-flanked DNA) bind target DNA and catalyze the insertion of ME-flanked DNA into the target DNA with high frequency [14]. When free synthetic ME adaptors are used instead (isolated from one another, in contrast to ME-flanked DNA in which two ME sequences are linked by the intervening DNA), transposase activity results in fragmentation and end-joining of the synthetic ME adaptor to the 5' end of target DNA. To generate fragment libraries compatible with massively parallel DNA sequencing, limited-cycle PCR is used to append platform-specific primers (Figure 1b).

Significant potential advantages of transposase-catalyzed adaptor insertion as a library preparation method, relative to conventional library preparation, include, firstly, many fewer steps, as the fragmentation, polishing, and ligation steps are replaced by a single 5-minute reaction and optional 10-minute pre-PCR clean-up (Figure 2). Libraries requiring particularly constrained insert size distributions (such as for *de novo* assembly) may optionally be subjected to chip- or gel-based size selection, increasing preparation time by 1 hour or 3-4 hours, respectively. The second advantage is greatly reduced input requirements while maintaining library complexity. This is expected to be possible because of a more efficient conversion of input DNA into sequencing-compatible material. However, these potential advantages are balanced by the competing concern that transposase-mediated fragmentation will introduce significant sequence-dependent biases relative to conventional library construction.

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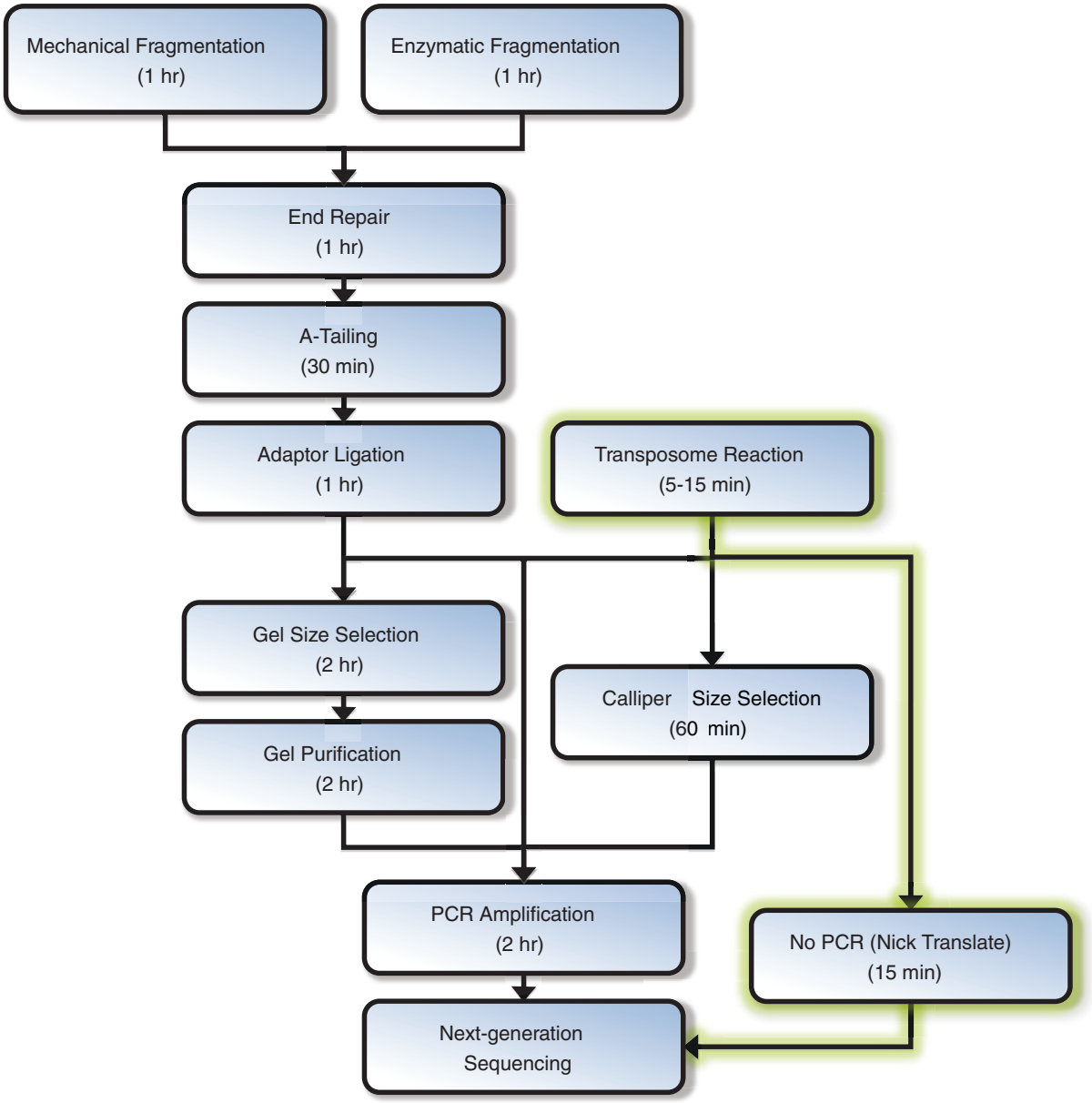


Figure 2 Schematic of steps associated with different library preparation methods. Transposase-catalyzed adaptor insertion significantly reduces the number of steps and time associated with library construction (green path).

Here, we report the results of an extensive comparison of transposase-catalyzed fragmentation with standard library construction protocols. We also describe the development of several derivative protocols for transposase-catalyzed fragmentation that significantly extend its capabilities. To evaluate performance with respect to key parameters including sequence-dependent biases, we compared methods across several organisms and sequencing platforms, including whole genome sequencing of a cell line derived from a previously sequenced human, YH1 [15], on a single flow-cell with the Illumina HiSeq platform. New protocols reported here that extend the utility of this method include: (1) a 96-plex sample indexing scheme, validated on 96 bacterial genomes; (2) capture and sequencing of the complete coding exon content (exome) from 50 ng of input human genomic DNA; (3) a protocol for the construction and sequencing of shotgun libraries from as little as 10 pg of starting material; (4) a PCR-free version of the method that mitigates associated G+C biases and decreases the

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total time for library preparation time to less than 30 minutes; and (5) a method analogous to 'colony PCR' for single-step preparation of genomic sequencing libraries directly from bacterial colonies.

Results

Comparison of standard versus transposase-based protocols

We performed a side-by-side comparison of three protocols: (1) standard library construction with mechanical fragmentation; (2) standard library construction with time-dependent endonuclease-based fragmentation ('dsDNA fragmentase', NEB); and (3) transposase-catalyzed adaptor insertion ('Nextera', Epicentre). To evaluate performance on the Illumina platform, sequencing libraries and technical replicates were prepared from two genomic DNA samples (*Homo sapiens* NA18507, *Escherichia coli* CC118) with each of the three methods. Paired-end, 36 bp reads were generated on an Illumina Genome Analyzer IIx (GAIIx). Reads were mapped using BWA [16] to the *E. coli* genome (K12) or human genome (hg18) as appropriate. To evaluate performance on the Roche (454) platform, sequencing libraries were constructed from two bacteriophage DNAs (CRW10 and PA1) with each of the three methods. Libraries were sequenced on a Roche (454) Genome Sequencer FLX, followed by *de novo* assembly (gsAssembler) and read mapping (gsMapper) to the appropriate reference genome. A summary of samples processed and sequence data generated on both platforms is provided in Table S1 in Additional file 1.

Sites of mechanical fragmentation, endonuclease fragmentation, and transposase-catalyzed adaptor insertion were characterized by calculating nucleotide composition in the vicinity of the mapping position of the first base of each sequence read (the fragmentation site; Figure S1 in Additional file 2). This revealed a slight but highly correlated bias for mechanical and endonuclease fragmentation, which suggests that most bias for these two methods is introduced after these protocols converge (for example with A-tailing or adaptor ligation), and that both mechanical fragmentation (here, either acoustic sonication or nebulization) and endonuclease fragmentation (with dsDNA fragmentase) have very low intrinsic biases. In contrast, a more extended signature is observed for sites of transposase-catalyzed adaptor insertion, weakly resembling the reported insertion preference of the native Tn5 transposase (AGNTYWRANCT, where N is any nucleotide, R is A or G, W is A or T, and Y is C or T) [17]. However, when calculated in terms of per-position information content, the bias of transposase-catalyzed adaptor insertion is low, and only slightly greater than the other protocols. For *E. coli* data, maxima of per-position information content over ± 10 bp, on a two-bit scale for fixed positions, are 0.10, 0.11, and 0.16 for mechanical fragmentation, endonuclease fragmentation, and transposase-catalyzed adaptor insertion, respectively. Average information content over ± 10 bp are 0.0056, 0.018, and 0.049, respectively. Equivalently low information contents were observed for human and phage libraries (Table S2 in Additional file 1). The effective bias associated with transposase-catalyzed adaptor insertion is thus greater than with standard library construction, but only modestly so. For *E. coli* and human libraries, signatures of bias were consistent in technical replicates for all three methods.

The greater insertion bias is problematic in a practical sense only if it has a significant impact on the distribution of genomic coverage. Consistent with the low calculated information content of the observed biases, the gross distributions of genomic coverage observed for the three methods are very similar (Figure 3a, b), the exception being the PA1 bacteriophage library, which may be skewed as a result of sequence context in a relatively small genome. Furthermore, similar biases in coverage are observed for different G+C content bins, with reduced representation at both extremes (Figure 3c). As PCR was used to prepare libraries constructed with all three methods, the consistent G+C bias probably arises at that step [11]. We initially predicted that the similar genomic coverage distribution associated with each method was due to factors introduced after the three protocols converge on common steps (solution phase PCR, cluster PCR, and sequencing). However, the correlation in coverage between methods at a per-base level was modest, with transposase-catalyzed adaptor insertion the least correlated with the other methods (Table S3 in Additional file 1).

In this comparative analysis, libraries generated by transposase-catalyzed adaptor insertion were sequenced directly after PCR (without size-selection), and the observed insert size distribution was considerably shorter than the other, size-selected, methods (transposase: 100 ± 47 bp, sonication: 256 ± 48 bp, endonuclease: 244 ± 56 bp; Figure S2 in Additional file 2). To evaluate whether a lower-bound on insert size exists, tails of long-read (101 bp) pairs were aligned to one another and a mapping-independent size distribution constructed, revealing a sharp decrease at about 35 bp that is probably a secondary consequence of steric hindrance of adjacent, attacking transposases (Figure 4). This phenomenon also explains the about 10 bp peaks at the lower end of the insert size distribution resulting from the helical pitch of the DNA as it extends away from the transposase.

With alternative buffer and reaction conditions, other target size ranges can be achieved. For example, the transposon method adapted for Roche (454) library

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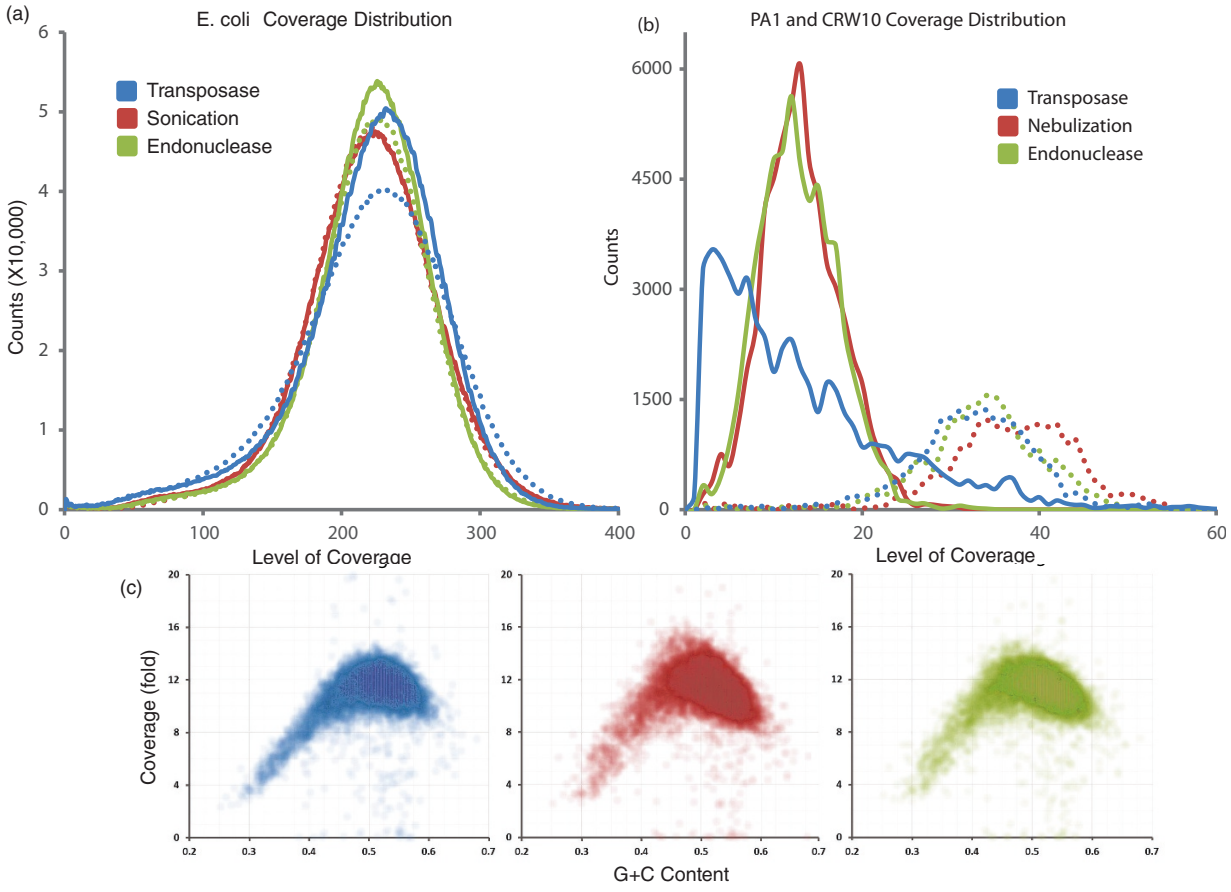


Figure 3 Comparison of coverage bias. **(a)** Coverage distribution across the *E. coli* genome with transposase (blue), sonication (red), and endonuclease (green) methods (solid lines) and replicates (dotted lines), normalized for total sequencing depth. **(b)** Coverage distribution across the PA1 and CRW10 bacteriophage genomes with transposase (blue), nebulization (red), and endonuclease (green) methods (dotted lines represent replicate libraries). **(c)** G+C bias for *E. coli* was assessed by calculating G+C content of the reference in 500 bp bins and plotting the coverage in each for transposase (blue), sonication (red), and endonuclease (green) methods, all of which show an approximately equivalent bias against the extremes.

construction resulted in significantly longer fragments (300-800 bp; Figure S3 in Additional file 2). To assess whether fragment size of libraries generated by transposase-catalyzed adaptor insertion could be constrained without resorting to gel-based size-selection, we evaluated alternative buffer and reaction conditions in combination with different approaches to post-PCR sample clean-up (Figure S4 in Additional file 2). Notably, an automated chip-based size-selection yielded well-constrained libraries (insert size 162 ± 28 bp).

Whole genome sequencing of human and *Drosophila* genomes

To assess performance further, we conducted whole genome sequencing on transposase-based libraries from *H. sapiens* and *Drosophila melanogaster*. Human genomic DNA from a previously sequenced individual, YH1 [15], was used to generate a series of libraries under different reaction conditions and size-selections that were then subjected to seven lanes of paired-end 90 bp (PE90) sequencing on the Illumina HiSeq platform. Of 934 million reads, 781 million were mapped [16] to the human genome (hg18) for 25× coverage. Although a total of seven libraries were constructed and sequenced to assess reproducibility, the complexity of each individual library was sufficient enough that whole genome sequencing could be carried out using a single library. Variant calling on mapped YH1 data was performed with *samtools* [18] requiring consensus Q30 at called positions (Figure S5 in Additional file 2). By these criteria, 3,556,679 SNPs were called (87% in dbSNP129; transition/transversion ratio (Ti/Tv) = 2.07), substantially greater than the 3,074,097 SNPs reported in initial sequencing of YH1. There were 2,922,525 SNPs shared between the analyses (91% in dbSNP129; Ti/Tv = 2.07), 634,154 SNPs unique to our analysis of this genome (70% in dbSNP129; Ti/Tv = 2.08), and 151,572 SNPs

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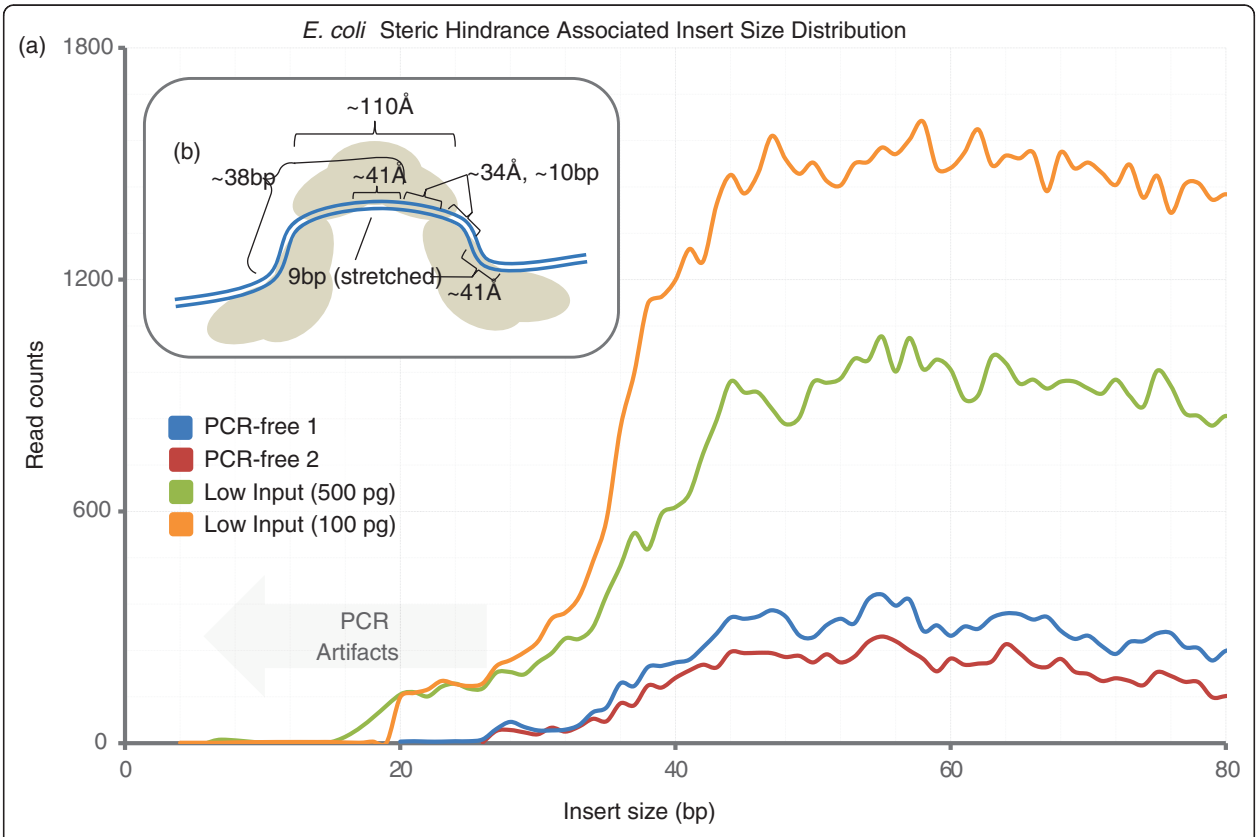


Figure 4 Insert size showing steric hindrance. **(a)** Insert size was generated from libraries spiked into a paired-end 101 bp run resulting in a large proportion of reads reading into the adaptor sequence. Tails of reads were then aligned to one another to discern the insert size between adaptors, resulting in a mapping-independent insert size at the lower extreme. All reads with an insert size less than 25 bp were PCR artifacts. **(b)** The noticeable drop below 40 bp is consistent with a model for complete saturation of transposition events on a given stretch of DNA. The roughly 110 Å transposase homodimer (grey) is bound to genomic DNA (blue), such that the core of the enzyme acts on a 9 bp region drawn out to 41 Å as well as approximately 10 additional bases of DNA flanking either side (~34 Å each) that are essentially protected from a subsequent transposase attack due to steric hindrance. Since the core region is duplicated in the process, the minimum spacing of transposition events is approximately 38 bp.

unique to the initial analysis of this genome (65% in dbSNP129; Ti/Tv = 1.18). The larger number of SNPs identified here may follow in part from greater mappability with longer read-lengths.

In this analysis, cell-line DNA derived from lymphoblasts was used; however, original sequencing of YH1 by Wang *et al.* (2008) [15] was carried out on blood DNA. Notably, 4,036 positions were called as mutations in the cell line and as the reference base in blood, both at a high quality score (30) and in uniquely mappable regions of the genome (see Methods). Of the 1,720 SNPs at a quality over 50 (Ti/Tv = 0.95), a randomly selected 100 were subjected to validation in DNA from blood, DNA from the primary culture used to generate the cell line, and DNA from the cell line. Interestingly, 63 were confirmed as mutations only in the cell line (Ti/Tv = 1.1; one failed assay in primary culture). Of the 37 positions that failed validation, 31 were confirmed as the reference base in blood, primary culture, and cell line (Ti/Tv = 0.48), and the remaining six positions were variant in all three (Ti/Tv = 1.0; one failed assay in primary cell culture). Further experimentation is required to determine whether the validated mutations observed in the cell line represent only mutations occurring during immortalization or propagation of the cell line, or the eventual fixation of somatic mutations present at very low frequencies in primary culture.

Importantly, coverage of YH1's genome in sequencing of libraries derived from transposase-catalyzed fragmentation was relatively uniform when compared with the data generated on this same individual from conventional libraries (Figure 5a). The observed GC bias in whole human genome sequencing data from the two methods was comparable (Figure 5c); however, a modest decrease (23%) in coverage at bins with high GC content (≥60%) was observed with the transposase method. This decrease can potentially be mitigated by a PCR-free version of this

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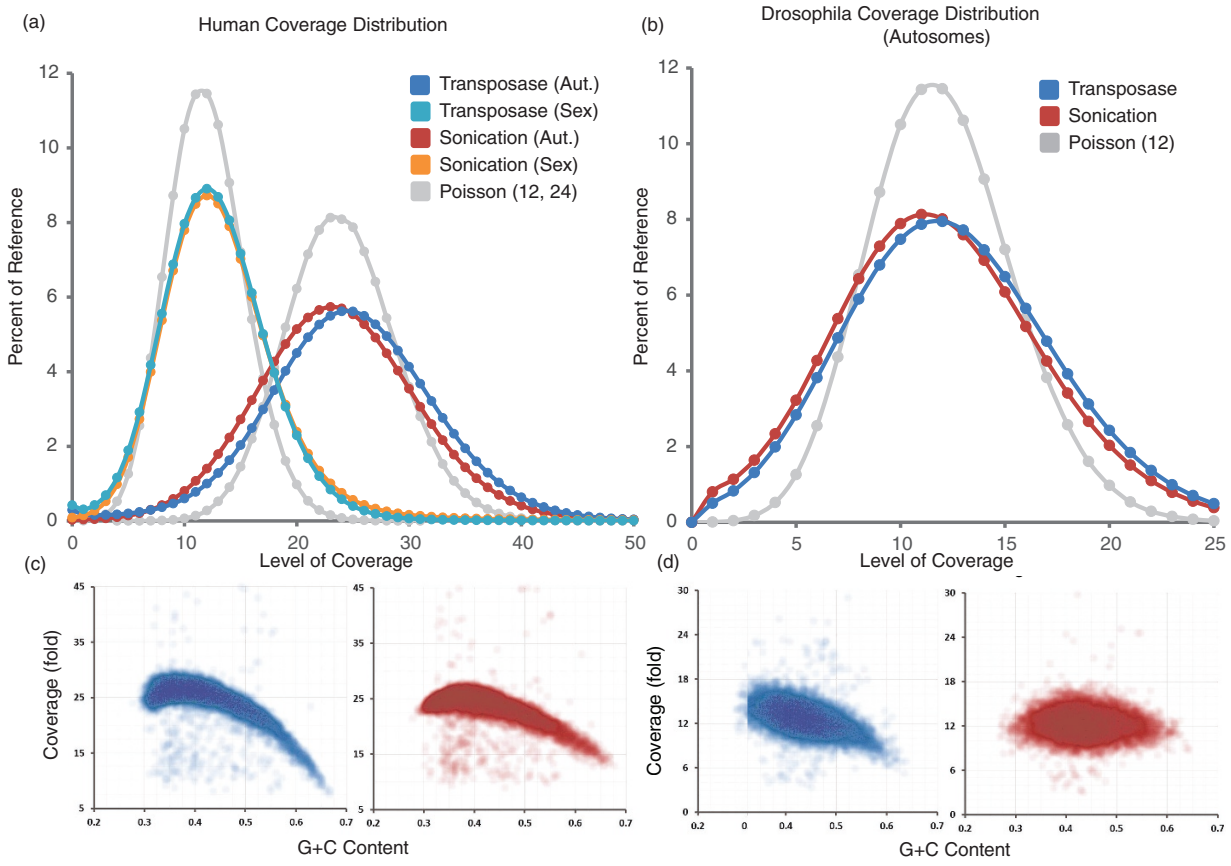


Figure 5 Sequence coverage of human and *Drosophila*. **(a)** Coverage distribution as a percentage of the genome for human (YH1) using transposase (dark blue, autosomes; light blue, sex chromosomes) and sonication [15] (down-sampled to equivalent coverage; red, autosomes; orange, sex chromosomes) methods. Poisson (no bias) distributions (gray) with $\lambda = 12$ (sex chromosomes) and $\lambda = 24$ (autosomes) are also shown. Poisson distribution is the expected if there were absolutely no bias. **(b)** Coverage distribution as a percentage of *Drosophila* autosomes using transposase (blue, down-sampled to equivalent coverage) and sonication (*Drosophila* Population Genomics Project (DPGP), red) methods, as well as Poisson distribution with $\lambda = 12$ (gray). **(c,d)** Coverage with respect to G+C content of the reference in 10 kb or 1 kb bins for (c) human (YH1) and (d) *Drosophila* genomes respectively, for transposase (blue) and sonication (red) methods at comparable global genomic coverage.

method (discussed below), or by alternative PCR conditions (data not shown). For the *Drosophila* genome (dm3), one lane of PE45 sequencing of a transposase-based library on an Illumina GAIIx yielded 16× mean coverage. As with the human genome, the distribution of coverage was largely equivalent to that observed in sequencing *Drosophila* with standard libraries (Figure 5b, d) along with the modest decrease in coverage for regions of high GC content. For both human and *Drosophila* genomes, the signature of bias in the vicinity of insertion sites was similar to that observed in the comparative analysis.

Complexity, that is, the number of molecules of distinct origin, is a critical aspect of shotgun library quality, especially for libraries that will be deeply sampled or subjected to further bottlenecking, such as hybrid capture or size selection. Low complexity manifests as an excess of duplicate reads with identical mapping coordinates, which arise from the same progenitor molecule and can thus skew downstream analyses including SNP calling and genotyping. The complexity of each prepared library here was analyzed by incremental sampling of 50,000 read-pairs without replacement and plotting the number of read-pairs sampled versus the number of unique read-pairs (as determined by mapping location) within the sample. In this analysis, the extent of deviation from linearity provides a measurement of sample complexity. All human genome and *Drosophila* genome libraries sequenced here were found to be highly complex, each comprising over 96% unique read-pairs, even as sequencing depths approached 100 million read-pairs, indicating a single such library could be used for whole genome sequencing (Figure 6). The high complexities achieved are consistent with a high efficiency of conversion of input mass into sequence-

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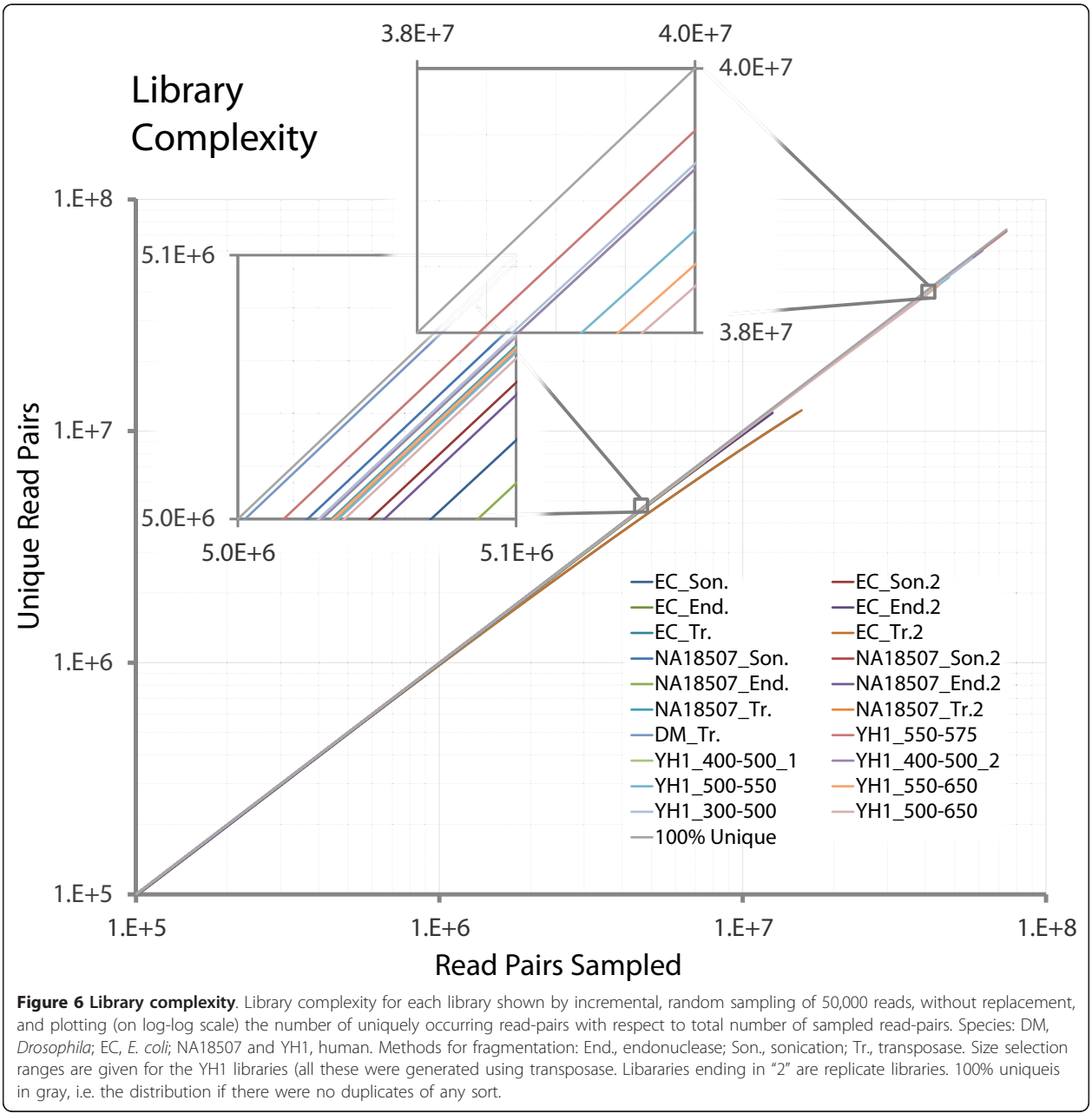


Figure 6 Library complexity. Library complexity for each library shown by incremental, random sampling of 50,000 reads, without replacement, and plotting (on log-log scale) the number of uniquely occurring read-pairs with respect to total number of sampled read-pairs. Species: DM, *Drosophila*; EC, *E. coli*; NA18507 and YH1, human. Methods for fragmentation: End., endonuclease; Son., sonication; Tr., transposase. Size selection ranges are given for the YH1 libraries (all these were generated using transposase. Libararies ending in "2" are replicate libraries. 100% uniqueis in gray, i.e. the distribution if there were no duplicates of any sort.

compatible material as compared with conventional library construction. Less complexity was consistently observed for all *E. coli* libraries, but this is likely to be because we are simply saturating the set of possibilities for start-point pairs that are used to identify PCR duplicates by deep sequencing of a small genome.

Low input targeted sequence capture of the human exome

Exome capture is an increasingly mature technology, but standard protocols require several micrograms of input genomic DNA, which can be problematic when sample is limiting (for example with tumor samples). We subjected a library from 50 ng human genomic DNA by transposome fragmentation to exome capture (Nimblegen SeqCap EZ Exome probes v1.0). Because the adaptor sequences are different from those in libraries prepared using mechanical shearing, custom blocking oligonucleotides were designed and used. After capture, the library was subjected to pre-sequencing real-time PCR with standard primers followed by sequencing on an Illumina GAIIx (SE36). The resulting reads were

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aligned to the human genome (hg18) with 78% mapping, of which 47% fell within 100 bp of a targeted exon (Figure S6 in Additional file 2). A direct comparison with an equivalent number of mapped SE36 reads from a standard library, after capture with the same kit, revealed nearly identical complexity for on-target reads (41% and 43% of an equivalent number of on-target SE36 reads with unique start sites for transposome-based and standard libraries, respectively), as well as comparable uniformity (87% and 82% of target bases covered with ≥1 reads for transposome-based and standard libraries, respectively). However, specificity was notably lower (47% of reads on or near target for transposome-based libraries versus 80% for standard libraries). Nonetheless, we note that the standard protocol has been extensively optimized in the context of production-level scaling, and it is likely that specificity in the capture of transposome-based libraries can also be improved upon. Furthermore, the disadvantage of lower specificity is balanced by the advantage of significantly lower input requirements for genomic DNA entering a targeted capture workflow (50 ng for transposase-based libraries versus 3 μg for standard libraries).

Sub-nanogram library construction

To push the limits on library construction using reduced starting material, *E. coli* libraries were generated from 500 pg and 100 pg genomic DNA and sequenced as part of a barcode pool. For each library, expected numbers of read counts were observed (0.5 and 0.6 million mapped reads, respectively) without a noticeable drop in complexity (both libraries over 98% at 0.5 million read-pairs), or coverage uniformity. Next, we generated a library from 10 pg human genomic DNA, or roughly three copies of the human genome, which produced over 2 million uniquely mapped read-pairs. Although complexity was reduced because of the significant decrease in progenitor molecules entering PCR, the potential advantages of sequencing from material approaching a single equivalent of the human genome are substantial.

PCR-free library construction

Standard sequencing libraries for the Illumina platform have been generated without the use of PCR amplification in order to reduce associated biases [11,12]. We developed a similar approach for transposase-based methods by including sequences corresponding to the primers used for cluster formation, i.e. the Illumina adaptor sequences, into the adaptors that are added during the transposition reaction, as opposed to incorporating them during PCR (See Methods). After transposition, a nick translation is performed resulting in Illumina-ready libraries. This method was used to sequence *E. coli* CC118 and human NA18507 with two replicates of each using 100 ng and 200 ng starting material. A noticeable decrease in G+C coverage bias was observed (Figure 7a). Furthermore, complexity for each of these libraries was over 98%. The development of PCR-free, transposase-based library construction reduces the full amount of time required for converting DNA to a sequencing-ready shotgun library to less than 30 minutes.

96-plex sample indexing

Second-generation sequencing platforms suffer from poor granularity. For example, in the data described above, a single PE90 lane on the Illumina HiSeq platform yielded 20 Gb of mappable sequence, which is far in excess of what is required for many projects. Sample indexing (or 'barcoding') is a useful solution, but reported protocols still require most steps of library

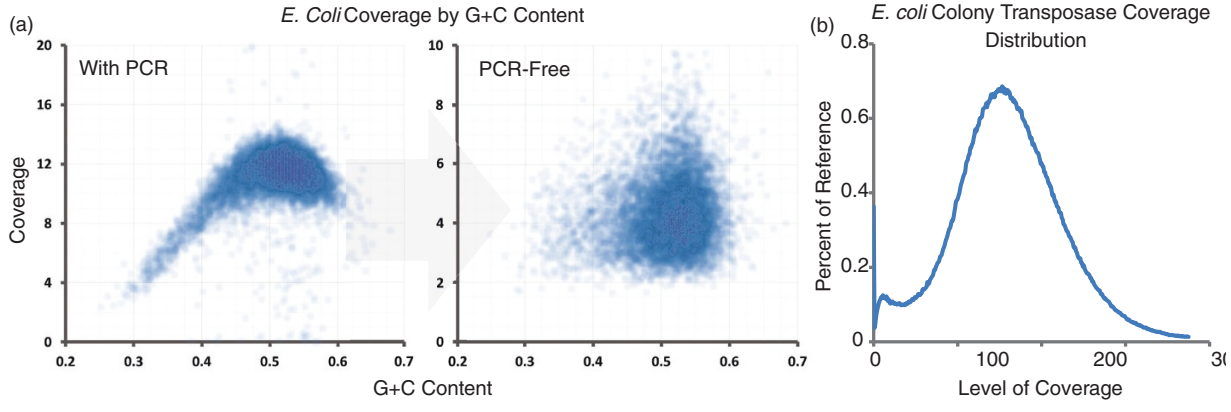


Figure 7 PCR-free reduction in G+C coverage bias and direct-from-colony coverage distribution. **(a)** Coverage with respect to G+C content in *E. coli* with and without PCR was assessed by calculating G+C content of the reference in 500 bp bins and plotting the coverage in each for transposase after PCR (left) and with the PCR-free method (right). A significant reduction in coverage bias at the extremes of G+C content is observed. **(b)** Coverage distribution for *E. coli* library prepared directly from cell lysate without purification.

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preparation to be carried out on individual samples prior to pooling, and also can suffer from non-uniform performance of individual barcodes [9]. With transposase-catalyzed adaptor insertion, sample indexing could potentially be introduced during adaptor insertion, or during the subsequent PCR step, that is, using a primer-embedded barcode sequence (Figure 1b). To evaluate the compatibility of this method with indexing, we attempted the latter approach. Ninety-six barcodes (9 bp) were designed with a minimal edit distance of four between all pairs and additional constraints on base composition (Table S4 in Additional file 3). Performance was evaluated by subjecting DNA from 96 evolved derivatives of *Pseudomonas aeruginosa* to independent library construction, each with a different barcode-embedded primer during PCR. Post-PCR amplicons were quantified and pooled, followed by several lanes of massively parallel sequencing (PE76, with a third read to collect the 9 bp index). Samples were deconvolved using 9 bp indexes: 92%, 3%, and 3% reads were assigned with 0, 1, and 2 mismatches, respectively, and only 4% could not be unambiguously assigned. With the exception of a few outliers, the distribution of barcode assignments across the 96 was relatively uniform with 90% within a fourfold range (Figure S7 in Additional file 2), as was the proportion of reads mapping to the reference, illustrating the robustness of the library protocol and the indexing scheme.

Constructing genomic libraries directly from bacterial colonies

In evaluating 96-plex sample indexing for 96-plex bacterial genome sequencing with transposase-catalyzed adaptor insertion, the burden of technical effort shifted from library preparation to the isolation of genomic DNA from each isolate. We speculated that integration of the transposase reaction into a 'colony PCR'-like workflow (in which cells from bacterial colonies are directly mixed into a PCR reaction without DNA isolation) could be used to further simplify library preparation for bacterial genome sequencing (Figure S8 in Additional file 2). A pipet tip was used to transfer a small number of cells directly from an *E. coli* colony transformed with pUC19 to a transposase fragmentation reaction (with heat-lysing prior to addition of enzyme). An aliquot of this reaction served as input for PCR amplification without any intervening clean-up step. Sequencing (SE36) yielded 27 million reads, for 170× coverage of the *E. coli* genome (81% of reads) and 37,000× coverage of pUC19 (10% of reads). The remaining 8% mapped to the F plasmid, or an insert cloned into pUC19, or remained unmapped. Coverage of the *E. coli* genome was uniform (Figure 7b). We propose that direct preparation of genomic sequencing libraries from bacterial colonies with no DNA isolation or intervening purification steps will be useful for rapidly preparing sequencing-ready, indexed fragment libraries from large numbers of bacterial isolates.

Discussion

Massively parallel sequencing platforms generally require the conversion of genomic DNA (or other nucleic acid sample) into a fragment library that includes common adaptor sequences that are used to mediate clonal amplification and/or the priming of sequencing reactions. Practical limitations of conventional approaches to generating these libraries include high requirements for labor, time and cost, as well as the low efficiency of mass conversion into sequencing-compatible material. Here, we evaluate an alternative approach in which transposase catalyzes the fragmentation of target DNA and insertion of adaptor sequences in a 5-minute, small-volume reaction. The workflow is thus markedly simpler than the conventional approach yielding significant savings in terms of time and labor (summarized in Figure 2). The input requirements are also over an order of magnitude lower than what is typically used with standard methods, and we demonstrate that high complexity libraries can be generated from as little as 100 pg of input DNA. Furthermore, input can be reduced to as low as just a few copies of the human genome and still produce a significant amount of sequence data. Taking advantage of the simplicity and low input requirements, we developed a method to construct libraries directly from bacterial colonies without DNA isolation or intervening clean-up steps.

Although there are significant advantages, this method nonetheless has its limitations. First, although there is a significant reduction in required steps and time, preparing very large numbers of libraries is still challenging without some degree of automation. Second, one has relatively limited control over the size distribution of fragmentation. In general, the trend is that the insert size distribution is smaller than desired when reacting to completion, and broader than desired when altering reaction conditions to increase the mean insert size. Third, genomes with high G+C contents show greater bias with this method than with conventional methods, although this is potentially correctable in part by the PCR-free approach or through modified PCR conditions. A related point is that we have also observed that performing transposase-catalyzed adaptor insertion on a single PCR product results in significantly greater bias than with shotgun libraries (J. Hiatt, personal communication), potentially secondary to a high molar concentration of a limited number of possible insertion sites. Fourth, we note that this library preparation does not solve an ongoing challenge in the field, which is how

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best to efficiently construct high-complexity, long-insert mate-paired libraries.

A comparison of sequence composition in the vicinity of fragmentation sites identified a bias signature that was larger and more extended than that associated with conventional methods. However, we found that this had little impact at the level of coverage for bacterial, human, and *Drosophila* genomes. Nonetheless, the observed impact may be greater in genomes with compositions that correlate with this bias pattern, or in smaller genomes such as bacteriophage. In our view, for most goals, the disadvantage of the slightly greater bias is offset by the large advantages that we observed with respect to speed, simplicity, and low input requirements.

A further reduction in preparation time was achieved in developing a PCR-free, transposase-based library construction method. This approach also decreased biases resulting from amplification, notably in coverage with respect to G+C content. Looking ahead, we anticipate that the partnership of extremely fast, simplified methods for library construction with third-generation 'real-time' sequencing methods may represent a critical path forward in reducing the time between acquiring biological material and obtaining analyzed sequence data to less than 1 hour.

Conclusions

Current methods for preparing *in vitro* DNA fragment libraries for massively parallel sequencing are suboptimal for projects involving limited amounts of starting material or large numbers of samples. Here we have characterized an alternative method of library construction in which highly active transposase catalyzes the simultaneous fragmentation and adaptor ligation in a single 5-minute incubation. Comparison with conventional methods of library preparation, relying on mechanical or endonuclease fragmentation, finds that although transposase-catalyzed adaptor insertion demonstrates a slightly greater insertion bias, this has little impact at the level of genomic coverage and is offset by large advantages with respect to speed, simplicity, and low input requirements.

To fully take advantage of the method and expand on transposase-catalyzed adaptor insertion we applied it on a larger scale, including sequencing a human genome on a single flow-cell of a massively parallel sequencing platform, and validating a 96-plex sample indexing scheme. We also show that transposase-catalyzed adaptor insertion can be integrated with exome sequencing workflows, with the advantage of significantly lower input requirements relative to conventional protocols for targeted sequence capture (50 ng versus several micrograms). In addition, we have generated libraries from as little as 10 pg of starting material and also developed a PCR-free library construction method in order to reduce associated biases and further reduce preparation time to less than 30 minutes. Finally, we demonstrate the direct preparation of genomic sequencing libraries from bacterial colonies with no DNA isolation or intervening purification steps.

Materials and methods

Genomic DNA

Genomic DNA for *H. sapiens* NA18507 (Yoruban) was prepared by the Coriell Institute. *E. coli* CC118 (MC1000 (*araD139* Δ(*ara leu*)*7697* Δ*lacX74 phoAΔ20 galE galK thi rpsE rpoB* $argE_{am}$ *recA1*)) [19], genomic DNA, and CRW10 and PA1 phage genomic DNA were extracted using Qiagen (Valencia, CA, USA) MidiPrep buffers P1, P2, P3 and were cleaned by phenol, purified from low melting point agarose and dissolved in Tris/EDTA (TE) buffer. YH1 genomic DNA was extracted from a lymphoblastoid cell line of Yanhuang [15] using protein K and phenol/chloroform [20] and further subjected to RNase treatment/purification. The *D. melanogaster* genomic DNA was extracted from whole bodies of several individuals by Puregene blood core kit B (Qiagen). The molecular weights of both *Drosophila* and YH1 genomic DNAs were confirmed to be larger than 23 kb by gel electrophoresis (not shown), with no detection of degradation or RNA/protein contamination, and quantified by Quant-iT dsDNA HS assay kit (0.2-100 ng; Invitrogen, Q32854, Carlsbad, CA, USA); of each, an aliquot was diluted to 25 ng/μl for use in library construction. *P. aeruginosa* PAO1 strains were selected for tobramycin resistance at 16 mg/l (41 strains), ciprofloxacin resistance at 4 mg/l (47 strains), or no antibiotic resistance (8 strains). DNA was then isolated using the Wizard® SV 96 genomic DNA purification system (Promega, Madison, WI, USA). Concentrations of isolated DNA were measured using a Nanodrop (Thermo Scientific, Waltham, MA, USA).

Mechanical shearing

Shearing was performed on two 5-μg aliquots of *E. coli* CC118 genomic DNA brought up to 200 μl with TE. Sonication was performed by two 15-min treatments with a Bioruptor sonicator (Wolf Laboratories, Pocklington, UK) at maximum settings in a cold room, switching out the water between treatments to keep samples cool. After sonication, each sample was cleaned up using a QIAquick PCR purification kit, eluting in 30 μl Buffer EB (Qiagen). CRW10 and PA1 phage DNA was fragmented using 5 μg aliquots brought to 100 μl with TE and mixed with 500 μl nebulization buffer (Roche/454, Branford, CT, USA) in a nebulizer cup. Nebulization was carried out using 45 psi (310 kPa) nitrogen for 1 min on ice. The sheared DNA was cleaned and

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concentrated using MinElute columns and eluting in Buffer EB (Qiagen).

Enzymatic fragmentation

Enzymatic fragmentation was performed on six 1 μg aliquots of genomic DNA from both *E. coli* CC118 and *H. sapiens* NA18507 added to 2 μl 10× fragmentation reaction buffer, 0.2 μl 100× BSA (NEBiolabs, Ipswich, MA, USA), 2 μl NEB fragmentase enzyme, and nuclease-free water (Ambion, Austin, TX, USA) to 20 μl. Reactions were gently vortexed and spun-down, then incubated on ice for 5 min followed by a time-course incubation at 37°C, removing samples at 15, 20, 30, 45, 60, and 120 min for each of the two sets. Reactions were stopped by placing on ice followed by purification using a QIAquick PCR purification kit, eluting in 30 μl buffer EB (Qiagen). 4 μl of each sample was run on a Novex TBE gel (Invitrogen) to observe size distribution. The 60-min time point showed the desired size range and duplicate samples were prepared by the same method for each organism. Fragmentation of phage DNAs were performed using 1 μg aliquots of PA1 and CRW10 in a 20 μl reaction volume including fragmentation reaction buffer to 1×, BSA, and 2 μl NEB fragmentase enzyme (NEB). The reaction was incubated on ice for 5 min followed by incubation at 37°C for 20 min and stopped with 5 μl 500 mM EDTA and cleaned using MinElute columns (Qiagen).

Post-fragmentation library preparation

Post-fragmentation library preparation on the duplicate samples of *E. coli* CC118 sonication and fragmentase (60 min), and *H. sapiens* NA18507 fragmentase (60 min), was carried out as per standard Illumina methods, including a size selection using Novex TBE gels (Invitrogen), excising the 400-500 bp band. Final PCR amplification was carried out on a Bio-Rad (Hercules, CA, USA) MiniOpticon using SYBR Green I as a dye to monitor amplification. 1 μl of each final library was run on a Novex TBE gel (Invitrogen) for library size confirmation. Nebulized or fragmentase-treated phage samples were size-selected using SPRI beads (Beckman Coulter, Danvers, MA, USA) and used to construct libraries according to standard protocols, including end-polishing, adaptor ligation, fill-in, and single-strand isolation. Adaptor sequences included multiplex identifiers (barcodes).

Transposase-based library preparation

Transposase-based library preparation for *E. coli* CC118 and *H. sapiens* NA18507 Illumina-compatible libraries used 50 ng of genomic DNA brought up to 15 μl in nuclease-free water (Ambion) followed by the addition of 4 μl 5× LMW Nextera reaction buffer and 1 μl Nextera enzyme mix (Illumina-compatible; Epicentre), followed by a gentle vortex and brief centrifugation. Each reaction tube was incubated at 55°C in a thermocycler with a heated lid for 5 min followed by placement on ice and immediate purification using a QIAquick PCR purification kit and elution in 20 μl buffer EB (Qiagen). Suppression PCR was then carried out using 10 μl of the eluate as template with 11.5 μl nuclease-free water (Ambion), 25 μl 2× Nextera PCR buffer, 0.5 μl SYBR Green, 1 μl 50× Nextera primer cocktail (Illumina-compatible), 1 μl 50× Nextera adaptor 2 (barcodes 1-2 for *E. coli* and 3-4 for *H. sapiens*), and 1 μl Nextera PCR enzyme. The reaction was cycled in a Bio-Rad MiniOpticon to monitor the reaction under the following conditions: (1×) 3:00 min at 72°C and (1×) 0:30 min at 95°C, followed by 13 cycles of [0:10 min at 95°C, 0:30 min at 62°C, 3:00 min at 72°C] for *E. coli* barcodes 1 and 2 and *H. sapiens* barcodes 3 and 4 (12 cycles for *H. sapiens* barcode 4). 1 μl of each post-PCR library was electrophoresed through a Novex TBE gel (Invitrogen) for library size confirmation. Size selection of the *E. coli* CC118 transposase libraries was carried out at Epicentre Biotechnology using Agencourt AMPure (> 300 bp size selection), Zymo DNA purification (no size selection), or Caliper (350 ± 10% bp size selection) methods. The *D. melanogaster* library was constructed by pooling two standard Nextera reactions following the manufacturer's protocol (Epicentre). For each reaction, 50 ng genomic DNA was initially tagmented (*in vitro* transposase-catalyzed adaptor insertion) at 55°C for 5 min, and then MinElute purified. This was followed by PCR amplification with same conditions as with *H. sapiens* and *E. coli* libraries for 12 cycles. 400-450 bp gel-based size selection was carried out prior to sequencing.

A total of seven *H. sapiens* YH1 libraries were constructed, differing in mass of DNA, number of PCR cycles, and selected DNA fragment size. These included two (about 500 bp and about 550 bp) produced from pooling five standard Nextera reactions, three (400-500 bp, 500-550 bp and 550-600 bp) produced from pooling two modified reactions with nine cycles of PCR enrichment, and another two libraries (300-500 bp and 500-650 bp) from a single tagmentation reaction using 500 ng starting DNA with five cycles of PCR enrichment. The insert-size distribution and final yields for the *Drosophila* and *H. sapiens* YH1 libraries were validated separately using a 2100 Bioanalyzer (DNA 1000 and 7500 kit; Agilent, Santa Clara, CA, USA) and quantitative PCR.

P. aeruginosa PAO1 Illumina-compatible shotgun libraries were prepared for each strain using Epicentre Biotechnologies' Nextera DNA sample preparation kits with a customized, unique 9 bp barcode sequence for each strain. The tagmentation reaction consisted of

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200 ng PAO1 DNA, 25 μl Nextera high molecular weight buffer, 1 μl Nextera transposase enzyme, and water to a total volume of 20 μl. The reaction was incubated for 5 min at 55°C, cleaned using Qiagen MiniElute columns, and eluted in 11 μl water. PCR reactions included 5 μl of the fragmented DNA, 17 μl water, 25 μl Nextera PCR buffer, 1 μl Nextera PCR enzyme, 1 μl of a Nextera primer cocktail containing two short primers (at 10 μM each) and one long Illumina-compatible adaptor (at 5 μM), and 1 μl of the barcode containing Illumina adaptor (at 5 μM). PCR conditions used were the same as above using 12 cycles of amplification, followed by MinElute clean-up as before. Samples were run on a Novex TBE polyacrylamide gel to confirm library quality, and DNA concentrations measured using a Nanodrop.

For the Roche (454)-compatible libraries, standard Nextera reaction conditions were used with 50 ng CRW10 (barcode 11) or PA1 (barcode 10) bacteriophage DNA, 454-Titanium compatible kit components and standard PCR methods, cycling 15 times. The PCR products were purified using Qiagen MinElute columns. Library fragment sizes were assessed using an Agilent Bioanalyzer DNA1000 chip.

Targeted sequence capture of the human exome

Libraries were prepared by transposase-catalyzed adaptor insertion by previously described methods using 50 ng genomic DNA (BK229.03, SFARI-SSC), 1 μl transposomes, 4 μl 5× HMW buffer, and water to 20 μl. Samples were incubated at 55°C for 5 min then cleaned up (AMPure) and eluted in 20 μl followed by the addition of 25 μl 2× Nextera PCR buffer, 1 μl 50× Nextera primer cocktail, 1 μl Nextera PCR enzyme, 0.5 μl 100× SYBR Green, and 1 μl of a barcoded adaptor (Table S4 in Additional file 3) with water to 50 μl. Reactions were carried out on a Bio-Rad MiniOpticon using recommended cycling conditions for 12 rounds. Each tube was cleaned up (AMPure, Agencourt, Boston, MA, USA) and checked for size and quantity on an Agilent Bioanalyzer DNA 1000 chip. One sample was selected for capture using all of the 414.6 ng for hybridization to Nimblegen (Madison, WI, USA) SeqCap EZ Exome probes v1.0 as per Nimblegen protocols using custom blockers (Nextera_Block1: 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACG CCT CCC TCG CGC CAT CAG AGA TGT GTA TAA GAG ACA G-3', Nextera_Block1_REV: 5'-CTG TCT CTT ATA CAC ATC TCT GAT GGC GCG AGG GAG GCG TGT AGA TCT CGG TGG TCG CCG TAT CAT T-3', Nextera_Block2: 5'-CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT GCC TTG CCA GCC CGC TCA GAG ATG TGT ATA AGA GAC AG-3', Nextera_Block2_REV: 5'-CTG TCT CTT ATA CAC ATC TCT GAG CGG GCT GGC AAG GCA GAC CGA TCT CGT ATG CCG TCT TCT GCT TG-3') for 72 h at 47°C. After hybridization, wash was performed as per Nimblegen protocols with streptavidin-coupled magnetic beads. Finally, PCR amplification was performed on exome captured library (Post_Cap_Short_For_Amp: 5'-AAT GAT ACG GCG ACC ACC GAG ATC T-3', Post_Cap_Short_Rev_Amp: 5'-CAA GCA GAA GAC GGC ATA CGA GAT-3'; 1× [0:30 min at 98°C], 17× [0:10 min at 98°C, 0:30 min at 65°C, 0:45 min at 72°C]) followed by clean up (AMPure) and sequencing on an Illumina GAIIx SE36 run.

PCR-free library preparation

Adaptor sequences (NoPCR1: 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACG CCT CCC TCG CGC CAT CAG AGA TGT GTA TAA GAG ACA G-3', and NoPCR2: 5'-CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT GCC TTG CCA GCC CGC TCA GAG ATG TGT ATA AGA GAC AG-3') were designed to contain the original 'Nextera' adaptor sequences, but with an additional 5' overhang of either P1 or P2 on adaptor 1 or adaptor 2, respectively (i.e. sequences to make compatible with cluster PCR on Illumina flowcell), thus eliminating the need to add them during a PCR step. The 5' phosphorylated reverse compliment of the 19 bp mosaic end (ME: 5'-Phos-CTG TCT CTT ATA CAC ATC T-3') sequence was hybridized to NoPCR1/2 by combining 5 μl of each NoPCR1 and NoPCR2 with 10 μl ME reverse complement all at 100 μM with 80 μl TE, followed by denaturation at 95°C for 5 min then slow cooling to room temperature for a final annealed adaptor concentration of 10 μM. Transposomes were assembled by incubating 5 μl annealed adaptors at 10 μM with 5 μl 100% glycerol and 10 μl Ez-Tn5 transposase (Epicentre) and allowed to incubate at room temperature for 20 min.

Tagmentation was carried out using previously prepared *E. coli* (CC118) or human (NA18507) genomic DNA using either 100 or 200 ng of DNA, 5 μl prepared transposomes, 2 μl 5× Nextera HMW buffer (Epicentre), and water to 10 μl. Reactions were incubated at 55°C for 5 min, followed by the addition of 25 μl 2× FailSafe PCR master mix (Epicentre), 1 μl FailSafe DNA polymerase (Epicentre), and 14 μl nuclease-free water (Ambion), and subsequent 5 min incubation at 72°C for nick translation. Tubes were then cleaned up using Qiaquick MinElute PCR purification columns (Qiagen), eluting in 12 μl buffer EB.

For each reaction, 2 μl was used as template for a real time PCR on a MiniOpticon (Bio-Rad) using 0.5 μl SYBR Green, 25 μl 2× Nextera PCR master mix, 1 μl Nextera PCR enzyme and nuclease-free water to 50 μl. Alongside the NoPCR reactions, libraries of known concentrations were used as template at successive dilutions

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to be used as a standard for rough library quantification. Standard Nextera cycling conditions were used, without the initial 72°C extension step. PCR reactions were cleaned up by Qiaquick PCR purification columns and run on a Noved 6% TBE PAGE gel (Invitrogen) for library size verification. After quantification, libraries were sequenced as per standard Illumina GAIIx protocol as a paired-end 36 bp run.

Low input transposase-based library preparation

For the 500 pg and 100 pg *E. coli* (CC118) libraries and 10 pg human library (NA18507, Coriell), genomic DNA (in 1 μl volume) was incubated with 1 μl Nextera Illumina-compatible transposomes (Epicentre) at a 1 to 50 dilution (1 μl Nextera enzyme, 24 μl TE, 25 μl 100% glycerol), 1 μl 5× Nextera HMW buffer, and 2 μl nuclease-free water (Ambion). To avoid contamination, all dilutions and reaction preparation was carried out in a PCR hood. Reactions were incubated at 55°C for 5 min followed by addition of 25 μl 2× Nextera PCR buffer, 0.5 μl SYBR Green, 1 μl 50× Nextera primer cocktail, and 1 μl 0.5 μM barcode adaptor 2 (barcodes A6, A9, or A4 for 500 pg and 100 pg *E. coli* DNA, or 10 pg human DNA, respectively) and cycled under standard Nextera conditions in a MiniOpticon (Bio-Rad) real-time PCR thermocycler. Both reactions were removed after 20 cycles and cleaned up using Qiaquick MinElute columns, eluting in 20 μl EB. Libraries were run on a 6% Novex TBE PAGE gel (Invitrogen) for size verification and sequenced as barcoded spike-ins as per standard Illumina GAIIx protocol as a paired-end 101 bp (plus 9 bp barcode) run for *E. coli* libraries and a paired-end 36 bp (plus 9 bp barcode) run for human.

Direct colony-based library preparation

Fusion-Blue chemically competent *E. coli* (Clontech, Mountain View, CA, USA) were transformed with pUC19 bearing a 2 kbp insert of human genomic DNA, and then plated on Luria broth (LB) + ampicillin. A small number of cells were picked from a single bacterial colony with a 10 μl pipette tip, and then transferred with dipping into 15 μl nuclease-free H_2O. The suspended cells were heat-lysed at 95°C for 5 min, then placed on ice for 2 min. Nextera 5× LMW reaction buffer and enzyme (Illumina-compatible; Epicentre) were added to the sample, followed by brief mixing and incubation at 55°C for 5 min. The reaction was then stopped by heating to 70°C for 15 min. Sequencing-compatible primer sites were added in a 50 μl PCR reaction using 5 μl of the transposase reaction directly as template without intervening purification. PCR was carried out with 31.6 μl H_2O, 10 μl Kapa 2G robust A buffer 5× (Kapa Biosystems, Cape Town, South Africa), 1 μl dNTP mix (10 mM each), 0.25 μl SYBR Green 100×, 1 μl 50× Nextera primer cocktail, 1 μl 50× Nextera adaptor 2, and 0.20 μl Kapa 2G robust polymerase; cycling conditions were as described by Epicentre. The amplification reaction was cleaned up with a Qiaquick PCR clean-up column (Qiagen) and eluted into 50 μl EB.

Sequencing

Sequencing of the *H. sapiens* NA18507, *E. coli* CC118, and *D. melanogaster* libraries was done on an Illumina Genome Analyzer IIx as paired-end 36, 36, and 45 bp runs, respectively, using standard read primers for sonication and fragmentase libraries, run in individual lanes, and Nextera read primers for Nextera libraries. *H. sapiens* YH1 libraries were run on an Illumina HiSeq2000 as paired-end 90 bp run using Nextera read primers. Phage libraries contained library-specific barcodes and were run as multiplexed samples using GS FLX Titanium sequencing protocols. *P. aeruginosa* libraries were pooled by combining 100 ng of each strain library, and sequenced on an Illumina Genome Analyzer IIx with a paired-end 76-cycle run.

Short read mapping

Short read mapping was done on the *E. coli* CC118, *D. melanogaster* and *H. sapiens* (NA18507 & YH1) Illumina GAIIx or HiSeq2000 sequenced samples by converting the raw sequence files to fastq format and then mapping to the hg18 (NCBI36) reference using the BWA [16] alignment software. After mapping, PCR duplicates were removed, as well as read-pairs with an insert size shorter than that of the read length.

Long read assembly

Long read assembly from bacteriophage samples sequenced on the Roche GS FLX Ti was done using Roche's newbler assembler under default parameters. Individual reads from each dataset were mapped against the assembled genome using gsMapper (Roche Software Release: 2.3 (091027_1459)).

Fragmentation site characterization

Fragmentation site characterization was carried out by stacking all regions of the genome flanking forward strand mapping start locations and the reverse complement of reverse strand start locations followed by calculating nucleotide frequencies at each position relative to the fragmentation site, thus generating a positional weight matrix (PWM). The PWMs then were imported into the SeqLogo (Oliver Bembom, Dept. of Biostatistics, University of California, Berkeley, 2008) package for Bioconductor in R and used to generate positional

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information content (IC) and sequence logos using the equation outlined by T. D. Schneider *et al.* [21,22]:

$$\mathrm{IC(w)} = \log_2(\mathrm{J}) + \Sigma_{j=1}^{\mathrm{J}}\, p_{wj}\, \log_2(p_{wj}) = \log_2(\mathrm{J}) - \mathit{entropy}(w)$$

where J is the number of variables in the alphabet (4; A, C, G, or T), and j is the base at position w. This equation does not factor in the background nucleotide frequencies.

Normalization of Illumina GAIIx coverage

Normalization of Illumina GAIIx coverage for the *E. coli* (non-size-selected) data was done by dividing the coverage at each position in the genome by the total number of mapped bases and then multiplying by a constant (the average number of mapped bases was close to 1 Gb, therefore 10^9 was used as the constant).

Coverage distribution histograms

Coverage distribution histograms were generated by calculating the number of times each base of the genome was sequenced and plotting the frequency of each level of coverage.

Coverage by G+C content

Coverage by G+C content plots were generated by binning the reference genome into 500 bp bins for *E. coli*, 10 kbp bins for human, and 1 kbp bins for *Drosophila* (other sizes were also investigated, resulting in very similar distributions) and calculating the G+C content of each bin, followed by plotting the coverage of that bin.

Library complexity

Library complexity was calculated by random sampling of 50,000 read-pairs without replacement and plotting the number of uniquely occurring read-pairs versus the total number of sampled read-pairs.

Insertion size distributions

Insertion size distributions were generated by taking the distance between the start mapping location of the first read and the end mapping location of the second read for every read-pair and plotting the frequency of occurrences of each insert size.

SNP calls

SNP calls for the YH1 genome were generated using the SAMtools [18] variant caller with a maximum coverage of 1,000 and minimum quality score of 30. Prior to variant calling, read-pairs with an insert size less than 90 bp and reads not properly paired were removed to reduce noise. Calls were then compared with the SNPs reported by Wang *et al.* (2008) [15] and to dbSNP build 129.

Cell-line-discordant SNP calls

In order to minimize false calls due to mapping errors, a repeat-masked version of hg18 was used along with further masking with respect to mappability according to the UCSC Genome Browser 'Rosetta 35mer uniqueness' and excluding all regions with a score of 0 (this score means that the sequence maps perfectly to multiple locations in the genome). This track was used because it was generated using the BWA aligner that was used in our analysis, and because the original YH1 sequence data is made up of 36 bp reads. Out of this newly masked genome, positions were called that had a SNP quality score in the cell line over 30, a reference call in blood over 30, and coverage less than 100× in both datasets. Of those with a quality score of 50 in both for their calls, 100 were randomly chosen for validation.

Validation was carried out using a mass spectrometry assay (Sequenom, San Diego, CA, USA). Primers for PCR amplification and extension were successfully designed for 100 mutation sites using the Sequenom MassArray Assay Design v3.1. PCR amplification, shrimp alkaline phosphatase treatment of unincorporated dNTPs, probe extension and resin desalting were carried out in sequence using the conditions described elsewhere [23]. Sequenom genotyping was performed in parallel for genomic DNA from YH1 blood and the same batch of lymphoblastoid cell lines as was used for sequencing. A negative control and technical replicate were also run in parallel for each typed position. Genotyping of all 100 testing sites passed the filter criteria of: (1) no failing extension, (2) no false positive in the negative control, (3) consistency between two technical replicates. Genotyping was further performed for the 100 positions in the YH primary lymphoblastoid cell lines using the same method, with 98 meeting the above filter criteria.

Exome analysis

Sequence reads from transposase-based libraries subjected to human exome capture were aligned to the human reference (hg18) using BWA [16]. Each aligned base was deemed to be on target if it was within 100 bases of a targeted sequence. At each position within target regions, coverage was assessed and any position with a depth of at least one was considered covered. Comparison to standard exome methods was made by trimming read 1 of a PE76 lane from a GAIIx down to 36 bases and aligning it the same way. Because the standard library had fewer reads mapped, 28 million reads were randomly taken from both libraries and above analysis performed. Complexity was interrogated by taking an equivalent number of on-target SE36 reads generated

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by each method and calculating the percentage with unique start-points.

Barcode design

Barcode design yielded a set of 96 × 9 bp sequences in which each 9 bp sequence contained no homopolymer run of three or more bases, had a GC content of ≤ 60%, was a edit distance of at least four away from all other members of the set of 96, and screened negative when compared with other adaptor and primer sequences used here. Also, we took care to ensure that each base (A, G, C, or T) was represented at least once in each 9 bp barcode, and at least once in each position along the 9 bp.

Barcode deconvolution

Barcode deconvolution for pooled, multiplexed *Pseudomonas* samples was carried out by computing the Levenshtein edit distance between the obtained index read and each of the 96 barcode sequences used. The corresponding read-pair was assigned to a barcode when that barcode was within edit distance of 2 of the index read, with the next closest matching barcode being at least two further edits away.

Data accessibility

All sequence data described here is being deposited in the NCBI Sequence Read Archive (SRA) under accession SRP004087.

Additional material

Additional file 1: Supplementary Tables 1-3.

Additional file 2: Supplementary Figures 1-8.

Additional file 3: Supplementary Table 4. S4: barcode adaptors.

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Authors' contributions

AA, HGM, Asan, XX, NCC, XZ, and JS conceived the study and planned experiments. AA analyzed all data, conducted comparison experiments with the Illumina platform, and developed the PCR-free protocol, the exome capture protocol, and the low-input protocol. HGM conducted comparison experiments and accompanying analyses with the 454 platform. Asan, XX, and XZ performed YH1 and *Drosophila* genome sequencing, and SNP validation. JOK developed the direct-from-colony library construction protocol and analyzed the resulting data. APM performed exome capture experiments. EHT, BS and AA developed and tested the 96-plex barcoding protocol. AA and JS wrote the manuscript. All authors reviewed drafts, contributed comments, and approved the final manuscript.

Competing interests

NCC is an employee of Epicentre Biotechnologies. The other authors declare that they have no completing interests.

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Expert Opinion

1. Mechanisms of ultrasonic-enhanced drug delivery
2. Ultrasonic-assisted delivery of therapeutic molecules
3. Expert opinion

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Ultrasonic drug delivery – a general review

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Ultrasound has an ever-increasing role in the delivery of therapeutic agents, including genetic material, protein and chemotherapeutic agents. Cavitating gas bodies, such as microbubbles, are the mediators through which the energy of relatively non-interactive pressure waves is concentrated to produce forces that permeabilise cell membranes and disrupt the vesicles that carry drugs. Thus, the presence of microbubbles enormously enhances ultrasonic delivery of genetic material, proteins and smaller chemical agents. Numerous reports show that the most efficient delivery of genetic material occurs in the presence of cavitating microbubbles. Attaching the DNA directly to the microbubbles, or to gas-containing liposomes, enhances gene uptake even further. Ultrasonic-enhanced gene delivery has been studied in various tissues, including cardiac, vascular, skeletal muscle, tumour and even fetal tissue. Ultrasonic-assisted delivery of proteins has found most application in transdermal transport of insulin. Cavitation events reversibly disrupt the structure of the stratus corneum to allow transport of these large molecules. Other hormones and small proteins could also be delivered transdermally. Small chemotherapeutic molecules are delivered in research settings from micelles and liposomes exposed to ultrasound. Cavitation appears to play two roles: it disrupts the structure of the carrier vesicle and releases the drug; and makes cell membranes and capillaries more permeable to drugs. There remains a need to better understand the physics of cavitation of microbubbles and the impact that such cavitation has on cells and drug-carrying vesicles.

Keywords: cavitation, chemopotentiation, chemotherapy, DNA, hyperthermia, liposomes, micelles, microbubbles, protein, targeted drug delivery, ultrasound

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1. Mechanisms of ultrasonic-enhanced drug delivery

Ultrasound has been employed to enhance the delivery and activity of drugs for the past two decades. In the past 6 years, however, research in ultrasound-activated drug delivery has blossomed with the introduction of gas bubbles. In order to understand the mechanisms of ultrasound-enhanced drug delivery, one must first understand the physics of ultrasound and of cavitation, the keystone of this novel approach to drug delivery.

1.1 Physics of ultrasound

Just as audio sound is the transmission of pressure waves through a medium, such as air or water, ultrasound is the same type of transmission of pressure waves, but at frequencies above human hearing, or ≥ 20,000 Hz. As with light waves, these ultrasonic waves can be reflected, refracted (bent), focused and absorbed. Unlike light waves, ultrasonic waves are very physical in nature; they are actual movement of molecules as the medium is compressed (at high pressure) and expanded (at low pressure), and thus ultrasound can act physically on biomolecules and cells.

Most importantly, unlike visible light waves, ultrasonic waves are absorbed relatively little by water, flesh and other tissues. Therefore, ultrasound can 'see' into the

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body (e.g., diagnostic ultrasound) and can be used to transmit energy into the body at precise locations. This safe, non-invasive and painless transmission of energy into the body is the key to ultrasonic-activated drug delivery.

1.2 Hyperthermia

The traditional use of ultrasound in medicine is for diagnostic imaging (which occurs at low average intensities and high frequencies) and for tissue heating (which occurs at higher intensities and higher frequencies). The intensity (also called power density) of an ultrasonic beam is measured in terms of power carried/cross-section area of the beam, typically having units of W/cm^2. If a beam is focused down to a small size on a targeted tissue, the power/area becomes very large and significant thermal energy can be absorbed from the beam by the tissue, resulting in heating. Such hyperthermia has been traditionally employed in physical therapy to warm tissues [1], in drug delivery to 'melt' drug-containing liposomes [2], and in medical therapy to kill or ablate tissue [3-5]. Thus, hyperthermia in targeted drug delivery accomplishes the role of heating the drugs, drug carriers and/or the tissues receiving the drugs.

1.3 Cavitation

1.3.1 Nature of cavitation

A relatively recent and novel application in drug delivery takes advantage of the remarkable ability of ultrasound to produce cavitation activity. Cavitation is the formation and/or activity of gas-filled bubbles in a medium exposed to ultrasound [6]. As the pressure wave passes through the media, gas bubbles of any size will expand at low pressure and contract at high pressure. If the resulting oscillation in bubble size is fairly stable (repeatable over many cycles), the cavitation is called 'stable' or 'non-inertial' cavitation. Such oscillation creates a circulating fluid flow (called microstreaming) around the bubble [7-9], with velocities and shear rates proportional to the amplitude of the oscillation. At high amplitudes, the associated shear forces are capable of shearing open red cells [10] and synthetic vesicles such as liposomes [7].

As the ultrasonic intensity increases, the amplitude of oscillation also increases to a point in which the inward moving wall of fluid has sufficient inertia that it cannot reverse direction when the acoustic pressure reverses, but continues to compress the gas in the bubble to a very small volume, creating extremely high pressures and temperatures [11,12]. This type of cavitation (called transient, inertial or collapse cavitation) can be detrimental to cells or vesicles because of the very high shear stresses in the region of the collapse, the shock wave produced by the collapse, and the free radicals produced by the high temperatures. The collapsed bubble often fragments into smaller bubbles that serve as cavitation nuclei, grow in size, and eventually collapse again [11,12]. **Figure 1** shows a streak optical photograph before, during and after a cavitation collapse [12]. On the left is the original microbubble. The middle frame is a high-speed streak photograph showing the boundaries of the bubble when subjected to a five-cycle driving pulse at 2.5 MHz and 1.6 MPa; the pressure measurement is recorded at the top of the photograph. The right frame shows the resulting fragments of the bubble. Cavitation is a violent phenomenon that concentrates the energy from ultrasound into a small volume.

If the collapse is near a solid surface, an asymmetrical collapse occurs that ejects a liquid jet at sonic speed toward the surface [11,13]. **Figure 2** illustrates this type of collapse. If the rigid surface is a blood vessel wall, skin, a large cell or a semi-rigid vesicle, then the jet can pierce the surface.

Collapse cavitation can be damaging to biological tissues and, therefore, there has been much research into the conditions under which it is produced. In general, the likelihood and intensity of collapse cavitation increases at higher intensities and lower frequencies, as has been demonstrated by experiments [11,14,15] and theory [16]. The size of the bubble and its physical properties (i.e., gas species, interfacial tension, surface rigidity) also affect the cavitation process [17-21].

1.3.2 Ultrasound-induced mechanisms of drug delivery

1.3.2.1 Enhanced transport

Ultrasonication and cavitation can be involved in drug delivery by several mechanisms. The simplest mechanism derives from the oscillatory motion of the insonated fluid and can occur in the absence of cavitation. The oscillating fluid increases the effective diffusivity of molecules; thus, the transport of any drug, whether free or bound to a carrier, will be augmented by the oscillatory motion of the fluid. Such ultrasonic-enhanced transport may occur within blood, cells or extracellular fluids.

When a strong ultrasonic beam is directed through a partially absorbing liquid, some momentum from the beam is transferred to the fluid, imparting a large-scale convective motion to the fluid that can also increase the overall rate of drug transport [22]. Although this so-called 'acoustic streaming' could enhance drug transport *in vitro*, we opine that it has little, if any, application for *in vivo* systems, because fluid convection in the vascular system is already very fast, and in tissues beyond the vascular system there are few reservoirs of fluid that can be accelerated by an acoustic beam.

In the presence of oscillating bubbles, drug transport is enhanced by orders of magnitude compared with transport by diffusion alone. At least two mechanisms are reported for creating convection in the presence of a stable oscillating bubble. The first phenomenon, called microstreaming, is the creation of circulating eddies around the oscillating bubble [7-9]. Such eddies can transport drugs at high velocities. For example, the velocity of water near the surface of a 10 μm (diameter) bubble, with a 2 μm oscillation amplitude, are in the order of 10 m/s [9]. Even more surprising are the extremely high viscous shear rates near the surface of the bubble, which, in this example, are on the order of 10^7/s [9]. This shear rate is equivalent to shearing water in a 1 mm gap between parallel plates in which one plate is stationary and the other moving at

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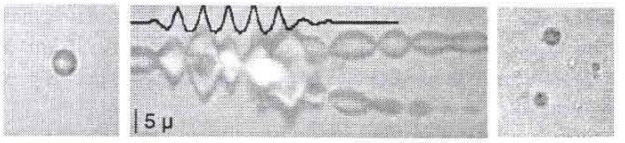


Figure 1. Optical images of a 2.5 μm radius microbubble exposed to five cycles of 2.5 MHz ultrasound at 1.6 MPa pressure amplitude. The left panel shows the bubble before exposure. The central panel shows a streak photograph (an optical M-mode image of a line through the centre of the bubble as a function of time) with the measured pressure superimposed at the top of the panel. The right panel shows the bubble fragments produced by the collapse of the cavitating bubble. Used with permission from MAY DJ, ALLEN JS, FERRARA KW: Dynamics and fragmentation of thick-shelled microbubbles. *Ieee Transactions on Ultrasonics Ferroelectrics and Frequency Control* (2002) **49**(10):1400-1410 [12], © 2002 IEEE.

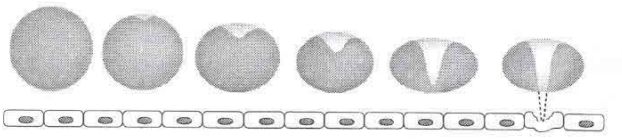


Figure 2. Illustration of an asymmetric collapse of a bubble near a surface, producing a jet of liquid toward the surface.

10^{10} m/s. This extremely high shear rate stresses cells and vesicles, as will be discussed later.

The second phenomenon related to oscillating bubbles is called acoustic pressure, which is a net force acting on other suspended bodies in the vicinity of an oscillating bubble. If the body is more dense than the suspending liquid, the body is pushed toward the bubble; if it is less dense, the body is repelled from the bubble [23]. Most drug-carrying liposomes and micelles are more dense than water, and will be convected toward the bubble, thus increasing the dispersive transport of the drug carrier, particularly if the vesicle is drawn into the microstreaming field around the bubble, and is sheared open by the high shear rate (releasing the drug). Marmottant and Hilgenfeldt show a fascinating example of this phenomenon [7]. If the vesicle is another microbubble, it will be dispersed away from the primary oscillating bubble because it is less dense. Therefore, a field of microbubbles, such as an injected bolus of contrast agent, will tend to spread itself in the ultrasonic field, and at the same time attract and shear more dense vesicles, such as suspended cells or introduced liposomes.

1.3.2.2 Perturbation of the drug carrier

This leads to the second major contribution of ultrasound toward drug delivery: the disruption of drug carriers by ultrasound. As mentioned above, vesicles more dense than the surrounding liquid will be sucked into the shear field surrounding an oscillating bubble. If the shear stress exceeds the strength of the vesicle, it will rupture and spill its contents. In the case of a liposome (and perhaps a micelle), the vesicle will probably reform, often at a smaller size than before the encounter with the shear field. The case of a liposome resizing into a number of smaller vesicles, with the same amount of surface area (assuming no phospholipids are lost), presents an interesting method of drug release. If a large spherical vesicle is fractured into a number of smaller vesicles and the surface area is conserved, the total volume of the smaller vesicles is less than the original volume. Thus, some interior liquid must be released during the fracture event. If a drug is soluble in the aqueous core of a liposome, some will be released following fracture, even if the liposomes reform into smaller vesicles. Collapse cavitation is not required for this method of drug release; a stable oscillating bubble is sufficient.

Other mechanisms of vesicle perturbation arise from collapse cavitation. The collapse event produces a shock wave that can be envisioned as an expanding thin shell of dense water. As this spike of dense fluid passes over a vesicle, a shear stress is produced at the vesicle surface that can rupture the vesicle if the critical stress is exceeded [24,25]. As the shock wave expands spherically, its energy density decreases, such that, beyond a certain distance from the bubble, there is no longer sufficient shear to disrupt vesicles [25].

As mentioned previously, the sonic jet of fluid produced by collapse cavitation near a solid surface also generates extreme shear stresses that can shear open or perhaps pierce nearby vesicles. Some of these methods of drug delivery are illustrated in **Figure 3**.

It is well known that low-frequency, high-intensity ultrasound (conditions promoting collapse cavitation) can reduce the molecular weight of soluble polymers [26-28]. Polymer chain scission is attributed to viscous shear stresses that pull the backbone apart and to the generation of free radicals that may react with and break the chain. In cases employing drugs bound to a polymeric carrier, such reduction in polymer molecular weight will increase the diffusivity of the polymer–drug fragments. In some cases, the drug might be sheared from the polymer backbone. An interesting example is given for the ultrasonically activated delivery of cobalamin (vitamin B_{12}) from a carrier. The release is attributed to free radicals, generated by collapse cavitation, that react with, rearrange, and break the bond connecting the cobalamin to the drug carrier [29].

1.3.2.3 Cell permeabilisation and capillary rupture.

The third major contribution of ultrasound to drug delivery relates to the stresses inflicted on cells and tissues as a result of cavitation events. Ultrasound by itself, in the absence of cavitation, is thought to have little, if any, effect on cells and tissues apart from some heating that may occur at higher frequencies and intensities [6,23,30,31]. As with vesicles, cells in an environment of cavitation events are subject to shear from

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microstreaming, shock waves and sonic jets. It is possible that a large semi-rigid cell, adjacent to a small cavitating bubble, could induce an asymmetric bubble collapse, by which a small jet of liquid would shoot directly into the cell at sonic speeds, probably rupturing the cell membrane. Similarly, a collapse of a microbubble near a capillary or blood vessel wall will cause the liquid jet to shoot right into the wall. Such a collapse may be the source of the large amount of extravasation that is caused in tissue exposed to ultrasound in the presence of microbubble contrast agents [32-35]. There is some concern that a blood vessel injury by sonic jets, shear stresses, or radicals could generate a nidus, initiating subsequent thrombus formation.

1.3.3 Biological and physical consequences of cavitation

Much has been written about the biological consequences of ultrasonic cavitation in living systems, and little, if any, is positive with respect to the health of the organism or tissue [6,23,30,31]. Obviously, a plethora of bubbles undergoing collapse cavitation is not a healthy environment for a cell. Shock waves and shear forces are shearing the cell membrane. Liquid microjets at sonic velocities may be shooting right through cells (and lysing them). Free radicals may be interfering with essential biochemical processes. At the other extreme, a field of quivering bubbles experiencing mild stable cavitation probably has no negative biological consequences to most cells, and may even be beneficial in terms of increasing convection of oxygen or nutrients to cells [36].

Targeted drug release is a very active process, and these same energetic cavitational phenomena, which may be detrimental to tissues, are requisite in promoting the physical or chemical release of drugs from a carrier, or in promoting the transport of drugs into cells. Low-intensity stable cavitation may not be adequate to effectively execute drug delivery. Therefore, some cavitational activity must be tolerated to accomplish the medicinal goal. Somewhere between these harsh and mild extremes lies the desired realm of ultrasonic drug delivery. This would be a cavitational level that produces bubble activity sufficient to permeabilise cell membranes without killing the cells, a level that creates a sufficient number of microjets to allow extravasation from capillary walls without killing the endothelial cells or causing thrombosis, or a level that generates sufficient microstreaming to break open liposomes, or other vesicles, without lysing red or other host cells.

1.4 Types of drug carriers

The delivery of free drug, not associated with a carrier system, can be enhanced by ultrasound, as will be discussed in Section 2.3.1. However, the disadvantage of free drug is that it often interacts with non-targeted tissues if it is delivered systemically.

To preclude the non-targeted interaction of free drug with tissues, the drug is sometimes attached to a polymeric carrier from which it is released at the target site by degradation of a linker, via enzymes or pH at the target site. Ultrasound has been used to release covalently-bound cobalamin from a carrier [29], but this was a very specific application and may be difficult to extend to drugs in general.

Liposomes and micelles can sequester hydrophobic drugs within their lipophilic membrane (liposome) or core (micelle), and liposomes can sequester hydrophilic drugs in their aqueous interior. These vesicles appear to prevent general and/or premature release of the drug [37-39]. In theory, liposomes and micelles should not be acoustically active if they do not contain any gas. However, they can be drawn towards and then sheared open by the action of cavitating bubbles. In addition, cell membranes can be sheared by cavitation events, rendering them more permeable toward liposome or free drug uptake. Some studies suggest that even carefully prepared liposomes contain some gas, and thus they can become acoustically active during insonation and release their payload following cavitation-induced fragmentation [40,41]. Unger has prepared a liposome–microbubble hybrid, in which gas and stabilising oil are introduced into the standard liposome [42]. This 'liposphere' can be considered a hybrid between a pure liposome and microbubble, and can also be used as an ultrasonic contrast agent.

Microbubbles are distinguished from liposomes in that they contain gas and do not necessarily have a bilayer structure; they are simply gas bubbles stabilised by a surfactant at their surface. Proteins such as albumin can be used in place of conventional phospholipid surfactants. As mentioned, when microbubbles of gas-carrying lipospheres also carry drugs, they are dually effective drug delivery vehicles, being both the carrier and the activator for the ultrasonic drug delivery.

Some drugs can spontaneously associate with the surface of liposomes and microbubbles. An example is the association of negatively-charged DNA and RNA with liposomes and microbubbles composed of cationic surfactants. When the liposome or microbubble is fragmented by cavitation events, the drug can be released, although it may yet retain some association with the cationic surfactant. The free drug can be taken up by normal mechanisms, whereas the drug associated with fragmented surface could be taken up by pinocytosis, or related mechanisms.

Having completed this basic introduction to ultrasound, cavitation and drug carriers, this review is organised according to the general type of drug delivered. First the delivery of the largest and most sensitive macromolecules, DNA, to targeted tissue will be discussed. The focus will then turn to smaller and/or globular macromolecules (i.e., proteins). Finally, strategies for the ultrasonic delivery of conventional small chemotherapeutic drugs will be presented.

2. Ultrasonic-assisted delivery of therapeutic molecules

2.1 DNA and gene delivery

Gene delivery is a topic of intense interest in targeted drug delivery [43-49]. The use of ultrasound in gene delivery has

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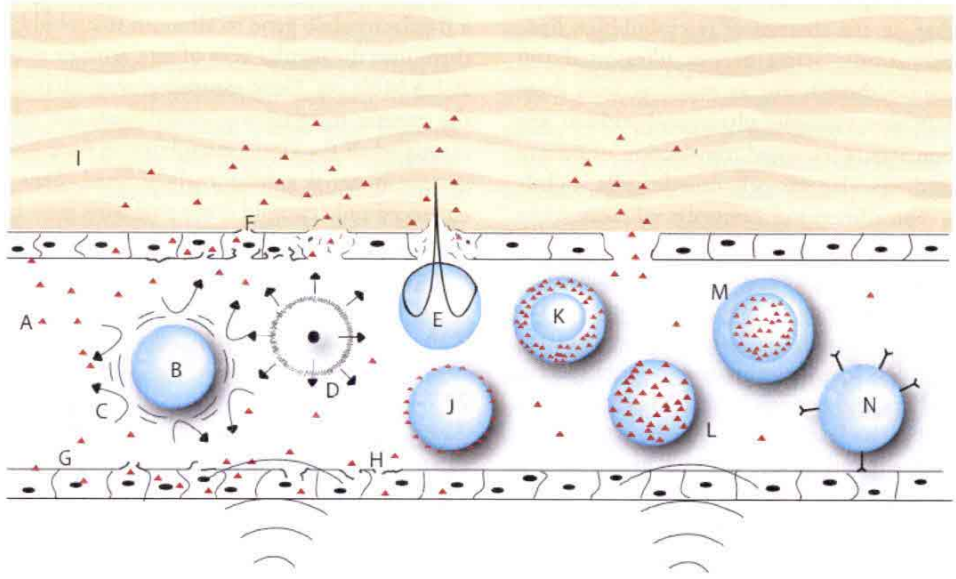


Figure 3. Schematic representation of various modes by which drug delivery can be enhanced by ultrasound. (A) Therapeutic agent (triangles); **(B)** gas bubble undergoing stable cavitation; **(C)** microstreaming around cavitating bubble; **(D)** collapse cavitation emitting a shock wave; **(E)** asymmetrical bubble collapse producing a liquid jet that pierces the endothelial lining; **(F)** completely pierced and ruptured cell; **(G)** non-ruptured cells with increased membrane permeability due to insonation; **(H)** cell with damaged membrane from microstreaming or shock wave; **(I)** extravascular tissue; **(J)** thin-walled microbubble decorated with agent on surface; **(K)** thick-walled microbubble with agent in lipophilic phase; **(L)** micelle with agent in lipophilic phase; **(M)** liposome with agent in aqueous interior; **(N)** vesicle decorated with targeting moieties attached to a specific target.

exceptional potential because the beam can be focused on a particular tissue. The trick is to release the genetic material only at the targeted site. A further complication is that the DNA (or RNA) must enter the targeted cells before it is degraded by DNase, or carried to other tissues by the blood. This section will first discuss gene carriers, and then review examples of targeting specific tissues.

2.1.1 Ultrasonically activated gene carriers

2.1.1.1 Microbubbles

Gene delivery using insonated microbubbles was first reported in 2000 [50,51] and has since become a topic of intense study. The popularity of this technique arises from the availability of commercial ultrasound contrast agents, combined with the versatility and ease of their use. The microbubbles can be injected upstream of the target region, which is easily imaged at low intensity by the presence of the bubbles. When such imaging demonstrates that the ultrasound is precisely focused on the target tissue, the ultrasonic intensity can be increased to create collapse cavitation. The collapse events appear to permeabilise the vessel walls and provide pathways for extravasation of DNA that is freely floating with the bubbles, or that is associated with the bubble surface [47,52]. Lawrie *et al.* [53] showed that transfection was not related to free radical production from collapse cavitation, but attributed transfection to transient holes in the cell membrane produced by other cavitation phenomena.

A convenient manner to prepare gene-carrying microbubbles is to insonate a mixture of surfactant, such as albumin, in the presence of gas (air or perfluorocarbon) and the plasmid or DNA fragment to be delivered [54]. If commercial contrast agents are available, the genetic material can be mixed directly with the contrast agent and injected [52,55,56]. This is often naked DNA, but one can also mix the genetic material with a cationic stabilising agent, such as poly(ethylene imine) or poly(L-lysine) [57].

2.1.1.2 Liposomes

Liposomes have been used for decades as drug carriers, and recently as gene carriers, particularly cationic liposomes. During the past decade, ultrasound applied in combination with gene-carrying liposomes has enhanced the transfection rate both *in vitro* [58-61] and *in vivo* [62].

Cationic liposomes are particularly effective in delivering DNA because the DNA stays associated with the liposome until it is acoustically activated. Most cationic liposomes contain dioleoylphosphatidylethanolamine, although some contain quaternary ammonium compounds or cationic derivatives of cholesterol [46,59,63].

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2.1.1.3 Free genetic material

It is noteworthy that, in the absence of microbubbles, liposomes, or other acoustically active agents, ultrasound still enhances transfection, although not as much as with them present [64]. As an *in vitro* example, ultrasound without any extraneous cavitation agents increased transfection of human endothelial cells and vascular smooth muscle cells slightly (compared with a non-ultrasound control), whereas when microbubbles were present, the transfection increased by ≥ 1000-fold [64]. Such transfection is usually attributed to sonoporation of cell membranes by acoustic activity, but the mechanisms leading to sonoporation are not proven in the absence of microbubbles. It is plausible that *in vitro* there is sufficiently dissolved gas and enough organic molecules with a surfactant nature that the insonation itself generates cavitation bubbles that subsequently grow, collapse, and shear cell membranes. On the other hand, postulating an *in vivo* source of cavitation nuclei is more problematic because the lungs are very efficient at clearing small bubbles from the circulatory system [6,23].

Another disadvantage of using free DNA is that ultrasound causes DNA fragmentation [28,65], which reduces the transfection efficiency [57]. Complexing the DNA plasmids with cationic polymers such as poly(ethylene imine) and poly(L-lysine) preserves the integrity of the DNA on exposure to 20 kHz ultrasound [57]. Another strategy to protect the DNA is to expose the tissues to ultrasound and subsequently perfuse the genetic material into the targeted region [66].

2.1.2 Examples of ultrasound-assisted gene delivery

Ultrasonically enhanced gene delivery to cultured cells *in vitro* has been abundantly reported. However, this review will focus on published examples of gene delivery to tissues *in vivo*.

2.1.2.1 Cardiac tissue

A major thrust in ultrasound-enhanced gene delivery is the combination of visualising coronary arteries or other heart structures, and then delivering genes or drugs to the diseased tissues [67]. In most published studies, a marker gene has been delivered to show efficacy of the concept. One of the long-term goals of cardiac gene delivery is to deliver genes that will inhibit, or reverse, stenosis of coronary arteries or to regrow missing or diseased tissue.

Bekeredjian *et al.* imaged and delivered a luciferase transgene marker to the left ventricle of rat hearts using both commercial and custom gas microbubbles mixed with the genetic material [68,69]. The sonography (1.3 MHz) was triggered by electrocardiographic signals to expose the microbubbles every four ventricular contractions. Genetic expression was significantly greater using triggered rather than continuous insonation [70]. The resulting gene expression was limited to the heart, with slight expression in the liver and pancreas, and no expression in the brain, muscles or lung, thus validating their goal of targeted delivery. The microbubble destruction caused very little change in the regulation of host genes in the heart [71].

Shohet *et al.* mixed an adenovirus delivery system encoding a β-galactosidase gene to albumin microbubbles and injected them into the jugular vein of rats, with or without insonation at 1.3 MHz [50]. Gene expression was enhanced 10-fold when the genetic material was mixed with the microbubbles and insonated. If the virgin microbubbles were insonated, and the genetic material subsequently infused, gene expression was enhanced only twofold compared with gene infusion without any insonation. This experiment indicates that gene delivery is most efficient when applied concomitantly with insonation; apparently the cell permeability to genetic material decreases with time after insonation.

2.1.2.2 Vascular tissue

There are many reports of ultrasound-enhanced gene delivery to arteries, with the goal of developing a delivery system that can be used to treat stenosis and other arterial diseases. Both endovascular ultrasound and extracorporeal ultrasound have been studied. An endovascular ultrasonic catheter was employed in a rabbit femoral artery model of overdilation [72]. An adenovirus delivery system expressing a blue fluorescent protein (*BFP*) gene or a non-viral plasmid with the *BFP* gene, was infused with or without application of 2 MHz endovascular ultrasound. Insonation increased plasmid-mediated gene expression 12-fold and viral-mediated gene expression 19-fold over their non-insonated controls.

Beeri *et al.* [55] mixed an adenovirus encoding a reporter gene with albumin microbubbles and delivered it to the aortic root of rats. Extracorporeal ultrasound was employed both to image and to disrupt the microbubbles. Genetic expression in the aortic tissue was increased if the blood flow was transiently stopped during insonation. Some incubation time appears to be helpful in allowing transport of the gene into the cells.

Also employing a rat overdilation model, Taniyama *et al.* [64] delivered a naked plasmid encoding a luciferase gene to the carotid artery. Neither plasmid DNA alone, nor plasmid DNA with ultrasound induced significant gene expression. However, when albumin microbubbles were mixed with the plasmid and insonated, and the data showed ≥ 1000-fold increase in luciferase activity compared with plasmid DNA alone.

Huber *et al.* [73] employed a rabbit carotid artery model and used high-intensity focused ultrasound at 0.85 MHz to deliver a plasmid reporter gene. Consistent with the above results, they found that gene expression after exposure to plasmid alone or plasmid plus microbubbles was minimal. Although insonation of the vessel with plasmid increased the gene expression, insonation with plasmid and albumin microbubbles increased gene expression even more.

In an *ex vivo* model, Teupe *et al.* [54] exposed excised porcine arteries to albumin microbubbles mixed with plasmid DNA with a reporter gene. Ultrasound alone, or microbubbles alone, produced a minimal gene expression, whereas the combination of 2.2 MHz diagnostic ultrasound with microbubbles significantly increased gene expression. The perfusion flow rate through the artery affected the expression,

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with a maximum expression at 2 ml/min (compared with no flow or higher flows).

In a slight twist of the conventional reports above, Du *et al.* [74] synthesised echogenic gas-filled solid polymer microspheres containing a reporter plasmid. These were injected into the femoral artery of pigs and subjected to diagnostic Doppler imaging. Gene expression was subsequently found (6 days later) in all experiments. Unfortunately, there were no control experiments in which genes were delivered without ultrasound. Solid echogenic microspheres appear to deliver genes, but at this point the transfection efficiency cannot be compared with microbubble delivery systems.

2.1.2.3 Tumour tissue

There is considerable interest in genetic targeting of tumours as an anticancer therapy, and several studies have examined the feasibility of such a therapy. For example, Manome *et al.* [75] grew MC38 murine colon carcinoma in mice and injected a naked plasmid with a reporter gene, directly into the tumour. Application of 1 MHz transcutaneous insonation increased the reporter activity threefold over the non-insonated control. Higher power densities increased reporter activity, as did increasing insonation time up to 30 s.

Using a cationic lipid–cholesterol transfection complex, Anwer *et al.* [66] delivered an *IL-12* gene to a mouse tumour model. Insonation significantly increased the gene expression, and the transfected tissue was limited to the tumour vasculature. The expression of *IL-12* was sufficient to inhibit tumour growth compared with the control conditions. Although the authors did not expressly introduce microbubbles, or mention their possible existence, it is possible that the synthesis of the DNA–lipid–cholesterol complex created some liposomes, some of which could contain gas and become acoustically active during insonation. McCreery describes a similar procedure for plasmid gene delivery to human adenocarcinoma grown in nude mice, with similar results [62].

Huber *et al.* [76,77] delivered a naked plasmid DNA reporter gene into subcutaneous Dunning prostate tumours in rats. Insonation at 0.85 MHz produced a 10- to 15-fold increase in reporter activity compared with non-insonated controls.

In a related procedure, Bao reports the use of a lithotripter to deliver a luciferase reporter to B16 melanoma grown in mice [78]. A lithotripter generates a shock wave containing a spectrum of acoustic frequencies and can produce cavitation phenomena. The plasmid was injected directly into the tumour, and in some cases, 10% air (relative to the tumour volume) was also injected. Various regimens of shock waves were subsequently applied, and the luciferase expression evaluated. The results showed that shock waves with direct injection enhanced expression ~ 15-fold relative to direct injection alone, and that application of air produced a further 7-fold increase in expression. More information on *in vitro* gene delivery by lithotripter shock waves is reviewed elsewhere [49].

2.1.2.4 Skeletal muscle and bone tissue

Skeletal muscle is one of the largest tissue systems in the body, and as such can express a large quantity of therapeutic protein into the circulatory system to eventually find another target tissue, or to produce a 'whole body' therapy. Other applications aim at promoting neovascularisation for tissue repair. Several articles have been written on ultrasound-enhanced gene delivery to skeletal muscles.

In some *in vivo* experiments in rats, the triceps brachii or gastrocnemius was exposed to diagnostic ultrasound. A mixture of plasmid encoding for luciferase and commercial microbubble contrast agents was injected into the muscle, followed immediately by insonation. The results showed that insonation in the presence of microbubbles and plasmid resulted in higher luciferase activity than injection of plasmid alone, plasmid plus microbubbles (without insonation), or plasmid lipofection. Virtually no luciferase activity was observed in other muscles or organs.

In similar experiments, Christiansen *et al.* injected a luciferase reporter coupled to cationic lipid microbubbles by intra-arterial or intravenous routes [79]. Only the rat hind limb skeletal muscle was insonated. Transfection was 200-fold greater with the intra-arterial than intravenous route, and was similar to intramuscular injection of plasmid. No transfection was observed if the plasmid was administered without microbubbles. These results indicated that insonation of plasmid–microbubbles at a remote site (away from the injection site) produced sufficient extravascular deposition and DNA incorporation leading to genetic expression. Other groups have also used luciferase and microbubbles to transfect rodent skeletal muscle [80,81].

Schratzberger *et al.* [82] investigated the effect of duty cycle and power density on naked DNA transfection in rabbit quadricep muscle. A plasmid reporter gene was injected via the intramuscular route, and insonation was immediately applied. Consistent with the above findings, the plasmid reporter gene showed very little expression when DNA, but not ultrasound, was applied. The group concluded that gene expression increased as the duty cycle (the fraction of time that ultrasound is activated in a pulse sequence) or the power density increases, consistent with the hypothesis that cavitation is involved in the gene delivery. No microbubbles were used in these experiments.

2.1.2.5 Fetal tissue

Gene transfection on a mouse fetus was carried out *in utero* using a plasmid encoding a fluorescent marker [56]. In this procedure, an incision was made on a pregnant mouse and the uterus was externalised. Then, plasmid mixed with microbubbles was delivered to specific locations by micropipette, the ultrasound applied, the uterus replaced, and the fetus developed for another 24 – 48 h. Gene expression with naked DNA alone, DNA with microbubbles, or DNA with ultrasound was low and showed no significant difference between these conditions. However, application of 1 MHz ultrasound to plasmid and

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microbubbles produced ~ 1000-fold enhancement in gene expression. Micrographs showed some disruption to the fetal skin under conditions of ultrasound with microbubbles.

2.1.2.6 Brain

It would be advantageous to express genes directly in the brain that may ameliorate debilitating brain diseases, such as Alzheimer's. So far, there are no examples of *in vivo* enhancement of gene delivery using ultrasound. However, there are some publications of *in vitro* delivery to neural tissue in culture [63,83], and reports of employing ultrasound to breach the blood–brain barrier [35,43,84-87].

2.1.2.7 Lung

Lung tissue contains gas and, therefore, reflects and scatters ultrasound; thus transcutaneous ultrasound cannot be used to deliver therapeutics to the lungs. An alternative use of ultrasound in gene delivery is to create an aerosol using an ultrasonic nebuliser. Cationic DNA complexes have been delivered to mice, rats and guinea-pigs, and their lung epithelial cells transfected [88,89].

2.1.3 Needs in gene delivery

As most of the recent progress in gene delivery involves microbubble cavitation, the most pressing need is a better understanding of cavitation physics and ultrasound–microbubble interactions. Of course, this will aid not just in gene delivery, but in all aspects of drug delivery.

Another need is to develop better protection for the genetic material so that it is not degraded by fluid shear forces or enzymes before it can be delivered to the cells. In addition, the loading of genetic material needs to be optimised such that the genes are delivered only to the target tissues and not to tissues downstream from the insonated site.

2.2 Protein delivery

Here, proteins will be differentiated from other macromolecules by their size and characteristic polypeptide backbone. This review will consider proteins as having a molecular weight of ≥ 2000 Da. Smaller polypeptides can be treated as low-molecular-weight drugs. Compared with low-molecular-weight drugs, proteins have very different transport and solubility characteristics in tissues, and, therefore, their delivery is usually much more complex. Specifically, proteins do not diffuse easily through solids and most gels. A simple bilayer lipid membrane is sufficient to preclude the transport of a protein. Two systems with large surface area, the skin and the gastrointestinal (GI) tract, are problematic. The skin is designed to be impermeable to protein transport, and the GI tract hydrolyses proteins into smaller peptides and amino acids for absorption. The high surface area of the lungs makes the pulmonary system attractive for protein delivery. However, lung tissue blocks ultrasound, as previously mentioned. These limitations relegate most protein delivery to injection with some applications employing inhalation.

Regulatory hormones are the centre of focus for controlled protein delivery; specifically, insulin delivery for diabetes therapy comprises the vast majority of protein delivery research, with some research effort in growth-related hormones and birth-control hormones. With respect to ultrasound-assisted protein delivery, nearly all research is focused on insulin delivery, and the great majority of that research is on transdermal delivery. Ultrasonic delivery is ideal, because a small transducer can be placed on the skin surface for a painless, non-invasive delivery route.

2.2.1 Transdermal protein delivery

There is a tremendous amount of literature on the use of ultrasound to enhance the permeability of skin for transdermal drug delivery, including several excellent reviews [90-97]. Therapeutic levels of ultrasound (1 – 3 MHz, 1 – 3 W/cm^2) have been used for years to drive small hydrophobic molecules, such as steroids, into or through the skin [91,92,96-101]. Chemical enhancers have been used to further increase the permeability [102-104]. However, no significant transport of protein could be achieved until 10 years ago when Mitragotri *et al.* [102-114] showed that low-frequency ultrasound was much more effective than higher frequencies and provided evidence as to the mechanism involved. Skin permeability increased with decreasing frequency, and with increasing time of exposure and intensity (beyond a threshold), thus identifying collapse cavitation as a causative mechanism [105-107,110,111,115].

The current theory is that cavitation events open reversible channels in the lipid layers of the stratum corneum, and provide less tortuous paths of transport for proteins, such as insulin [90,105-107,110]. Electron microscopy on skin exposed to low frequency ultrasound revealed the removal of surface cells and the formation of large pores and pockets (~ 20 µm), large enough to accommodate transport of proteins and other large molecules [116-119]. Tezel and Mitragotri [24,120] have formulated a model of the shock wave and of microject cavitation events and their impact on skin permeability. Although this model can be fit to the data of this group, it is based on many assumptions and parameters, and more direct evidence is needed to conclusively reveal the mechanisms of ultrasound-enhanced transdermal protein delivery.

The future of ultrasound-enhanced transdermal protein delivery is brimming with potential, but it has not yet appeared in the clinic. Because large pores and channels are opened through the natural skin barriers, many hormones and proteins could be candidates for transdermal delivery [105,106,121-127]. The effect of the ultrasound on the protein conformation and/or activity needs to be addressed in more detail.

2.2.2 Other protein delivery

2.2.2.1 Activated protein depot

Kwok *et al.* [128] studied insulin-loaded drug depots of poly(2-hydroxyethyl methacrylate) and poly(ethylene glycol) with a surface layer of C18 alkyl chains. In the absence of

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insonation, the alkyl chains appeared to form an organised and less permeable barrier to proteins; on insonation the surface organisation was apparently disrupted, and protein within the depot matrix escaped. In this research very little insulin was released until 1.1 MHz ultrasound was applied. On termination of insonation, the low permeability of the lipid-like surface layer was restored. Such a device is envisioned as a subcutaneous depot with an external transducer positioned over the depot that can be activated either automatically or on demand as insulin is required.

2.2.2.2 Thrombolytic enzymes

The transport of tissue plasminogen activator (tPA) and other lytic proteins, such as urokinase, into clots is beneficial in increasing the rate of fibrinolysis of clots [129,130]. In most studies, the protein is delivered to the clot via intravenous catheter, and the ultrasound is applied transdermally.

Francis *et al.* [131] showed that 1 MHz ultrasound increased the rate of uptake and the depth of penetration of tPA into clots. They speculated that enhanced transport could be due to fluid motion from shock waves, microstreaming, or penetration of the clot by microjets. Later studies showed that pulsed low frequency ultrasound (27 kHz) is more effective than higher frequencies in enhancing fibrinolysis by tPA, again supporting a non-thermal cavitation-related transport into the clots [132].

The same group also showed that water permeability through a fibrin gel was increased by ultrasound (in the absence of any lytic enzymes) [133]. This increase was reduced when the fibrin gels were degassed, and was thus attributed to cavitational activity. Other authors have shown that high-intensity, focused ultrasound produced echo-dense material in the clot, most probably by creating cavitation bubbles [134].

Although ultrasound by itself is beneficial in enhancing thrombolysis, the addition of microbubbles appears to enhance thrombolysis even more [135-139]. In 1995, it was shown that application of ultrasound with albumin-based contrast agent significantly increased fibrinolysis by urokinase of thrombus *in vitro* [135-137]. *In vivo* studies using tPA and other enzymes led to the same conclusions [138,139].

Another advance in this technology is to attach receptors for the thrombus material to the microbubbles, thus attaching the bubble to the thrombus surface during the ultrasonic exposure. These ligands often bind to the glycoprotein IIb/IIIa receptor of platelets, which are expressed when platelets coalesce into a clot, and are used with or without a thrombolytic protein present [137,140]. The authors envision that bubbles cavitating on the thrombus surface will produce microjets that can mechanically disrupt the clot.

2.2.2.3 Pulmonary delivery by ultrasonic nebulisation

There are several reports of protein delivery to the lungs via inhalation of ultrasonically aerosolised protein solutions. Nebulisation requires rather severe cavitation and, therefore, it has been found beneficial to protect the proteins by the addition of certain surfactant stabilisers [141,142]. These additives appear to complex with the proteins, and protect them from degradative shear stresses and perhaps from free radical attack. It is possible that the surfactants may form micelles or liposomes that sequester the proteins and protect them from cavitation stresses. A short list of ultrasonically nebulised protein delivery includes IFN [143], platelet-activating factor [144], lactate dehydrogenase [141], superoxide dismutase [145], α_1-protease inhibitor [146,147], urokinase plasminogen activator [148] and aviscumine [142].

2.2.3 Needs in protein delivery

As mentioned at the beginning of this section, protein delivery is fairly limited at present by very slow diffusion of proteins through skin and polymeric depots, and by the proteinolysis that occurs in the upper GI tract. The authors foresee a need for technology in which a polymeric depot can be implanted, perhaps in subcutaneous, intraperitoneal or intramuscular locations. Timed drug release could then be activated by transdermal ultrasound. Polymers in a depot can be degraded by ultrasound [27,149], but this is a fairly slow process, and the same physicochemical mechanisms that degrade the polymers may degrade the proteins in the depot. What is needed is technology that will open and close the depot to protein transport. The technology of Kwok *et al.* [128] is a good start in this direction, but the depot needs to completely shut off protein delivery when not insonated.

It would also be advantageous if the depot were activated by stable cavitation or some other low intensity phenomenon related to ultrasound. Collapse cavitation may be capable of opening depots, but repeated ultrasonic exposure over long periods may start to have adverse effects on the healthy tissue in the region of the depot. Finally, it would be beneficial if the depot were eventually degradable, so that it did not need to be surgically removed when it was emptied. A refillable depot would also be useful.

2.3 Ultrasound-enhanced small chemical delivery

In the last two decades, ultrasound has been investigated as a delivery mechanism for a variety of therapeutic agents to diseased cells throughout the body. Section 2.3.1 will discuss the use of ultrasound in the delivery of conventional chemotherapy agents for vertebrate tissues, whereas Section 2.3.2 will focus on the enhanced activity of antibacterials in the presence of ultrasound.

2.3.1 Traditional chemotherapy

The use of ultrasound as a drug release and potentiation mechanism in traditional chemotherapy has been studied extensively. This section will discuss ultrasonic chemotherapy delivery in free, micellar and liposomal forms.

2.3.1.1 Free drug

A synergistic effect between the pharmacological activity of chemotherapeutic drugs and ultrasound has been reported for a variety of agents. Loverock *et al.* [150] have shown that 1 h of

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exposure to ultrasound (2.3 W/cm^2 at 2.6 MHz) rendered doxorubicin significantly more toxic to Chinese hamster lung fibroblasts. Exposure to ultrasound alone did not affect cell viability. Flow cytometry revealed an increase in doxorubicin concentration inside the cells, but the authors did not attribute the increased uptake to any particular mechanism.

Tachibana *et al.* [151] studied the effect of 0.3 W/cm^2 and 48 kHz ultrasound on the cytotoxicity of cytosine arabinoside (Ara-C) towards human leukaemia (HL60) cells. Insonation for 120 s in the presence of Ara-C, reduced the number of observed colonies 100-fold when compared with cells incubated with the same concentration of the drug. This increase in cell death was not caused by hyperthermia, as the temperature increase was ≤ 0.2°C throughout the experiments. Scanning electron microscopy of insonated cells revealed a decrease in the total number of microvilli and 'a slight disrupted cell surface with flap-like wrinkles' under the action of ultrasound. They concluded that low intensity ultrasound altered the cell membrane, which resulted in the increase of Ara-C cell uptake.

In another study, Tachibana *et al.* [152] have shown that the exposure of HL60 cells to 255 kHz ultrasound and MC 540 for 30 s formed pores in the cell membrane. They claimed that sonoporation caused the cytoplasm of HL60 cells to extrude through pores formed in their cell membrane. The same effect was not observed when cells were exposed to ultrasound alone, thus implicating a synergism between the drug and ultrasound.

Saito *et al.* [152] have shown that exposure to ultrasound increased the permeability of corneal endothelium cells. The increase in permeability appeared to be reversible and the cells regained their membrane integrity after several minutes.

Rapoport *et al.* [154] investigated the increase in intracellular drug uptake by HL60 cells as a result of ultrasonic irradiation (67 kHz and 2.5 W/cm^2) using fluorescence techniques. It was found that the amount of doxorubicin that intercalated the DNA increased after 1 h of insonation. In another related study, Munshi *et al.* reported that the IC_{50} (inhibitory concentration 50%) for doxorubicin was reduced substantially when HL60 cells were sonicated for 1 h at 80 kHz in the presence of doxorubicin [155]. In both studies, the temperature of the cell suspensions was kept at 37°C using a thermostatic bath and, therefore, hyperthermia was not part of the mechanism of this ultrasonic enhanced killing.

Yu *et al.* [156,157] investigated drug-ultrasound synergism applied to doxorubicin-resistant and cisplatin-resistant human ovarian carcinoma cell. They concluded that the synergism reported was not due to the decrease of the multidrug resistance (*mdr1*) gene level by insonation. Thus, multidrug resistance gene expression is not inhibited by ultrasound. The group did not report whether any changes in drug accumulation occurred.

Some investigators attribute this synergism between drug activity and ultrasound to an increase in the local temperature of the sonicated area (hyperthermia) [158-160]. Saad and Hahn [159] exposed Chinese hamster cells to ultrasound (average intensities in the range 0.5 – 2 W/cm^2) and to several drugs at temperatures in the range 37 – 43°C. The study showed that at lower intensities (0.5 W/cm^2) the cytotoxicity of doxorubicin was significantly enhanced when the temperature was raised from 37 to 41°C. At higher ultrasonic power densities (1 W/cm^2), the cytotoxic effect of doxorubicin increased threefold when the temperature reached 41°C. The study concluded that the 'temperature threshold' decreases as the power intensity of ultrasound increases.

In summary, ultrasound has been used in combination with chemotherapeutic agents for increased efficacy. Insonation appears to enhance the transport of drugs into cells and tissues. Considering the physics of cavitation processes, it is the authors' opinion that ultrasound produces transient micropores in the cell membrane, which would increase the passive accumulation of the drugs in the cells and tissues. Although the cytotoxic effect of chemotherapeutic agents has been shown to increase with insonation, this effect has not been shown to be independent of any increase in drug uptake.

Most studies attribute ultrasonic enhanced killing of cancer cells in the presence of drugs to a phenomena called chemopotentiation. The use of the term chemopotentiation is rather misleading, as it suggests that ultrasound alters the structure of the drug and renders the chemical more potent. Most data reported in the literature support the hypothesis that ultrasound permeabilises the membrane so that more chemotherapeutic drug molecules are able to diffuse into the cells. In some cases, the permeabilisation appears truly synergistic in that both drug and ultrasound are required simultaneously to render the cell membrane more permeable. In general, at sufficiently high intensities the ultrasound permeabilises the membrane, most probably through shear stresses in the membrane from microstreaming or shock waves.

A major problem associated with whole body chemotherapy is not totally alleviated by ultrasound; the drug is still delivered systemically, thus causing systemic side effects. For this reason, research in recent years has focused on developing molecular vehicles that can sequester the drug inside a package, and release it using ultrasound stimulus at the tumour site. Two types of drug delivery molecules have been developed for this purpose: polymeric micelles and liposomes.

2.3.1.2 Micelles

Polymeric micelles have been used to improve site-specific drug delivery in cancer therapy. The technique relies on the small size of these carriers to extravasate at the tumour site where the drug can diffuse into the tumour and carry out its therapeutic effect [161-164]. Although several groups have investigated the use of polymeric carriers to deliver chemotherapeutic as well as other drugs, the only group that has reported the use of polymeric micelles in conjunction with ultrasound is Rapoport, Pitt and colleagues at the University of Utah and Brigham Young University. Their micelles are made from the Pluronic® (BASF Corporation) family of block copolymers.

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Pluronic polymers are triblock copolymers of poly(ethylene oxide) (PEO)–poly(propylene oxide)–PEO, which form micelles at sufficiently high aqueous concentrations [165-170]. These micelles have several advantages as drug delivery vehicles. They are stable in blood and other biological fluids, and are large enough to escape renal excretion, while being small enough to extravasate at the tumour site. Antineoplastic agents can be easily sequestered inside the core of these polymeric micelles by the simple act of mixing [154,171], thus avoiding the complexities involved with covalently bonding the drug to the polymeric carrier [172]. Several studies have reported the effect of Pluronic surfactants in overcoming multi-drug resistance [173-175]. Furthermore, the PEO chains on the micelle exterior prevent its recognition by cells of the reticuloendothelial system (RES).

The feasibility of using ultrasound with Pluronic micelles to deliver anticancer agents *in vitro* was first reported by Munshi *et al.* [155] and Husseini *et al.* [39], who reported that a combination of 70 kHz ultrasound and Pluronic–P105-encapsulated doxorubicin substantially increased the cytotoxicity of the drug. The enhanced toxicity on insonation was attributed to the release of the agent from micelles under the action of ultrasound [176,177].

Using fluorescent microscopy and flow cytometry, they also reported that insonation enhanced the intracellular uptake of Pluronic micelles and their internalisation into the nucleus of HL60 cells [178-183].

The main challenge facing the use of micelles to deliver chemotherapy drugs is in maintaining the concentration of the polymer above the critical micellar concentration, to guarantee that the micellar structures remain intact and do not dissolve and prematurely release the drug before reaching the target site. Pruitt *et al.* [184] have stabilised Pluronic micelles with an interpenetrating network using *N,N*-diethylacrylamide to form Plurogels™. The network expands at room temperature, allowing the drug to accumulate inside its hydrophobic core, and contracts ≥ 31°C, thus trapping the drug at body temperature. The network-micellar structure is eventually degraded after a few days (the half-life is ~ 17 h [185]). Plurogel micelles have also been shown to release doxorubicin after exposure to ultrasound [186].

Recently, *in vivo* studies examined the feasibility of acoustically-activated drug delivery from Plurogel. Nelson *et al.* [187] showed that exposure to 70 kHz ultrasound of doxorubicin encapsulated in Plurogel significantly decreased the size of colorectal cancer tumours in rats. When unencapsulated doxorubicin was administered, the same dose was lethal to the rats within 2 weeks of injection. Gao *et al.* [188] studied the intracellular distribution of fluorescently-labelled non-stabilised Pluronic P105 and Pluronic P105 stabilised using PEG-diacylphospholipid. The study showed that insonation at 1 MHz was able to enhance the accumulation of these labelled micelles at the tumour site in ovarian cancer-bearing *nu-nu* mice. Using the same *in vivo* mouse model, Rapoport *et al.* [189] showed that micelle accumulation was significantly higher in the ultrasonicated tumour than in the non-insonated tumour in the same mouse.

The mechanisms of this acoustically-activated micellar drug delivery system are still being investigated, and there is a strong correlation with insonation frequency and power density that suggests a strong role of stable and transient cavitation.

2.3.1.3 Liposomes

Unlike micelles, liposomes can sequester both hydrophilic and hydrophobic drugs in their aqueous interior and lipid bilayer membrane, respectively. Liposomes are also larger than the polymeric micelles; the average diameters of liposomes are in the range of 150 – 200 nm, compared with 5 – 30 nm for micelles. Herman *et al.* [190] showed that liposomes are able to encapsulate doxorubicin, and reported a decrease in the cardiotoxicity of the drug. Zvi *et al.* [191] reported that liposome-encapsulated doxorubicin accumulates preferentially in cancerous muscle tissues when compared with tumour-free muscles. The studies mentioned above rely on the increased accumulation of liposomes at the tumour site due to increased extravasation (passive targeting). Recently, studies have been reported in the literature in which ultrasound is used as an active targeting mechanism to release drugs from liposomes [32,42,101,192].

Ning *et al.* [37] demonstrated that ultrasound-induced hyperthermia, in addition to enhancing drug antitumour activity, accelerated the release of doxorubicin from long-circulating liposomes. The group reported that by increasing the temperature from 37 to 41°C, the rate of release of doxorubicin was increased sixfold after 1 h of sonication at 2 W/cm^2. The accumulation of doxorubicin in RIF-1 tumour cells was 10-times higher when introduced in liposomes at 42°C compared with when introduced as free drug at 37°C. Several other reports have shown that other drugs can be released from liposomes using ultrasonic hyperthermia [2,193-195].

2.3.2 Antibacterial chemotherapy

Rediske *et al.* [198-200] showed that ultrasound increased the killing of bacteria both in planktonic suspension [196,197] and biofilm forms in the presence of antibiotics. This synergistic killing effect was most pronounced at lower frequencies and decreased as the frequency of insonation increased [201,202]. The effect was more pronounced in *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria) than in *Staphylococcus epidermidis* (Gram-positive bacteria). They found that this enhanced killing synergistic effect is prevalent with certain antibiotics (the aminoglycosides), but does not exist when others are used. They hypothesised that stable cavitation or sonoporation might be involved in increasing the transport of antibiotics into the bacteria either by reducing the mass transfer boundary layer around the cells, or by altering the cell membrane, thus allowing the antibiotics to diffuse through newly formed membrane pores.

In vivo experiments in a rabbit model of an implant infection confirmed the increased toxicity of gentamicin

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against *E. coli* biofilms in the presence of low frequency ultrasound (28.48 kHz and 0.3 W/cm^2) [203]. Pulsed ultrasound applied for 48 h with gentamicin was effective in eliminating *E. coli* infections [204], but was less effective against *P. aeruginosa* [205]. Vancomycin combined with ultrasound was somewhat effective against *S. epidermidis* infections in the same rabbit model [206].

2.3.3 Needs in ultrasonic-enhanced small-chemical delivery

The role of ultrasound in small-chemical delivery involves permeabilisation of the cell membrane, such that these molecules can enter more easily. At present, very little is known about how ultrasound permeabilises the cell membrane. Are small transient holes formed for µs timescales, allowing diffusive entry? Or are larger, more permanent holes or disorganised regions formed? Is a different treatment needed for molecules of different sizes?

Neither is much known about how ultrasound may regulate gene expression and protein production. Does ultrasound cause a stress response (similar to heat shock) that may enhance or interfere with the action of drugs? Is this response similar for all cells, and does it differ for various ultrasonic frequencies and intensities? These and other questions about cell physiology need to be addressed.

Finally, liposomes used in drug delivery are thermodynamically stable, but are cleared by the RES. Alternatively, micelles may not be cleared by the RES, but they are thermodynamically unstable when diluted in blood. Neither system is ideal and, thus, there is a need to make the liposomes more stealthy and the micelles more stable.

3. Expert opinion

3.1 The future of ultrasonic-activated drug delivery

It is clear that the field of ultrasonic-enhanced drug delivery has expanded tremendously during the past decade, and we expect that this trend will continue as our understanding and technology increases. Furthermore, the use of microbubbles to assist drug delivery has exploded in the past 5 years, and we expect that much of the technological growth in the next decade will be in the clever use of microbubbles and ultrasonic pulse sequences.

The published literature regarding ultrasound and microbubble in gene delivery is solid, makes important contributions, and is definitely worth studying. This review has cited many innovations, and more are sure to come. We propose that ultrasound-enhanced gene delivery will experience the greatest growth and provide the greatest medical contribution because ultrasound and microbubbles allow genes to be delivered through non-viral technologies to specific locations. Such genetic therapy can make monumental contributions to the treatment of heart disease, vascular disease, cancer, autoimmune diseases, and much more. As microbubble collapse is controlled and fine-tuned, we speculate that it can be applied to deliver genetic therapy to the brain to treat neurological diseases.

The area of protein delivery has been limited in the past to insulin delivery technology, and will probably remain so, with some small expansion into delivery of a few other small regulatory hormones. Despite 10 years of solid research on transdermal delivery of proteins, there are still limitations in the rate at which a protein can pass through the skin without inflicting permanent damage. Transport can be increased by increasing the surface area, but a larger treatment area and heavy transducers may lead to difficulty with patient compliance. We foresee some growth in this area, but not wide clinical application until the size of the transducer is reduced, such that it can be easily attached and carried at work or at home.

Technological developments for small chemical delivery will follow those of gene delivery. For cancer chemotherapy or antibiotic therapy, delivery to specific tissues will be targeted by decorating the carriers or microbubbles with antibodies or other site-specific adhesive molecules.

3.2 What are the critical needs?

The basis of future technological advancement requires a better understanding of the behaviour of microbubbles in ultrasonic fields. This will require more modelling and experimental understanding of the physics of microbubble oscillation and collapse as a function of microbubble size, internal gas composition, membrane or wall thickness, mechanical properties (i.e., modulus, shear strength, viscosity), acoustic frequency and pressure amplitude. At present, most ultrasound/microbubble drug delivery is done with imaging transducers at high frequencies that are not necessarily optimised for cavitation or drug delivery. What are the optimal bubble sizes and ultrasonic frequencies for drug delivery? The goal of such research should be to develop the acoustic parameters and perhaps the pulse sequences that can excite a bubble to cavitate without imposing mechanical or thermal damage to tissues. Some modelling effort has commenced [17,207], but much more is needed.

If bubble physics dictate that the optimal frequencies are different than those frequencies available in imaging transducers, then a new generation of transducers must be developed for use in ultrasound/microbubble drug delivery. Even better would be a multi-frequency transducer that can perform three functions, perhaps all at different frequencies: target imaging, bubble destruction, and cell membrane permeabilisation.

As mentioned, in conjunction with transdermal drug delivery, small-sized low-frequency transducers need to be developed so that patients can wear them for continuous insulin delivery. Although some intra-luminal transducers are available in a catheter format, at present, these are designed for imaging; catheter-based transducers also need to be developed for drug delivery.

Finally, we must not forget the issue of biological response to these new applications of ultrasound in drug delivery. Is

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the frequency for optimum drug release from a carrier also the optimum frequency for permeabilising a cell membrane, without destroying the cell? It is very probable that frequencies that optimise membrane permeability are different than frequencies that optimise drug release. Thus, the response of cells and their membranes to ultrasound must be studied, so that in our efforts to optimise drug release, we do not produce collateral damage to the target or adjacent tissues.

3.3 What are the best technologies?

It is our opinion that the best technologies at present are those that employ gas bubbles, with the therapeutic agent in or on the bubble, such as DNA decorating the exterior of a surfactant-stabilised microbubble, or a thick-shelled microbubble [12,42] that can carry the drug inside, either in an oil or aqueous phase. In these systems the drug and cavitation agent are intimately mixed, and the drug is, therefore, released at the location where tissues are stressed by cavitation. Micelles and liposomes without gas are less useful because the cavitation is not necessarily produced at the same time or place as the drug. Application of free drug or free DNA is least efficient because it is not sequestered, and can, therefore, interact with non-targeted tissue, or be cleared (or degraded) before it can reach therapeutic concentrations. Gas liposomes or microbubbles constructed from native proteins (such as albumin), or with PEO chains on the surface, may have an advantage in that they will not be recognised and cleared as fast from the circulatory system [208,209].

Another current and innovative technology in which we anticipate more growth is the attachment of targeting molecules to microbubbles or drug carriers. The targeting molecules may be different for each application (such as unique or individualised antibodies), and, therefore, it may be advantageous to attach a generic binder to the microbubbles. The specific antibodies could then be attached to a complementary binder. The target-specific antibodies could then be mixed with the generic microbubbles to create custom drug delivery systems with the drug attached to the bubbles. Although biotin and streptavidin have been suggested for such a system [210], these proteins may elicit an antigenic response. Thus, other systems should be investigated, such as a self-assembled biological system or a chemical system, such as maleimide–thiol bond formation.

Without a crystal ball, one cannot predict what specific technology will be in place 10 years from now. However, we can predict that ultrasonic-activated drug delivery will play an ever-increasing role in targeted drug therapies.

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Attachment 1A

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072 _7 $aMED107000$2bisacsh

082 04 $a591.3$223

245 00 $aEpigenetics protocols /$cedited by Trygve O. Tollefsbol.

250 $a2nd edition

264 _1 $aNew York :$bHumana Press,$c[2011]

264 _4 $c©2011

300 $a1 online resource (xiv, 332 pages) :$billustrations (some color)

336 $atext$btxt$2rdacontent

337 $acomputer$bc$2rdamedia

338 $aonline resource$bcr$2rdacarrier

347 $atext file$2rda

490 1_ $aMethods in molecular biology,$x1940-6029 ;$vv. 791

504 $aIncludes bibliographical references and index.

505 0_ $aAdvances in epigenetic technology -- DNA methylation detection: Bisulfite genomic sequencing analysis -- M
Methyl-typing -- SIRPH: An HPLC-based SNuPE for quantitative methylation measurement at specific CpG sites -
Inhibition of DNA methylation in somatic cells -- DNA methyltransferase assays -- A chromatin immunoprecipit
- Combined chromatin immunoprecipitation and bisulfite methylation sequencing analysis -- Studying RNA-prote
elements -- Inhibition of histone deacetylases -- Computational methods for epigenetic analysis: The protocc

588 0_ $aOnline resource; title from PDF title page (Springer, viewed Sept. 22, 2011).

520 $aThe field of epigenetics has played a major role at the forefront not only of molecular biology, but also
highlight select techniques that have been mainstays in the field as well as to cover methods that are espec
epigenetic analyses which is essential to a complete understanding of the vast body of information that is b
and reagents, step-by-step, readily reproducible laboratory protocols, and notes on troubleshooting and avoi
investigators who wish to continue exploring this exciting and progressive research field.

546 $aEnglish.

650 _0 $aChromatin$vLaboratory manuals.

650 _0 $aTelomere$vLaboratory manuals.

650 _0 $aADP-ribosylation$vLaboratory manuals.

PARSE EX.1051.114

650 _0 $aEpigenesis$vLaboratory manuals.

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650 _2 $aAmino Acid Sequence.

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650 _2 $aDNA Packaging.

650 _2 $aNuclear Proteins.

650 _2 $aGenetic Structures.

650 _2 $aNucleoproteins.

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650 _2 $aCell Physiological Phenomena.

650 _2 $aAlkylation.

650 _2 $aMolecular Structure.

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650 _2 $aChemical Phenomena.

650 _2 $aPhenomena and Processes

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650 _2 $aChemicals and Drugs

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650 _2 $aCells.

650 _2 $aAnatomy.

650 _2 $aDNA Methylation.

650 _2 $aHistone Code.

650 _2 $aChromosome Structures.

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700    1_    $aTollefsbol, Trygve O.
776    08    $iPrint version:$tEpigenetics protocols$s2nd ed.$dNew York : Humana/Springer, ©2011$z9781617793158
830    _0    $aMethods in molecular biology (Clifton, N.J.) ;$vv. 791.
856    40    $uhttp://link.springer.com/10.1007/978-1-61779-316-5
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060 10 QU 25
082 00 572.8/7 $2 23
090 $b
245 00 Epigenetics protocols / $c edited by Trygve O. Tollefsbol.
250 2nd edition.
264 1 New York, NY : $b Humana Press, $c ©2011.
300 xi, 332 pages : $b illustrations ; $c 26 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
490 1 Springer protocols
490 1 Methods in molecular biology, $x 1940-6029 ; $v v. 791
504 Includes bibliographical references and index.
505 0 1. Advances in epigenetic technology / Trygve O. Tollefsbol -- 2. DNA methylation detection: bisulfite genomic sequencing analysis / Yuanyuan Li and Trygve O. Tollefsbol -- 3. Methylation-specific PCR / Ja-Lok Ku, You-Kyung Jeon, and Jae-Gabb Park -- 4. Analysing DNA methylation using bisulphite pyrosequencing / Thomas Mikeska, Jörg Felsberg, Chelsee A. Hewitt, and Alexander Dobrovic -- 5. Closed-tube PCR methods for locus-specific DNA methylation analysis / Ida L.M. Candiloro, Thomas Mikeska, and Alexander Dobrovic -- 6. A combined bisulfite restriction analysis bioinformatics tool: methyl-typing / Cheng-Hong Yang, Yu-Huei Cheng, Li-Yeh Chuang, and Hsueh-Wei Chang -- 7. SIRPH: an HPLC-based SNuPE for quantitative methylation measurement at specific CpG sites / Heike Singer, Nicole Nüsgen, and Osman El-Maarri -- 8. Restriction landmark genome scanning / Hisato Okuizumi, Tomoko

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650 0 Chromatin $v Laboratory manuals.
650 0 Telomere $v Laboratory manuals.
650 0 ADP-ribosylation $v Laboratory manuals.
650 0 Epigenesis $v Laboratory manuals.
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700 1 Tollefsbol, Trygve O.
830 0 Springer protocols (Series)
830 0 Methods in molecular biology (Clifton, N.J.) ; $v v. 791.
856 41 $3 SpringerLink $u https://doi.org/10.1007/978-1-61779-316-5
856 41 $3 Table of contents $u http://catdir.loc.gov/catdir/enhancements/fy1312/2011935043-t.html

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856 4 $u http://www.springerprotocols.com/BookToc/doi/10.1007/978-1-61779-316-5
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Attachment 1C

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906 __ |**t** 64-- |**u** ---- |**v** 0
953 __ |**a** xx00

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260 __ |**i** subdivision |**a** Laboratory manuals |**i** under scientific and technical headings for workbooks containing concise background information and directions for performing work, including experiments, in the laboratory, e.g. |**a** Cancer--Diagnosis--Laboratory manuals; Radio--Laboratory manuals

906 __ |**t** 8716 |**u** fk05 |**v** 0

952 __ |**a** 0 bib. records to be changed

953 __ |**a** fj26

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150 __ |**a** Telomere

450 __ |**a** Telomeres

550 __ |**w** g |**a** Chromosomes

670 __ |**a** Work cat.: 94-33104: Kipling, D. The telomere, 1995.

670 __ |**a** MeSH browser, Mar. 31, 2020 |**b** (Telomere. SN A terminal section of a chromosome which has a specialized structure and which is involved in chromosomal replication and stability. Its length is believed to be a few hundred base pairs. UF Telomeres. BT Chromosome Structures)

670 __ |**a** Cerchiara, J.A. Telomere dynamics in Magellanic penguins (Spheniscus magellanicus), 2015: |**b** p. 3 (Telomeres, tandem repeating, non-coding sequences that protect the coding regions of DNA during cell replication, are linked to survival. Telomeres shorten with age in most species)

670 __ |**a** A dictionary of biology, 2019 |**b** (telomere: The end of a chromosome, which consists of tandemly repeated short sequences of DNA that perform the function of ensuring that each cycle of DNA replication has been completed. Each time a cell divides some sequences of the telomere are lost; eventually (after 60-100 divisions in an average cell) the cell dies (the telomere theory of ageing (see senescence) is based on this phenomenon). Thus for a limited period telomeres provide protection for the genes located at the ends of the chromosome.)

670 __ |**a** Oxford dictionary of biochemistry and molecular biology, 2008 |**b** (telomere: the DNA-protein structure that seals either end of a chromosome. Telomeric DNA consists of simple tandemly repeated sequences specific for each species)

PARSE EX.1051.127

670 __ |**a** Merriam-Webster dictionary online, Mar. 31, 2020 |**b** (telomere: the natural end of a eukaryotic chromosome composed of a usually repetitive DNA sequence and serving to stabilize the chromosome)

906 __ |**t** 9434 |**u** te04 |**v** 1

910 __ |**a** Record saved by td14 on 08/05/2020 at 07:53:38

910 __ |**a** Proposal saved by tc03 on 04/02/2020 at 12:28:38

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Attachment 1F

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550 __ |**w** g |**a** Cellular signal transduction
670 __ |**a** Work cat.: ADP-ribosylating toxins and G proteins, c1990 |**b** (ADP-ribosylation)
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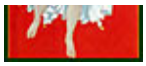


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Attachment 1G

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550 __ |**w** g |**a** Embryology

550 __ |**w** g |**a** Evolution (Biology)

550 __ |**w** g |**a** Genetics

670 __ |**a** McGraw-Hill dictionary of scientific and technical terms, 2003 |**b** (Epigenesis [embryology] development in gradual stages of differentiation)

670 __ |**a** Wikipedia, accessed Oct. 24, 2011: |**b** (Epigenesis (biology): in biology, epigenesis has at least two distinct meanings: [1] the unfolding development in an organism, and in particular the development of a plant or animal from an egg or spore through a sequence of steps in which cells differentiate and organs form; [2] the theory that plants and animals develop in this way, in contrast to theories of preformationism)

906 __ |**t** 9037 |**u** fk07 |**v** 0

910 __ |**a** Proposal saved by tc03 on 10/31/2011 at 11:09:25

952 __ |**a** 550 (Evolution (Biology)) prompted by TxU; 550 (Evolution) deletion prompted by TxU

953 __ |**a** xx00 |**b** fh12

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Type of Work: Text
Registration Number / Date: TX0007451979 / 2011-11-23
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Title: Epigenetics Protocols, Editor Trygve O. Tollefsbol.
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Copyright Claimant: Springer Science+Business Media, LLC. Address: 233 Spring Street, New York, NY, 10013, United States.
Date of Creation: 2011
Date of Publication: 2011-09-15
Nation of First Publication: United States
Authorship on Application: Springer Science+Business Media, LLC, employer for hire; Domicile: not known; Citizenship: not known. Authorship: text, editing.
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```

```
997    ##$aNot considered title change 20150731
998    ##$aERMSOA 118128 $b20180912
999    ##$aAUTH
035    ##$a(OCoLC)45320094
```

Attachment 2B

Biology MeSH Descriptor Data 2023

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading
Biology
Tree Number(s)
H01.158.273
Unique ID
D001695
RDF Unique Identifier
http://id.nlm.nih.gov/mesh/D001695
Annotation
the discipline (education, history, etc) only; use BIOLOGICAL PHENOMENA or specifics treed under it for biological characteristics and processes of organisms and of the biosphere; note broader heading BIOLOGICAL SCIENCE DISCIPLINES is also available
Scope Note
One of the BIOLOGICAL SCIENCE DISCIPLINES concerned with the origin, structure, development, growth, function, genetics, and reproduction of animals, plants, and microorganisms.
Entry Version
BIOL
Date Established
1966/01/01
Date of Entry
1999/01/01
Revision Date
2008/11/26

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Web Policies
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HHS Vulnerability Disclosure

NLM Support Center
Accessibility
Careers

NLM | NIH | HHS | USA.gov

Attachment 2C

OCLC 45320094 **No holdings in XXX - 53 other holdings**

Continuing Resources

								Rec Stat		**Entered**	20007 21	**Replaced**	20220809165851.6		
Type	a	**ELvl**		**Srce**		**GPub**		**Ctrl**		**Lang**	eng				
BLvl	s	**Form**		**Conf**	0	**Freq**	m	**MRec**		**Ctry**	enk				
S/L	0	**Orig**		**EntW**		**Regl**	r	**Alph**							
Desc	a	**SrTp**	p	**Cont**		**DtSt**	d	**Dates**	2000	,	2001				

010 00243520
040 NLM $b eng $c NLM $d CAS $d DLC $d EYM $d CGU $d OCLCQ $d IUL $d UQ1 $d OCLCQ $d HEBIS $d GBVCP $d UKMGB $d OCLCQ $d OCLCF $d OCLCO $d CUS $d OCLCQ $d OCLCO $d OCLCQ $d NLW $d PUL
015 021606609 $2 dnb
015 GBA053211 $2 bnb
016 7 021606609 $2 DE-101
016 7 2042577-6 $2 DE-600
016 7 100960660 $2 DNLM
016 7 011647534 $2 Uk
016 7 bA053211 $2 Uk
019 1275070782
022 1465-6906 $l 1465-6906
030 GNBLFW
037 $b Genome Biology Ltd., Middlesex House, 34-42 Cleveland Street, London W1P 6LB, UK (England)
042 pcc
050 00 QH447 $b .G465
060 00 W1 $b GE336FQ
090 $b
245 00 GenomeBiology.com.
246 3 Genome biology.com
246 13 GenomeBiology
246 3 Genome biology
246 13 GB
260 London : $b Genome Biology Ltd., $c ©2000-©2001.
300 volumes : $b illustrations ; $c 28 cm
310 Monthly
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 Vol. 1, no. 1 (2000)-v. 2, issue 2 (2001).
500 Title from cover.
510 0 Chemical abstracts $x 0009-2258
530 The original and complete version of the journal is the electronic version available and updated continuously on the world wide web. While everything in the printed version of the journal is also available online, parts of the electronic version will not be included in the printed journal.
650 0 Genomes $v Periodicals.

650 0 Biology $v Periodicals.
650 0 Molecular biology $v Periodicals.
650 12 Biology.
650 22 Genetics.
650 22 Genome.
650 7 Biology. $2 fast $0 (OCoLC)fst00832383
650 7 Genomes. $2 fast $0 (OCoLC)fst00940225
650 7 Molecular biology. $2 fast $0 (OCoLC)fst01024734
650 17 Génomique. $2 rasuqam
650 7 Génome. $2 rasuqam
650 7 Génétique. $2 rasuqam
650 7 Biologie. $2 rasuqam
655 2 Periodicals.
655 7 Periodicals. $2 fast $0 (OCoLC)fst01411641
655 7 Periodicals. $2 lcgft $0 http://id.loc.gov/authorities/genreForms/gf2014026139 $0 (uri)
http://id.loc.gov/authorities/genreForms/gf2014026139
655 7 Ressource Internet (Descripteur de forme) $2 rasuqam
655 7 Périodique électronique (Descripteur de forme) $2 rasuqam
776 1 $t GenomeBiology.com (Online) $w (DLC) 2002262016 $w (OCoLC)45225582
785 00 $t Genome biology $w (DLC) 2002262018 $w (OCoLC)49212711
850   DLC $a DNLM
856 41 $u http://bibpurl.oclc.org/web/37 $u http://genomebiology.com/ $7 0
856 41 $u http://bibpurl.oclc.org/web/281 $u http://www.biomedcentral.com/1465-6906/ $7 0
856 41 $u http://www.ncbi.nlm.nih.gov/pmc/journals/7/

Attachment 2D

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2009125753
LCCN Permalink: https://lccn.loc.gov/sh2009125753
HEADING: Genomes Periodicals

000 00447cz a2200169n 450
001 8060248
005 20110729162708.0
008 091020|| anannbabn |n ana
010 __ |**a** sh2009125753
035 __ |**a** (DLC)442122
035 __ |**a** (DLC)sh2009125753
040 __ |**a** DLC |**b** eng |**c** DLC
150 __ |**a** Genomes |**v** Periodicals
667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: Mammalian genome, c1991-
906 __ |**t** 8888 |**u** tc00 |**v** 0
953 __ |**a** tc00

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Attachment 2E

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2007101986
LCCN Permalink: https://lccn.loc.gov/sh2007101986
HEADING: Biology Periodicals

000 00427cz a2200169n 450
001 7416978
005 20110729155828.0
008 071129|| anannbabn |n ana
010 __ |**a** sh2007101986
035 __ |**a** (DLC)373502
035 __ |**a** (DLC)sh2007101986
040 __ |**a** DLC |**b** eng |**c** DLC
150 __ |**a** Biology |**v** Periodicals
667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: TIP,
906 __ |**t** 8888 |**u** te00 |**v** 0
953 __ |**a** te00

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Attachment 2F

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2008107896
LCCN Permalink: https://lccn.loc.gov/sh2008107896
HEADING: Molecular biology Periodicals

000 00494cz a2200169n 450
001 7458543
005 20110729160534.0
008 080304|| anannbabn |n ana
010 __ |**a** sh2008107896
035 __ |**a** (DLC)384071
035 __ |**a** (DLC)sh2008107896
040 __ |**a** DLC |**b** eng |**c** DLC
150 __ |**a** Molecular biology |**v** Periodicals
667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: Zhongguo sheng wu hua xue yu fen zi sheng wu xue bao =, 1998-
906 __ |**t** 8888 |**u** te00 |**v** 0
953 __ |**a** te00

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Attachment 3A

```
001    9912284213406676
005    20230310094239.0
007    cr|cn#||||||||
008    040830c20049999enkbr p       0    0eng
010    ##$a  2005243226
022    ##$a1742-5247 $l1742-5247 $9PY $7(Print)
022    ##$a1744-7593 $9EN $7(Electronic)
035    ##$9101228421
040    ##$aDNLM $cDNLM
041    0#$aeng
042    ##$alcd
044    ##$9England
060    00$aW1 $bEX52LF
210    10$aExpert Opin Drug Deliv $2DNLM
245    00$aExpert opinion on drug delivery.
260    ##$aLondon : $bAshley Publications, Ltd.
260    3#$aLondon : $bInforma Healthcare
300    ##$av. : $bill.
310    ##$aBimonthly
336    ##$atext $btxt $2rdacontent
337    ##$aunmediated $bn $2rdamedia
338    ##$avolume $bnc $2rdacarrier
362    1#$aBegan with: Vol. 1, no. 1 (Nov. 2004). $zCf. Publisher's
web site.
500    ##$aDescription based on: Vol. 2, no. 1 (Jan. 2005); title
from cover.
510    1#$91 $aMEDLINE $bv1n1, Nov. 2004-
510    1#$91 $aPubMed $bv1n1, Nov. 2004-
510    1#$aIndex medicus
530    ##$aAlso issued online.
650    12$aDrug Delivery Systems
655    #2$aPeriodical
856    4#$uhttp://www.tandfonline.com/loi/iedd20
911    ##$aElectronic and print, print canceled 2017
914    ##$aPortico $bCurrent to 2022 as of 20220627
$cISSN_17425247_21
935    ##$a(DNLM)1228421-nlmdb
992    ##$pP2 $eE4 $a20050204
993    ##$aSDB $b20050217
994    ##$aCDN $b20050217
995    ##$aAUTH $b20050223 $cREV $d20220629
996    ##$aCONSER $b20050223
997    ##$aOMR
998    ##$aERMSLC 118145 $b20150827
999    ##$aAUTH
035    ##$a(OCoLC)57731789
```

Attachment 3B

Drug Delivery Systems MeSH Descriptor Data 2023

Details | Qualifiers | MeSH Tree Structures | Concepts

MeSH Heading
Drug Delivery Systems
Tree Number(s)
E02.319.300
Unique ID
D016503
RDF Unique Identifier
http://id.nlm.nih.gov/mesh/D016503
Annotation
coordinate with drug / admin; do not confuse with MEDICATION SYSTEMS, an organizational concept in patient care for providing medication; note entry term DRUG TARGETING: do not confuse with MOLECULAR TARGETED THERAPY
Scope Note
Systems for the delivery of drugs to target sites of pharmacological actions. Technologies employed include those concerning drug preparation, route of administration, site targeting, metabolism, and toxicity.
Entry Term(s)
Drug Targeting
See Also
Dosage Forms
Drug Administration Routes
Drug Carriers
Public MeSH Note
92
History Note
92
Date Established
1992/01/01
Date of Entry
1991/06/21
Revision Date
2010/06/25

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PARSE EX.1051.155

NLM | NIH | HHS | USA.gov

Attachment 3C

OCLC 57731789 **No holdings in XXX - 27 other holdings**

Continuing Resources

						Rec Stat		**Entered**	20040830	**Replaced**	20200403202533.2
Type	a	**ELvl**		**Srce**		**GPub**		**Ctrl**		**Lang**	eng
BLvl	s	**Form**		**Conf**	0	**Freq**	b	**MRec**		**Ctry**	enk
S/L	0	**Orig**		**EntW**		**Regl**	r	**Alph**			
Desc	a	**SrTp**	p	**Cont**		**DtSt**	c	**Dates**	2004 ,	9999	

010 2005243226
040 NLM $b eng $c NLM $d OCLCQ $d NLE $d OCLCQ $d GBVCP $d UKMGB $d MYG $d OCLCQ $d OCLCF $d OCLCO $d OCLCQ $d CUS $d OCLCQ $d OCLCO $d OCLCA
015 GBA547474 $2 bnb
016 7 026672103 $2 DE-101
016 7 2167286-6 $2 DE-600
016 7 101228421 $2 DNLM
016 7 012915682 $2 Uk
019 1062164524
022 1742-5247 $l 1742-5247
042 pcc
050 4 RS199.5
060 00 W1 $b EX52LF
090 $b
210 0 Expert opin. drug deliv. $b (Print)
222 0 Expert opinion on drug delivery $b (Print)
245 00 Expert opinion on drug delivery.
260 London : $b Ashley Publications
300 volumes : $b illustrations
310 Bimonthly
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 1 Began with: Vol. 1, no. 1 (Nov. 2004). $z Cf. Publisher's web site.
530 Also issued online; subscription required for full-text online access.
588 Description based on: Vol. 2, no. 1 (Jan. 2005); title from cover.
650 0 Drug delivery devices $v Periodicals.
650 0 Drug delivery systems $v Periodicals.
650 12 Drug Delivery Systems. $0 (DNLM)D016503
650 7 Drug delivery devices. $2 fast $0 (OCoLC)fst00898666
650 7 Drug delivery systems. $2 fast $0 (OCoLC)fst00898667
655 2 Periodical. $0 (DNLM)D020492
655 7 Periodicals. $2 fast $0 (OCoLC)fst01411641
776 08 $i Online version: $t Expert opinion on drug delivery $x 1744-7593 $w (DLC) 2011242064 $w (OCoLC)60630038
850 DNLM
856 41 $u http://www.tandfonline.com/loi/iedd20

856 41 $u http://informahealthcare.com/journal/edd

Attachment 3D

LC control no.: sh 87007331
LCCN Permalink: https://lccn.loc.gov/sh87007331
HEADING: Drug delivery devices

000 00707cz a2200241n 450
001 4812291
005 20120329073110.0
008 871124i| anannbabn |a ana
010 __ |**a** sh 87007331
035 __ |**a** (DLC)sh 87007331
035 __ |**a** (DLC)158976
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** RS210
150 __ |**a** Drug delivery devices
450 __ |**a** Devices, Drug delivery
550 __ |**w** g |**a** Drug delivery systems
550 __ |**w** g |**a** Medical instruments and apparatus
670 __ |**a** Work cat.: Drug delivery devices : fundamentals and applications, c1988.
670 __ |**a** NIH |**b** (Drugs, Drug delivery systems)
675 __ |**a** MESH
906 __ |**t** 8849 |**u** fk04 |**v** 1
952 __ |**a** 0 bib. record(s) to be changed
953 __ |**a** ff01 |**b** ff01

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Attachment 3E

LC control no.: sh 88007108
LCCN Permalink: https://lccn.loc.gov/sh88007108
HEADING: Drug delivery systems

000 00834cz a2200277n 450
001 4819817
005 20120329074529.0
008 881114i| anannbabn |a ana
010 __ |**a** sh 88007108
035 __ |**a** (DLC)sh 88007108
035 __ |**a** (DLC)166502
040 __ |**a** DLC |**c** DLC |**d** DLC
053 _0 |**a** RS199.5 |**b** RS210
150 __ |**a** Drug delivery systems
450 __ |**a** Delivery systems, Drug
450 __ |**a** Drug administration technology
450 __ |**a** Drug delivery technology
450 __ |**a** Drugs |**x** Delivery systems
550 __ |**w** g |**a** Pharmaceutical technology
670 __ |**a** Work cat.: Drug delivery systems, 1987.
670 __ |**a** NIH |**b** (Drugs, Drug delivery systems)
670 __ |**a** LC database, Nov. 10, 1988.
670 __ |**a** MESH |**b** (Drug delivery systems)
906 __ |**t** 9843 |**u** te07 |**v** 1
952 __ |**a** LC pattern: Drug delivery devices
953 __ |**a** ff01 |**b** ta25

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PARSE EX.1051.165

APPENDIX 1

CURRICULUM VITAE SYLVIA D. HALL-ELLIS

EDUCATION

Ph.D., University of Pittsburgh, Pittsburgh, Pennsylvania, 1985
M.P.S., University of Denver, Denver, Colorado, 2014
Post Graduate Studies, University of Texas – San Antonio, Texas, 1975-1976
M.L.S., University of North Texas, Denton, Texas, 1972
B.A., Rockford University, Rockford, Illinois, 1971

PROFESSIONAL EXPERIENCE

1981- **Consultant for higher education, non-profit organizations, and corporations.**

2002- **Adjunct Professor, School of Information, San José State University, San José, California.** Serve as part-time faculty member teaching graduate students in technical services (cataloging, bibliographic control, classification), "core courses," and special topics.

2014-2016 **Director, Grants and Resource Development, Colorado Community College System.** Provided leadership and vision to foster the continued growth of rigorous scholarship, innovative projects, and creative work for statewide system, 13 campuses, and 50 teaching sites serving 155,000 students. Responsible for leadership and ensured efficient functioning of contract and grants in compliance with state & federal requirements and successful implementation and management. Served as a subject matter expert and liaison for college Grant Directors for all issues relating to grants and subcontracts.

2010-2014 **Senior Grant Administrator, Morgridge College of Education, University of Denver (Colorado).** Provided leadership and vision to foster continued growth of rigorous scholarship, innovative research, and creative work in the Morgridge College of Education. Ensure that contract and grants processes function effectively and efficiently for 60 faculty and researchers with a focus on the successful progression and efficient management of grants totaling $13M. Worked effectively and collegially with Department Chairs and Program Coordinators on operational grant-related management activities and with a broad range of internal and external constituencies. Supported the dissemination and promotion of faculty research and scholarship to outside constituents at conferences and through publications. Assisted Principal Investigators and grant project teams by coaching, mentoring, and financial management.

2011-2013 **Interim Director & Assistant Dean, Westminster Law Library, Sturm College of Law, University of Denver.** Planned, organized, and directed all administrative activities for the library serving students, faculty, and alumni; oversaw the employment, retention, promotion, transfer and termination of library personnel; represented the library at professional conferences and public meetings; created and promoted a climate and culture of acceptance for new programs and services, a positive high-quality image of the law library, and that reflect the organization's values, encourage excellent performance, and reward high productivity and innovation; provided leadership and set strategic direction of the organization; ensured that the library provided excellent customer service through solution-oriented staff response to patron needs and by responsiveness and continuous improvement of the organization; promoted, developed, and maintained positive working relationships with colleagues and customers including key stakeholders and groups, higher education institutions, the legal community, other regional libraries and districts statewide, and national library organizations.

2007-2014 **Associate Professor, Library & Information Science, Morgridge College of Education, University of Denver (Colorado).** Served in leadership role and worked collaboratively in

PARSE EX.1051.167

program, college, campus and community environments. Advised and supervised students, taught core and specialized courses at the graduate level in an integrative, student-centered learning environment. Served on LIS, College, and University committees, and maintained working relationships with colleagues in other academic units and information professionals in the Rocky Mountain region and beyond. Served on and chair doctoral student dissertation committees. Oversaw and facilitated the College and LIS graduate student association.

2002-2007 **Assistant Professor, Library & Information Science, College of Education, University of Denver (Colorado).** Served as tenure-track faculty member teaching graduate students in "core courses," resource description and access, service learning, and independent studies. Advised graduate students, participate on LIS and College committees, and serve on doctoral student dissertation committees. Oversaw and facilitated the LIS graduate student association and alumni association.

2000-2002 **Affiliate Faculty, Library & Information Science, College of Education, University of Denver (Colorado).** Served as part-time faculty member teaching graduate students in technical services (cataloging, bibliographic control, classification), "core courses," and special topics. Oversaw and facilitated the LIS graduate student association and alumni association.

2000-2001 **Special Assistant to the Secretary's Regional Representative, U.S. Department of Education, Region VIII, Denver, Colorado.** Served as the principal advisor and representative of the U. S. Secretary of Education's Regional Representative (SRR). Ensured the implementation of major goals of the SRR and the Secretary. Provided leadership on behalf of the SRR in contacts with high-level officials in Region VIII requiring sensitive policy interpretation in communication with senior Department officials to solve problems and resolve issues raised by State and local education officials. Served as the primary contact for School-to-Work/Career, Children's Health Insurance Program, and Safe and Drug-Free Schools. Delivered technical assistance to local education agencies and institutions of higher education in technology, professional development, and school construction.

1999-2000 **Catalog Librarian, Jefferson County Public Library, Lakewood, Colorado.** Performed original, copy cataloging and classification of library materials (English and Spanish) using standard library protocols; completed original descriptive cataloging and subject analysis; enhanced brief catalog and authority records in III.

1997-1999 **Development Officer, McREL International, Aurora, Colorado.** Served as senior member of corporate management team in strategic planning, development of proposals and contracts, implementation, and evaluation of new services, products, and programs for educational agencies. Provided creative leadership to corporate committees to solicit ideas, identify goals and objectives, plan, develop, present, and evaluate professional development opportunities.

1995-1997 **Education Specialist, Education Service Center, Region One, Edinburg, Texas.** Served as member of Administrative Cabinet team in strategic planning, development of proposals and contracts, implementation, and evaluation of telecommunications capabilities, services, products, and programs for 40 school districts serving 283,000 students in 7 counties. Provided creative leadership to regional and state committees to solicit ideas, identify strategic goals and objectives, plan, develop, present, and evaluate funding opportunities and professional development for 400 librarians.

1993-1996 **Assistant Professor of Library Science, Sam Houston State University, Huntsville, Texas.** Served a faculty member teaching 400 graduate students in technical services (cataloging,

bibliographic control, classification), automation, and networking. Participated in distance education program and coordinated annual conference. Conducted university and Texas Library Association-funded field research focused on library collection development and academic achievement.

1992-1993 **Head Librarian, Rocky Mountain College of Art & Design, Denver, Colorado.** Responsible for the daily operation, selection and acquisition of materials, formulation of policies for library operations, media center, and photography/slides archives. Designed and implemented library automation and delivery of electronic resources to college community.

1981-1985 **Development Officer, PRLC, Inc., Pittsburgh, Pennsylvania.** Served as senior member of corporate management team in strategic planning, development of proposals and contracts, implementation, and evaluation of new services, products, and programs for 100 institutional member organizations. Coordinated the development of proposals and contracts totaling $4,000,000 annually. Provided creative leadership to corporate committees to solicit ideas, identify goals and objectives, plan, develop, present, and evaluate professional development opportunities.

1981 **Director of Library Development, Pennsylvania Department of Education, Harrisburg, Pennsylvania.** Responsible for statewide development, technical assistance, professional development, resource sharing, children's services, institutional library services, networking, and state aid program for all libraries throughout the Commonwealth. Functioned as liaison to Governor's Advisory Council, LSCA Advisory Council, District Administrators, private colleges, universities, consortia managers, and network directors. Supervised $14,000,000 formula-based state aid program and $3,000,000 grant awards to individual libraries, consortia, and networks.

1978-1981 **Assistant Director, Southern Tier Library System, Corning, New York.** Coordinated operation of system-wide programs (technical assistance, professional development, resource sharing, technical services, outreach) to 40 public libraries in 5 counties serving 500,000 residents. Solicited ideas, identified goals, sponsored, and evaluated professional development opportunities and technical assistance sessions.

1976-1978 **Division Librarian for Technical Services, Corpus Christi Public Libraries, Corpus Christi, Texas.** Provided leadership in acquisitions, cataloging, serials control, and processing for main library and 4 branches serving 250,000 residents. Participated as senior member of library management team. Compiled and prepared technical evaluations, reports, and statistical analyses of Division operations to measure the achievement and cost of annual goals, objectives, and staff performance.

1975-1976 **System Coordinator, San Antonio Major Resource Center, San Antonio, Texas.** Served as senior member of the management team for District X Office, charged to provide technical assistance, resource sharing, media services, and professional development to librarians and staff representing 30 public library jurisdictions in 21 counties serving 1,500,000 residents. Functioned as liaison to System Director, staff, and members of governing bodies with the System Board of Directors and the Texas State Library and Historical Commission. Prepared LSCA grant applications and monitored awards totaling $1,100,000 annually.

1973-1975 **Bilingual Branch Librarian, San Antonio Public Library, San Antonio, Texas.** Worked as librarian providing reference, information, and readers' advisory services in branch serving 50,000 Spanish-speaking residents in southwest San Antonio. Participated in collection development and resource acquisition activities, specializing in children's work, Spanish language resources, and multicultural studies.

1972-1973 **Librarian, Holding Institute, Laredo, Texas.** Worked as high school librarian serving 500 boarding students in Spanish-speaking environment of private school. Provided reference, research assistance, and library instruction to students and 35 faculty members.

1966-1971 **Rockford Public Library, Rockford, Illinois.** Worked in branches as part-time as a Library Assistant, Clerk, and Page in city library serving 150,000 residents.

PUBLICATIONS

Editor-reviewed Monographs (Completed and in Progress)

Hall-Ellis, Sylvia D., and Mary Beth Weber. *Contemporary Cataloging in an RDA Environment: A Handbook for Students and Practitioners.* Chicago, IL: American Library Association. Under contract & In development.

RDA Testing: Lessons Learned and Challenges Revealed. Sylvia D. Hall-Ellis and Robert O. Ellett, Jr., eds. Binghamton, N.Y.: Haworth, 2012. 128 p.

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Bardach, Ann Louise. *Cuba Confidential: Love and Vengeance in Miami and Havana.* New York: Random, 2002. 0-375-50489-3, $25.95 [*Library Journal*, 2002]

Swan, James. *Fundraising for Libraries: 25 Proven Ways to Get More Money for Your Library.* New York: Neal Schuman, 2002. 1-55570-433-6, $69.95 [*Colorado Libraries,* 2002]

Puíg de Lange, Victoria. *Sol Con Agua.* Nashville, Tenn.: Editorial Vistazo/Ediciones Reio Negro, 2002. 0-9724506-0-2, $17.75 [*Críticas*, 2002]

The Encyclopedia of Latin American Politics. Edited by Diana Kapiszewski; assistant editor, Alexander Kazan. Westport, Conn.: Oryx Press, 2002. 1-57356-306-4, $74.95 [*Library Journal*, 2002]

Vargas Lizano, Isabel. *Y Si Quieres Saber de Mi Pasado.* Con la colaboración de J.C. Vales. Madrid: Santillana Ediciones Generales, S.L., 2002. 84-03-09278-4, $18.95 [*Críticas*, 2002]

Ruy Sánchez, Alberto. *Los Jardines Secretos de Mogador: Voces de Tierra.* México, D.F.: Alfaguara, S.A., 2001. 968-19-0879-1, $16.95 [*Críticas*, 2002]

Chávez, Ricardo and Celso Santajuliana. *El Final de las Nubes.* Barcelona: RBA Libros, S.A., 2001. 84-7901-760-0, $11.95 [*Críticas*, 2002]

The Power of Language / El Poder de la Palabra: Selected Papers from the Second REFORMA National Conference. Edited by Lillian Castillo-Speed and the REFORMA National Conference Publications Committee. Englewood, Colo.: Libraries Unlimited, 2001. 1-563089459, $35.00 [*Colorado Libraries,* 2001]

Martínez, Rubén. *Crossing Over: A Mexican Family on the Migrant Trail.* Metropolitan: Holt, 2001. 0-8050-4908-8, $26.00 [*Library Journal*, 2001]

Guevara, Ernesto "Che." *The African Dream: the Diaries of the Revolutionary War in the Congo.* Translated from the Spanish by Patrick Camiller. With an introduction by Richard Gott and a foreword by Aleida Guevara March. New York: Grove Press, 2001. 0-8021-3834-9, $13.00 [*Library Journal*, 2001]

Cooper, Gail and Garry Cooper. *New Virtual Field Trips.* Englewood, Colo.: Libraries Unlimited, 2001. 1-56308-887-8, $27.50 [*Colorado Libraries,* 2001]

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NATIONAL SERVICE & PROFESSIONAL AFFILIATIONS

American Library Association, Life Member
Association for Library Collections & Technical Services (now CORE)
Cataloging, Classification, and Metadata Section
ALCTS Editorial Board, Member, 2015-2019
Committee on Cataloging: Description and Access, Member, 2008-2012
Test Site for RDA, Manager, 2009-2011
Nominating Committee, Member, 2007-2008
Committee for Education and Training of Catalogers, Chair, 2006-2007, Member, 2003-2007
Competencies & Education for a Career in Cataloging Interest Group, Chair, 2010-2012; Member, 2010-
President's Program Committee, 2015-2016
Education Committee, Member, 2005-2007
Task Force on Competencies and Education for a Career in Cataloging, Chair, 2007-2009
2007 Annual Meeting Pre-conference, "What They Don't Teach in Library School: Competencies, Education and Employer Expectations for a Career in Cataloging," Steering Committee Chair
Fundraising Committee, Member, 2004-2006
Office of Diversity
Committee on Diversity, Chair, 2010-2011
Diversity Research Advisory Committee, Member, 2009-2010
Spectrum Scholar Mentor, 2009
Diversity Grants Review Committee, Member, 2007, 2010, 2012
Office of Statistics and Research
Research and Statistics Committee, Member, 2005-2007; Intern, 2003-2005
Library Research Round Table
Nominating Committee, 2022-2023
Membership Committee, 2019-2021
Board of Directors, Member At-Large, 2009-2012
Office of Accreditation
Accreditation Panel Member (training completed 2004)
Accreditation Review Panel Member (training completed 2004)

REFORMA, Life Member
National Board of Directors, 2005-2013
National Fundraising Chair, 2005-2013
National Recruiting and Mentoring Committee, 2008-2010
Colorado Chapter, Secretary, 2004-2005
Colorado Chapter Liaison to National Board of Directors, 2004-2013

American Association of Law Libraries (AALL), Life Member Online Audiovisual Catalogers Association (OLAC) Colorado Association of Law Librarians (CoALL)

American Association of University Women Association for Library and Information Science Education (ALISE)
University of Denver ALISE Representative, 2003-2008; 2010-2011
Membership Advisory Committee, 2007-2010
Technical Services Education Special Interest Group, 2003-
Garfield Doctoral Dissertation Award Reviewer, 2012
Garfield Doctoral Dissertation Scholarship Reviewer, 2014
American Association of University Professors (AAUP), 2006-

American Grant Writers Association, 2014- Grant Professionals Institute, 2016- National Grants Management Association, 2014-
Grant Reviewer (National and Regional Team Leader), U.S. Department of Education, 1998- Grant Reviewer, Broadband Technologies Opportunity Program, U.S. Department of Commerce, 2009
Grant Reviewer, Institute of Museum and Library Services, 1998-2000
Grant Reviewer, Colorado Department of Education, 2014-
Grant Reviewer, Colorado State Library, 1998-
Grant Reviewer, American Association of Community Colleges *Working Connections* program, 1999
Peer Reviewer, *Journal for Library and Information Science Education,* 2005-
Peer Reviewer, *Journal of Library Metadata,* 2009
Peer Reviewer, *International Journal of Library and Information Science,* 2011
Regular Columnist, *The Bottom Line*, 2013-2016
Book Reviewer, *Library Journal*, 2001
Book Reviewer, *Críticas*, 2002-2009
Book Reviewer, *Colorado Libraries,* 2000-2012

REGIONAL SERVICE & PROFESSIONAL AFFILIATIONS

Mountain Plains Library Association
Professional Development Grants Committee, Member, 2005-2006
Professional Development Policy and Guidelines Sub-committee Chair, 2006

Colorado Association of Libraries
"Student Voices" Column Editor, *Colorado Libraries,* 2005-2006
Conference Planning Committee, Member, 2002
Technical Services and Automation Division, Chair, 2002-2003; Member, 2000-
Academic Libraries Division, Peer Review Conference Papers Committee, Member, 2007-2009
Education Committee, Member, 1988-1993
Diverse Populations Committee, Member, 2009-2013

SERVICE TO THE UNIVERSITY OF DENVER
Center for Teaching and Learning Faculty Advisory Board, 2007-2008; 2010-2012
Center for Community Engagement and Service Learning Advisory Board, 2007-2012
Faculty Senate
Executive Committee, 2008-2013
Nominations, Rules & Credentials Committee, Chair, 2008-2013; Member 2007-2013
Appointment, Promotion, and Tenure Revision Committee, 2010-2012
Grievance Policy Committee Member, 2007-2010
Law Library Director Search Committee Chair, 2012-2013
PROF Grant Review, College Representative to the University Review Team, 2006, 2008
Project Homeless Connect Evaluations, Principal Investigator, 2006-2009
University Technology Council, 2007-2009
University of Denver Hyde Interviews for Incoming Freshmen, 2003-2012

SERVICE TO THE MORGRIDGE COLLEGE OF EDUCATION
Appointment, Tenure & Review Committee
Member, voting, 2003-2005; 2007- 2009
Chair, Clinical Faculty Promotion & Tenure Policy Subcommittee, 2008-2009
Member, Community Engagement Subcommittee, 2007-2009
Member, Tenure Review Panel, 2003
Advancement and Alumni Relations Committee, Chair, 2003-2007; Member, 2002-2007
College Building Committee Member, 2004-2010

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College of Education Student Association, Faculty Advisor, 2004-2007; 2009-2010
Faculty Senator, 2007-2013
Research and Scholarship Committee, Chair, 2008-2009; Member, 2002-2003, 2008-2009
Research and Grant Mentoring Committee, Chair, 2009-2010
Research Task Force Member, 2010-2012
Search Committee Member, Assistant Professor for Curriculum & Instruction, 2010-2011
Workload Task Force Member, 2010-2011

SERVICE TO THE LIBRARY & INFORMATION SCIENCE PROGRAM

Library and Information Science Student and Alumni Association, Faculty Advisor, 2002-2008
ALA Student Chapter, Faculty Advisor, 2005-2008
Beta Phi Mu Phi Chapter, Faculty Advisor, 2004-2014
Steering Committee Member, Accreditation by the American Library Association, 2001-2004
Search Committee Member, Associate Professor for LIS, 2006
Search Committee Chair, Assistant Professors for LIS, 2003, 2005, 2006, 2007
Search Committee Ex-Officio Member, Director for LIS, 2004

SERVICE TO SAM HOUSTON STATE UNIVERSITY

Rio Roundup: South Texas Literature Conference, Conference Coordinator, 1993
External Relations, Fund Raising, Grants Committee, Chair, 1993-1995
Advisory Council Committee, Chair, 1993-1995
Students, Admissions, and Advisement Committee, Chair, 1994
Institutional Effectiveness Committee, Member, 1993-1995

SERVICE TO THE COLLEGE OF EDUCATION AND APPLIED SCIENCE

Faculty Affairs Committee, Member, 1993-1995
Curriculum Committee, Member, 1993-1995
Continuing Education Committee, Member, 1993-1995

SERVICE TO THE COMMUNITY

French Teacher & Student Exchange with Laon, France, Monarch High School, Boulder Valley School District (Boulder, Colorado), 2017-
Denver/Boulder Games 2022, Board of Directors, Secretary-Treasurer, 2015-2018
United Way Campaign Committee, College of Education, 2006
Arapahoe County, Election Judge for the County Clerk and Recorder, 2000-2005
Arthritis Foundation, Rocky Mountain Chapter, Certified Educator & Trainer, 1999-2008
Bonfils Blood Center, Silver Level Donor, 2000-2016
Denver Museum of Natural History, Docent, 1992
Tech Prep of the Lower Rio Grande Valley, Inc. (Harlingen, Texas) Board of Directors, 1995-1997
Executive Committee, 1996-1997; Chair, Development Committee, 1995-1997; Chair, Fiscal Agency Committee, 1995-1996; Chair, Colleges and Universities Committee, 1996-1997
Gilpin County (Colorado) Public Library Board of Trustees, 1986-1989; Vice President, 1987-1989
City of Central (Colorado) Economic Development Committee, 1987-1989
Columbine Family Health Centers, Inc. (Nederland and Black Hawk, Colorado) Board of Directors, 1988-1989

CERTIFICATION

Permanent Public Librarian Certificate - Pennsylvania, New York, Texas
Westlaw Expert Witness, 2008-
Certified Grant Writer®, 2016-

AWARDS AND HONORS

Advanced Practitioner for Service Learning and Community Engagement, University of Denver, 2011

Platinum Star Alumnae, College of Information, Library Science & Technologies, University of North Texas, 2009
Commendation for Integration of Technologies in Teaching & Learning Environment, University of Denver, 2006
Outstanding Adjunct Faculty Member Award, College of Education, University of Denver, 2002
Beta Phi Mu, Pi, University of Pittsburgh, 1985
Alpha Lambda Sigma, University of North Texas, 1972
Albert Nelson Marquis Lifetime Achievement Award, Who's Who in America
Who's Who in American Women
Who's Who of Women Executives
Dictionary of International Biography
Who's Who in the East
Who's Who in the South and Southwest
Who's Who in the World
Who's Who of Online Professionals
Who's Who in Library and Information Science
2,000 Notable Women
Who's Who of Emerging Leaders
Who's Who in Professional and Executive Women
Who's Who in American Education
International Who's Who of Professional and Business Women
International Leaders in Achievement
International Educator of the Year
Who's Who in Finance and Industry
Who's Who in Finance and Business

Invited International and National Conference Presentations

Driscoll, Margaret, Christy Confetti Higgins, Scott Brown, and Sylvia D. Hall-Ellis. *A View from Within: Open House Tour of Three Canvas Core Courses*. Presentation to be delivered at the School of Information, San José State University Professional Development Seminar, San José, California, October 17, 2019.

Hall-Ellis, Sylvia D. *Invest in Me -- I'm Your Future: Succession Planning for Libraries.* Keynote presentation delivered at the ALCTS President's Symposium, Boston, Mass., January 8, 2016.

Seidel, Kent E. and Sylvia D. Hall-Ellis. *Making Grants Work for You: Strategies for Doctoral Students and Early Career Scholars.* Presentation delivered at the University Council for Educational Administration, Early Career Scholars Session, Indianapolis, Ind., November 9, 2013.

Hall-Ellis, Sylvia D. *So You Want to be a Manager; Leadership Skills and Competencies for Technical Services Managers and Administrators.* Presentation delivered at the 138th Annual Conference, American Library Association, Chicago, Ill., June 29, 2013.

Seidel, Kent E., Karen S. Riley, Lyndsay Agans, Susan Korach, and Sylvia D. Hall-Ellis. *Making Grants Work for You (Instead of Just Working for Grants).* A panel discussion delivered at the University Council for Educational Administration, Early Career Scholars Session, Denver, Colo., November 17, 2012.

Hall-Ellis, Sylvia D. *After the Great TS Reorganization: The Westminster Law Library.* Presentation delivered at the 137th Annual Conference, American Library Association, Anaheim, Calif., June 23, 2012.

Hall-Ellis, Sylvia D. *Conversations with Catalogers in the 21st Century.* A panel discussion sponsored by the ALCTS Competencies for a Career in Cataloging Interest Group, delivered at the 137th Annual Conference, American Library Association, Anaheim, Calif., June 22, 2012.

Hall-Ellis, Sylvia D., moderator. *Mid-Career Leaders Program.* A panel discussion sponsored by the ALA Committee on Diversity delivered at the 136th Annual Conference, American Library Association, New Orleans, La., June 26, 2011.

Hall-Ellis, Sylvia D., moderator. *Diversity Town Hall.* A community conversation sponsored by the ALA Committee on Diversity delivered at the 136th Annual Conference, American Library Association, New Orleans, La., June 24, 2011.

LaBarre, Kathryn, Sylvia D. Hall-Ellis, Karen Anderson, Rick Hasenyager, Christopher Cronin, and Penny Baker. *Briefings from RDA Test Participants.* A panel discussion delivered at the Midwinter Conference, American Library Association, San Diego, Calif., January 7, 2011.

Miksa, Shawne, Marjorie Bloss, and Sylvia D. Hall-Ellis. *Educating the Next Generation of Catalogers: Teaching RDA.* A panel discussion delivered at the 97th Annual Conference, Association for Library and Information Science Education, San Diego, Calif., January 7, 2011.

Hall-Ellis, Sylvia D., Robert Maxwell, John Hostage, and George Prager. *RDA Panel: What Cataloging Managers Need to Know.* Presentation delivered at the 103rd Annual Conference, American Association of Law Librarians, Denver, Colo., July 12, 2010.

Hall-Ellis, Sylvia D. and Stacey L. Bowers. *Catalogers in the RDA Environment: Skill Sets, Expectations and Challenges.* Presentation delivered at the 103rd Annual Conference, American Association of Law Librarians, Denver, Colo., July 11, 2010.

Hall-Ellis, Sylvia D. *Comfortable in Your Cataloging and Metadata Specialist Skin? Or, So You Want to Hire a Cataloger.* Presentation delivered to the ALCTS Research Group at the 134th Annual Conference, American Library Association, Chicago, Ill., July 11, 2009.

Perez, Megan, Sylvia D. Hall-Ellis, and Denise Anthony. *From Novice to Expert: Collaboration for Succession Planning.* A "hot topic" presentation delivered at the 14th ACRL Conference, Seattle, Wash., March 13, 2009.

Hall-Ellis, Sylvia D. *Cataloging in the RDA Environment: Skill Sets, Expectations and Challenges.* Presentation delivered to the ALCTS Research and Publications Committee at the Midwinter Conference, American Library Association, Denver, Colo., January 24, 2009.

Hall-Ellis, Sylvia D. *LIS Cataloging Education for the 21st Century: Expectations and Challenges.* A panel discussion held at the 95th Annual Conference, Association for Library and Information Science Education, Denver, Colo., January 23, 2009.

Chu, Clara, Sylvia D. Hall-Ellis, and Mark Winston. *The Doctoral Degree & Building a Career.* A panel discussion delivered at the ALA Office of Diversity Spectrum Doctoral Fellows E.J. Josey Leadership Institute, Midwinter Conference, American Library Association, Denver, Colo., January 20, 2009.

Hall-Ellis, Sylvia D. and Robert O. Ellett, Jr. *Fundamentals of Cataloging Course: An Overview of the ALCTS Online Course.* Presentation delivered to the ALCTS Big Heads Group, 133rd Annual Conference, American Library Association, Anaheim, Calif., June 30, 2008.

Hall-Ellis, Sylvia D. *Employers' Expectations for Technical Services Librarians: What We Don't Know.* Presentation delivered to the ALCTS Research and Publications Committee Program, 133rd Annual Conference, American Library Association, Anaheim, Calif., June 28, 2008.

Hall-Ellis, Sylvia D., Virginia R. Maloney, and Mary Stansbury. *Institutional Responses to Engaged Scholarship: The Carnegie Foundation Engaged University Classification at Two Universities.* Presentation delivered to the 94th Annual Conference, Association for Library and Information Science Education, Philadelphia, Pa., January 11, 2008.

Hall-Ellis, Sylvia D. *Puzzles, Problems, and Predicaments.* Presentation delivered to the ALCTS Research Discussion Group, 132nd Annual Conference, American Library Association, Washington, D.C., June 23, 2007.

Hall-Ellis, Sylvia D. *Cataloging Education: A New Emphasis for the Library and Information Science Curriculum.* Presentation delivered to the ALCTS Pre-conference, 132nd Annual Conference, American Library Association, Washington, D.C., June 22, 2007. http://www.loc.gov/catdir/cpso/careercat.html

Ellett, Jr., Robert O. and Sylvia D. Hall-Ellis. *Copy Cataloging Done Smarter.* Presentation delivered to the International Conference on Interdisciplinary Information Sciences and Technologies (InSciT2006), October 25-28, 2006.

Hall-Ellis, Sylvia D. and Robert O. Ellett, Jr. *Cooperative Cataloging: Challenges and Opportunities for Defense Libraries.* Presentation delivered to the 1st Annual Conference of Defense Libraries, Spanish Ministry of Defense, Madrid, Spain, July 7, 2006.

Hall-Ellis, Sylvia D. *Cataloger Competencies: Do the Employers Require What the Professors Teach?* Presentation delivered to the ALCTS CCS Heads of Cataloging Discussion Group, 131st Annual Conference, American Library Association, New Orleans, La., June 26, 2006.

Grealy, Deborah S. and Sylvia D. Hall-Ellis. *From Research to Practice: The Scholarship of Teaching and Learning in LIS Education.* Presentation delivered to the at the 92nd Annual Conference, Association for Library and Information Science Education, San Antonio, Tex., January 18, 2006.

Hall-Ellis, Sylvia D. *Employers' Expectations for Entry-Level Catalogers: What Position Announcement Data Indicate.* Research paper delivered to the Technical Services Special Interests Group, 91st Annual Conference, Association for Library and Information Science Education, Boston, Mass., January 12, 2005. http://dlist.sir.arizona.edu/

Hall-Ellis, Sylvia D. *Common Errors in MARC Records Prepared by LIS Students: What Does It Mean?* Research paper delivered to the ALCTS CCS Cataloging Norms Discussion Group, Mid-Winter Conference, 90th American Library Association, San Diego, Calif., January 10, 2004.

Hall-Ellis, Sylvia D. *Visual Arts as Foundation for Successful Library Automation: The Rocky Mountain College of Art & Design Experience.* Paper delivered at the 6th Annual Conference of Higher Education, Charleston, S. C., 1993.

Hall, Sylvia D. *Design Elements for Bibliographic Databases: An Overview.* Paper delivered at the 14th Online National Conference, New York, 1983.

Invited Regional Conference Presentations

Hall-Ellis, Sylvia D., Hudson, Christopher D., Brittany Cronin, and Kathryn Michaels. "The Colorado Law Project: Meeting the Public's Need for Legal Information." Panel discussion delivered at the Mountain Plains Library Association Conference, Billings, Mont., April 9, 2011.

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Grealy, Deborah S. and Sylvia D. Hall-Ellis. *Education for Information Professionals in New Mexico: Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the New Mexico Library Education Summit, Las Vegas, N.M., September 26, 2005.

Hall-Ellis, Sylvia D. *Public Library-School Library Partnerships.* Presentation delivered at the 3rd Annual Colorado Association of Libraries Conference with the Mountain Plains Library Association, Denver, Colo., October 22, 2004.

Hall-Ellis, Sylvia D. *Learn All You Can – Educational Partnership Opportunities for the Lewis and Clark Bicentennial Commemoration.* Paper delivered at the 3rd Annual Lewis and Clark Bicentennial Council National Planning Conference, Bismarck, N.D., April 26, 1998.

Invited State Conference Presentations

Grealy, Deborah S. and Sylvia D. Hall-Ellis. *Academic Library Leadership Changes: Using Succession Planning and Mentoring*. Presentation delivered at the Minnesota Library Education Conference, St. Cloud, Minn., October 10, 2013.

Hall-Ellis, Sylvia D., Merrie Valliant, and Melissa Powell. *RDA: What Is It and What Do You Need To Do With It At Your Library?* Presentation delivered at the Colorado Library Consortium Spring Conference, Ft. Morgan, Colo., April 26, 2013.

Hall-Ellis, Sylvia D. *Service Learning and the Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 4th LEADS Scholars Orientation, Denver, Colo., August 5, 2009.

Hall-Ellis, Sylvia D. *Service Learning: Enhancement to Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 3rd LEADS Scholars Orientation, Denver, Colo., June 2008.

Hall-Ellis, Sylvia D. *Law Librarianship: A Community Conversation.* Sponsored by the Colorado Association of Law Libraries. Paper delivered at the Colorado Supreme Court Library, Denver, Colo., May 14, 2008.

Hall-Ellis, Sylvia D. *Opportunities and Challenges in Law Librarianship: A Community Conversation.* Presentation delivered at the Sturm College of Law, Denver, Colo., November 7, 2007.

Ellis, Sylvia D. *Grant Writing Resources for Nursing Professionals.* Presentation delivered at the Presbyterian / St. Luke's Health One Medical Center 1st Annual Research Symposium, Denver, Colo., October 17, 2007.

Hall-Ellis, Sylvia D. *Project Homeless Connect 4 Event Evaluation – Insights and Lessons Learned.* Presentation delivered at the Homelessness Research Symposium: What is DU Doing about Homelessness in Denver, Denver, Colo., September 14, 2007.

Hall-Ellis, Sylvia D. *Service Learning and the Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 2nd LEADS Scholars Orientation, Denver, Colo., August 10, 2007.

Hall-Ellis, Sylvia D. *Education for Information Professionals in a Digital Environment: Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 15th Spring Mountains and Plains Parapros Conference, Denver, Colo., February 24, 2007.

Hall-Ellis, Sylvia D. *Public Library Service to Spanish-Speaking and Latino Residents in Denver: A Case Study.* Presentation delivered at the 4th Annual Colorado Association of Libraries Conference, Denver, Colo., November 10, 2005.

Hall-Ellis, Sylvia D. *Education for Information Professionals in a Digital Environment: Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 14th Annual Mountains and Plains Parapros Conference, Aurora, Colo., July 29, 2005.

Hall-Ellis, Sylvia D. *Educational Opportunities: Library & Information Science Graduate Education at the University of Denver.* Presentation delivered at the 13th Annual Mountains and Plains Parapros Conference, Centennial, Colo., August 6, 2004.

Hall-Ellis, Sylvia D. *Library Education & Training: Focus on the West: An LIS Faculty Member's Personal Response*. Presentation delivered at the 1st Annual Colorado Association of Libraries Conference, Keystone, Colo., October 18, 2002.

Hall-Ellis, Sylvia D. *Grant Writing for School Librarians.* Presentation delivered at the 2002 Annual Colorado Education Media Association Conference, Colorado Springs, Colo., February 15, 2002.

Hall-Ellis, Sylvia D. *Grants – Opportunities for the Future.* Paper delivered at the Southeast Regional Accountability Annual Conference, Lamar, Colo., November 12, 1998.

Hall-Ellis, Sylvia D. *The Texas Library Connection and Interlibrary Loan: An Experiment in Resource Sharing.* Paper delivered at the Texas Computer Educators' Association Annual Conference, Austin, Tex., February 6, 1997.

Hall-Ellis, Sylvia D. *Finding Grant Sources on the Internet: A Guide for Librarians.* Paper delivered at the 2nd Annual Institute for School Library Personnel, Pharr-San Juan-Alamo North High School, Pharr, Tex., July 29, 1996**.**

Hall-Ellis, Sylvia D. *Mathematical and Logical Thinking: A Critical Intelligence.* Paper delivered at the 3rd Annual Paraprofessional Conference at the University of Texas - Pan American, Edinburg, Tex., March 8, 1996.

Hall-Ellis, Sylvia D. *Cataloging Trends and Issues: Update Session.* Paper delivered at the 1st Annual Institute for School Library Personnel, South Texas Community College, McAllen, Tex., July 19, 1995.

Hall-Ellis, Sylvia D. *Grant Writing: Tips and Encouragement for School Librarians.* Paper delivered at the 1st Annual Institute for School Library Personnel, South Texas Community College, McAllen, Tex., July 18 and 19, 1995.

Hall-Ellis, Sylvia D. *How to Become an Expert Grant Writer.* Paper delivered at the 3rd Annual High School Principals' Academy, South Padre Island, Port Isabel, Tex., June 19, 1995.

Texas Library Study: Results from Regions I and II. Paper delivered at the 3rd Annual Technology Conference, Texas A&M University, College Station, Tex., November 18, 1994.

Hall-Ellis, Sylvia D. *Academic Achievement and Middle School Students.* Paper delivered at the 8th Annual Young Adult Conference, Sam Houston State University, Huntsville, Tex., November 5, 1994.

Hall-Ellis, Sylvia D. *Multimedia Resources for Library Leaders.* Paper delivered at the Institute for Librarians in A Multicultural Environment, Sam Houston State University, Huntsville, Tex., June 10, 1994.

Hall-Ellis, Sylvia D. *Finding the Resource: Empowering the User, or, the Case for Curriculum Based Subject Access to Learning Resource Center Collections.* Paper delivered at the 81st Annual Texas Library Association Conference, Corpus Christi, Tex., April 12-16, 1994.

Hall-Ellis, Sylvia D. and William H. Pichette. *Sam Houston State University Makes Use of OCLC/AMIGOS Collection Analysis CD.* Paper delivered at the 81st Annual Texas Library Association Conference, Corpus Christi, Tex., April 12-16, 1994.

Hall, Sylvia D. *Funding and Library Development in Pennsylvania: A Symbiotic Relationship.* Paper delivered at the Annual Graduate Student Colloquia, University of Pittsburgh, School of Library and Information Science, 1982.

Hall, Sylvia D. *Leadership for Public Library Trustees.* Paper delivered for the Trustees Division, Pennsylvania Library Association Annual Conference, Lancaster, Penn., 1981.

Seminar and Professional Development Presentations

Taylor, Meredith, and Sylvia D. Hall-Ellis. *Talent Management and Succession Planning.* ALCTS eForum, held March 22, 2017.

Hirsh, Sandra, Heather O'Brien, Michelle Holschuh Simmons, Michael Krasulski, and Sylvia D. Hall-Ellis. *Information Services Today: An Introduction. Part 3: Information Services: Roles in the Digital Age*. Rowan and Littlefield in partnership with Library Journal webinar, recorded February 5, 2015.

Hall-Ellis, Sylvia D. and Jennifer Sweda. *Copy Cataloging in an RDA Environment.* ALCTS eForum, held May 14 and 15, 2013.

Hall-Ellis, Sylvia D. *Law Librarianship: A Community Conversation.* Sponsored by the Colorado Association of Law Libraries, presented at the Colorado Supreme Court Library, Denver, Colo., May 14, 2008.

Hall-Ellis, Sylvia D. and Beatrice Z. Gerrish. *Reading and Libraries: Recent Research in Reading.* Presentation at the Ricks Center for Gifted Education, Denver, Colo., March 4, 2008.

Hall-Ellis, Sylvia D. *Cataloger Competencies: Do the Employers Require What the Professors Teach?* Presentation for the School of Library and Information Science, San José State University, February 12, 2008.

Hall-Ellis, Sylvia D. and Robert O. Ellett, Jr. *Cooperative Cataloging: Rules, Tools, and Conventions for Building a Multi-institutional Catalog.* Sponsored for the Spanish Ministry of Defense, Madrid, Spain, July 10, 2006.

Hall-Ellis, Sylvia D. *Cash for Kids: Grant Writing Opportunities for Youth Services Librarians.* Sponsored by the Colorado Young Adult Librarians; presented at Bemis Memorial Library, Littleton, Colo., November 13, 2002.

Hall-Ellis, Sylvia D. *MARC Records and Authority Control: Planning for Bibliographic Database Migration.* Sponsored and held at the Douglas County Public Library, Castle Rock, Colo., May 28, 2002.

Grant Writing: A Refresher for Librarians. Sponsored by Library and Information Science Program, College of Education, University of Denver; presented at University Center at Chaparral, August 12, 2000.

Hall-Ellis, Sylvia D. *Shaking the Money Tree – Basic Grant Writing for Colorado Educators.* Sponsored by the Office of Educational Telecommunications of the Colorado Department of Education.
Pikes Peak Community College, Colorado Springs, Colo., November 16, 1998.
Pueblo School District 60, Pueblo, Colo., November 12, 1998.
University of Northern Colorado, Greeley, Colo., November 10, 1998.
United Technology Educational Partnership, Grand Junction, Colo., November 9, 1998.

Hall-Ellis, Sylvia D. *Cataloging Multimedia, Kits, Globes and Map Materials in USMARC.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., April 24, 1997 and December 12, 1996.

Hall-Ellis, Sylvia D. *New Standards for School Library Media Centers.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., April 15, 1997.

Hall-Ellis, Sylvia D. *The School Library Media Specialist in the 21st Century: Visions for the Future.* Sponsored and hosted by Pharr-San Juan-Alamo Independent School District, Pharr, Tex., April 4, 1997.

Hall-Ellis, Sylvia D. *Cataloging Sound Recordings and Audio Materials in USMARC.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., March 20, 1997 and November 21, 1996.

Hall-Ellis, Sylvia D. *Evaluating and Selecting CD-ROMS for School Library Media Collections.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., March 18, 1997.

Hall-Ellis, Sylvia D. *Cataloging Audiovisual Materials in USMARC.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., February 27, 1997, November 7, 1996 and October 24, 1996.

Hall-Ellis, Sylvia D. *Introduction to Dialog: Basic Searching Strategies.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., February 14, 1997.

Hall-Ellis, Sylvia D. *Cataloging Books in USMARC.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., January 30, 1997, October 10, 1996, September 26, 1996 and August 8, 1996.

Hall-Ellis, Sylvia D. *Advanced Internet Searching Techniques for Librarians.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., January 21, 1997.

Hall-Ellis, Sylvia D. *Texas Library Connection Full-Text Searching.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., December 13, 1996 and October 25, 1996.

Hall-Ellis, Sylvia D. *Developing Evaluation Strategies for Grants and Proposals.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., December 10, 1996.

Hall-Ellis, Sylvia D. *Developing Needs Assessment for Grants and Proposals.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., November 12, 1996.

Hall-Ellis, Sylvia D. *Texas Library Connection Union Catalog Searching.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., November 8, 1996 and September 6, 1996.

Hall-Ellis, Sylvia D. *Grant Resources on the Internet.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., October 23, 1996.

Hall-Ellis, Sylvia D. *Preparing a Response to the Telecommunications Infrastructure Fund Board: Needs Assessment, Professional Development Framework, and Evaluation Strategies.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., October 16, 1996.

Hall-Ellis, Sylvia D. *Texas Library Connection: Building the Districtwide Bibliographic Database.* Sponsored and hosted by Mercedes Independent School District, Mercedes, Tex., September 13, 1996 and August 8, 1996. Sponsored and hosted by Los Fresnos Consolidated Independent School District, Los Fresnos, Tex., August 12, 1996.

Hall-Ellis, Sylvia D. *School-to-Work and Special Education: An Inclusive Partnership for Success.* Sponsored and hosted by the Office of Special Education, Region One Education Service Center, Edinburg, Tex., September 11, 1996.

Hall-Ellis, Sylvia D. *Telecommunications Infrastructure Fund Board: An Overview of Funding for Secondary Schools.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., August 31, 1996.

Hall-Ellis, Sylvia D. *Cataloging Books, Multimedia, and Realia in USMARC.* Sponsored and hosted by Edinburg Consolidated Independent School District, Edinburg, Tex., August 6, 1996.

Hall-Ellis, Sylvia D. *Internet Resources for Grant Writers.* Sponsored and hosted by Region One Education Service Center, Edinburg, Tex., April 20, 1996.

Hall-Ellis, Sylvia D. *Enhanced Grant Writing Skills for Mathematics Educators: Writing Skills for Campus Teams.* Sponsored and hosted by the Office of General Education, Region One Education Service Center, Edinburg, Tex., March 22, 1996.

Hall-Ellis, Sylvia D. *Internet Resources for Grant Writers.* Sponsored and hosted by the College of Education and Applied Science, Sam Houston State University, Huntsville, Tex., March 9, 1996.

Hall-Ellis, Sylvia D. *Grant Writing for Mathematics Educators: A Development Process for Campus Teams.* Sponsored and hosted by the Office of General Education, Region One Education Service Center, Edinburg, Tex., March 4, 1996.

Hall-Ellis, Sylvia D. *Shaking the Money Tree: Preparing Successful Technology Grant Applications.* Sponsored and hosted by the Office of Technology and Media Services, Region One Education Service Center, Edinburg, Tex., February 29, 1996 and April 29, 1996.

Hall-Ellis, Sylvia D. *District-wide Technology Planning: Technical Assistance for the Texas Education Agency Initiative.* Sponsored and hosted by the Office of Technology and Media, Region One Education Service Center, Edinburg, Tex., February 2, 1996.

Hall-Ellis, Sylvia D. *Enhanced Grant Writing Skills.* Sponsored and hosted by Pharr-San Juan-Alamo Independent School District, Pharr, Tex., January 27, 1996.

Hall-Ellis, Sylvia D. *United States Copyright Act of 1976, Video Transmissions, Computer Software and the Internet.* Sponsored and hosted by the Office of Technology and Media, Region One Education Service Center, Edinburg, Tex., January 24, 1996.

Hall-Ellis, Sylvia D. *The Grant Writing Development Process.* Sponsored and hosted by Pharr-San Juan-Alamo Independent School District, Pharr, Tex., January 20, 1996.

Hall-Ellis, Sylvia D. *MARC Cataloging of Materials for Library Media Centers.* Sponsored and hosted by the Office of Media Services, Region One Education Service Center for the Donna Independent School District, Donna, Tex., August 17, 1995.

Hall-Ellis, Sylvia D. *Classification.* Sponsored by AJ Seminars, Rockville, Maryland; presented at University Hilton Hotel, Houston, Tex., May 10, 1995.

Hall-Ellis, Sylvia D. *Library Technical Services.* Sponsored by AJ Seminars, Rockville, Maryland; presented at University Hilton Hotel, Houston, Tex., April 26, 1995.

Hall-Ellis, Sylvia D. *Grant Writing: An Introduction for Public School Administrators.* Sponsored and hosted by the Office of Administrative Services, Region One Education Service Center, Edinburg, Tex., April 19, 1995.

Hall-Ellis, Sylvia D. *The School Library Media Specialist in the 21st Century: Visions for the Future.* Sponsored and hosted by the United Independent School District, Laredo, Tex., March 31, 1995.

Hall-Ellis, Sylvia D. *Using USMARC.* Sponsored by AJ Seminars, Rockville, Maryland; presented at University Hilton Hotel, Houston, Tex., March 29, 1995.

Hall-Ellis, Sylvia D. *Library Media Center Policies and Guidelines: How to Prepare for School Board Adoption.* Sponsored and hosted by the Office of Technology and Media Services, La Joya Independent School District, La Joya, Tex., March 10, 1995.

Hall-Ellis, Sylvia D. *Developing District-wide Policies and Guidelines for Library Media Centers.* Sponsored and hosted by the Office of Technology and Media Services, La Joya Independent School District, La Joya, Tex., January 10, 1995.

Hall-Ellis, Sylvia D. *MARC Cataloging of Audiovisual Materials for Library Media Centers.* Sponsored and hosted by the Office of Library Media and Technology Services, Cypress-Fairbanks Independent School District, Houston, Tex., December 1, 1994.

Hall-Ellis, Sylvia D. *The School Library Media Specialist in the 21st Century: Visions for the Future.* Sponsored and hosted by United Independent School District, Laredo, Tex., October 14, 1994.

Hall-Ellis, Sylvia D. *The School Library Media Specialist in the 21st Century: Visions for the Future.* Sponsored and hosted by the Laredo Independent School District, Laredo, Tex., October 7, 1994.

Hall-Ellis, Sylvia D. *MARC Cataloging for Library Media Centers.* Sponsored by the Office of Library Media and Technology Services, Cypress-Fairbanks Independent School District, Houston, Tex., October 5, 1994.

Hall-Ellis, Sylvia D. *Jump to the Head of the Class: Undergraduate Library Resources Available at Sam Houston State University.* Sponsored by the Office of the Associate Vice President for Student Services, Sam Houston State University, Huntsville, Tex., October 4, 1994.

Hall-Ellis, Sylvia D. *CD-ROMs - 1994's Newest and the Best for Secondary Level Media Centers.* Sponsored and hosted by Clear Lake Independent School District, Houston, Tex., June 3, 1994.

Hall-Ellis, Sylvia D. *Introduction to Classification.* Sponsored by AJ Seminars, Rockville, Maryland; presented at Holiday Inn, Market Center, Dallas, Tex., May 18, 1994.

Hall-Ellis, Sylvia D. *Basic Descriptive Cataloging.* Sponsored by AJ Seminars, Rockville, Maryland; presented at University Hilton Hotel, Houston, Tex., May 4, 1994.

Hall-Ellis, Sylvia D. *Shaking the Money Tree - Part II: Writing Successful Grant Applications.* Sponsored by Donna Independent School District, Donna, Texas, and Region One Education Service Center Edinburg, Texas; presented at South Texas Community College Library, McAllen, Tex., April 29, 1994.

Hall-Ellis, Sylvia D. *Using MARC.* Sponsored by AJ Seminars, Rockville, Maryland; presented at Holiday Inn, Market Center, Dallas, Tex., April 6, 1994.

Hall-Ellis, Sylvia D. *Automated Authority Control.* Sponsored by AJ Seminars, Rockville, Maryland; presented at University Hilton Hotel, Houston, Tex., March 23, 1994.

Hall-Ellis, Sylvia D. *Automating the District School Library Media Centers: Choices and Opportunities.* Sponsored and hosted by the Office of Technology and Library Media Services, Fort Bend Independent School District, Sugar Land, Tex., March 4, 1994.

Hall-Ellis, Sylvia D. *Shaking the Money Tree - Part I: Preparing Successful Grant Applications.* Sponsored and hosted by the Office of Library Media Services, Donna Independent School District, Donna, Tex., February 4, 1994.

GRADUATE COURSES TAUGHT

San José State University, School of Library and Information Science
INFO 249 – Advanced Cataloging and Classification (Fall 2015, 2016; Summer 2016)
INFO 287 – Special Topics in Cataloging and Classification (Spring 2017, 2018, 2019, 2020, 2021, 2022, 2023; Fall 2020, 2021, 2022, 2023)
LIBR 248 – Beginning Cataloging and Classification (Summer 2002, 2003, 2004, 2005, 2006)
LIBR 249 – Advanced Cataloging and Classification (Summer 2003; Fall 2014; Summer 2015)

University of Denver, University College
Grant Writing Certificate Seminar for Non-Profit Organizations (Fall 2020, 2021; Spring 2021)

University of Denver, Morgridge College of Education HED
5991 – Grant Writing in Higher Education (Spring 2011)
LIS 4010 – Organization of Information (Fall 2002-2009; Winter 2005; Spring 2004-2006)
LIS 4020 – Professional Principles and Ethics (Summer 2000)
LIS 4040 – Management of Libraries and Information Centers (Fall 2010; Spring 2003, 2009 (DS); Winter 2005)
LIS 4070 – Cataloging and Classification (Winter 2008, 2009, 2010, 2011, 2012; Fall 2009 (DS)) LIS
4321 – Collection Management (Spring 2005)
LIS 4326 – LIS Research (Winter 2009 (DS); Spring 2009 (DS))
LIS 4350 – Adult Materials and Services (Summer 2006, 2009)
LIS 4379 – Social Sciences Resources (Spring 2009)
LIS 4400 – Cataloging and Classification (Spring 2000, 2001; Winter 2002-2009; Summer 2005, 2006 – course renumbered LIS 4070, September 2007)
LIS 4401 – Descriptive Cataloging (Winter 2001; Spring 2002, 2003, 2006-2009; Summer 2005)
LIS 4402 – Subject Cataloging (Spring 2001, 2009; Summer 2005, 2006, 2007, 2008)
LIS 4403 – Classification Schemes (Fall 2007, 2008, 2009 (DS)) LIS
LIS 4405 – Authority Control (Winter 2009)
LIS 4510 – Materials and Services for Children (Winter 2004; Summer 2005)
LIS 4620 – Grant Writing and Fundraising (Summer 2000, 2002, 2004, 2010; Winter 2006; Fall 2006-2009)
LIS 4700 – Seminar in Technical Services (Fall 2001)
LIS 4700 – Seminar in Public Libraries (Summer 2002)
LIS 4804 – Management of Electronic Records (Spring 2004; Fall 2005)
LIS 4902 – Capstone Projects (Winter 2003, Spring 2008, 2009, 2011)
LIS 4910 – Independent Study (every quarter Winter 2000 through Spring 2011)
LIS 4920 – Service Learning (every quarter Summer 2004 through Spring 2011)
RMS 4954 – Grant Writing (Summer 2013, 2014)
RMS 4959 – Content Analysis Methodology (Spring 2015)
QRM 4978 – Grant Writing (Summer 2011, 2012)

Rutgers University, School of Communication and Information
SC&I 522 – Cataloging and Classification (Summer 2013)

University of Arizona, College of Behavioral and Social Sciences
LIS 602 – Cataloging and Classification (Summer 1995)
LIS 612 – Advanced Online Search and Retrieval (Summer 1995)

Sam Houston State University, College of Education & Applied Science
LIS 532 - Cataloging and Classification (Fall 1993, 1994, 1995)
LIS 563 - Advanced Cataloging and Classification (Summer 1994)
LIS 567 - Research Methods (Spring 1994, Spring 1995)
LIS 591 - Educational Technology (Spring 1994, 1995)
LIS 596 - Networking and Computer Technologies in Education (Fall 1993, Summer 1994, Fall 1995)

Dissertations at the University of Denver

Bowers, Stacey L. *Library Anxiety of Law School Students: A Study Utilizing the Multidimensional Library Anxiety Scale.* Chair, May 5, 2010.

Fattor, Melissa M. *Student Engagement Differences by Ethnicity and Scale for Ninth Grade Students.* Chair, November 1, 2010.

Fulton, Roseanne. *A Case Study of Culturally Responsive Teaching in Middle School Mathematics.* Kent Seidel, Chair, June 18, 2009, Outside Chair.

Grealy, Deborah S. *Tribes and Territories in Library and Information Studies Education.* Bruce Uhrmacher, Chair, June 10, 2008, Committee Member.

McCord, J. Michael. *Developing a Standard of Care for Educational Malpractice.* Chair, April 15, 2011.

Priebe, Sarah J. *Distinguishing Effects of Domain and General Knowledge on Passage Fluency and Comprehension.* Jan Keenan, Chair. July 21, 2011, Outside Chair.

Snyder-Mondragon, Sandra M. *Institutional Factors that Impact the Retention of Graduate Students of Color in Schools of Library and Information Science: A Metaregression of Accredited Library School Statistics on Student Retention and Graduation Rates.* Kathy Green, Chair, July 24, 2009, Committee Member.

Taylor, Karen Pickles. *Effective Teaching.* Elinor Katz, Chair, July 15, 2009, Outside Chair.

Thompson, Jennifer. *Distinguishing a Western Women's College: A History of the Curriculum and Student Experience at Colorado Women's College.* Edith W. King, Chair, July 16, 2010, Committee Member.

Walker, Emelda. *Influence of Organizational Factors on Job Satisfaction of Disability Service Providers at Postsecondary Institutions.* Chair, April 29, 2010.

Dissertation Proposals at the University of Denver

Bowers, Stacey L. *Library Anxiety of Law School Students: A Study Utilizing the Multidimensional Library Anxiety Scale.* Chair, November 5, 2009.

McCord, J. Michael. *Developing a Standard of Care for Educational Malpractice.* Edith W. King, Chair. April 23, 2010.

Thompson, Jennifer. *History of Colorado Women's College.* Edith W. King, Chair, October 30, 2008. Committee Member.

Walker, Emelda. *Influence of Organizational Factors on Job Satisfaction of Disability Service Providers at Postsecondary Institutions.* Chair, July 21, 2009.

Dissertations at Other Institutions

Rodríguez-Mori, Howard. *The Information Behavior of Puerto Rican Immigrants to Central Florida, 2003-2009: Grounded Analysis of Six Case Studies Use of Social Networks during the Migration Process.* Kathleen Burnett, Chair, April 10, 2009, Florida State University, Committee Member.

Schwartz, Brian *More than a Look-up Skill: Medical Information Literacy Education in Osteopathic Medical Schools.* D. Mirah Dow, Chair, July 18, 2017. Emporia State University, Committee Member.

Snow, Karen. *A Study of the Perception of Cataloging Quality among Catalogers.* Shawne D. Miksa, Chair, August 1, 2011. University of North Texas, Committee Member.

Dissertation Proposals at Other Institutions

Schwartz, Brian *More than a Look-up Skill: Medical Information Literacy Education in Osteopathic Medical Schools.* D. Mirah Dow, Chair, December 4, 2015. Emporia State University, Committee Member.

Snow, Karen. *A Study of the Perception of Cataloging Quality among Catalogers.* Shawne D. Miksa, Chair, May 11, 2010. University of North Texas, Committee Member.

Master's Thesis at the University of Denver

Hemingson, Jeff. *Recital Paper.* Lamont School of Music, February 2010, Outside Chair.

Capstones at the University of Denver

Anthony, Alisa. *Correlation of Library and Information Science Program outcomes and Vacant Position Qualifications Listed on the Colorado State Library Jobline by Employers During the Period September 1, 2000 through August 31, 2002.* Chair, Winter Quarter, 2003.

Borden, Donna M. *Improving Emergency Communications Systems: Is a Radio Communications Network the Answer?* Fall Quarter, 2014.

Bowden, Heather L.M. *Exploring Biological Models for Long-term Data Preservation.* Chair, Spring Quarter, 2008.

Casenada, Cassandra Y. *Challenges to the Recruitment, Education, and Retention of Librarians of Color*. Chair, Spring Quarter, 2008.

Chang, Jennifer C. *Legal Research Practice and Preference: A Law Firm Perspective.* Chair, Spring Quarter 2011.

Ellis, Megan S. Fitzgerald. *Design of a Public Library Adult Volunteer Recruitment Program and Training Curriculum.* Chair, Winter Quarter, 2003.

Kircher, Kathy. *Development of a Library Pathfinder for Exobiology and Posting it on the Internet.* Chair, Winter Quarter, 2003.

Melhado, Loretta. *Design of St. John's Episcopal Church Library.* Chair, Spring Quarter, 2008.

Radcliff, Kathy. *Original Cataloging of Archival materials in the HERS Collection in Penrose Library.* Chair, Winter Quarter, 2003.

Sass, Carol Ann. *ACT Periodical Index: Access to Catholic Thinking Periodical Index: Web Index to Select Catholic Periodicals.* Spring Quarter 2000.

Stone, Sergio D. *Conducting Community Analysis for the Bemis Public Library (Littleton, Colorado) Using 2002 U.S. Census Data and the Online Outcome-Based Evaluation Toolkit.* Chair, Winter Quarter, 2003.

Tureson, Tamara. *Design and Use of an Information Audit Tool for Use in a Law Library.* Chair, Winter Quarter, 2003.

Tweed, Beth. *Evaluation of Email Reference Service in a Consumer Health Library Environment.* Chair, Winter Quarter, 2003.

GRANTS AND CONTRACTS

Student Learning in Agriculture STEM through Teacher Professional Development. Research team: Stanton Gartin (PI) and Cyndi Hofmeister, Northeast Junior College; Jeff Cash, Cheryl Sánchez, Anne-Marie Crampton, Lamar Community College; Suzanna Spears, Morgan Community College; Jack Wiley, Kerry Gabrielson, Trinidad State Junior College; Michael J. Miller, Colorado State University; Michael Womochil (Co-PI), Casey Sacks, and Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Agriculture, Agriculture and Food Research Initiative, Food, Agriculture, Natural Resources and Human Sciences Education and Literacy Initiative, $404,460 (2017-2020)

Leading and Achieving: the Colorado Agriculture Regional Consortium. Research team: Jack Wiley (PI), Kerry Gabrielson, Trinidad State Junior College; Jeff Cash (PI), Cheryl Sánchez, Anne-Marie Crampton, Lamar Community College; Suzanna Spears, Morgan Community College; Cyndi Hofmeister, Northeast Junior College; Michael Womochil (Co-PI), Casey Sacks, and Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Agriculture, Hispanic Serving Institutions Education Grants Program, $504,414 (2017-2020)

Cyber Prep Program Planning Grant. Research team: Debbie Sagen (PI), Brenda Lauer, Pikes Peak Community College; Gretchen Martin, Koiosa Insights; and, Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Commerce, Regional Alliances and Multi-stakeholder Partnerships to Stimulate (RAMPS) Cybersecurity Education and Workforce Development, $199,681 (2016-2018)

Career and Technical Education in Colorado: Pathways to Education and Employment. Research team: Heather McKay (PI), Rutgers University; Sarah Heath, Casey Sacks, Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Education, Institute of Education Sciences, $1,400,000 (2017-2021)

Pre-Alliance Planning Grant Colorado Community College Alliance. Research team: Victor Vialpondo (PI) and Janel Highfill, Community College of Aurora; Heidi Loshbaugh (Co-PI), Community College of Denver; Rick Reeves, Bill McGreevy, Liz Cox, and Kristin Aslin, Red Rocks Community College; Cathy Pellish, Front Range Community College; Samuel DeVries, Arapahoe Community College; Sylvia D. Hall-Ellis, Colorado Community College System. National Science Foundation, Louis Stokes Alliance for Minority Participation (LSAMP), $86,817 (2016-2017)

Towards Scalable Differentiated Instruction Using Technology-enabled, Competency-based, Dynamic Scaffolding. Research team: Karen Wilcox (PI) and Vijay Kumar, Center for Computational Engineering, Massachusetts Institute of Technology; Flora McMartin, Broad-based Knowledge; Quinsigamond Community College; and, Casey Sacks, and Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Education, First in the World Developmental Grant, $2,891,882 (2015-2019)

Colorado Strategic Partnerships Emergency Grant. Research team: Elise Lowe-Vaughn (PI), Amy Hodson, Celia Hardin, Barbara McBride, James Newby, Christopher Dewhurst, Nina Holland, Kate Anderson, MaryAnn Roe, Chrystalynn Chrystalynn, Elaine Edon, Mona Barnes, Tom Morgan, Rob Hanni, and Marie Valenzuela, Colorado Department of Labor and Employment; Steve Anton, Joelle Brouner, and, Katie Griego, Colorado Department of Human Services; Rebecca Holmes, Judith Martinez, and Jennifer Jirous, Colorado Department of Education; Emily Templin Lesh, Colorado Workforce Development Council; Cory Everett, Colorado Department of Regulatory Agencies; and, Casey Sacks and Sylvia D. Hall-Ellis, Colorado Community College System. U.S. Department of Labor, Strategic Partnerships Emergency Grant, $5,000,000 (2015-2017)

CAEL Jump Start Program: Competency-Based Education. Research team: Jerry Migler (PI), Casey Sacks, Debra Cohn, Thomas Hartman, and Sylvia D. Hall-Ellis, Colorado Community College System; Matt Jamison, Front Range Community College; Mike Coste, Red Rocks Community College; Amanda Corum, Pueblo Community College; Janet Colvin, Pikes Peak Community College; and, MaryAnn Matheny, Community College of Denver. Council for Adult and Experiential Learning, $15,000 (2015)

PARSE EX.1051.197

Summit on the Redesign of Developmental Education. Research team: Jerry Migler (PI), Casey Sacks, and Sylvia D. Hall-Ellis, Colorado Community College System; Chip Nava, Pueblo Community College; Kim Moultney, Arapahoe Community College; Debbie Ulibarri, Trinidad State Junior College; and, Kris Bernard, Front Range Community College. American Association of Community Colleges, $15,000 (2015-2016)

Equity in Excellence at Colorado Community Colleges. Research team: Keith Howard (PI) and Sylvia D. Hall-Ellis, Colorado Community College System; Estela Mata Bensimon, Center for Urban Education, University of Southern California; and Kerry Gabrielson, Trinidad State Junior College. Colorado Department of Higher Education, Colorado Opportunity Scholarship Program, $150,000 (2015-2016)

MBA High School of Business in Colorado. Research team: Laurie Urich (PI) and Sylvia D. Hall-Ellis, Colorado Community College System; Rudolph Sumpter and Beatrice Gerrish, Boulder Valley Schools; Keith Curry Lance, RSL Research Group. Colorado Department of Higher Education, Colorado Opportunity Scholarship Program, $501,295 (2015-2016)

Fullbridge Program in Colorado. Research team: Keith Howard (PI) and Sylvia D. Hall-Ellis, Colorado Community College System; Suzanna Spears, Fort Morgan Community College; and, Cheryl Sánchez and Anne-Marie Compton, Lamar Community College. Colorado Department of Higher Education, Colorado Opportunity Scholarship Program, $300,000 (2015-2017)

Colorado Community College System Alternative Credit Program. Research team: Jerry Migler (PI), Keith Howard, and Sylvia D. Hall-Ellis, Colorado Community College System. American Council on Education, $13,000 (2015)

Internationalization in Higher Education. Researcher: Samuel D. Museus. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $8,000 (2014-2015)

International Perspectives on Bilingual Education. Researcher: Sharolyn Pollard-Durodola. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $3,700 (2014-2015)

Cultivating Culturally Relevant and Responsive Curriculum and Pedagogy in College. Researcher: Samuel Museus. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, iRise Grant, $5,000 (2014-2015)

Project EMERGE (Educational Model for Evaluation and Replicability in Gifted Environments). Research team: Norma Hafenstein (PI) and Bruce Uhrmacher. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Lynde and Harry Bradley Foundation, $235,000 (2014-2015)

Collecting Asian American Refugee Stories. Researcher: Samuel Museus. Technical reviewer for MCE: Sylvia D. Hall-Ellis. American Educational Research Association, Research Grant, $5,000 (2014-2015)

Developing Expertise in Teaching K-5 Mathematics. Research team: Julie Sarama (PI) and Douglas H. Clements (Co-PI), in partnership with the School of Education at the University of Michigan. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, $130,344 (2013-2015)

Investigation of the Long-Term Outcomes for Special Education Students. Researcher: Antonio Olmos-Gallos. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Jefferson County School District, Office of Assessment, $40,000 (2014)

Refugee Community Collaboration. Researcher: Vicki Tomlin (PI) in partnership with the African Community Center and Jewish Family Service. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, $14,854 (2014-2015)

Access to Mathematics for All. Research team: Richard S. Kitchen (PI), Nicole M. Russell (Co-PI), and Terrence Blackman (Co-PI), Curriculum Studies and Teaching Program, Morgridge College of Education; Álvaro Árias (Co-PI, Department of Mathematics); and, James Gray (Co-PI), Department of Mathematics, Community College of Aurora. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, The Robert Noyce Scholarship Program, Capacity Building Project, $349,926 (2014-2016)

Cognitive Test Battery for Intellectual Disabilities. Research team: Karen Riley (PI), Lyndsay Agans, Jessica Lerner, and Karin Dittrick-Nathan in partnership with David Hessl (PI), The MIND Institute at the University of California – Davis, and Elizabeth Berry-Kravis, Rush University Medical Center. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Institutes of Health, Outcome Measures for Use in Treatment Trials for Individuals with Intellectual and Developmental Disabilities (R01), $2,499,996 ($588,672 at DU) (2014- 2019)

Broadening Participation in Engineering among Women and Latino/as: A Longitudinal, Multi-Site Study. Researcher: Patton Garriott (PI) in partnership with the University of North Dakota and the University of Missouri. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, HER Core Research, $677,390 ($69,992 at DU) (2014-2019)

Designing a Teacher Evaluation System to Improve Teacher Effectiveness for Culturally and Linguistically Diverse Learners. Research team: María del Carmen Sálazar (PI), Jessica Lerner, and Kathy Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Professional Research Opportunities for Faculty, $29,988 (2014-2016)

Developing a College-Going Culture in Latina/O Families: Exploring the Influence of Funds of Knowledge on Family Outreach Programs. Researcher: Judy Marquez Kiyama. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Professional Research Opportunities for Faculty, $18,720 (2014-2016)

Assessment of Quality of Life in Neutral Implantation Surgery for the Treatment of Parkinson's Disease. Researcher: Cynthia McRae. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $3,967 (2014-2015)

Pura Vida: Cloud Forest, Curriculum and Cross-Cultural Study. Research team: Norma Hafenstein (PI) and Bruce Uhrmacher (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $8,000 (2014-2015)

Online Course Development for Curriculum and Instruction. Researcher: Ruth Chao. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $3,000 (2014)

Online Course Development for Curriculum and Instruction. Researcher: María del Carmen Sálazar. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $3,000 (2014)

Online Course Development for Curriculum and Instruction. Researcher: Jessica Lerner. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $3,000 (2014)

Online Course Development for Curriculum and Instruction. Researcher: Duan Zhang. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $3,000 (2014)

The Mathematics Education of African Americans, 1866 – 1954. Researcher: Nicole M. Russell. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Academy of Education, Spencer Foundation Postdoctoral Fellowship, $55,000 (2014-2016)

PARSE EX.1051.199

Early Childhood Care and Education Study. Research team: Carrie Germeroth (PI), Melissa Mincic, and Douglas H. Clements. Technical reviewer for MCE: Sylvia D. Hall-Ellis. State of North Dakota, Department of Public Instruction, $73,500 (2013-2014)

Graduate Level Specialty in Addiction Counselor Training with Emphasis on Integration of Native American Specific Content. Research team: Ruth Chao (PI) and Michael J. Faragher (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. The Galena Foundation, $289,732 (2013-2016)

The Collecting Asian American and Pacific Islander Refugee Stories (CARS) Project. Researcher: Samuel Museus. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, $15,000 (2014)

Parents in Transition: A Multiple Case Study of Parent and Family Orientation Programs. Researcher: Judy Marquéz Kiyama. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Faculty Research Fund, $1,500 (2014-2015)

The Sistah Network: Black Women Graduate Students Supporting and Retaining Each Other. Researcher; Nicole M. Russell. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Faculty Research Fund, $2,708 (2014-2015)

Evaluation of the Northeast Denver Babies Ready for College Program. Research team: Carrie Germeroth (PI), Melissa Mincic, and Douglas H. Clements. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Mile High Montessori, $9,922 (2013-2014)

Developing Teaching Expertise in K-5 Mathematics. Research team: Julie Sarama (PI) and Douglas Clements (University of Denver), in partnership with Timothy Boerst (PI), Meghan Shaughnessy, Deborah Ball, Hyman Bass (School of Education at the University of Michigan). Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, $449,827 ($130,344 at the University of Denver (2013-2015)

Early Learning Care and Education Study Program Grant for the State of North Dakota. Research team: Carrie Germeroth (PI), Melissa Mincic, and Sheridan Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. State of North Dakota Department of Public Instruction, $73,500 (2013-2014)

Local Professional Learning Community for School Leaders. Research team: Susan Korach (PI), Kristina Hesbol, and Rebecca McClure. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Education Development Center, $10,950 (2013)

Healthy Eaters, Lifelong Movers 2: Implementing Evidence-Based School Environment, Policy, and Curricular Changes to Increase Opportunities for Healthy Eating and Physical Activity in Low Income, Rural Colorado. Research team: Elaine Berlansky, University of Colorado at Denver (PI), Nicholas Cutforth, University of Denver (Co-PI), and Allison Reeds. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Health Foundation, $3,103,108 (2013-2017)

Hughes Rare Books Library Room Renovation in the Sturm College of Law. Project team: Patti H. Marks, Sylvia D. Hall-Ellis, and Leigh Elliott. Mabel T. Hughes Charitable Trust, $34,000 (2013-2014)

The Promise Center Partnership with the Marsico Institute for Early Learning and Literacy and the City and County of Denver. Research team: Karen Riley (PI), Douglas H. Clements (Co-PI), and Sheridan Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. The Piton Foundation, $223,468 (2013-2014)

Center of Excellence for Problem Gambling. Research team: Ruth Chao (PI) and J. Mike Faragher. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Department of Behavioral Health, $68,021 (2012-2013)

United Way Implementation and Validation Review. Research team: Douglas H. Clements (PI), Amanda Moreno, and Sheridan Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Mile High United Way, $19,737 (2013)

PARSE EX.1051.200

Math/Science Partnership in Rural Districts. Research team: Kristen Bunn (PI, Eagle County Schools), Paul Michalec (Co-PI), and Alegra Reiber. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Department of Education, Colorado's Mathematics and Science Partnership Program, $750,000 (2013-2014)

Mathematics and Science Education of African Americans. Research team: Nicole Russell (PI), Sylvia D. Hall-Ellis, Steve Fisher (Penrose Library). Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Professional Research Opportunities for Faculty, $29,994 (2013-2015)

Online Course Development for Curriculum and Instruction. Researcher: Nicole Russell. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $3,000 (2013)

Discourse and Opportunity: Undocumented Students and Higher Education Policy. Researcher: Ryan Gildersleeve. Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Academy of Education, Spencer Foundation Postdoctoral Fellowship, $55,000 (2012-2014)

An Anthropological Study of the Latino Graduation Ceremony. Researcher: Ryan Gildersleeve. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Spencer Foundation, $39,900 (2012-2013)

Fragile-X and Pharmaceutical Company: Clinical Trial of AFQ056. University of California – Davis Children's Hospital MIND Institute (Sacramento), Children's Hospital Denver, and Rush Children's Hospital at Rush University Medical Center (Chicago). Karen Riley, PI. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Novartis Pharmaceuticals Corporation, $394,206 (2012-2014)

Project Engage, Phase 2, a DAPRA Grant in partnership with Total Immersion Systems, Inc., and Texas A&M University. Research team: Karen Riley (PI) and Lyndsay Agans (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. U.S. Department of Defense, $60,000 (2011-2013)

International School Psychology Practicum Exchange. Researcher: Gloria L. Miller. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, $14,951 (2012-2013)

Online Course Development for Curriculum and Instruction. Research team: Bruce Uhrmacher (PI) and Norma Hafenstein (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Teaching and Learning, $18,193 (2012-2014)

Writers in the Schools. Research team: Karen Riley (PI) and Amanda Moreno (Co-PI).Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Humanities, $4,965 (2012-2013)

Learning Ecosystem Validation Grant. Research team: Karen Riley (PI), Lyndsay Agans, Kent Seidel, and Shimelis Assefa. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Bill & Melinda Gates Foundation, $315,000 (2012-2013)

Project Words of Oral Reading and Language Development (WORLD). Research team: Jorge E. Gonzalez (PI), Texas A&M University; Laura Saenz (Co-PI), University of Texas – Pan American; and, Sharolyn Pollard-Durodola (Co-PI), University of Denver. Technical reviewer for MCE: Sylvia D. Hall-Ellis. U.S. Department of Education, Institute of Education Sciences, $53,354 (2012-2015); award $640,718, transfer from Texas A&M University

PARSE EX.1051.201

Increasing the Efficacy of an Early Mathematics Curriculum with Scaffolding Designed to Promote Self-Regulation. Research team: Douglas H. Clements (PI) and Julie Sarama (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. U.S. Department of Education, Institute of Education Sciences, $1,445,315 (2008-2014); award $4,541,975, transfer from University at Buffalo, The State University of New York

Using Rule Space and Poset-based Adaptive Testing Methodologies to Identify Ability Patterns in Early Mathematics and Create a Comprehensive Mathematics Ability Test. Research team: Douglas H. Clements (PI) and Julie Sarama (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, $323,791 (2010-2014); award $1,194,944, transfer from University at Buffalo, The State University of New York

Comprehensive Postdoctoral Training in Scientific Education Research. Researcher: Julie Sarama (PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. U.S. Department of Education, Institute of Education Sciences, $133,458 (2010-2014); award $613,353, transfer from University at Buffalo, The State University of New York

Longitudinal Study of a Successful Scaling Up Project: Extending TRIAD. Research team: Douglas H. Clements (PI), Julie Sarama (Co-PI), and Abt Associates (Carolyn Layzer, Fatih Unlu, Laurie Bozzi, Lily Fesler, Alina Martinez, Cristofer Price, James van Orden). Technical reviewer for MCE: Sylvia D. Hall-Ellis. Institute of Education Sciences, $384,940 (2011-2015; award $1,250,286, transfer from University at Buffalo, The State University of New York

Early Childhood Education in the Context of Mathematics, Science, and Literacy. Research team: Julie Sarama (PI), Douglas H. Clements (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. National Science Foundation, $990,020 (2010-2014; award $2,285,228, transfer from University at Buffalo, The State University of New York

Early Childhood Clearinghouse Information Center Redesign. Research team: Karen Riley (PI) and Amanda Moreno (Co-PI).Technical reviewer for MCE: Sylvia D. Hall-Ellis. Office of the Lt. Governor of Colorado, $12,000 (2012-2013)

Morgridge Rural Educational Leadership Initiative. Research team: Lyndsay Agans (PI), Linda Brookhart (Co-PI), Susan Korach, and Rebecca McClure. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Morgridge Family Foundation, $100,000 (2012-2014)

Center of Excellence for Problem Gambling. Research team: Patrick Sherry (PI) and J. Mike Faragher. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Department of Behavioral Health, $55,000 (2012-2013)

Intermodal Transportation Institute Research Initiatives. Researcher: Patrick Sherry. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University Transportation Centers Program, Research and Innovative Technology Administration, U.S. Department of Transportation. $600,000 (2012-2014)

Mile High United Way Social Innovation Fund Early Literacy Initiative. Gloria L. Miller, PI, Amanda Moreano, Kim Hartnett-Edwards, and Sheridan Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Mile High United Way, $89,992 (2012-2013)

International School Psychology Practicum Exchange. Researcher: Gloria L. Miller. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, $1,000 (2012-2013)

Refugee Student Art Outreach. Researcher: Karin Dittrick-Nathan. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Faculty Research Fund, $3,000 (2012-2013)

Resistance, Resilience, and Reciprocity: Centering the Voices of Black Doctoral Women with Faculty Aspirations. Researcher: Nicole M. Russell. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Faculty Research Fund, $2,965 (2012-2013)

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Assessing Learning through Student Notebooks. Research team: Keith Miller, Nancy Sasaki, and Kathy Green. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Professional Research Opportunities for Faculty, $30,000 (2012-2014)

International School Psychology Practicum Exchange. Researcher: Gloria L. Miller. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $2,000 (2012-2013)

ELO in Colorado. Research team: Cynthia Hazel and Duan Zhang. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Legacy Foundation, $150,000 (2012-2013)

Creating Online LIS Courses. Research team: Mary C. Stansbury (PI), Shimelis Assefa, Denise Anthony, Xiao Hu, and Krystyna Matusiak. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Teaching and Learning, $15,000 (2011-2013)

Maritime Piracy Seminar. Researcher: Ved Nanda, The Nanda Center, Sturm College of Law. Technical reviewer for Sturm College of Law: Sylvia D. Hall-Ellis. Arsenault Family Foundation, $15,000 (2012-2013).

Early Childhood Librarianship: An Interdisciplinary, Experiential Learning MLIS. Researcher: Mary C. Stansbury (PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. Laura Bush's 21st Century Librarians Program, Institute for Museums and Library Services, $249,066 (2012-2014)

Learning Ecosystem Planning Grant. Research team: Karen Riley (PI), Lyndsay Agans, Kent Seidel, and Shimelis Assefa. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Bill & Melinda Gates Foundation, $281,217 (2011-2012)

Advanced Service Learning Practitioner Faculty Grant, Sylvia D. Hall-Ellis, University of Denver, Center for Community Engagement and Service Learning, $400 (2011)

Evaluating and Enhancing the EspeciallyMe Program. Research team: Lori D. Patton and Nicole Russell. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, Public Good Fellowship Grant, $24,780 (2012)

Choosing Excellence: Let Every Child Bloom. Research team: Shimelis Assefa (PI) and Mary C. Stansbury (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Community Engagement and Service Learning, Public Good Grant, $7,657 (2012)

K-8 STEM Content Specific Professional Development to Improve Elementary Student Achievement. Research team: Kent Seidel (PI), Nicole Russell (Co-PI), Kimberly Hartnett-Edwards, Paul Michalec, Jeff Farmer, Keith Miller, Alegra Reiber, and Nancy Sasaki. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Department of Education, Improving Teacher Quality Grant, (Title II ESEA), $307,299 (2011-2012)

Building a Better Principal Pipeline to Boost Student Achievement. Researcher: Susan Korach. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Denver Public Schools; a subcontract from the Wallace Foundation Grant, $170,000 (2011-2017)

Center of Excellence for Problem Gambling. Research team: J. Mike Faragher (Co-PI) and Bobbie Vollmer (PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Department of Behavioral Health, $79,999 (2011-2012)

An Exploration of Novice Teachers' Core Competencies: Impacts on Student Achievement, and Effectiveness of Preparation. Research team: Kent Seidel (PI), Kathy Green (Co-PI), Kimberly Hartnett-Edwards, and Duan Zhang. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Institute of Education Sciences, Effective Teachers and Effective Teaching, $990,987 (2012-2015)

Project Engage, a DAPRA Grant in partnership with Total Immersion Systems, Inc., and Texas A&M University. Research team: Karen Riley (PI) and Lyndsay Agans (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. U.S. Department of Defense, $49,964 (2011-2014)

User-centered Evaluation of Music Search Engines. Researcher: Xiao Hu. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Faculty Research Fund, $2,931 (2011-2012)

Educational Practicum in Vietnam and China to Promote the Inclusion of Young Children with Disabilities. Researcher: Gloria L. Miller. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Office of Internationalization, $5,000 (2011-2012)

Evaluation of Colorado's Enhancing Quality in Infant-Toddler (EQIT) Initiative. Research team: Virginia R. Maloney and Amanda Moreno. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Buell Foundation, $395,884 (2011-2013)

Creating Engaging Environments to Teach Pre-Algebra Mathematics to Elementary Students. Research team: Álvaro Arias, Mario López, María del Carmen Salazar, and Lyndsay Agans. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver Interdisciplinary Grant, $60,000 (2011-2012)

Morgridge Education Technology Accessible (META) Resource Project. Research team: Lyndsay Agans (PI) and Shimelis Assefa (Co-PI). Technical reviewer for MCE: Sylvia D. Hall-Ellis. Morgridge Family Foundation, $36,000 (2011)

MCE Connect: A 21st Century Framework for Faculty Development. Research team: Bruce Uhrmacher (PI), Shimelis Assefa (Co-PI), Lyndsay Agans (Co-PI), Kimberly Hartnett-Edwards, Norma Hafenstein, Xiao Hu, Paul Michalec (Co-PI), María del Carmen Salazar (Co-PI), and Sandra Snyder-Mondragon. Technical reviewer for MCE: Sylvia D. Hall-Ellis. University of Denver, Center for Teaching and Learning, $22,355 (2011-2012)

Parent Education and Parent Leadership and Advocacy. Research team: Virginia Maloney (PI) and Amanda Moreno, Marsico Institute for Early Learning and Literacy. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Health Foundation, $27,358 (2010-2011)

Intentional School Culture. Researcher: Cynthia Hazel. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Denver Public Schools, $38,040 (2010-2011)

Healthy Eaters, Lifelong Movers: Implementing Evidence-Based School Environment, Policy, and Curricular Changes to Increase Opportunities for Healthy Eating and Physical Activity in Low Income, Rural Colorado. Research team: Elaine Berlansky, University of Colorado at Denver (PI), Nicholas Cutforth, University of Denver (Co-PI), and Allison Reeds. Technical reviewer for MCE: Sylvia D. Hall-Ellis. Colorado Health Foundation, $1,683,277 (2011-2014)

Second Life Learning Community. Research team: Don McCubbrey (PI), Sylvia D. Hall-Ellis (Co-PI), Walter LaMendola, and Paul Novak. University of Denver, Center for Teaching and Learning, $13,000 (2010-2011)

Reintroducing the Value of Law Librarians to Academic and Public Librarians in Colorado through the Identification and Use of Emerging Technologies. Research team: Sylvia D. Hall-Ellis (Co-PI), Stacey L. Bowers (PI), and Christopher Hudson, in partnership with Denver Public Library, Arapahoe Library District, and the Colorado State Supreme Court Library. University of Denver, Center for Community Engagement and Service Learning, $5,773 (2010-11)

Reintroducing the Value of Law Librarians to Public Librarians through the Identification and Use of Emerging Technologies and Resources. Research team: Stacey Bowers (PI) and Sylvia D. Hall-Ellis (Co-PI). American Association of Libraries, Wolters Kluwer Law & Business Grant Program, $2,725 (2010-2011)

Faculty Service Learning Pod. Research team: María del Carmen Salazar and Nicholas Cutforth (Curriculum and Instruction Program), Frank Tuitt (Higher Education Program), Cynthia Hazel and Gloria Miller (Child, Family, and School Psychology Program), and Sylvia D. Hall-Ellis (Library and Information Science Program). University of Denver, Center for Community Engagement and Service Learning, $8,000 (2010-2011)

Lincoln Collaborative. Research team: Antonio Esquibel (Principal, Lincoln High School), María del Carmen Salazar (Curriculum and Instruction Program), and. Sylvia D. Hall-Ellis. School Improvement Grant, Denver Public Schools, $375,000 (2010-2012)

Future LEADers of America: Leaders III. Research team: Denver Public Library (Kristen Svendson, PI), the University of Denver (Sylvia D. Hall-Ellis), the Colorado Chapter of REFORMA (Orlando Archibeque), and the Colorado Association of Libraries (Martin Garnar). Laura Bush's Recruiting Librarians for the 21st Century Program, Institute for Museums and Library Services, $988,366 (2009-2012)

Teaching for Success in the Library Environment: LIS 4030 in Library 2.0. Research team: Deborah S. Grealy (PI) and Sylvia D. Hall-Ellis (Co-PI). University of Denver, Center for Teaching and Learning, $ 9,350 (2009-10)

Writing Group Faculty Grant, Sylvia D. Hall-Ellis, University of Denver, Center for Community Engagement and Service Learning, $750 (2008-2009)

Collaborative Learning Faculty Grant, Sylvia D. Hall-Ellis, University of Denver, Center for Teaching and Learning, $1,500 (2008)

Connecting Information Literacy to Learning. Research team: Lori Micho, Merrie Valliant, Amanda Samland, and Sylvia D. Hall-Ellis. Colorado State Library, LSTA Discretionary Grant Program, $19,548 (2008-2009)

Law Librarianship Fellows Program. Research team: Sylvia D. Hall-Ellis (PI), Stacey Bowers (Co-PI), and Christopher Hudson, Westminster Law Library. Laura Bush's 21st Century Librarians Program, Institute for Museums and Library Services, $999,370 (2008-2012)

Grant Writing in a Cooperative Learning Environment. Sylvia D. Hall-Ellis. University of Denver, Center for Teaching and Learning, $2,000 (2008-2009)

Project Homeless Connect 6 Event Evaluation. Research team: Sylvia D. Hall-Ellis and Duan Zhang. University of Denver Center for Community Engagement and Service Learning, $9,785 (2008)

Project Ecuador: International Learning Service Libraries – A Faculty Development Experience in Ecuador. Sylvia D. Hall-Ellis, Office of Internationalization, University of Denver, $600 (2007)

Project Ecuador: International Learning Service Libraries – A Faculty Development Experience in Ecuador. Sylvia D. Hall-Ellis, International Service Learning Office, University of Denver, $400 (2007)

Project Homeless Connect 5 Event Evaluation. Research team: Sylvia D. Hall-Ellis and Duan Zhang. University of Denver Center for Community Engagement and Service Learning, $1,000 (2007)

Project Homeless Connect 4 Event Evaluation. Research team: Sylvia D. Hall-Ellis and Duan Zhang. University of Denver Center for Community Engagement and Service Learning, $4,595 (2007)

Future LEADers of America: Leaders II. Research team: Denver Public Library (Kristen Svendson, PI), the University of Denver (Sylvia D. Hall-Ellis), and the Colorado Chapter of REFORMA (Orlando Archibeque). Laura Bush's Recruiting Librarians for the 21st Century Program, Institute for Museums and Library Services, $988,518 (2007-2010)

Strategic Planning Assistance for the Denver Medical Library. Sylvia D. Hall-Ellis. Denver Medical Library, Inc., $30,000 (2006-2007)

Destiny Software for JMAC Student Lab. Sylvia D. Hall-Ellis, gift from the Sagebrush Corporation, Minneapolis, Minnesota, $10,000 (2006)

Libraries: Tools for Education and Development Worldwide: A Faculty Development Experience in France. Sylvia D. Hall-Ellis, Office of Internationalization, University of Denver, $800 (2005)

Denver Public Library: Opportunities for Change. Sylvia D. Hall-Ellis, Colorado Community Based Research Network, $2,000 (2005)

Future LEADers of America. Research team: Denver Public Library (Letty Icolari and Steve Taylor, PIs), Emporia State University (Jim Agee), and the University of Denver (Sylvia D. Hall-Ellis). Recruiting Librarians for the 21st Century Program, Institute for Museums and Library Services, $670,315 (2005-2008)

American Association of University Professors Summer Institute Professional Development Grant. Sylvia D. Hall-Ellis, American Association of University Professors, $300 (2005)

Beta Phi Mu Alumni Tea. Sylvia D. Hall-Ellis, gift from the Office of Alumni and Parent and Relations, University of Denver, $2,000 (2005)

Spectrum Software for LIS Student Lab. Sylvia D. Hall-Ellis, gift from the Sagebrush Corporation, Minneapolis, Minnesota, $5,000 (2005)

Developing Research Capacity in Community Organizations and Residents through Training and Technical Assistance. Nicholas J. Cutforth (PI) and Sylvia D. Hall-Ellis. Piton Foundation, $25,000 (2005-2006)

Libraries: Tools for Education and Development Worldwide: A Faculty Development Experience in Argentina. Sylvia D. Hall-Ellis, Office of Internationalization, University of Denver, $500 (2004)

Developing Research Capacity in Community Organizations and Residents through Training and Technical Assistance. Research team: Nicholas J. Cutforth (PI), Gary Lichtenstein and Sylvia D. Hall-Ellis, Piton Foundation, $25,000 (2003-2004)

Increasing Spanish-Speaking and Hispanic Diversity among Library and Information Science Students at the University of Denver: Development of a Student Recruitment Model. Researcher: Sylvia D. Hall-Ellis (PI), Office of Multicultural Excellence, University of Denver, $2,750 (2003-2004)

Collection Development Enrichment to Support the Cataloging & Classification Specialization and School Librarianship within the Library & Information Science Program at the University of Denver. Research team: Sylvia D. Hall-Ellis (PI) and Deborah S. Grealy. Women's Library Association, University of Denver, $4,000 (2003)

Collection Development Enrichment to Support the Library & Information Science Program at the University of Denver. Research team: Deborah S. Grealy (PI) and Sylvia D. Hall-Ellis. Women's Library Association, University of Denver, $4,000 (2001)

Wireless LAN for Library Education. Research team: Deborah S. Grealy (PI) and Sylvia Hall-Ellis. University of Denver, Center for Teaching and Learning, $24,000 (2001-2002)

Upward Bound – A Program for South Texas Youth. Research team: Monte Churchill, Executive Director of Community Relations; Gary R. Sauceda, Outreach Coordinator; Raymond Hernandez, (PI) Associate Dean for Student Success; and Sylvia D. Hall-Ellis, South Texas Community College (McAllen, Texas). U.S. Department of Education, Office of Postsecondary Education, $2,672,003 (1999-2003)

Write Now! Improving Elementary Students' Writing Skills. Research team: Caryl G. Thomason, Assistant Superintendent (PI); Karen Tankersley, Principal; and, Hal Anderson, Director of Technology, Cheyenne Mountain School District 12 (Colorado Springs, Colorado); and Sylvia D. Hall-Ellis. Colorado Department of Education, Educational Telecommunications Unit, $189,453 (1999-2001)

Operation Quick Start -- Distance Learning in Rural Colorado. Research team: Randal Weigum, Technology Coordinator (PI); Bonnie Barns, Director of Federal Programs and Staff Development; and, Adam "Joe" Raskop, Executive Director, Southeastern Board of Cooperative Educational Services (Lamar, Colorado); and Sylvia D. Hall-Ellis. Colorado Department of Education, Educational Telecommunications Unit, $400,000 (1999-2001)

Advanced Technological Training for Information Professionals for the 21st Century. Research team: Mario Reyna, Division of Business Director (PI) and Sylvia D. Hall-Ellis, South Texas Community College (McAllen, Texas). Phi Delta Kappa and the National Science Foundation, $250,000 (1999-2001)

Working Connections – Training Information Technologies Professionals for the 21st Century. Research team: Mario Reyna, Division of Business Director (PI) and Sylvia D. Hall-Ellis, South Texas Community College (McAllen, Texas). Microsoft Corporation and the American Association of Community Colleges, $1,147,775(1999-2001)

The ROAD (Research Oriented Amplification of Development to Literacy) Program. Research team: Deborah J. Leong (PI), Metropolitan State University; Elena Bodrova, Metropolitan State University; Dmitri Semenov, Robert J. Marzano, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). Hewlett-Packard Foundation, $25,000 (1998-1999)

Press to Literacy. Research team: Deborah J. Leong (PI), Metropolitan State University; Elena Bodrova, Metropolitan State University; Dmitri Semenov, Robert J. Marzano, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). The Denver Post and the Robert S. McCormick Foundation, $49,818 (1998-1999)

International Telementor Center. Research team: David Neils, David B. Frost (PI), and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). Hewlett-Packard Philanthropy, $100,000 (1998-1999)

America Reads: Providing Tutor Training for the America Reads Challenge. Research team: Louis F. Cicchinelli (PI) and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). U.S. Department of Education, Office of Educational Research and Improvement, $306,000 (1998-1999)

Comprehensive School Reform. Research team: Louis F. Cicchinelli (PI), J. Timothy Waters, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). U.S. Department of Education, Office of Educational Research and Improvement, $285,000 (1998-1999)

Sustainable Energy Education (SEE) Program for Grades 4-8: Preparing Today's Youth for Lifelong Learning and Responsible Actions in Energy Conservation. Research team: Mary Gromko, Colorado Department of Education; Gene McCarthy, Rocky Flats Field Office, U.S. Department of Energy; Gina Kissell, National Renewable Energy Laboratory; and Barbara L. McCombs (PI), Janet L. Bishop, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). State of Colorado Governor's Office for Energy Conservation, $499,936 (1997-1999)

Review of Kansas Curriculum Standards in Mathematics and Language Arts. Research team: Robert J. Marzano

(PI), John S. Kendall, David B. Frost, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). Submitted to the Kansas State Department of Education, $25,903 (1998)

International Telementor Center. Research team: David Neils, John Kuglin, Chris Rapp, David B. Frost (PI), and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). Hewlett-Packard Company, $54,752 (1998)

Genesis Mission: Education Public Outreach. Developed in partnership by the Jet Propulsion Laboratory, California Institute of Technology, Los Alamos National Laboratory, Lockheed Martin Astronautics, and the Mid-continent Regional Educational Laboratory (Aurora, Colorado). Research team: John T. Sutton (PI), Alice Krueger, Martha Henry, Greg Rawls, Shae Isaacs, Jeff Johnson, Deb Jordan, Arlene Mitchell, David B. Frost, Jana Caldwell, J. Timothy Waters, and Sylvia D. Hall-Ellis. National Aeronautics and Space Administration, $139,700,000; subcontract award, $4,750,000 (1997-2007)

North Dakota Mathematics Assessment Project. Developed in partnership by the North Dakota Department of Education and Mid-continent Regional Educational Laboratory (Mid-continent Regional Educational Laboratory). Research team: Ann Clapper, Clarence Bina, Greg Gallagher, North Dakota Department of Education; Don Burger (PI), Hillary Michaels, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). U.S. Office of Education, $1,618,214; subcontract award, $389,076 (1997-2001)

Pacific Resources for Education and Learning Distance Education: Project Evaluation. Developed in partnership by the Pacific Educational Community, the Pacific Resources for Education and Learning (PREL), and Mid-continent Regional Educational Laboratory (McREL). Research team: John W. Kofel (PI), Executive Director, PREL; J. Timothy Waters, Executive Director; Joan Buttram, Robert Keller, and Sylvia D. Hall-Ellis, Mid-continent Regional Educational Laboratory (Aurora, Colorado). U.S. Office of Education, Star Schools Program, $10,000,000; subcontract award, $500,000 (1997-2002)

Identification of Bilingual Gifted and Talented Children: A Comprehensive School Grants for Bilingual Education. Developed in partnership by Hidalgo (Texas) ISD, Los Fresnos (Texas) CISD, Progreso (Texas) ISD, University of Texas – Pan American (Edinburg, Texas), and Education Service Center, Region One (Edinburg, Texas). Research team: Hilda Medrano, Dean, College of Education, University of Texas – Pan American; Linda Phemister (PI), Janie Navarro, and Sylvia D. Hall-Ellis, Education Service Center, Region One. U.S. Department of Education, Office of Bilingual Education and Minority Languages Affairs, $1,670,633 (1997-2002)

Academics 2000: First Things First -- The Texas Goals 2000. Developed for Jim Hogg (Hebbronville, Texas) County ISD, Mirando City (Texas) ISD, and San Isidro (Texas) ISD. Research team: Hilda Medrano, Dean, College of Education, University of Texas - Pan American (Edinburg, Texas); Angie Lehmann, Amy Mares, Ellen Gonzalez (PI), and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg, Texas). Texas Education Agency, $339,987 (1997-2000)

Southwestern Bell's Learning Communities Initiative. Developed for Tech Prep of the Rio Grande Valley, Inc. (Harlingen, Texas); the Center for Professional Teacher Development, University of Texas – Brownsville; Teach for America – Rio Grande Valley (McAllen, Texas); and Education Service Center, Region One (Edinburg, Texas). Research team: Patricia G. Bubb, Executive Director (PI), Tech Prep of the Rio Grande Valley; Martin Winchester, Executive Director, Teach for America – Rio Grande Valley; Aileen Johnson, Director, School of Education, University of Texas – Brownsville; and Sylvia D. Hall-Ellis. Southwestern Bell Foundation, $100,000 (1997-1998)

Texas School to Work: Regional Implementation. Developed for Tech Prep of the Rio Grande Valley, Inc. (Harlingen), South Texas Community College (McAllen), Texas State Technical College (Harlingen), Texas Southmost College (Brownsville), Empowerment Zone of the Rio Grande Valley (Mercedes), Project VIDA (Weslaco), Youth Fair Chance (McAllen), and Education Service Center, Region One (Edinburg). Research team: Patricia G. Bubb (PI), Tech Prep of the Rio Grande Valley; Stephen Vassberg, Texas State Technical College; Ellen Trevino, Youth Fair Chance; Wanda Garza, Project VIDA; Leonardo Olivares, University of

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Texas – Pan American; Michael Bell, South Texas Community College; and Sylvia D. Hall-Ellis. Texas Workforce Commission, $4,250,000 (1997-2002).

Comprehensive Bilingual Education Grant for Hidalgo ISD and Roma ISD. Developed for Hidalgo (Texas) ISD, Roma (Texas) ISD, College of Education, University of Texas – Pan American (Edinburg, Texas), and Education Service Center, Region One (Edinburg, Texas). Research team: Tomas Thomas (PI), Director, Office of Bilingual Education and Sylvia D. Hall-Ellis. U.S. Department of Education, Office of Bilingual Education and Minority Languages Affairs, $1,531,361 (1997-2002)

Lopez High School, Porter High School, Rivera High School, Central Middle School, and Perkins Middle School, Brownsville (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $1,473,611 (1997-1998)

Donna High School, Todd Middle School, and Solis Middle School, Donna (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $704,052 (1997-1998)

Memorial Middle School and Nellie Schunior Middle School, La Joya Independent School District (Texas). Telecommunications Infrastructure Fund Board, $293,641 (1997-1998)

Martin High School, Christen Middle School, Cigarroa Middle School, Lamar Middle School, Laredo (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $1,200,000 (1997-1998).

Lasara Middle School, Lasara (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $246,210 (1997-1998)

Los Fresnos High School and Resaca Middle School, Los Fresnos (Texas) Consolidated Independent School District. Telecommunications Infrastructure Fund Board, $500,000 (1997-1998)

Lyford Junior High School, Lyford (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $354,997 (1997-1998)

Travis Middle School, McAllen (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $269,139 (1997-1998)

Mission High School, Mission (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $297,010 (1997-1998)

Progreso High School, Progreso (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $300,000 (1997-1998)

San Perlita High School, San Perlita (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $293,490 (1997-1998)

Myra Green Junior High School, Raymondville (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $225,000 (1997-1998)

Rio Grande City High School, Ringgold Middle School, Gruella Middle School, Rio Grande City (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $809,934 (1997-1998)

Rio Hondo Junior High School, Rio Hondo (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $297,024 (1997-1998)

Roma High School, Roma (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $333,178 (1997-1998)

San Benito High School, Miller Jordan Junior High School, San Benito (Texas) Consolidated Independent School District. Telecommunications Infrastructure Fund Board, $547,000 (1997-1998)

Santa Maria High School and Santa Maria Middle School, Santa Maria (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $400,000 (1997-1998)

Salvador High School, United Independent School District (Laredo, Texas). Telecommunications Infrastructure Fund Board, $250,879 (1997-1998)

Weslaco High School, Cabaza Middle School, Cuellar Middle School, Weslaco (Texas) Independent School District. Telecommunications Infrastructure Fund Board, $874,242 (1997-1998)

Principals' Assessment and Training Center. Developed for Education Service Center, Region One (Edinburg, Texas). Research team: Roberto Zamora, Leonel Barrera, William H. Parry, and Sylvia D. Hall-Ellis. Texas Principals' Leadership Initiative, Texas Association of Secondary School Principals, Texas Association of Elementary School Principals, and the Sid Richardson Foundation. $461,439 (1996-1998)

The Texas Library Connection -- Integrating and Sharing Resources. Developed for Hidalgo County Library System (McAllen), Cameron County Library System (Brownsville), South Texas Community College (McAllen), the University of Texas - Pan American (Edinburg), University of Texas – Brownsville, and Education Service Center, Region One (Edinburg). Research team: William R. McGee, Coordinator, Hidalgo County Library System; Joe Garcia, Director, Cameron County Library System; Michael D. Bell, Library Director, South Texas Community College; Eleanor Folger Foster, University Library, University of Texas - Pan American; Jaime Vela (PI), Director of Instructional Technology and Media Services, Ron Pontius, Instructional Technology, and Fabiola Fuentes, Media Services, Education Service Center, Region One; and Sylvia D. Hall-Ellis. Office of Library Media Services, Technology Services, Texas Education Agency. $8,000 (1996-1997)

Developing Leadership Communities for Improving Algebra I for All Students. Developed for a partnership of the Region One Statewide Systemic Initiative Team. Research team: Noel Villarreal (PI), Eduardo Cancino, and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Statewide Systemic Initiative for Reform in Mathematics, Science and Technology Education to The Charles A. Dana Center for Mathematics and Science Education, The University of Texas at Austin. $25,000 (1996-1997)

Academics 2000: First Things First -- The Texas Goals 2000. Developed for Edinburg (Texas) CISD. Research team: Hilda Medrano, Dean, College of Education, University of Texas - Pan American (Edinburg); Helen Jones, Director (PI), Gifted and Talented Education, Edinburg CISD; and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Education Agency, $750,000 (1996-2001)

Texas Teachers Empowered for Achievement in Mathematics (TEXTEAM) Institute for Algebra I. Developed for Hidalgo ISD, Jim Hogg (Hebbronville) County ISD, La Joya ISD, La Villa ISD, Lyford ISD, Mission CISD, Pharr-San Juan-Alamo ISD, Rio Hondo ISD, San Isidro ISD, San Perlita ISD, Santa Maria ISD, Santa Rosa, Sharyland ISD, Valley View ISD, Webb (Laredo) CISD, Weslaco ISD, and Zapata County (Zapata) ISD. Research team: Noel Villarreal (PI), Chuck McInteer, Ellen M. Gonzalez, Education Service Center, Region One (Edinburg); and Sylvia D. Hall-Ellis. Charles A. Dana Center for Mathematics and Science Education, University of Texas - Austin, $15,975 (1996)

Community Learning Center for La Villa, Texas. Developed for Edcouch-Elsa Independent School District. Research team: Noe Gonzalez (PI), Assistant Superintendent, Edcouch-Elsa Independent School District, and Sylvia D. Hall-Ellis. Delta Region Subzone, Rio Grande Valley (Texas) Rural Empowerment Zone, $325,000 (1996-1997)

Monte Alto (Texas) Community Learning Center. Developed for Monte Alto Independent School District. Research team: Homero A. Diaz (PI), Superintendent, Monte Alto Independent School District, and Sylvia D. Hall-Ellis. Delta Region Subzone, Rio Grande Valley (Texas) Rural Empowerment Zone, $300,000 (1996-1997)

Community Learning Center for La Villa, Texas. Developed for La Villa Independent School District. Research team: Sam Gonzalez (PI), Assistant Superintendent, La Villa Independent School District; and Sylvia D. Hall-Ellis. Delta Region Subzone, Rio Grande Valley (Texas) Rural Empowerment Zone, $325,000 (1996-1997)

Building Project for Taylor Elementary. Developed for Mercedes (Texas) Independent School District. Research team: Mrs. Denise Rivera, Librarian, and Eduardo Infante, Principal, Taylor Elementary School; Ismael S. Cantu (PI), Federal Programs Director, Mercedes Independent School District; and Sylvia D. Hall-Ellis. Delta Region Subzone, Rio Grande Valley (Texas) Rural Empowerment Zone, $375,000 (1996-1997)

Border Education Network (BEN) - Distance Education through Cable Television. Developed for Edinburg (Texas) Consolidated Independent School District. Research team: Noe Torres (PI), Library Media Specialist, Magdalena
Rosas, Library Media and Technology Coordinator, Edinburg Consolidated Independent School District; and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Edinburg (Texas) Consolidated School District, $250,000 (1996-1997)

Multiservice One-Stop Open Enrollment Charter School. Developed for the Information Referral Resource Assistance, Inc. (McAllen, Texas). Research team: Pablo Perez and Aguie Pena (PI), Executive Director, Information Referral Resource Assistance, Inc.; Roberto Zamora and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Education Agency, $3,066,000 (1996-2001)

Project OK: A Community Youth Opportunities Grant for Summer, 1996. Developed for McAllen (Texas) Independent School District, St. Joseph the Worker Catholic Church, and the Office of Adult Education, Education Service Center, Region One. Research team: Father Bart Flatt, St. Joseph the Worker Catholic Church; Noe Calvillo (PI), Office of Adult Education; Maria Louisa Garcia, McAllen Independent School District; and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Protective and Regulatory Agency through the Office of the Mayor, City of McAllen, $350,000 (1996-2001).

Early Childhood: a Time of Discovery. Developed for a partnership of Lasara ISD, Rio Hondo ISD, San Perlita ISD, the School of Education, University of Texas - Brownsville, and Education Service Center, Region One (Edinburg). Research team: Hugo Rodriguez, Dean, School of Education, University of Texas - Pan American; Leonel Barrera and Jack Damron, Field Service Agents, Ellen M. Gonzalez (PI), Administrator for Student Instructional Services, Ruth Solis, Education Specialist in Special Education, and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Education Agency, $722,090 (1996-2001)

Innovative Gifted and Talented Programs for Early Childhood and Elementary Education Students. Developed for a partnership of Edinburg (Texas) CISD, the School of Education, University of Texas - Pan American (Edinburg), and Education Service Center, Region One (Edinburg). Research team: Hilda Medrano, Dean, School of Education, University of Texas - Pan American; Helen de la Garza (PI), Director of Elementary Curriculum and Instruction, Edinburg CISD; and Sylvia D. Hall-Ellis. Texas Education Agency, $750,000 (1996-2001)

Reading Recovery in Early Elementary Grades. Developed for a partnership of La Villa (Texas) ISD the School of Education, University of Texas - Pan American (Edinburg), and Education Service Center, Region One (Edinburg). Research team: Hilda Medrano, Dean, School of Education, University of Texas - Pan American; Marcario Salinas (PI), Supervisor of Elementary Curriculum and Instruction, La Villa ISD; and Sylvia D. Hall-Ellis, Education Service Center, Region One. Texas Education Agency, $750,000 (1996-2001)

Connected Mathematics Project for Middle and Junior High Students. Developed for a partnership of Michigan State University, The Charles A. Dana Center for Mathematics and Science Education, The University of Texas at Austin and Region One Statewide Systemic Initiative Team. Research team: Jack Damron, Chuck McInteer, Noel Villarreal (PI), and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). National Science Foundation through Michigan State University to the Charles A. Dana Center at the University of Texas - Austin, $539,610 (1996-1999)

Sharing Resources: Testing the Interlibrary Loan Potential of the Texas Library Connection -- Integrating Media Resources. Developed under the sponsorship of the Hidalgo County Library System (McAllen) and Education Service Center, Region One (Edinburg). Research team: William H. McGee, Hidalgo County System Coordinator; Fabiola Fuentes, Library Media; Ronald Pontius (PI), Instructional Technology, Education Service Center, Region One; and Sylvia D. Hall-Ellis. Library Media Services Program, Office of Technology Services, Texas Education Agency, $25,000 (1996-1997)

State and Federal Adult Education JOBS Program. Developed for Education Service Center, Region One (Edinburg). Research team: Noe Calvillo (PI), Director, Adult Education Program, and Sylvia D. Hall-Ellis. Adult Education Program, Texas Education Agency, $532,846 (1995-1996)

Creating Safe and Drug-Free Schools and Communities. Developed for a partnership of the Region One Consortium for Safe and Drug-Free Education Environments. Research team: Clara Contreras (PI), Health Specialist, and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Education Agency, $150,000 (1995-1996)

Developing Leadership Communities for Improving Mathematics Performance for All Students on Title I Campuses. Developed for a partnership of the Region One Statewide Systemic Initiative Team. Research team: Jack Damron, Chuck McInteer, Noel Villarreal (PI), and Sylvia D. Hall-Ellis, Education Service Center, Region One (Edinburg). Texas Statewide Systemic Initiative for Reform in Mathematics, Science and Technology Education to The Charles A. Dana Center for Mathematics and Science Education, The University of Texas at Austin, $50,669 (1995-1996)

The Impact of Library Resource Centers on Academic Achievement in Selected Public Schools in South Texas. Sylvia D. Hall-Ellis (PI). Developed under the sponsorship of the Department of Library Science, College of Education and Applied Science, Sam Houston State University (Huntsville). Texas Association of School Librarians, Children's Services Round Table, and Young Adults Round Table (Austin, Texas), $2,500 (1995)

School Library Media Specialists Fellowship Program. Sylvia D. Hall-Ellis (PI). Developed under the sponsorship of the Department of Library Science, College of Education and Applied Science, Sam Houston State University (Huntsville, Texas). U.S. Department of Education, HEA Title II-B, Library Education and Human Resource Development Program, $44,000 (1995-1996)

Planning for Educational Technology. Research team: Ruth Ann Riggins (PI), Director of Library Media and Technology Services, Donna (Texas) Independent School District; Noe Torres, Education Service Center, Region One; Patricia G. Bubb, Tech Prep of the Rio Grande Calley, Inc; Michael D. Bell, South Texas Community College; and Sylvia D. Hall-Ellis, Sam Houston State University. Developed under the sponsorship of Donna (Texas) Independent School District, Education Service Center, Region One (Edinburg), Tech Prep of the Rio Grande Valley, Inc. (Harlingen), South Texas Community College (McAllen), and the Department of Library Science, Sam Houston State University (Huntsville). Funded through Infusion of Educational Technology Planning Grant Program (H.B. 18: Models). Texas Education Agency, $18,000 (1993-1994)

The Impact of Library Resource Centers on Academic Achievement in Selected Public Schools in South Texas. Sylvia D. Hall-Ellis (PI) and Mary Ann Berry. Developed under the sponsorship of the Department of Library Science, College of Education and Applied Science, Sam Houston State University (Huntsville). Sam Houston State University Research Enhancement Fund, $7,500 (1993-1994)

Electronic Mail Resource Sharing System: Management and Operation of the Iowa Computer-Assisted Network. State Library of Iowa, $294,000 (1986-1993)

The Iowa Locator: A CD-ROM Resource Sharing Tool. State Library of Iowa, $567,000 (1986-1992)

Statewide Database Development: An OCLC Tape Analysis To Determine Feasibility. State Library of South Dakota, $15,000 (1987)

The Iowa Locator: A Feasibility Study. State Library of Iowa, $50,000 (1986)

Electronic Mail Resource Sharing System: A Feasibility Study for Libraries in the State of Iowa. State Library of Iowa, $5,000 (1985)

Access Pennsylvania: A Feasibility Study. State Library of Pennsylvania, $50,000 (1984)

Automating Library Administrative and Management Tasks: Procurement and Distribution of Microcomputer Systems for District Library Centers in the Commonwealth of Pennsylvania. State Library of Pennsylvania, $495,000 (1982)

Sharing Serial Titles Resources: Procurement and Distribution of Microfiche Readers for 550 Libraries in the Commonwealth of Pennsylvania. State Library of Pennsylvania, $500,000 (1982)

Electronic Mail System: A Pilot Project for Libraries throughout the Commonwealth of Pennsylvania. State Library of Pennsylvania, $125,000 (1982)

Literacy Program for Adults in Rural Upstate New York State: Program Outreach and Evaluation - Phase 3. Appalachian Regional Commission, $50,000 (1981)

Database Building: A Cooperative Project of the Finger Lakes Library System (Ithaca), Four County Library System (Binghamton), and the Southern Tier Library System (Corning). New York State Library, $450,000 (1980-1985)

Faces of the Southern Tier: A Professional Photographer-in-Residence. New York State National Endowment for the Humanities, $25,000 (1980)

Literacy Program for Adults in Rural Upstate New York State: Program Implementation - Phase 2. Appalachian Regional Commission, $50,000 (1980)

Small Business Resources Center: A Pilot Project for Rural Public Libraries. New York State Library, $50,000 (1980)

Media Programming: A Professional Development Program for Public Librarians in Upstate New York: A Program in Allegheny, Chemung, Schuyler, Steuben, and Yates Counties. New York State Library, $35,000 (1979)

Information and Library Resources for Inmates and Prisoners in Selected Upstate New York Facilities: A Cooperative Program in Allegheny, Chemung, Schuyler, Steuben, and Yates Counties. New York State Library, $25,000 (1979)

PARSE EX.1051.213

Library Resources for Homebound Adults in Upstate New York: An Outreach Program in Allegheny, Chemung, Schuyler, Steuben, and Yates Counties. New York State Library, $40,000 (1979)

Literacy Program for Adults in Rural Upstate New York State: Program Initiation and Establishment - Phase 1. Funded by the Appalachian Regional Commission. Awarded to the Corning (New York) Public Library, $50,000 (1979)

Information Reference Services to Homebound Adults. New York State Library, $31,000 (1978)

Books-By-Mail Services to Homebound Adults. New York State Library, $31,000 (1978)

System Headquarters Services and Programs for Public Libraries in District 10. Funded through Library Services and Construction Act Title I. Texas State Library and Historical Commission, $880,000 (1976)

County Library Development Grant for Atascosita County. Funded through Library Services and Construction Act Title I. Texas State Library and Historical Commission, $15,000 (1976)

Spanish Language Materials Collection Development Program. Texas State Library and Historical Commission, $40,000 (1975)

Establishment of System Headquarters for Alamo Regional Library System (District 10) Headquartered at the San Antonio (Texas) Public Library. Texas State Library and Historical Commission, $800,000 (1975)

SELECTED CONSULTANTSHIPS

Project Strategic Planning & Funding Proposal Development

Walden University, 2015.
Pikes Peak Library District, 2014.
Douglas County Public Libraries, 2011-2012.
Denver School for Science and Technology, 2008-2011.
Johnson & Wales University, Denver Campus, 2007-2009.
Challenges, Choices, and Images K-12 Charter School, Denver Public Schools, 2007.
Denver Medical Library, Inc., 2006-2010.
Bemis Public Library (Littleton), Ergonomic Design and Facilities Enhancement Consultant, 2003.
Curtis Park Community Center (Denver), Community Technology Center Evaluation and Proposal Development Consultant, 2003.
Colorado Community Based Research Network (Denver), Funding Research Associate, 2002.
Our Lady of the Rosary Academy (Edgewater), Learning Resource Center Development Project Consultant, 2001-2003
University of Southern Colorado (Pueblo). Technology Integration and Curriculum Enhancement into Higher Education Learning Environment. Proposal Development Consultant, 2000.
Jefferson County Library System (Lakewood), 1999-2000.
Southeastern BOCES (Lamar, CO). Distance Learning Curriculum Content Development Project. Proposal Development Consultant, 1998-2004.
Cheyenne Mountain School District 12 (Colorado Springs, CO). Technology Integration into Elementary Writing Curriculum Project. Proposal Development Consultant, 1998.
Telecommunications Infrastructure Fund Board Round #2 Application, 1997. Technical assistance to the following: La Villa (Texas) Independent School District; Mirando City (Texas) Independent School District; Monte Alto (Texas) Independent School District; San Isidro (Texas) Independent School District.
Telecommunications Infrastructure Fund Board Round #1, 1996. Technical assistance to the following: Brownsville (Texas) Independent School District; Donna (Texas) Independent School District; Edinburg (Texas) Consolidated Independent School District; Harlingen (Texas) Consolidated Independent School District; Jim Hogg County Independent School District (Hebbronville, Texas); La Feria (Texas) Independent School District;

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La Joya (Texas) Independent School District; La Villa (Texas) Independent School District; Los Fresnos (Texas) Consolidated Independent School District; Progreso (Texas) Independent School District; Raymondville (Texas) Independent School District; Rio Hondo (Texas) Independent School District; San Benito (Texas) Independent School District; Sharyland Independent School District (Mission, Texas); Weslaco (Texas) Independent School District; Zapata (Texas) County Independent School District.

South Texas Community College (McAllen). Strategy Development to Meet the Technology Instructional Needs for Vocational, School-To-Work and Academic Programs, 1994-2000. Development Consultant.

Donna (Texas) Independent School District. Planning for the Infusion of Technology in Middle Schools Serving High At-Risk Students, 1994-1995. Research Associate.

Pettus (Texas) Independent School District. Planning for District-wide Automation in School Library Media Centers: Preparation of Data, and CD-ROM Hardware/Software Configuration Evaluation, 1994. Principal Investigator.

Fort Bend Independent School District (Sugar Land, TX). Planning for District-wide Automation in School Library Media Centers, Preparation of Data, and CD-ROM Hardware/Software Configuration Evaluation, 1994-1995. Principal Investigator.

Northern Waters Library Services (Ashland, WI). Professional Consulting Services to Make Recommendations on the Development of System-Wide Efforts of the Northern Waters Library Service, 1988-1989. Research Associate.

State Library of Iowa (Des Moines). The Iowa Locator Compact Disc to Support Multi-Type Libraries Resource Sharing including Database Design, Building, Production, and Distribution, 1986-1991. Project Director.

State Library of Iowa (Des Moines). Iowa Computer-Assisted Network Development, Enhancement, and Operation, 1985-1992. Technical Director.

State Library of South Dakota (Pierre). Statistical Sampling and Analysis of Multi-Institutional OCLC Archive Tape for Statewide Online Database Building, 1985-1986. Research Associate.

District of Columbia Public Library (Washington, DC). Planning Document for the Retrospective Conversion of Bibliographic Records and Automation Issues in the 1990's, 1984-1985. Research Associate.

State Library of Pennsylvania (Harrisburg). Database Development for High School Libraries, 1984-1985. Research Associate.

MINITEX (Minneapolis, MN). Workshops for Retrospective Conversion, Bar Coding, Library Statistics, and OCLC Serials Format, 1985. Presenter and Research Associate.

ABC Film Consortium (Altoona, Bellefonte, Johnstown, PA). Database Building, System Design, and Implementation of Online Film Booking System, 1983-1985. Database Manager.

Library Automation -- System Design & Implementation

Douglas County Library District (Castle Rock, CO). Library Automation Technical and System Performance Specifications, 2001.

Pharr-San Juan-Alamo Independent School District (Pharr, TX). Library Automation Technical and System Performance Specifications, 1997. Project Development Consultant.

Harlingen (Texas) Independent School District. Planning for District-wide Automation in Junior High School Library Media Centers: Preparation of Data, and CD-ROM Hardware/Software Configuration Evaluation, 1996. Principal Investigator.

Sharyland (Texas) Independent School District. Design and Construction of New Elementary School Library and Technology Resources Center, 1995-1996. Principal Investigator.

South Texas Independent School District (Mercedes, TX). Design and Construction of a New High School Library and Technology Resources Center, 1995-1996. Principal Investigator.

Citizens' Library (Washington, PA). Online Community Resources Files: Design and Implementation, 1984. Research Associate.

Altoona (Pennsylvania) Hospital. Integrated Online System Upgrade Study, 1984. Research Associate.

State Library of South Dakota (Pierre). Automation Plan and State Database Development, 1982-1983. Principal Investigator.

State Library of Pennsylvania (Harrisburg). COM Production and Preparation of the Technical Specifications Document, Procurement, and Distribution of Microcomputers in the Commonwealth of Pennsylvania, 1983-1984. Research Associate.

Altoona (Pennsylvania) Area Public Library. Integrated System (Circulation/Online Catalog) Study, 1982-1983. Principal Investigator.

COPSCAULD (Council of Pennsylvania State College and University Library Directors, Edinboro, PA). Design of Online Media Catalog, 1982. Principal Investigator.

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Erie (Pennsylvania) County Library System. Operations Research and Design for Automated Circulation, Hardware Upgrade and Re-Retrospective Conversion, 1982. Research Associate.
Michigan Library Consortium (Lansing, MI). Tape Management System Design and Implementation, 1982-1984. Research Associate.
State Library of Pennsylvania (Harrisburg). Preparation of the Technical Specifications Document, Procurement and Distribution of Microfiche Readers in the Commonwealth of Pennsylvania, 1982. Research Associate.
Community College of Allegheny County, Allegheny Campus (Pittsburgh, PA). Integrated Systems Study, 1981. Principal Investigator.
Community College of Allegheny County, South Campus (West Mifflin, PA). Retrospective Conversion Training Program for Handicapped Students, 1981. Technical Director.
Southern Tier Library System (Corning, NY). OCLC Acquisitions Subsystem Evaluation, 1981. Principal Investigator.
South Central Reference and Research Council (Ithaca, NY). South Central Regional Delivery System, 1980. Principal Investigator.
Southern Tier Library System (Corning, NY). Newspapers on Microfilm in the Chemung-Southern Tier Library System, 1979-1981. Principal Investigator.

Cataloging & Bibliographic Database Building

Challenges, Choices, and Images K-12 Charter School, Denver Public Schools, 2007.
Denver Medical Library, Inc., 2006-2010.
Ricks Gifted and Talented School Library, 2005-
American Humane Society (Englewood), 2004-2006.
Fisher Early Learning Center Library, 2004-
Colorado Community Based Research Network (Denver), 2004-
Boulder Valley School District (Boulder), 2002-2003.
Douglas Public Library District (Castle Rock), 2001-2002.

Bibliographic Center for Research, Inc. (Denver). Original Cataloging, 2001-2003 & 1992-1993. Professional Cataloger. Libraries include Arapahoe Library District (Centennial); Clarke College (Clarke, IA); Kaiser Permanente Center for Health Research (Portland, KS); Douglas County Library System (Carson City, NV); Grinnell College (Grinnell, IA); Kansas State University (Manhattan, KS); Kansas State Library (Topeka, KS); McPherson College (McPherson, KS); Newman University (Wichita, KS); Montana Technical University (Helena, MT); Montana State Library (Helena, MT); University of Texas Southwest Medical Center Library (Dallas, TX); Community College of Southern Nevada (Las Vegas, NV); Pikes Peak Library District (Colorado Springs); Mesa Community College (Grand Junction); Coe College (Cedar Rapids, IA); Briar Cliff College (Sioux City, IA); Tri-Care Health Systems (Aurora); The Penrose-St. Francis Healthcare System (Colorado Springs); Westminster Public Library (Westminster); Lutheran Medical Center Medical Library (Wheat Ridge); American Heritage Center, University of Wyoming (Laramie, WY); National Wildlife Research Center Library (Lakewood); University of Colorado Health Sciences Center, Denison Memorial Library (Denver); Water Resources Library, Denver District Office, Bureau of Land Management (Lakewood); Mesa State Community College Learning Resources Center (Pueblo); Front Range Community College Learning Resources Center (Westminster).

Colorado Historical Society Library (Denver). Original Cataloging of Serials for Multi-Institutional Union Listing Project, 1993. Technical Services Associate. Institutional partners: Denver Art Museum, Denver Museum of Natural History Library, and Denver Botanical Gardens Library.

Arapahoe Library District (Centennial). Original Cataloging of Major Media, 1991.

Original Cataloging Projects: Davis and Elkins College (Elkins, WV), 1989-1990; Waldorf College (Forest City, IA), 1989-1990; Kennametal Corporation (Latrobe, PA), 1984-1985; Pennsylvania Public Libraries Film Center (Harrisburg), 1982-1985.

Retrospective Conversion Projects: North Central Regional Library Service (Mason City, IA), 1987-1988; Southeastern Library Services (Davenport, IA), 1986-1987; Virginia Theological Seminary (Alexandria), 1984-1985; California University of Pennsylvania (California, PA), 1984-1985; Kennametal Corporation (Latrobe, PA), 1984-1985; West Virginia University (Morgantown), 1983-1985; Altoona Area Public Library (Altoona, PA), 1983-1984; George Washington University (Washington, DC), 1983-1984; Indiana University of Pennsylvania (Indiana, PA), 1983-1985; Health Education Center (Pittsburgh), 1983-1984; Central Pennsylvania District Library Center (Bellefonte, PA), 1982-1985; Duquesne University Law School Library (Pittsburgh), 1982-1983; Allegheny County Law Library (Pittsburgh), 1982-1984; Calgon Technical Information Center (Pittsburgh), 1982; Dow Corning Corporation, Technical Information Center (Midland, MI), 1982-1983; Tri-System Public Library Retrospective Conversion Project (Binghamton, Corning, and Ithaca, NY), 1978-1981, Technical Project Director.

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