Robotic Scripts, Methods, Reagents, and Devices for High Throughput Automated Production of Next Generation Sequencing Libraries

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ABSTRACT

Recent technological advances have greatly increased both the speed and throughput of genome and transcriptome sequencing. The pace of sequencing has further increased with target-enrichment and library barcoding techniques that allow multiplexing of sequencing runs. However, current manual methods for creating libraries do not scale well, limiting the practical investigation of large numbers of samples. To ease the library-creation bottleneck, we describe here a set of protocols, robotics scripts, bulk reagents and instrumentation developed to automate the production of up to 96 DNA fragment sequencing libraries at once.

Current protocols for creating sequencing libraries are lengthy, laborious, and not amenable to automation. We describe here new magnetic bead based methods that produce libraries with yield, purity, and size-selection comparable or superior to current column and gel-based protocols. Using these bead-based methods we developed a unique combination of optimized adaptor concentrations and clean-up techniques which increase the yield of libraries from small amounts of DNA by several fold. We also describe robotic scripts for producing 1-96 libraries simultaneously on two commonly used robotic platforms. These protocols accept 10-8000 ng of sheared DNA, calculate and dilute barcode adaptors for each library as necessary, automate all intermediate processing steps, and deliver purified libraries ready for amplification off-instrument. An additional script for post-PCR purification of the libraries is also provided. We further describe the Library Builder™ System, comprising a benchtop device and kits of plastic tips, tubes and sealed cartridges prefilled with reagents necessary for producing 1 to 13 DNA fragment libraries either with or without size selection. Our analysis of sequencing data shows that libraries produced by all of the above methods are free of excess adaptors which interfere with quantitation, are unbiased, of high complexity, and free from cross-contamination. The protocols herein are provided to the community for use or customization.

INTRODUCTION

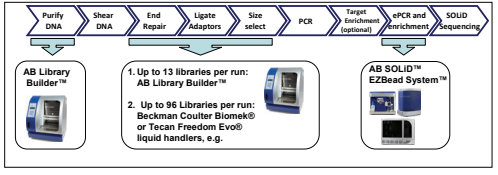
As high-throughput sequencing becomes more widely adopted in labs and clinics, there will be increasing demand for scalable techniques for creating robust sequencing libraries. Ultimately, the entire sequencing workflow will become completely automated, and significant steps have already been taken towards this goal (Fig 1.) Current manual protocols for creating SOLiD™ sequencing libraries contain several steps that are not amenable to automation (Fig. 3). We describe here a set of modifications using optimized adaptor concentrations and magnetic bead-based clean up and size selection techniques that can be automated for library creation. Creation of up to 13 libraries per run can be automated with the AB Library Builder™ System. For higher throughput needs, up to 96 libraries can be produced on existing liquid handling robotic systems using the scripts and protocols described herein.

MATERIALS AND METHODS

Libraries were created using reagents from the Applied Biosystems SOLiD™ Fragment Library Construction Kit (4443473 and 4443471) and adaptors from the SOLiD™ Fragment Library Oligo Kit (4401151) and the SOLiD™ Fragment Library Barcode kit 1-96 (4449637). E. coli DH10b genomic DNA was used for Beckman Coulter Genomics Agencourt® AMPure® XP magnetic beads were used for clean-up and size selection steps, following optimized protocols described herein. Applied Biosystems SOLiD™ Library TaqMan® Quantitation Kit (4449639) and Library qPCR Standard (A12126) were used for quantitating libraries. Emulsion PCR was performed on the SOLiD™ EZBead™ system, and templated beads sequenced on SOLiD™ 4 System. Bioinformatics analysis was done with BioScope™ Software and other tools.

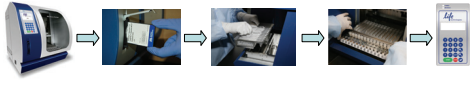
RESULTS

Figure 1. Automation in the SOLiD™ System Sequencing Workflow

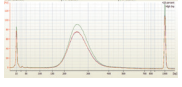


Top row: workflow from purifying DNA to obtaining SOLiD sequencing. Bottom row: available instruments for automating specific steps in the workflow. The Applied Biosystems Library Builder™ device can both purify DNA from various source materials, as well as automate library creation for up to 13 libraries, including end repair, size selection, ligation, and cleanup steps. For creation of up to 96 libraries at a time, the Tecan Freedom Evo® or Beckman Coulter Biomek® series of instruments can be used with protocols described in this poster. The Applied Biosystems SOLiD™ EZBead™ System automates all steps in the creation of templated beads for SOLiD™ System sequencing.

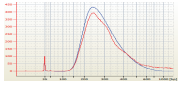
Figure 2. SOLiD™ DNA Fragment Library Construction with the AB Library Builder™ System

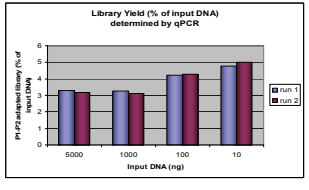


SOLiD DNA Fragment with Size Selection



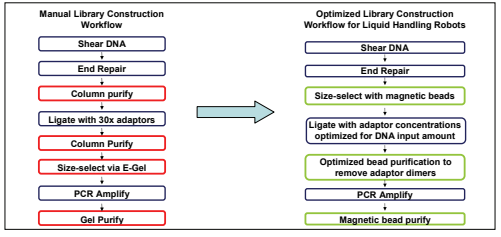
SOLiD DNA Fragment without Size Selection





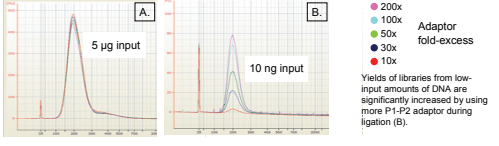
The AB Library Builder™ System comprises the Library Builder™ instrument and cartridge kits (top row) containing reagents for making SOLiD 4.0 or 5500 DNA Fragment Libraries. The Library Builder™ can automate creation of libraries either with size selection (middle row, left) for a tight size distribution, or without size selection (middle row, right) for a more broad size distribution. The Library Builder™ produces consistent high yields of libraries from 10 to 5000 ng of input DNA (bottom row, yields expressed as a percentage of input DNA).

Figure 3. DNA Fragment Library Construction Workflow Optimized for Liquid Handling Robots



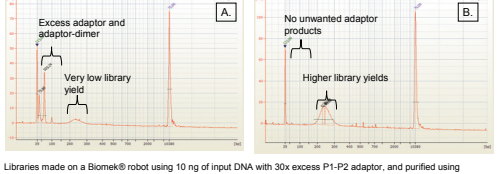
Steps in the existing workflow for creating SOLiD™ 4.0 libraries are shown in the column on the left. Steps that are difficult to automate are shown in red. Column on the right details the automated workflow incorporating optimized steps for size-selection of the library, increased yields for low-input libraries, and removal of adaptor-dimers.

Figure 4. Increasing adaptor concentrations increases library yields from very low DNA inputs



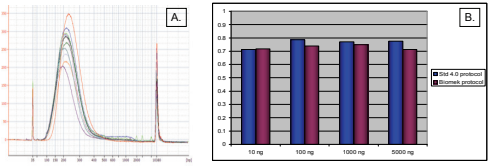
Yields of libraries from low-input amounts of DNA are significantly increased by using more P1-P2 adaptor during ligation (B).

Figure 5. Optimized workflow increases yield and purity of libraries made from low-input DNA



Libraries made on a Biomek® robot using 10 ng of input DNA with 30x excess P1-P2 adaptor, and purified using standard magnetic bead protocols have low yields of library and high contamination with adaptors and adaptor dimers [A, on left]. Libraries made on a Biomek® robot using 10 ng of input DNA with 200x excess P1-P2 adaptor, and purified with the optimized magnetic bead protocols have much higher library yields, and are free from adaptor or adaptor dimer contamination [B, on right]. Failure to completely remove adaptors and adaptor dimers can lead to incorrect quantitation of libraries by qPCR, contributing to uneven pooling of multiple libraries into one emulsion PCR.

Figure 6. Automated protocols produce consistent high quality sequencing libraries.



A. BioAnalyzer trace from 12 separate libraries produced from 1 ug DNA input. Libraries are consistently high yield and purity (Avg yield 0.46±0.17 pmol. No cross-contaminating libraries were detectable by qPCR in the plate, data not shown).

B. Libraries made by the automated protocols are of high sequencing complexity. Libraries were made from 10-5000 ng of DNA following either the manual SOLiD™ 4.0 protocol using columns, or using the Biomek® protocol. Data are starts/unique starts from a random subsample of ~2.7M unique starts.

CONCLUSIONS

We present here the AB Library Builder™ System for producing up to 13 SOLiD™ DNA Fragment libraries in an ~2.5 hour run from as little as 10 ng of input DNA. We also describe a set of optimized protocols for automating construction of up to 96 SOLiD™ 4 DNA Fragment libraries on Biomek® and Freedom EVO® robotic platforms, from as little as 10 ng of input DNA in ~3 hours. The optimized protocols are particularly effective at producing high yield libraries from low DNA inputs that are free from contaminating adaptors or adaptor dimers, which can skew qPCR quantitation. Robotic scripts are available upon request.

REFERENCES

1. Applied Biosystems SOLiD™ 4 System Library Preparation Guide.
2. Applied Biosystems SOLiD™ 4 System Standard and Barcoded Fragment Library Preparation Using the Beckman Coulter Biomek® FX/FX^P. AB Demonstrated Protocol and associated instrument scripts.
3. Applied Biosystems SOLiD™ 4 System Express Fragment Library Preparation Using the Tecan Freedom EVO® 75. AB Demonstrated Protocol and associated instrument scripts.

ACKNOWLEDGEMENTS

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For additional information on any of the library construction techniques or robotics scripts referenced herein, please contact your local Field Application Specialist.

TRADEMARKS