Methods and Reagents for Automating DNA Fragment Library Construction on Commonly Available Liquid Handling Robotic Platforms

John Bishop, Michael Allen, Karen Poulter, Adam Harris, Rob Bennett. Life Technologies, 5791 Van Allen Way, Carlsbad, California, USA, 92008

ABSTRACT

Techniques for producing sequencing libraries have not kept pace with recent advances in the ability to multiplex large numbers of barcoded or target-enriched libraries onto the latest high-throughput sequencing platforms. To support these high-throughput sequencing capabilities, we describe here a set of protocols, robotics scripts, and bulk reagents that have been developed to automate the production of up to 96 DNA fragment sequencing libraries at once.

Current manual protocols for creating DNA Fragment sequencing libraries require performing a series of sequential enzymatic steps on sheared DNA, punctuated by multiple column and gel-based buffer-exchange and size-selection steps which are not amenable to automation. We demonstrate here magnetic bead-based methods that replace column and gel-based DNA clean-up and size selection steps. These bead-based methods produce DNA yields, purity, and size-selection comparable or superior to typical manual results. We also describe a unique combination of optimized adaptor concentrations and clean-up techniques which increase the yield of libraries from low DNA inputs by several fold, while effectively removing excess adaptors which can skew library quantitation and pooling. We further describe automated scripts for producing 1-96 libraries simultaneously on two commonly used robotic fluidic-handling platforms. These protocols accept 10-8000 ng of sheared DNA input, calculate and perform dilutions of barcode adaptors for each library as necessary, automate all intermediate processing and cleanup steps, and depending on user-specification, deliver purified libraries either in buffer for quantitation, or in PCR mastermix ready for amplification off-instrument. We also provide an additional script for post-PCR purification of the libraries. Our analysis of sequencing data shows that libraries produced by automation are free of excess adaptors which interfere with quantitation and pooling, are unbiased, of high complexity, and free from cross-contamination. The protocols and robotics scripts are provided to the community for use or for further customization as necessary.

INTRODUCTION

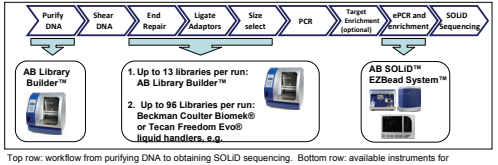
As high-throughput sequencing becomes more widely adopted in labs and clinics, there will be increasing demand for scalable techniques for creating robust sequencing libraries. Ultimately, the entire sequencing workflow will become completely automated, and signficant steps have already been taken towards this goal (Fig 1.) Current manual protocols for creating SOLiD™ sequencing libraries contain several steps that are not amenable to automation (Fig. 2). 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.

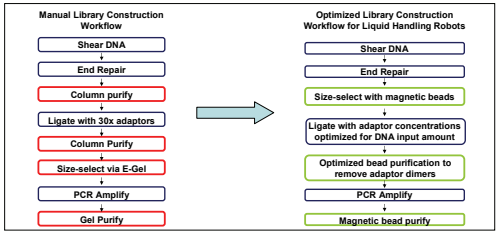
RESULTS

Figure 1. Automation in the SOLiD™ System Sequencing Workflow



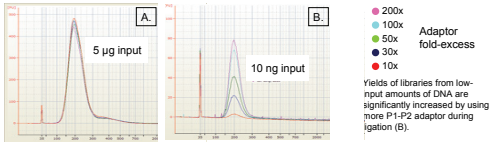
Top row: workflow from purifying DNA to obtaining SOLiD sequencing. Bottom row: available instruments for automating specific steps in the workflow. The Applied Biosystems Library Builder™ device can both purify DNA from various source materials, as well as automate library creation for up to 13 libraries, 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. DNA Fragment Library Construction Workflow Optimized for Liquid Handling Robots



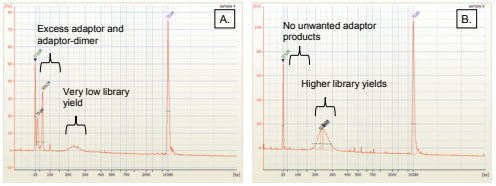
Steps in the existing workflow for creating SOLiD™ 4.0 libraries are shown in the column on the left. Steps that are difficult to automate are shown in red. Column on the right details the automated workflow incorporating optimized steps for size-selection of the library, increased yields for low-input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

Figure 3. Increasing adaptor concentrations increases library yields from very low DNA inputs



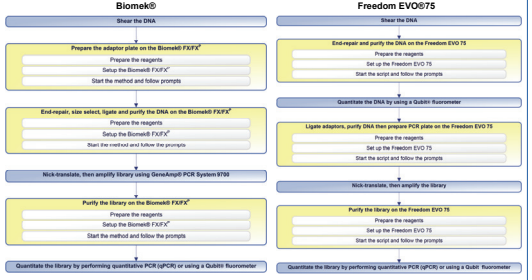
Yields of libraries from low-input amounts of DNA are significantly increased by using more P1-P2 adaptor during ligation (B).

Figure 4. Optimized workflow increases yield and purity of libraries made from low-input DNA



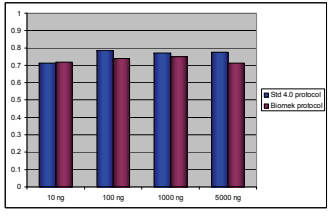
Libraries made on a Biomek® robot using 10 ng of input DNA with 30x excess P1-P2 adaptor, and purified using standard magnetic bead protocols have low yields of library and high contamination with adaptors and adaptor dimers [A, on left]. Libraries made on a Biomek® robot using 10 ng of input DNA with 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 5. SOLiD™ DNA Fragment library workflows for Biomek® and Freedom EVO® platforms



Steps in the robotics scripts for creating SOLiD™ DNA Fragment libraries on Biomek FX/FXP and Tecan Freedom EVO®75 platforms. Scripts and instructions can be obtained from your local SOLiD™ Field Application Specialist.

Figure 6. Library complexity



Libraries were made from 10-5000 ng of DNA following either the manual SOLiD™ 4 protocol using columns, or using the Biomek® protocol. Data are starts/unique starts from a random subsample of ~2.7M unique starts.

CONCLUSIONS

We show here a set of optimized protocols for automating construction of SOLiD™ 4 DNA Fragment libraries on Biomek® and Freedom EVO® robotic platforms. 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. Using the optimized protocols and robotics scripts, up to 96 libraries can be created in one ~ 3 hour run from 10-8000 ng of input DNA. 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

We acknowledge George Marnellos and Tony Xu for bioinformatics support, as well as Kamini Varma and Antje Taliana for support with robotics platforms.

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

TRADEMARKS