Roche GS FLX Titanium* Rapid Library Preparation Kit Automation Using Biomek® FXp Laboratory Automation Workstation

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ABSTRACT

Next-generation sequencing (NGS) revolutionized the field of genomic sequencing since 2005 due to its massively-parallel nature. However, many research labs, including most genome centers, still manually prepare NGS libraries using laborious and expensive process. Automating those processes could improve next-generation sequencing library preparation throughput, consistency and accuracy; and enable genomics research to address population-level questions and large-scale screening for DNA polymorphisms in the fields of medicine, evolutionary biology, environmental toxicology, and others.

This poster presents the automated Roche GS FLX Titanium* "Rapid Library Preparation Kit" using Beckman Coulter's Biomek* FXp dual hybrid laboratory automation workstation. The protocol can generate up to 96 MID-tagged libraries using standard Biomek ALPs, an on-deck thermocycler, a static cooling peltier ALP and an orbital shaking ALP for the process that can generate 96 libraries from sheared DNA samples takes approximately 3 hours on the Biomek FXp Workstation. Random sheared DNA (500 ng) from clonal isolates of *Daphnia pulex* provided by Indiana University were used to generate libraries for sequencing on the Roche/454 Life Science* platform. The DNA sequencing data generated from this model species for testing environmental water quality both from manual and automated processes are described and compared.

Note: The Biomek FXp is for Laboratory Use only; not for use in diagnostic procedures.
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INTRODUCTION

Library preparation is a major bottleneck in next generation sequencing (NGS) sample preparation due to the long and complicated process, which includes DNA fragmentation, size selection, enzymatic reactions and reaction cleanup steps. This long process can lead to not only significant DNA fragment loss but also limits the number of samples can be processed manually.

To improve the NGS library construction throughput, increase its process accuracy and reduce human error, the "Rapid Library Preparation Protocol" from Roche was automated on Beckman Coulter's Biomek FXp Laboratory Automation Workstation (**Figure 1**). The automated method features: on-deck temperature-controlled incubations, variable input sample number (1-96), and variable magnetic bead dry-time in a 96-well plate format. Reaction volumes were optimized to be automation-friendly eliminating small volume transfers. As a result, the automated Roche rapid library preparation method is simple to set-up, fast, and very precise in all steps. The time to process a full 96-well plate of samples is approximately 3 hours with as little as 500ng starting gDNA per library.

The resulting libraries were conveniently eluted into a half-area black flat bottom plate which can be used in (1) Library Quantitation using the Roche RL Standard on the DTX 880 Multimode Detector (excitation 485nm and emission 535nm, Beckman Coulter); and (2) Library Assessment using the Agilent 2100 Bioanalyzer with a high sensitivity DNA Chip (see **Figure 2,** and **Figure 3**).

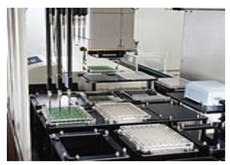
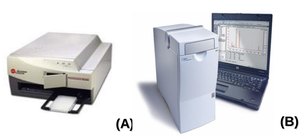


Figure 1: Biomek® FXp Laboratory Automation Workstation**:** Dual Arm System with Mulitchannel Pipettor and Span-8 Pipettor (Beckman, PN: A31844)



(A) (B)

Figure 2: NGS Rapid Library Quantitation (A) and Quality Assessment (B). **A**: DTX 880 Multimode Detector (Beckman, PN:987921); **B**: Agilent 20 Bioanalyzer (Agilent, GCA)2

REAGENTS & METHOD

Reagents

1. **GS FLX Titanium* Rapid Library Preparation Kit (Roche, 05608228001)**
2. **GS FLX Titanium* Rapid Library MID Adaptors Kit (Roche, 05619211001)**
3. **GS FLX Standard Nebulizers Kit (Roche, 05160570001)**
4. **Agencourt AMPure XP (Beckman, A63881)**
5. **Ethanol (200 Proof Absolute, Sigma, E7023-6x500mL)**
6. **UltraPure™ Distilled Water (GIBCO, 10977)**
7. **gDNA *Daphnia pulex* (500ng/Library, Indiana University)**
8. **Agilent High Sensitivity DNA Kit (Agilent, 506-4626)**

Method

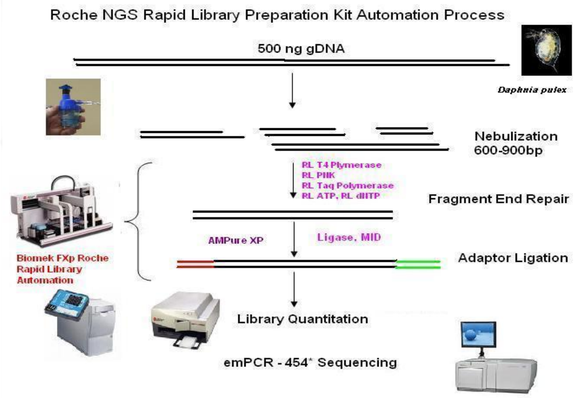


Figure 3. *GS FLX Titanium** Rapid Library Preparation Method (Roche)

The overall process for Roche Rapid Library Preparation Method is shown in Figure 3. The detailed steps are as follows:

Step 1. **DNA Fragmentation by Nebulization** (Manual process as directed by manufacturer), i.e.

DNA Sample: Add 500ng DNA in 1.7mLmicrocentrifuge tube was diluted with TE to a final volume of 100uL, which is transferred to a Nebulizer cup.

Nebulization: Add 500uL Nebulization buffer to cup and apply 30 psi (2.1 bar) of nitrogen for 1min; add 2.5mL PBI buffer.

Purification and Elution: Purify DNA by the MinElute PCR Purification Kit (Qiagen, Cat#:28006) eluting in 16uL TE buffer.

Step 2. **Biomek FXp Library Preparation Automation**

Library preparation occurs in a 96-well reaction plate with every library individually tagged by MID adaptors. The libraries from this automated process are ready to be emulsified using Roche *GS FLX Titanium** emPCR kits and sequenced on Roche Genome Sequencer FLX* System.

Deck Setup: Put Tips, Plate, Reagent Tube and Reagent Reservoir on deck as shown on "Instrument Setup" Steps (Figure 4B)

Tips: 4 P50 sterile tip boxes, 1 AP96 P250 sterile tip box, 1 Span-8 P250 sterile LLS tip box, 1 Span-8 P1000 barrier LLS sterile tip box

Plates and Lid: 1 MJ PCR Rxn Plates, 1 Rxn Plate Lid, 1 half-height Wash Plate, 1 half-area black flat bottom Elution Plate

Reagent Block and Reagent Reservoir: Put "end repair" reaction mix at Reagent Block (A1 Position); and add reagents to Reagent Reservoir as directed.

Adding reagents after bead preparation (Figure 4B): After AMPure Bead Preparation, a "Pause step" is provided to put a tube of diluted Ligase at Reagent Block (B1) and a plate of diluted MID tags on Deck (P5 Position).

Collect Eluates: At the end of the process, libraries (50 uL) were eluted in a black half-area plate and are ready for the library quantity and quality assessment process.

Step 3: **Library Quantitation** (Beckman, DTX 880 Multimode Detector, Manual)

RL Standard (50uL/well): Prepare RL standard dilution series in 8 tubes (Figure 5A), and aliquot 50uL of each concentration in duplicates into 96-well black half-area flat bottom plate. Scan the plate using DTX 880 multimode detector using 485nm excitation/535nm Emission to generate RL Standard (Figure 5B).

Sample Plate: Scan the elution plate for the library preparation process under the same conditions as above. Use this data and the standard curve equation to determine the concentration of the libraries.

Step 4: **Library Quality Assessment** (Bioanalyzer 2100, Manual): Analyze 1uL of Library from the library elution plate on the Bioanalyzer 2100. The qualified library shall be between 600bp and 900bp fragment length with <10% below 350bp.

Biomek® FXp Roche Rapid Library Preparation Method Deck Set-up

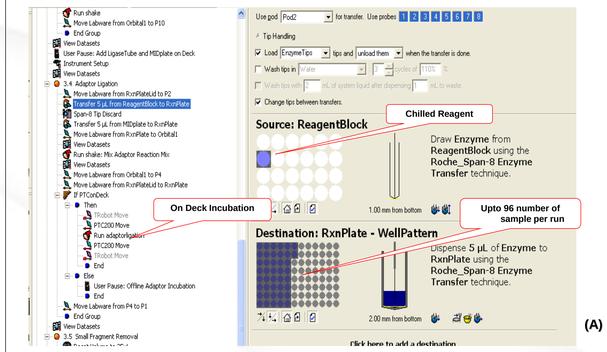
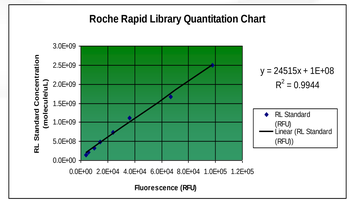


Figure 4. Biomek® FXp Roche Rapid Library Deck Set-up Method. Use WellPatern to choose the number of samples to be processed (A). Starting deck setup (B) and setup after adding fresh diluted Ligase tube in Reagent Block and diluted MID plate on deck (C).

Tube #	Dilution Factor	RL Standard (molIuL)
1	no dilution	2.50E+09
2	2/3rd dilutuion of Tube 1	1.67E+09
3	2/3rd dilutuion of Tube 2	1.11E+09
4	2/3rd dilutuion of Tube 3	7.41E+08
5	2/3rd dilutuion of Tube 4	4.94E+08
6	2/3rd dilutuion of Tube 5	3.29E+08
7	2/3rd dilutuion of Tube 6	2.19E+08
8	2/3rd dilutuion of Tube 7	1.46E+08

(A)



(B)

Figure 5: Roche RL Standard: Using Beckman Coulter's DTX 880 Multimode Detector**,** Roche RL Standard dilution series that prepared manually can be detected in the linear range (Excitation 485nm; Emission 535nm).

Hardware and Labware

Part No	Manufacture	Materials
Hardware		
A31844	Beckman Coulter	Biomek® FXP Laboratory Automation Workstation
719368	Beckman Coulter	Biomek® FXP, 200uL Multichannel Head
719356	Beckman Coulter	Biomek® FXP, Tip Loader
719654	Beckman Coulter	Biomek® NXP, FXP, Span-8 Wash
719948	Beckman Coulter	4x3 ALP
719357	Beckman Coulter	1X1 ALP
379448	Beckman Coulter	Orbital Shaker
969125	Inheco	Static Peltier Device
PTC-0200G	BioRad	PTC-200 DNA Engine Cycler (Single Block Model)
Labware		
717251	Beckman Coulter	AP96 P250uL, Sterile, 240/220uL
373688	Beckman Coulter	AP96 P1000uL Barrier Sterile LLS
A21582	Beckman Coulter	P50 Sterile (90uL)
394627	Beckman Coulter	P250 Sterile, Span-8, LLS (220uL)
372790,372795	Beckman Coulter	Modular Reservoir and Frame
609681	Beckman Coulter	BC Deep 96well Square
HSP9901	BioRad	MJ Hard Shell 96well Plate
3993	Corning Inc.	Half-area, Black, Flat Bottom
A83050	Beckman Coulter	Reagent Tube Block
A32782	Beckman Coulter	Agencourt® SPRIPlate®, 96R-Ring. Super Magnet Plate
AB-1127	Marsh Bio Production	Half-deep 96well plate

Acknowledgements: *Thanks Mary Blair, Amy Yoder, and Kelly Marshall for their valuable input during this method development.*

RESULTS

I. Roche Rapid Library Quantification Results

(A) Manual Libraries

Sample	Total Molecules	Molecules/µl
A	1.22E+11	2.45E+09
B	1.06E+11	2.12E+09
C	7.32E+10	1.46E+09
D	5.57E+10	1.11E+09
E	3.64E+10	7.28E+08
F	6.18E+10	1.24E+09
G	6.40E+10	1.28E+09

(B) Automated Libraries

Sample	Total Molecules	Molecules/µl
A	1.23E+11	2.47E+09
B	1.52E+11	3.04E+09
C	9.91E+10	1.98E+09
D	1.11E+11	2.22E+09
E	7.44E+10	1.49E+09
F	9.69E+10	1.94E+09
G	9.55E+10	1.91E+09

Figure 6: DNA Quantification of the manual and automated Roche Rapid Libraries using RL Standard (TBS380 Fluorometer). Mean recovery for automated libraries was slightly better (Difference of 3.326E+10, p < 0.05). Samples A & B of manual libraries were quantified separately.

II. Roche Library Quality Assessment Results

Red peaks are size standards

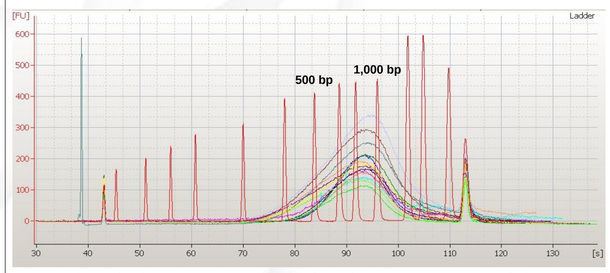


Figure 7: Size distributions of manual and automated Roche Rapid Libraries, measured by DNA high-sensitivity Agilent Bioanalyzer 2100 chips (Agilent). Automated libraries are significantly shorter (mean of 689 bp compared to 779 bp) and have a broader distribution (Stand. Dev. of 186 bp compared to 155 bp).

III. Roche Rapid Library Sequencing Results

(A) Amount of Sequences Obtained from Manual and Automated Libraries

	Manual	Automated
Number of Reads	990,295	996,686
Number of Filtered Reads	634,432	699,910
Average Read Length	391 bp	361 bp
Number of Bases (bp)	248,062,912	252,667,510

(B) Distribution of Data Among Manual and Automated Libraries

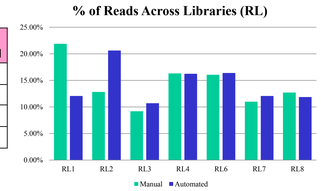


Figure 8: Sequencing results comparing (A) the total data produced from manual and automated libraries and (B) the distribution of the data across each of the 14 libraries. Although greater average read lengths were obtained from manual libraries, more reads were generated from the automated libraries

DISCUSSION & CONCLUSIONS

There are many factors that can impact the quality of libraries, therefore effect the quantity and quality of next generation sequencing results. Accurate enzyme reaction incubation condition, optimum magnetic beads drying, and precise washing steps to avoid magnetic beads loss are the top 3 factors among all others.

We compared the libraries that were made by our Biomek FXp automation method to the libraries made manually to discover that automated protocols produced sequencing data that were equal in quantify and quality to standard protocols. First, the recovered DNA, ready for sequencing, was proportionally equal across libraries but the yield was better using automation (Result I). The size distribution of DNA fragments all fit within acceptable boundaries. Yet a significant downward shift in the mean size of fragments and a broadening of the size distribution are seen from automation (Result II). However, there is only a minor impact on the average length of the reads (Result IIIA). Moreover, discounting the uneven representation of reads from the two first libraries – because these were quantified separately – we find very even fractions from each library in the sequence data (Result IIIB).

Refinements are underway to maximize sequence with sample throughput.

Disclaimer: The Biomek FXp Method Cited here is for laboratory use only; not for use in diagnostic purpose.