

Automated GS FLX Titanium emPCR Enrichment

using the REM e System on a Hamilton MICROLAB® STARlet Liquid Handler



INTRODUCTION

The Genome Sequencer FLX System is a versatile sequencing platform suitable for a wide range of research applications, including *de novo* sequencing and assembly of genomic DNA, transcriptome sequencing, small RNA analysis, and amplicon sequencing.

The Genome Sequencer FLX workflow consists of three primary processes: library preparation, emPCR amplification, and sequencing (Figure 1). The REM e System is a liquid handling accessory designed to fully automate the enrichment and sequence primer annealing steps of the GS FLX Titanium emPCR process. When integrated with a liquid handling platform, the REM e System dramatically simplifies the emPCR workflow by replacing 5 hours of dedicated manual lab work with an automated walk-away procedure.

A number of common liquid handlers have been tested for compatibility with the REM e System, including the Hamilton MICROLAB® STARlet liquid handler. Results from various samples and formats using the STARlet liquid handler, performed at the 454 Life Sciences Sequencing Center, demonstrate that together the systems achieve sequencing results comparable to those of manual emPCR enrichment. One such experiment is presented in this application note.

Note: All REM e System and GS FLX Titanium Manuals, Protocols and Quick Guides mentioned in this document are available to all 454 Sequencing customers on the my454 Site at www.454.com/my454.

GS FLX Titanium Workflow

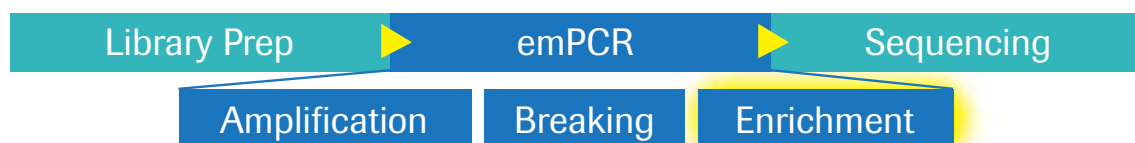


Figure 1. The REM e System is a liquid handler accessory designed to fully automate the enrichment and sequence primer annealing steps of the GS FLX Titanium Series emPCR process.

METHODS

The Hamilton MICROLAB STARlet liquid handler at the 454 Life Sciences Sequencing Center has been outfitted with the following components:

- (8) Hamilton 1000 µl independent pipet channels
- (1) 454 Life Sciences REM e System, Main Assembly, Cat No.: 05 976 120 001
- (1) Hamilton REM e System Tube Rack Carrier*
- (1) Hamilton REM e System Deck Module Carrier*
- (1) Hamilton 3x120 ml Reagent Carrier, RGT CAR 3R A01 carrier, PN 185290
- (3) Hamilton 120 ml Reagent Trough, Reagent Carrier 1T 120mL, PN 182703
- (1) Hamilton Tip Carrier, TIP CAR 480 A00, PN 182085
- (1) Hamilton 1000 µl CO-RE disposable pipet tips (96), conductive, non-filtered, PN 235904

*Hamilton part number forthcoming. Use part name when contacting Hamilton about these carriers.

The REM e System was installed using the configuration described in the REM e System Quick Install Guide.

The programming of the “Large Volume 4 Emulsion Cups per Run” method was performed as described in the REM e System Protocol document using Hamilton Vector 4.2.0.6425 software.

Large Volume emPCR amplification reactions were created for (4) GS FLX Titanium Rapid Libraries as described in the GS FLX Titanium Series emPCR Method Manual — Lib-L LV . Following thermocycling the LV emPCR method was broken using the protocol described in the GS FLX Titanium Series emPCR Method Manual — Lib-L LV up to step 13 of Section 3.5.3. At this point, the beads of each LV sample were combined into a single 50 ml conical tube and the volume of the sample was raised to 5 ml by the addition of Enhancing Fluid XT from the emPCR Kit (Cat No.: 05 233 542 001). These samples were then placed on the Hamilton STARlet as described in the REM e System User Manual and processed using the REM e System Protocol, LV, 4 Emulsion Cups per Run method.

RESULTS

After enrichment on the REM e System, the beads were counted on a Beckman Coulter Counter, following the manufacturer’s recommended protocol. The expected enrichment of 5-20% was achieved in all four samples. The beads were sequenced using a PicoTiterPlate device with a 4-Medium regions gasket, following the recommended protocol described in the GS FLX Titanium Series Sequencing Method Manual.

Name of Library	Emulsion Size	Final Volume (µL)	Bead Count	% Recovery	Bead Yield
Sample 1	LV	696	3,898	8	2,713,008
Sample 2	LV	746	3,952	8	2,948,192
Sample 3	LV	820	3,048	7	2,499,360
Sample 4	LV	800	3,960	9	3,168,000

Table 1. Enrichment results from four samples processed on the REM e System. The bead counts for all samples are approximately 35 million prior to enrichment. All samples achieved an enrichment between 5% and 20%, indicating that the samples are suitable for sequencing.

Region	Raw Wells	Keypass	Passed Filter	Avg. Read Length	Total Bases
		(% Raw Wells)	(% Keypass)		
Sample 1	423,043	421,750	312,480	398.42	124,499,665
		(99.69%)	(75.11%)		
Sample 2	417,639	416,499	285,115	397.05	113,203,662
		(99.73%)	(69.47%)		
Sample 3	420,444	419,269	302,955	395.92	119,945,258
		(99.72%)	(73.34%)		
Sample 4	411,832	410,428	283,726	394.07	111,807,987
		(99.66%)	(70.25%)		
ALL	1,672,958	1,667,946	1,184,276	396.41	469,456,572
		(99.70%)	(72.07%)		

Table 2. Sequencing results of samples processed using the REM e System for emPCR enrichment. Sequencing was performed with the GS FLX Titanium series chemistry on a PicoTiterPlate device using a 4-Medium regions gasket.

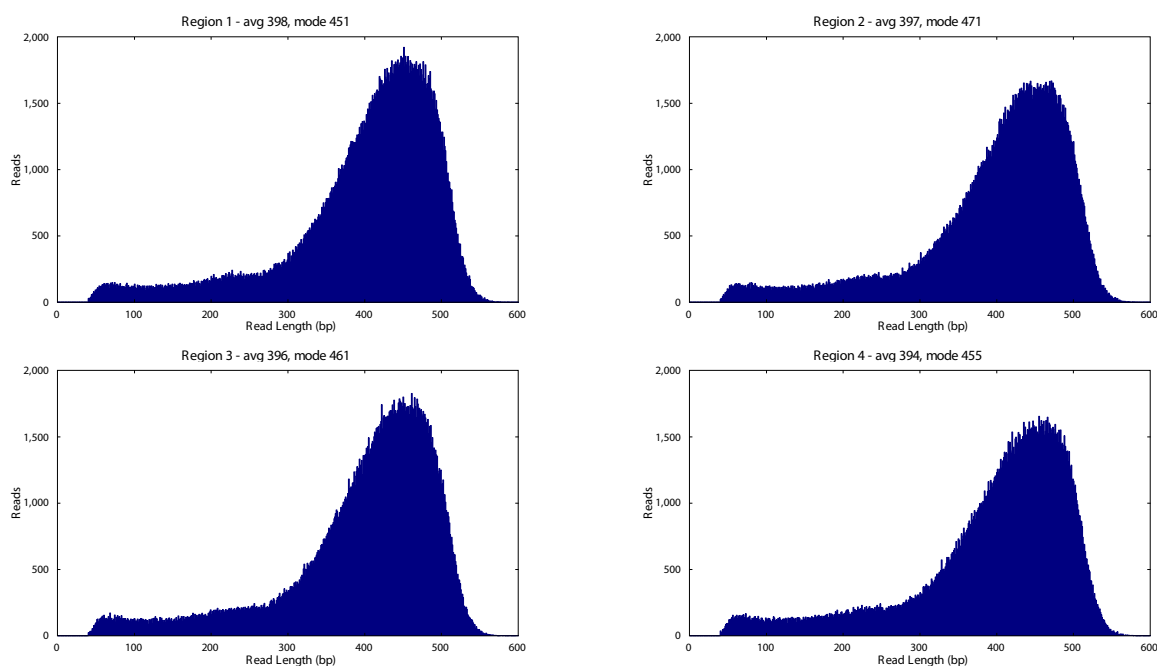
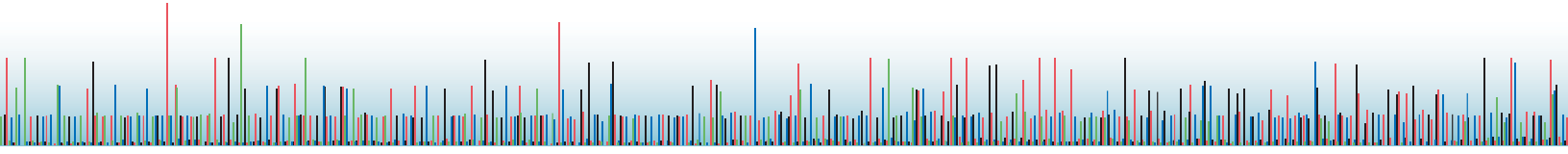


Figure 2. Read length distributions for the 4-Medium region gaskets of the GS FLX Titanium PicoTiterPlate device. All regions yielded acceptable read length, with a mode read length of approx. 450 bp.

CONCLUSIONS

The enrichment data along with the sequencing data confirms that the REM e System is capable of performing emPCR enrichment with results that are consistent with expectations. Importantly, the system significantly decreases the amount of hands-on time necessary to perform sequencing. In addition, the REM e System

helps prevent errors due to mispipetting. While the data displayed here is for a shotgun library sample using the GS FLX Titanium LV emPCR method, the system is compatible with all GS FLX Titanium library types and emulsion formats.



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Published by:
Roche Diagnostics GmbH
Roche Applied Science
Werk Penzberg
82372 Penzberg
Germany

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