

Simplifying Next Generation Sequencing Workflows

SPRIworks Fragment Library System II Fully Automated Fragment Library System for the Roche GS FLX* DNA Sequencer

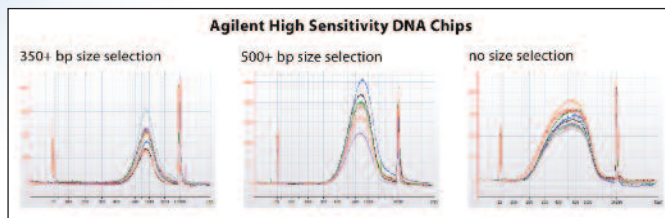
The emergence of the Roche 454 FLX next generation sequencing platform has enabled scientists to generate vastly increased data sets from their sequencing experiments. This dramatic increase in throughput, however, is supported by workflows that are relatively complicated and labor intensive, requiring skilled labor and significant hands-on time to prepare samples for each instrument. Beckman Coulter has created a simplified automated workflow for the leading second generation sequencing platforms. Utilizing Solid Phase Reversible Immobilization (SPRI) paramagnetic bead technology, the SPRIworks Fragment Library System II is an automated system for 454 library construction. Data highlights performance from multiple libraries prepared on the platform with only five minutes hands-on time.

Key Features:

- Easy to use bench-top liquid handling system
- Reagents pre-dispensed in a disposable cartridge
- Automated purification of fragmented or sheared DNA
- Optimized size selection chemistry – no more gels or columns
- Process up to 10 samples in parallel per system in under 4 hours
- Reproducible and consistent library output
- Reduced sequencing cost/sample
- Improved sequencing service turnaround time

Consistent and Reproducible Size Selection

Ten SPRIworks Fragment Library System II libraries were constructed with each of the size selection options (500+ bp, 350+ bp, and no size selection), using Roche RL MID adaptors and 500 ng sheared E.Coli DNA. Libraries were assayed with the Agilent High Sensitivity DNA Chip.



SPRIworks Fragment Library System II consistently performs the desired size selection range using SPRI magnetic particles on the SPRI-TE system without any user intervention.

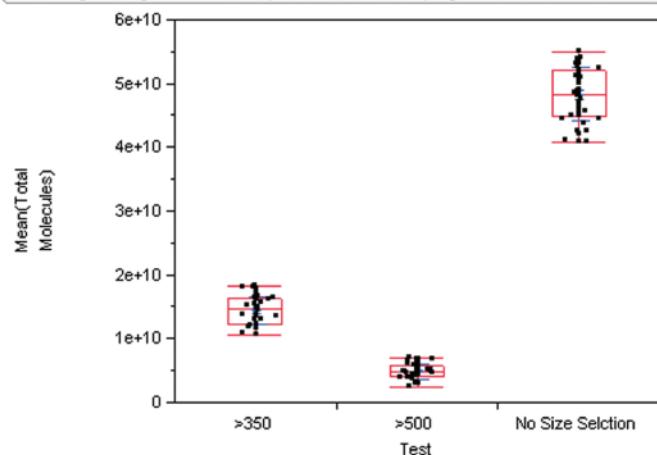


The SPRIworks Fragment Library System II has three components; The SPRI-TE Nucleic Acid Extractor, a method card that controls liquid handling, and a cartridge containing all the reagents required to prepare a single library.

Genomics
Proteomics
Cell Analysis
Particle Characterization
Centrifugation
Lab Automation
Bioseparation
Lab Tools

Consistent and Reproducible Library Yield

Oneway Analysis of Mean (Total Molecules) By Test



Means and Std Deviations

| Level | Number | Mean | Std Dev |
|-------------------|--------|-----------|-----------|
| >350 | 40 | 1.438e+10 | 2.2035e+9 |
| >500 | 40 | 4.9027e+9 | 1.1835e+9 |
| No Size Selection | 40 | 4.828e+10 | 4.2026e+9 |

SPRIworks Fragment Library System II consistently yields total molecules in the range of 5E9 to 5E10. Total molecules, as measured by KAPA Biosystems KAPA SYBR[®] FAST qPCR[®] kit, are confirmed as having correctly ligated adapters, and are therefore all usable in emPCR.

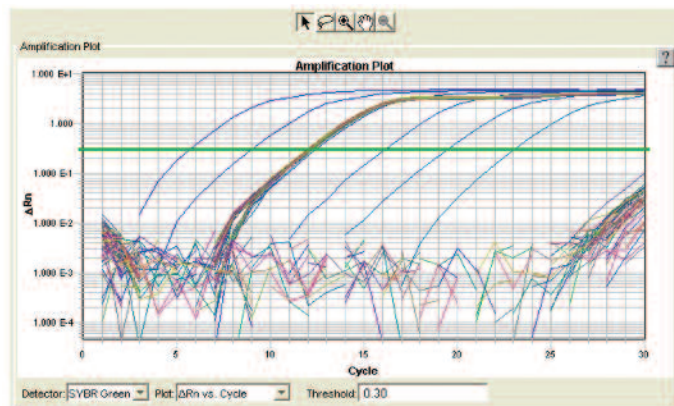
Performance

The libraries were then sequenced on the Roche GS FLX DNA Sequencer. Sequencing output and data quality metrics are shown in Table.

| | 350 + | 500 + |
|-------------------------------|-------------|-------------|
| # of Raw Wells | 1,087,101 | 1,064,381 |
| # Key Pass Wells | 1,059,999 | 1,033,016 |
| # of Filter Pass Wells | 772,005 | 751,678 |
| Total Bases | 285,991,291 | 275,159,393 |
| % Filter Pass | 72.83% | 72.77% |
| pct_dot failed | 5.42% | 4.62% |
| pct_mixed_failed | 3.53% | 3.53% |
| pct_trimmed_too_short_quality | 17.42% | 18.53% |
| pct_trimmed_too_short_primer | 0.79% | 0.55% |
| read_length_average | 370.51 | 366.2 |
| read_length_stdev | 110.76 | 120.42 |
| key_signal_per_base_average | 940.3 | 838.77 |
| Reads Assembled/Passing | 92.55% | 90.58% |
| Data Assembled | 257 Mb | 242 Mb |
| Reads Assembled | 714 k | 681 k |
| contigs | 90 | 83 |
| assembled length | 4.5 Mb | 4.5 Mb |

No Cross Contamination

Ten libraries were constructed using the SPRIworks Fragment Library System II over two instrument runs with the no size selection option and 500 ng of a PCR amplified Titanium library. In the first instrument run, the PCR product was input into the odd rows of the instrument and negative control was present in the even rows. In the second instrument run, the positions of the samples and negative controls were swapped. All method outputs were assayed with the KAPA Biosystems KAPA SYBR FAST qPCR kit.

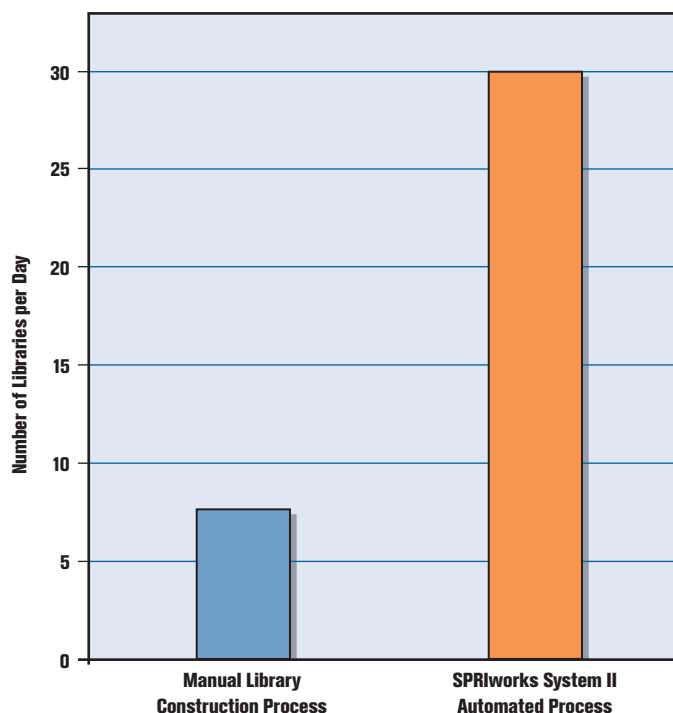


The samples amplified consistently at ~12 Ct units, while the negative controls are not detected by the assay within 30 Ct units. The SPRIworks Fragment Library System II constructs each library within a self-contained, pre-aliquoted reagent cartridge with isolated tips that never cross over other samples, thereby greatly reducing the possibility of cross-contamination between samples.

Maximizing Roche GS FLX Sequencing Workflow

On average, it would take a highly qualified scientist one day to prepare up to four libraries manually. The SPRIworks System II enables the user to prepare up to 30 libraries in a working day, a significant increase in throughput with a corresponding decrease in labor.

Throughput Comparison

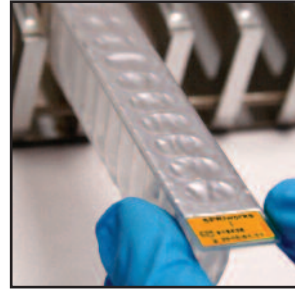
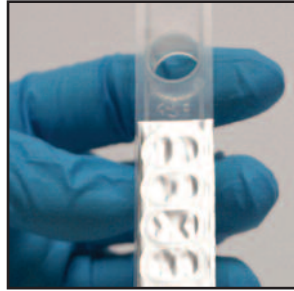


SPRIworks Fragment Library System II throughput vs. manual library construction process for Roche GS FLX DNA Sequencer.

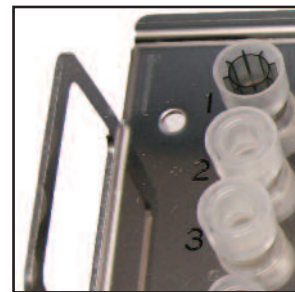
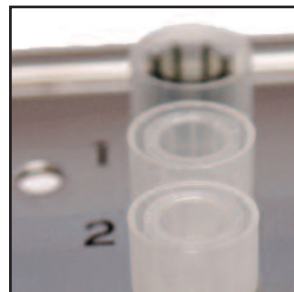
Four Simple and Easy Basic Setup Steps

The setup process to prepare up to 10 libraries takes less than 5 minutes. The SPRIworks end-user provides input DNA and adaptors. All other reagents and consumables are included in the SPRIworks Fragment Library Kit II.

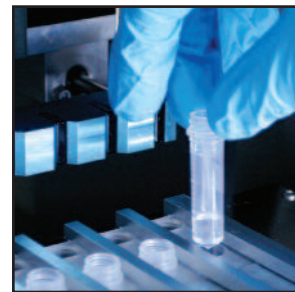
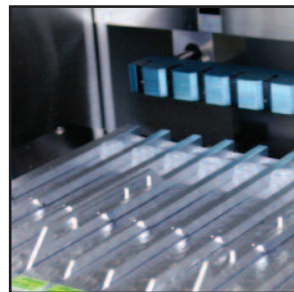
1 – Load SPRIworks Fragment Library Kit II Cartridge into the Rack



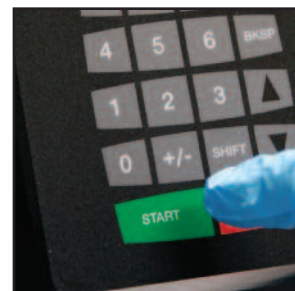
2 – Transfer Input Sample: Sheared DNA, cDNA or Amplicons



3 – Load SPRI-TE with Cartridges, Input Samples, Adaptors and Labware



4 – Insert Method Card, Select Size Range and Press START



Summary

SPRIworks System II automated library construction system enables users to prepare libraries for the Roche DNA Sequencer in under 4 hours with minimal hands on time. Up to ten samples per system can be processed in parallel with pre-dispensed reagents in SPRIworks cartridges. The finished libraries are ready for emPCR. SPRIworks systems simplify next generation sequencing workflows.

SPRIworks Fragment Library System II (For Roche GS FLX DNA Sequencer)



Ordering Information

In the United States and Canada call **+1 800 526 3821**

Product

SPRIworks Fragment Library System II (For Roche GS FLX DNA Sequencer)

Product #

A88268

Includes:

SPRI-TE instrument (A50100)

SPRI-TE Operator Manual

SPRIworks Accessories Kit (A88270)

Utility Card

SPRI-TE Operator Manual CD

SPRI-TE Step Feeler Gauge

Tubes and Tips

SPRIworks Fragment Library Kit II (For Roche GS FLX DNA Sequencer) (A84801)

10 cartridges for the preparation of 10 libraries

SPRIworks Nebulization Buffer

Tubes and Tips

SPRIworks Method Card Kit for Fragment Library System II (For Roche GS FLX DNA Sequencer) (A84869)

Instructions For Use

| Recurring Customer | Size | Product # |
|--|------|-----------|
| SPRIworks Fragment Library Kit II (For Roche GS FLX DNA Sequencer) | 10 | A84805 |
| 10 cartridges for the preparation of 10 libraries | | |
| SPRIworks Fragment Library Kit II (For Roche GS FLX DNA Sequencer) | 50 | A84806 |
| 50 cartridges for the preparation of 50 libraries | | |
| Accessories | Size | Product # |
| SPRIworks Nebulizer Kit | 10 | A84871 |
| 10 SPRIworks nebulizers each suitable for a single nebulization | | |
| SPRIworks Nebulizer Kit | 50 | A84872 |
| 50 SPRIworks nebulizers each suitable for a single nebulization | | |
| SPRIworks Method Card Kit for Fragment Library System II (For Roche GS FLX DNA Sequencer) | | A85410 |

† The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.

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