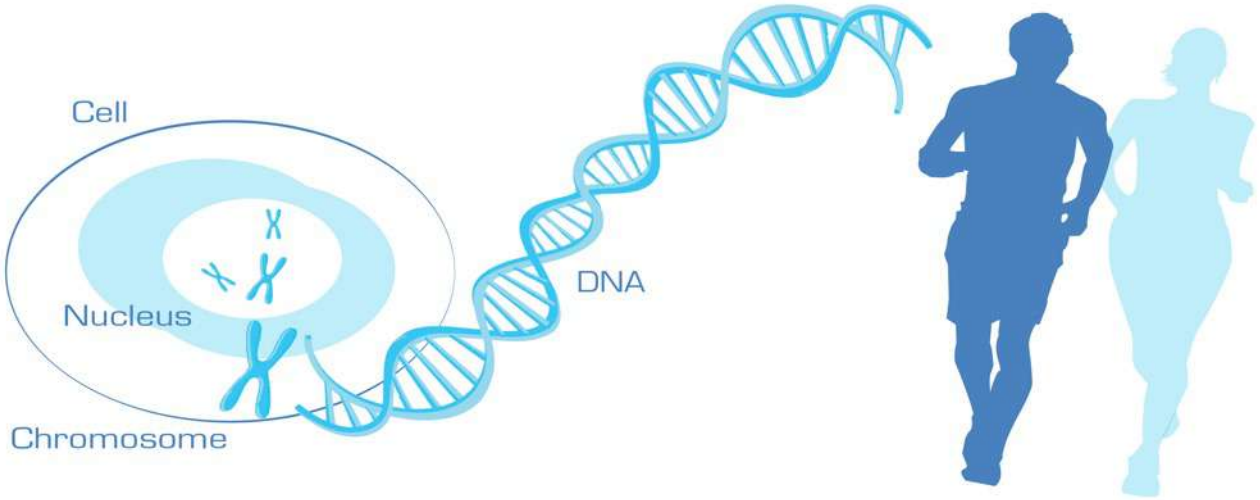
The RevoluGen vision is to deliver the purest form of unbroken DNA & revolutionize rapid diagnostics

Fire Flower

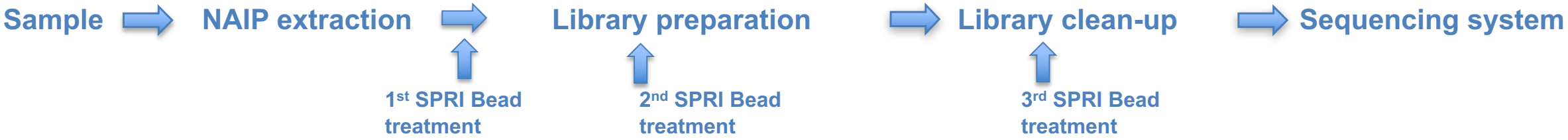
– Size selection clean-up removes small DNA fragments



17th November 2018

Yiaseme technology makes the Fire Flower size selection product – size selection of molecules is an essential part of the sequencing process

All genomic sequencing systems follow similar basic steps as illustrated below;



Solid Phase Reversible Immobilization (SPRI) beads selectively bind DNA fragments of various lengths. Typically SPRI beads are used at three points during the sample preparation process for sequencing as illustrated above and described below;

1 Firstly, just after NAIP extraction step in order to clean up much of the debris left after extraction,
2 secondly, after the fragmentation step during the Library preparation process to again remove much of the small DNA fragment debris, and
3 thirdly after the completed library preparation process in a library clean-up step.

RevoluGen is developing a library clean-up technology called YIASEME

Yiaseme is a clean up technology in the sense that it will remove most of the small DNA molecules below 10,000 base pairs in length. Yiaseme technology will be incorporated into the Fire Monkey and Fire Flower range of products.

Sequencing is greatly improved by the removal of much of the shorter DNA fragments because;

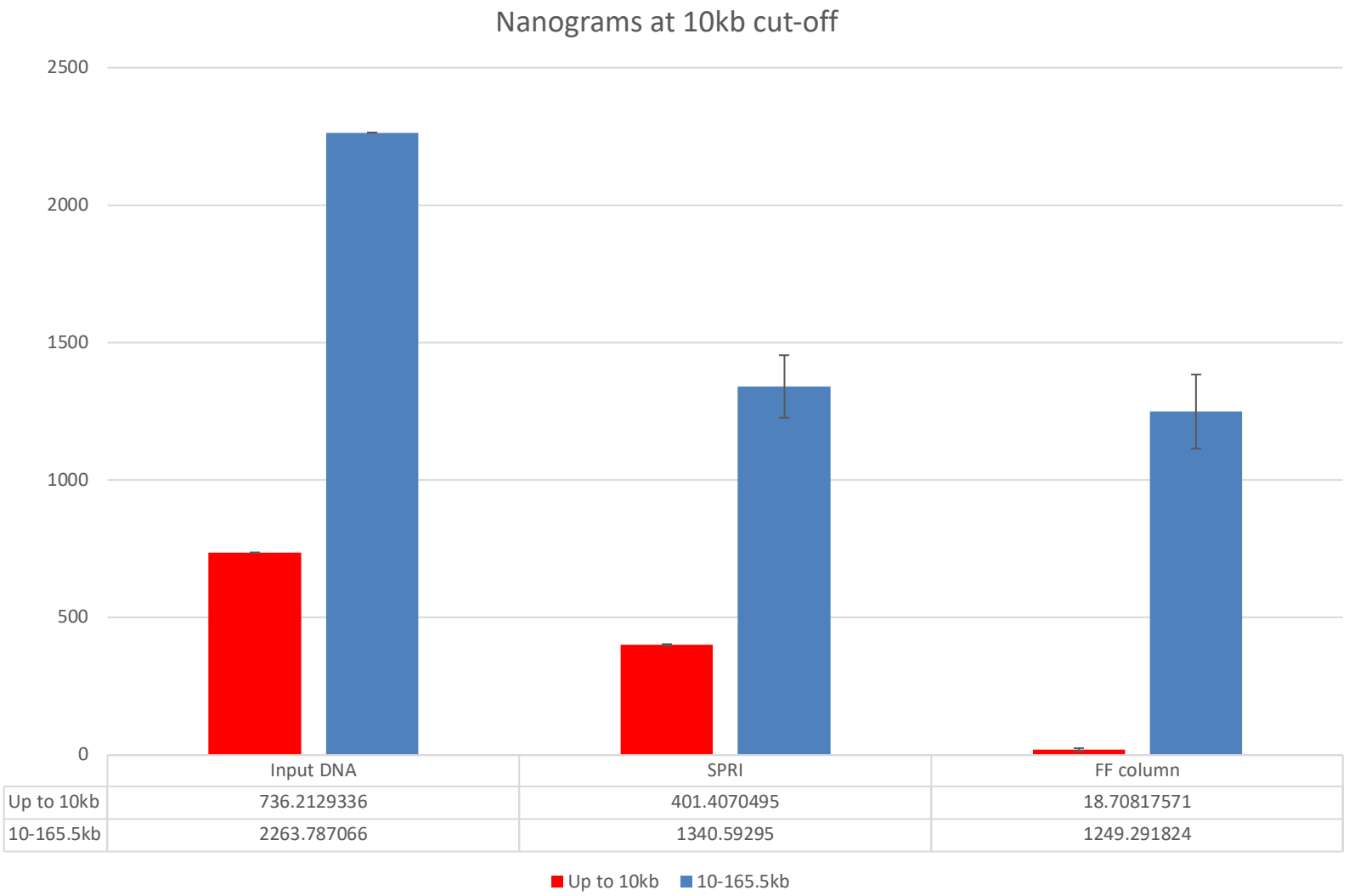
1 The very small DNA molecules such oligos and adapters that have a size of a few tens of bases interfere with accurate sequencing.
2 DNA molecules under 20kb are genomic DNA library fragments that will be preferentially sequenced over the larger molecules dropping the average read length.

The present solutions available are

1 SPRI beads (Beckman Coulter). They come in different formulations and are designed to deplete small molecules (~20 to 40bp) such as oligos/adapter by retaining larger fragments with a cut-off between 100bp to ~1kb and longer. They are relatively economical and protocols work within 10 to 20mins.
2 BluePippin (Sage Science) extraction which can deplete up to tens of kb, but it is a very expensive machine (~$15,000) and it takes hours to run (up to 16hrs*).

Fire Flower mass output comparison

FF works with all DNA samples made by all extraction processes and in all sequencing processes



	Input DNA	SPRI	FF column
Up to 10kb	736.2129336	401.4070495	18.70817571
10-165.5kb	2263.787066	1340.59295	1249.291824

Better mass of DNA output with FF.

In this experiment, a 3µg synthetic mixture of Low Molecular Weight (LMW) and High Molecular Weight (HMW) DNA was used as input for size-exclusion comparison between the 0.7x SPRI product and the Fire Flower (FF) protocol, in duplicate.

After size exclusion, Nanodrop and FEMTOpulse analysis of the DNA output were used to calculate the number of nanograms below and above 10kb.

Both SPRI and FF reduce the mass of over 10kb at a similar rate. However FF has a much greater efficiency in reducing the under 10kb mass than SPRI.

Fire Flower is a:

1. 10 minute spin-column process that depletes small DNA fragments much more efficiently than SPRI beads (which cut-off of 2kb vs 10kb)
2. Quick process that can be fitted in at the end of all NAIP kits

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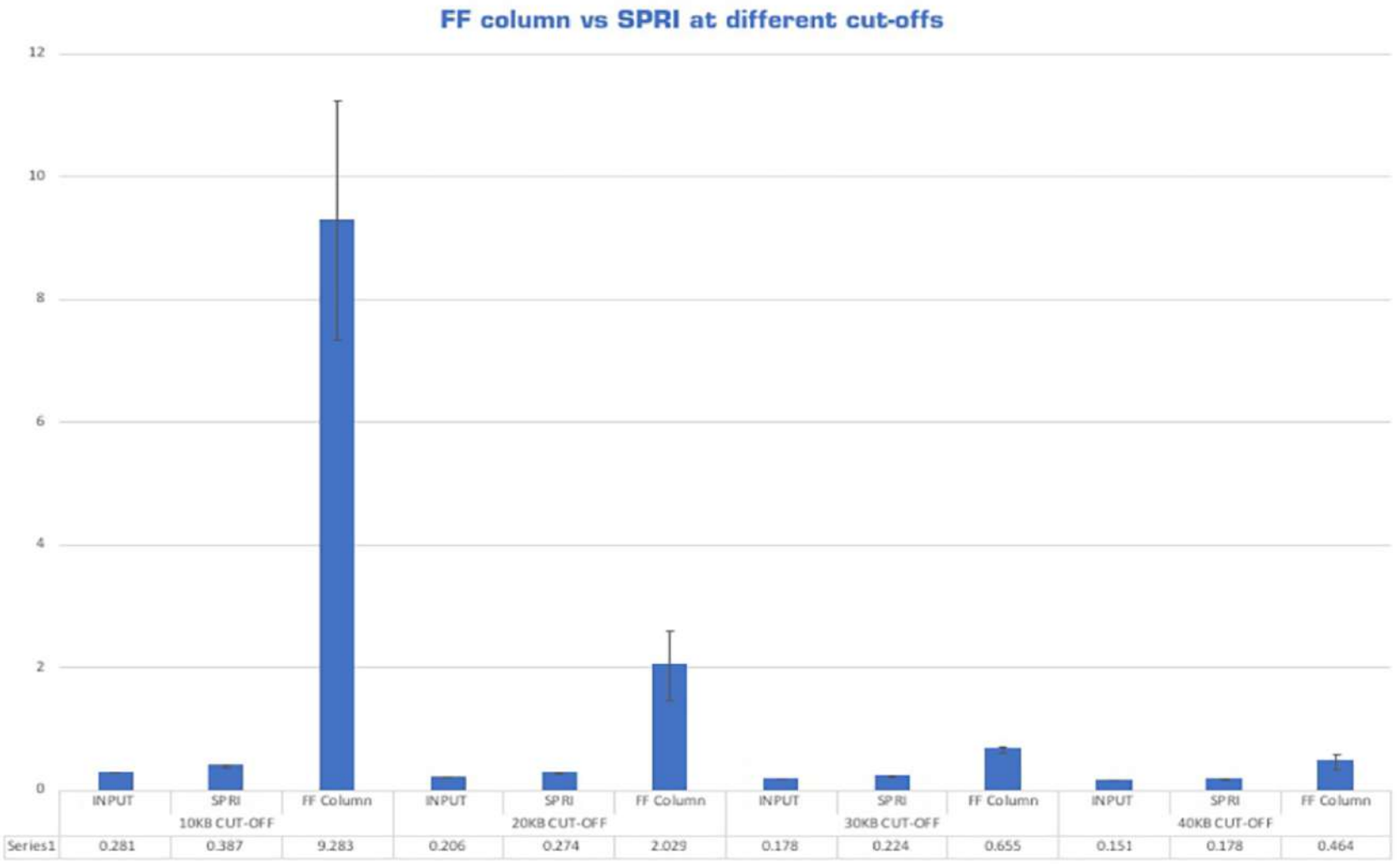
Fire Flower outperforms SPRI beads comprehensively in terms of number of molecules and the molecular ratio



The result in terms of number of molecules

A 3µg synthetic mixture of Low Molecular Weight (LMW) and High Molecular Weight (HMW) DNA was used as input for size-exclusion comparison between the 0.7x SPRI product and the Fire Flower (FF) protocol.

After size exclusion, FEMTOpulse analysis of the DNA output was measured and used to calculate the ratio of the number of molecules of long DNA to short DNA.

The ratio is just the number of long molecules divided by the number of short molecules. The cut off point is the DNA length at which the long/short DNA ratio was measured.

Long was defined as all molecules from cut-off up to 165.5kb and short was all molecules from 1.3kb up to cut-off.

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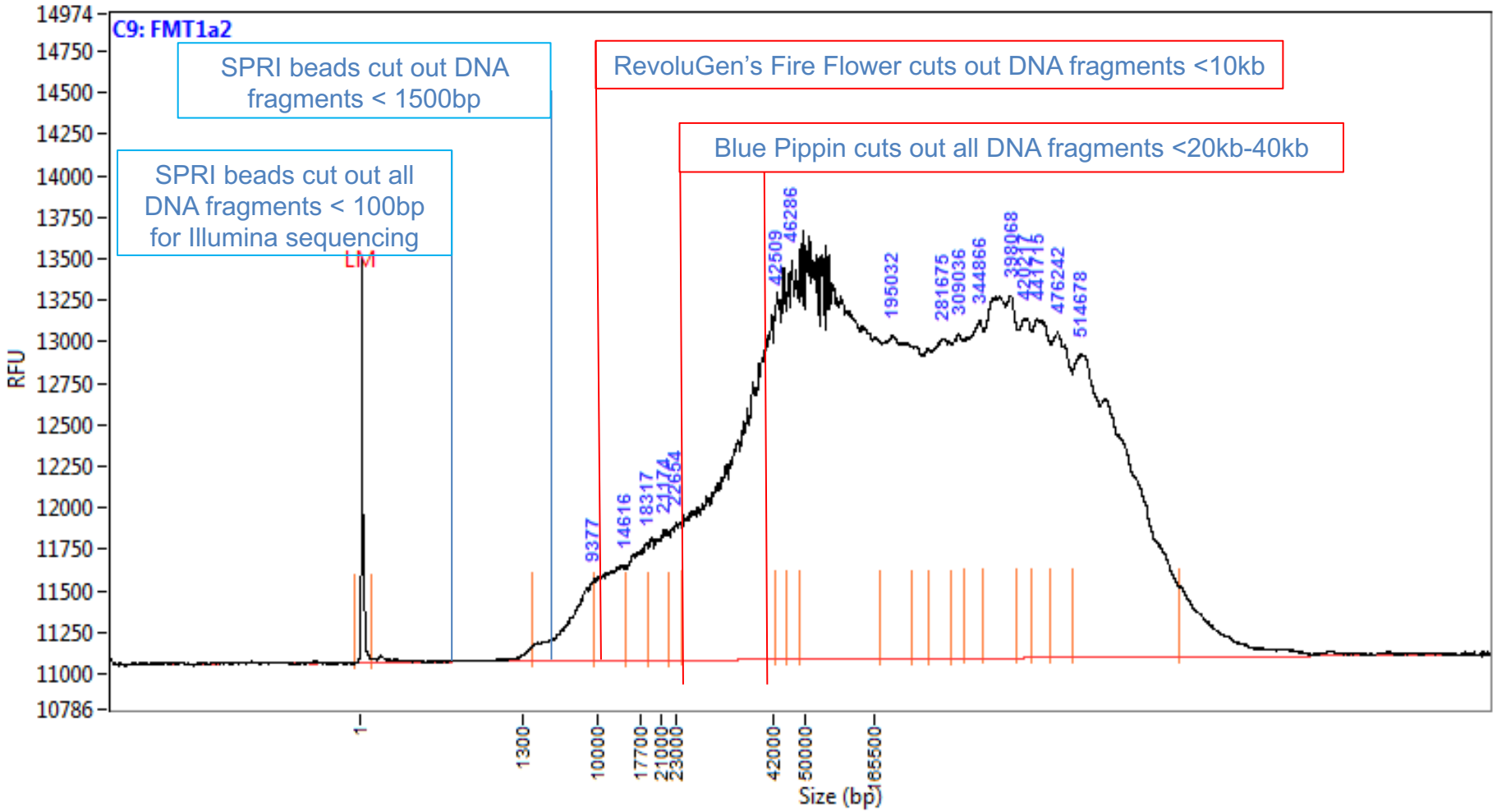
Fire Flower size selection clean up

Blue Pippin, SPRI beads & Fire Flower remove more smaller DNA fragments than larger ones



All genomic sequencing needs firstly a NAIP extraction process followed by a library preparation step which is then followed by a clean-up step prior to the actual sequencing step.

Library clean-up basically involves removing the short DNA fragments that block-up the actual sequencing process itself.

All sequencing processes have to use a library clean-up step.

Fire Flower has competitive advantages – offering simple & effective size selection - improving the sequencing results

Faster Speed

Fire Flower is a 10 minute procedure
SPRI Beads is a 20 minute procedure
Blue Pippin is a several hour procedure

More Ease of Use

Fire Monkey is a simple spin column based procedure that requires no special equipment above what is found in any molecular diagnostics research laboratory.

Higher useable Yield

All size selection techniques reduce DNA yield. Fire Flower produces higher usable DNA yield than the SPRI beads Blue Pippin is known to sometimes destroy whole samples

Universal application

Fire Flower is an independent process that can fit at the end of all NAIP processes from all types of samples and perform well for all sequencing systems.

Improved ratio of small / long DNA

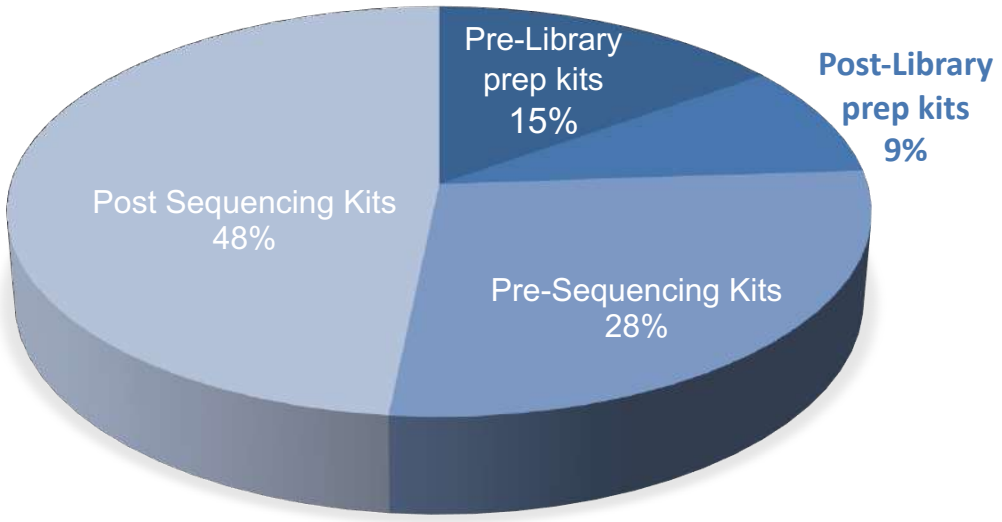
Fire Flower reduces all DNA to some extent but reduces the small DNA fragments in preference to the long DNA fragments. The ratio of the number of molecules of long DNA divided by the number of molecules of small DNA improves dramatically more than with SPRI beads.

Good Price Performance

Fire Flower is very competitive on a price performance basis Fire Flower is better, simpler and faster than SPRI beads. Fire Flower requires no capital equipment cost unlike Blue Pippin

Size Selection market is near $400m & Library clean up kit market is over a quarter of a billion dollars per year

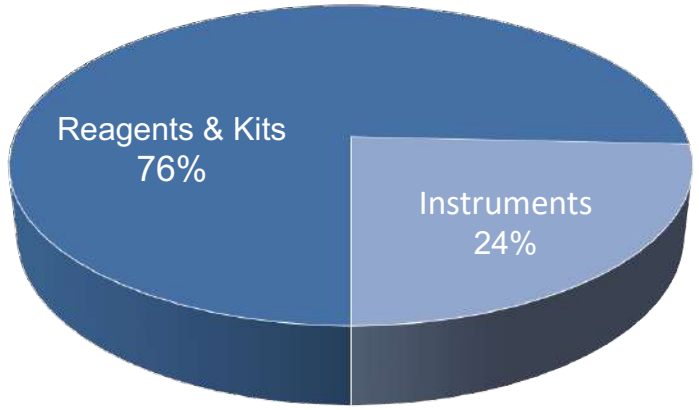
DNA Fragment Size Selection Market 2017 ($397m)



Sequencing procedures (x/year)	257m/year
Library Clean up Kits Market 2017 ($m)	$254m

With over a quarter of a million whole genome sequencing procedures carried out each year there is considerable scope for improvements in the size selection market and library clean up markets, neither of which is restricted to proprietary processes in the way that the actual library preparation market itself is. The companies selling automated sequencing machines generate the bulk of their revenue and profits from the sale of the specific library preparation kits each individual sequencing process requires.

NAIP Market 2017 by consumables / Instruments



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