SageELF and BluePippin Updates for Iso-Seq

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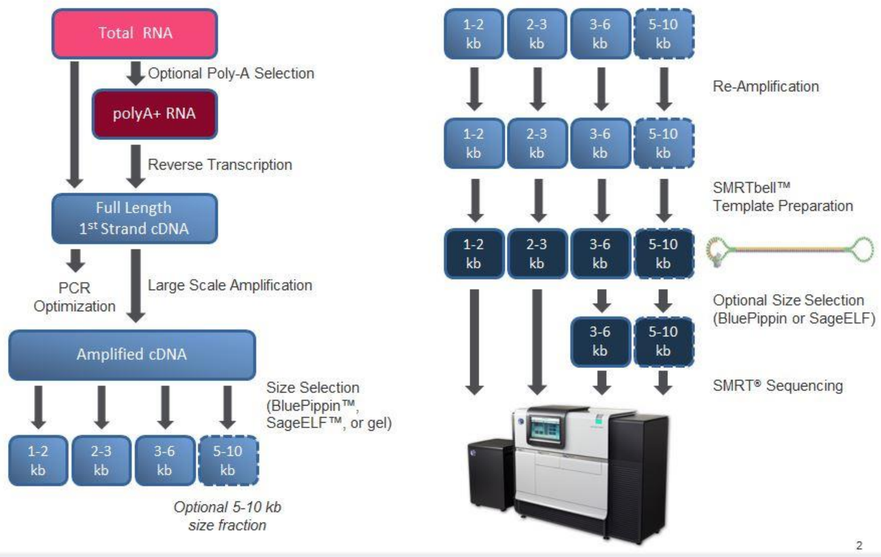
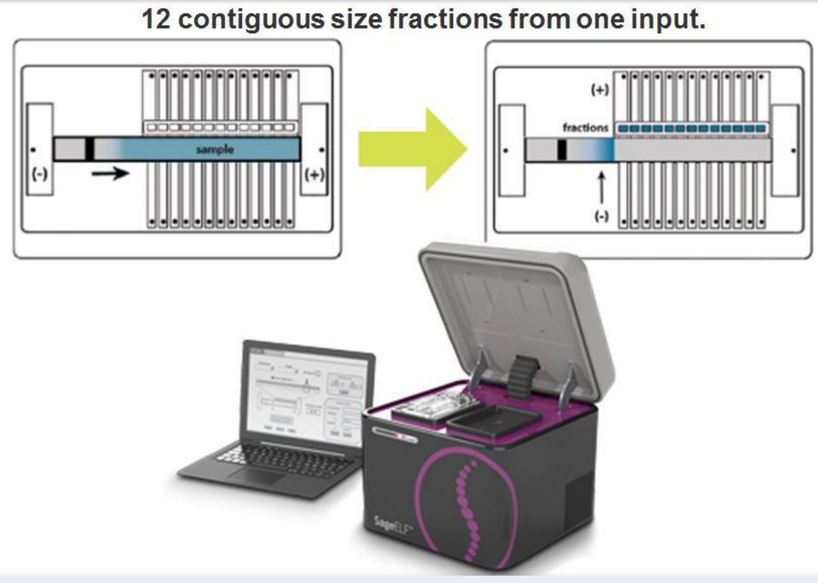
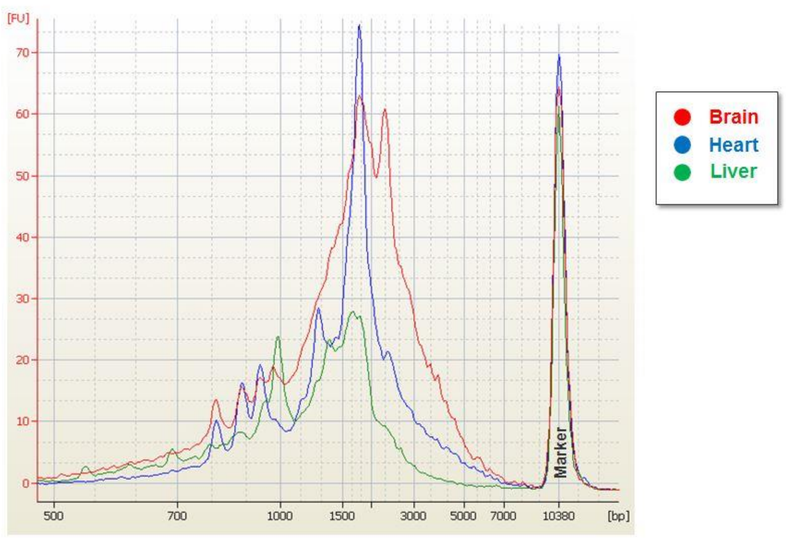
Detailed Clontech Workflow for Conversion of cDNA into SMRTbell™ Libraries



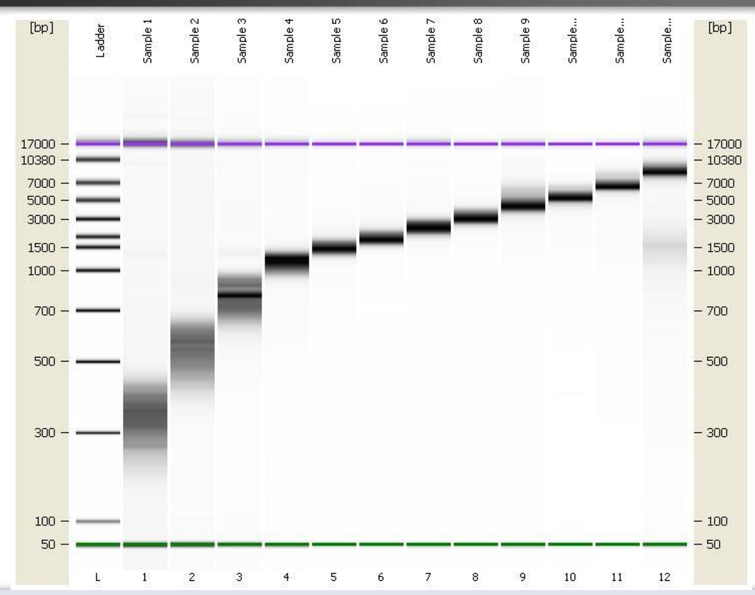
Size Fractionation with SageELF™ System



Size Distribution of Amplified cDNA from Multiple Tissues

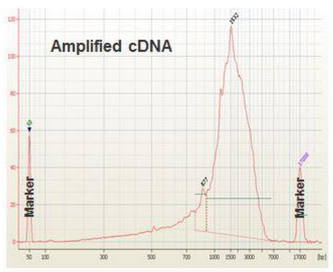


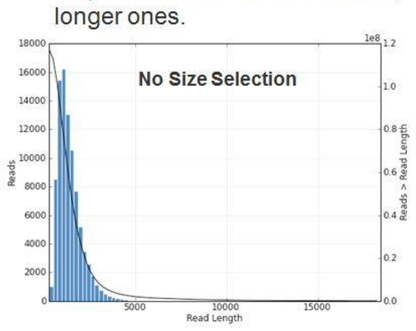
SageELF – 12 size bins (Amplified cDNA)

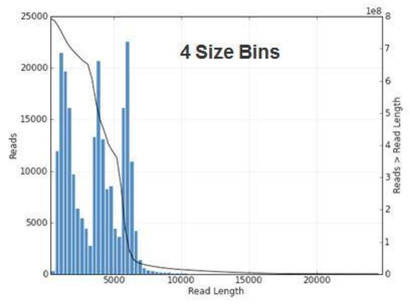


Issues with Size Selection

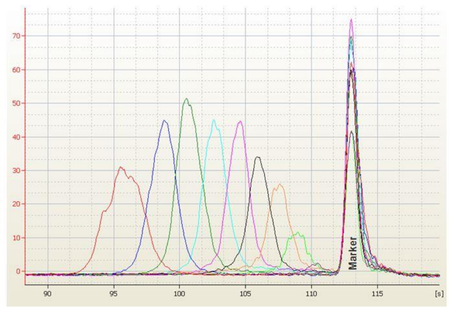
- Without Size Selection
  - Decreased sample prep time and effort
  - **Full-length sequences will be predominately from smaller transcripts**
- With Size Selection
  - Increased number of full-length mRNA sequences from all size classes, especially longer ones.







Amplified cDNA After Size Fractionation on SageELF System



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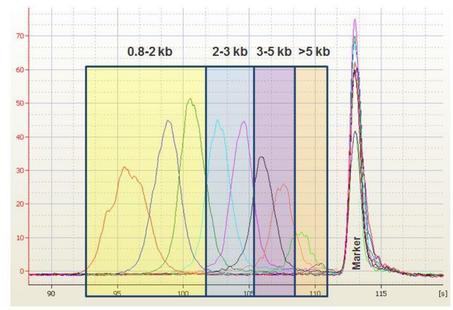

ELF	SIZE
11	1160
10	1650
9	1900
8	2400
7	2900
6	3700
5	5100
4	6050
3	7000

SageELF SMRTbell Libraries

Distribution of FL Reads from SageELF Fractionated Libraries

BluePippin™ Size Selection

Size Distribution of Size-selected SMRTbell™ Libraries

Size Fraction	BluePippin Settings	
	Start	Stop
1-2 kb	0.5 kb	2.0 kb
2-3 kb	1.5 kb	3.0 kb
3-6 kb	2.5 kb	6.0 kb
5-10 kb	4.5 kb	10.0 kb

SMRTbell Template Size Selection

3-6 kb SMRTbell Library Before BP Size Selection

3-6 kb SMRTbell Library After BP Size Selection

SMRTbell Template Size Selection

3-6 kb SMRTbell Library Before BP Size Selection

3-6 kb SMRTbell Library After BP Size Selection

[Slides from PacBio Iso-Seq webinar by T. Clark 2/11/15

For more info, see http://www.pacificbiosciences.com/applications/isoseq/]

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