IsoSeq Details

**Total RNA Input** to Clontech SMARTer cDNA synthesis – 500ng \* 3 reactions = 1.5ug

**Large Scale PCR for Size Selection on the SAGE Elf System** – 14 cycles

Kapa HiFi PCR Master Mix - 200ul

5’ PCR Primer IIA (12uM)- 25.6ul  
Diluted 1st strand cDNA- 80ul

H20 – 94.4ul

Divide into 8 tubes (50ul each)

**Sage ELF** Pool Fractions 5-7 (3-5kb), 8-9 (2-3kb), 10-12 (1-2kb)

**Large Scale PCR for SMRTbell Library Prep**

KAPA HiFi PCR Master Mix - 100ul

Pooled cDNA + H20 - 87.2ul

5’ PCR Primer IIA (12uM) - 12.8ul

Divide into 4 tubes (50ul each)

1-2kb (Fractions 10-12) - 10 cycles, extension 2min 30 seconds

2-3kb (Fractions 8-9) – 12 cycles, extension 3 min 30 seconds

3-5kb (Fractions 5-7) – 15 cycles, extension 5 min 30 seconds

**cDNA SMRTbell Template Prep Input**

1-2kb 500ng

2-3kb 1ug

3-5kb 1.5ug