

Cronn, Richard, Brian J. Knaus, Aaron Liston, Peter J. Maughan, Matthew Parks, John V. Syring, and Joshua Udall. 2012. Targeted enrichment strategies for next-generation plant biology. *American Journal of Botany* 99(2): 383-396.

**Appendix S3.** Estimated costs for targeted enrichment from 96 samples for different enrichment strategies.

**PCR-BASED ENRICHMENT, SHORT TARGETS (0.5 kb targets), DIRECT SEQUENCING**

**Assumptions:** (1) Sequencing uses Roche/454 Titanium chemistry for long reads to read two MID adapters; (2) 90% of sequence Reads on-target; (3) hole-filling and repeated reactions not included; (4) DNA sequencing includes cost of titration and sequencing run.

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>				
454/Roche Library titration = \$500	-	\$500				
AMPure beads (clean up)	-	\$100				
Barcoded (MID) adapters (assume 96-plex maximum)	-	\$700				
Gene-specific primers per locus = \$10 per pair	\$10	-				
DNA Sequencing costs (Roche/454 Titanium chemistry)	Variable: 1/8 PTP = \$2100; 1/2 PTP = \$5000					
PCR costs = \$0.80/sample (NEB Phusion; plastic etc)	\$0.80	\$77				
<b>Costs for 96 samples, different sized targets</b>	<b>prep costs</b>	<b>PCR costs</b>	<b>sequencing costs</b>	<b>oligo costs<sup>1</sup></b>	<b>total</b>	<b>per sample cost</b>
100 loci, 50 kb	\$50	\$7,680	\$2,600	\$1,000	\$11,330	118
300 loci, 150 kb	\$50	\$23,040	\$2,600	\$3,000	\$28,690	299
1000 loci, 0.5 Mb	\$50	\$84,480	\$2,600	\$10,000	\$97,130	1012
2000 loci, 1 Mb	\$50	\$153,600	\$2,600	\$20,000	\$176,250	1836

**Notes:**

<sup>1</sup> oligo costs assume small scale synthesis for gene-specific primers (e.g., 25 nmol), and larger synthesis for barcoded (MID) adapters (e.g., 100 nmol)

**PCR-BASED ENRICHMENT, SHORT TARGETS (0.5 kb targets), DIRECT SEQUENCING, AMPLIFICATION WITH 48.48 ACCESS ARRAY**

**Assumptions:** (1) Sequencing uses Roche/454 Titanium chemistry for long reads to read two MID adapters; (2) 90% of sequence reads on-target; (3) hole-filling and repeated reactions not included; (4) sequencing costs include cost of titration and sequencing run.

	<i>Cost/Unit</i>	<i>Cost/96</i>				
454/Roche Library titration = \$500	-	\$500				
AMPure beads (clean up)	-	\$100				
Barcoded (MID) adapters (assume 96-plex maximum)	-	\$700				
Gene-specific primers per locus = \$10 per pair	\$10	-				
DNA Sequencing costs (Roche/454 Titanium chemistry)	Variable: 1/8 PTP = \$2100; 1/2 PTP = \$5000					
PCR costs = \$15 NEB Phusion; \$325 Access Array; \$10 supplies	-	\$350				
<b>Costs for 96 samples, different sized targets</b>	<b>prep costs</b>	<b>PCR costs</b>	<b>sequencing costs</b>	<b>oligo costs<sup>1</sup></b>	<b>total</b>	<b>per sample cost</b>
100 loci, 50 kb (4 arrays)	\$50	\$1,400	\$2,600	\$1,000	\$5,050	53
300 loci, 150 kb (12 arrays)	\$50	\$4,200	\$2,600	\$3,000	\$9,850	103
1000 loci, 0.5 Mb (40 arrays)	\$50	\$14,000	\$2,600	\$10,000	\$26,650	278
2000 loci, 1 Mb (80 arrays)	\$50	\$28,000	\$2,600	\$20,000	\$50,650	528

**Notes:**

<sup>1</sup> oligo costs assume small scale synthesis for gene-specific primers (e.g., 25 nmol), and larger synthesis for barcoded (MID) adapters (e.g., 100 nmol)

**PCR-BASED ENRICHMENT, LONG TARGETS (5 kb targets), SEQUENCING FROM SHEARED LIBRARIES**

**Assumptions:** (1) sample preparation and sequencing reagents use Illumina GAllx chemistry; (2) 90% of reads are on target; (3) hole-filling and repeated reactions are not included; (4) shearing costs not included (assumes availability of sonicator or similar)

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>				
Illumina TruSeq V 48 sample preparation kit	\$2,600	\$5,200				
AMPure beads (clean up)	-	\$500				
Barcoded Adapters (assumes 48-plex)	\$70	\$6,720				
Gene-specific primers per locus = \$10 per pair	\$10	-				
DNA Sequencing costs (1 lane, GAllx, 1 x 100)	\$2,200	\$2,200				
PCR costs = \$1/sample (NEB Phusion; plastic etc)	\$1.00	\$96				
<b><i>Costs for 96 samples, different sized targets</i></b>	<b><i>prep costs</i></b>	<b><i>PCR costs</i></b>	<b><i>sequencing costs</i></b>	<b><i>primer costs<sup>1</sup></i></b>	<b><i>total</i></b>	<b><i>per sample cost</i></b>
10 loci, 50 kb	\$12,420	\$960	\$2,200	\$100	\$15,680	163
30 loci, 150 kb	\$12,420	\$2,880	\$2,200	\$300	\$17,800	185
100 loci, 0.5 Mb	\$12,420	\$9,600	\$2,200	\$1,000	\$25,220	263
200 loci, 1 Mb	\$12,420	\$19,200	\$2,200	\$2,000	\$35,820	373

**Notes:**

<sup>1</sup> oligo costs assume small scale synthesis for gene-specific primers (e.g., 25 nmol), and larger synthesis for barcoded adapters (100 nmol)

## HYBRIDIZATION-BASED ENRICHMENT

**Assumptions:** (1) 2 Mb synthesis of oligonucleotide probes; (2) hybridization reactions include two samples each; (3) sample preparation and sequencing reagents use Illumina HiSeq chemistry; (4) assumes 50% of reads derive from enriched target; (5) Desired sequencing depth per target is > 50X.

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>			
Illumina TruSeq V 48 sample preparation kit	\$2,600	\$5,200			
AMPure beads (clean up)	-	\$500			
Barcoded Adapters (assumes 48-plex)	\$70	\$3,360			
Oligonucleotide probes for 96 samples (duplex hybridization)	\$5,760 <sup>1</sup>	\$5,760			
DNA Sequencing (1 lane, HiSeq 2000, 1 x 101 bp)	\$3,000	\$3,000			
<b><i>Costs for 96 samples, different sized targets</i></b>	<b><i>prep costs</i></b>	<b><i>probe costs</i></b>	<b><i>sequencing costs</i></b>	<b><i>total cost</i></b>	<b><i>per sample cost</i></b>
100 loci, 50 kb	\$9,060	\$5,760	\$3,000	\$17,820	\$186
300 loci, 150 kb	\$9,060	\$5,760	\$3,000	\$17,820	\$186
1000 loci 0.5 Mb	\$9,060	\$5,760	\$3,000	\$17,820	\$186
2000 loci, 1 Mb <sup>2</sup>	\$9,060	\$5,760	\$3,000	\$17,820	\$186

**Notes:**

<sup>1</sup> probe costs are identical across different target sizes because 2Mb is the minimum synthesis size.

<sup>2</sup> At 50% of on-target specificity, 1 Mb targets translate to 2 Mb of sequence complexity/individual. If 50X depth is required, then this is 200 Mb total sequence per individual. For 96 individuals, this adds up to 9.6 Gb, which is within the capacity of one lane of the HiSeq 2000

**TRANSCRIPTOME-BASED ENRICHMENT (standard mRNA sequencing)**

**Assumptions:** (1) assumes 12-plex multiplexing; (2) that > 1 Mbp of transcriptome can be assembled from 12-plex sequencing reactions, which is reasonable for the HiSeq 2000; (3) Desired sequencing depth per target is > 50X.

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>		
Illumina TruSeq V mRNA Sequencing Kit	\$3,350	\$6,700		
AMPure beads (clean up)		\$500		
Barcoded Adapters (assumes 12-plex)	\$70	\$840		
DNA Sequencing (8 lanes, HiSeq 2000, 1 x 101bp)	\$3,000	\$24,000		
<b><i>Costs for 96 samples, different sized targets</i></b>	<b><i>prep costs<sup>1</sup></i></b>	<b><i>sequencing costs</i></b>	<b><i>total cost</i></b>	<b><i>per sample cost</i></b>
96 samples	\$8,040	\$24,000	\$32,040	\$334
300 loci, 150 kb	\$8,040	\$24,000	\$32,040	\$334
1000 loci 0.5 Mb	\$8,040	\$24,000	\$32,040	\$334
2000 loci, 1 Mb	\$8,040	\$24,000	\$32,040	\$334

**Notes:**

<sup>1</sup> Does not include cost of RNA isolation, which is typically higher than DNA isolation

### RAD-BASED ENRICHMENT

**Assumptions:** (1) one lane of Illumina sequencing, HiSeq 2000; (2) 96-plex sample multiplexing

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>		
Enzymes (Restriction enzyme; blunting enzymes; ligase; polymerase)	\$11	\$1,100		
AMPure beads or Quick-Spin (clean up)	-	\$100		
Barcoded Adapters (assumes 962-plex)	-	\$7,667		
DNA Sequencing (1 lane, HiSeq 2000, 1 x 101bp)	\$3,000	\$3,000		
<b>Costs for 96 samples</b>	<b>prep costs</b>	<b>sequencing costs</b>	<b>total cost</b>	<b>per sample cost</b>
96 samples	\$8,867	\$3,000	\$11,867	\$124

### GBS-BASED ENRICHMENT

**Assumptions:** (1) one lane of Illumina sequencing, HiSeq 2000; (2) 96-plex sample multiplexing

<i>Item</i>	<i>Cost/Unit</i>	<i>Cost/96</i>		
Enzymes (Restriction enzyme, ligase, polymerase)	\$3	\$280		
AMPure beads or Quick-Spin (clean up)	-	\$100		
Barcoded Adapters and oligonucleotides (assumes 96-plex)	-	\$500		
DNA Sequencing (1 lane, HiSeq 2000, 1 x 101bp)	\$3,000	\$3,000		
<b>Costs for 96 samples</b>	<b>prep costs</b>	<b>sequencing costs</b>	<b>total cost</b>	<b>per sample cost</b>
96 samples	\$880	\$3,000	\$3,880	\$40

**Note:** Prices are comparable with both GBS and GR-RSC