

Supplemental Data Table S2. Sequencing conditions and results of each optimization condition

Condition	Condition #1 (0.25% reference material)			Condition #2			Condition #3			Condition #4 (dilution VAF 0.125%)			
	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Expected VAF	Adaptor ×1	Adaptor ×2	Adaptor ×3	
Input DNA	300	300	100	300	300	300	300	300	300	300	300	300	
Pre-PCR cycles	8	8	8	6	6	6	6	6	6	6	6	6	
Post-PCR cycles	13	10	10	10	10	10	12	12	12	12	12	12	
PISeq QC Parameters													
Mapped reads	304,418,814	230,059,328	279,893,590	250,126,976	76,024,451	81,040,396	78,613,492	60,080,618	69,683,253	76,024,451	69,683,253	99,804,968	92,928,536
Mean depth, x	181,028	134,368	176,464	161,907	89,103	95,501	92,363	70,767	81,881	89,130	81,881	109,624	101,977
Duplicate (%)	96.0%	94.2%	97.7%	70.2%	91.5%	91.4%	91.5%	88.9%	90.6%	91.5%	90.6%	89.8%	91.1%
On target (%)	67.9%	66.3%	71.2%	70.2%	69.9%	69.5%	69.8%	70.1%	69.4%	69.9%	69.4%	64.3%	65.3%
Total number of molecular barcodes	4,548,699	4,678,736	2,701,291	8,604,644	2,477,941	2,836,163	2,671,283	2,666,453	2,684,426	2,477,941	2,684,426	3,739,511	2,871,491
Duplex matched molecular barcodes (%)	58.4%	60.6%	72.5%	50.0%	32.6%	38.0%	40.3%	29.0%	33.9%	32.6%	33.9%	59.6%	63.6%
Median number of duplicates per molecular barcode	20	14	36	10	9	9	9	7	9	9	9	8	10
Mutation													
<i>IDH2</i> c.515G>A	0.41%	0.40%	0.44%	0.29%	0.21%	ND	0.17%	0.16%	0.08%	0.16%	0.08%	0.19%	0.13%
<i>KIT</i> c.2447A>T	0.32%	0.29%	0.39%	0.30%	0.25%	0.13%	ND	0.22%	ND	ND	ND	0.16%	0.11%
<i>KRAS</i> c.34G>T	0.19%	0.14%	ND	0.16%	0.22%	0.13%	0.14%	0.38%	0.22%	0.16%	0.22%	0.12%	ND
<i>KRAS</i> c.35G>A	0.41%	0.40%	0.27%	0.32%	0.06%	0.09%	0.11%	0.22%	0.15%	0.06%	0.15%	0.08%	0.13%
<i>KRAS</i> c.183A>C	0.26%	0.31%	0.20%	0.31%	0.20%	0.05%	0.07%	0.39%	0.19%	0.06%	0.19%	0.13%	0.10%
<i>NRAS</i> c.182A>G	ND	0.09%	ND	0.32%	0.14%	0.09%	ND	0.44%	0.10%	0.08%	0.10%	0.06%	0.18%

Abbreviations: VAF, variant allele frequency; ND, not detected