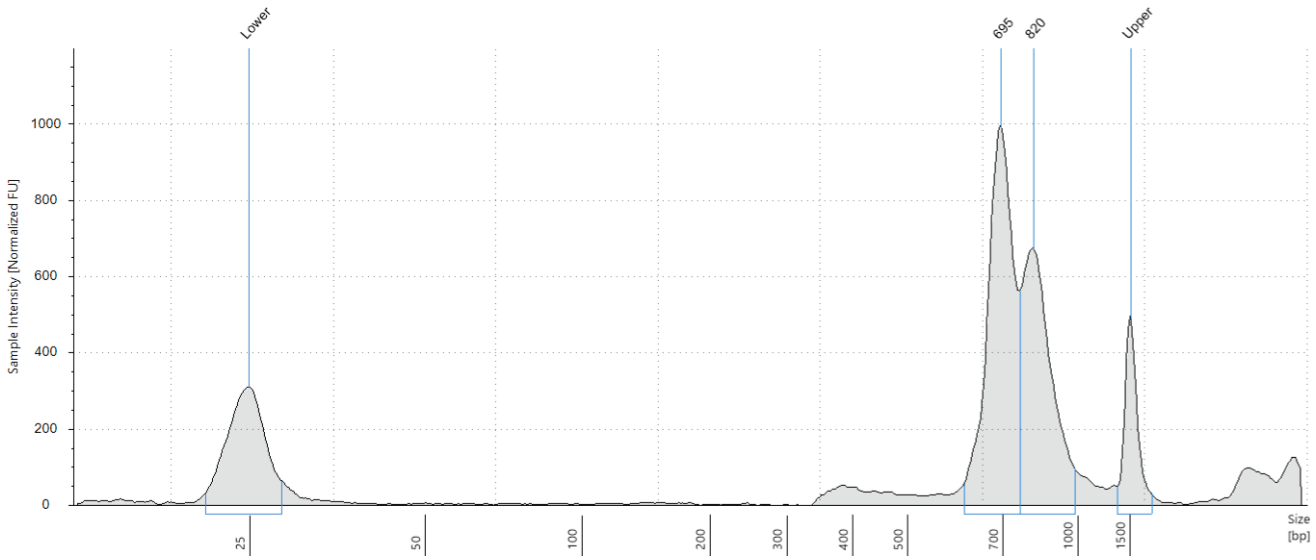
TCR Seq QC Report

SMARTER TCR a/b Profiling Kit



Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
F2	1890	control RNA-Kit		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment	Observations
25	485	-	29900	-		Lower Marker
695	1060	-	2340	56.11		
820	828	-	1550	43.89		
1500	250	250	256	-		Upper Marker

Figure 1: *a) Representative electrophoregram obtained from Tapestation from TCR a+b libraries generated using 10 ng of Jurkat total RNA from the SMARTer Human TCR Profiling Kit (Takara Bio USA Inc). Expected peak is 700-800 bp shows enrichment of TCR a/b transcripts. b) Table 1 shows results from the quantification of the libraries.*

Sample Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
TOTAL_READS	96194	100528	18	1960922	32506	7434832	4855764
TOTAL_DATA	28.86(Mb)	30.16(Mb)	0.01(Mb)	588.28(Mb)	9.75(Mb)	2.23(Gb)	1.46(Gb)
AVERAGE_READ_LENGTH	300	300	300	300	300	300	300
GC_PERCENTAGE	50.6	50.5	51.04	53.95	50.8	50.68	49.93
AVERAGE_BASE_QUALITY	33.91	32.73	30.48	33.44	33.78	33.35	33.77
TOTAL_DATA_MORE_THAN_Q30	83.22	78.94	71.72	81.52	82.69	81.4	82.82
TOTAL_READS_AFTER_PRE-PROCESSING	96104	99418	18	1951504	32428	7419352	4847816
TOTAL_DATA_AFTER_PRE-PROCESSING	25.78(Mb)	23.88(Mb)	0.00(Mb)	461.23(Mb)	8.57(Mb)	1.81(Gb)	1.27(Gb)
AVERAGE_READ_LENGTH_AFTER_PRE-PROCESSING	268.21	240.17	154.83	236.35	264.33	244.01	260.96
AVERAGE_BASE_QUALITY_AFTER_PRE-PROCESSING	34.51	34.65	36.94	35.28	34.54	35	34.65
TOTAL_DATA_MORE_THAN_Q30_AFTER_PRE-PROCESSING	85.46	86.08	96.23	88.53	85.53	87.6	86.08
Total sequencing reads	48052	49709	9	975752	16214	3709676	2423908
Successfully aligned reads	34934 (72.7%)	25709 (51.72%)	0 (0%)	215115 (22.05%)	11373 (70.14%)	1961435 (52.87%)	1907037 (78.68%)
Chimeras	3 (0.01%)	0	0	0	0	21(0%)	4 (0%)
Paired-end alignment conflicts eliminated	2605 (5.42%)	1768 (3.56%)	0	9946 (1.02%)	856 (5.28%)	131991(3.56%)	118268 (4.88%)
Alignment failed, no hits (not TCR/IG?)	3330 (6.93%)	5871(11.81%)	3 (33.33%)	205357 (21.05%)	1195 (7.37%)	439484 (11.85%)	121993 (5.03%)
Alignment failed because of absence of V hits	292 (0.61%)	186 (0.37%)	0	5959 (0.61%)	79 (0.49%)	12885 (0.35%)	3283 (0.14%)
Alignment failed because of absence of J hits	8972 (18.67%)	17354 (34.91%)	6 (66.67%)	528327 (54.15%)	3410 (21.03%)	1252992 (33.78%)	380173 (15.68%)
No target with both V and J alignments	148 (0.31%)	82 (0.16%)	0	2871(0.29%)	41(0.25%)	6634 (0.18%)	1875 (0.08%)
Alignment failed because of low total score	376 (0.78%)	507 (1.02%)	0	18123 (1.86%)	116 (0.72%)	36246 (0.98%)	9547 (0.39%)
Overlapped	42024 (87.46%)	38148 (76.74%)	9 (100%)	916814 (93.96%)	14090 (86.9%)	3321785 (89.54%)	2018687 (83.28%)
Overlapped and aligned	30042 (62.52%)	15786 (31.76%)	0 (0%)	183812 (18.84%)	9558 (58.95%)	1631355 (43.98%)	1521278 (62.76%)
Alignment-aided overlaps	1595 (5.31%)	3547 (22.47%)	0 (锟?)	10177 (5.54%)	515 (5.39%)	111321(6.82%)	68126 (4.48%)
Overlapped and not aligned	11982 (24.94%)	22362 (44.99%)	9 (100%)	733002 (75.12%)	4532 (27.95%)	1690430 (45.57%)	497409 (20.52%)
V gene chimeras	5 (0.01%)	122 (0.25%)	0	23 (0%)	1(0.01%)	610 (0.02%)	403 (0.02%)
J gene chimeras	6 (0.01%)	0	0	85 (0.01%)	0	161(0%)	56 (0%)
TRA chains	24360 (69.73%)	16980 (66.05%)	0	99900 (46.44%)	9270 (81.51%)	1313407 (66.96%)	1364794 (71.57%)
TRB chains	10481(30%)	8624 (33.54%)	0	107870 (50.15%)	2073 (18.23%)	637608 (32.51%)	540312 (28.33%)
TRD chains	1(0%)	0	0	5 (0%)	0	73 (0%)	18 (0%)
TRG chains	0	0	0	4 (0%)	0	12 (0%)	2 (0%)
IGH chains	1(0%)	3 (0.01%)	0	34 (0.02%)	0	92 (0%)	16 (0%)
IGK chains	0	1(0%)	0	7 (0%)	0	118 (0.01%)	18 (0%)
IGL chains	88 (0.25%)	101(0.39%)	0	7295 (3.39%)	30 (0.26%)	10104 (0.52%)	1872 (0.1%)
Final clonotype count	4628	1372	0	4231	1900	7254	3998
Average number of reads per clonotype	6.98	16.73	锟?34.67	5.45	237.77	318.78	0
Reads used in clonotypes, percent of total	32315 (67.25%)	22954 (46.18%)	0 (0%)	146688 (15.03%)	10352 (63.85%)	1724785 (46.49%)	1274466 (52.58%)
Reads used in clonotypes before clustering, percent of total	33200 (69.09%)	24010 (48.3%)	0 (0%)	152393 (15.62%)	10799 (66.6%)	1825907 (49.22%)	1353727 (55.85%)
Number of reads used as a core, percent of used	32824 (98.87%)	23728 (98.83%)	0 (锟?)	150368 (98.67%)	10716 (99.23%)	1813907 (99.34%)	1342656 (99.18%)
Mapped low quality reads, percent of used	376 (1.13%)	282 (1.17%)	0 (锟?)	2025 (1.33%)	83 (0.77%)	12000 (0.66%)	11071(0.82%)
Reads clustered in PCR error correction, percent of used	885 (2.67%)	1056 (4.4%)	0 (锟?)	5705 (3.74%)	447 (4.14%)	101122 (5.54%)	79261(5.86%)
Reads pre-clustered due to the similar VJC-lists, percent of used	0 (0%)	0 (0%)	0 (锟?)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Reads dropped due to the lack of a clone sequence	1270 (2.64%)	1425 (2.87%)	0 (0%)	61302 (6.28%)	404 (2.49%)	126412 (3.41%)	544876 (22.48%)
Reads dropped due to low quality	1(0%)	0 (0%)	0 (0%)	22 (0%)	0 (0%)	166 (0%)	139 (0.01%)
Reads dropped due to failed mapping	463 (0.96%)	274 (0.55%)	0 (0%)	1398 (0.14%)	170 (1.05%)	8950 (0.24%)	8295 (0.34%)
Reads dropped with low quality clones	0 (0%)	0 (0%)	0 (锟?)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Clonotypes eliminated by PCR error correction	204	391	0	3230	181	13221	10058
Clonotypes dropped as low quality	0	0	0	0	0	0	0
Clonotypes pre-clustered due to the similar VJC-lists	0	0	0	0	0	0	0

Table 1: *Representative example of top clonotypes identified using the TCR-sequencing libraries and CDR3 sequences for the top clones. The report shared with the customer will have the information on all clonotypes that were identified in the form of an excel document.*

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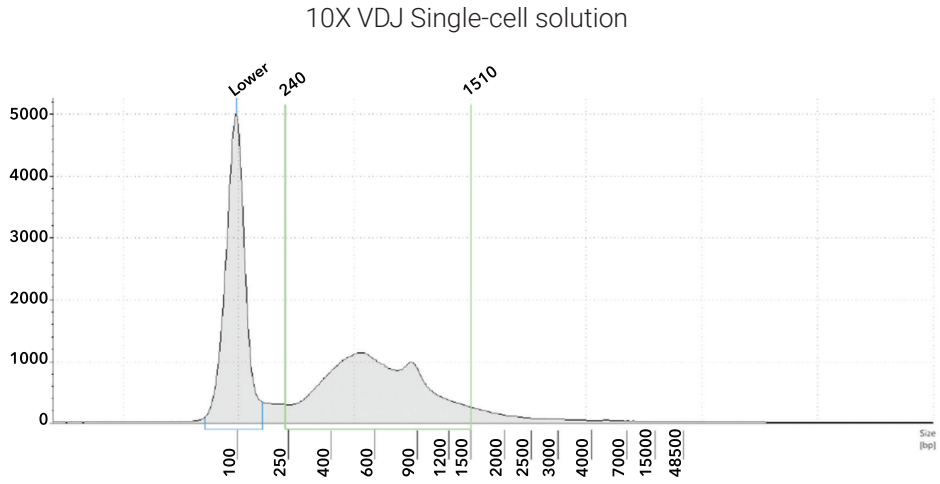

B **Region Table**

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/ µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
240	1510	657	9.96	27.8	82.91		

Figure 2: *a) Representative chromatogram of single-cell TCR libraries generated using the 10X V(D)J TCR Profiling Kit. b) Table shows quantification of the libraries.*

Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell	Number of Reads	Valid Barcodes	Sequencing Saturation	Q30 Bases in Barcode	Q30 Bases in RNA Read	Q30 Bases in RNA Read 2	Q30 Bases Sample Index	Q30 Bases in UMI	Reads Mapped to Genome	Reads Mapped Confidently to Genome	Reads Mapped Confidently to Intergenic Regions	Reads Mapped Confidently to Intronic Regions	Reads Mapped Confidently to Exonic Regions	Reads Mapped Confidently to Transcriptome	Reads Mapped Antisense to Gene	Fraction Reads in Cells	Total Genes Detected	Median UMI Counts per Cell
12,365	37,275	2,747	460,906,190	89.70%	78.20%	96.70%	93.70%	88.40%	94.70%	96.70%	95.80%	91.20%	6.60%	11.40%	75.60%	66.00%	5.30%	95.40%	20,761	8,557
13,246	30,341	2,676	401,896,981	91.10%	72.40%	96.80%	93.70%	87.90%	94.10%	96.70%	95.60%	92.20%	5.70%	10.70%	78.30%	69.20%	4.80%	94.40%	21,291	8,190
11,775	33,218	2,850	391,145,184	91.70%	74.00%	96.70%	93.70%	87.90%	94.30%	96.70%	95.70%	92.20%	5.70%	11.00%	78.00%	69.50%	4.30%	94.90%	20,899	8,937
6,972	57,698	2,841	402,274,499	93.10%	83.60%	96.80%	93.70%	87.70%	94.50%	96.70%	94.60%	88.80%	5.00%	19.40%	67.30%	61.50%	1.60%	81.70%	20,127	7,814
14,448	25,756	2,350	372,133,710	91.60%	74.40%	96.70%	93.70%	87.60%	94.40%	96.70%	95.20%	91.90%	6.30%	10.70%	77.20%	68.50%	4.40%	95.10%	20,974	6,902
11,139	34,794	2,738	387,580,252	91.30%	76.70%	96.70%	93.70%	88.10%	95.00%	96.70%	95.40%	91.80%	6.10%	10.50%	77.60%	68.90%	4.40%	95.00%	21,076	8,708
9,760	43,299	3,002	422,599,869	91.70%	78.20%	96.80%	93.70%	88.80%	95.00%	96.70%	94.90%	91.60%	6.50%	10.90%	76.40%	67.80%	4.40%	94.60%	20,879	10,109

Table 2: *Representative sequencing metrics obtained from the 10X Genomics Cell Ranger and Loupe Browser Analysis of libraries generated by sequencing purified T cells using the 10X Chromium Immune Profiling Single-cell TCR Profiling Approach.*

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