TCR sequencing solutions at MedGenome

At MedGenome, we provide TCR repertoire profiling using bulk input (from cells, RNA and FFPE tissue) using the SMARTerTCR Profiling Kit (Takara Bio USA Inc) and single-cell inputs using the SMARTer single-cell TCR Profiling kit and the Chromium Immune Profiling solutions (10X Genomics). We have tested and optimized the commercially available library preparation methods to work for a wide range of input types to obtain TCR alpha, beta, gamma & delta clonotypes (for bulk), and TCR alpha and beta clonotypes from single-cell inputs. The sample types we have validated include fresh and frozen PBMCs, FACS purified CD8 and CD4 T cells, purified tumor infiltrating lymphocytes, and T-cell lines. We follow the manufacturer's recommended guidelines for our quality control at every step of the process and provide customers with accurate QC reports.

Table 1 *: Shows a summary of all the TCR-Sequencing solutions that are offered at MedGenome*

Name of Offering	Input Type	Amount of Material Needed	Analysis Method	Information Obtained
SMARTer TCR Profilling Kit (Takara Bio USA)	Isolated cells or RNA	10 ng-3 ug/50-10,000 cells (Human, Mouse)	MiXCR	CDR3, V(D)J sequences α/β pairing (from SC Kit)
Single Cell Immune Profiling (10X Genomics)	Isolated cells	Single-cells (Human, Mouse)	Cell Ranger	CDR3, α/β pairing and clonotypes V(D)J sequences
Gamma Delta TCR Profiling	Isolated cells or RNA	10 ng-3 µg/50-10,000 cells	MiXCR	CDR3, V(D)J sequences
FFPE TCR Profiling	RNA (DV200>20)	> 10 ng total RNA	MiXCR	CDR3, V(D)J sequences

Sample and Library QC

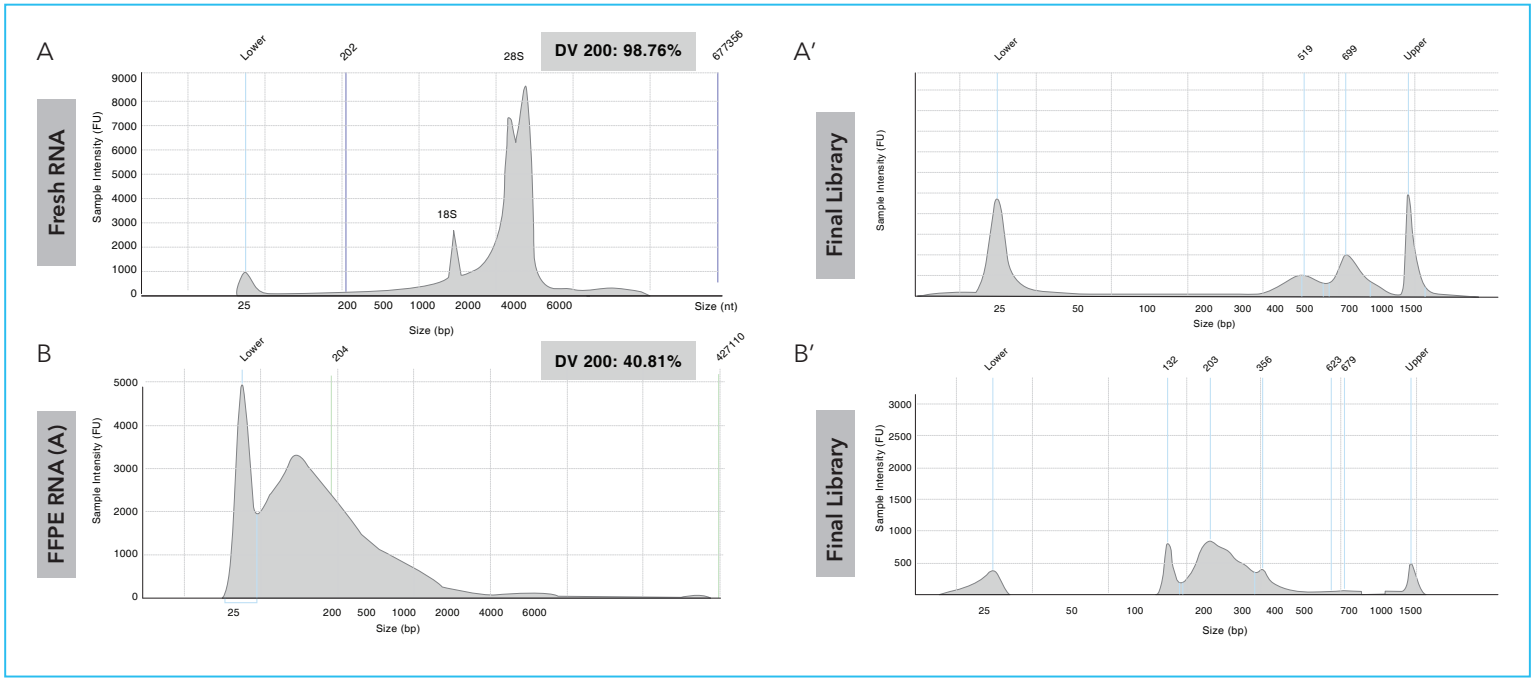


Figure 1: *TapeStation profiles to evaluate RNA quality and TCR-Seq libraries generated from FFPE tissues in comparison to fresh tissue: The panels on the left show the RNA profiles of total RNA isolated from PBMCs (top panel) and 2 different FFPE tumor tissue samples. DV 200 values for each sample shows the RNA integrity of the samples (inset). Panels on the right shows profiles of TCR-Seq libraries generated using SMARTer TCR Profiling Kit with modifications to the protocol from the corresponding input RNAs.*

Table 2: *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.*

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 3: *TCR alpha/ beta/ gamma/ delta clonotypes identified*

cloneId	cloneCount	cloneFraction	allVHitsWithScore	allDHitsWithScore	allJHitsWithScore	allCHitsWithScore	aaSeqCDR3
0	1736	0.007594316511513963	TRBV9*00(1308)	TRBD1*00(35)	TRBJ2-1*00(209.5)	TRBC2*00(281), TRBC1*00(244.9)	CASSVAGGDEQFF
1	1052	0.004602085812276895	TRBV28*00(1313.4)	TRBD1*00(45)	TRBJ2-7*00(224.4)	TRBC2*00(280.9)	CASGRQGAYEQYF
2	852	0.003727164555190033	TRAV12-2*00(1221.9)		TRAJ42*00(319.6)	TRAC*00(174)	CALNYGGSQGNLIF
3	721	0.0031540911317981383	TRBV12-3*00(1299.6), TRBV12-4*00(1263.8)	TRBD1*00(26)	TRBJ1-4*00(194.6)	TRBC1*00(278.9)	CASSFGQAAQLFF
4	659	0.002882865542101211	TRBV4-1*00(1312.5)	TRBD1*00(40)	TRBJ1-5*00(214.7)	TRBC1*00(279.4)	CASSLRTGDGQPQHF
5	651	0.0028478686918177363	TRBV7-9*00(1240.6)	TRBD1*00(26), TRBD2*00(25)	TRBJ2-7*00(215.4)	TRBC2*00(280.4)	CASSLIGEGFSDEQYF
6	648	0.0028347448729614333	TRBV4-3*00(1297.8), TRBV4-2*00(1251.2)	TRBD1*00(41)	TRBJ2-1*00(234.5)	TRBC2*00(279.7)	CASSQDGTGGYNEQFF
7	543	0.0023754112129908307	TRAV21*00(1308.9)	TRDD3*00(25)	TRAJ31*00(254.6)	TRAC*00(172)	CAAPGLDNARLMF
8	543	0.0023754112129908307	TRAV4*00(1310.1)		TRAJ33*00(284.6)	TRAC*00(173.8)	CLVDSNYQLIW
9	536	0.0023447889689927905	TRBV12-4*00(1292.1), TRBV12-3*00(1244.4)		TRBJ1-1*00(220)	TRBC1*00(279.6)	CASSFMVVQADSTEAFF
10	528	0.002309792118709316	TRAV26-2*00(1269.7)		TRAJ49*00(285)	TRAC*00(174)	CILRDPNTGNQFYF
11	515	0.00225292223699867	TRAV21*00(1306.8)		TRAJ15*00(250.6)	TRAC*00(172.2)	CAVKGQAGTALIF
12	490	0.0021435570798628125	TRAV21*00(1281.1)		TRAJ4*00(319.2)	TRAC*00(173.6)	CAGPMFSGGYNKLIF
13	478	0.0020910618044376007	TRBV7-9*00(1269.8)	TRBD1*00(25), TRBD2*00(25)	TRBJ2-1*00(259.7)	TRBC2*00(281.2)	CASSLIGISSYNEQFF
14	468	0.0020473157415832575	TRAV21*00(1312.7)		TRAJ52*00(329.7)	TRAC*00(173.7)	CAVMDAGGTSYGKLTF
15	466	0.002038566529012389	TRAV38-2DV8*00(1350.4)	TRDD3*00(30)	TRAJ49*00(259.6)	TRAC*00(173.9)	CAYRSPPTGNQFYF
16	466	0.002038566529012389	TRAV13-1*00(1333.7)		TRAJ11*00(264.3)	TRAC*00(174.2)	CAAHEGYSTLTF
17	431	0.001885455309022188	TRBV7-9*00(1260.2)		TRBJ2-1*00(259.8)	TRBC2*00(280.6)	CASSLIGVSSYNEQFF
18	400	0.0017498425141737244	TRAV12-2*00(1215.5)		TRAJ20*00(259.6)	TRAC*00(172.3)	CAVNINDYKLSF
19	399	0.00174546790788829	TRAV2*00(1250.9)		TRAJ18*00(309.8)	TRAC*00(172.6)	CASRGSTLGRLYF
0	1151	0.00424535261138979	TRAV21*00(1338.6)		TRAJ9*00(279.5)	TRAC*00(173.3)	CAVTTGGFKTIF
1	929	0.003426526999114783	TRAV21*00(1304.6)		TRAJ33*00(274.5)	TRAC*00(173.4)	CAVPDSNYQLIW
2	925	0.0034117733844791975	TRAV21*00(1306.1)		TRAJ4*00(309.6)	TRAC*00(173)	CALYQFSGGYNKLIF

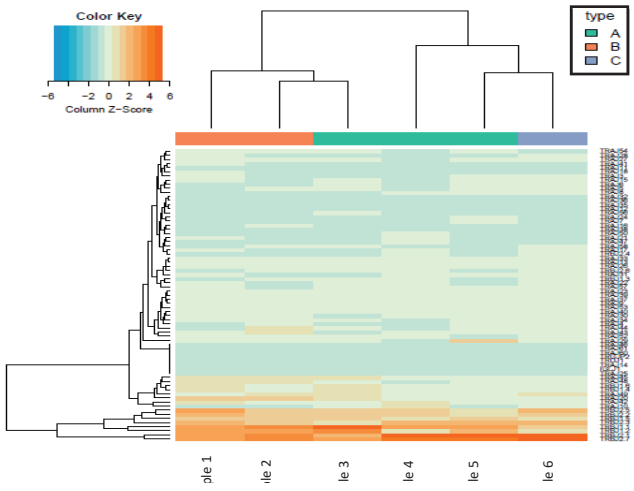
Table 4: *Clonotype comparison output, donor 1, 2 , 3 etc to sample 1, 2, 3*

cdr3nt	cdr3aa	V	D	J	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AACGAGGGGAAGTCTGGAAACACCATATATTTT	NEGKSGNTIYF	TRBV10-3	TRBD1	TRBJ1-3	0	0	0	0	0
AGCAGCGCCGACTCTGGGGCCAATGTCCTGACTTTC	SSADSGANVLTF	TRBV29-1		TRBJ2-6	0	0	0	0	0
AGCAGCGTTGAGGGAAGGAAGGGAAACTACGAGCAGTACTTC	SSVEGRKGNYEQYF	TRBV29-1		TRBJ2-7	0	0	0	0	0
AGCAGCGTTGAGTGGCGGGAGAGGGCGAACACCGGGGAGCTGTTTTTT	SSVEWRERANTGELFF	TRBV29-1	TRBD2	TRBJ2-2	0	0	0	0	0
AGCAGCGTTGTGGCCAACACCGGGGAGCTGTTTTTT	SSVVANTGELFF	TRBV29-1		TRBJ2-2	0	0	0	0	0
AGCAGTGCTAACGGGGGCACTGAAGCTTTCTTT	SSANGGTEAFF	TRBV20-1	TRBD1	TRBJ1-1	0	0	0	0	0
AGCAGTGCTAGACACGGACTAGCGGGAGGTAGGAATGAGCAGTTCTTC	SSARHGLAGGRNEQFF	TRBV20-1	TRBD2	TRBJ2-1	0	0	0	0	0
AGCAGTGCTAGACCCCCCAGTTCCGCGTTTATCTACCGGGGAGCTGTTTTTT	SSARPPSSA_LSTGELFF	TRBV20-1	TRBD1	TRBJ2-2	0	0	0	0	0
AGCAGTGCTAGAGAAGTGCTGGGGATTGGATACAATGAGCAGTTCTTC	SSAREVLGIGYNEQFF	TRBV20-1		TRBJ2-1	0	0	4.5940470338535325E-6	0	0
AGCAGTGCTAGAGATCGAGCGGGGGCTTTTTTATACGAGCAGTACTTC	SSARDRAGAFLYEQYF	TRBV20-1	TRBD1	TRBJ2-7	0	0	0	0	4.290261963395485E-6
AGCAGTGCTAGAGATTTGAAGACTGACGAGCAGTACTTC	SSARDLKTDEQYF	TRBV20-1		TRBJ2-7	0	0	0	3.627578301277633E-6	0
AGCAGTGCTAGAGATTTTGCCAGCCGGGAGGCTTATGAAAAACTGTTTTTT	SSARDFASREAYEKLFF	TRBV20-1	TRBD2	TRBJ1-4	0	0	0	0	0
AGCAGTGCTAGCAGCGGGAGAGGTAATGAGCAGTTCTTC	SSASSGRGNEQFF	TRBV20-1	TRBD2	TRBJ2-1	0	0	0	0	0
AGCAGTGCTAGCCCCGGGACAGAGTATGGCTACACCTTC	SSASPGTEYGYTF	TRBV20-1	TRBD1	TRBJ1-2	0	0	0	0	0
AGCAGTGCTAGCTCGGGGGTTAGCACAGATACGCAGTATTTT	SSASSGVSTDTQYF	TRBV20-1	TRBD1	TRBJ2-3	0	0	0	0	0
AGCAGTGCTAGTAGACTAGCGGTTACCTACGAGCAGTACTTC	SSASRLAVTYEQYF	TRBV20-1	TRBD2	TRBJ2-7	0	0	0	0	4.290261963395485E-6
AGCAGTGCTAGTGAGGATCAGTTGGGCTCCTACAATGAGCAGTTCTTC	SSASEDQLGSYNEQFF	TRBV20-1		TRBJ2-1	0	0	0	0	4.290261963395485E-6
AGCAGTGCTTGGCACCCCGGGACAGGGGGCAAACGGGATGGCTACACCTTC	SSAWHPGTGGKRDGYTF	TRBV20-1	TRBD1	TRBJ1-2	0	0	0	0	4.290261963395485E-6

Advanced deliverables

In addition to the standard deliverables using the MixCR pipeline and MedGenome's pipeline for clonotype comparisons, we also offer advanced visual outputs for data comprehension using the V(D)J tools by MiLabs. The outputs include a) Dendrograms and clustering diagrams showing usage of the V(D)J genes across multiple samples, b) chord-diagram showing the usage and pairing of the TCR α/β chains c) spectratype plot of the CDR3 usage for the samples d) clonotype diversity index across multiple samples .

A



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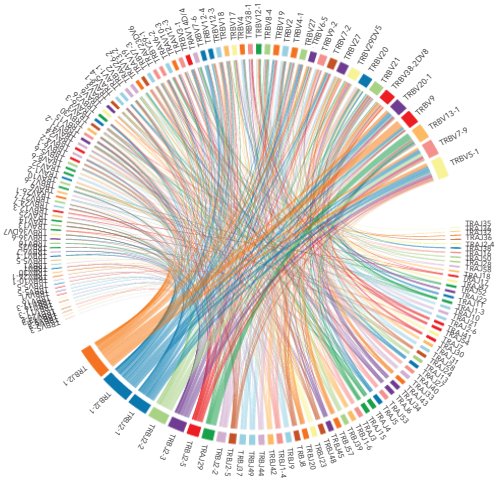

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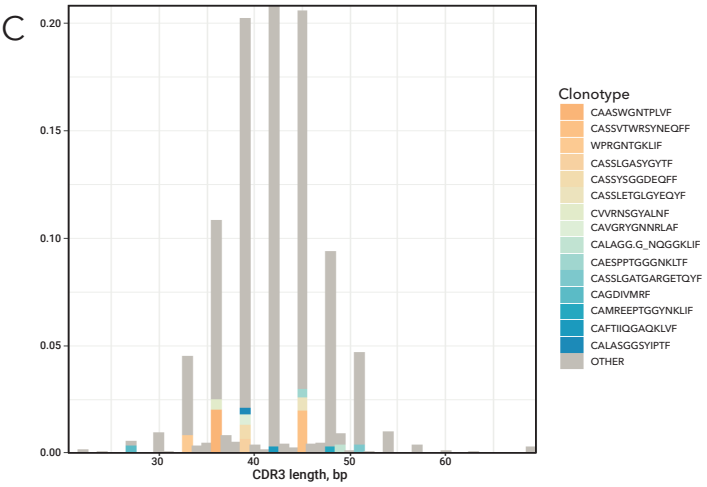

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