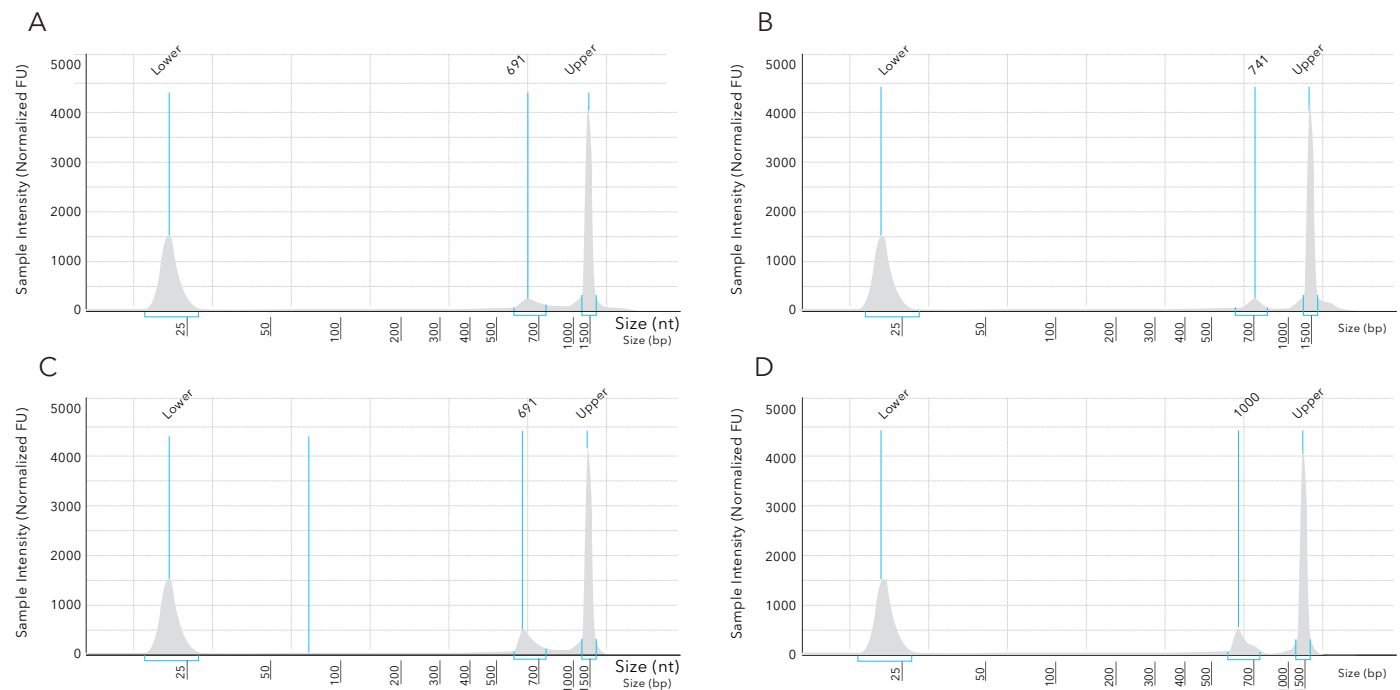


## Human Bulk BCR Profiling Technical Sheet

At MedGenome, we provide BCR repertoire profiling from bulk input (from cells & RNA) using the SMARTer BCR Profiling Kit (Takara Bio USA Inc) and single-cell inputs using the Chromium Immune Profiling solutions (10X Genomics). The sample types we have validated include frozen PBMCs, hybridoma and spleen. We follow the manufacturers' recommended guidelines for our quality control at every step of the process and provide customers with accurate QC reports. In addition, we support experimental design and basic and advanced analysis.

**Table 1:** BCR - Sequencing services offered at medGenome.

Technology	Source & Input Type	Required Amount	Sequencing Method	Analysis Method	Information Obtained
Single Cell BCR Immune Profiling (10X Genomics)	Isolated Cells (Fresh, Frozen, Fixed) (Human, Mouse)	Single cell suspension 100,000 cells	Illumina NovaSeq PE150	Cell Ranger, Loupe browser	CDR3, H/K/L pairing and clonotypes V(D)J sequences
Bulk SMARTer IgG/M BCR Profiling Kit (Takara Bio USA)	Isolated Cells RNA (Human)	10 ng-3 µg RNA 1000-10,000 cells	Illumina MiSeq PE300	MiXCR VDJ Tools	Full length V(D)J Region sequences with hits, clone count and frequencies
Bulk SMARTer IgG BCR Profiling Kit (Takara Bio USA)	Isolated Cells RNA (Mouse)	10 ng-3 µg RNA 1000-10,000 cells	Illumina MiSeq PE300	MiXCR VDJ Tools	Full length V(D)J Region sequences with hits, clone count and frequencies



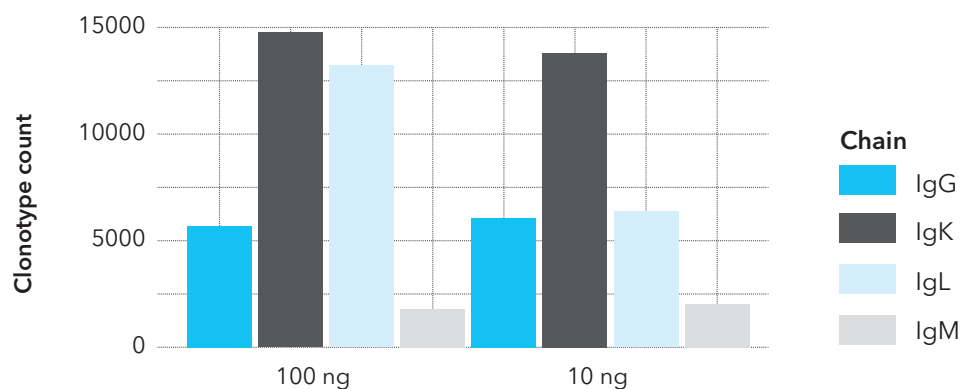
**Figure 1:** TapeStation traces show representative profiles of B-cell receptor repertoire libraries generated from 10 ng of total RNA from human spleen to specifically amplify the IgG heavy chain (A), IgM heavy chain (B) Kappa (C) and Lambda (D) chains respectively. The libraries were generated using the Takara SMARTer Human BCR IgG IgM H/K/L Profiling Kit and following manufacturers' instructions. Sequencing was performed using the Illumina MiSeqV3 600 cycle kit, and analysis was performed using the immune profiler pipeline provided by Takara.

**Table 2.** Mapping statistics shows specificity of amplification of heavy and light chains in BCR libraries from 10 ng of RNA from spleen

Sample	% reads assigned to IGG	% reads assigned to IGM	% reads assigned to IGK	% reads assigned to IGL	Final clonotype count	% undetermined reads	Total reads (%)
10ng IgG	139813 (97.0%)	53 (0.0%)	93 (0.1%)	263 (0.2%)	5900	3953 (2.7%)	144175 (100.0%)
10ng IgM	55 (0.1%)	51847 (97.1%)	45 (0.1%)	75 (0.1%)	1904	1393 (2.6%)	53415 (100.0%)
10ng IgK	109 (0.1%)	44 (0.0%)	152162 (97.1%)	93 (0.1%)	13734	4257 (2.7%)	156665 (100.0%)
10ng IgL	144 (0.1%)	80 (0.1%)	261 (0.2%)	101928 (96.0%)	6327	3783 (3.6%)	106196 (100.0%)
100ng IgG	2235787 (97.3%)	238 (0.0%)	540 (0.0%)	1988 (0.1%)	5517	59298 (2.6%)	2297851 (100.0%)
100ng IgM	274 (0.0%)	644372 (97.6%)	582 (0.1%)	291 (0.0%)	1748	14750 (2.2%)	660269 (100.0%)
100ng IgK	453 (0.0%)	186 (0.0%)	1155708 (97.4%)	334 (0.0%)	14619	30286 (2.6%)	1186967 (100.0%)
100ng IgL	674 (0.0%)	183 (0.0%)	293 (0.0%)	1546070 (96.7%)	13060	51057 (3.2%)	1598277 (100.0%)

**Table 3.** Shows representative outputs after assembly of final clonotypes from BCR profiling libraries generated using the Takara Human BCR IgG/IgM H/K/L Profiling Kit

Read Count	Clonal Sequence	CDR3 Sequence	CDR3 Amino Acid Sequence	Clonal Type	V segment	all V hits	D segment	all D hits	J segment	all J hits	C segment	all C hits
10	TGTGCGAGATACAGTA- ACTACGGTTGGACCTT- GACAACCTGG	TGTGCGAGATACAGTAAC- TACGGTTGGACCTTTGA- CAACTGG	CARYSNYGWTFDNW	IGG	IGHV4-34	IGHV4-34	IGHD4-11	IGHD4-11;IGHD4-4	IGHJ4	IGHJ4	IGHG3	IGHG3;IGHG4
10	TGTGCGAGACGGATAC- CCCTCTACGGTATGGAC- GTCTGG	TGTGCGAGACGGATAC- CCCTCTACGGTATGGAC- GTCTGG	CARRIPLYGMDWW	IGG	IGHV3-48	IGHV3-48	IGHD4-17	IGHD4-17;IGHD4-23;IGHD5-24	IGHJ6	IGHJ6	IGHG1	IGHG1;IGHG2;IGHG3
8	TGTGCAAGGTTTAACTG- GGGATCCGGCTCCTGG- TACTTCGATCTCTGG	TGTGCAAGGTTTAACTG- GGGATCCGGCTCCTGG- TACTTCGATCTCTGG	CARFNWGSWSWYF- DLW	IGM	IGHV6-1	IGHV6-1	IGHD7-27	IGHD7-27	IGHJ2	IGHJ2	IGHM	IGHM
5	TGTGTGAGAGATGGGG- GAGTATTACGATGATA- ATAGTGTTTATCAGTAT- TACCAGTACTACATG- GACGCTCTGG	TGTGTGAGAGATGGGGAG- TATTACGATGATAATAGT- GTTTATCAGTATTACCAG- TACTACATGGACGCTCTGG	CVRDGEYYDDNSVY- QYYQYMDWW	IGM	IGHV3-11	IGHV3-11;IGHV3-7	IGHD3-22	IGHD3-22	IGHJ6	IGHJ6	IGHM	IGHM
123	TGTCAACACCTTAA- AAACTTATGCTCCCTG- GACGTTTC	TGTCAACACCTTAAACT- TATGCTCCCTGGACGTTTC	COHLKYAPWTF	IGK	IGKV1-9	IGKV1-9			IGKJ3	IGKJ3;IGKJ1	IGKC	IGKC
123	TGTCAACACCTTAA- AAACTTATGCTCCCTG- GACGTTTC	TGTCAACACCTTAAACT- TATGCTCCCTGGACGTTTC	COHLKYAPWTF	IGK	IGKV1-9	IGKV1-9			IGKJ1	IGKJ1	IGKC	IGKC
30	TGCCAGTCTTTGACAG- CAGGCTGAATGCTGC- GATTTTC	TGCCAGTCTTTGACAG- CAGGCTGAATGCTGC- GATTTTC	CQSFDSRLNAAIF	IGL	IGLV1-40	IGLV1-40			IGLJ2	IGLJ2;IGLJ3	IGLC7	IGLC7;IGLC2;IGLC3;IGLC6
24	TGTCAGGCGTGGGA- CAGCAGCACTGTGG- TATTC	TGTCAGGCGTGGGACAG- CAGCACTGTGGTATTC	CQAWDSSTVVF	IGL	IGLV3-1	IGLV3-1			IGLJ2	IGLJ2	IGLC7	IGLC7;IGLC2;IGLC3;IGLC6



**Figure 2.** Bar chart shows the total number of clonotypes identified per Ig chain with different concentrations of RNA from spleen