

Appendix

Table S1 Primers for reverse transcription and PCR

Name	Sequence ¹
RTC _μ	gatgacttcagtggtgt
J558-1	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGagRtYcagctgcaRcagtct
J558-2	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGaggtccaactgcagcagcc
Q52	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGtctgcctggtgacWttccca
36-60	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGgtgcagcttcaggagtcag
X24	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGgaggtgaagcttctcagtc
7183	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGgaagtgaagctggtggagtc
J606	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGatgKacttgggactgaRctgt
S107	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGcagtgtaggtgaagctggt
3609	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGccaggttactctgaaagagtc
VGAM3.8	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGtgtggaccttctattctga
V _H 10	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGtgttgggctgaagtggttt
V _H 11	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGatggagtggaactgagctta
V _H 12	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGagcttcaggagtcaggacc
3609N	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGcaggtgcagctttagagac
SM7	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGatgcagctgggtcattctt
V _H 15	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGgactggatttgatcacKctc
V _H 16	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGtggagtttgacttagttggg
C _μ 0	CCATCTCATCCCTGCGTGTCTCCGACTCAGcatggccaccagattcttat
C _μ 1 ²	CCATCTCATCCCTGCGTGTCTCCGACTCAG <u>gt</u> catggccaccagattcttat
C _μ 2	CCATCTCATCCCTGCGTGTCTCCGACTCAG <u>gagcatg</u> catggccaccagattcttat
C _μ 3	CCATCTCATCCCTGCGTGTCTCCGACTCAG <u>tctgtcagtc</u> atggccaccagattcttat

Note: ¹ Capital letters correspond to 454-adaptor sequence, and small letters to mouse V_H family-specific upstream primers. All sequences are written 5' to 3';

² the underlined letters indicate different barcoding index sequences.

Table S2 Chi-square comparisons of individual V_H genes between three different populations of B cells.

Comparison ¹	Pre-B vs. IM-B	IM-B vs. S-B	Pre-B vs. S-B
J558.1	-	-	-
J558.2	-	-	-
J558.3	$P < 10^{-9}$	-	$P < 10^{-18}$
J558.4	-	-	-
J558.6	-	-	-
J558.8	-	-	-
J558.9	N ²	N	N
J558.12	-	-	-
J558.13	-	N	-
J558.16	-	-	-
J558.18	$P < 10^{-12}$	-	-
J558.19	-	-	-
J558.22	-	-	$P < 10^{-7}$
J558.23	-	-	-
J558.26	-	-	-
J558.31	-	-	-
J558.34	-	-	-
J558.36	-	-	-
J558.37	-	-	-
J558.39	-	-	-
J558.42	-	-	-
J558.43	-	-	-
J558.47	-	-	-
J558.49	-	-	-
J558.50	$P < 10^{-9}$	-	$P < 10^{-6}$
J558.52	$P < 10^{-60}$	-	$P < 10^{-70}$
J558.53	$P < 10^{-30}$	$P < 10^{-40}$	$P < 10^{-155}$
J558.54	-	-	-
J558.55	$P < 10^{-11}$	-	$P < 10^{-19}$
J558.56	$P < 10^{-5}$	-	$P < 10^{-7}$
J558.58	-	-	-
J558.59	-	$P < 10^{-7}$	$P < 10^{-6}$
J558.61	$P < 10^{-9}$	-	-
J558.64	N	N	N
J558.65	-	-	-
J558.66	$P < 10^{-8}$	-	$P < 10^{-8}$
J558.67	$P < 10^{-18}$	$P < 10^{-8}$	-
J558.69	-	-	-
J558.72	$P < 10^{-40}$	-	$P < 10^{-46}$
J558.74	-	-	-
J558.75	$P < 10^{-19}$	$P < 10^{-32}$	-
J558.77	$P < 10^{-5}$	$P < 10^{-25}$	$P < 10^{-56}$

J558.78	-	-	-
J558.79	-	-	-
J558.80	-	-	--
J558.81	-	-	-
J558.83	$P < 10^{-9}$	-	$P < 10^{-19}$
J558.84	$P < 10^{-19}$	-	$P < 10^{-15}$
J558.85	-	-	-
J558.86	-	-	-
J558.87	-	-	-
J558.88	-	-	-
Q52.2	$P < 10^{-7}$	$P < 10^{-10}$	-
Q52.3	-	$P < 10^{-8}$	$P < 10^{-22}$
Q52.5	-	-	-
Q52.7	-	-	-
Q52.8	$P < 10^{-6}$	$P < 10^{-6}$	$P < 10^{-25}$
Q52.9	-	-	-
Q52.10	-	-	-
Q52.11	N	N	N
Q52.13	-	-	-
36-60.1	$P < 10^{-26}$	-	$P < 10^{-13}$
36-60.3	-	$P < 10^{-11}$	$P < 10^{-23}$
36-60.4	-	-	-
36-60.5	-	$P < 10^{-7}$	$P < 10^{-14}$
36-60.6	$P < 10^{-88}$	-	$P < 10^{-108}$
36-60.8	-	$P < 10^{-7}$	$P < 10^{-18}$
X24.2	-	-	-
7183.2	$P < 10^{-30}$	$P < 10^{-16}$	$p < 10^{-77}$
7183.4	-	-	-
7183.7	$P < 10^{-22}$	$P < 10^{-13}$	-
7183.9	-	-	-
7183.12	$P < 10^{-122}$	$P < 10^{-18}$	$P < 10^{-207}$
7183.14	-	-	$P < 10^{-6}$
7183.16	-	N	-
7183.18	$P < 10^{-46}$	-	$P < 10^{-72}$
7183.19	$P < 10^{-9}$	-	$P < 10^{-12}$
7183.20	-	$p < 10^{-20}$	$P < 10^{-48}$
J606.1	-	-	-
J606.2	N	N	N
J606.3	N	N	N
J606.4	-	$P < 10^{-6}$	-
J606.5	-	N	-
S107.1	-	$P < 10^{-83}$	$P < 10^{-112}$
S107.3	$P < 10^{-23}$	$P < 10^{-35}$	-
S107.4	-	-	-

3609.1	N	N	N
3609.3	N	N	N
3609.4	-	-	-
3609.5	-	-	-
3609.7	-	-	-
3609.9	-	-	-
3609.11	N	N	N
3609.12	-	-	-
VGAM3.8-1	-	-	$P < 10^{-9}$
VGAM3.8-2	$P < 10^{-13}$	$P < 10^{-6}$	$P < 10^{-34}$
VGAM3.8-3	$P < 10^{-8}$	$P < 10^{-7}$	-
VGAM3.8-4	N	N	N
V _H 10.1	$P < 10^{-34}$	$P < 10^{-29}$	-
V _H 10.3	-	-	-
V _H 11.1	-	-	-
V _H 11.2	-	-	-
V _H 12.1	$P < 10^{-15}$	$P < 10^{-15}$	$P < 10^{-52}$
3609N.2	$P < 10^{-32}$	$P < 10^{-12}$	$P < 10^{-77}$
SM7.1	-	$P < 10^{-7}$	$P < 10^{-22}$
SM7.2	-	$P < 10^{-19}$	$P < 10^{-34}$
SM7.3	-	-	-
SM7.4	$P < 10^{-19}$	$P < 10^{-5}$	$P < 10^{-38}$
V _H 15.1	$P < 10^{-34}$	$P < 10^{-34}$	$P < 10^{-34}$
V _H 16.1	N	N	N

Note: ¹Pre-B, IM-B, and S-B cell populations were compared with each other. For each comparison, P value is shown if it is significantly different ($P < 10^{-5}$), or the P value is replaced with “-”.

² “N” indicates that the corresponding V_H gene segment was not found to participate in any recombination in two compared B cell populations.

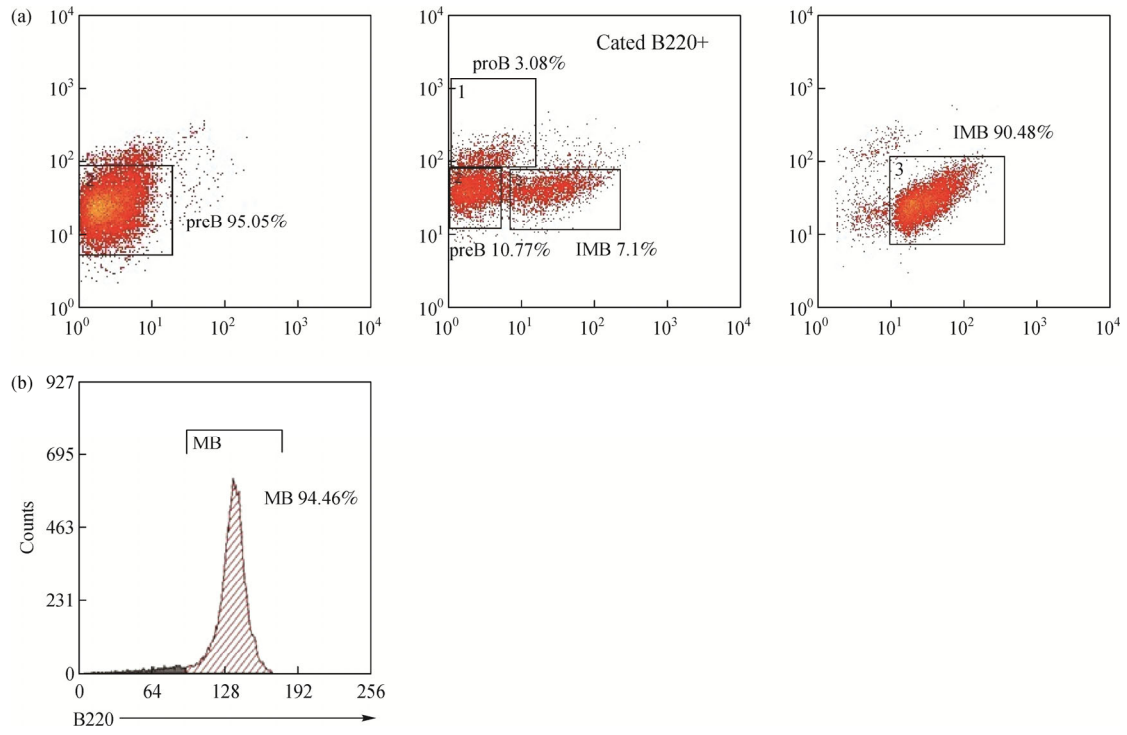


Fig. S1 Identification of B cell populations in bone marrow (a) and spleen (b) by FACS

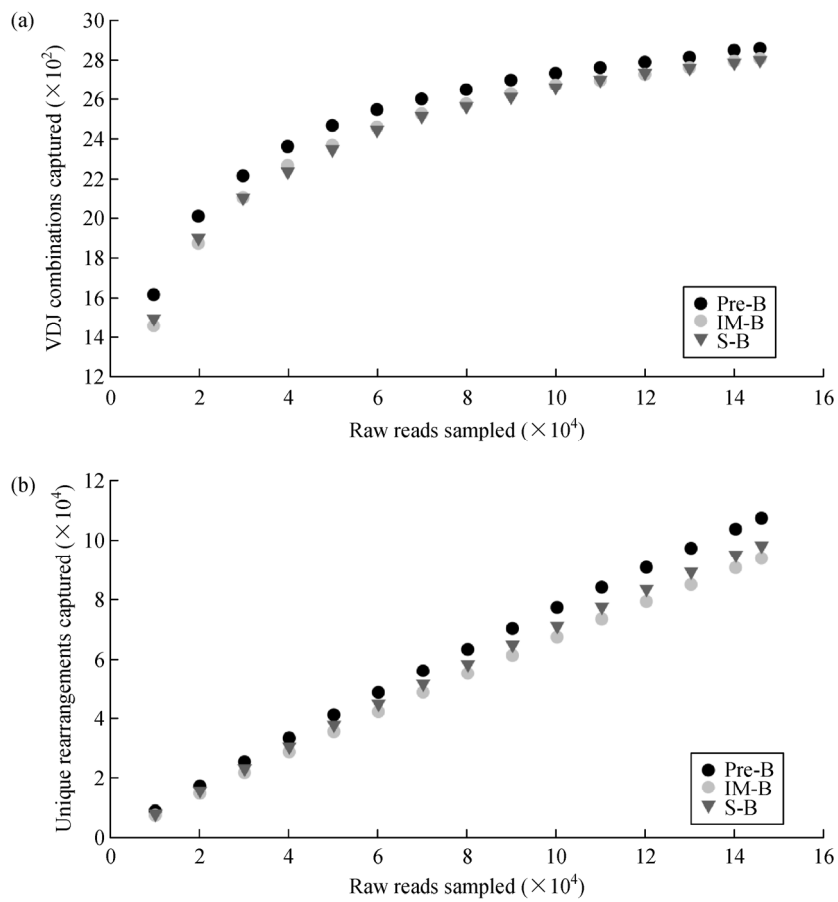


Fig. S2 Saturation analysis of Ig heavy-chain VDJ combination repertoire (a) and antibody repertoire (b)

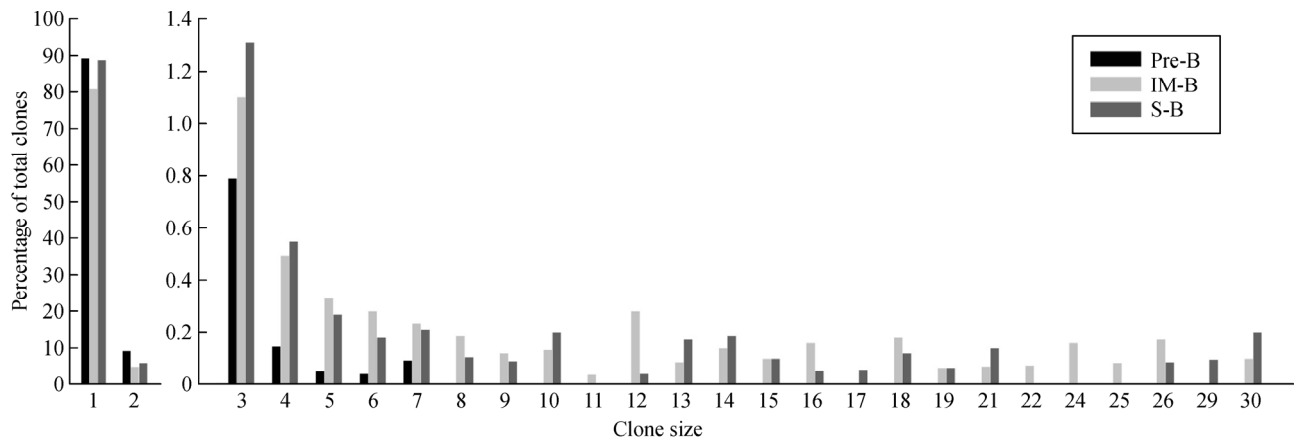


Fig. S3 Frequency of clonal expansion in different B cell populations