SUPPLEMENTAL MATERIAL

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IgM^{a+b-} cells do not harbor cryptic but productive V_H7183– D_H-J_H rearrangements on the WT^b allele

To test the possibility that the presence of $E\mu$ on the $V_H E\mu^a$ allele was in some way prohibiting surface expression of a µ-chain from the other productively rearranged Ighb allele, we sorted IgM^{a+b-} cells (single producers) from both $V_H \Delta^a / WT^b$ and $V_{H}E\mu^{a}/WT^{b}$ mice and cloned $V_{H}7183-D_{H}-J_{H}$ rearrangements that had taken place on the WT^b allele. In an initial experiment focusing only on $V_H \Delta^a / WT^b$ mice, four out of five $V_H 7183$ -D_H-J_H rearrangements on the WT^b allele cloned from IgM^{a+b-} cells were unproductive, whereas seven out of seven V_H7183 - D_H - J_H rearrangements cloned from the same WT allele but from IgM^{a+b+} cells (double producers) were productive (unpublished data). In a second experiment involving both $V_H \Delta^a / WT^b$ and $V_{\rm H} E \mu^a / W T^b$ mice, all $V_{\rm H} 7183\text{-}D_{\rm H}\text{-}J_{\rm H}$ rearrangements on the WT^b allele cloned from IgM^{a+b-} cells (single producers) were unproductive (14 unique clones from $V_H\Delta^a/WT^b$ cells and 18 unique clones from V_HEµ^a/WT^b cells; unpublished data). Surface expression, therefore, faithfully reflects VDJ assembly status on the WT Igh^b allele, providing no support for a model of E μ dependent and allele-specific silencing.



Figure S1. PCR analysis of WT and mutant F_1 mice. Top, primers generate 322-bpproduct from WT *lgh* alleles and 848-bp product from V_H knockin alleles. Bottom, primers generate ~1,500-bp product before and 400-bp product after deletion of neo^R.

Double-producers in $V_H\Delta^a/WT^b$ mice are enriched among marginal zone B cells in spleen and B1 B cells in the peritoneal cavity As shown in Fig. 3, double producers were found in large numbers in the peritoneal cavity of $V_H\Delta^a/WT^b$ mice. Three kinds of phenotypically and functionally distinct B cell populations have been described in both mice and people: follicular B cells (also known as conventional B cells or B2 B cells), marginal zone (MZ) B cells, and B-1 B cells. Follicular and MZ B cells, as their names imply, occupy distinct regions in the spleen and can be distinguished by surface phenotype (Martin, F., and J.F. Kearney. 2002. *Nat. Rev. Immunol.* 2:323–335). Follicular B cells make up the majority of B cells in blood, lymph nodes, and spleen. The peritoneal cavity, on the other hand, is largely populated by B-1 B cells, although B-1 B cells are also found in small numbers elsewhere. B-1 B cells are subdivided into those that do (B1a) and those that do not (B1b) express CD5.

Using markers to distinguish marginal zone and follicular B cells, we examined $V_H\Delta^a/WT^b$ mice and found that the singleproducers were predominantly of the follicular cell phenotype (CD23^{hi}, CD21^{lo}, IgD^{hi}, IgM⁺; Supplemental Fig. 2). In contrast, the double-producers in these animals were almost equally divided between those with the follicular and those with the MZ phenotype (CD23^{lo}, CD21^{hi}, IgD^{lo}, IgM^{hi}). In the peritoneal cavity, both $V_H\Delta^a/WT^b$ and $V_HE\mu^a/WT^b$ mice had reduced numbers of cells with the B1 phenotype (relative to WT^a/WT^b mice; Fig. S2). When we analyzed the peritoneal cells for IgM^a and IgM^b, however, IgM^{a+b-} single producers were almost uniformly of the B2 phenotype (IgD^{hi}CD5⁻IgM⁺B220⁺), whereas a large proportion of the double producers had a B1 phenotype (IgD^{dull} CD5^{+/-}IgM^{hi}B220^{lo}) (Fig. S2). In fact, among CD5⁺ cells in the peritoneal cavity, almost all proved to be double producers.

Double producers in fetal liver and newborn spleen

To test the hypothesis that double producers were arising more frequently within progenitors to B1 B cells, fetal livers from six $V_H\Delta^a/WT^b$ embryos (day 16–18, approximately) were examined for IgM⁺ cells. B220⁺ cells were gated for analysis of the two *Igh* allotypes. As shown in Fig. S1 A, IgM^{a+b-} cells were detected in these fetal livers, but there were no obvious double producers, lending no support for the idea that B1 B cell progenitors are enriched for double producers.

To determine whether double producers dominated among the early migrants to spleen, spleen cells were isolated from newborn mice. As illustrated in Fig. S3 (and quantified in Fig. 3C), double producers in newborn spleen made up a mean of 4.4% IgM⁺ cells, much as in the immature B cells of BM. Taken together, these data suggest that the large number of double producers consistently found in the spleens of $V_H \Delta^a / WT^b$ mice (~20%) results from peripheral expansion of a small pool of cells arising in the BM. The double producers found in the peritoneal cavity may similarly result from peripheral expansion of a small pool arising in fetal liver.



Figure S2. Surface phenotype of double-producers in spleen and peritoneal cavity of $V_H\Delta^a/WT^b$ mice. (A) CD23, CD21, IgD, and IgM expression on splenic IgM^{a+b-} cells (single producers; shaded curve) and IgM^{a+b+} cells (double producers; dark line). Non-B cells were included as controls (IgM^{a-b-}; dotted line). Cells were gated on the basis of IgM^a and IgM^b expression and histograms generated for expression of the third surface marker. (B) IgD expression on peritoneal cells (plotted versus forward scatter [FSC]). Percentage of total cells in three IgD gates (negative, dull, high) provided. (C) IgD levels on peritoneal cells gated as IgM^{a-b-}, IgM^{a+b-}, and IgM^{a+b+}. Left, contour plot of anti-IgM^a and anti-IgM^b staining, with gates and percentage of total cells indicated. Right, histogram of IgD levels on gated populations as in A. (D) IgM allotype expression on peritoneal cells. Cells were stained with antibodies to CD5, IgM^a, and IgM^b. Cells gated on the basis of CD5 and IgM^a expression (left) were analyzed for IgM^a and IgM^b expression (middle and right).



Figure S3. Ontogeny of double-producers in heterozygous V_H\Delta^a/WT^b mice. (A) IgM allotype expression in adult BM and fetal liver of V_H Δ^a /WT^b mice. Lymphocytes isolated from one adult BM or pooled from six fetal livers (~16–18-d embryos) were stained with FITC-conjugated anti-mouse IgM^b, PE-conjugated anti-mouse IgM^a, and APC-conjugated anti-mouse B220. Plots shown are gated for B220⁺ cells. Numbers shown are IgM^{a+b+}/IgM cells × 100. (B) IgM allotype expression in splenic lymphocytes of a newborn C57BL/6 (WT^b/WT^b) mouse, a V_H Δ^a /V_H Δ^a homozygous mouse, and a V_H Δ^a /WT^b heterozy-gous mouse (representative of two mice analyzed). Spleen cells from ~2–3-d-old newborn mice were stained with FITC-conjugated anti-mouse IgM^b and PE-conjugated anti-mouse IgM^{a+b+}/IgM cells × 100. *, In these cases, the number shown is IgM^{a+b+}/(IgM^{a+b-} + IgM^{a+b+}) × 100 because the signals in the bottom right quadrant are a result of background staining that is present in mice that lack *Igh*^b allele (middle).

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Figure S4. Quantitative analysis of V–DJ rearrangements. (A) Representative blot of HS4 PCR products using twofold serially diluted DNA samples. Dilutions of four independent samples are shown. PCRs were carried out as described in Materials and methods except that the 30-cycle PCR was reduced to 20 cycles. (B) Representative blots of VDJ PCR products using two-fold serially diluted DNA samples as template. Methods were as described in Fig. 5B. Dilutions of three independent samples are shown. (C) Quantitative analysis (ImageQuant) of HS4 blots like that shown in A. Dilutions of a total of eight independent samples were included in these analyses. For each set of twofold serially diluted samples, the amount of template in the first sample was assigned a value of 1, the second a value of 1/2, the third 1/4, and so on. Relative amount of PCR product for each dilution point in a series was calculated as follows: sum of template amount used in the dilution series (e.g., 1 + 1/2 + 1/4 for a series of three dilutions) × (ImageQuant reading of the PCR product for the sample in question)/ (ImageQuant reading of the sum of the products in the dilution series). The graph is the best-fit line for data collected from eight sets of twofold serially diluted samples (Microsoft Excel trendline). (D–F) Quantitative analysis (ImageQuant) was performed as described in C for VDJ rearrangements using the promiscuous V_H primer (D), the V_HJ558 family primer (E), and the V_H7183 family primer (F). D–F are each plots of data collected from 14 independent twofold serial dilutions. Error bars show SD.



Figure S5. Pie charts showing relative usage of J κ gene segments in splenic B cells from WT and mutant mice. Each PCR product was quantified (e.g., V κ -J κ 4 products) and proportion for each PCR product was assigned relative to the summed value for all of the products (e.g., V κ -J κ 4/(V κ -J κ 1 + V κ -J κ 2 + V κ -J κ 4 + V κ -J κ 5). V κ -J κ 1 and V κ -J κ 2 PCR product values were pooled for these analyses.

Table S1. Absolute cell numbers	s in WT and mutant mice
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	$lgh^{b}(n = 3)$	$V_{\mu}E\mu^{a}/V_{\mu}E\mu^{a}(n = 3)$	$V_{\mu}\Delta^{a}/V_{\mu}\Delta^{a}(n = 3)$
BM (\times 10 ⁶)	-		
Total cells	25.83 <u>+</u> 2.84	21.83 ± 6.45	21.50 ± 1.00
Lymphocytes	7.22 ± 0.24	4.94 ± 1.17	5.10 ± 0.56
B220+	4.20 ± 0.12	$1.84 \pm 0.44^{*}$	$2.22 \pm 0.37^*$
Pro-B	0.49 ± 0.07	$0.27 \pm 0.06^{*}$	0.35 ± 0.10
Pre-B	2.09 + 0.26	0.78 + 0.17*	0.88 + 0.31*
Immature B	0.44 ± 0.05	$0.21 \pm 0.07^*$	$0.19 \pm 0.07^*$
Mature B	0.88 ± 0.13	$0.40 \pm 0.14^{*}$	$0.53 \pm 0.20^{*}$
Spleen (\times 10 ⁶)	—	—	_
Total cells	33.00 ± 2.18	31.67 ± 4.80	36.50 ± 5.07
Lymphocytes	26.85 <u>+</u> 2.88	25.46 ± 4.09	29.59 ± 3.59
B cells	14.36 ± 2.68	12.97 ± 2.32	14.60 ± 0.40
T cells	9.28 + 0.67	9.61 + 1.35	11.16 + 1.95

Cells recovered from BM and spleen of individual mice were counted (total cells). Aliquots were stained for B-lineage markers (B220, CD43, IgD, and IgM in BM) and for CD3- ε (T cells) and B220 (B cells) in spleen. Cell number for lymphocyte subpopulations were calculated based on total cell count and the percentage of total cells comprising each subpopulation (determined by flow cytometry). *, P < 0.001 (vs. Igh^b/Igh^b) by two-tailed Student's t-test. *n* = number of animals.

Table S2. $V_H - D_H - J_{H4}$ junction sequences on the WT *Igh*^b allele of $V_H \Delta^a / WT^b$ and $V_H E \mu^a / WT^b$ pre-B cells.

v _H Eh ~/wT~	pre-s cells	$\Lambda^{\rm H}$ \183- $\Pi^{\rm H}$ - $\Pi^{\rm H}$	4 re	arrangements				
V name	3'V-REGION	Nl	P	D-REGION	N2	D name	Productivity	CDR3 length
IGHV5-2	tgtgcaaga	ctt		ctacq	tgc		Р	12
TGHV5-2	tatacaada	C		atagtaaccac	ttaaca	TGHD3-1	P	12
TOUVE 0	tetees	+			ccgucg	101105 1	-	14
IGHV5-2	tgtgcaaga	Cat		gtetatge	cacggggggg		P	14
IGHV5-2	tgtgcaaga	caag		taactggg		IGHD4-1	Р	10
IGHV5-2	tgt	ccc		tggtt		IGHD2-3	P	8
IGHV5-2	tgtgcaaga	ca		taactggg		IGHD4-1	P	11
IGHV5-2	tgtgcaaga	ctt		ctacg	tac		P	12
TCHV5-2	tatacaada	<i>c</i>		actacggtagtagc	2	TCHD1-1	NP	
TOURIE 2						101121	ND	
IGHV5-2	tgtgcaaga	cgactg		ggtggt			NP	
IGHV5-2	tgtgcaaga			tggttactac	a	IGHD2-3	NP	
IGHV5-2	tgtgcaaggac			ctcccgcta	t		NP	
IGHV5-2	tgtgcaaga	с		atagtaactac	aacgagct	IGHD3-1	NP	
IGHV5-2	tatacaada	c		atagtaactac	gaggaget	IGHD3-1	NP	
TOWNE 2	tataa	6			gaegagee	TCHD2-1	ND	
IGHV5-2	LgLgC			LaCLALA	L	IGHD3-I	IN F	
IGHV5-2	tgtgcaaga			tggttacga	gagaggggggggggt	IGHD2-7	NP	
IGHV5-2	tgtgcaaga	С		tagtaactac	gtat	IGHD3-1	NP	
IGHV5-2	tgtgcaaga	catgggc		ccaaataggg	g		NP	
IGHV5-2	totocaaga	c		atgg	aa		NP	
TCHV5-2	tatacaada	-			, , , , , , , , , , , , , , , , , , ,	TOWD3-1	ND	
IGHVJ-2		C		actatagtaact	aaaaa .	IGHD5-1	NE	
IGHV5-2	tgtgcaaga	c		atagtaactac	gacgaget	IGHD3-1	NP	
IGHV5-2	tgtgcaaga			ctactatagtaac	cctgt	IGHD3-1	NP	
IGHV5-4	tgtgcaaga	ga		tctccgggggtg	gtet		P	13
IGHV5-4	totocaaga	a		atggtaactac	at	IGHD2-8	P	13
TCHV5-4	tatacaada	5		atac	5-		NP	
IGHVJ-4				gtac			IN E	
IGHV5-4	tgtgcaag			ctactatagtaac	ga	IGHD3-1	NP	
IGHV5-4	tgtgcaaga	gat		ctatgattacgac	gacggcccc	IGHD2-4	NP	
IGHV5-4	tgtgcaaga	gagagttct	t	agacageteagg	gacggg	IGHD3-2	NP	
IGHV5-4	tgtgcaaga	gatgg		ggtagtage	aatctc	IGHD1-1	NP	
TCHV5-6	tatacaagaca	5 55		taagacg	-		P	12
TOUVE 6	tataaaaaaa					TOUDI 1	-	11
IGHV5-6	tgtgcaagaca			ttattaetaeg	agggetae	IGHDI-I	P	11
IGHV5-6	tgtgcaagac	cg		gggaggttcta	g		NP	
IGHV5-12	tgtgcaaga	catg		actatgactacgac	ggttgg	IGHD2-4	P	15
IGHV5-12	tgtgcaaga	с		atgtctatgac	eccet	IGHD2-3	P	14
IGHV5-12	totocaaga	catecte		atggttactac	atee	IGHD2-3	NP	
TOWNE-9-1*	(Crea 114 is a	niccing)			9000	101102 0	ND	
IGHV3-9-1.	(CYS 114 15 1	urssing)					IN F	
IGHV5-17	tgtgcaagg	Cgaatcagecag	ſ	attactacggtagtag	ggaag	IGHDI-I	P	17
IGHV5-17	tatacaaaa	aggaacggg			330	TGHD1-1	P	15
	ogogodagg			actacggtagtag	aay	IONDI I	-	13
	ogogeaagg			actacggtagtag	aay	101121	-	15
v Aa/wmb	pre-B cells V	7183-D -T 4	rea	actacggtagtag	aay		-	13
V _H ∆ ^a /WT ^b]	pre-B cells V		rea	actacggtagtag	aay		-	
V _H ƻ/WT ^b] V name	pre-B cells V 3'V-REGION		rea P	actacggtagtag rrangements D-REGION	N2	D name	Productivity	CDR3 length
V _H ∆ ^a /WT ^b] V name IGHV5-1	pre-B cells V 3'V-REGION tgtttgaga	2 _H 7183-D _H -J _H 4 N1 cat	rea P	actacggtagtag rrangements D-REGION cctact	N2 tt	D name	Productivity NP	CDR3 length
$V_{H}\Delta^{a}/WT^{b}$] V name IGHV5-1 IGHV5-1	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga	$T_{\rm H}$ 7183- $D_{\rm H}$ - $J_{\rm H}$ 4 N1 cat	P P	actacggtagtag p-REGION cctact tggtaac	N2 tt	D name IGHD2-8	Productivity NP NP	CDR3 length
V _H Δ ^a /WT ^b j V name IGHV5-1 IGHV5-1 IGHV5-2	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga tgtttgcaag	$r_{\rm H}7183-D_{\rm H}-J_{\rm H}4$ N1 cat c c cat	P P	actacggtagtag D-REGION cctact tggtaac cttcqq	N2 tt	D name IGHD2-8	Productivity NP NP P	CDR3 length
$V_{H} \Delta^{a} / W T^{b}$] V name IGHV5-1 IGHV5-2 IGHV5-2	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaag	2 _H 7183-D _H -J _H 4 N1 cat c cat	P P	actacggtagtag mrangements D-REGION cctact tggtaac cttcggg	N2 tt	D name	Productivity NP NP P P	CDR3 length
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$V_{H}\Delta^{a}/WT^{b}$] V name IGHV5-1 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	r_{H} 7183- D_{H} - J_{H} 4 N1 cat c cat gaccg cat cat cat cat cat g	p	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg tactaggtacgac gacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac	N2 tt cccc ggg ac cct agga gggg	D name IGHD2-8 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4	Productivity NP P P P P P P NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$V_{H} \Delta a / WT^{b}$] <u>V name</u> IGHV5-1 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	$\frac{1}{1}$	p	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac ggacagctcaggctac ggggcagctcaggc tgattacga ctgagt tctactatgattacgac cctactatgattacgac cctactatgattacgac	N2 tt cccc ggg ac cct agga gggg	D name IGHD2-8 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD2-1	Productivity NP P P P P P NP NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactaggtagg tactatgattacgac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag	N2 tt cccc ggg ac cct agga gggg	D name IGHD2-8 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD2-1	Productivity NP P P P P P NP NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$V_{H}\Delta^{a}/WT^{b}$] V name IGHV5-1 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2 IGHV5-2	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	r_{H} 7183- D_{H} - J_{H} 4 N1 cat c cat gaccg cat cat cat cat cat cat cat	P P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac gacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatgattacgac actgaagt	N2 tt cccc ggg ac cct agga gggg	D name IGHD2-8 IGHD2-8 IGHD2-4 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1	Productivity NP NP P P P P P NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$V_{H} \Delta a / WT^{b}$] <u>V name</u> IGHV5-1 IGHV5-2 IGHV	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	$\frac{1}{1}$	P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatggtacgac ggacgctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatgattacgac cctactatag tagc	N2 tt cccc ggg ac cct agga gggg gggagg	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1	Productivity NP NP P P P P P NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$\begin{array}{c} V_{H}\Delta \ ^{a}/WT^{b} \ \\ \hline V_{H}\Delta \ ^{a}/WT^{b} \ \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	P P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac gagagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacga cctacatag tactatagattacga catagtatacga cctactatag	N2 tt cccc ggg ac cct agga gggg gggg gggg	D name IGHD2-8 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1	Productivity NP P P P P P NP NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$\begin{array}{c} V_{H}\Delta \ ^{a}/WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-2 \\$	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	⁷ _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	l rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac gacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatgattacgac ggggcagctcaggc tgatacca ggggcagctcaggc tgataccac ggggcagctcaggc tgataccac ggggagt tctactatgattacgac cctactatgattacgac cctactatgattacgac cctactatag tagc atagtaactac gtaactac gtaactac aaagggg	N2 tt cccc ggg ac cct agga gggg ggagg ggaagg ggaagg g	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1	Productivity NP P P P P P P NP NP NP NP NP NP NP NP	CDR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-2 \\ IGHV5$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat ca cat ca cat ca cat ca cat cat	l rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatggtacgac ggacagctcaggc tgattacga ctgagt tctactatgattacgac cctactatag tactatggttacgac cctactatag tagtactac ggacagctaggc atagtactac adagtactac gtactaca cctactatag tagc	N2 tt cccc ggg ac cct agga gggg gggagg ggaagg ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	CDR3 length 7 12 9 7 14
$\begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5-$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	$_{\rm H}$ 7183- $D_{\rm H}$ - $J_{\rm H}$ 4 N1 cat cat cat cat cat cat cat cat	p p	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctacatag tactatagattacgac cctactatag tagtaactac gtaactac aagggg ctatgaatacgac gtaactac aagggg ctatgattacgac gtaactac aagggg	N2 tt cccc ggg ac cct agga gggg gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-2 IGHD3-2	Productivity NP P P P P P P NP NP NP NP NP NP NP NP	2DR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Lambda \ ^{a} / WT^{b} \ \\ y \ name \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5-2$	pre-B cells V 3'V-REGION tgtttgaga tgtttgaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga tgtgcaaga	$_{H}$ 7183 $-D_{H}$ - J_{H} 4 N1 cat cat cat cat cat cat cat cat cat cat	i rea	actacggtagtag arrangements D-REGION cetact tggtaac ettegg categgg tactatgattacgac gacageteaggetac ggggeageteagge tgattacga etgaagt tetactatgattacgac cetaetatgattacgac cotaetatag tage atagtaactac gtaaetac aaagggg etatgatacgac agtaaetac aaagggg	N2 tt cccc gggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8	Productivity NP NP P P P P P NP NP NP NP NP NP NP NP	2DR3 length 7 12 9 7 14
$V_{H} \Delta a / WT^{b}$] V name IGHV5-1 IGHV5-2 IGHV	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat ca cat ca cat ca cat ca cat ca cat ca cat ca cat ca cat cat	p	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatggtacgac ggacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatggattacgac cctactatag tagc atagtaactac gtaactac ggaaggg ctatgattacgac aagggg ctatgattacgac atagtaactac aagggg ctatgattacgac	N2 tt cccc ggg ac cct agga gggg gggagg gggaagg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	CDR3 length 7 12 9 7 14
$\begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V_{II} \Delta \ ^{a} / WT^{b} \ \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 $	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	$\begin{array}{c} \begin{array}{c} {}_{H} 7183 - D_{H} - J_{H} 4 \\ \hline \\ N1 \\ \\ \hline \\ cat \\ c \\ cat \\ \\ cat \\ c$	p P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tactatagattacgac cctactatag tagtaactac ggtaactac aaagggg ctatgattacgac ggtaactac aaagggg	N2 tt cccc ggg ac cct agga gggg gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-4	Productivity NP P P P P P P NP NP NP NP NP NP NP NP	2DR3 length 7 12 9 7 14
$\begin{array}{c} V_{H} \Lambda \ ^{a} / WT^{b} \ \\ y \ name \\ \hline \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat cat cat cat cat cat cat cat cat cat	P P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg tactatgattacgac gacagctcaggctaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac agggaagtagtactac ggtaactac aggtaactac tgattacga	N2 tt cccc gggg ac cct agga gggg ggga ggga	D name IGHD2-8 IGHD2-8 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-4 IGHD2-4 IGHD2-1	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	20R3 length 7 12 9 7 14
$\begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ \end{array}$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	p P	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatggttacgac ggacagctcaggc tgattacga ctgagg tactatggttacgac cctactatgg tactatggttacgac cctactatag tagtactac ggaagtac atagtaactac aagggg ctatgattacgac ggtaactac atggtaactac tggtagtacgac ggtagtagtac acggtagtagcac acggtagtagcac	N2 tt cccc ggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-1 IGHD2-1 IGHD3-1	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	13 CDR3 length 7 12 9 7 14
$\begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V_{II} \Delta \ ^{a} / WT^{b} \ \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-4 \\ \end{array}$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgttgcaaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	a rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg tactatgattacgac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagtaactac ggtaactac aaagggg ctatgatacgac ggtaactac aagggaactac ggtaactac aaagggg ctatgattacgac ggtaactac aagggaactac tgattacgac tgattacgac ggtaactac atggtaactac tgattacga tgattacgac tgattacg	N2 tt cccc ggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	13 <u>CDR3 length</u> 7 12 9 7 14 14
$ \begin{array}{c} V_{H} \Lambda \ ^{a} / WT^{b} \ \\ y \ name \\ \hline \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-4 \\ \end{array} $	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat gaccg cat cat cat cat cat cat cat cat cat cat	a rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac gacagctcaggctag ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac aggtaactac aggtaactac aggtaactac tgattacga ctatgattacgac ggtaactac adagtaactac tgattacga dggtaactac tgattacga ggtagtagctac acgatgtaactac tgattacga	N2 tt cccc ggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-4 IGHD2-8 IGHD2-7 IGHD2-7 IGHD2-8	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	2DR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ IGHV5$	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	runt representation of the second sec	a rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac ggacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac gtaactac aagggg ctatgattacgac ggtaactac atggtaactac tggtagtaccac tgatagtaccac cgtagtagcac atggtagtaccac atggtagtaccac acgatagtaccac acgatagtaccac	N2 tt cccc ggg ac cct agga gggg gggagg gggaagg g ggaagg g ggga g ggga g ggga g gggg g gcgacccct	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7 IGHD2-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD3-1 IGHD2-8 IGHD2-8 IGHD2-8 IGHD3-1 IGHD3-2 IGHD	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	2DR3 length 7 12 9 7 14
V _H Δ ^a /WT ^b IGHV5-1 IGHV5-2 IGHV5-4	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	run 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	a rea	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg catcgggg tactatgattacgac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac agggagtagtagca ctatgatacgac ggtaactac aggtagtagcac tgattacga ggtaactac aggtagtagcac tgattacga ggtaactac tgattacga ggtaactac aggtagtagcac tgattacga ggtagtagcac tgattacga ggtagtagcac tctactatggttacga ggtagtagcac tctactatggttacga tctactatggttacga tgatactac tgattacga ggtagtagcac tctactatggttacga ggtagtagcac tctactatggttacga	N2 tt cccc ggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-2 ZGHD3-1 ZGHD3-1 ZGHD3-2 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-2 ZGHD3-1 ZGHD3-1 ZGHD2-4 ZGHD2-4 ZGHD2-4 ZGHD2-4 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-7 ZGHD3-1 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-7 ZGHD3-1 ZGHD2-7 ZGHD2-8 ZGHD2-8 ZGHD2-7 ZGHD3-1 ZGHD3-1 ZGHD2-8 ZGHD2-8 ZGHD2-8 ZGHD2-7 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-1 ZGHD3-2 ZGHD	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	2DR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Lambda \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline \\ \hline IGHV5-1 \\ IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-6 \\ \end{array} $	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	a a a	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg tactatgattacgac gacagctcaggctagge tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac aggtaactac aggtaactac aggtaactac tgattacga ctatgattacgac ggtaactac adggtaactac tgattacga ggtagtagtaccac tgattacga ggtagtagctac acgatagtaactac tgattacga ggtagtagctac acgatagtaactac tgattacga ggtagtagctac acgatagtaactac tctactatggttacga ggtagtagctac acgatagtaacgac tctactatggttacga ggatagtacga tctactatggttacga ggatagccagg tttattactacgg	N2 tt cccc gggg ac cct agga gggg ggga ggga	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD3-2 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-4 IGHD2-7 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-7 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-7 IGHD2-7 IGHD3-1 IGHD2-7 IGHD2-7 IGHD2-7 IGHD2-7 IGHD2-7 IGHD2-7 IGHD2-7 IGHD3-1 IGHD2-7 IGHD2-7 IGHD3-1 IGHD2-7 IGHD2-7 IGHD2-7 IGHD3-1 IGHD2-7 IGHD7	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	2DR3 length 7 12 9 7 14
$ \begin{array}{c} V_{H} \Delta \ ^{a} / WT^{b} \ \\ \hline V \ name \\ \hline IGHV5-1 \\ IGHV5-2 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-4 \\ IGHV5-6 \\ IGHV5-9 \\ \end{array} $	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	runt representation of the second sec	a a	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg catcgggg tactatgattacgac gggcagctcaggctac ggggcagctcaggc tgattacga ctgagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac aagggg ctatgattacgac ggtaactac atagtaactac aggtagtacca tgattacga ggtagtagcac atggtaactac tgattacga ggtagtagcac acggtagtagcac acgtagtacga tctactatggttacga ggatggtaagg gatagcacgg ttattaccacgg	N2 tt cccc ggg ac cct agga gggg gggagg gggagg g ggaagg g ggaagg g ggga g ggga g ggga g gggg g gggg g ggggg g g ggggg g g gggg	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7 IGHD2-7 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	13 CDR3 length 7 12 9 7 14 14 14
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$ \begin{array}{c} V_{H} \Lambda \ ^{a} / WT^{b} \ \\ y \ name \\ \hline \\ $	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	r _H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	a a	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcggg tactatgattacgac gacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac ggtaactac aggtaactac tgattacga dggtaactac tgattacga ggtagtactac tgattacga ggtagtactac tgattacga ggtagtactac tgattacga ggtagtactac tgattacga ggtagtagtacac acgatgtaactac tctactatggttacga ggaatggtaacga tctactatggttacga ggaatggtaacga tctatgatgtacga ggaatggtaacga tctatgatgtacga ggaatggtaccac acgatagtaacga tctatgatgtaccac tctactatggttaccac tctactatggttaccac acgatagtaacga ggaatggtaccac acgatagtaacga tctatgatgtaccac acgatagtaacga ggaatggtaccacac acgatagtaacacac tctactatggttaccac acacacacacacacacacacacacacacacaca	N2 tt cccc gggg ac cct agga gggg ggga ggga	D name IGHD2-8 IGHD2-8 IGHD2-4 IGHD3-2 IGHD3-2 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7 IGHD2-8 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-8 IGHD	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	13 CDR3 length 7 12 9 7 14 14 14 8
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	pre-B cells V 3'V-REGION tgtttgaga tgttgaga tgtgcaaga	'H 7183-D _H -J _H 4 N1 cat c cat cat cat cat cat cat cat cat c	a	actacggtagtag arrangements D-REGION cctact tggtaac cttcgg catcgggg catcgggg tactatgattacgac ggacagctcaggctac ggggcagctcaggc tgattacga ctgaagt tctactatgattacgac cctactatag tagc atagtaactac ggtaactac aggtagtagctac aggtagtagctac acggtagtagctac acgatgtaactac tgattacga ggtagtagctac acgatgtaactac tgattacga ggtagtagctac acggtagtaggt tctactatggttacga ggtagtagctac acgatgtaactac tctactatggttacga ggtagtagctac acgatgtaactac tctactatggttacga ggtagtagctac acgatgtactac atagtaactac tctactatggttacga ggaacggtagtacg tctactatgatgttactac ataa ggtaaccac ataa ggtaaccac acgatgtactac acgatgtactac ataa ggtaaccacgg tctatgatggttactac ataa ggtaaccacg gagggtact acgatgg aactrorr	N2 tt cccc gggg ac cct agga ggggg gggagg gggagg g gggagg g ggggg g gcgaccct gacgggg g gcgaccct gacgggg g gcgaccct g gggg g gcgaccct	D name IGHD2-4 IGHD2-4 IGHD3-2 IGHD3-2 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-4 IGHD2-8 IGHD2-8 IGHD2-8 IGHD2-7 IGHD2-7 IGHD2-7 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-4 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-4 IGHD2-4 IGHD2-3 IGHD2-3 IGHD2-3 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-4 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD3-1 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-4 IGHD2-8 IGHD2-7 IGHD	Productivity NP NP P P P P NP NP NP NP NP NP NP NP N	13 CDR3 length 7 12 9 7 14 14 14 14 8 12 14 14 14 12 14 14 12 14 14 14 12 14 14 14 14 14 14 14 14 14 14

 V_H 7183D_µJ_{H4} rearrangements were cloned by PCR from genomic DNA of isolated pre-B cells using a V_H 7183 family primer and J_{H4} primer. DNA sequences were analyzed by the IMGT/V-QUEST program (http://imgt.cines.fr). Because of ambiguity, assigned D_H genes were manually revised where necessary, using IMGT/V-QUEST results as guidance, and junctions without a perfect alignment of seven or more nucleotides to a C57BL/6 D_H gene were not assigned a D_H gene. P, productive; NP, non-productive. CDR3 lengths were given for productive clones (the IMGT/V-QUEST program assigns the CDR3 to sequences between Cys 104 in the V_H and Trp/Phe in the conserved Trp/Phe-Gly-X-Gly motif in J_H). Clones were isolated in two separate experiments: in one experiment, clones were obtained from pre-B cells of an individual $V_H E \mu^a/WT^b$ and an individual $V_H \Delta^a/WT^b$ mouse; in the second experiment, pre-B cells were isolated from a pool of two mice for each genotype.*, in this rearrangement, the V_H and J_H genes can be identified, but the Cys 104 was missing so the junction was not analyzed.**, the V_H used in this rearrangement is from another (non 7183) V_H family.