Barcoded RT-PCR B-cell receptor IsoTyper amplification from RNA from mice

Primers

Reverse RT primer mix (10uM per primer): these should be mixed in an equimolar manner to a final concentration of 10uM per primer for the PCR reaction.

Primer ID	Primer
MusM_IGHGA_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNCAGGGACCAAGGGATAGAC
MusM_IGHGB_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNCAGGGGCCAGTGGATAG
MusM_IGHA_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNTGTCAGTGGGTAGATGGTG
MusM_IGHM_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNCATGGCCACCAGATTCT
MusM_IGHE_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNAAGGGGTAGAGCTGAGGG
MusM_IGHD_BC	GATACGGCGACCAATGTNNNNTNNNNTNNNNGGCTTTGCACTCTGAGAG

PCR reverse primer:

Primer ID	Primer
UNIB	GATACGGCGACCAATGT

Forward primer mix (10uM per primer): this refers to the current forward primers used in the PCR reaction that bind to FR1 region of human IgH sequences. These can be mixed in an equimolar manner to a final concentration of 10uM per primer for the PCR reaction:

Primer ID	Primer sequence*	
VH-for11	CAGATKCAGCTTMAGGAGTC	
VH-for13	CAGGTTCACCTACAACAGTC	
VH-for15	GARGTGMAGCTGKTGGAGAC	
VH-for2	CAGGTGCAAMTGMAGSAGTC	Group 1 forward primers
VH-for5	GAKGTGCAGCTTCAGSAGTC	
VH-for8	GAGGTGMAGCTASTTGAGWC	
VH-for1	GAGGTTCDSCTGCAACAGTY	
VH-for12	CAGGCTTATCTGCAGCAGTC	
VH-for14	CAGGTGCAGCTTGTAGAGAC	Group 2 forward primers
VH-for3	GAVGTGMWGCTGGTGGAGTC	
VH-for7	CAGRTCCAACTGCAGCAGYC	

* Using standard ambiguity codes

Reagents:

Manufacturer	Reagent	Cat No.
KAPA Biosystems	Real-time PCR library amplification	KK2702
Life technologies	SuperScript III	18080-044
Thermo Scientific	RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777-019

And library preparation kit for MiSeq sequencing.

Reaction volumes are given per reaction.

RT-PCR

Add the following reagents using between up to 500ng RNA, where the total RNA volume is diluted to give 14ul:

Reagent:	Volume (ul)
Barcoded reverse primer, 10uM	1
10mM dNTP Mix	1
Lymphocyte RNA template (Xug)	14
Total volume	16

Heat to 65°C for 5 minutes and incubate on ice for at least 1 minute. Then add the following to the tube/plate well:

Reagent:	Volume (ul)
5X First-Strand Buffer	4
1ul 0.1 M DTT	1
1ul RNaseOUT	1
1ul SuperScript III RT	1
Aliquot	7

Incubate 50'C for 60mins and 70'C for 15mins.

cDNA clean up

1. Shake AMP XP beads.
2. Add 36uL (or corrected amount, see table below) of beads to cDNA and pipette mix 10 times.

RT Reaction Volume (µL)	AMPure Volume (µL)
10	18
20	36
50	90

3. Incubate for 8 minutes at RT.
4. Place plate/tubes on the Magnet plate. Wait for 2 minutes.
5. Aspirate the cleared solution from the reaction plate and discard.
6. Take plate from the Magnet. Spin plate.
7. Place it in the Magnet. Aspirate and discard flow through.
8. Add 50uL of H2O. Pipette up and down 10 times.
9. Place plate on the Magnet. Wait for 2 minutes. Take the cDNA (eluted from beads).

PCR reaction:

Split cDNA into two equimolar aliquots, and perform two PCRs using the the following reaction mixture:

	Group 1 forward primers	Group 2 forward primers
	Volume (ul)	Volume (ul)
2x KAPA buffer	25	25
Forward primer mix A or B (10uM per primer)	1	1
Universal const. reverse (UNIB) (10uM)	1	1
Aliquot of PCR mix	27	27
cDNA template	23	23
Total volume	50	50

PCR program:

1 cycle:	95oC	5min
5 cycles:	98oC	5sec
	72oC	2min
5 cycles:	98oC	5sec
	65oC	10sec
	72oC	2min
30 cycles:	98oC	20sec
	60oC	1min
	72oC	2min
Final extension:	72oC	7min

Combine the group 1 and group 2 primer PCR products per sample, and can proceed for library preparation and sequencing, performing the DNA cleanup first:

cDNA clean up

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