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

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

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.

Chain	Primer sequence
IGHA_human_BC	TGTCCAGCACGCTTCAGGCTNNNNTNNNNTNNNNGAYGACCACGTTCCCATCT
IGHM_human_BC	TGTCCAGCACGCTTCAGGCTNNNNTNNNNTNNNNTCGTATCCGACGGGGAATTC
IGHD_human_BC	TGTCCAGCACGCTTCAGGCTNNNNTNNNNTNNNNGGGCTGTTATCCTTTGGGTG
IGHE_human_BC	TGTCCAGCACGCTTCAGGCTNNNNTNNNNTNNNNAGAGTCACGGAGGTGGCATT
IGHG_human_BC	TGTCCAGCACGCTTCAGGCTNNNNTNNNNTNNNNAGTAGTCCTTGACCAGGCAG

PCR reverse primer

Chain	Primer sequence
CNU Barcode primer_S	TGTCCAGCACGCTTCAGGCT

PCR 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 should be mixed in an equimolar manner to a final concentration of 10uM per primer for the PCR reaction.

Chain	Primer sequence
VH1-FR1	GGCCTCAGTGAAGGTCTCCTGCAAG
VH2-FR1	GTCTGGTCCTACGCTGGTGAAACCC
VH3-FR1	CTGGGGGGTCCCTGAGACTCTCCTG
VH4-FR1	CTTCGGAGACCCTGTCCCTCACCTG
VH5-FR1	CGGGGAGTCTCTGAAGATCTCCTGT
VH6-FR1	TCGCAGACCCTCTCACTCACCTGTG

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)
Reverse RT primer mix, 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:

Set up the following reaction mixture:

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

Note: this uses half of the cDNA product, allowing for repeats if necessary.

PCR program:

1 cycle:	95oC	5min
5 cycles:	98oC	5sec
	72oC	2min
5 cycles:	98oC	5sec
	65oC	10sec
	72oC	2min
25-30 cycles:	98oC	20sec
	60oC	1min
	72oC	2min
Final extension:	72oC	7min

DNA clean up

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PCR product may then be used directly for library preparation and MiSeq sequencing (300bp PE).