Clonality analysis and spectratyping of B cell populations

A normal polyclonal B cell repertoire exhibits a bell-shaped curve of individual size-peaks according to a Gaussian-type distribution of length diversity within the VDJ junction. Conversely, clonal expansions or monoclonal B cell populations have only few or one single size-peak corresponding to few or only one dominating clone(s). To analyze the B cell repertoire on clonality/diversity V_H-DJ_H gene rearrangements from cDNAs of mouse peripheral blood, were amplified using PCR primers specific for the J558 V_H region gene together with a primer specific for the Cμ constant region gene.

1	μL	dNTP (10 mM)
5	μL	10 x PCR buffer
2.5	μL	V_HJ558 forward primer(10 μM)
2.5	μL	Cμ reverse primer (10 μM)
1.5	μL	50 mM $MgCl_2$
1	μL	Platinum *Taq* DNA polymerase (Invitrogen)
35.5	μL	H_2O
49	μL	
1	μL	cDNA
50	μL	

The following program was used:

1	95°C	2 min
2	95°C	1 min
3	60°C	1 min
4	72°C	1 min
5	72°C	5 min
6	4°C	pause

30-34x

Using a FAM-labeled Cμ constant region gene-specific primer in a run-off reaction, PCR products were labeled and subsequently analyzed on an ABI3100 capillary sequencer by

fragment length analysis. In additional experiments, J_H-specific primers were used instead of the Cμ constant region gene-specific primer. Whereas J558-Cμ fragment analysis generates an overview over the repertoire of B cell clones using this V_H gene segment, J558-J_H1-4 amplifications have a higher level of resolution for individual V_H-J_H combinations.

1	μL	dNTP (10 mM)
5	μL	10 x PCR buffer
2.5	μL	V_HJ558 forward primer(10 μM)
2.5	μL	Cμ-FAM/J_H(1-4)-FAM reverse primer (10 μM)
1.5	μL	50 mM $MgCl_2$
1	μL	Platinum *Taq* DNA polymerase (Invitrogen)
35.5	μL	H_2O
49	μL	
1	μL	1st round
50	μL	

The following program was used:

1	95°C	2 min	
2	95°C	1 min	10x
3	60°C	1 min	
4	72°C	1 min	
5	72°C	5 min	
6	4°C	pause	

Note: The annealing temperature will depend on your primer sets.