CD21 PCR (Mark Zabel 03.08.2004)

mz116:
Neo primer near the 3' terminus (~100bp from end (3849-3869) of pIR2) :
5' **GAC CGC TAT CAG GAC ATA GCG** 3'

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Current Oligo, 21-mer [33]:
Td = 66.6° [nearest neighbor method]
Tm = 72.8° [%GC method]
Tm = 66° [2°*(A+T) + 4°*(G+C) method]
Mr     =     6.5 k (one strand)
Mr     =    13.0 k (two strands)
µg/OD =    46.1    (dsDNA)
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Base	Number and %	
A	6	[28.6]
C	6	[28.6]
G	6	[28.6]
T	3	[14.3]
A + T	9	[42.9]
G + C	12	[57.1]

mz117b:
forward CD21 intron 1 primer from 1418-1435 :
5' **CAG TGT TAG TCA CTA CTC CG** 3'

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Current Oligo, 20-mer [15]:
Td = 54.3° [nearest neighbor method]
Tm = 68.2° [%GC method]
Tm = 60° [2°*(A+T) + 4°*(G+C) method]
Mr     =     6.1 k (one strand)
Mr     =    12.4 k (two strands)
µg/OD =    47.4    (dsDNA)
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Base	Number and %	
A	4	[20.0]
C	6	[30.0]
G	4	[20.0]
T	6	[30.0]
A + T	10	[50.0]
G + C	10	[50.0]

mz118br:
reverse CD21 intron 1 primer from 1866-1845 :
5' **GCC AAA ATG AAG CAA GAA TCA G** 3'
use with mz 116 to generate ~800 bp band in the knockout, and with mz117 to generate a 400 bp band in wt.

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Lower Primer, 22-mer [54]:
Td = 63.8° [nearest neighbor method]
Tm = 71.3° [%GC method]
Tm = 66° [2°*(A+T) + 4°*(G+C) method]
Mr     =     6.9 k (one strand)
Mr     =    13.6 k (two strands)
µg/OD =    47.4    (dsDNA)
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Base	Number and %	
A	8	[36.4]
C	4	[18.2]
G	7	[31.8]
T	3	[13.6]
A + T	11	[50.0]
G + C	11	[50.0]

Thermocycle:
5 min @ 95C for 1 cycle

30 sec @ 95C,
30 sec @ 58C,
2 min @ 72C;
for 33 cycles

5 min @ 72C for 1 cycle