

PCR villin-Cre genotyping

Patrick Kaiser, April 2011

Use ~100 ng Template DNA for PCR in a 25ul volume reaction.

Primers

MyD88A: 1ul from 10nm substock

5'-aga cag gct gag tgc aaa ctt gtg ctg-3'

MyD88B: 1ul from 10nm substock

5'-ccg gca act aga aca gac aga cta tcg-3'

VilCre1: 2.5ul 10nm from 10nm substock

5'-gtg tgg gac aga gaa caa acc-3'

VilCre2: 2.5ul 10nm from 10nm substock

5'-aca tct tca ggt tct gcg gg-3'

Products in one-pot reaction:

MyD88 internal (positive ctrl): lower band ca. 400 bp

VilCre transgen: upper band (only if tg⁺) ca. 1100 bp

PCR Cycles (Touchdown):

Step 1: 5 min 94°C

Step 2: 1 min 94°C

Step 3: 1 min 67°C

Step 4: 1 min 72°C

**cycle 4x through step 2-4, decreasing
annealing temperature by 1°C per step (=down to 63°C)**

Step 5: 1 min 94°C

Step 6: 1 min 62°C

Step 7: 1 min 72°C back to Step 5, 30 cycles

Step 8: 5 min 72°C

4°C ∞