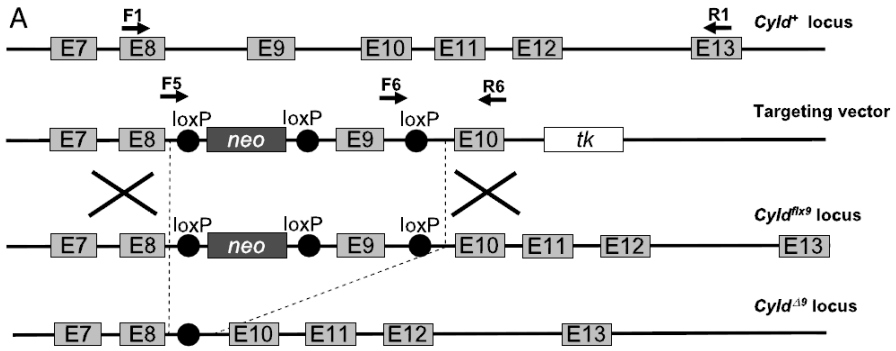
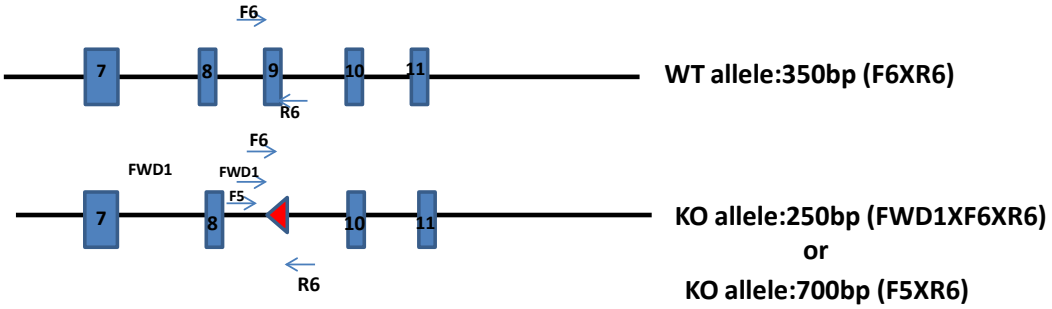
Genotyping protocol for Δ9 mice:



Targeted genomic locus of mouse Cyld



Primers used

FWD1: 5'-GATGGCTCTTGTCACCACTT-3'
F5: 5'- GCA GGC TGT ACA GAT GGA AC- 3'
F6: 5'-CGT GAA CAG ATG TGA TGA AGG-3'
R6: 5'-CTA CCA TCC CTG CTA ACC AC-3'

For the detection of WT allele, we use primer combination F6 x R6. For the detection of KO allele, we use either primer combination FWD1 x F6 x R6 (new protocol) or primer combination F5/ R6 (old protocol)

WT allele Protocol

F6:5'-CGT GAA CAG ATG TGA TGA AGG-3'
R6: 5'-CTA CCA TCC CTG CTA ACC AC-3'

PCR program

1) T=94°C 3 min
2) T=94°C 45 sec
3) T=54°C 45 sec (depending on the PCR machine, range 52-56°C)
4) T=72°C 1min 30 sec
5) Go to step 2 35x
6) T=72°C 7 min
7) T=16°C forever

Expected size: 350 bp (WT allele), 450bp (Floxed allele). For Δ9 heterozygotes we detect only the 350bp band, whereas for homozygote Δ9 /Δ9 mice F6XR6 combination doesn't give any product.

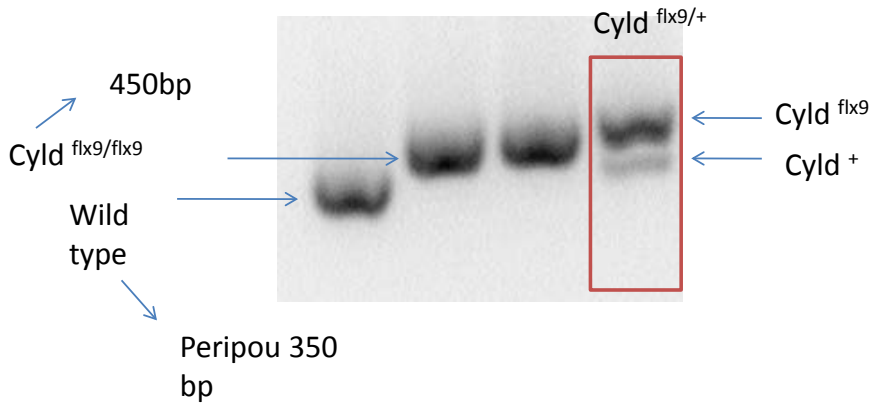
Reaction

Template: we use 2 µl DNA from 1/20 dilution of 30 µl genomic DNA preparation from 2mm mouse ear punch. (Each mouse ear punch yields typically 2-3µg genomic DNA)

2,5 µl dNTPs (2 mM stock)
5 µl 5x reaction buffer
1,5 µl MgCl2 (stock 25 mM)
1 µl primer F6 (10 pmol/µl)
1 µl primer R6 (10 pmol/µl)
0,25 µl Taq
H20 up to 25 µl

Analyze PCR product in 2% agarose gel.
Controls: C57BL/6 genomic DNA, Fl9/wt genomic DNA

Expected image for Floxed/WT bands, using F6-R6 primer combination.



KO allele genotyping protocol, using primer combination FWD1 x F6 x R6 (NEW protocol)

FWD1: 5'-GATGGCTCTTGTCACCACTT-3'
F6: 5'-CGT GAA CAG ATG TGA TGA AGG-3'
R6: 5'-CTA CCA TCC CTG CTA ACC AC-3'

PCR program (Biorad machine)

1) T=94°C 3 min
2) T=94°C 45 sec
3) T=57°C 45 sec
4) T=72°C 1min 30 sec
5) Go to step 2 35x
6) T=72°C 3 min
7) T=4°C forever

Expected size: 250 bp for KO allele and 350bp for WT

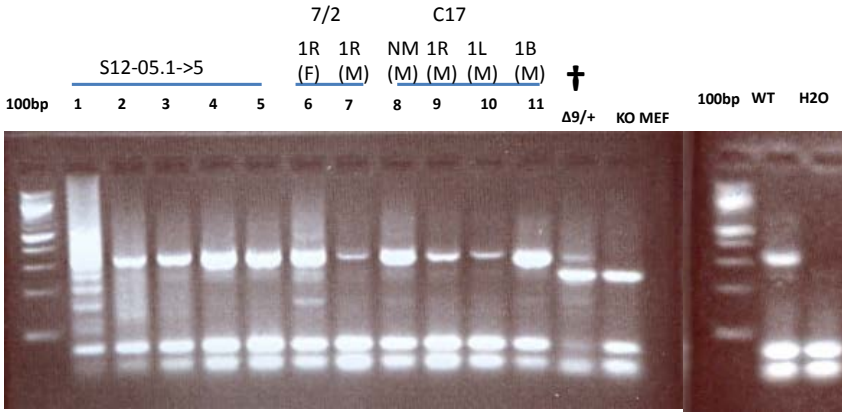
Reaction

Template: we use 2 µl DNA from 1/20 dilution of 30 µl genomic DNA preparation from 2mm mouse ear punch. (Each mouse ear punch yields typically 2-3µg genomic DNA)
2,5 µl dNTPs (2 mM stock)
5 µl 5x reaction buffer
1,5 µl MgCl2 (stock 25 mM)
1 µl primer F6 (10 pmol/µl)
1 µl primer FWD1 (10 pmol/µl)
2 µl primer R6 (10 pmol/µl)
0,25 µl Taq
1 µl DMSO
H20 up to 25 µl

Analyze PCR product in 2% agarose gel.
Controls: C57BL/6 genomic DNA, KO MEF DNA (Δ9/Δ9) and or Δ9/+ genomic DNA.
There is a preference for KO allele using the triple primer combination, when having heterozygotes.

Expected image for WT/KO bands using F6-FWD1-R6 primer combination.

PCR for screening of Δ9 and WT alleles, using a set of 3 primers (F6-R6-KO1)



KO allele genotyping protocol, using primer combination F5 x R6 (OLD protocol)

F5: 5'- GCA GGC TGT ACA GAT GGA AC- 3‘

R6: 5’-CTA CCA TCC CTG CTA ACC AC-3’

PCR program

1. T=94°C 3 min
2. T=94°C 45 sec
3. T=52°C 45 sec
4. T=72°C 90 sec
5. Go to 2 35x
6. T=72°C for 3 min
7. Hold at 16°C

Expected size: 700 bp

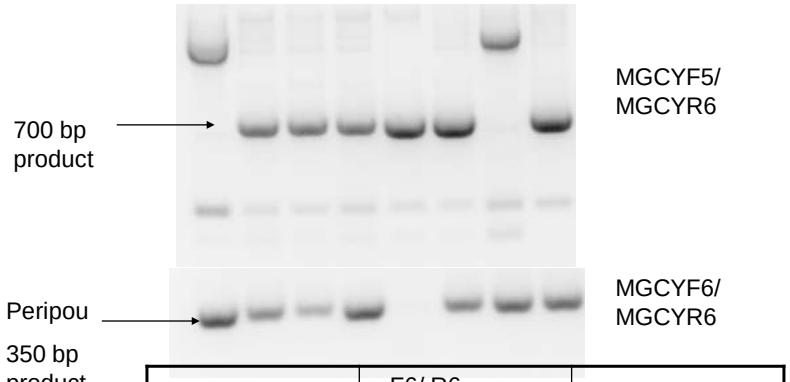
Reaction

Template: we use 1 µl DNA from 50 µl genomic DNA preparation from mouse tails. (Dissolve @ 56°C for 1-2h before use.)

2,5 µl dNTPs (2 mM stock)
5 µl 5x reaction buffer
1,5 µl MgCl2 (stock 25 mM)
1 µl primer F5 (10 pmol/µl)
1 µl primer R6 (10 pmol/µl)
0,25 µl Taq
H20 up to 25 µl

Analyze PCR product in 2% agarose gel.
Controls: C57BL/6 genomic DNA, KO MEF DNA (Δ9/Δ9) or Δ9/+ genomic DNA.

Expected image for WT/KO bands, using F5-R6 primer combination.



F5/ R6	F6/ R6	
+	+	**eterozygo**
+	-	**Cyd ko (perinatally lethal)**
-	+	**Wild type**