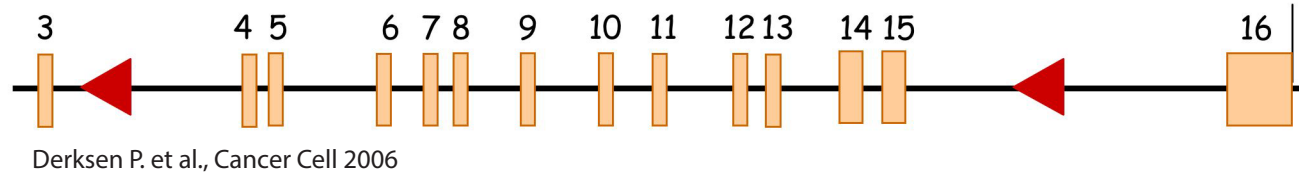


Poly(A)

Cdh1^F

or E-cadherin Flox



Ecad JV38 PCR.

Primer 1 Ecad JV 38 **ACA TGT TTG TAT CGA TCT CAG**
Primer 2 Ecad JV 39 **CCA TAC ACT GAT AAT GTC AGA**

- DNA 1 µl
- PCR buffer 10 x 2 µl
- dNTPs 2 mM 2 µl
- MgCl₂ 1 µl
- LZ1 10 µmol 1 µl
- LZ2 10 µmol 1 µl
- Taq 0.2 µl
- H₂O 13 µl

PCR program:

- 5 min 94 ° C
- 30 sec 94 ° C \
- 30 sec 58 ° C > 29 cycles
- 50 sec 72 ° C /
- 5 min 72 ° C

Bands:

Pos: +/- **300 bp**
Neg: +/- **200 bp**

Southern analysis of Cdh1-ex.4-15-flox mice:

- Detection of 5' loxP site (located in intron 3):
Digest: *EcoRV*
Probe: exons 7+8 PCR-probe
Germ-line band: 23.5 kbp.
5' loxP band: 13.5 kbp.
- Detection of 3' loxP site (located in intron 15):
Digest: *ScaI*
Probe: exon 16 PCR-probe
Germ-line band: ±21 kbp.
3' loxP band: ±17 kbp.
- Detection of deletion of exons 4-15:
Digest: *ScaI*
Probe: exon 16 PCR-probe
Germ-line band: ±21 kbp.
Del. ex. 4-15 band: ±14 kbp.

Primer-sets for (radioactive) PCR-amplification of Cdh1 exon probes:

- Cdh1 exon 7+8 PCR-probe: Forward primer (JV-15): 5'- GAA CCT CCG TGA TGA A TCT -3'
Reverse primer (JV-18): 5'- CGT GCT TGG GTT GAA GA AGG -3'
Product: 305 nt.
- Cdh1 exon 16 PCR-probe: Forward primer (JV-46): 5'- ACC TGA AGG CAG CCG A GCG -3'
Reverse primer (JV-47): 5'- CCC TAG TCG TCC TCA CC CCG -3'
Product: 211 nt.

PCR analysis of Cdh1-ex.4-15-flox mice:

Four primers have been developed for detection of the different alleles:

- A: Cdh1-int3-fwd (JV-38): 5'- ACA TGT TTG TAT CGA TCT CAG -3'
- B: Cdh1-int3-rev (JV-39): 5'- CCA TAC ACT GAT AAT GTC AGA -3'
- C: Cdh1-int15-fwd (JV-42): 5'- TCA ATC TCA GAG CCC CAC CTA -3'
- D: Cdh1-int15-rev (JV-43): 5'- CCT GCC ATG ATT GTC ATG GAC -3'