NETHERLANDS CANCER INSTITUTE
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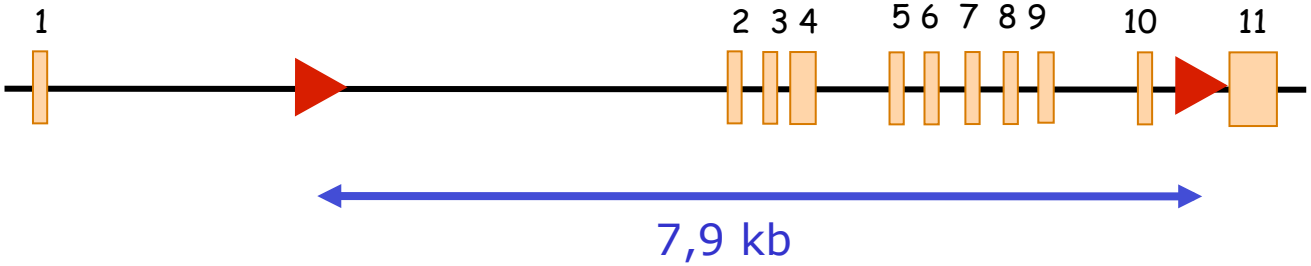
Trp53F



Jonkers et al., Nature Genetics 2001

Southern analysis of p53-ex.2-10-flox mice:

Detection of 5' loxP site (located in intron 1):	Digest: *Eco*RV Probe: 5' *Xba*I PCR-probe Germ-line band: 13 kbp. 5' loxP band: 8 kbp.
Detection of 3' loxP site (located in intron 10):	Digest: *Bam*HI Probe: exon 11 PCR-probe pseudogene band 10 kbp. Germ-line band: 10 kbp. 3' loxP band: 8 kbp.
Detection of deletion of exons 2-10:	Digest: *Bgl*II Probe: 5' *Xba*I PCR-probe Germ-line band: 18 kbp. D ex. 2-10 band: 9.4 kbp.

Primer-sets for (radioactive) PCR-amplification of p53 5' and exon 11 probes:

p53 5' *Xba*I PCR-probe:	Forward primer: T3 Reverse primer: T7 Product: plm. 700 nt.
P53 exon 11 PCR-probe:	Forward primer: 5'-CTA CCT GAA GAC CAA GAA GG-3' Reverse primer: 5'-TGG AGG ATA TGG ACC CTA TG-3' Product: 390 nt.

PCR analysis of p53-ex.2-10-flox mice:

A: p53-int1-fwd: 5'- CAC AAA AAC AGG TTA AAC CCA G -3'
B: p53-int1-rev: 5'- AGC ACA TAG GAG GCA GAG AC -3'
C: p53-int10-fwd: 5'- AAG GGG TAT GAG GGA CAA GG -3'
D: p53-int10-rev: 5'- GAA GAC AGA AAA GGG GAG GG -3'

- DNA	1	µl	
- PCR buffer 10 x	2	µl	
- dNTPs 2 mM	2	µl	
- $MgCl_2$	1	µl	
- primer 1 10 µmol	1	µl	
- primer 2 10 µmol	1	µl	
- Taq		0.2	µl
- H_2O	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		

Detection of 5' loxP site (located in intron 1):	primers: A + B	
	Germ-line product:	288 nt.
	5' loxP product:	370 nt.
Detection of 3' loxP site (located in intron 10):	primers: C + D	
	Germ-line product:	431 nt.
	3' loxP product:	584 nt.
Detection of deletion of exons 2-10:	primers: A + D	
	Germ-line product:	none
	D ex. 2-10 product:	612 nt.