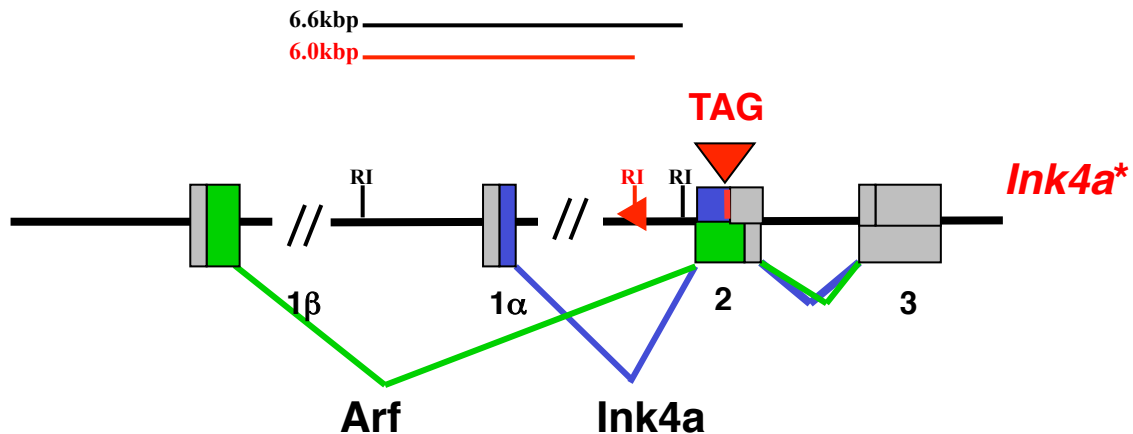


# Cdkn2a\*



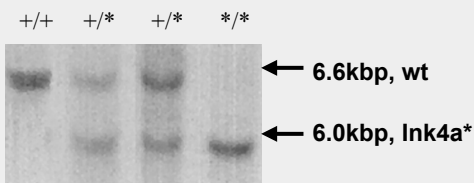
*Krimpenfort et al., Nature 2001*

## Souther blot analysis: presence LoxP

Digest: EcoRI

Probe: 500bp fragment 5'Xho site 5' exon1

### Results:



## PCR analysis P16B (Ink4a\*) mutant: presence LoxP

Primers around LoxP site inserted in BamHI site in first intron

Forward pr. (1): GCAGTGTTCAGTTTGAACCC

Reverse pr.: (2) TGTGGCAACTGATTCAGTTGG

### Conditions

DNA	1	μl
PCR buffer 10x	2	
dNTP 2mM	2	
MgCl 50mM	1	
Primer 1 (10 μM)	2	
Primer 2 (10 μM)	2	
Taq	0,2	
H2O	9,8	

### PCR reaction

5 min.	94	C
30 cycles:		
30 sec	94	
30 sec	59	
50 sec	72	

5 min

72

### Expected products:

Wild type:	500bp
Mutant:	600

## PCR analysis P16B (Ink4a\*) mutant: presence mutation related StyI site

Primers around LoxP site inserted in first intron and 3' exon 2

Forward pr. (1): AGGCGGGACTAGGAGCCTTTATG

Reverse pr.: (2) CGTGCTTGAGCTGAAGCTATGCC

### Conditions

DNA	1	μl
PCR buffer 10x	2	
dNTP 2mM	2	
MgCl 50mM	1	
Primer 1 (10 μM)	2	
Primer 2 (10 μM)	2	
Taq	0,2	
H2O	9,8	

### PCR reaction

5 min.	94	C
30 cycles:		
30 sec	94	
30 sec	60	
50 sec	72	

5 min

72

Subsequent digestion product with StyI

### Expected products:

Wild type:	600bp
Mutant:	480 and 120