Identification of MR-alleles by PCR

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1. NID-DNA preparation

- cut tail (2-5 mm)
- add 200 µl NID-buffer (50 mM KCl; 10 mM Tris-HCl, pH 8,3; 2 mM $MgCl_2$; 0,1 mg/ml gelatin, 0,45% NP40; 0,45% Tween20)
  2 µl Proteinase K (10 mg/ml)
- incubate O/N, 56°C
- incubate 10 min., 95°C
- use 2 µl for PCR

NID-buffer stocks:	for 500 ml:
1 M KCl	25 ml
1 M Tris-HCl pH 8,3	5 ml
1 M $MgCl_2$	1 ml
100 mg/ml gelatin	0,5 ml
10% NP40	22,5 ml
10% Tween20	22,5 ml
	424 ml H_2O

2. 3-Primer PCR (Roche-Taq: 1U/µl)

2,5 µl	10x buffer with $MgCl_2$ (15 mM)
1,0 µl	dNTPs (5 mM)
0,5 µl	Primer A (10 µM)
0,5 µl	Primer B (10 µM)
0,5 µl	Primer C (10 µM)
0,5 µl	Taq-DNA-polymerase (1 U/µl)
17,5 µl	H_2O
2,0 µl	NID-DNA prep.

95°C, 30'' / 63°C, 1' / 72°C, 1' // 35 cycles

Primer A (MRflox-7): 5'-CTGGAGATCTGAACTCCAGGCT-3'
Primer B (MRflox-10): 5'-TAGAAACACTTCGTAAAGTAGAGCT-3'
Primer C (MRflox-8): 5'-CCTAGAGTTCCTGAGCTGCTGA-3'

MR^{null}: 390 bp / MR^{flox}: 335 bp / MR^{wt}: 285 bp