

Preparation of Tail DNA for PCR Using Non-Ionic Detergents

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(Adapted from Jackson Laboratory's Protocol)

1. Cut the last 2 mm of the mouse tail and place it in a 1.5 ml microfuge tube.
2. Add 200 μ l of PBNB buffer containing 100 μ g/ml Proteinase K.
3. Incubate in 56°C water bath with occasional vortexing until the tissue is lysed (1-3 hours). If necessary, the incubation can be allowed to proceed overnight.
4. Heat samples at 95°C for 5 min in heat block to inactivate the Proteinase K. Make sure to perforate the lid of the tubes with a small needle to avoid exploding tubes.
5. Use 0.5 μ l of sample for a 20 μ l PCR reaction.

PBNB (PCR buffer with non-ionic detergents) Preparation

(50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, 0.1 mg/ml Gelatin, 0.45% v/v IGEPAL and 0.45% v/v Tween 20).

To 450 ml of ddH₂O add:

5.0 ml	1M Tris-HCl Stock, pH 8.3
1.87 g	KCl
0.255 g	MgCl ₂ .6H ₂ O
2.25 ml	IGEPAL
2.25 ml	Tween 20
0.05 g	Gelatin

Bring volume to 500 ml with ddH₂O and autoclave.

Prepare 10 ml aliquots and freeze at -20°C.

Note: Gelatin will dissolve only after autoclaving.