

DNA extraction from tail biopsies- “rapid method”

Notes: When preparing samples of genomic DNA, use only those materials and solutions reserved for genomic use. These include Eppendorf tubes, proteinase K, dH₂O, ethanol, and TE.

When pipetting genomic DNA samples, only use pipette tips from which the tips have been snipped off (or purchased large-bore tips). This prevents shearing of the DNA, maintaining an average molecule size > 80 – 100 kb.

For PCR analysis after genomic extraction, use non-ionic detergents such as Triton X100 in the Lysis buffer, as SDS can inhibit PCR reactions.

Procedure will work for subsequent Southern analysis, depending upon the enzyme, but an additional phenol-chisam extraction and the use of SDS in the lysis buffer is more highly recommended (see DNA extraction- Jones lab protocol).

Procedure:

1. Using a new razor blade, cut approximately ¼ to ½ inch from the tip of the tails and place in sterile eppendorf tube. Add 600 µl of **Rapid Lysis Buffer** to each tube (see below).
2. Add 20 µl of freshly prepared Proteinase K (20mg/ml, in sterile water) to each tube. Cap tightly.
3. Rock or shake the tubes gently overnight @55 - 65° C.
4. Pellet the hair and other cellular debris (as needed) by spinning the tubes for 2 minutes @ maximum speed in a microfuge.
5. Transfer supernatant to a clean 1.5 ml eppendorf tube (recovery is ~ 500µl).
6. Add 1/10th volume (50µl) of 3M sodium acetate.
7. Add 600µl absolute ethanol (ambient temperature). Cap tightly, and precipitate the DNA by gentle inversion.
8. Spin down DNA at maximum speed in microfuge for 30 seconds.
9. Rinse pellet gently with 80% ethanol and let air dry completely. Add 100µl TE and dissolve overnight at room temperature (or 4 hours @55 - 65° C.). Flick only, do not vortex the samples.

Rapid Lysis Buffer

50mM Tris pH 7.5
50mM EDTA pH 8.0
100mM NaCl
5mM DTT
0.5mM Spermidine
1% Triton x-100 (v/v)

To make 100mls of buffer:

5ml 1M Tris, pH 7.5
10ml 0.5M EDTA, pH 8.0
10ml 1M NaCl
0.5ml 1M DTT
50µl 1M Spermidine
1ml Triton x-100