

DNA Extraction Protocol: large tadpoles and post-metamorphic juveniles

(modified from Sparrow, D. B., Latinkic, B., and Mohun, T. J. (2000). A simplified method of generating transgenic *Xenopus*. *Nucleic Acids Res.* 28, E12.).

Sample Preparation:

Euthanize tadpoles and juveniles by prolonged immersion in 0.05% benzocaine. Transfer to 15 ml tubes (1 individual/tube) and freeze in an ethanol-dry ice bath. Store at -80°C .

Proteinase K and RNase Treatment

Incubate each sample in 3 ml Proteinase K solution at 55°C overnight (Proteinase K solution: 10 mM Tris pH 8.0; 0.1% EDTA; 0.5% SDS; 100 $\mu\text{g/ml}$ Proteinase K. Inactivate Proteinase K by incubation at 90°C for 10 minutes. Cool to room temperature. Add RNase A (10 mg/ml stock) to a final concentration of 50 $\mu\text{g/ml}$ and incubate at 37°C for 90 minutes.

Extraction and Precipitation

Extract twice with an equal volume of buffered phenol, then once with chloroform. Add 0.2 volumes 10 M ammonium acetate, followed by 2 volumes 100% ethanol. Precipitate at -20°C .

DNA collection

Centrifuge at 10,000 rpm for 45 minutes. Wash pellet 2X with 70% ethanol and air dry. Resuspend in 1 ml TE at 37°C overnight.

Yield

Expected DNA yields vary with the size of the individual. Medium-sized premetamorphic tadpoles yield approximately 100 μg DNA, while larger postmetamorphic juveniles can yield over 700 mg DNA.