

DNA extraction from mouse tail to genotyping

(NO ORGANIC SOLVENTS EXTRATION)

- 1) Obtain the last 2 mm of the ear or tail tissue and place directly into 75 μ l **alkaline lyse reagent** in a PCR tube. (Tails can be stored at frozen in PBS or PBND until use).
- 2) Samples heated in 95°C 10 min –1 h. I actually let them until the tissue is dissolved. After heating, samples are cooled to 4°C and 75 μ l **Neutralization reagent** are added to each sample.
- 3) One to five μ l of the final preparation are used per each PCR reaction.

Alkaline lysis reagent FINAL VOLUME = 50 ml
25mM NaOH (from 1M) 1/40 --> 1.25ml
0.2mM Na₂-EDTA 2H₂O (from 1M) 1/5000 --> 10ul
(410,31g/mol; 1M = 20.5g in 50ml)
pH: around 12 (not adjust)

Neutralization reagent FINAL VOLUME = 50 ml
40mM Tris-HCl (from 1M) 1/25 --> 2ml
(157,60g/mol; 1M = 7.88g in 50ml)
pH: around 5 (not adjust)

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