

# TheOnyx

## Genomic DNA Extraction from Mouse Tails on TheOnyx

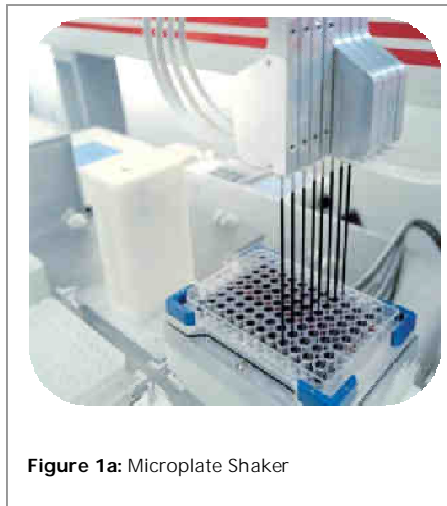
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### Overview

The manual extraction of genomic DNA from mouse tails is a laborious and time consuming laboratory routine. Traditional phenol-chloroform based extraction methods can be difficult to automate due to the involvement of centrifugation steps. Here we describe a fully automated solution for the purification of genomic DNA from mouse tail lysates with TheOnyx, using magnetic beads coated with a DNA binding matrix. The isolated DNA samples were used in downstream PCR applications with excellent results.

### Modules required for this application

- Non-cooled reagent rack
- 96well pipetting position, non-cooled
- 96well pipetting position, heated to 55°C
- Microplate shaker (**Figure 1a**)
- Magnetic separator (**Figure 1b**)
- Magnetic bead stirrer (**Figure 1c**)
- Disposable tip racks
- Sample tube rack / plate (according to the vessel format for collecting mouse tails)



**Figure 1a:** Microplate Shaker



**Figure 1b:** Magnetic Separator



**Figure 1c:** Magnetic Bead Stirrer

## Method

The method uses AGOWA's mag Midi DNA isolation kit, and is optimized for 50 µl of tissue lysate.

### *a) Digestion of mouse tails*

Mouse tails (sections weighing 2 – 40 mg approx.) are digested by adding 50 µl lysis buffer (10 mM Tris-HCl, pH 7.5; 10 mM EDTA; 150 mM NaCl; 0.5% SDS) and 20 µl Proteinase K (Sigma Cat. No. P-2308; 20 mg/ml dissolved in water or lysis buffer). After incubating overnight (60°C with shaking at 200 rpm) the mouse tails should be digested. If not, an additional 10 µl Proteinase K is added and the tails shaken for a further 2 hours. Samples are then centrifuged to remove hairs, and the supernatants transferred to a fresh deep well plate or Eppendorf tubes.

### *b) Automated DNA extraction from lysates*

The genomic DNA is separated from tissue lysate by binding to magnetic particles. Impurities are removed during washing steps. Finally, the purified DNA is eluted from the magnetic particles in presence of a suitable elution buffer while incubating at an increased temperature.

## Robotic protocol in detail

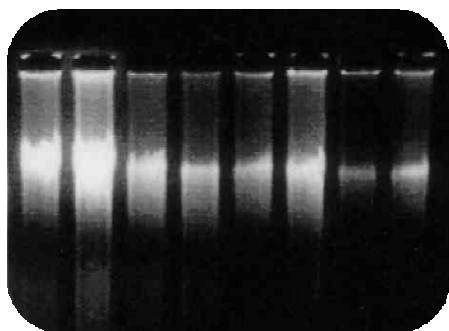
1. 100 µl "Binding buffer" (1:1 mixture of Lysis buffer BLM : ethanol) and 16 µl magnetic bead suspension are pipetted into each well of a 96 PCR microtiter plate on an ambient 96well pipetting position.
2. 50 µl of lysate (digested mouse tail) is transferred into each well and mixed thoroughly.
3. The samples are incubated at room temperature for 2 minutes.
4. After incubation the microtiter plate is transferred to the magnetic separator and left for 1 minute, allowing the beads to gather as a pellet at the side of each well.
5. Supernatant is discarded by gentle pipetting.
6. The microtiter plate is moved back to the pipetting position, and 170 µl of wash buffer BLM1 added to each well. To wash the beads the plate is moved from side to side on the separator for 10 minutes.
7. The beads are then gathered at the side of each well by resting the microtiter plate on the separator for 1 minute.
8. Wash buffer BLM1 is removed by gentle pipetting. Special care is taken to remove all residual liquid.
9. For the second washing step the plate is moved back to the pipetting position and 175 µl wash buffer BLM2 is added to each well.

10. To wash the beads the plate is again moved from side to side on the separator for 10 minutes and then rested for 1 minute.
11. Wash buffer BLm2 is removed from each well by gentle pipetting.
12. The washing procedure with wash buffer BLm2 is repeated.
13. To dry the magnetic bead pellets (removing all traces of acetone) the microtiter plate is transferred onto a heated pipetting position (55°C) for 30 minutes.
14. The beads are then resuspended in 100 µl elution buffer BLm.
15. Genomic DNA is removed from the beads by incubating the resuspended samples at 55°C for 10 minutes. During this time the microtiter plate is agitated 3 times on the integrated shaker, each time for 10 seconds.
16. Purified genomic DNA is separated from the beads by positioning the microtiter plate on the magnetic bead separator. The cleared DNA supernatant is finally transferred into a clean plate.

## Results

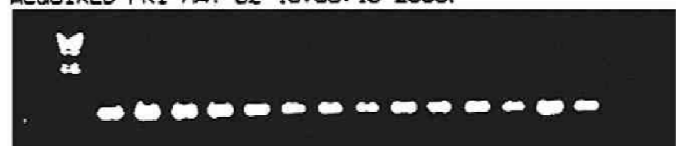
We processed customer samples (kindly provided by Pekka Kallunki, Lundbeck A/S, Copenhagen) of different tail sizes on TheOnyx. Results are shown in **Figure 2**. The quality and purity of the genomic DNA is checked by measuring the OD260/280 ratio of the samples (**Table 1**), and in downstream PCR experiments. The results are presented in **Figure 3**.

- Time per run for a plate of 96 samples is approximately 2 hours 45 minutes.
- Less than 1 µl of the extracted genomic DNA typically is needed for downstream PCR reactions.



**Figure 2:** Agarose gel image of genomic DNA samples extracted on TheOnyx

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**Figure 3:** Agarose gel of mouse beta-globin PCR products obtained from genomic DNA samples extracted on TheOnyx

Sample ref.	OD260	OD280	260 / 280	Conc. (mg/μl)	Yield (μg)
1-3-3	0.207	0.116	1.78	113.7	9.1
90	0.433	0.250	1.73	237.9	19.0
95	0.345	0.185	1.86	189.5	15.2
11	0.040	0.023	1.77	22.17	1.8
31	0.586	0.331	1.77	29.3	2.3
29	0.346	0.199	1.74	17.3	1.4
30	1.306	0.735	1.76	65.3	5.9
33	0.377	0.225	1.68	18.9	1.7
1-3-9	1.951	1.092	1.79	97.6	8.8
MWG	0.573	0.329	1.74	28.6	2.6

Table 1: Yield and concentration of extracted samples. OD measurements are made with a Discovery HT microplate reader from AVISO. The variations in yield result from the different sizes of mouse tails.

## Conclusions

Magnetic bead separation provides a simple, cost-effective and automation-friendly procedure for the extraction of genomic DNA. Here the procedure has been optimized for mouse tail lysates. No manual intervention steps are required from start to finish. The yields of DNA obtained are more than sufficient for the downstream application. Following extraction, the DNA sample concentration can be normalized on TheOnyx to a user-defined concentration via the appropriate dilution command within RoboManager operating software. PCR reactions performed using the purified DNA produced clean products as expected.

## Acknowledgement

We thank Pekka Kallunki (Lundbeck A/S, Copenhagen) for providing mouse tails and experimental data.

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