

TheOnyx

Genomic DNA Extraction from Bird Blood

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Overview

The manual extraction of genomic DNA from bird blood 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 bird blood with TheOnyx, using magnetic beads coated with a DNA binding matrix. The isolated DNA samples can be used in downstream PCR applications.

Modules required for this application

- Disposable reagent trough rack
- Non-cooled reagent racks for sample tubes
- Tube Rack for 16 x 2 ml & 6 x 14 ml tubes, temperature-controlled
- Flat pipetting position for MTP, temperature-controlled
- 96well pipetting position, heated to 55°C
- Microplate shaker heated to 55°C (**Figure 1a**)
- Magnetic separator (**Figure 1b**)
- Magnetic bead stirrer (**Figure 1c**)
- Disposable tip racks (200 µl and 1000 µl)
- Stacker for MTP, temperature-controlled
- Universal MTP carrier

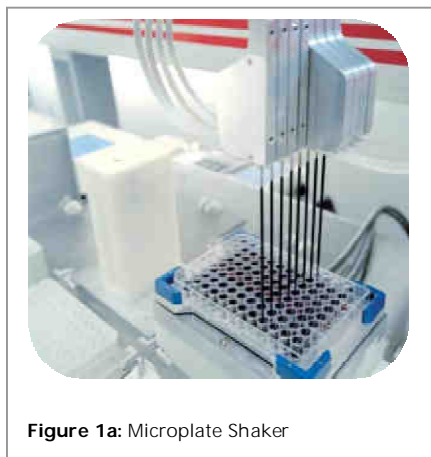


Figure 1a: Microplate Shaker



Figure 1b: Magnetic Separator



Figure 1c: Magnetic Bead Stirrer

Method

The method uses AGOWA's mag Midi DNA isolation kit (Cat-Nr. 40402), and is optimized for 50 µl of bird blood.

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

Robotic protocol in detail

1. 250 – 350 µl of dilution buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M Na-EDTA, 1% n-lauroylsarcosine, pH 8.0) is distributed to the blood. Samples are mixed thoroughly to avoid any clogging.
2. 50 µl of binding buffer BLm and 5 µl of protease are distributed to an empty PCR plate.
3. 50 µl of diluted blood sample is transferred to each well of the PCR plate and mixed to obtain a homogeneous solution.
4. Plate is moved to the preheated shaker and incubated for 10 minutes at 55°C.
5. 50 µl of ethanol and 16 µl of magnetic beads are transferred to the samples.
6. Plate is shaken for 15 seconds and incubated for 2 minutes at room temperature.
7. After incubation the PCR 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.
8. Supernatant is discarded by gentle pipetting.
9. The PCR plate is moved back to the pipetting position, and 170 µl of wash buffer BLm1 added to each well.
10. To wash the beads the PCR plate is moved from side to side on the separator for 10 minutes.
11. The beads are then gathered at the side of each well by resting the PCR plate on the separator for 1 minute.
12. Wash buffer BLm1 is removed by gentle pipetting. Special care is taken to remove all residual liquid. For the second washing step the PCR plate is moved back to the pipetting position and 175 µl wash buffer BLm2 is added to each well.
13. To wash the beads the PCR plate is again moved from side to side on the separator for 10 minutes and then rested for 1 minute.
14. Wash buffer BLm2 is removed from each well by gentle pipetting.
15. The washing procedure with wash buffer BLm2 is repeated.
16. To dry the magnetic bead pellets (removing all traces of acetone) the PCR plate is transferred onto the heated shaker (55°C) for 30 minutes.
17. The beads are then resuspended in 100 µl elution buffer BLm.
18. Genomic DNA is removed from the beads by incubating the resuspended samples at 55°C for 10 minutes. During this time the PCR plate is agitated 3 times on the heated shaker, each time for 30 seconds.
19. Purified genomic DNA is separated from the beads by positioning the PCR plate on the magnetic bead separator. The cleared DNA supernatant is finally transferred into a clean plate (approx. 90 µl).

Results

We processed blood samples from birds with different dilutions 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 sample (**Table 1**).

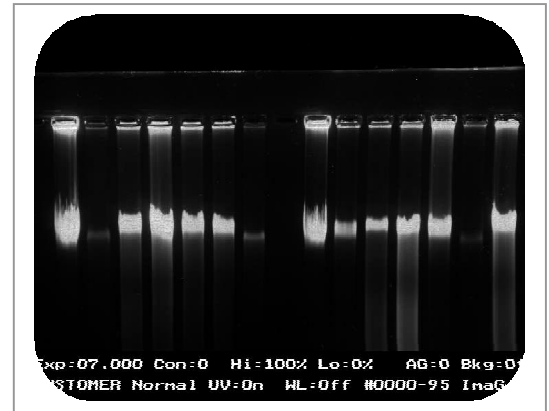


Figure 2: Agarose gel image of genomic DNA samples extracted on TheOnyx

Sample ref.	OD260	OD280	260 / 280 corr.	Conc. (mg/μl)	Yield (μg)
A1	0.184	0.102	1.80	37.0	3.4
A2	0.024	0.012	1.92	5.0	0.5
A3	0.105	0.056	1.80	21.1	1.9
A4	0.192	0.113	1.73	38.4	3.5
A5	0.103	0.055	1.85	20.8	1.9
A6	0.048	0.026	1.82	9.7	0.9
B1	0.034	0.019	1.76	6.9	0.6
B2	0.060	0.034	1.68	12.9	1.2
B3	0.097	0.060	1.81	16.7	1.5
B4	0.068	0.034	1.88	14.4	1.3
B5	0.100	0.055	1.74	20.8	1.9
B6	0.025	0.011	1.94	5.9	0.6

Table 1: Yield and concentration of gDNA after extraction from 50 μl blood samples. OD measurements are made with a Discovery HT microplate reader from AVISO. The variations in yield result from differences in predilutions of blood samples.

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 bird blood. 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.

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