Collar thickness of 2.82–2.85 mm was noticed in the regenerated seedlings and was not affected by the pre-treatments studied. Seed treatment had a profound influence on seedling vigour index, as it improved from 298.67 in untreated control to almost double in seeds treated with $KNO_3$ (Figure 1 *b*).

Substrate is known to have profound influence on nursery success of a species; however, limited information is available in case of the genus *Musa*[9]. In experiment 2, significantly improved germination percentage (from 28.7% to 90.7%) and seedling vigour index (from 513.73 to 1703.3) were recorded when vermicompost was compared with coir pith as substrate (Figure 2).

Considering improved germination characteristics, seed treatment with 0.1% $KNO_3$ for 24 h and vermicompost as substrate could be recommended for raising vigorous seedlings. This could help in conservation of this threatened species and its subsequent utilization. Further, habitat enrichment would also be helpful for island wildlife, including bats, birds, rats, elephants and other unknown fauna, which are dependent on fruits of this species for food.


1. Murugan, C., Prabhu, S., Sathiyaseelan, R. and Pandey, R. P., *A Checklist of Plants of Andaman and Nicobar Islands* (eds Singh, P. and Arisdason, W.), ENVIS Centre on Floral Diversity, Botanical Survey of India, Kolkata, 2016.
2. Singh, S., Waman, A. A., Bohra, P., Gautam, R. K. and Dam Roy, S., *Genet. Resour. Crop Evol.*, 2016, **63**, 1431–1445.
3. Singh, L. J., *Nordic J. Bot.*, 2017, **35**(1), 77–84.
4. Abdul Baki, A. A. and Anderson, J. D., *Crop Sci.*, 1973, **13**, 630–633.
5. Waman, A. A., Bohra, P. and Norman, A., *J. For. Res.*, 2018, **29**, 1283–1289; https://doi.org/10.1007/s11676-017-0562-9.
6. Burgos-Hernandez, M., Castillo-Campos, G., Mata-Rosas, M., Gonzalez, D., Vovides, A. P. and Murguia-Gonzalez, J., *Seed Sci. Technol.*, 2014, **42**, 16–27.
7. Dayarani, M., Dhanarajan, M. S., Arun, K., Uma, S. and Narayani, P., *J. Appl. Hortic.*, 2014, **16**(2), 126–130.
8. Puteh, A. B., Aris, E. M., Sinniah, U. R., Rahman, M. M., Mohamad, R. B. and Abdullah, N. A. P., *Afr. J. Biotechnol.*, 2011, **10**(65), 14373–14379.


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# Gelatin-coated magnetic nanoparticles for genomic DNA isolation from biological samples

The selection of an appropriate DNA isolation method for a specific biological sample (whole blood, saliva, stool, urine, fresh tissue or paraffin-embedded tissue) is a prerequisite for any molecular testing. This selection is dependent on many factors, including yield, purity, time, safety, requirement of specialized equipment, trained personnel, intended downstream applications, cost and sample source.

The use of magnetic nanoparticles (MNPs) provides a number of advantages in biotechnological applications, including nucleic acid isolation[1,2]. Even though uncoated MNPs can bind to DNA and can be used for its isolation, polymer-coated MNPs provide higher DNA recovery. MNPs coated with different polymers such as agarose and silica have been previously described and used in bacterial cells[3–5]. In this study, we synthesized gelatin-coated magnetic nanoparticles (GMNPs) for use in the isolation of genomic DNA from biological samples.

GMNPs were synthesized by chemical co-precipitation of $Fe^{+3}$ and $Fe^{+2}$ in an alkaline solution under hydrothermal conditions[6,7]. Briefly, GMNPs were prepared by mixing 1 M $FeSO_4 \cdot 7H_2O$ and 2 M $FeCl_3 \cdot 6H_2O$ with 1 ml of gelatin solution (0.15 g/ml, previously melted), followed by the addition of 8 M ammonium hydroxide. The $Fe_3O_4$ (magnetite) nanoparticles were formed at approximately pH 10. Co-precipitation was done at 60°C for 30 min. In the gelatin solution, iron nanoparticles interact electrostatically with amide bonds of the gelatin chain[8]. GMNPs were purified by washing three times with deionized water and using a high-gradient magnetic field to remove impurities. To estimate the total yield, GMNPs were first dried at 100°C for 2 h. Then they were resuspended in deionized water at a concentration of 50 mg/ml. The nanoparticles obtained exhibited a strong magnetic response.

The particles were characterized, and the mean particle size determined by transmission electron microscopy (TEM). Briefly, GMNPs were suspended in 500 µl distilled water. Next, 5 µl of the suspension was dropped into a Formvar/Carbon 200 mesh grids and left to dry at room temperature. The particles were examined using TEM (Hitachi 7500 Transmission Electron Microscope operating at 80 kV). Images were obtained using an AMT camera equipped with AMT image capture engine (V602). Images were analysed using FIJI. For analysis, each image was scaled, and each particle was measured by drawing a line across the diameter. One hundred particles per sample were measured and used for analysis. GMNPs were stored at 4°C.

The procedure for DNA isolation was standardized for whole blood, saliva and stool samples. The samples were collected from 30 healthy participants. Written informed consent was obtained from all of them. For DNA standardization, whole blood samples were collected in ethylenediaminetetra acetic acid (EDTA)

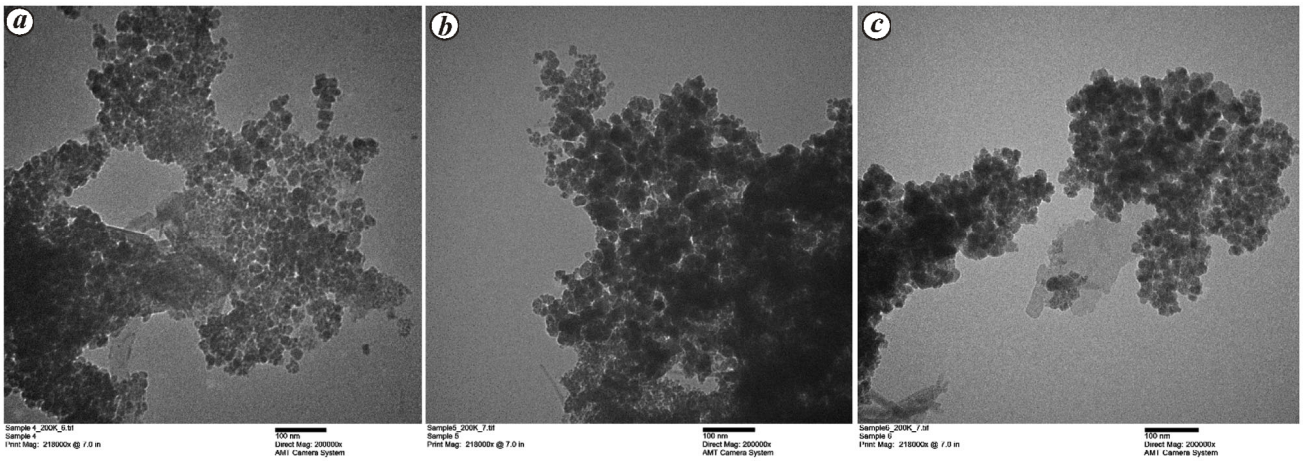


**Figure 1.** Gelatin-coated magnetic nanoparticles observed under transmission electron microscope (200,000 X). Storage temperature (***a***) 25°C, (***b***) 4°C, (***c***) –20°C.

**Table 1.** Quantity and quality of genomic DNA isolated using gelatin-coated magnetic nanoparticles from biological samples

| Sample type | Sample quantity | Mean concentration (ng/µl) | Mean $A_{260}/A_{280}$ | Mean $A_{260}/A_{230}$ | Total amount of DNA isolated (ug) |
|---|---|---|---|---|---|
| Whole blood ($n$ = 30) | 100 ul | 22.5 ± 5.17 | 1.73 ± 0.12 | 1.74 ± 0.92 | 2–4 |
| Saliva ($n$ = 30) | 200 ul | 53.2 ± 12.23 | 1.94 ± 0.10 | 1.85 ± 0.98 | 2–6 |
| Stool ($n$ = 30) | 20 mg | 96.2 ± 22.10 | 1.51 ± 0.10 | 0.54 ± 0.29 | 2–10 |

tubes. Next, 100 ul of whole blood was mixed with 100 ul of cell lysis buffer (10 mM Tris, 26 mM EDTA and 0.5% sodium dodecyl sulphate (SDS) by pipetting. The solution was incubated at room temperature for 5 min. After incubation, 10 ul of GMNPs (50 mg/ml) and 100 ul of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000) were added to the lysate. The solution was mixed and incubated at room temperature for 5 min. Magnetic pellet was immobilized in a magnetic rack (made with neodymium rare earth permanent magnets; New England Biolabs, Inc., MA, USA) and the supernatant was removed. Magnetic pellet was washed three times with wash buffer (10 mM Tris-HCl, 1 mM EDTA, 70% ethanol, pH 8) and dried for 5 min. Then, it was resuspended in 100 ul of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and incubated at 65°C for 10 min. The supernatant containing DNA was transferred to a fresh tube. The yield and quality of DNA isolated were analysed (QIA expert, Qiagen, USA).

Saliva sample was collected in a sterile 50 ml centrifuge tube, of which 200 ul was used for DNA isolation. The sample was mixed with 200 ul of cell lysis buffer mix by pipetting. The solution was incubated at room temperature for 5 min. After incubation, 20 ul of GMNPs and 200 ul of binding buffer were added to the lysate. The rest of the procedure was the same as for whole blood. Stool samples were collected in a sterile tube, of which 20 mg was used to isolate DNA. The sample was mixed with 500 ul of cell lysis buffer and incubated at room temperature for 5 min. After incubation, 20 ul of GMNPs was added to the lysate followed by 200 ul of binding buffer. The rest of the procedure was the same as for whole blood.

Quality of genomic DNA isolated from whole blood, saliva and stool samples was analysed by PCR amplification of X-specific *ATL1* and Y-specific *SRY* gene sequences. All PCRs were performed in a 50 ul reaction volume (Platinum *Taq* Polymerase Invitrogen, USA) according to the manufacturer's procedure and using a thermal cycler (GTQ Cycler 24 from Hain Lifescience (Germany)). *ALT1*-specific sequence (301 bp) and *SRY*-specific sequence (239 bp) were co-amplified first by two sets of PCR primer pairs: X1 (5′-CCCTGATGAAGAACTTGTATCTC-3′) and X3 (5′-GAAATTACACACATAGGTGGCACT-3′) and Y1.5 (5′-CTAGACCGCAGAGGCGCCCAT-3′) and Y1.6 (5′-TAGTACCCACGCCTGCTCCGG-3′) respectively[9–11] using 100 ng of DNA template. Thermal cycling was performed at 94°C for 3 min, followed by 45 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec. Next, 20 ul of the first PCR was used on the nested PCR with the following primers: X2 (5′-TCGCCTTTCTCAAATTCCAAG-3′) and X3 (5′-GAAATTACACACATAGGTGGCACT-3′) and Y1.7 (5′-CATCCAGAGCGTCCCTGGCTT-3′) and Y1.8 (5′-CTTTCCACAGCCACATTTGTC-3′). Thermal cycling for this PCR was performed at 94°C for 3 min, followed by 20 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec. PCR products were analysed using 1.5% agarose gel electrophoresis and visualized with blue light.

GMNPs were analysed by TEM, demonstrating well-separated and evenly distributed spherical nanoparticles. The mean diameter was of ~13 nm and size distribution ($\sigma$) was ~0.27 (Figure 1). GMNPs exhibited a strong magnetic response. There was no sedimentation of particles and no oxidation observed in GMNPs stored at 4°C and –20°C even after six months, thus confirming the long-time stability (Figure 2).

The yields of recovered genomic DNA using GMNPs as a solid-phase support were ~2–4 ug per 100 ul of whole blood sample, ~2–6 ug per 200 ul of saliva, and ~2–10 ug per 20 mg of stool sample (Table 1). The average optical density (OD) ratio, viz. $OD_{260}/OD_{280}$ was 1.7, indicating that the DNA obtained was of

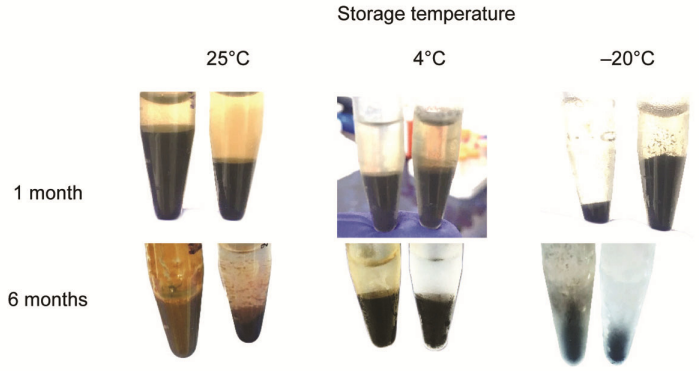


**Figure 2.** Photographs showing GMNPs stored at different temperatures.

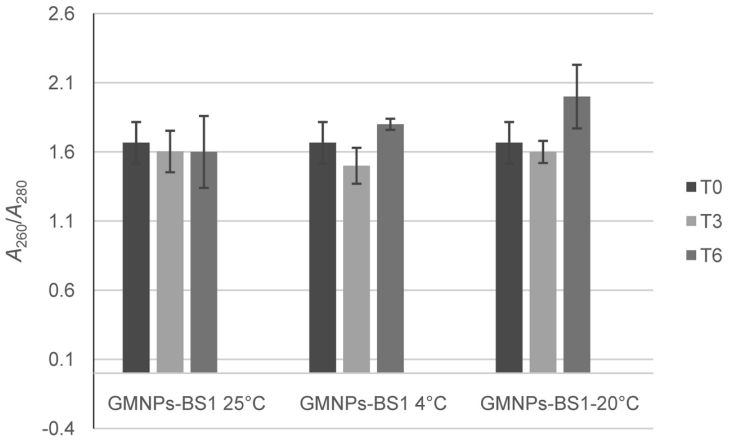


**Figure 3.** Long-term storage of GMNPs evaluated by analysing the quality of genomic DNA isolated ($OD_{260}/OD_{280}$ ratio between 1.7–2.0), sample number ($n = 3$). T0, Initial temperature; T3, Three months; T6, Six months.

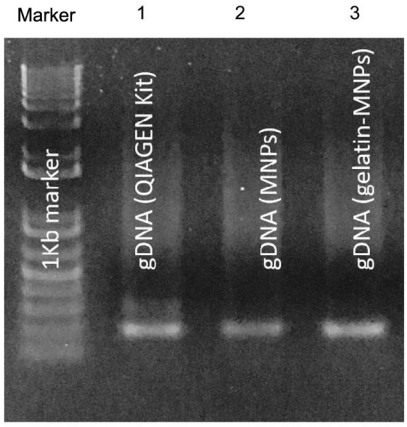


**Figure 4.** Gel electrophoresis of PCR products amplified from genomic DNA extracted using silica column from QIAGEN kit (lane 1), MNPs (lane 2) and GMNPs (lane 3).

good quality with negligible protein contamination ($OD_{260}/OD_{230}$ ~1.7) (Figure 3).

Successful PCR amplification of *ATL1* gene-specific sequence on X chromosome and the SRY gene-specific sequence on Y chromosome was achieved using genomic DNA extracted using GMNPs. As shown in Figure 4, amplification pattern was consistent for all samples, and was further confirmed by direct sequencing. These GMNPs were used to develop genomic DNA isolation with INBIOMag Genomic DNA Kit (INBIOMEDIC, Peru). In summary, GMNPs for genomic DNA isolation developed in this study provide a rapid, simple, and inexpensive method that does not require the use of organic solvents.

*Conflicts of interest:* All authors declare no conflict of interest

1. Borlido, L., Azevedo, A. M., Roque, A. C. A. and Aires-Barros, M. R., *Biotechnol. Adv.*, 2013, **31**, 1374–1385; doi:10.1016/j.biotechadv.2013.05.009.
2. Intorasoot, S., Srirung, R., Intorasoot, A. and Ngamratanapaiboon, S., *Anal. Biochem.*, 2009, **386**, 291–292; doi:10.1016/j.ab.2008.12.032.
3. Taylor, J. I., Hurst, C. D., Davies, M. J., Sachsinger, N. and Bruce, I. J., *J. Chromatogr. A*, 2000, **890**, 159–166; doi:10.1016/S0021-9673(00)00107-2.
4. Yoza, B., Matsumoto, M. and Matsunaga, T., *J. Biotechnol.*, 2002, **94**, 217–224; doi:10.1016/S0168-1656(01)-00427-8.
5. Chiang, C. L., Sung, C. S., Wu, T. F., Chen, C. Y. and Hsu, C. Y., *J. Chromatogr. B*, 2005, **822**, 54–60; doi:10.1016/j.jchromb.2005.05.017.
6. Mehta, R. V., Upadhyay, R. V., Charles, S. W. and Ramchand, C. N., *Biotechnol. Tech.*, 1997, **11**, 493–496; doi:10.1023/a:1018457915380.
7. Saiyed, Z. M., Ramchand, C. N. and Telang, S. D., *J Phys. Condens. Matter.*, 2008, **20**, 204153; doi:10.1088/0953-8984/20/20/204153.
8. Gaihre, B., Aryal, S., Khil, M. S. and Kim, H. Y., *J. Microencapsul.*, 2008; doi:10.1080/02652040701737697.
9. Tungwiwat, W., Fucharoen, G., Ratanasiri, T., Sanchaisuriya, K. and Fucharoen, S., *Clin. Chim. Acta*, 2003, **334**, 173–177; doi:10.1016/S0009-8981(03)00224-9.
10. Gunter, C. *et al.*, *Hum. Mol. Genet.*, 1998, **7**, 1935–1946; doi:10.1093/hmg/7.12.1935.
11. Lo, Y. M. D., Patel, P., Sampietro, M., Gillmer, M. D. G., Fleming, K. A. and Wainscoat, J. S., *Lancet*, 1990, **335**, 1463–1464; doi:10.1016/0140-6736(90)-91491-R.

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