

Genomic DNA extraction (via bead beater) #1 – original 'FastPrep' method

Hazards: Binding matrix contains conc. guanidine thiocyanate which is an irritant.

Notes: This protocol is versatile and can be used for any kind of cells/samples (animal, plant, microbe, soil, faeces etc). Note that the DNA obtained is of lower quality than that obtained via chemical/enzymatic lysis (e.g. above protocol), and it will be sheared to ~ 1-10 kb in size. This is fine for PCR, but may not be ok for making clone libraries. Reference: Yeates and Gillings (1998)

Protocol

1. Prepare bead-beater tubes ahead of time: Get glass beads plus the scoop and funnel from the chemicals cabinet (under 'G' for Glass) and put 2 large beads and two scoops each of the medium and small size beads into each tube. Sterilise by autoclaving.
2. (Optional, for tough G+ bacteria: Scrape a large loopful of growth to 500 μ L TE buffer and add 0.5 mg lysozyme. Mix well, then incubate at 37°C for 1 hour)
3. Add 122 μ l of MT buffer and 780 μ l sodium phosphate buffer to the bead beater tube, then add your sample (200-500 μ l volume). Leave a little bit of headspace for good beating action. For pure cultures, the pellet from a 5 ml broth or a large loopful (~half of a streak plate) is good.
4. Place tubes in bead beater and process for 30 sec at speed 5.5
5. Centrifuge ~ 10000 g for 5 min to pellet beads and debris
6. Transfer supernatant to 1.5ml microfuge tube
7. Add 125 μ l PPS and mix
8. Centrifuge ~ 10000 g for 5 min to pellet the protein precipitate
9. Transfer 700 μ l of supernatant to a new 1.5 ml microfuge tube. If high yield is important, you can transfer the remaining liquid (~300-600 μ l) to a second tube, and process in parallel.
10. Add 700 μ l Binding Matrix Suspension and mix gently for 5 minutes
11. Centrifuge ~ 10000 g for 1 min to pellet matrix-bound DNA
12. Remove supernatant (avoid Binding matrix)
13. Add 500 μ l of salt/ethanol wash solution and resuspend pellet by vortexing
14. Centrifuge ~ 10000 g for 1 min and discard supernatant
15. Repeat steps 12-13
16. Invert tube with lid open on paper towel and drain off excess salt/ethanol (~5 min), then transfer tubes to 60°C incubator for ~20 mins to evaporate residual ethanol. When fully dry, the pellet should look white, not grey/brown.
17. Resuspend matrix by vortexing in 200 μ l TE buffer. Incubate 60°C for 10 min to dissolve DNA.
18. Centrifuge ~ 10000 g for 1 min and transfer supernatant to new tube.
19. Store DNA cold (4°C) or frozen (-20°C). Cold is better if you are going to use it a lot (repeated freeze/thaw cycles are bad for DNA), but frozen is better for very long-term storage.

Solutions:

MT buffer: 1% SDS, 0.5% Teepol, 5% PVP40, 10 mM Tris (pH 8), 10 mM EDTA (pH 8). Autoclaved.

Sodium phosphate: 0.1M Na₂HPO₄ (pH 7.0), sterilise by autoclaving

PPS: 7.5M potassium acetate, sterilise by autoclaving

Binding Matrix Suspension: (bought from MPBio = silica in guanidine solution). Can be diluted 2:1 in guanidine thiocyanate (6 M) solution to make stock go further.

Salt/ethanol wash solution: 70% ethanol, 100 mM sodium acetate
(add ethanol to autoclaved 100 mM sodium acetate after cooling)

TE buffer: 10 mM Tris-HCl, 1 mM EDTA. pH 8.0, sterilise by autoclaving

Genomic DNA extraction (via bead beater). #2. Neil Wilson modified version

Hazards: Binding matrix contains conc. guanidine thiocyanate which is an irritant.

Notes: as for above protocol; this version was optimised for DNA from compost, but is broadly useful

1. Prepare the beadbeater tubes by adding two scoops* of tiny beads, two scoops of small beads, and one large bead to each 2 ml screw cap tube. Autoclave with lids off, loosely covered in foil. Autoclave lids separately, also loosely wrapped in foil. Put caps on tubes aseptically once cooled. * one 'scoop' is approx 100 ul volume. tiny beads are ~100-200 micron, small beads are ~500 micron, and large bead is 5 mm. All 'beads' are acid washed glass beads. eg. Sigma G1145-100G
2. Add 300 µl or 300 mg of sample (soil, cells etc.) per tube, then 150 µl of **SH solution**. Mix by vortexing until the sample is evenly mixed with the solution.
3. Add 750 µl of **Lysis Buffer** per tube, vortex again, then freeze at -80C for at least 20 min (prep can be indefinitely stored at this point for later processing).
4. Thaw samples. Ensure fully thawed and cap is on tightly. Bead beat 30 seconds in beadbeater, then incubate samples at 50°C for 1 hr with occasional mixing, then freeze again at -80°C for >20 min.
6. Add 120 µl of **K-acetate** solution, incubate on ice for 10 min, centrifuge at ~10,000 g for 5 min.
7. Transfer 700 µl of supernatant to a new (labelled) 1.5 ml tube. Find the '**binding matrix**' and vortex the bottle/tube to resuspend the silica particles, then immediately add 500 µl of matrix to DNA sample. Incubate 5 minutes at room temp, mixing occasionally to keep the matrix suspended.
8. Centrifuge at ~10,000 g for 30 seconds to pellet the matrix-bound DNA, then pour off the supernatant into the discard bottle. Be careful not to back-contaminate your DNA sample from the discard bottle ! Tap gently on paper towel to remove last drops of supernatant.
10. Add 750 µl of **SEWS** and resuspend the pellet by vigorous vortexing/flicking (approx 30 sec). Allow matrix to sit in SEWS buffer ~1 minute, with occasional mixing.
11. Centrifuge at ~10,000 g for 30 seconds, then discard supernatant. Leave the tubes open for 15 min in heating block at 60°C to allow residual ethanol (in SEWS buffer) to evaporate. Its very important to remove all the ethanol or DNA will misbehave later ! When fully dry, the matrix pellet should be a uniform bright white colour, with no darker patches.
12. Add 200 µl **EB** to the pellet, vortex/flick vigorously, heat 60°C for 5 min (lid closed this time!).
13. Centrifuge at ~10,000 g for 1 min, then transfer the DNA-containing supernatant to a new labelled tube. Note that you will only recover ~100 µl liquid. Be careful to avoid the matrix material.
14. Store the DNA cold (4°C) or frozen (-20°C). Cold is better if you are going to use it a lot (repeated freeze/thaw cycles are bad for DNA), but frozen is better for very long-term storage.

Solutions:

SH solution: Sodium hexametaphosphate (200 mM). Sterilised by autoclaving.

Lysis buffer: Tris base (50 mM), EDTA (50 mM), guanidine-HCl (1 M), Triton X-100 (0.5% w/v), adjust to pH 10 with NaOH. Sterilised by autoclaving.

K-acetate: 7 M potassium acetate. Sterilised by autoclaving.

Binding matrix: MP Biomedicals™ Binding Matrix (silica in 6M guanidine salt). Note: the commercial product can be diluted 1:2 with 6 M guanidine-HCl before use to make it go further. The raw product has more binding capacity than is needed.

SEWS (salt/ethanol wash solution): 100 mM sodium acetate in 70% ethanol. Make 30 ml of 333 mM sodium acetate, autoclave this, then after cooling, add 70 ml of 100% ethanol.

EB: 10 mM Tris HCl, pH 8