



Extraction with FastPrep kit (Bio 101)

1. Subject

The FastPrep System, which includes both the FastPrep Instrument and FastDNA kit, has the ability to lyse cells with minimal shearing of the nucleic acids. The procedure eliminates the major concerns in isolation of nucleic acids from cells that are difficult to lyse without enzymes, manual grinding, or homogenizing. It is these laborious and time consuming lysing steps which allow nuclease to act and can make nucleic acid isolation a chore. The FastPrep System, by use of highly energetic mechanical means and careful choice of reagents, disrupts whole tissues, lyses cells, and stabilizes nucleic acid from any source, thus eliminating the need for lysing enzymes or grinding and homogenizing equipment.

2. Principal

The FastPrep Instrument shakes a tube up and down (and with a slight twisting motion) at very high speeds. The rotor holds 12x2 ml tubes enabling 12 samples to be processed simultaneously.

The FastDNA kits include 2 ml tubes with 1/4" ceramic spheres and garnet matrix. In addition, 1/4" ceramic cylinders are supplied. The cylinders can be added to the tubes after the addition of reagents and sample. For extremely difficult to homogenize samples, all three matrices (sphere, garnet, and cylinder) can be used. For soft tissues (liver, brain, tissue culture cells, etc.) the sphere is sufficient to homogenize the sample.

The 2 ml tubes that contain the lysing matrix and sample also contain a chaotropic DNA stabilizing solution which is a proprietary mixture of detergents and salts. The detergents have been found to serve two functions. One, they contribute to inactivate nucleases. Two, they provide lubrication during the lysing step to control the degree of shearing of the DNA.

When processing samples in the FastPrep Instrument complete lysis occurs in 10-40 sec.. The energy generated by friction during the lysis step is considerable causing a temperature increase in the tube. This rise in temperature facilitates the inactivation of nucleases and does not harm the DNA.

3. Material and reagents

- 3.1 FastDNA Kit (OmniLabo 6050073, Bio 101 catalog# 6540-400)
(the kit contains all the necessary material and reagents)
- 3.2 FastPrep Instrument
- 3.3 pipets
- 3.4 pipettips
- 3.5 centrifuge (Sigma 112, Eppendorf 5417)
- 3.6 round bottom eppendorfcups 2.0 ml (Eppendorf 0030.120.094)
- 3.7 eppendorfcups 1.5 ml (Sarstedt 72.690)
- 3.8 Tris(hydroxymethyl)-aminomethane (Merck 8382)
- 3.9 Na-EDTA (Titriplex III, Bio Rad 161-0729)
- 3.10 ultrapure water
- 3.11 1N HCl (Ferak 11448)

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4. Solutions

- 4.1 TE-buffer, pH 8.0:
Add 0.12 g Tris [3.8] and 0.04 g Na-EDTA [3.9] to 80 ml ultrapure water. Adjust pH to 8.0 with 1 N HCl [3.11]. Heat the solution if EDTA doesn't dissolve very well. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Autoclave the solution during 15 min. at 121°C. Store at roomtemperature.

5. Protocol

- 5.1 Prepare a tube with lysing matrix for every sample.
5.2 Add 50-100 mg of fresh fungal material or \pm 30 mg dried or lyophilized material.
5.3 Add 1 ml CLS-Y buffer [6.1].
5.4 Incubate 15-20 min. at roomtemperature.
5.5 Homogenize samples in FastPrep Instrument, speed 4.0-5.0 for 5-30 sec. [6.2].
5.6 Incubate again at roomtemperature for 15 min. to 2 hours [6.3].
5.7 Spin in microcentrifuge for 5 min. at 14.000 rpm (20.400 rcf) to pellet protein and cell debris [6.4].
5.8 Transfer 600 μ l of the supernatant to a clean microcentrifuge tube [3.6].
5.9 Add 600 μ l binding matrix, mix gently and incubate at roomtemperature for 5 min. [6.5].
5.10 Spin for 1 min. at 11.500 rpm (14.000 rcf) and discard supernatant.
5.11 Resuspend the pellet gently in 500 μ l SEWS-M.
5.12 Spin for 1 min. (14.000 rcf) and discard supernatant.
5.13 Spin for 10 sec. and remove residual liquid with a small bore pipet tip.
5.14 Elute DNA from binding matrix by gently resuspending in 100 μ l TE-buffer [4.1], and not DES-buffer which is supplied with the package.
5.15 Incubate for 2-3 min. at roomtemperature.
5.16 Spin for 1 min. at 14.000 rcf.
5.17 Transfer supernatant carefully containing the DNA, to a new clean microcentrifuge tube [3.7] without transferring any particles of the binding matrix. This should be avoided in all cases.
5.18 Store DNA samples at 4°C or -20°C for longer periods.

6. Remarks

- 6.1 The volumes are calculated to leave a minimum air space of approximately 0.25 cc. If less air space is present, there is a likelihood of sample loss due to tube failure or deformation around the cap allowing sample to bubble out; this is caused by an increase in pressure with temperature increase during FastPrep runs. The presence of 0.25 cc of air space in the tube is sufficient to prevent sample loss during routine FastPrep runs.
6.2 Optimal conditions depends on the material used. To start the operation try speed 5 for 15 sec..
6.3 This step is optional, but to improve chances of optimized recovery of DNA from extremely dry samples or fibrous material, an incubation of 15 min. to 2 hours is recommended after processing in the FastPrep Instrument.
6.4 Extending spin to 15 min. can enhance elimination of excessive debris from large samples or from cells with complex cell walls.
6.5 The binding matrix contains guanidine thiocyanate. Use with proper caution. Before use, the solution should be homogenized by vortexing.

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