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Protocol status: Working
We use this protocol and it's working

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Single strain culture DNA extractions with polyzyme

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ABSTRACT

Protocol for DNA extractions from single strain bacterial cultures using the MetaPolyzyme Multilytic Enzyme mix (SIgma)

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Bacterial culturing

1 - Place cells from agarose plates with single strain growing on it (preferably from single colony) into liquid culture.

- Culture cells at appropriate temperature (27-30 C traditionally) for 1-4 days, as appropriate.

Pelleting cells

2 - Centrifuge 1 mL of liquid bacterial culture for 2 minutes at 10,000 RPM in a 1.5+ mL Eppendorf tube.

- If concerned about the amount of cells being too low, you can repeat this a second time by discarding supernatant, and then adding in 1mL of extra culture and repeating the process.

- This can be repeated again, though at a certain point, consider re-culturing strains to generate denser cultures.

PBS wash

3 - Discard supernatant, and resuspend cells in 1 mL PBS and vortex.

- Centrifuge and discard supernatant.

- Repeat above 2 steps.

- Repeat first 2 steps again (3 PBS washes total).

- Discard supernatant, and resuspend pellet in 150 µL PBS and vortex.

Polyzyme digestion

4 - Add 2 µL of Polyzyme solution.

- Incubate at 35 C for 4-24 hours.

Cell lysis

5 - Add 600 µL of lysis solution.

- Pipette up and down to suspend cells.

- Heat at (up to) 80 C for (up to) 10 minutes. Especially if samples are dense or cloudy.

RNAase treatment

6 - Add 1.2 µL of 10 mg/mL RNase A.

- Invert gently, ~ 2-5 times.

- Incubate at 37 C for 1 hour.

Protein precipitation

7 - Cool to room temperature.

- Add 250 µL of protein precipitation solution.

- Invert gently, ~ 2-5 times to mix.

- Place on ice for 5 minutes.

- Centrifuge at 10,000 RPM for 10 minutes.

- Transfer as much supernatant as possible (~900 µL works) to fresh sterile tubes using pipettes with tips removed.

- Repeat the protein precipitation steps (addition of solution through centrifugation) again.

DNA precipitation

8 - Transfer 900 µL to 2 mL Eppendorf tubes with pre-aliquoted 600 µL 100% isopropanol. Use pipettes with tips removed.

- Invert gently 50 times!

- Pellet DNA at 13,000 RPM for 2 minutes.

- Discard isopropanol supernatant.

- Wash DNA with 1 mL 70% ethanol.

- Centrifuge at 13,000 RPM for 2 minutes.

- Pour off as much ethanol as possible. Be careful, the pellet may be loose!

- Air dry pellet for at least 15 minutes. This can be done in the safety cabinet, by placing the opening of the tubes over the air flow beneath the glass barrier.

- Rehydrate DNA in ~25 µL (depending on volume of DNA, can be increased) Elution buffer (Qiagen) or TE buffer. Use pipettes with tips removed to suspend DNA by pipetting up and down.

Measuring DNA

9 - Measure DNA concentration using Qubit or other instrument