Project created on 13.04.2018 18:39.

Report for project Pseudomonas (MIRA)

Experiment created on 13.04.2018 14:56.

ARES0001

Extraction of DNA from pseudomonas bacterial culture utilizing chemical lysis and phenol:chloroform separation

Task created on 13.04.2018 18:47.

Phenol:Chloroform DNA Extraction

No due date Completed on 13.04.2018 20:05

Completed 04.04.18

Task tags: dnaextraction

Created by Emily Reesey on 13.04.2018 18:48.

Step 1: Materials **Uncompleted**

- TE buffer
- Genomic DNA solution
- Fresh lysozyme solution
- 20% Sarkosyl solution
- 10mg/mL RNase A
- 10mg/mL proteinase K
- Phenol:chloroform:isoamyl alcohol: 25:24:1
- Chloroform:isoamyl alcohol, 24:1
- 3 M sodium acetate
- 70% and 100% ethanol
- Phase-lock light gel tubes

Created by Emily Reesey on 13.04.2018 18:48.

Step 2: Bacterial Culture **Uncompleted**

1. Record sample info in results.
2. From a frozen stock, plate pseudomonas on MacConkey agar and grow overnight.
3. Select an individual colony and plate onto a new MacConkey plate. Grow overnight.

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Step 3: Lyse Bacteria **Uncompleted**

1. Scrape plates with sterile loop and add into tube with 200uL of genomic DNA solution.
2. Add 50uL lysozyme solution (50mg/mL) and incubate for 2hr at 37C
3. Add 100uL of 20% Sarkosyl solution and 15uL RNase A (10mg/mL) and incubate at 37C for 1hr or until solution clears (up to overnight).
4. Add 15uL of proteinase K (10mg/mL) and incubate at 37C for 30 minutes.
5. Use TE buffer to bring the volume up to 600uL and transfer to a 2mL phase lock tube.

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Step 4: Phenol:Chloroform Extraction **Uncompleted**

1. Add 600uL of phenol phenol:chloroform:isoamyl alcohol to phase lock tube.

2. Gently mix on rotator until emulsion forms – do not vortex.
3. Spin at high speed for 10 minutes.
4. Remove aqueous phase to new tube phase lock tube.
5. Add 600uL TE to original phase-lock gel tube. Mix until emulsion forms and centrifuge at high speed for 10 minutes.
6. Add aqueous phase into the new phase lock tube from step 4.
7. Add 600uL of chloroform:isoamyl alchocol (24:1) to extracted aqueous phase.
8. Gently mix on rotator until emulsion forms, then spin at high speed for 10 minutes.
9. Remove aqueous phase to new tube.

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Step 5: Precipitate DNA **Uncompleted**

1. Add 50uL of 3M sodium acetate (pH 5.2) and 3 volumes of cold 100% ethanol.
2. Spin at full speed for 5 minutes.
3. Decant off supernatant then wash pellet with 500uL of 70% EtOH
4. Spin at full speed for 5 minutes.
5. Remove ethanol and dry pellet in 40C dry block for 10 minutes – do not over dry the pellet.
6. Resuspend DNA in 50-100uL of TE or EB buffer and store at 4C – do not freeze DNA.
7. Quantify DNA on Qubit and Nanodrop; record in results.