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INNOSMART® DNEX Plasmid Extraction Experiment (LV004)

Introduction

INNOSMART® DNEX is an automated workstation designed for plasmid preparation, enabling a fully automated, one-stop extraction process. The equipment integrates various process units, including automated pipetting, sterile liquid pumping, centrifugation, lid opening, shaking modules, and integrated shake flask operations. It offers flexible process editing capabilities, supports rapid process switching during the R&D phase, and can handle a throughput of over 200 samples per day. The workstation features a Class 100 laminar flow environment, compliant with international standards such as 21 CFR Part 11, and includes intelligent anomaly handling to ensure experimental safety. Additionally, it boasts low consumable costs.

Experimental Objectives and Plan

The objective of this experiment is to transform, select, and amplify the previously stored LV004 plasmid. After confirming the correct identification of the plasmid through mini-preparation, we will use the DNEX device for plasmid extraction from the LV004 culture. The concentration will then be measured, and the plasmid will be stored at -80°C for use in subsequent experiments.

Experimental Procedure

1. Preparation of Culture Media

1.1 Preparation of LB Solid Medium

Dissolve 1 g of peptone, 0.5 g of yeast extract, 1 g of sodium chloride, and 1.5 g of agar powder in 100 mL of distilled water.

Stir the mixture until homogeneous and sterilize at 121°C for 30 minutes.

Allow the solution to cool to 50-60°C, then add ampicillin to achieve a final concentration of 100 ng/µL and mix well.

Dispense approximately 20 mL of the medium into sterile Petri dishes and place them in a laminar flow hood until completely solidified. Store in the refrigerator for future use.

1.2 Preparation of LB Liquid Medium

Combine 30 g of peptone, 15 g of yeast extract, and 30 g of sodium chloride in 3000 mL of distilled water.

Mix thoroughly and sterilize at 121°C under high pressure for 30 minutes.

Once cooled, store the liquid medium in the refrigerator for future use.

2. Experimental Procedure

2.1 Plasmid Transformation

Prepare the experiment by turning on the ice maker and water bath in advance.

Retrieve one tube (100 µL) of DH5α competent cells from the -80°C freezer and allow it to thaw on ice.

Add 2 µL of the previously stored LV004 plasmid to the competent cells, gently mix by flicking the tube, and let it sit on ice for 30 minutes.

Transfer the tube to a 42°C water bath for heat shock for 90 seconds.

Move the tube back to ice and incubate for 150 seconds.

Add 500 µL of LB medium to the centrifuge tube, then incubate at 37°C with shaking at 150 rpm for 1 hour to achieve turbidity.

Transfer 100 µL of the cultured cells to an LB agar plate, spread the cells evenly with a spreader, and incubate the plate in a 37°C incubator for 30 minutes before inverting and incubating for an additional 12–15 hours.

2.2 Selection and Expansion of Colonies

Take a clean 50 mL centrifuge tube, add 20 mL of LB liquid medium, and supplement with ampicillin to a final concentration of 100 ng/µL. Pick a single colony from the plate and inoculate it into the LB liquid medium. Incubate overnight at 37°C with shaking at 150 rpm.

Use a plasmid mini-prep kit to extract the LV004 plasmid in small quantities.

Validate the extracted plasmid using double restriction enzyme digestion.

Once the double digestion validation is confirmed, expand the culture: add 6 ml of the cell culture to 600 mL of LB liquid medium, supplement with ampicillin, and distribute into six 250 mL shaking flasks (100 mL each). Incubate overnight at 37°C with shaking at 150 rpm.

2.3 Plasmid Extraction

Use DNEX equipment to extract plasmid DNA from the expanded LV004 culture. Measure the concentration of the extracted plasmid and perform agarose gel electrophoresis for identification. Finally, store the plasmid at -80°C.

Results

1. Concentration Measurement Results

The concentration measurements of six plasmid samples extracted with DNEX are as follows. (Elution volume is 1 mL)

Plasmid name concentration (ng/µL)A260/280A260/230A1820.61.82.11A2805.4	concentration (ng/µL)A260/280A260/230A1820.61.82.11A2805.4	A260/280A260/230A1820.61.82.11A2805.41.92.14A3812.41.932.26A4788.21.772.05A5776.91.84	A260/230A1820.61.82.11A2805.41.92.14A3812.41.932.26A4788.21.772.05A5776.91.842.18A6794.41.862.22
A1820.61.82.11A2805.41.92.14A3812.41.932.26A4	820.61.82.11A2805.41.92.14A3812.41.932.26A4788.21.772.05A5776.91.842.18A6794.41.862.22	1.82.11A2805.41.92.14A3812.41.932.26A4788.2	2.11A2805.41.92.14A3
A2805.41.92.14A3812.41.932.26A4788.21.772.05A5776.91.842.18A6794.41.862.22	805.41.92.14A3812.41.93	1.92.14A3812.41.932.26	2.14A3812.41.932.26A4788.21.772.05A5776.91.842.18A6794.41.86
A3812.41.932.26A4788.2	812.41.932.26A4788.21.772.05A5776.91.842.18A6	1.932.26A4788.21.772.05A5776.91.842.18A6794.41.862.22	2.26A4788.21.772.05A5776.91.842.18A6794.41.862.22
A4788.21.772.05A5776.9	788.21.772.05A5776.91.842.18A6794.41.862.22	1.772.05A5776.91.842.18A6794.41.862.22	2.05A5776.91.842.18A6794.41.862.22
A5776.91.842.18A6794.4	776.91.842.18A6794.41.862.22	1.842.18A6794.41.862.22	2.18A6794.41.862.22
A6794.41.862.22	794.41.862.22	1.862.22	2.22

2. Electrophoresis Results

The six extracted samples were diluted tenfold and analyzed using agarose gel electrophoresis. The results are as follows.

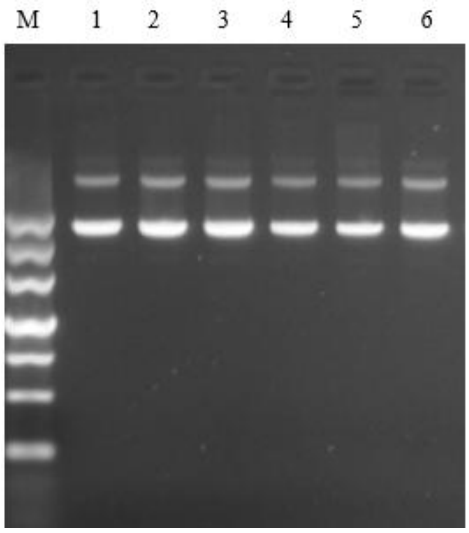

Figure 1: Electrophoresis results of plasmid LV004.
M: DNA Marker III**; 1-6:** Samples A1 to A6 (diluted 10-fold).

Conclusions

- Plasmids were extracted using DNEX from 100 ml of bacterial culture, yielding a plasmid quantity between 0.7 mg and 0.9 mg, which meets the experimental requirements.
- The agarose gel electrophoresis analysis of the extracted plasmids showed band sizes consistent with expectations, confirming that DNEX is suitable for plasmid extraction experiments.