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Bacterial Transformation Protocol

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Transformation of bacteria to amplify DNA for cloning, virus production, or other molecular biology techniques.

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Bacteria, molecular biology

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MATERIALS

NAME	CATALOG #	VENDOR
LB agar plates with the proper antibiotic (e.g. Kanamycin)		
MACH1 or DH5a or TOP10 or NEB stable or etc for DNA purification/miniprep		
Luria Broth Base (Miller's LB Broth Base)™, powder	12795027	Thermo Fisher

MATERIALS TEXT

14-956-9C Culture Tubes

BEFORE STARTING

- Prepare Luria broth (LB) agar plates and allow to set.
- If pre-poured plates are being used, ensure the plates are warmed to 37° C.
- Depending on the antibiotic marker present in the plasmid DNA, incorporate appropriate antibiotic in the LB agar.
- Heat the water bath or heat block to 42° C.
- Warm the sterile LB medium to room temperature (or 20-25° C in water bath).

1 Transfer competent cells (DH5a) from -80 to wet ice for 5-10 min or until thawed.

2 Add 1ng to 50ng of DNA directly to cells. Incubate for 10 minutes on ice.

3 Heat shock cells for 45 seconds at 42C in a heat block. (Do not go over 45 seconds!) You can kill bacteria by keeping them at high temps for too long.

4 Transfer cells to ice and incubate for 5 minutes.

5 Add cells to sterile culture tubes with 5ml of LB + selection antibiotic. Ensure lid is not tight to ensure proper aeration of the cultures.

6 Incubate starter culture for 1h at 230rpm in a shaker at 37C.

7 Add 100ul of starter culture after incubation to warmed LB plate. Spread using roller beads.

8 Incubate plates o/n at 37C with the plate facing down to avoid desiccation of the agar.

9 In the morning, select colonies and culture in 3-5ml of Luria Broth (LB) overnight for Mini-prep in following morning.

10 Isolate the DNA from the culture.

11 Digest the plasmid with appropriate restriction enzyme and visualize with gel electrophoresis to determine if the plasmid insert is correct.

12 Send out the plasmid for sequencing before using to make virus, or functional studies.