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

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Metagenomic library plates

Bonnie Evans[1,2]

[1]MRC Laboratory of Medical Sciences; [2]Imperial College London

Bonnie Evans
MRC Laboratory of Medical Sciences, Imperial College London

ABSTRACT

Arraying Canadian MetaMicroBiome Library (Neufeld *et al.* 2011) clones into multi-well plates for screening.

ATTACHMENTS

PIXL_user_guide_v2.9[Clea ver1].pdf

MATERIALS

Tetracycline hydrochloride (Sigma-Aldrich, T3383-25G)
ColiRollers Plating Beads (Novagen, 71013-3)
384 Well Clear Flat Bottom Polystyrene Not Treated Microplate with Lid Sterile (Corning, 3680)

BEFORE START INSTRUCTIONS

Make up 30% glycerol in sterile water and autoclave.

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Plate pooled library sample

1 Prepare tetracycline LB agar plates

Protocol

NAME

Making tetracycline LB agar plates

CREATED BY

Bonnie Evans

PREVIEW

2 Dilute 2 uL glycerol stock in 20 mL sterile PBS (1/10000 dilution).

Note

Keep sample on ice and return to -80 °C before proceeding with next steps.

3 Pipette 100 uL of diluted sample into centre of 90 mm agar plate

4 Put 4-5 plating beads onto the agar. With lid on, move the plate back and forth so the beads spread the liquid evenly across the agar surface.

5 To remove the beads, turn the plate so the beads collect in the side of the lid. Carefully open the lid and drop the beads into a glass beaker.

6 Incubate the inoculated plates at 37° for ~25.5hrs.

Expected result

Individual white colonies. Large enough to be detected by the PIXL but not touching neighbouring colonies.

7 Clean plating beads

7.1 Add sterile water to the glass beaker.

7.2 Place 70 uM cell strainer on another beaker to collect beads from liquid. Put beads back into the empty beaker. Dispose of water in waste bottle for treatment with Virkon.

7.3 Add ethanol to beads. Repeat above step. Dispose of liquid in ethanol waste bottle.

7.4 Wash beads with sterile water as above. Put beads in a bottle and send for autoclaving.

Prepare growth media

8 Make up 15 mg/ml tetracycline hydrochloride in sterile water, and add 1/000 dilution to LB broth (fi nal conc. 15 ug/ml)

9 Using the VIAFILL, dispense 50 uL of tetracycline LB per well into 384 well plates

Pick colonies with PIXL

10 Before using the PIXL, sterilise the instrument with UV.

Note

See attachment for PIXL colony picker (Sanger) user guide,

10.1 Interlock door guard into slot on the inside of the instrument door Prepare growth media Pick colonies with the PIXL 4

10.2 On the Home Screen, select UV Lamp and run for 30 minutes

11 Set up instrument via display screen.

11.1 Home Screen -> Run Workfl ows -> Random Colony Picking -> Blank

11.2 Select Source Plate: Petri dish -> 90 mm

11.3 Capture settings -> White Light -> Select colonies

> **Note**
>
> PIXL will now analyse the image to detect colonies. You can manually select or deselect colonies, or change the algorithm to optimise detection.

11.4 Select Target Plate: SBS -> 384

11.5 Check the Project Summary -> Start Picking

> **Note**
>
> Check the pick up line is touching the colony and then the LB media in the well to ensure proper inoculation.

Make glycerol stock plates

12 Incubate 384 well plates at 37 °C overnight.

13 Measure OD600 of the overnight culture using a plate reader.

14 Using the VIAFILL, dispense 50 uL of 30% glycerol per well into 384 well plates (final conc: 15% glycerol).

15 Incubate plate for 2 hours at 37 °C.

16 Seal using aluminium seals and store at -80 °C.