Conjugation on filters

Nat Brown

Abstract

A simple procedure to conjugate plasmids between bacteria. Conjugation is performed on sterile filters to reduce the number of plates required (5 can easily be fit on one 10 cm plate) and to make suspending the bacteria in liquid simple following conjugation.

Citation: Nat Brown Conjugation on filters. **protocols.io**
https://www.protocols.io/view/Conjugation-on-filters-eqybdxw
Published: 22 Mar 2016

Before start

Don't fuck anything up.

Materials

- 1X PBS (Phosphate-buffered saline) by Contributed by users
- Lysogeny agar by Contributed by users
- Lysogeny broth by Contributed by users

Protocol

Step 1.
Inoculate small (2 or 3 ml) cultures of your donor and recipient strains, with appropriate antibiotics, in lysogeny broth (LB). Do this late in the day, the day before you want to do the conjugation.

Step 2.
First thing in the morning on the day of the conjugation, place sterilised 0.2 um pore size filters on an L-agar plate without antibiotics. Use aseptic technique. You will need one filter for each conjugation you're setting up, plus one for each strain on its own as a control.

NOTES
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Almost any type of filter material will do, and if the filters you have are too big, cut them into useable sizes (say 1 cm^2) with scissors. Place the filters in a small bottle or container and sterilise by autoclaving. Dry in a low temperature oven or incubator if necessary following autoclaving.

Step 3.
Take 1 ml of donor and recipient culture and centrifuge at 10,000 rcf for 2 minutes to pellet the bacteria. Aspirate and discard the supernatant and resuspend the bacterial pellet in fresh LB with no antibiotics.

Step 4.
Pipette 10 ul of the resuspended donor and recipient strains in the same place on a filter, sitting on the agar plate. Do the same for each strain on its own as a control. Incubate the plate upside down (agar suface facing up) at 37 degrees Celsius.

Step 5.
Near the end of your work day, take the plate out of the incubator. Using aseptic technique, pick each filter off the plate using forceps and place it in a 1.5 ml tube containing 1 ml of sterile PBS. Vortex the tubes to free the bacteria from the filter. Remove and discard the filters from the tubes using aseptic technique. Plate desired volumes of the bacterial suspension on selective agar medium and incubate over night.

REAGENTS
- 1X PBS (Phosphate-buffered saline) by Contributed by users