Dot blot to verify microinjection efficacy in Euplotes crassus

Version 3

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Abstract

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Protocol

Step 1.

Grow cells up to a density of 1000 cells/ml, and transfer 400 µl of each culture to an Eppendorf tube.

Step 2.

Pellet the cells at max speed for 5 minutes, and resuspend them in 400 µl of ddH_2O.

Step 3.

Add 50 µl of 0.5 M EDTA, pH 8.0 and 50 µl of 4 M NaOH to the cells.

Step 4.

Lyse the cells at 68°C for 30 minutes, and centrifuge them for 1 minute at 13000 rpm, then chill them on ice.

Step 5.

Soak a membrane for nucleic acid binding and a sheet of Whatmann paper in 0.4 M of NaOH, and place both in a Dot blot apparatus.

Step 6.

Switch on the vacuum pump, and transfer all the lysates into the chamber.

Step 7.

Soak the membrane in 0.4 M NaOH for 15 minutes, and wash it in 2X SSC buffer for 10 minutes, then drain it on a sheet of Whatmann paper.

Step 8.

Pre-hybridize the membrane in 20 ml of Church buffer at 60°C for 2 hours, then hybridize it at constant temperature (annealing temperature) o/n in 10 ml of Church buffer containing the radioactive probe.

Step 9.

Wash the membrane twice with 2X SSC, 0.1% SDS for 15 minutes each time.

Step 10.

Place the membrane in a plastic foil, expose it overnight and scan it to determine which clones have sufficient DNA from the microinjection (compared to suitable DNA standards used as positive controls).