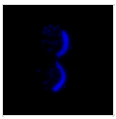

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Working

Dot blot to verify microinjection efficacy in Euplotes crassus

Forked from Dot blot to verify microinjection efficacy in Euplotes crassus

RACHELE CESARONI[1], Rachele Cesaroni[2]

[1]University of Bern, Institute of Biology, [2]Universität Bern

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Protist Research to Optimize Tools in Genetics (PROT-G)

Angela Piersanti
University of Camerino

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

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

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

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

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.

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

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.

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.

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

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).