Dot blot to verify microinjection efficacy in Euplotes crassus

Version 2

RACHELE CESARONI

Abstract

Citation: RACHELE CESARONI Dot blot to verify microinjection efficacy in Euplotes crassus. **protocols.io** dx.doi.org/10.17504/protocols.io.hizb4f6
Published: 01 Apr 2017

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 oWhatmann 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 60ºC for 2 hours and then hybridize it at constant temperature (annealing temperature) overnight 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).