# Dot blot to verify microinjection efficacy in Euplotes crassus

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

### 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 acids and a 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 Whatmann paper.

### Step 8.

Pre-hybridize the membrane in 20 ml of Church buffer at 60ºC for 2 hours and then hybridize it at

costant 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 o/n and scan it to determine the positive clones.