Directed yeast two hybrid screening for MEKK1 interaction partners

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Introduction

This protocol utilizes the Y190 yeast strain, that characteristically turns from white to red during growth due to ade- mutation. Baits were subcloned into the pAS1cyh2 vector, and Preys subcloned into pACT2 (5).

Reagents

Y190 yeast strain

YPAD medium

0.1M LiAc (Sigma)

ssDNA (2.0 mg/ml)

PEG 3350 (Sigma)

Sterile H_2O

42 ˚C waterbath

SC minus plates (-LEU, -TRP)

SC minus plates (-LEU, -HIS, -TRP), supplemented with 50 mM 3AT and 20 µg/ml X-gal

Procedure

Transformation

1 Inoculate 10 ml of YPAD medium with a single Y190 colony and incubate overnight at 30 ˚C. 55 ml of fresh YPAD are then cultured overnight to an OD_{600} of 0.3. Cells are then incubated by shaking at 200 RPM and 30 ˚C until they reach an OD_{600} of 0.7.

2 Harvest cells at 2000 RPM for 5 min at room temperature.

3 Aspirate the media, wash cells in sterile water, and spin at 2000 RPM at 25 ˚C for 5 min.

4 Aspirate water and resuspend cells in 1 ml of 0.1 M LiAc and transfer suspension to a fresh microfuge tube. Pellet cells at 6000 RPM for 15 s and aspirate the supernatant. Next, resuspend the pellet in 500 µl of 100 mM LiAc.

5 Boil a 1 ml sample of ssDNA (2.0 mg/ml) and chill on ice. Mix the yeast cell suspension and transfer 50 µl samples into fresh tubes for the interactions to be tested.

6 Pellet cells at 6000 RPM and aspirate supernatant.

7 Add the following gently over the cell pellet: 240 µl of PEG 3350 (50% w/v), 36 µl of 1.0 M LiAc, 25 µg of ssDNA (2.0 mg/ml), and 50 µl sterile water containing plasmid DNA for the interaction screen (0.1 – 2.0 µg). Vortex mixture until cell pellet is completely mixed into suspension.

8 Incubate the suspension at 30 ˚C for 30 min. Transform the yeast by heat shock at 42 ˚C for 15 min, mix suspension carefully and pellet cells at 6000 RPM for 15 s.

9 Finally, resuspend yeast cells in 200 µl of sterile water for plating.

Plating and selection

10 Plate yeast onto SC minus plates (-LEU, -TRP) and grow at 30 ˚C for 3 – 5 days.

11 Streak single colonies onto SC minus plates (-LEU, -HIS, -TRP) supplemented with 50 mM 3AT and 20 µg/ml X-gal.

12 Yeast are then grown for 3 – 5 days and scored for growth and color change.

Timing

5 d minimum

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Kinase MEKK1 is required for CD40-dependent activation of the kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production

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