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Protein interaction analysis of KaiC3 with various Kai homologs via yeast two-hybrid experiments (β-Galactosidase Assay)

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ABSTRACT

This protocol can be used to investigate protein-protein interaction via yeast two-hybrid experiments. It describes the yeast-two hybrid method relying on a color change using β-galactosidase activity.

MATERIALS TEXT

List of Materials

- Yeast cells (Y190)
- Frozen-EZ Yeast Transformation II Kit (Zymo Research)
- Yeast Nitrogen Base without amino acids (Formedium, CYN0401)
- Drop-out mixture (-Leu -Trp; MP Biomedicals, 114520012)
- Adenine-hemisulfate (Sigma-Aldrich, A9126)
- Bacto Agar (BD Diagnostics, 214010)
- Filter papers (MN 615, Macherey-Nagel)
- X-β-Gal (Roth, 2315.X)
- D-Glucose (Roth, X997.2)
- Na_2HPO_4 (Roth, 4984.1)
- NaH_2PO_4 (Merck, 10049-21-5)
- KCl (Merck, 7447-40-7)
- $MgSO_4$ (Roth, P027.1)
- Parafilm

Complete supplement medium (CSM)

Components for CSM Agar	
Yeast Nitrogen Base with ammonium /without amino acids	6.7 g/L
D-Glucose	20 g/L
Bacto Agar	20 g/L
Drop-Out-Mix (amino acid mixture)	0.60-0.64 g/L
Adenine Hemisulfate	50 mg/L
dd H_2O	

- Autoclave 15 min, 121°C or filter sterilize before using

Z-Buffer

Components Z-buffer	final conc.	Stock solution	50 ml
Na_2HPO_4	60 mM	0.5 M	6 ml
NaH_2PO_4	40 mM	0.5 M	4 ml
KCl	10 mM	1 M	0.5 ml
$MgSO_4$	1 mM	1 M	0.05 ml
			39.45 ml H2O

- Check pH, if not 7.0 adjust with 200 mM Na_2HPO_4 or 200 mM NaH_2PO_4
- Can be prepared as either 1x or 5x Solution and autoclaved
- Note Mg will fall out of solution on autoclaving, redissolve once cool by shaking

X-Gal Solution

- Prepare a 20 mg/ml stock solution of X-Gal in N,N-dimethylformamide (DMF) or dimethylsulfoxide (DMSO)
- Store stock solution protected from light at -20°C. Solutions may be stored at -20°C for 6-12 months.

Preparation of buffers

1 Prepare
- CSM -Leu -Trp agar (complete supplement mixture lacking leucine and tryptophan)
- Z-Buffer
- X-Gal solution

Transformation of yeast cells

2 Perform the transformation of yeast cells according to manufacturer's guidelines using the Frozen-EZ Yeast Transformation II Kit (Zymo Research) and select transformed cells on complete supplement mixture lacking leucine and tryptophan (CSM -Leu -Trp) at 30 °C for 3–4 days.

Re-plating of colonies

3 Spot formed colonies on a second plate (CSM -Leu -Trp)
- Resuspend 3 colonies in 100 µl H_2O
- Spot 5 µl on the master plate
- Wrap parafilm around the plate
- Incubate at 30°C for 2 days

β-galactosidase assay

4 Prepare Z-Buffer/X-Gal solution
- 5 ml for each plate (10 cm circula)
- 10 ml Z-Buffer + 27 µl β-Mercaptoethanol + 42 µl X-Gal solution

For each plate to be assayed, pre-soak sterile filters in a petri-dish
- Stack 3 filters in a petri-dish and add 5 ml Z buffer/X-Gal solution
- Store in a plastic bag to prevent evaporation

β-galactosidase activity
- Prepare a petri-dish with 1 sterile and dry filter and mark the orientation of the filter (e.g. the top with a pencil line)
- Using a pair of tweezers place the dry filter in the correct orientation onto the plate with the colonies. Gently rub the filter with a sterile glass spreader to mediate clinging of the colonies to the filter (the filter becomes slowly wet) until spots are visible for all colonies
- Carefully lift the filter off the agar plate and transfer it to a pool of liquid nitrogen. Submerge the filter for ≈ 10 s
- After the filter is frozen completely replace it to the petri dish (colonies facing up) and let it thaw at RT
- Repeat freezing and defreezing for 3-5 times
- Remove excess liquid from the pre-soaked 3 stack filters

- Place the filter (colony side up) on the pre-soaked stack

- Avoid trapping air bubbles under or between the filters
- Colonies are supposed to look glossy

- Store the plates in a plastic bag and incubate at RT (if possible in the dark, since X-Gal is light sensitive)
- Check periodically for the appearance of blue colonies

- To stop the reaction remove the filter from the pre-soaked stack and let it dry open under the fume hood
- Scan the plates to record the results
- Result: Interaction of two proteins is positive if there is a blue color change of the respective colony, but without any color change of the controls