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

Anika Wiegard[1], Christin Köbler[2], Katsuaki Oyama[3], Anja K. Dörrich[4], Chihiro Azai[5,3], Kazuki Terauchi[5,3], Annegret Wilde[2], Ilka Maria Axmann[6]

[1]Karolinska Institutet, Department of Cell and Molecular Biology, Stockholm, Sweden, [2]Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany, [3]Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan, [4]Institute for Microbiology and Molecular Biology, Justus-Liebig University, 35392 Giessen, Germany, [5]College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan, [6]Institute for Synthetic Microbiology, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany

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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 the activity of the histidine-synthetase. If the proteins interact, the yeast cells are able to grow on selective medium without histidine.

MATERIALS TEXT

List of Materials

- Yeast cells (AH109)
- Frozen-EZ Yeast Transformation II Kit (Zymo Research)
- Yeast Nitrogen Base without amino acids (Formedium, CYN0401)
- Drop-out mixture (-Leu -Trp; MP Biomedicals, 114520012)
- Drop-out mixture (-Leu -Trp -his; MP Biomedicals, 114530112)
- Adenine-hemisulfate (Sigma-Aldrich, A9126)
- 3-amino-1,2,4-triazole (3-AT, Sigma-Aldrich, A8056)
- Bacto Agar (BD Diagnostics, 214010)
- D-Glucose (Roth, X997.2)

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	20g/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

Preparation of buffers and media

1 Prepare
- CSM -Leu -Trp agar (complete supplement mixture lacking leucine and tryptophan)
- CSM -Leu -Trp -His agar (complete supplement mixture lacking leucine, tryptophan and histidine)
- 3-AT solution, 1M

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.

Restreaking of colonies

3
- Prepare plates: CSM -Leu -Trp -His + 3-AT (interaction plate)
- Determine the appropriate concentration of 3-AT by adding different concentrations of 3-AT to the interaction plate
  - Possible concentrations of 3-AT: 0 mM, 3 mM, 6.25 mM, 12.5 mM, 25 mM and 50mM
  - Let the agar cool down before adding 3-AT, it is heat sensitive
- Mark and restreak 1-3 formed colonies from step 2 onto the interaction plates
  suggestion: only use a small amount of cells for restreaking to reduce false positives
- Wrap parafilm around the plates
- Incubate plates at 30°C for 3-6 days
- Use the lowest concentration of 3-AT that allows only small (<1-mm) colonies to grow after 3-4 days for further interaction plates

Growth assay

4
- Prepare plates: CSM -Leu -Trp (control) and CSM -Leu -Trp -His + 3-AT (interaction plate), use determined concentration of 3-AT
- Restreak 1 colony from step 2 onto the control plate and afterwards onto the interaction plate (reduces amount of cells and therefore false positives)
- Wrap parafilm around the plates
- Incubate plates at 30°C for 3-4 days
- Result: Interaction of two proteins is positive if there is growth on both, the control and interaction plate, but without any growth of the controls on the interaction plate.