JUL 11, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.wcnfave

Protocol Citation: Anika Wiegard, Christin Köbler, Katsuaki Oyama, Anja K. Dörrich, Chihiro Azai, Kazuki Terauchi, Annegret Wilde, Ilka Maria IM Axmann 2019. Protein interaction analysis of KaiC3 with various Kai homologs via yeast two-hybrid experiments (Growth Assay). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.wcnfave>

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Protocol status: Working
 We use this protocol and it's working

Created: Dec 11, 2018

Last Modified: Jul 11, 2019

PROTOCOL integer ID:
 18542

🌐 Protein interaction analysis of KaiC3 with various Kai homologs via yeast two-hybrid experiments (Growth 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 the activity of the histidine-synthetase. If the proteins interact, the yeast cells are able to grow on selective medium without histidine.

MATERIALS

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 ₂ O	

- 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.