Protocol

Prion Transfection of Yeast

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Transfection of yeast with amyloid filaments, made from recombinant protein or prepared from extracts of cells infected with a prion, has become an important method in characterizing yeast prions. Here, we describe a method for transmission of [URE3] with Ure2p amyloid that is based on a previously published protocol for transfection with Sup35p filaments to make cells [PSI⁺]. This method may be used for other prions by changing just the amyloid source, host strain, and plating medium.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Complete synthetic medium without leucine and containing adenine (CS + A.1-L) <R>
Complete synthetic medium without leucine and containing adenine sulfate (CS + A5-L) <R>
Lyticase solution <R>

Protein extract or amyloid filaments

Amyloid filaments prepared from bacterially expressed Ure2p (e.g., see Taylor et al. 1999; Brachmann et al. 2005) should be sonicated as described in Step 5 immediately before addition to spheroplasts. Amyloid filaments have been used at concentrations of 0.04–6 µg/µL.

pRS425 plasmid (2 µg/µL; Christianson et al. 1992)

PTC buffer <R>

Single-stranded DNA (10 mg/mL; Worthington-Biochemicals)

SOS medium <R>

ST buffer <R>

STC buffer <R>

Tris-Cl (1 M, pH 7.4)

Yeast strains

BY241 (MATa *leu2 trp1 ura3 P_{DAL5}:ADE2 P_{DAL5}:CAN1 kar1*)

BY256 (MATa *his3 trp1 leu2 P_{DAL5}:ADE2 P_{DAL5}:CAN1 kar1*)

YPAD medium <R>

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Equipment

Air supply for blowing cell lysates to new tube after breakage (see Step 2)
Branson 250 Sonifier equipped with a microtip
Conical screw cap microtubes (2 mL)
Glass beads (0.5 mm)
Incubator at 30°C and 50°C
Microcentrifuge
Mini-BeadBeater-8 (Biospec)
Spectrophotometer
Vortex mixer (e.g., Vortex Genie 2)

METHOD

1. Grow [URE3]-containing cells overnight at 30°C in 20 mL of complete synthetic medium without adenine (i.e., CS + A.1-L without any adenine) to select for maintenance of the prion. Collect the cells, resuspend them in 50 mL of YPAD to an OD₆₀₀ of 0.2–0.5, and grow them for 2–3 doublings at 30°C. Collect the cells, and wash the cell pellets twice with water.

At this point, the pellets can be stored frozen at –80°C.

2. Resuspend pellets of [URE3]-containing cells in 600 µL of water, and transfer them to a 2-mL conical screw cap microtube. Fill the tubes with 0.5-mm glass beads, and break the cells for 3 min in a Mini-BeadBeater-8 at 4°C. Briefly centrifuge the tubes, pierce them at the top and bottom, and blow the cell lysates into a clean microcentrifuge tube using an air hose. Clear the lysates by centrifugation for 5 min at 4°C, and store them at –80°C until recipient yeasts are ready.
3. Grow recipient [ure-o] cells overnight at 30°C in YPAD. Then use 1 mL of culture to inoculate 50 mL of YPAD, and grow the cells for 2–3 doublings at 30°C. Wash the cells once with 20 mL of water and twice with 25 mL of ST buffer, and then resuspend the cells in 5 mL of ST buffer.
4. Convert the cells into protoplasts by incubation for 40 min at 30°C with 4 µL of lyticase solution. Collect the protoplasts by centrifugation at 250g for 3 min, wash them two times with 10 mL of STC buffer, and then resuspend them in 1 mL of STC buffer.

To reduce shearing, perform all manipulations of protoplasts using pipette tips from which the ends are trimmed to have a wide bore size.

5. Sonicate lysates of [URE3]-containing cells (from Step 2) three times for 45 sec (duty cycle 20%, output 4) using a Branson 250 Sonifier equipped with a microtip. Keep the lysates on ice between sonications.
6. Add the following to 100 µL of protoplasts.

Single-stranded DNA (10 µg/µL)	1 µL
pRS425 plasmid (2.0 µg/µL)	2 µL
Protein extract or amyloid filaments	9 µL

7. Incubate the protoplast/DNA/protein mixture for 10 min at room temperature. Add 900 µL of PTC buffer, and incubate the mixture for 20 min at room temperature.
8. Collect the protoplasts by centrifugation at 400g for 3 min in a microcentrifuge. Add 200 µL of SOS buffer to the pellet, and leave the protoplasts to recover for 30 min at 30°C.
9. Pipette the recovered protoplasts into 10 mL of either CS + A.1-L or CS + A5-L medium kept at 50°C. Mix each solution by inverting the tubes and directly pour them into Petri dishes containing 20 mL of the same solidified medium.

10. Incubate the plates for 6 d at 30°C.

CS + A5-L medium allows all transformants to grow and thus provides a measurement for the transformation efficiency. CS + A.1-L medium is selective for [URE3] cells. Although [URE3] cells do not need adenine-supplemented medium to grow, primary transformants need it to allow phenotypic establishment of the prion (Brachmann et al. 2005).

DISCUSSION

The first yeast proteins identified genetically to form prions, Ure2p and Sup35p (Wickner 1994), are also the first yeast prion forming proteins shown to form amyloid (King et al. 1997; Taylor et al. 1999). The first attempt to infect yeast with a protein extract from a prion containing strain was made by Sparrer et al. (2000). However, the liposome fusion method was inefficient, and it was not clear if the prions detected were formed in the recipient cell following the introduction of prion domain protein—in effect induction of prion formation by overproduction of the prion domain. Using ballistic transformation Maddelein et al. (2002) showed that introduction of HET-s amyloid into the filamentous fungus *Podospora anserina* results in infection of mycelium with the [Het-s] prion; introduction of heat-aggregated HET-s did not result in infection. This method has not been used for *S. cerevisiae*. In 2004 both Tanaka et al. and King et al. created more efficient methods to deliver prion particles into yeast. King et al. used a protoplast fusion system (King and Diaz-Avalos 2004). In this system, protoplasts from two genetically marked strains are incubated with protein extracts. Cell fusion also introduces species present in the medium, including prion particles, into the cells. This method has not found wide application although, in addition to [PSI⁺], it was shown to work for [PIN⁺] (Sharma and Liebman 2013). Tanaka et al. created a method that uses plasmid uptake to identify yeast protoplasts that have taken up media contents (Tanaka et al. 2004). In addition to [PSI⁺], this method has been used to infect cells with prion particles and amyloid of several prion proteins (Ure2p, Rnq1p, Cyc8p, and Swi1p) (Brachmann et al. 2005; Patel and Liebman 2007; Patel et al. 2009; Du et al. 2010), and it is our minor modification (for [URE3]) of this method that we describe here.

Yeast strains used for protein transformation experiments aimed at [URE3] contain an *ADE2* ORF controlled by the *DAL5* promoter. The *DAL5* promoter is tightly controlled by Ure2p activity and is activated in [URE3] cells when soluble Ure2p levels drop because of sequestering in amyloid filaments (Schlumpberger et al. 2001; Brachmann et al. 2005). Cells also contain at least one nutritional marker to allow selection for cells that have obtained a yeast replication plasmid. Plasmid uptake is used to identify cells that have taken up buffer contents including Ure2p amyloid filaments (Brachmann et al. 2005). Dramatic differences in transfection efficiency have been noted among yeast strains examined with BY241 (*MATa leu2 trp1 ura3 P_{DAL5}:ADE2 P_{DAL5}:CAN1 kar1*) and BY256 (*MATa his3 trp1 leu2 P_{DAL5}:ADE2 P_{DAL5}:CAN1 kar1*) providing particularly good results (Brachmann et al. 2005).

RECIPES

Complete Synthetic Medium without Leucine and containing Adenine (CS + A.1-L)

Yeast nitrogen base without amino acids (Difco)	6.7 g/L
Dextrose	20 g/L
Agar (for solid medium)	20 g/L
Sorbitol	1 M
L-Tyrosine	50 mg/L
L-Arginine HCl	50 mg/L
L-Aspartic acid	80 mg/L

L-Histidine HCl	20 mg/L
L-Isoleucine	50 mg/L
L-Lysine HCl	50 mg/L
L-Methionine	20 mg/L
L-Phenylalanine	50 mg/L
L-Threonine	100 mg/L
L-Tryptophan	50 mg/L
Uracil	20 mg/L
L-Valine	140 mg/L
Adenine hemisulphate	0.1 mg/L

Dissolve the amino acid L-tyrosine first. (When it is added according to its alphabetical position it will not go into solution.) Autoclave. For solid medium, add 20 mL to each Petri plate. Store at 4°C (this is stable for at least 6 mo).

Complete Synthetic Medium without Leucine and containing Adenine Sulfate (CS + A5-L)

Yeast nitrogen base without amino acids (Difco)	6.7 g/L
Adenine sulfate	5 mg/L
Dextrose	20 g/L
Agar (for solid medium)	20 g/L
Sorbitol	1 M
L-Tyrosine	50 mg/L
L-Arginine HCl	50 mg/L
L-Aspartic acid	80 mg/L
L-Histidine HCl	20 mg/L
L-Isoleucine	50 mg/L
L-Lysine HCl	50 mg/L
L-Methionine	20 mg/L
L-Phenylalanine	50 mg/L
L-Threonine	100 mg/L
L-Tryptophan	50 mg/L
Uracil	20 mg/L
L-Valine	140 mg/L
Adenine hemisulphate	0.1 mg/L

Dissolve the amino acid L-tyrosine first. (When it is added according to its alphabetical position it will not go into solution.) Autoclave. For solid medium, add 20 mL to each Petri plate. Store at 4°C (this is stable for at least 6 mo).

Lyticase Solution

Reagent	Final concentration
Lyticase (Sigma-Aldrich L2524)	25 units/μL
Glycerol	20%
Tris-Cl (pH 7.4)	10 mM

Store at 4°C (this is stable for at least 6 mo).

PTC Buffer

Reagent	Final concentration
PEG8000	20% (w/v)
Tris-HCl (pH 7.4)	10 mM
CaCl ₂	10 mM

Store at 4°C (this is stable for at least 6 mo).

SOS Medium

Reagent	Final concentration
Sorbitol	1 M
CaCl ₂	7 mM
Yeast extract	3.3 g/L
Peptone	6.7 g/L
Glucose	6.7 g/L
Store at 4°C (this is stable for at least 6 mo).	

ST Buffer

Reagent	Final concentration
Sorbitol	1 M
Tris-HCl (pH 7.4)	10 mM
Store at 4°C (this is stable for at least 6 mo).	

STC Buffer

Reagent	Final concentration
Sorbitol	1 M
Tris-HCl (pH 7.4)	10 mM
CaCl ₂	10 mM
Store at 4°C (this is stable for at least 6 mo).	

YPAD Medium

Reagent	Final concentration
Yeast extract	10 g/L
Peptone	20 g/L
Dextrose	20 g/L
Adenine sulfate	0.4 g/L
Agar (for solid plates)	20 g/L
Store at 4°C (this is stable for at least 6 mo).	

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REFERENCES

- Brachmann A, Baxa U, Wickner RB. 2005. Prion generation in vitro: Amyloid of Ure2p is infectious. *EMBO J* **24**: 3082–3092.
- Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P. 1992. Multi-functional yeast high-copy-number shuttle vectors. *Gene* **110**: 119–122.
- Du Z, Crow ET, Kang HS, Li L. 2010. Distinct subregions of Swi1 manifest striking differences in prion transmission and SWI/SNF function. *Mol Cell Biol* **30**: 4644–4655.
- King CY, Diaz-Avalos R. 2004. Protein-only transmission of three yeast prion strains. *Nature* **428**: 319–323.
- King C-Y, Tittmann P, Gross H, Gebert R, Aeibi M, Wuthrich K. 1997. Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments. *Proc Natl Acad Sci* **94**: 6618–6622.
- Maddelein ML, Dos Reis S, Duvezin-Caubet S, Coulary-Salin B, Saupe SJ. 2002. Amyloid aggregates of the HET-s prion protein are infectious. *Proc Natl Acad Sci* **99**: 7402–7407.
- Patel BK, Liebman SW. 2007. “Prion proof” for [PIN⁺]: Infection with in vitro-made amyloid aggregates of Rnq1p-(132-405) induces [PIN⁺]. *J Mol Biol* **365**: 773–782.

- Patel BK, Gavin-Smyth J, Liebman SW. 2009. The yeast global transcriptional co-repressor protein Cyc8 can propagate as a prion. *Nat Cell Biol* 11: 344–349.
- Schlumpberger M, Prusiner SB, Herskowitz I. 2001. Induction of distinct [URE3] yeast prion strains. *Mol Cell Biol* 21: 7035–7046.
- Sharma J, Liebman SW. 2013. Exploring the basis of [PIN⁺] variant differences in [PSI⁺] induction. *J Mol Biol* 425: 3046–3059.
- Sparrer HE, Santoso A, Szoka FC, Weissman JS. 2000. Evidence for the prion hypothesis: Induction of the yeast [PSI⁺] factor by in vitro-converted Sup35 protein. *Science* 289: 595–599.
- Tanaka M, Chien P, Naber N, Cooke R, Weissman JS. 2004. Conformational variations in an infectious protein determine prion strain differences. *Nature* 428: 323–328.
- Taylor KL, Cheng N, Williams RW, Steven AC, Wickner RB. 1999. Prion domain initiation of amyloid formation in vitro from native Ure2p. *Science* 283: 1339–1343.
- Wickner RB. 1994. [URE3] as an altered URE2 protein: Evidence for a prion analog in *S. cerevisiae*. *Science* 264: 566–569.

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