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Cytoduction and Plasmiduction in Yeast

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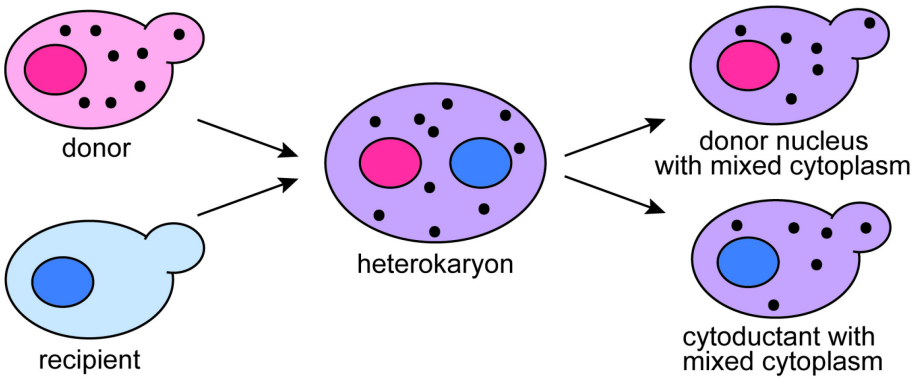
[Abstract] Cytoduction, and a related technique referred to as plasmiduction, have facilitated substantial advancements in the field of yeast prion biology by providing a streamlined method of transferring prions from one yeast strain to another. Prions are cytoplasmic elements consisting of aggregated misfolded proteins, and as such, they exhibit non-Mendelian patterns of inheritance. While prion transfer through mating and sporulation, or through protein transformation, is possible, these approaches yield non-isogenic strains or are technically complex, respectively. Cytoduction is a mating-based technique that takes advantage of a *kar1* mutation with impaired nuclear fusion (karyogamy). It is a straightforward method for introducing a prion to any yeast strain (referred to as the recipient) by mating it with a donor strain containing the prion of interest. The only absolute requirement is that one of these two strains (donor or recipient) must carry the *kar1-1* mutation to limit nuclear fusion. The resulting cytoductant contains the original nucleus of the recipient strain, but a cytoplasm reflecting a mix of all elements from the donor and the recipient. Modifications to the basic cytoduction strategy provide several options for successful cytoduction, including when working with slow growing or respiratory deficient strains. A significant advantage of the plasmiduction protocol presented is the ability to transfer a plasmid encoding a fluorescently tagged version of the prion protein, which allows for the direct verification of the prion state through visual protein aggregates.

Graphic abstract:



Transfer of Yeast Cytoplasmic Elements such as Prions using Cytoduction

Keywords: Cytoduction, Plasmiduction, *kar1*, Prion, Yeast, Karyogamy, Heterokaryon

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[Background] Cytoduction is a technique developed in yeast during the 1970s to study cytoplasmic inheritance (Conde and Fink, 1976; Zakharov and Yarovoy, 1977). Cytoduction takes advantage of a karyogamy mutant, *kar1-1*, in which nuclear fusion during mating is limited (Conde and Fink, 1976; Dutcher, 1981; Livingston, 1977). In a mating between *KAR1* and *kar1-1* cells, the cytoplasm is mixed but two distinct nuclei are maintained (Figure 1). The resulting daughter cells from this heterokaryon contain a mix of cytoplasm from both parents, yet only one of the original parental nuclei. This technique has been used as a tool to study and track other cytoplasmic elements such as mitochondria (Lancashire and Mattoon, 1979), killer toxins (Seki *et al.*, 1985), and oligomycin resistance (Matsuoka *et al.*, 1982). Cytoduction can also be used to replenish *rho*^{0}, or mitochondrial DNA deficient, strains with fresh mitochondria, thus restoring respiration deficiencies associated with the loss of mitochondrial DNA (Merz and Westermann, 2009; Stenger *et al.*, 2020). Because many terms associated with cytoduction may not be universally familiar, definitions are available for reference in Table 1.

Cytoduction has also been extremely helpful in the study of yeast prions. Yeast prions exhibit non-mendelian inheritance (Cox, 1965; Young and Cox, 1972), in which traditional mating and sporulation show a 4:0 pattern of inheritance. Cytoduction was used to show that several prions, including [*PSI*$^+$], can be inherited through cytoplasmic transfer (Cox *et al.*, 1980; Derkatch *et al.*, 2001; Brachmann *et al.*, 2005). Since these early studies, cytoduction and several other variant protocols have not only provided solid evidence that prions are cytoplasmic elements, but have facilitated important insight into the genetic mechanisms underlying prion formation (Masison *et al.*, 1997; Manogaran *et al.*, 2011) and how prions are maintained in populations (Manogaran *et al.*, 2010; Bateman and Wickner, 2013; Keefer and True, 2016; Dorweiler *et al.*, 2020).

Despite impaired nuclear fusion in *kar1-1/KAR1* heterokaryons, rare exchange of genetic information between nuclei occurs at a frequency that is inversely proportional to the size of the chromosome (Dutcher, 1981). To circumvent this downside of unintentional chromosomal transfer to cytoductants, the Rothstein lab has developed a universal donor strain in which each chromosome contains a galactose-inducible conditional centromere that can be destabilized after mating (Manogaran *et al.*, 2010; Reid *et al.*, 2011). Even if a rogue chromosome is transferred into the recipient strain, growth on galactose containing media results in chromosome destabilization and loss.

Plasmiduction is a closely related process that uses the principles of cytoduction and the rare exchange of nuclear information to transfer plasmids between strains. Originally used to transfer plasmids into strains that were difficult to transform, plasmiduction can be used to transfer centromeric, 2 μ, and yeast artificial chromosomes into recipient strains (Brown *et al.*, 1981; Natsoulis *et al.*, 1994; Spencer *et al.*, 1994). While the ability to transfer extrachromosomal DNA between strains is a valuable tool of its own right, when transferring prions between strains, plasmiduction offers the ability to include additional plasmid-associated selectable markers to help identify cytoductants. Additionally, plasmiduction can circumvent the need for subsequent plasmid transfer into cytoductants as part of the prion verification process.

Here, we present three different cytoduction strategies. We begin by presenting the classic protocol that relies upon using a cycloheximide resistant, *rho*^{0} recipient strain, and provides the most direct

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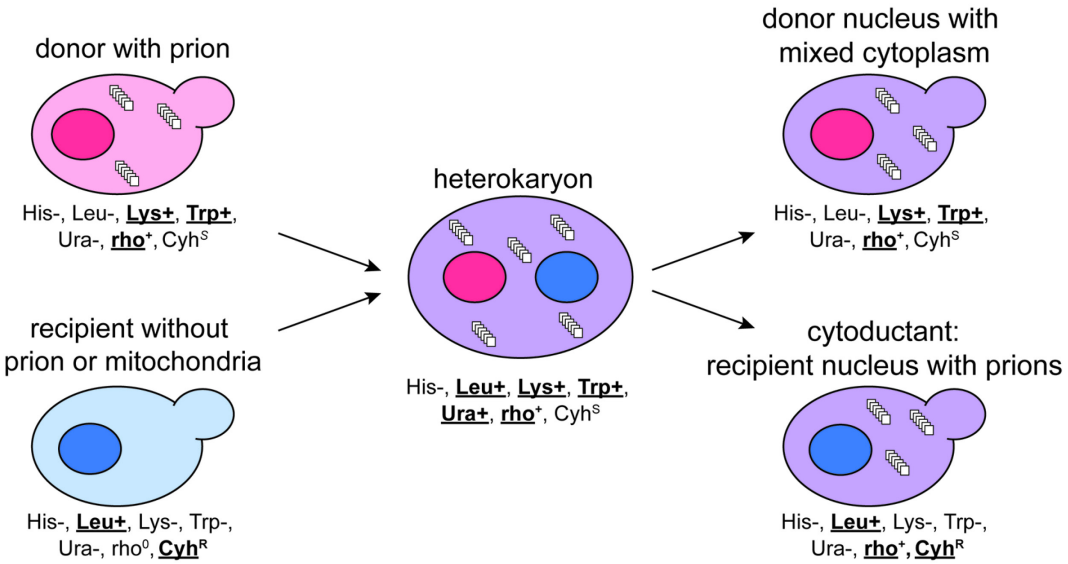
selection of cytoductants. Despite this significant advantage, this classic protocol is not always the most appropriate for growth sensitive or respiratory deficient strains. We also present two additional cytoduction strategies for generating cytoductants, including plasmiduction.



Genotypes:
donor: *MAT**a** kar1-1 his3 leu2 ura2* high [*PIN*$^{+}$]
recipient: *MAT*α *his3 lys9 ura3 trp1 cyh*^{R} *rho*^{0}

Figure 1. Schematic example of cytoduction. A donor yeast strain carrying the prion of interest is mated with a recipient strain that lacks the prion. Successful mating only happens when the two strains are of opposite mating type (*MAT**a*** vs. *MAT*α), and fusion between the two nuclei is limited only if one of the original strains carries the karyogamy deficient *kar1-1* mutation. Using the specific example genotypes provided for the donor and recipient, phenotypic labels are assigned to all cells within the schematic, with wild type or cycloheximide resistance phenotypes in bold. This practice reveals those phenotypes that can be used to select the desired cytoductants from all other cells in the process. Note that donor (*ura2)* and recipient (*ura3*) contain mutations in different genes within the uracil biosynthesis pathway. The heterokaryon is heterozygous for each of these genes, and therefore **Ura+** confers growth on SD-Ura media.

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Table 1. Terminology associated with prion cytoduction

Donor	Yeast strain containing the prion of interest that will 'donate' copies of that prion to the recipient strain.
Recipient	Yeast strain containing no prion that will receive copies of the prion from the donor.
Karyogamy	Fusion of parental nuclei upon mating. The *kar1-1* mutant exhibits impaired karyogamy.
Heterokaryon	A cell containing two distinct nuclei, commonly formed in a *kar1-1* mutant.
Cytoductant	Recipient that received the prion from the donor.
Plasmiductant	Recipient that has received both a plasmid and prion from the donor.
rho^0, (also known as petite)	Yeast cells that have lost mitochondrial DNA and are respiratory deficient (no growth on glycerol carbon source).
Cyh^R vs. Cyh^S	Cyh^S are cycloheximide sensitive strains. Cyh^R strains are cycloheximide resistant, usually through acquiring spontaneous recessive mutations in the RPL28 gene (Stocklein *et al.*, 1982; Kaufer *et al.*, 1983). Mating between Cyh^S strain and Cyh^R strains results in cycloheximide sensitive heterokaryons.

Materials and Reagents

1. Petri Dishes with Clear Lid (Fisherbrand™, catalog number: FB0875712)
2. Toothpicks (flat, sterilized)
3. Slides and coverslips (*e.g.*, Fisherbrand, catalog numbers: 12-550-A3 and 12-542A)
4. Yeast strains
   a. Donor and recipient strains of complementary mating type; *MAT**a*** and *MATα*.
   b. One of these, ideally the donor, must contain the *kar1-1* mutant allele.
   c. Unique auxotrophic or antibiotic markers that differentiate donor and recipient strains to significantly streamline isolation and confirmation of cytoductants.
   d. To use the plasmiduction protocol described in Section D below, the donor and recipient strains should share at least one auxotrophic marker that will be complemented by the plasmid. Alternatively, a plasmid conferring antibiotic resistance (such as G418 resistance) could be used.
   e. One additional yeast strain (referred to generically throughout this protocol as your "tester strain," for instance BY4741, BY4742, or another favorite strain; it must meet the following conditions).
      i. Must be of opposite mating type relative to your recipient strain.
      ii. Should be wild type for the *KAR1* gene.
      iii. Should include unique auxotrophic markers relative to your recipient strain to facilitate diploid selection.
      iv. Use of this strain to screen cytoductants for fluorescent aggregates (as described in Step B4a) demands two additional requirements. First, the strain must share at least

one auxotrophic marker relative to your recipient strain that facilitates maintenance of a plasmid necessary to confirm prion status of your cytoductants (as outlined below in Step B4a). For example, if using a *URA3* plasmid, both your recipient and tester strain must be *ura3.* Second, the strain should be devoid of all prions, but minimally must be devoid of the prion being cytoduced. If prion status of this tester strain is uncertain, cure the strain using guanidine hydrochloride (as described below in Step B4b). Regardless of whether you suspect that the tester strain is free of prions or have cured the strain yourself, it is prudent to verify that the strain contains no prions by visualizing cells via fluorescent microscopy as described (Step B4a, focusing on parts i., and v.-vii. only, as mating will not be required). If properly cured, these cells will have diffuse fluorescence under the conditions described in Step B4a.

Note: This protocol focuses predominately on Hsp104-dependent prions and thus describes the guanidine hydrochloride method for curing these prions. If you are working with prions that require an alternative curing method, such as [GAR$^+$] or [SMAUG$^+$], then curing of your tester strain should be completed using your prion specific curing method rather than that described in Step B4b.

5. Plasmids for prion identification or plasmiduction
   a. To detect [*PSI*$^+$] using fluorescence microscopy, plasmid with fluorescently tagged Sup35 protein (*e.g.*, pCUP1-Sup35-GFP, with *URA3*, Addgene.org #1087)
   b. To detect [*PIN*$^+$] using fluorescence microscopy, plasmid with fluorescently tagged Rnq1 protein (*e.g.*, pCUP1-Rnq1-YFP, with *URA3*, Addgene.org #15596)
   c. Plasmids that provide optional antibiotic or auxotrophic selection (for the plasmiduction protocol).
6. Media/Agar plates
   a. Dehydrated Agar for solid media (Bacto™, catalog number: 214030; 2 kg, or smaller quantity)
   b. YPD (ingredients, recipes in separate section below)
      i. Yeast Extract, low-dusting (LD) (Gibco™, Difco™, catalog number: 210933)
      ii. Peptone (Gibco™, Bacto™, catalog number: 211677)
      iii. Dextrose Glucose, Anhydrous, 2 kg (BD™ Difco™, catalog number: 215510 or smaller quantity)
   c. Components for Synthetic Dropout plates
      i. Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate (Difco™, catalog number: 233520)
      ii. Ammonium sulfate, 99.5%, for analysis (ACROS Organics™, catalog number: 205870010)
      iii. Nutrients for dropout powders (for an exhaustive list, including relative proportions, see Sherman, 2002)
         1) Amino acids: L-arginine-HCl, L-histidine, L-leucine, L-lysine mono-HCl, L-

methionine, L-phenylalanine, L-tryptophan, L-tyrosine, L-valine (Sigma-Aldrich)

2) Adenine hemisulfate salt (Sigma-Aldrich, catalog number: A-9126)
3) Uracil (Sigma-Aldrich, catalog number: U-0750)
4) Some yeast strains may have additional unique auxotrophic markers and require corresponding nutrients (Sherman, 2002).

d. Cycloheximide solution (for detailed protocol B only, see Recipes)
   i. Cycloheximide (Sigma, catalog number: C7698)
   ii. DMSO (Sigma, catalog number: D8418)
e. Ethidium bromide (if recipient strain needs to be rendered *rho*^{0} for appropriate cytoduction strategy (Sigma, catalog number: E8751)
f. Glycerol (Sigma, catalog number: G-5516)
g. Possibly one or more yeast antibiotics dependent upon cytoductant selection strategy. Two common yeast antibiotics are geneticin (Sigma, catalog number: A-1720; Winzeler *et al.*, 1999) and nourseothricin (GoldBio, catalog number: N-500-1; Haarer *et al.*, 2007).

7. Glass beads for spreading yeast cells (Zymo Research, catalog number: S1001)
8. Reagents for yeast transformation (Gietz, 2014)
   a. ssDNA (Sigma, catalog number: D7656)
   b. PEG 3350 (Sigma, catalog number: 88276)
   c. Tris ($C_4H_{11}NO_3$, GoldBio, catalog number: T-400-1)
   d. EDTA (EMD, catalog number: EX0539-1)
   e. Lithium Acetate (Sigma, catalog number: L4158)
9. Guanidine Hydrochloride (Sigma, catalog number: G4505)
10. Copper Sulfate (Sigma, catalog number: C1297)
11. Media (see Recipes)
12. 10 mg/ml Cycloheximide stock solution (see Recipes)
13. YPD (or YPGlycerol) plates with 10 µg/ml Cycloheximide (see Recipes)
14. 2.5 mg/ml Ethidium Bromide (ETBr; see Recipes)
15. YPD plates with 25 µg/ml EtBr (see Recipes)
16. 5 M Guanidine Hydrochloride (GuHCl; see Recipes)
17. YPD plates with 5 mM GuHCl (see Recipes)
18. SGlycerol-selective plates (see Recipes)

Equipment

1. Fluorescent microscope (to monitor the presence of prion via fluorescent tag)
2. Replica plater/velveteen squares (Fisher Scientific, catalog number: 09-718-1)
3. Autoclave
4. Incubator (30°C)
5. Erlenmeyer Flasks (1 L for media preparation)

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(All equipment is available from any standard scientific supply distributor; specific brands/models are not required)

Procedure

A. Selecting a cytoduction strategy

Before attempting cytoduction, it is important to consider the genotypes and phenotypes of your donor and recipient strains, and subsequent cells in the procedure (see Figure 1), to determine which cytoduction strategy will work best. The objective is to select *for* the cytoductants, and *against* as many other cell types as possible because mating is never 100%, and all cell types depicted in Figure 1 will co-exist after the 10-16 h mating period. The cycloheximide resistant/*rho*^{0} strategy, in which the recipient strain is made cycloheximide resistant and *rho*^{0}, is the most common strategy used for cytoduction of prions. However, if recipient strains have slow growth phenotypes or are respiratory deficient, making these strains cycloheximide resistant or *rho*^{0}, respectively, can be difficult. Therefore, we provide two alternative strategies that can be used: a *rho*^{0} only approach or use of a plasmid for selection (plasmiduction). It should also be acknowledged that the process of plasmiduction can be used to simultaneously introduce prions and a plasmid into a recipient strain, avoiding the need for a separate mating step to introduce the plasmid necessary for verifying prion status. Table 2 summarizes cytoduction strategies and the benefits and drawbacks of each approach.

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

Table 2. Three cytoduction strategies

Selection strategy	Recipient strain requirements	Donor strain requirements	Pros	Cons	Selection for cytoductant
Cycloheximide/ *rho*^{0}	Viable in the *rho*^{0} state Must survive selection for spontaneous cycloheximide resistant recessive mutants.	rho$^+$ Cannot be cycloheximide resistant.	Direct selection for cytoductants on YPGlycerol+cycloheximide media.	Does not work well with slow-growing or respiratory deficient strains. Confirmation of prion transfer and maintenance requires subsequent introduction of plasmid encoding fluorescently tagged prion protein.	YPGlycerol+cycloheximide Full protocol detailed in Section B.
***rho*^{0}**	Viable in the *rho*^{0} state Must have unique selectable markers from the donor.	rho$^+$	Transfer of mitochondria ensures cytoplasmic mixing has occurred.	Does not work well with slow-growing or respiratory deficient strains. Selection after mating requires further screening for cytoductants' markers. Confirmation of prion transfer and maintenance requires subsequent introduction of plasmid encoding fluorescently tagged prion protein.	Glycerol and donor counter selective media. Resulting colonies screened for recipient specific markers. Full protocol detailed in Section C.
Plasmiduction	Contains no plasmid but can maintain the plasmid to be transferred from donor strain.	Contains plasmid	Compatible with sensitive strains Desired method if a plasmid is required in the recipient. Can be used to introduce plasmids containing fluorescently tagged genes for the confirmation of prions.	Selection after mating requires further screening for cytoductants' markers.	Plasmid selective, donor counter selective media. Resulting colonies screened for recipient specific markers. Full protocol detailed in Section D.

Bio-protocol 11(17): e4146.
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B. Selecting cytoductants using the Cycloheximide/*rho*^{0} strategy

1. Strain selection
   a. The donor strain or the recipient strain must contain the *kar1* mutation, but this protocol works best when the donor is *kar1*.
   b. For the cytoduction of prions, the donor strain must contain the prion (such as high [*PIN*$^+$] or weak [*PSI*$^+$]).
   c. Map out the full genotypic information for donor and recipient (Figure 1).
      Example Donor: *MAT***a**, *kar1-1, his3, leu2, ura2*, *URA3, LYS9, TRP1,* high [*PIN*$^+$]
      Example Recipient: *MAT*α, *KAR1, his3-1, LEU2, URA2, ura3-14, lys9Δ, trp1-1*, no prions
   d. Recipient must be able to withstand selection for spontaneous cycloheximide resistance and loss of mitochondrial DNA (*rho*^{0}).
      Note: It may not be feasible to use this strategy with slow growing or temperature sensitive strains.
2. Preparing the recipient strain
   a. Generating cycloheximide resistant recessive strains.
      i. Grow the desired recipient strain on several YPD plates to generate a thick lawn of actively dividing cells. Incubate plates at 30°C overnight.
      Note: This thick lawn is best achieved by plating ~150 µl of a 10^7 suspension of cells on each of several YPD plates using sterile glass beads.
      ii. Replica plate (see Figure 2 for diagram of replica plating) each independent lawn of cells onto a fresh YPD plate containing 10 µg/ml cycloheximide, and incubate at room temperature (RT) for up to two weeks until rare colonies appear.
      Note: To avoid desiccation, make sure plates are approximately 6-7 mm thick, and incubation is done in a 'humidity chamber' containing several soaked paper towels in a plastic container with a breathable or unsealed lid. Use of replica plating from a thick lawn of actively dividing cells substantially increases the likelihood of obtaining cycloheximide resistant cells relative to directly plating even a dense quantity of log-phase cell culture onto the YPD+cycloheximide plates.

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

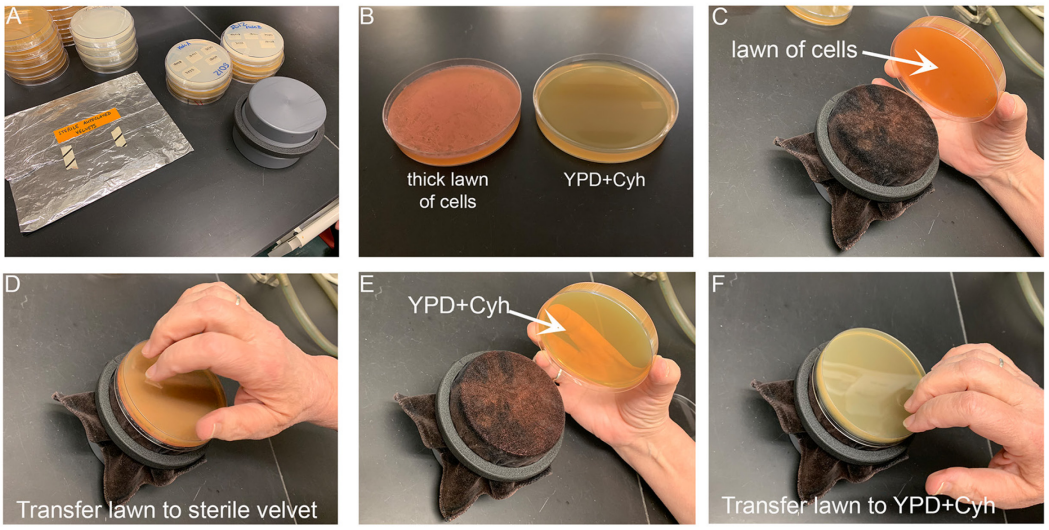

Figure 2. Diagram of replica plating. A. Replica plating requires sterile velvets, a replica plater, and sterile plates. Use of replica plating elsewhere in this protocol is analogous to the process shown here, with key differences indicated in those locations. B. Grow cells as a thick lawn (a red-colored yeast strain is shown as an example). A thickly poured fresh YPD+Cycloheximide (abbreviated YPD+Cyh within the figure) plate will also be needed. C. The thick lawn of cells will be placed onto the sterile velvet and lightly transferred with gentle pressure on the top of the plate (D). E. The fresh YPD+Cyh plate will be placed onto the velvet, and gentle pressure on top of the plate will transfer the cells (F).

iii. Colonies that grow on cycloheximide plates should represent rare mutations.

iv. Verify cycloheximide resistance by re-streaking colonies on a new YPD+cycloheximide plate. Incubate plates at 30°C for 2-3 days. Time required for colony development at this verification step should be comparable to that normally observed for your recipient strain.

v. To verify that the cycloheximide resistant mutation is recessive, the mutation must be scored in the diploid state. To generate diploids, your strain of interest and tester strain (see Materials and Reagents, 4.e.) must be freshly grown. On separate YPD plates, use a sterile toothpick to spread a thin film of each strain into a 2 × 2 cm “patch.” Incubate plates at 30°C overnight. To mate the strains, use a sterile toothpick to pick a small amount of each strain and mix in one patch on a fresh YPD plate. Incubate plates at 30°C overnight. To select for diploids, streak mated cells for single colonies onto plates that will select against each of the haploids, but maintain the diploid. Using the example recipient strain as defined above, and BY4741 (*Mat**a***, *his3, leu2, ura3, met15*) as the tester, diploids could be selected on SD-Leu. Incubate plates at 30°C for 2-3 days until individual colonies develop. To score for cycloheximide resistance, patch a few colonies onto YPD+cycloheximide vs. YPD. Diploids should grow on YPD, but not on

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

YPD+cycloheximide, because recessive mutations will not confer resistance to the diploid.

b. Generating *rho*^{0} recipient cells

i. Grow recipient strains, preferably after making strains cycloheximide resistant, by streaking for a single colony on YPD plates containing 25 µg/ml ethidium bromide. Ethidium bromide is a mutagen and potential carcinogen, so handle plates with gloves. Incubate plates at 30°C for 3-5 days.

Note: Ethidium bromide treatment will induce mitochondrial DNA loss, and colonies will be small.

ii. Verify strains are *rho*^{0} by streaking on YPD and YPGlycerol. Colonies will be small or "petite" on YPD and will not grow on YPGlycerol.

3. Mating and selection of cytoductants

a. Using a sterile toothpick, spread the cycloheximide resistant *rho*^{0} recipient strain in one or more long horizontal lines on a YPD plate, as shown in Figure 3. Incubate at 30°C 1-2 days.

Note: The recipient strain will grow very slowly; it is best to make thick patches or grow for two days.

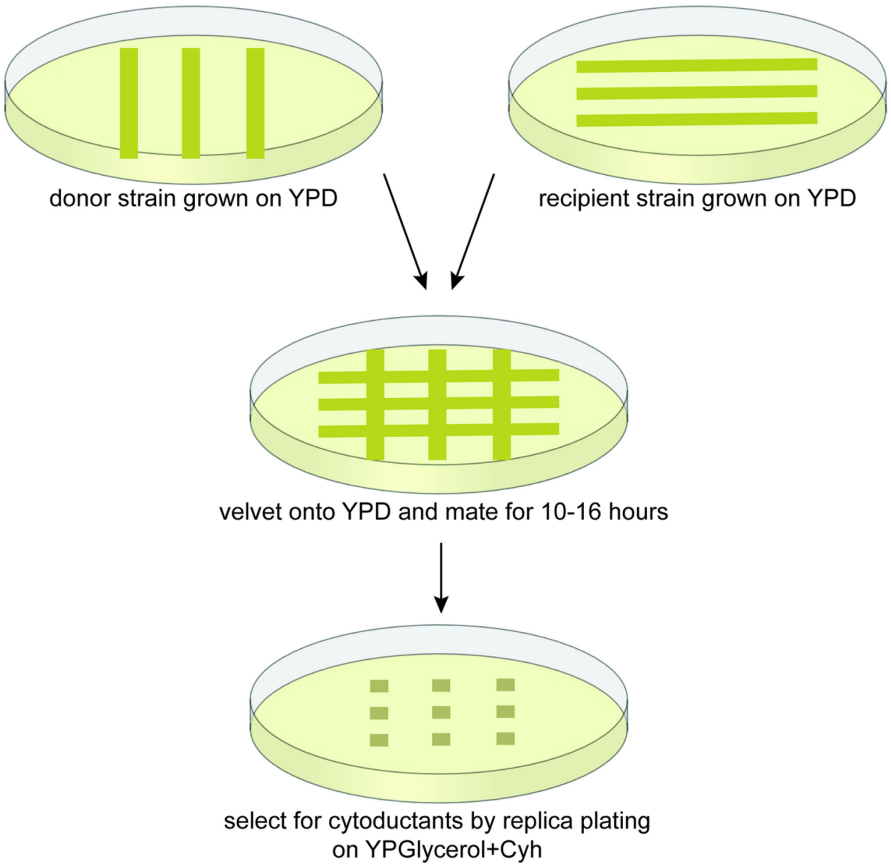

Figure 3. Schematic of mating strategy using a *rho*^{0}, cycloheximide resistant recipient strain. Donor strains are spread on plates in one or more vertical lines, and recipient strains are

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

spread on a separate plate in one or more horizontal lines. After growth, use a sterile velveteen to replica plate donor strains onto a fresh YPD plate. Use a second sterile velveteen to replica plate the recipient strain in a crisscross pattern over the donor. Allow strains to mate, and then replica plate onto a YPGlycerol+cycloheximide plate. Only cytoductants are able to grow and will appear at the intersections where donor and recipient mating occurred. Colonies usually appear after 7 days of growth.

b. Spread a thin film of the donor strain in one or more long vertical lines on another YPD plate, as shown in Figure 3. Incubate at 30°C overnight.
c. Using a replica plater and a sterile velveteen, velvet the donor strain onto a fresh YPD plate.
d. Using a fresh sterile velveteen, velvet the recipient strain in a crisscross pattern over donor strain as shown in Figure 3.
e. Incubate plates at 30°C for approximately 10-16 h.
Optional: Microscopically monitoring for "shmoos" or directional projection morphology can be performed at approximately 6 h to provide a positive indicator that the strains will mate successfully; for more detail on shmoo morphology, see Merlini et al. (2013).
f. Replica plate the mated cells onto a YPGlycerol 10 µg/ml cycloheximide plate, and incubate at 30°C.
Note: Because of selection pressures, colony appearance often takes up to seven days, as described above in Step B2a ii.
g. Verify colonies are cytoductants by replica plating or streaking on selective media to confirm the presence of recipient specific auxotrophic markers, and the ability to grow on YPGlycerol plates. Using the example provided above and depicted in Figure 1, cytoductants will grow on YPGlycerol, YPD+cycloheximide, and SD-Leu, but not on SD-Lys, SD-Ura, or SD-Trp.

4. Confirming the presence of the prion
The presence of a prion should be confirmed in two different ways: the presence of fluorescent aggregates and prion loss by growth on guanidine hydrochloride. These confirmations can be pursued simultaneously.
a. Determining the presence of a prion through fluorescent aggregates.
i. Transform your tester strain (Materials and Reagents, 4.e., meeting all conditions i.-iv.) using standard transformation protocols (Gietz, 2014) with a plasmid that encodes the prion domain or full-length prion protein, fused to a fluorescent tag (GFP, RFP, *etc.*), under a conditional promoter.
1) Several plasmids are available through addgene.org, such as pCup1-Sup35-GFP and pCUP1-Rnq1-YFP; see Materials and Reagents 5 for plasmid numbers.
2) For the purposes of this example, we will use a prion-free version of BY4741 (*Mat***a**, *his3, leu2, ura3, met15*) as the tester strain and a plasmid containing a *URA3* selectable marker. Additionally, BY4741 has been shown to carry a [*PIN*$^{+}$] variant (Manogaran *et al.*, 2010), so for use as a tester, it was cured of all prions on YPD

Bio-protocol 11(17): e4146.
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containing guanidine hydrochloride.

ii. Prepare cytoductants and the transformed tester strain for mating by generating fresh plates of actively growing cells. Use a sterile toothpick to spread a thin film of plasmid containing tester cells into a 2 × 2 cm “patch” on a fresh plasmid selective plate (such as SD-Ura). Do the same with the recipient strain on a YPD plate. Incubate plates at 30°C overnight.

iii. On a fresh YPD plate, use a sterile toothpick to mix both the recipient and tester strain in one patch. Incubate plates at 30°C overnight.

iv. After mating, streak cells for a single colony on media that selects for the diploid and the plasmid. Using the example recipient strain, as well as a BY4741 tester carrying a *URA3* selectable plasmid, cells can be streaked on SD-Leu-Ura to select for diploids retaining the plasmid. Incubate at 30°C for 2-3 days until single colonies appear.

v. Pick single colonies from selective plates and grow at 30°C overnight in liquid culture that will maintain the plasmid (*e.g.*, SD-Ura in the example given). As a control, grow a liquid culture of haploid tester containing the plasmid (from Step B4a i above). This will repeat confirmation that the tester strain does not carry the prion and provide cells grown in parallel as a visual control when scoring cytoductants for fluorescent aggregates in Step B4a vii. below.

vi. To induce expression of the fluorescently tagged prion protein via the *CUP1* promoter, add copper sulfate to a final concentration of 50 µM, and grow at 30°C for an additional 4-6 h.

vii. Use a fluorescent microscope to observe cells for fluorescent aggregates (Figure 4).
Note: Cells expressing the plasmid but that do not contain the prion, such as your haploid tester strain control, will have diffuse fluorescence (Figure 4).

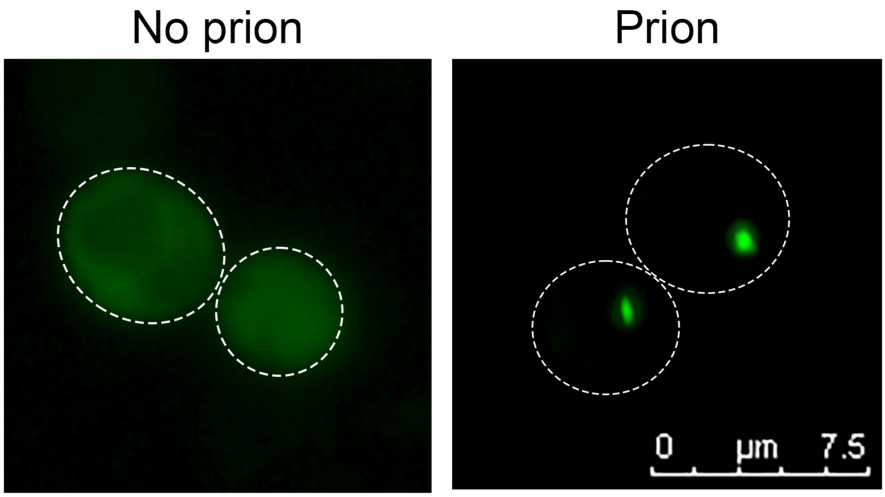

Figure 4. Fluorescent microscope images of cells with diffuse fluorescence versus aggregates indicating presence of the prion. Transient expression of Sup35-GFP or Rnq1-GFP in cured strains exhibit diffuse fluorescence, similar to the image on the left. In the presence

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

of a prion, fluorescent puncta are observed. The right panel shows transient expression of Rnq1-GFP in a BY4741 strain with high [*PIN*$^+$] [from Dorweiler *et al.* (2020)].

b. Curing of Hsp104-dependent prions by guanidine hydrochloride.
Note: If you are working with prions that require an alternative method for curing, such as [GAR$^+$] or [SMAUG$^+$], that curing method must be used instead of the method described here. Consult literature describing your prion of interest to determine the appropriate curing method to use.
   i. Patch haploid cytoductants (rather than diploids generated in Step B4a) on YPD media containing 5 mM guanidine hydrochloride. Incubate for 1-2 days at 30°C.
   ii. Repeat by taking patched cells and making another patch on a fresh YPD 5 mM guanidine hydrochloride plate. Incubate for 1-2 days at 30°C.
   Note: While two successive patches often results in curing most cells, it is recommended to repeat the patching for a total of three times.
   iii. Using a fresh toothpick, streak cells on a YPD plate and incubate at 30°C until single colonies appear.
   iv. To verify loss of the prion, score for the presence of fluorescent aggregates (as described in Step B4a).

C. Selecting cytoductants using the *rho*^{0} strategy
1. Strain selection
   a. The donor strain or the recipient strain must contain the *kar1* mutation.
   b. For the cytoduction of prions, the donor strain must contain the prion (such as high [*PIN*$^+$] or weak [*PSI*$^+$]).
   c. Map out the full genotypic information for the donor, recipient, and all subsequent cells in the cytoduction scheme / diagram (see Figure 1).
   Example Donor: *MAT**a**, kar1-1, his3, leu2, ura2*, *URA3, LYS9, TRP1,* high [*PIN*$^+$].
   Example Recipient: *MAT*α, *KAR1, his3-1, LEU2, URA2, ura3-14, lys9Δ, trp1-1*, no prions.
   Donor should differ from the recipient by 1 or more auxotrophic or antibiotic markers (*leu2* in the example given) to successfully distinguish cytoductants from other cells in the scheme.
   d. Recipient strain must not be respiration deficient (unless already *rho*^{0}).
2. Generate *rho*^{0} recipient cells as described in Step B2b
3. Mating and selection of cytoductants
Note: Because this strategy offers no direct selection against the heterokaryon, it is advised not to use the crisscross method described in Figure 3. Instead, mated cells will be streaked for single colonies in Step C3d.
   a. Prepare cells for mating by generating fresh plates of actively growing cells. Use a sterile toothpick to spread a thin film of donor cells into a 2 × 2 cm “patch” on a fresh YPD plate. Do the same with the recipient strain on another YPD plate. Incubate plates at 30°C

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

overnight.

Note: The recipient strain will grow slowly, and it is best if more cells are patched for growth.

b. On a fresh YPD plate, use a sterile toothpick to mix both the recipient and donor strain in one patch.

c. Incubate plates at 30°C for approximately 10-16 h.

Notes:

i. The presence of shmoos can be verified as described in Step B3e.

ii. Mating of strains beyond 16 h generates an excessive amount of heterokaryons that take over the culture, which may reduce the frequency of cytoductants obtained in Step C3e below.

d. Streak mated cells on synthetic glycerol (SGlycerol) plates that select against the donor strain (Using the example -leu donor strain, SGlycerol-Leu), and incubate at 30°C for 3-5 days until single colonies appear.

e. Pick single colonies and patch onto fresh selective SGlycerol plates (SGlycerol-Leu for example donor). Incubate at 30°C.

Note: It is advisable to pick at least 20 colonies, given that both heterokaryons and cytoductants will be present on the selective SGlycerol plates. When selecting colonies, avoid large colonies; these large colonies tend to be heterokaryons.

f. To screen for cytoductant specific markers, replica plate patches from Step C3e to the appropriate media. This process is analogous to that shown in Figure 2, with the exception that the plate used in Figure 2D would contain individual patches of several cytoductants (prepared in Step C3e), and several selective plates would be sequentially printed from that velveteen (Figure 2F). Importantly, mark the orientation of all replicate plates to ensure consistent scoring of each independent cytoductant. The cytoductants will be *rho*$^+$. Therefore, using the example above, cytoductants will grow on YPGlycerol and SD-Leu, but not on SD-Lys, SD-Ura, or SD-Trp. If strains grow on SD-Ura, these are heterokaryons.

4. Confirming the presence of the prion

To confirm the presence of the prion, follow Step B4.

D. Selecting cytoductants using the plasmiduction strategy

1. Strain selection

a. The donor strain or the recipient strain must contain the *kar1* mutation.

b. For the cytoduction of prions, the donor strain must contain the prion (such as high [*PIN*$^+$] or weak [*PSI*$^+$]).

c. Map out the full genotypic information for donor, recipient, and all subsequent cells in the cytoduction scheme/diagram (see Figure 1).

Example Donor: *MAT***a***, kar1-1, his3, leu2, ura2*, *URA3, LYS9, TRP1,* high [*PIN*$^+$].

Example Recipient: *MATα, KAR1, his3-1, LEU2, URA2, ura3-14, lys9Δ, trp1-1*, no prions.

Note: Donor and recipient must share at least one auxotrophic marker such that both strains

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

can maintain selection for a plasmid encoding that specific marker (e.g., his3 in this example). While neither of the above strains can grow on media lacking uracil, a URA3 plasmid can only complement this deficiency in the recipient strain.

2. Preparing the donor strain

   Transform the donor strain with a plasmid that can also be maintained with selection in the recipient (in the example above, it would be a *HIS3* plasmid), using standard transformation protocols (Gietz, 2014).

3. Mating and selection of plasmiductants

   Note: Because this strategy offers no direct selection against the heterokaryon, it is advised not to use the crisscross method described in Figure 3. Instead, mated cells will be streaked for single colonies in Step D3d.

   a. Prepare cells for mating by generating fresh plates of actively growing cells. Use a sterile toothpick to spread a thin film of plasmid containing donor cells into a 2 × 2 cm “patch” on a fresh plasmid selective plate (such as SD-His). Do the same with the recipient strain on a YPD plate. Incubate plates at 30°C overnight.

   b. On a fresh YPD plate, use a sterile toothpick to mix both the recipient and donor strain in one patch.

   c. Incubate plates at 30°C for approximately 10-16 h.

      Note: The presence of shmoos can be verified as described in Step B3e. Mating of strains beyond 16 h can result in plasmid loss due to the absence of selective pressure, and can generate excessive proportions of heterokaryons that take over the culture, both of which may reduce the frequency of cytoductants obtained in Step D3e below.

   d. After mating, streak cells for a single colony on a media that selects against the recipient strain and the donor strain. In the case above, because all but the recipient strain will now contain a *HIS3* plasmid, and the donor strain is leu^-, matings can be streaked on SD-Leu-His. Incubate at 30°C for 2-3 days until single colonies appear.

   e. Pick single colonies and patch onto fresh media that again selects against the donor and recipient strain. Incubate at 30°C.

      Note: It is advisable to pick at least 20 colonies, given that both heterokaryons and cytoductants will be present on the selective plates. When selecting colonies, avoid large colonies; these large colonies tend to be heterokaryons.

   f. To screen for cytoductant specific markers, replica plate patches to media providing both positive and negative selection. This process is analogous to that shown in Figure 2, with the exception that the plate used in Figure 2D would contain individual patches of several cytoductants (prepared in Step D3e), and several selective plates would be sequentially printed from that velveteen (Figure 2F). Importantly, mark the orientation of all replicate plates to ensure consistent scoring of each independent cytoductant. Using the example provided above, along with a HIS3 plasmid, cytoductants will be His^+ and Leu^+, whereas any strains that grow on SD-Ura are heterokaryons. Therefore, cytoductants will grow on

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

SD-Leu and SD-His, but not on SD-Lys, SD-Ura, or SD-Trp.

4. Confirming the presence of the prion

   To confirm the presence of the prion, follow Step B4.

   If the plasmid used for plasmiduction carries the fluorescently tagged prion, it is not necessary to mate cytoductants with a tester strain (as described in Steps B4a i-iv), but immediately proceed with screening cytoductants for the presence of fluorescent aggregates (as described in Steps B4a v-vii). Cuing the prion can be performed on YPD+GuHCl (as described in Step B4b). However, it is important to recognize that curing can also lead to plasmid loss. Therefore, screening afterwards via fluorescent aggregates may require subsequent selection for rare cells still carrying the plasmid, transformation, or mating with the tester strain carrying the plasmid to verify loss of prion aggregates (as described in Step B4b iv). An alternative option involves curing the prion on synthetic media that will maintain the plasmid, but note that this requires using a lower concentration of GuHCl (3 mM instead of 5) and thus requires additional passes for successful curing.

Data analysis

The objective of this cytoduction protocol is to generate prion containing cells in your genetic background of interest. These cells can be used in any number of downstream experiments of your choosing or design. Cytoduction and plasmiduction can be used to determine whether genetic mutants or certain prion variants influence prion maintenance. For example, introduction of the μdot [*PIN*$^+$] variant is cytoduced in 100% of wild-type cells but is not faithfully transmitted in some *act1* mutants (Dorweiler *et al.*, 2020). To interpret experiments of this nature, it is important to run cytoduction controls that include prions that are easily transmitted through cytoduction and recipient cells that are wild type.

Recipes

1. Media

   All basic media prepared as described by Sherman (2002).

2. 10 mg/ml Cycloheximide stock solution (for cytoduction involving cycloheximide resistance)

   Mix the following in a sterile 15 ml conical tube:

   20 mg Cycloheximide (***Caution: Highly toxic, wear PPE; weigh in fume hood to avoid inhaling dust.)

   2 ml of 100% DMSO (cycloheximide is also soluble in ethanol but takes longer to fully dissolve)

   Mix well until cycloheximide is fully dissolved

   Note: We have not found it necessary to filter-sterilize this solution, but please note that optional sterilization would require a DMSO-compatible filter.

   Wrap tube in foil and store remaining volume at -20°C.

Bio-protocol 11(17): e4146.
DOI:10.21769/BioProtoc.4146

3. YPD (or YPGlycerol) plates with 10 µg/ml Cycloheximide
   Cool freshly autoclaved YPD (or YPG) to ~50°C.
   Add cycloheximide to a final concentration of 10 µg/ml (for example, 0.5 ml of cycloheximide per 500 ml of YPD).
   Mix thoroughly but carefully before pouring plates.
4. 2.5 mg/ml Ethidium Bromide stock solution (for generating *rho*^{0} strains)
   Mix the following in a sterile 15 ml conical tube:
   25 mg Ethidium Bromide powder (***Caution: mutagen)
   10 ml sterile dH_2O
   Vortex vigorously until EtBr powder is dissolved.
   To store remaining volume, wrap tube in foil and place at 4°C.
5. YPD plates with 25 µg/ml EtBr (two plates, as this is sufficient for most instances)
   In a disposable, sterile 50 ml conical tube, combine 500 µl of the EtBr 2.5 mg/ml stock solution and 50 ml YPD + agar from a freshly autoclaved batch (the remainder can be used to pour standard YPD plates).
   Distribute between two sterile Petri plates.
6. 5 M Guanidine Hydrochloride
   For 10 ml of 5M guanidine hydrochloride solution, dissolve 4.78 g guanidine hydrochloride in 10 ml sterile distilled water.
   Filter sterilize using a 0.2 µm filter.
   Store unused solution at 4°C.
7. YPD plates with guanidine hydrochloride (5 mM)
   Cool freshly autoclaved YPD to ~50°C.
   Add guanidine hydrochloride to a final concentration of 5 mM (for example, 0.5 ml of 5M guanidine hydrochloride per 500 ml of YPD).
   Mix thoroughly but carefully before pouring plates.
8. SGlycerol-selective plates (for 600 ml – approximately 16-18 plates)
   1 g Yeast Nitrogen Base without amino acids and ammonium sulfate
   3 g Ammonium sulfate
   18 ml Glycerol
   12 g Agar
   All nutrients, as described in Materials and Reagents 6.c.iii., except those which provide the desired selection.
   600 ml dH_2O
   Autoclave, allow to cool, and pour plates.

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Competing interests

The authors declare no competing interests.

Ethics

None of the protocols described herein require use of human or animal subjects.

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