kar-mediated Plasmid Transfer between Yeast Strains: an Alternative to Traditional Transformation Methods

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Introduction

The budding yeast *Saccharomyces cerevisiae* is an excellent system for the study of many problems in eukaryotic biology. Despite the diverse nature of the questions asked, almost all of these studies use molecular tools and often require the transfer of exogenous DNA into the yeast cell. Several techniques for high-efficiency transformation of plasmid DNA into yeast have been developed and used successfully over the years. These include spheroplast transformation, transformation by electroporation and lithium acetate transformation.[1-3] One of the major drawbacks of these methods is the many and often time consuming steps that are necessary to prepare the host strain, introduce the exogenous plasmid and identify successful transformants. This becomes an important issue when the transformation of a plasmid into a large number of host strains is desired. Modifications that scale down the lithium acetate method have been successfully used for large-scale transformation of the same plasmid into a set of strains.[4] However, transformation efficiency is compromised and this method may not be useful for all purposes.

The problem becomes even more acute when multiple distinct plasmids need to be transferred from one strain to another since this requires the extra steps of DNA isolation from yeast and plasmid rescue in bacteria. In the age of genome-scale

manipulations of yeast, an easier and less time consuming method of plasmid introduction or transfer is desirable. Here we describe a method of *kar*-mediated transfer that allows the quick and efficient transfer of plasmids from one yeast strain to another or to a number of different strains. The method is based on yeast mating and uses a particular mutation in the karyogamy pathway, *kar1*.[5] *kar1* mutant strains initiate mating and proceed through conjugation without nuclear fusion, and therefore haploid progeny can be recovered.[5] During a *kar* mating heterokaryons with mixed cytoplasm are formed and nuclear DNA such as plasmids and whole chromosomes can be transferred between the two parents.[6,7] With the proper selection of nuclear genetic markers, plasmid transfer can be made directional.

Background and Principles of the Method

Glossary

karyogamy: the process of nuclear congression and fusion during conjugation

kar mutants: mutants defective in the karyogamy pathway

kar mating: a mating in which one of the parents carries a particular *kar* mutation, in this context, *kar1Δ15*

recipient strain: in a *kar* mating, the haploid parent (nucleus) that is selected as a host for the transferred material (plasmid)

donor strain: the haploid strain that carries the material (plasmid) that is transferred to the recipient via a *kar* mating

cytoductants: haploid progeny from a *kar* mating with the nucleus of the recipient and mixed cytoplasm from both parents

chromoductants: haploid progeny from a *kar* mating with the nucleus of the recipient and a chromosome(s) transferred from the donor

plasmoductants: haploid progeny from a *kar* mating with the nucleus of the recipient and a plasmid from the donor

Most laboratory yeast strains exist as *MAT***a** or *MAT*α haploids. A mating between *MAT***a** and *MAT*α yeast produces diploids. This process begins when haploid cells of the opposite mating type adhere to one another after the mutual exchange of pheromones. This is followed by the orderly removal of cell walls and plasma membrane to complete cell fusion.[8] One characteristic feature of all fungi including yeast is that during the process of conjugation, the nuclear envelope remains intact and the resulting diploid nucleus is the product of the direct fusion of the two parental nuclei.[9]

A number of mutations that block the karyogamy pathway have been identified and studied. One such mutation is *kar1-1,* which prevents nuclear fusion in approximately 90-95% of matings.[5,10] *KAR1* is essential for mitotic growth because of

its role in the initial stage of spindle pole body duplication.[10] Kar1 has three functional domains that separate its mitotic function from its role during nuclear fusion.[11] An amino-terminal domain is important for the nuclear fusion function and a deletion of this protein domain gives an allele, *kar1Δ15*, that is viable and defective only in nuclear fusion.[11] *kar1Δ15* mutation is unilateral in that its defect is observed even in a mating to a wild type (WT) strain.[11]

Matings in which either parent is *kar1Δ15* mutant are unproductive since they generate diploid nuclei at a very low frequency.[11] The majority of the products from such matings are cytoductants and, in practice, they can be selected when the nuclei and the cytoplasm of each parent are marked genetically.[12,13] Another characteristic of *kar* mating, of particular interest to the technique described here, is the occasional transfer of genetic material from one nucleus to the other.[6,7] This property of *kar* mating has been used for the directional transfer of yeast artificial chromosomes from one strain to another host of interest.[14,15] We have developed this technique further for the directional transfer of plasmids from one yeast strain to another.

The most important prerequisite for a directional transfer of plasmids is the proper design of the strains. The goal is to select haploid progeny that contain the desired parental nucleus and that have acquired the plasmid of interest. One consideration is that the recipient is auxotrophic for the marker selecting the plasmid. The other consideration

is that the parental genotypes allow selection for the recipient and against the donor nucleus. The *CAN1* and *CYH2* genes are suitable for this purpose. *CAN1* encodes the arginine permease and mutations that disrupt its function confer recessive resistance to canavanine, a toxic arginine analogue.[16] *CYH2* encodes the essential L29 ribosomal protein and is the target of the peptidyltransferase inhibitor, cycloheximide.[17-19] Recessive mutations in *CYH2* confer resistance to high doses of this drug.[17] The recipient strain must carry recessive drug-resistance markers, e.g. $can1^R$ and $cyh2^R$ while the donor strain must have the corresponding dominant drug-sensitive alleles, $CAN1^S$ and $CYH2^S$. Selection on cycloheximide and canavanine-containing medium ensures that only the $can1^R$ $cyh2^R$ recipient nucleus survives in a *kar* mating. The configuration of markers just described allows selection not only of plasmoductants but also allows counter-selection of the plasmid donor nucleus and the rare diploids that arise during the *kar* mating. Use of two drug-sensitive markers is most efficient in selecting against the donor nucleus since the frequency of spontaneous mutation to double drug resistance is extremely low. Either parent can provide the *kar1Δ15* allele because of the unilateral nature of this mutation.

Strains, media and drugs

Most of the experiments described here use strains with the W1588 background that is isogenic to W303 (*MAT***a** or α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) [20] but is *RAD5*. However, any haploid yeast strain that mates well and contains the appropriate genetic markers can be used. For example, the two-hybrid strain PJ69-4A (*MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 ade2-101::GAL2-ADE2 met2::GAL7-lacZ KAR1 CYH2 CAN1 RNR1*)[21] was successfully used as the donor strain in the experiment described in Figure 1. Introduction of the genetic markers can be achieved through standard genetic manipulations or through allele replacement methods.[22-25] In addition, spontaneous canavanine or cycloheximide resistant mutants can be selected after plating $CAN1^S$ or $CYH2^S$ strains on drug-containing medium. This approach is quite easy for selecting can^R colonies as the frequency of mutation from $CAN1^S$ to $can1^R$ is approximately 10^{-6}. Selecting cyh^R colonies is not as easy because the mutation frequency from $CYH2^S$ to cyh^R is approximately 10^{-8}. This is due to the fact that spontaneous cyh^R mutants are almost always altered at a single amino acid in Cyh2 protein, Q38.[26-28] Selection for spontaneous cyh^R colonies occasionally results in slow-growing colonies and such candidates should not be used.

Because of the mating defect of the *kar1Δ15* mutant, genetic manipulations with this allele will be explained in some detail. Since *kar* matings are inefficient in generating

diploids, one can "enrich" for diploids by extending the mating time during strain construction. Instead of the normal "overnight" incubation for matings of WT haploids, the incubation period for *kar1Δ15* strains should be extended to at least 2 days at 30°C on rich medium. It is important that diploids are selected by complementation of recessive auxotrophic markers in the haploid parents. When a strain construction scheme requires several crosses for the introduction of genetic markers, it is helpful to add the *kar1Δ15* allele last. This avoids successive crosses requiring extended periods of mating due to the *kar1* mutation.

Compared to WT, the mating deficiency of the *kar1Δ15* mutant provides an easy phenotype for scoring this mutation in tetrad analysis. In a time-limited mating assay,[10] plates with dissected tetrads are mated to appropriately marked *MAT***a** and *MAT*α mating testers for 2-3hrs at 30°C on YPD and subsequently replica plated on synthetic dextrose (SD) plates to select diploids. Because of the short incubation, only the *KAR1* spores undergo a robust mating and give rise to patches of complementing diploid clones on the SD plates. The *kar1Δ15* spores rarely produce diploids in this short mating and these are seen as occasional papillae on the SD plate. The mating type of the *kar1* mutant spores can be determined separately by mating the spore clones to the mating testers for 1-2 days before replicating them to the SD plates. After extended incubation, the *kar1Δ15* spores produce enough diploids to allow scoring of mating type by growth on SD plates.

In the experiments described in this chapter, all plasmids used in the transfers are 2μ-based vectors. In other experiments, we have shown that *CEN-ARS*-based vectors also transfer efficiently between strains via *kar* mating.

Standard yeast media and techniques for strain manipulations were used as described previously.[22] Since the method is based solely on mating and replica plating, the only equipment needed is a replicating block and sterile velvets. All matings are done at 30°C on YPD medium. Freshly grown strains, 2-3 days old, give best results.

Cycloheximide and canavanine at final concentrations of 1μg/ml and 60μg/ml, respectively, are added to autoclaved medium after cooling to 55°C. Canavanine is a toxic arginine analog and it should be added to synthetic complete medium that lacks arginine (SC-Arg).

General Protocol

Depending on the experiment, *kar* transfer can be done from many donors into one recipient, or from one donor into many recipients. In either case, the multiple strains are patched on a master plate of the appropriate medium and incubated for 1-2 days until patches, approximately 6-8 mm in diameter, are formed. In the case of uneven growth, strains can be grown for a longer period of time to produce enough cells in each patch.[28] The single strain, regardless of whether it is the recipient or donor, is spread on the

appropriate medium to create a uniform lawn that covers the entire surface of the plate. Subsequently this lawn can be propagated via replica-plating. One lawn is used for mating to each master plate containing multiple strains. Mating is performed by replica-plating the two plates together on a fresh YPD plate and incubating for 6-12 hrs at 30°C. The time of mating can be varied such that the number of papillae seen on the drug selection medium is optimized. Since *kar1* mutants are impaired, but not completely deficient for mating, the shortest mating time that efficiently gives plasmoductants should be used. The matings are next replica-plated to SC-Arg+Can+Cyh medium that also lacks the nutrient for the prototrophic marker of the transferred plasmid, and are grown for 4-5 days at 30°C, or until plasmoductants are seen at the mating control patch. A second replica to selective medium is important to ensure that the non-plasmoductant cells on the plates are dead. This step eliminates background and it is very important if the plasmoductants are tested for another phenotype by an additional replica-plating.

Transfer of a library or a set of plasmids to a strain of interest: test for complementation

In this section, we describe the *kar*-mediated transfer of multiple distinct plasmids to a single strain in a complementation experiment. A library of PCR-mutagenized *RNR1* in a *TRP1*-marked 2μ plasmid was initially tested for altered protein interactions in the

two-hybrid host PJ69-4A.[29] In the next step, each individual plasmid from the mutagenized *rnr1* library was analyzed for functional complementation in a new host that carried a temperature-sensitive allele of the *RNR1* gene (Figure 1). The two-hybrid plasmid donor strain was *MAT***a** *trp1 CYH2*^{S} *CAN1*^{S}, and the recipient strain was *MAT*α *kar1Δ15 trp1 cyh2*^{R} *can1*^{R} *rnr1*^{ts}. To perform the *kar* transfer of the mutagenized *rnr1* plasmids, the plasmid donor strains were patched on SC-Trp master plates and grown for 1-2 days. The recipient strain was spread as a uniform lawn on YPD plates. By replica-plating, each donor plate was mated for 6 hrs at 30°C to the recipient on a fresh YPD plate. The mating plates were next replica-plated to SC-Trp-Arg+Can+Cyh medium and grown for 4-5 days at 30°C until papillae of plasmoductants were seen at the donor patches. These plasmoductants were replica-plated once more to SC-Trp-Arg+Can+Cyh medium and subsequently tested for functional complementation by analysis of their ability to grow after a temperature shift to 37°C, the non-permissive temperature for the *rnr1*^{ts} allele.

The technique described above is a generic method to transfer any set of distinct plasmids into a single assay strain. For example, this method can be utilized for cloning by complementation. In this case, the donor is a set of strains carrying an ordered library of yeast sequences. The single recipient strain is a newly isolated unknown mutant. The arrayed library could then be used for complementation cloning of the mutant if its

phenotype can be scored by replica-plating. Since the initial step of arraying such a library is time consuming and laborious, it is worthwhile only if it will be used multiple times. Otherwise traditional cloning by transformation is more efficient.

Transfer of the same plasmid to a set of strains: test for synthetic phenotype

This section describes the use of *kar*-mediated plasmid transfer when moving the same plasmid into a number of different strains. This is very useful in mutant screens that are based on a plasmid-borne assay or phenotype, e.g., mutant searches for synthetic lethal[30] (SL) or "synthetic viable" (SV) interactions.[31] *S*ynthetic *l*ethal *m*utations (*slm*) cause a synergistic growth defect under certain conditions when combined with a pre-existing mutation, for example *y*our *f*avorite *g*ene *(yfgΔ*). Typically such screens rely on a strain that lacks the chromosomal chopy of *YFG* (*yfgΔ*) but contains a plasmid that harbors the WT copy of *YFG* and a marker that is both selectable and counter-selectable, e.g., *URA3* (p{*YFG URA3*}). After mutagenesis and the initial round of selection, colonies that are incapable of losing p{*YFG URA3*} are isolated. However, it is important to show that the *slm*-dependent plasmid retention is due to *YFG* and not to any other elements on the plasmid. This requires demonstration that the *slm* phenotype can be reproduced with a new plasmid carrying *YFG* and marked with something other than *URA3*, e.g., p{*YFG LEU2*}. The new p{*YFG LEU2*} as well as the empty vector

p{*LEU2*} must be introduced in parallel in each *slm* candidate. Upon counter-selection against p{*YFG URA3*}, a true *slm* strain can lose p{*YFG URA3*} in the presence of p{*YFG LEU2*}, but not in the presence of p{*LEU2*}. Traditionally, p{*YFG LEU2*} and p{*LEU2*} are introduced through transformation methods. Depending on the scale of the screen, the number of transformation reactions can be several hundred thus making the analysis quite laborious.

The method of *kar*-mediated plasmid transfer simplifies the problem of multiple transformations of the same plasmid by providing an alternative means for plasmid introduction. Through two parallel matings and a few steps of replica-plating, both the p{*YFG LEU2*} plasmid and the empty vector p{*LEU2*} can be transferred from two different donor strains to a large number of *slm* candidates. A scheme that depicts the *kar* plasmid transfer as a step in a SL screen is shown in Figure 2. The *URA3* gene in the starting plasmid p{*YFG URA3*} allows selection on SC-Ura and counterselection on 5-fluoro-orotic acid (5-FOA).[32] Each master plate with *slm* candidates is mated independently to two donor strains that carry either the p{*YFG LEU2*} plasmid or the p{*LEU2*} empty vector. Two successive rounds of replica-plating on SC-Leu-Arg+Cyh+Can medium selects plasmoductants. To test the synthetic lethal interaction, the two plates with plasmoductants of either the p{*YFG LEU2*} or the empty vector, are replica-plated to SC-Leu+5-FOA to shuffle out the starting p{*YFG URA3*} plasmid.[33]

There are several classes of mutant candidates: First, since true *slm* candidates require the function of *YFG*, only plasmoductants that transferred the p{*YFG LEU2*} plasmid, but not the empty vector, grow on 5-FOA-containing medium. On the other hand, plasmoductants of false *slm* candidates grow regardless of whether they transferred the p{*YFG LEU2*} plasmid or the empty vector. Any candidates that successfully transferred the p{*YFG LEU2*} plasmid and the empty vector but fail to grow on SC-Leu+5-FOA plates after counter-selection of the p{*YFG URA3*} plasmid, represent mutants that have SL interaction with other elements on the starting p{*YFG URA3*} plasmid and are of no interest. Finally, it is possible that a *slm* or a non-specific mutation renders the candidate mating-incompetent and plasmoductants can not be recovered. In this case traditional transformation methods are used.

A similar scheme can be employed to facilitate screening for mutations that show a SV interaction. In many cases a loss-of-function mutation in an essential gene is inviable unless another mutation in a different gene bypasses its defect. This second mutation is called a suppressor when it has been identified *after* the original mutation in the essential gene. However, if a potential suppressor mutation is *first* introduced into a strain and mutations of essential genes are sought that are now viable, this interaction is said to be "synthetic viable." Mutations in essential genes that show such interaction are called *s*ynthetic *v*iable *m*utation, *svm,* mutants. Another way of thinking about SV

interactions is that the removal of one gene's function permits growth of an otherwise inviable mutation in an essential gene.

An experiment to identify *svm* mutants can be based on a plasmid assay. In this scheme, mutants are sought that show synthetic viable interaction with deletion of a potential suppressor, e.g., *y*our *f*avorite *s*uppressor (*yfsΔ*). The starting strain is *yfsΔ can1*^{R} *cyh2*^{R} and carries a plasmid with *YFS* and a color marker such as *ADE3,* which allows plasmid visualization in an *ade2 ade3* background. For example, in one common design, yeast cells that maintain the plasmid grow to form red colonies while those that lose the plasmid are white.[30] After mutagenesis of the starting strain, *yfsΔ svm* candidates must lose the plasmid with *YFS* to be alive. Since the plasmid is not essential for viability, it could be lost for many other reasons following mutagenesis. Random loss versus obligatory loss of the plasmid is determined based on colony color. Red-white sectored colonies denote random loss while solid white colonies indicate obligatory loss. However, it is important to show that the observed obligatory plasmid loss is due to a SV interaction with *YFS*. If mutant candidates are transformed with a new plasmid with *YFS,* e.g., p{*YFS URA3*}*,* or the corresponding empty vector, p{*URA3*}, true *yfsΔ svm* mutants give transformants only with the empty vector and not with the p{*YFS URA3*}plasmid. This analysis requires two transformation reactions per *yfsΔ svm* candidate.

A *kar*-mediated plasmid transfer simplifies the introduction of the new p{*YFS URA3*} plasmid and the empty vector plasmid described above. In contrast to the SL mutants, true *svm* candidates will not grow when provided with a WT copy of *YFS* but will grow when provided with the empty vector (Figure 3). In this experiment, recipient *yfsΔ svm* candidates are patched in duplicate on the master plate. The two donor strains of the p{*YFS URA3*} plasmid and of the vector control are spread as separate lawns on SC-Ura plates. Matings between the recipient and the two donors are made on YPD for 12-14hrs at 30°C. Plasmoductants are analyzed for growth after two successive rounds of selection on SC-Ura-Arg+Cyh+Can. Acting as a control, patches that give rise to p{*URA3*} plasmoductants demonstrate the candidate's ability to mate and to stably maintain a plasmid. Furthermore, patches that fail to give plasmoductants with the p{*YFS URA3*} plasmid identify true *yfsΔ svm* strain. On the other hand, strains that give plasmoductants with both plasmids represent false *yfsΔ svm* candidates.

In Figure 3, only the control patches show a true *yfsΔ svm* mutant phenotype since these strains give plasmoductants only with the empty vector but not with p{*YFS URA3*}. All other patches are false *yfsΔ svm* candidates since they grow after transferring either plasmid. The duplicate patches that did not give plasmoductants with either plasmid may represent a non-mating *yfsΔ svm* candidate or a strain with a plasmid stability mutation. Such candidates can be examined using traditional transformation methods (for the non-maters) or by standard genetic analysis after a cross to a *YFS* strain. In this experiment,

kar-mediated transfer was used successfully to rigorously test the validity of a presumptive synthetic viable interaction with *yfsΔ*. This analysis helped eliminate further examination of false *yfsΔ svm* candidates.

Considerations

The process of *kar*-mediated plasmid transfer has one inherent problem. Transfer of yeast chromosomes from the donor nucleus to the recipient nucleus is observed at a frequency of 2-5% for individual chromosomes.[7,15] Therefore plasmoductants may also contain extra chromosomes. This is only a problem if single plasmoductant colonies are picked, propagated and used in further experiments. For the applications described in this chapter, any potential chromosome transfer is irrelevant for several reasons. First, in all cases, the experimental conditions provide an opportunity to look at multiple independent plasmoductants in a patch. Second, in all cases, plasmoductants in at least two individual patches are observed to score the desired phenotype. By meeting these two criteria, the possibility that the observed phenotype is affected by potential chromosome transfer events is virtually eliminated. Third and most importantly, the resulting plasmoductants are not used for further experimentation since the plasmids of interest are rescued from the original donor strain.

Summary and concluding remarks

We have described a method, based entirely on yeast mating and replica-plating, that allows the transfer of plasmids from one strain to another and eliminates the need for DNA isolation and transformation. Because this method uses a *kar1* mutation defective only in nuclear fusion, as well as markers that select against rare diploids, only haploid progeny are recovered. The method of *kar*-mediated plasmid transfer is simple, fast, efficient and does not require any special equipment or elaborate technical manipulations. In addition, it allows the transfer of plasmids into strains that normally do not transform well. Furthermore, depending on the strain background, only minor genetic changes of the strain may be necessary to meet the required marker criteria. Since the donor and recipient strains do not have to be in a common genetic background, this provides the flexibility to transfer plasmids between different laboratory strains. Because of these features, *kar*-mediated transfer is an excellent solution for plasmid library transfers or for the repeated transfer of the same plasmid into a number of host strains.

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Figure Legends

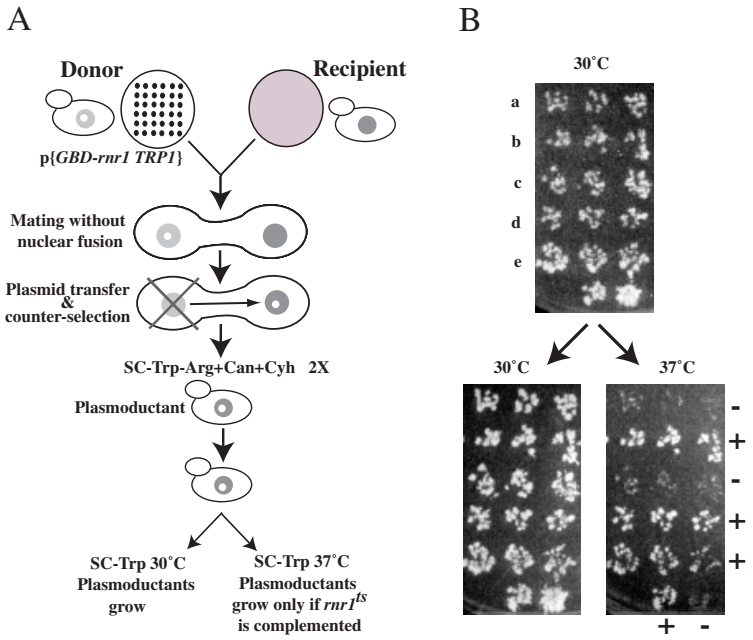
Figure 1. *kar*-mediated plasmid library transfer and functional complementation test. A. Schematic representation of the experimental design. The donor strain, PJ69-4A, is *MAT***a** *CAN1*^{S} *CYH2*^{S} and carries a library of PCR-mutagenized *rnr1* plasmids. The recipient strain is *MAT*α *kar1Δ15 can1*^{R} *cyh2*^{R} *rnr1*^{ts}. The plasmid is represented as a white dot. For complete experimental details see the text. B. Complementation of a *rnr1*^{ts} allele. Each row, a-e, contains papillae of plasmoductants of three isolates of the same *rnr1* mutant candidate. The two patches on the bottom are controls: the left patch, indicated by "+" symbol, is a donor strain that carries a plasmid with the WT *RNR1* gene, a positive control for complementation; the right patch, indicated by a "-" symbol, is a donor strain with the empty vector, a negative control for complementation. The rows marked with a "+" show complementation (b, d and e), while the rows marked with a "-" indicate lack of complementation (a and c).

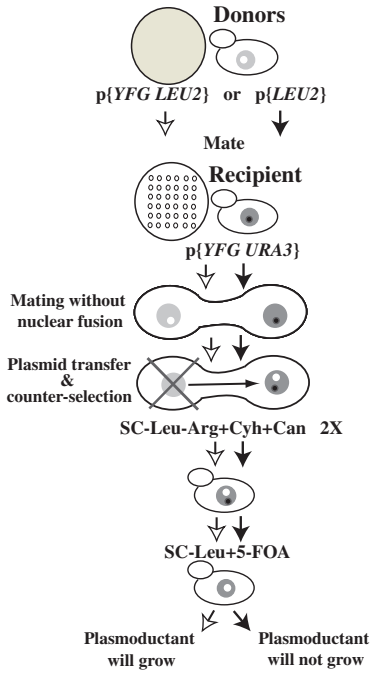
Figure 2. Schematic representation for use of *kar*-mediated plasmid transfer as a step in a synthetic lethal (SL) screen. The recipient strains are SL candidates of the genotype *yfgΔ slm can1*^{R} *cyh2*^{R}. After mutagenesis, these candidates require the plasmid p{*YFG URA3*} for viability (represented as a black dot). The genotype of the donor strain is *kar1Δ15 CAN1*^{S} *CYH2*^{S}. In this experiment there are two different donor strains, one that carries

the new plasmid p{*YFG LEU2*} and another one that carries the empty vector p{*LEU2*} (both represented as a white dot). Two separate matings between the recipients and each donor strain are made on YPD. One mating is to the donor strain carrying the p{*YFG LEU2*} plasmid and the other is to the donor strain with the vector p{*LEU2*} (designated by the open and filled arrowheads, respectively). Successful plasmoductants are selected after two rounds of replica plating to SC-Leu-Arg+Cyh+Can. Plasmoductants from each mating are counter-selected on SC-Leu+5-FOA medium to assay for their ability to grow in the absence of the original plasmid p{*YFG URA3*}. If the SL interaction is real, only plasmoductants that contain the new plasmid p{*YFG LEU2*} but not the empty vector p{*LEU2*} can grow after shuffling out p{*YFG URA3*}.

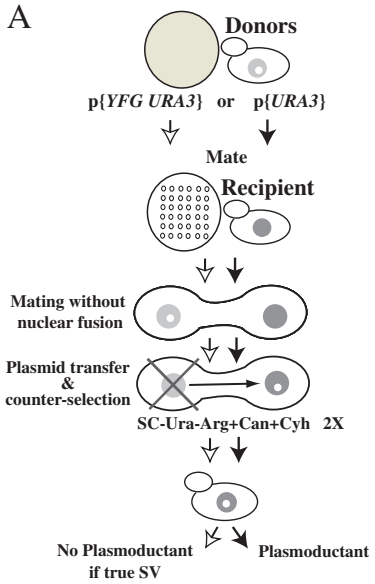
Figure 3. Schematic representation of *kar*-mediated transfer in a screen for SV mutants. A. Experimental design. The recipient strains are SV candidates of the genotype *MAT***a** *yfsΔ svm can1*^{R} *cyh2*^{R} that showed obligatory loss of a plasmid containing *YFG*. These candidates were patched in duplicate and mated independently to donor strains carrying either the p{*YFG URA3*} plasmid (open arrowhead) or the empty vector p{*URA3*} (filled arrowhead; both plasmids are represented as a white dot). The genotype of the donor strains was *MAT*α *kar1Δ15 CAN1*^{S} *CYH2*^{S}. Matings were done for 12-14 hrs at 30°C on YPD. After two rounds of selection on SC-Ura-Arg+Can+Cyh, plasmoductants were analyzed for their ability to grow after transfer of the p{*YFG URA3*} plasmid or the

empty vector. True *yfsΔ svm* candidates cannot grow after transferring p{*YFG URA3*}, while they can grow after transferring the empty vector. B. Results from an experiment of *kar*-mediated plasmid transfer in a screen for SV mutants. In this experiment *yfsΔ svm* candidates were tested for their ability to grow when given the plasmid p{*YFG URA3*} (right photo) or the empty vector p{*URA3*} (left photo). There are two patches for each *yfsΔ svm* candidate. In addition, the two patches on the top and the bottom of the plate are controls for the experiment. The left control patch is a WT strain that tests for mating and plasmoduction and that grows when either plasmid is transferred. The control patch on the right shows a strain with a known SV interaction with *yfsΔ*. This is a true *svm* candidate because it can grow after transfer of the empty vector (photo on the left), but not after transfer of the p{*YFG URA3*} plasmid (photo on the right). The two patches unable to form plasmoductants with either plasmid (left middle of the photo) probably represent a strain with a mating defect or plasmid stability mutation.

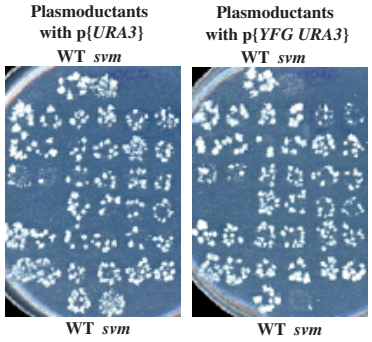

A
Donor
Recipient
p{GBD-rnr1 TRP1}
Mating without nuclear fusion
Plasmid transfer & counter-selection
SC-Trp-Arg+Can+Cyh 2X
Plasmoductant
SC-Trp 30˚C Plasmoductants grow
SC-Trp 37˚C Plasmoductants grow only if rnr1ts is complemented
B
30˚C
a
b
c
d
e
30˚C
37˚C
-
+
-
+
+
+ -


Donors
p{YFG LEU2} or p{LEU2}
Mate
Recipient
p{YFG URA3}
Mating without nuclear fusion
Plasmid transfer & counter-selection
SC-Leu-Arg+Cyh+Can 2X
SC-Leu+5-FOA
Plasmoductant will grow
Plasmoductant will not grow

A


Donors
p{YFG URA3} or p{URA3}
Mate
Recipient
Mating without nuclear fusion
Plasmid transfer & counter-selection
SC-Ura-Arg+Can+Cyh 2X
No Plasmoductant if true SV
Plasmoductant

B


Plasmoductants with p{URA3}
WT svm
Plasmoductants with p{YFG URA3}
WT svm
WT svm
WT svm