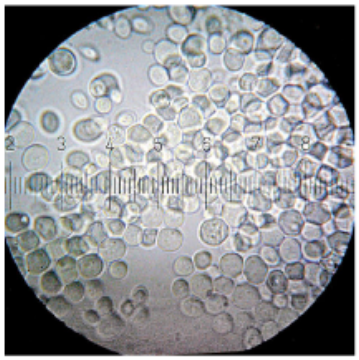
Plasmids 101: Yeast Vectors

By Marcy Patrick

In our first few Plasmids 101 posts, we focused mainly on the elements required for plasmid maintenence within an *E. coli* cell, but vectors can be widely utilized across many different cell types and each one requires different elements for vector propogation. This post, along with a future companion post on mammalian vectors, will catch you up on the core replication and resistance features of yeast vectors and explain how they differ from the bacterial elements previously described.



Source: Wikipedia, accessed 2/23/2014.
Author: Bob Blaylock

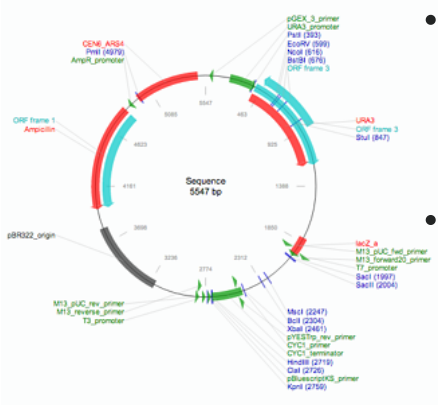
Why do scientists use yeast vectors?

Yeast are eukaryotes and thus contain complex internal cell structures similar to those of plants and animals. Unlike bacteria, yeast can post-translationally modify proteins yet they still share many of the same technical advantages that come with working with prokaryotes. This includes but is not limited to: rapid growth, ease of replica plating and mutant isolation, a well-defined genetic system, and a highly versatile DNA transformation system.Unlike most other microorganisms, yeast have both a stable haploid and diploid state which is useful for genetic analysis, as well as an efficient mechanism of homologous recombination to facilitate simple gene replacement/mutation. Yeast expression plasmids used in the lab typically contain all the necessary components to allow shuttling between *E. coli* and yeast cells. To be useful in the lab, the vectors must contain a yeast-specific origin of replication (ORI) and a means of selection in yeast cells, in addition to the bacterial ORI and antibiotic selection markers.

The yeast origin of replication

(Please note: This first section primarily pertains to ORIs in budding yeast, *Saccharomyces cerevisiae*; however, we've also noted some features required for the replication of fission yeast, *Schizosaccharomyces pombe,* vectors at the end.)

In a previous post, we briefly discussed how the regulation of bacterial ORIs determines plasmid copy number within the bacterial cell. Similary, the specific ORI elements included within a yeast vector determine how the plasmid is replicated and maintained within the yeast cell. These elements control not only the number of plasmids found in each cell, but also whether the plasmid gets integrated into the host DNA or is independently replicated as an episome. *The four main types of yeast plasmids are defined below*:



- ***Yeast Integrating plasmids (YIp)***: These plasmids lack an ORI and must be integrated directly into the host chromosome via homologous recombination.

- ***Yeast Replicating plasmids (YRp)***: These vectors contain an Autonomously Replicating Sequence (ARS) derived from the yeast chromosome. As the name suggests, these vectors can replicate independently of the yeast chromosome; however, they tend to be unstable and may be lost during budding.

- ***Yeast Centromere plasmids (YCp)***: These are considered low copy vectors and incorporate part of an ARS along with part of a centromere sequence (CEN). These vectors replicate as though they are small independent chromosomes and are thus typically found as a single copy. Unlike the ARS vectors, CEN vectors are stable without integration.

- ***Yeast Episomal plasmids (YEp)***: These are most similar to bacterial plasmids and are considered "high copy". A fragment from the 2 micron circle (a natural yeast plasmid) allows for 50+ copies to stably propogate per cell. The copy number of these vectors can also be controlled if specific regulatable elements are included (reviewed here).

Plasmids for use in *S. pombe*, on the other hand, do not require a well defined ORI. Instead, the size and A-T content of the DNA (apparently independent of a known specific sequence) dictate the replication of these vectors. *S. pombe* plasmids oftentimes utilize an ARS to aid in high transformation efficiency; however, this region does not necessarily promote replication.

Selection markers for yeast

Historically, scientists have utilized auxotrophic selection rather than antibiotic selection when working with yeast, due to high rates of spontaneously occuring resistant mutants and the

insensitivity of yeast strains to some antibiotics. Auxotrophy is defined as the inability of an organism to synthesize a particular organic compound required for its growth. Many auxotrophic strains of yeast exist which can be easily maintained when grown on media containing the missing nutrient. Scientists can exploit these host mutations by including a copy of a functional gene which complements the host's auxotrophy. When grown on media NOT containing the nutrient, the host cells will die unless they have incorporated the plasmid carrying the required gene.

The table below lists some of the most commonly used selection markers in yeast and provides the element needed to overcome the auxotrophy as well as additional uses for said element. This link provides a more extensive list of yeast auxotrophic markers and includes the associated references.

Yeast selection marker	What does the marker help synthesize?	Counterselection? (growth-based positive selection for the loss of the marker gene)	For use in:	Can this be used in other yeast species?	Can this be used for auxotrophic selection in E. coli?	Additional considerations
HIS3	L-hisitidine	no	*S. cerevisiae*	no	yes	
URA3	pyrimidine (uracil)	yes - Grow with 5-FOA.	*S. cerevisiae*	yes - This can complement *ura4-S. pombe*, but the complementation is weak.	yes	
LYS2	L-lysine	yes - Grow on plates containing alpha-aminoadipate in the absence of a nitrogen source.	*S. cerevisiae*	no	no	
LEU2	L-leucine	no	*S. cerevisiae*	yes - This can complement leu1-*S. pombe*, but mulitple copies are required.	yes	
TRP1	L-tryptophan	yes - Grow with 5-FAA.	*S. cerevisiae*	no	no	*TRP1* alters some yeast phenotypes. This marker should not be used in gene disruption experiments.

MET15	L-methionine and overproduces hydrosulfide ions	yes - Grow with methylmercury.	*S. cerevisiae*	no	no	Can be used for color and growth selection if divalent lead ions are used in the growth media.
ura4+	pyrimidine (uracil)	yes - Grow with 5-FOA*.	*S. pombe*	no	no	*FOA in fission yeast induces mutation in the *ura5+* gene in addition to *ura4+*.
leu1+	L-leucine		*S. pombe*	no	no	
ade6+	purine (adenine)		*S. pombe*	no	no	

Considerations when using auxotrophic selection

Of course, there are some drawbacks to using auxotrophic markers as a means of selection:

1. A specific selection marker needs to be used with a yeast strain deficient in that compound. Therefore known auxotrophic strain/ selection element pairs must be utilized or a new combination needs to be created in advance of the experiment.

2. The marker provided by the plasmid may be expressed at higher than normal physiological levels due to high copy numbers. This creates a potential metabolic burden on the yeast cells.

3. Some phenotypes may be altered due to the presence of the selection marker at non-physiological levels.

Scientists have tried varied approaches to combat these issues. One method to reduce the amount of marker gene expression is to use a partially defective promoter to drive expression of the selection marker. This reduces the amount of gene product present in the cell, thus allowing the yeast to maintain higher copy numbers. Additionally, improvements in antibiotic selection have made utilizing the more traditional drug selection methods feasible in yeast as a complement or alternative to using auxotrophic markers. The MX series of antibiotic resistance cassettes is most commonly employed, with the KanMX being the most prevalent due to its versility and ability to be used in bacterial, yeast, and mammalian cells.

Topics: Yeast, Plasmids 101, Plasmids

Leave a Comment

Mohamad Farhan Mohamad Sobri 2015/7/2 下午3:04:18

Hello there, first of all thank you for such an informative post! :) I would like to ask regarding a specific case in particular and I hope you'd be able to help. I intend to use a yeast integrative plasmid and the issue of copy number has come to my attention. Since referring to the fact that YIPs don't have an origin of replication, should this new info bring any matter of significance to be? In addition, as I intend to work with native yeast strains (non-auxotrophic), I have been made aware as to the possibility of introducing my gene of interest heterologously in Saccharomyces cerevisiae by using integrative vectors containing drug resistance markers as opposed to auxotrophic markers instead (based on Taxis and Knop, 2006). Can you give suggestions as to the whether this is a feasible alternative?

Reply to *Mohamad Farhan Mohamad Sobri*

Marcy Patrick (Addgene) 2015/7/7 上午4:59:51

Dear Mohamad,

Thank you for your question--I am glad this post has been helpful!

According to PMID 2199782, YIps usually integrate as a single copy in the yeast genome; however, it is possible for multiple copies of the plasmid to be tandemly integrated, so you may want to do some diagnostic tests if a single integration event is required for your experiments. In general YIps must have a dominant selectable marker, but I cannot comment as to whether an auxotrophic or a drug resistance marker would work best. Presumably, as long as the maker is dominant in yeast, you should be able to select for positive integration.

I hope this helps!

Reply to *Marcy Patrick (Addgene)*

Stan MORAND 2016/7/8 下午6:01:43

Thanks for this 101 course. I have a little question: I heard we should not transform yeast cells w/ multiple CEN6ARS4 plasmids but rather diversify the CEN/ARS combinations if we have to transform multiple plasmids. Do you confirm ? If it's indeed the case, what are the other well known CEN/ARS used combinations ? Thanks in advance. Cheers, Stan

Reply to *Stan MORAND*

Marcy Patrick (Addgene) 2016/7/9 上午1:35:51

Dear Stan,

Thanks for your question--I am glad to hear the 101 series has been useful to you! This paper indicates that having too many CEN plasmids in a yeast cell may be toxic: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC367762/. That being said, I think scientists can successfully co-transform 2 plasmids with the same origin without any major issues.

The YCp series of plasmids mentioned in this post use ARS1/CEN4 so that would be one known alternative combination to try.

I hope this helps!

Reply to *Marcy Patrick (Addgene)*

Babar Ali 2019/3/22 下午9:30:51

How CEN gene control copy number??

Reply to *Babar Ali*

Andrew Hempstead (Addgene)

2019/3/25 下午11:18:43

Dear Barbar,

Thank you for your question regarding the role of CEN sequences in control of copy number.

Copy number of yeast centromeric plasmids is controlled by both the ARS and CEN sequences. During S-phase, replication is initiated once from the ARS sequence. Kinetochore complexes then bind to the CEN sequences to direct their segregation during division.

Please see PMID: 28072905 for additional information.

I hope this information helps!

Reply to *Andrew Hempstead (Addgene)*

Sarah Stainbrook 2017/6/13 上午1:32:46

Thanks for the helpful post! I have a gene on a centromeric plasmid with an auxotrophic marker (Ura3), and I would like to integrate it into the chromosomal locus. I know that typically, integrative plasmids are used for this, but I'm wondering if I could integrate the centromeric plasmid. Would putting part of the centromeric sequence (contained in the plasmid) in the Ura3 locus be problematic? Thanks!

Reply to *Sarah Stainbrook*

Jane Dorweiler 2017/7/7 上午2:25:34

Hi Sarah,
My understanding of the key difference for integrating plasmids is the fact that they do not have an origin of replication, so cannot be maintained unless they integrate. If you are using a CEN plasmid, the likelihood of integration would be minimal, even with a reasonable amount of homologous DNA for recombination. It may be possible to 'double digest' your plasmid, such that the CEN sequence is separated from the remainder of the plasmid that you want to integrate, particularly if you gel-purify, and then attempt integration.

Reply to *Jane Dorweiler*

Pankaj Chaturvedi 2017/6/28 上午4:18:56

Hi, Thanks for compiling all the useful info into one page. I am planning to introduce long (about 50-60 kb) human genomic DNA inserts using yeast vectors. Can I eliminate ARS from YCps and force the cells to using origin of replication from human genomic DNA. This may help me in cutting down the background and getting colonies only if successful integration has taken place. Any suggestions?

Reply to *Pankaj Chaturvedi*

Purnima K 2017/8/26 下午11:29:57

Hi,
This is super useful. Thank you!
I have a few questions:
1) Do you know labs don't usually make glycerol stocks of yeast strain transformed with centromeric plasmids? I understand labs typically make the stocks in rich medium and that unless the selection pressure is maintained, the plasmid may be lost but I am wondering if stocks can be made in the drop-out medium, or why we can't just test to make sure that plasmid is maintained by streaking out the strain+plasmid stock on drop-out medium?
2) Related, do you know what the plasmid loss rate is for centromeric plasmids?
3) Would you mind sharing a reference for this "[YCp] vectors replicate as though they are small independent chromosomes and are thus typically found as a single copy."
Thank you so much!
Purnima

Reply to *Purnima K*

Sarah stainbrook 2017/8/27 上午11:44:03

1) For our strains containing centromeric plasmids, we make glycerol stocks in dropout medium and they are maintained just fine- simply streak them onto dropout plates from the freezer stock. They take about 3 days to grow when streaked out from -80.
2) The paper referenced below has a 10^-2 rate of centromeric plasmid loss. I would guess that the rate of plasmid loss depends on how much it costs the cell to maintain it- for example, if it's a large plasmid or has a high-strength constitutive promoter or a toxic protein, it would be lost more quickly. For us an overnight growth in

nonselective medium tends to make most cells lose the plasmid. You can make this happen even faster and more completely with a counterselection, such as 5-FOA for the Ura3 marker.
3) V. L. LarionovT. S. KarpovaN. Y. KouprinaG. A. Jouravleva. 1985. "A mutant of Saccharomyces cerevisiae with impaired maintenance of centromeric plasmids." Current Genetics 10(1):15-20. https://link.springer.com/article/10.1007%2FBF00418488?LI=true

Reply to *Sarah stainbrook*

Rahul S 2017/12/4 下午6:09:24

Hi,
Thank you for the useful post.
I don't have prior experience with yeast transformation. I would like to know about the localization of yeast plasmid.
Any transformed plasmid is maintained only in the nucleus of yeast cell? Is it possible that some copies can remain in cytoplasm also?

Reply to *Rahul S*

Marcy Patrick (Addgene) 2017/12/5 上午12:04:58

Dear Rahul,

Thanks for your questions. To my knowledge, unless a standard plasmid enters the nucleus, no replication or gene expression can occur. Therefore, any plasmids that are not properly transported to the nucleus after transformation will likely be degraded by cytoplasmic endonucleases. That being said, there appears to be a separate group of yeast plasmids beyond the scope of this blog that are known to be cytoplasmic. These "linear DNA plasmids" are unique from the circular plasmids that reside in the nucleus are not meant for gene expression as far as I can tell. I've included a few references below that may be useful to you:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4403635/
https://www.biotechniques.com/multimedia/archive/00010/01304rv02_10885a.pdf
https://www.ncbi.nlm.nih.gov/pubmed/7557303
https://www.ncbi.nlm.nih.gov/pubmed/16448513

I hope this is helpful!

Reply to *Marcy Patrick (Addgene)*

Sarah Stainbrook 2018/4/8 上午12:05:35

Hi Ibtisam,
You must have a separate resistance or auxotrophy marker for each plasmid you want to maintain in the host. If you put in two plasmids with the same selectable marker, the cell will only need to keep one of them to survive. Based on the plasmid map, it seems that this backbone has a Ura3 auxotrophy marker. This means that you will need to put one of the two genes into a plasmid with a different selectable marker, or put both genes on the same plasmid.
It looks like pYes-DEST52 is a Gateway cloning vector. You may be able to use the same cloning strategy with pDest27 (link: https://tools.thermofisher.com/content/sfs/vectors/pdest27_map.pdf), which has a Neomycin selectable marker for yeast.
Once your two plasmids have two different resistance markers, you can transform them both at the same time (select on plates with neomycin, lacking uracil) and get colonies with both plasmids.

Reply to *Sarah Stainbrook*

Sarah Stainbrook 2018/4/10 上午1:31:07

I've never used an IRES before, I've always worked with traditional yeast promoters from the Mumberg collection (for example, the GPD promoter.) If expressing both off the same plasmid, be sure to put a terminator between them so you can control expression of each gene independently without polymerase read-through from the first gene. Then you can use different promoters for each gene. If you already have both genes synthesized, "PCR stitching" or Splicing by Overlap Extension (SOEing) may be the easiest way to join them together for insertion into a single backbone. See the links below for info about this method. I've used this method to successfully assemble four pieces together and amplify the resulting piece for Gibson assembly into a backbone.
Good luck!

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC373371/
https://openwetware.org/wiki/PCR_Overlap_Extension

Reply to *Sarah Stainbrook*

Omer Sabach 2020/10/27 下午11:17:47

There is a way to cure the 2-micron plasmid (exogenous DNA) of S. Cerevisiae?

Reply to *Omer Sabach*

Andrew Hempstead (Addgene) 2020/10/28 下午9:37:53

Dear Omer,

Thank you for your question! While we don't perform any experiments with yeast at Addgene, I was able to find the following publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC217149/), which may provide some insight into curing a plasmid from Saccharomyces cerevisiae.

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

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jamshaid afridi 2021/2/14 下午4:44:26

You are given the task to manufacture a variety of tomatoe plant. For this purpose, gene is provided to you. Which methods will you use for transformation and what are the advantages and disadvantages of each method? Which one is the best in your opinion and why..

please help me any one

Reply to *jamshaid afridi*

Yogesh Sharma 2021/2/22 下午5:08:39

Hi,
I have been struggling to cure a yeast vector after gene editing. I am using FOA for negetive selection against URA in the vector. I have a question, people usually recommend minimal media for FOA, but my strain is already URA negative. how will i use FOA in this case. cells without vector will obviously die even with added URA. NO? please correct my concept. where am i going wrong?

Reply to *Yogesh Sharma*

Andrew Hempstead (Addgene) 2021/2/22 下午10:40:37

Hi Yogesh,

Thank you for your question. As a nonprofit repository, we unfortunately have not performed any experiments with yeast in our laboratory, but yeast will require uracil for growth, either through the activity of URA3 (ODCase), or supplemented in the growth medium. 5-FOA could be used to counterselect against the URA3, but the medium would likely need to be supplemented with uracil or uridine.

I'd recommend the following publication for additional information (https://pubmed.ncbi.nlm.nih.gov/3323810/).

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Yogesh Sharma 2021/3/5 上午3:43:39

Thanks Andrew,

I have one more curiosity. Since I am trying to counterselect a URA vector i.e selecting colonies with lost URA plasmid. Can you provide me any information regarding vector loss efficiency in yeast? Or some protocol? How much population should I screen to get a desired single colony? Any idea?

Reply to *Yogesh Sharma*

Andrew Hempstead 2021/3/9 上午6:11:09

Dear Yogesh,

Thank you for your follow up comment. I was able to find the following publication (https://pubmed.ncbi.nlm.nih.gov/31213518/) that you may find helpful. Figure 2 describes experiments investigating 5-

FOA resistance.

Best,
Andrew

Reply to *Andrew Hempstead*

Jingde Qiu 2021/3/31 上午12:47:17

Is there any plasmid with GAL1, GAL2 or other GAL promoter driven URA3 as selection marker and can be used as negative selection marker plus 5-FOA in yeast?

Reply to *Jingde Qiu*

Nyla Naim (Addgene) 2021/3/31 下午9:28:13

Hello Jingde,

For plasmid search requests, please send your questions to help@addgene.org. I searched our repository for Gal-driven URA3 vectors but was unable to find any. If you know of a publication describing such a construct, please fill out the "Suggest a plasmid" form at the bottom of our main webpage (https://www.addgene.org/deposit/suggest-plasmid/).

Best regards,
Nyla

Reply to *Nyla Naim (Addgene)*

Add Comment

Marcy Patrick

February 25, 2014

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