

Guidelines for Generating Yeast-Adapted Cloning Vectors

- Use vector with a single- or low-copy-number origin for a final construct of >15 kb, if the final plasmid construct will be transferred into *E. coli*.
- Avoid spectinomycin and chloramphenicol selection markers on the custom vector.
- If spectinomycin or chloramphenicol selection markers cannot be avoided, remove or disrupt the spectinomycin or the chloramphenicol marker on the custom vector using restriction enzyme(s) and gel purify the desired vector backbone.
- If you use restriction enzymes that produce overhangs (i.e., non-blunt-end enzymes) to disrupt the spectinomycin or chloramphenicol selection marker, fill-in the ends with DNA polymerase I (Klenow fragment) before gel purifying the desired vector backbone.

Generating Yeast-Adapted Cloning Vectors

1. Linearize your vector with blunt-ended restriction enzyme(s).
Note: If cannot avoid using a vector containing spectinomycin and chloramphenicol selection markers, cut out or disrupt the selection marker(s) using restriction enzymes that produce blunt-ends and gel purify the vector backbone. If you use non-blunt-end restriction enzymes, fill in the overhangs using DNA polymerase I (Klenow fragment) before purifying the vector backbone.
2. Clean up the restriction reaction with a PCR cleanup kit (e.g., PureLink® PCR Purification Kit) or by phenol/chloroform extraction and ethanol precipitation.
3. Ligate ~10 ng of your linearized vector backbone with the GeneArt® Vector Conversion Cassette with Sapphire™ Technology at a 1:10 (vector:insert) molar ratio at 14°C overnight using T4 ligase.
4. Transform competent *E. coli* cells with the ligation mixture and plate on double selection LB plates (chloramphenicol plus the antibiotic marker on your custom vector backbone). Incubate the plates at 37°C overnight.
5. Pick the resultant colonies (i.e., transformants) and grow them overnight at 37°C in LB medium supplemented with chloramphenicol and the appropriate selection antibiotic for your custom vector.

6. The next day, harvest the cells and isolate the yeast-adapted vector using PureLink® Quick Plasmid Miniprep Kit or equivalent.
7. Analyze the yeast-adapted vector by restriction enzyme digestion and/or sequencing for verification.
8. Prior to use, linearize the adapted vector using *AscI* restriction enzyme. If there are additional *AscI* recognition sites on your custom vector backbone, use *AsiSI*.
Note: As a last resort, you can use *NotI*, *PacI*, *I-SceI*, or *I-CeuI* restriction enzymes for linearizing your yeast-adapted vector. However, the recognition sites for these enzymes are saved for mapping the assembled construct.
9. Clean up the digestion reaction with a PCR cleanup kit (e.g., PureLink® PCR Purification Kit) or by phenol/chloroform extraction and ethanol precipitation.

Related Products

Product	Amount	Cat. no.
GeneArt® High-Order Genetic Assembly System	1 kit	A13285
GeneArt® High-Order Genetic Assembly System (with Yeast Growth Media)	1 kit	A13286
GeneArt® pYES1L Vector with Sapphire™ Technology	10 reactions	A13287
CSM Media for MaV203 Yeast Cells	1 kit	A13292
PureLink® Quick Plasmid Miniprep Kit	50 preps	K2100-10
PureLink® PCR Purification Kit	50 preps	K3100-01
PureLink® Quick Gel Extraction Kit	1 kit	K2100-12
T4 DNA Ligase (5 U/μL)	250 units	15224-017
DNA Polymerase I (Klenow fragment)	100 units	18012-021

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Limited Use Label License No. 359: Transformation-Associated Recombination Methods

The linear yeast recombinant element included in this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform the transformation-associated recombination ("TAR") methods. No other rights are conveyed expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com
lifetechnologies.com

