CASdesigner manual

CASdesigner is a web tool to help you design donor DNA cassettes for genomic integration. These cassettes can be used to delete or replace existing genes, or to introduce genes into well-characterized empty loci. In all cases, CASdesigner provides primers which can be used to construct cassettes by Gibson assembly, PCR overlap, or homologous recombination. The markerless cassette can be integrated with the aid of a Cas9/gRNA plasmid (pCut) specifying the chromosomal target of the desired edit. These plasmids are available from the JBEI Public Registry.

We also provide experimental data on ~100 parts including integration sites, promoters, and protein tags that can be used with CASdesigner. When possible, we have standardized the construction and definition of parts for ease of use. These data are available from the JBEI Public Experimental Data Depot.

This web version of CASdesigner will be updated sporadically. There may be newer, jupyter notebook-based versions of CASdesigner at https://github.com/Leodespaux/CASdesigner with additional features including changing promoter and terminator lengths, bidirectional cassettes, etc.

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Acknowledgements, licensing, and citing our tool

CASdesigner is part of the following study, which we kindly ask you cite in any articles that employ it:

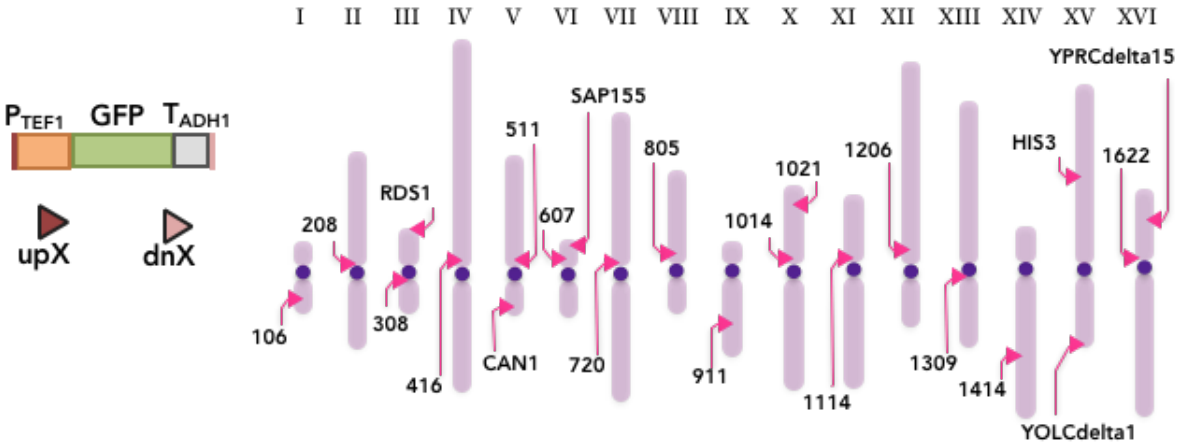
> A Cas9-based toolkit to program expression context in *Saccharomyces cerevisiae*.
> Amanda Reider Apel*, Leo d'Espaux*, Maren Wehrs*, Daniel Sachs, Rachel A. Li, Gary J. Tong, Megan Garber, Oge Nnadi, William Zhuang, Nathan J. Hillson, Jay D. Keasling, and Aindrila Mukhopadhyay. *Nucleic Acids Research (2017)*

The software tool was created and coded by Leo d'Espaux, William Zhuang, and Oge Nnadi. The data included in this manual were collected by Maren Wehrs, Leo d'Espaux, Amanda Reider Apel, and Rachel Li. CASdesigner is provided open-source under a BSD license, and is available from github. We kindly thank Gos Micklem for permission to use Intermine to fetch yeast sequences.

Integration Loci

We have characterized 23 integration loci. The integration deletes the 20-nt guide sequence and the subsequent 14 bp. Below are data for a GFP cassette integrated into various loci and tested in different media and time points. For each integration site, we have a pCut plasmid available from the JBEI Public Registry.

While the 23 cut sites were chosen from a BY *S. cerevisiae* strain background, all 23 sites are also present in both CEN.PK and W303 backgrounds with two exceptions: (1) 208a is mutated at the 4th nucleotide in CEN.PK, and (2) His3b is missing in W303. Therefore, in addition to the two low efficiency sites, 208a should not be used for strain engineering in a CEN.PK background and His3b should be avoided when working in W303.



	YPD		CSM		Int	Guide Sequence
	8hr	24hr	8hr	24hr	Eff	
208a	1.00	0.27	0.67	0.47	0.99	GTCCGCTAAACAAAAGATCT
1622b	0.80	0.17	0.61	0.23	1.00	TAAAGCCACCACATCGCAAA
YPRCd15c	0.79	0.18	0.66	0.34	0.87	AATCCGAACAACAGAGCATA
308a	0.78	0.23	0.57	0.40	0.98	CACTTGTCAAACAGAATATA
1021b	0.78	0.16	0.50	0.29	0.72	CCTCTGTGTGGTGGTAATTG
911b	0.77	0.15	0.60	0.32	1.00	GTAATATTGTCTTGTTTCCC
1014a	0.74	0.16	0.56	0.31	0.42	TTATGTGCGTATTGCTTTCA
HIS3b	0.71	0.13	0.54	0.30	0.98	AATATAGAGTGTACTAGAGG
YOLCd1b	0.70	0.10	0.50	0.39	0.33	CTAGAATTTCCATTTTGCGT
416d	0.70	0.28	0.54	0.30	0.99	TAGTGCACTTACCCCACGTT
1309a	0.69	0.17	0.54	0.22	0.99	CCTGTGGTGACTACGTATCC
SAP155b	0.62	0.15	0.66	0.29	0.83	GGTTTTCATACTGGGGCCGC
805a	0.60	0.20	0.51	0.33	0.01	TTATTTGAATGATATTTAGT
CAN1y	0.59	0.23	0.63	0.35	0.40	GATACGTTCTCTATGGAGGA
1206a	0.58	0.15	0.52	0.26	0.01	CGAACATTTTTCCATGCGCT
SAP155c	0.57	0.21	0.56	0.29	0.51	ATGAAAGACAACTATAGGGC
106a	0.54	0.13	0.39	0.22	1.00	ATACGGTCAGGGTAGCGCCC
607c	0.53	0.18	0.78	0.23	0.02	CTATTTTTGCTTTCTGCACA
1114a	0.52	0.12	0.51	0.26	0.46	CTTGTGAAACAAATAATTGG
720a	0.52	0.19	0.56	0.28	0.98	CAACAATTGTTACAATAGTA
1414a	0.47	0.10	0.75	0.27	0.82	GCGCCACAGTTTCAAGGGTC
RDS1a	0.46	0.17	0.42	0.23	0.16	ATTCAATACGAAATGTGTGC
511b	0.33	0.17	0.45	0.25	0.96	CAGTGTATGCCAGTCAGCCA

Promoters

We have characterized 37 promoters. Below are data for a GFP expressed from a fixed locus (1021b) tested in different media and time points. You can also use promoters from any native *S. cerevisiae* gene by referring to its common name, *e.g.*, GPD1. In all cases, CASdesigner includes as the promoter the 600 bp upstream the start codon of that gene.

	YPD				CSM				YPG			Fold
	4hr	8hr	24hr	48hr	4hr	8hr	24hr	48hr	8hr	24hr	48hr	
TDH3	1.00	0.96	0.66	0.86	1.07	0.84	0.60	0.49	0.70	0.66	0.89	0.86
CCW12	0.90	0.89	0.21	0.18	0.87	0.68	0.30	0.18	0.93	0.30	0.32	0.20
ENO2	0.68	0.50	0.18	0.18	0.63	0.43	0.23	0.15	0.34	0.14	0.27	0.27
TEF1	0.55	0.43	0.14	0.15	0.48	0.37	0.16	0.12	0.41	0.14	0.10	0.27
TEF2	0.48	0.38	0.19	0.29	0.50	0.31	0.27	0.19	0.56	0.26	0.24	0.61
RPL3	0.45	0.32	0.11	0.13	0.33	0.21	0.13	0.07	0.44	0.15	0.12	0.29
PGK1	0.43	0.37	0.46	0.46	0.38	0.45	0.43	0.21	0.37	0.40	0.38	1.07
HHF2	0.37	0.37	0.25	0.22	0.34	0.29	0.19	0.13	0.44	0.22	0.16	0.60
TPI1	0.30	0.25	0.11	0.15	0.29	0.21	0.12	0.09	0.26	0.13	0.11	0.51
YEF3	0.26	0.29	0.21	0.29	0.33	0.29	0.20	0.17	0.26	0.25	0.23	1.11
HHF1	0.24	0.25	0.12	0.13	0.27	0.23	0.12	0.08	0.28	0.13	0.11	0.54
ASC1	0.22	0.19	0.05	0.04	0.18	0.11	0.06	0.03	0.20	0.05	0.04	0.20
SSA1	0.20	0.14	0.32	0.45	0.22	0.30	0.25	0.24	0.28	0.39	0.52	2.26
HSP26	0.20	0.06	0.79	0.91	0.24	0.30	0.39	0.28	0.13	0.81	1.07	4.56
PDC1	0.16	0.12	0.02	0.02	0.19	0.11	0.06	0.07	0.05	0.02	0.06	0.15
PCK1	0.15	0.01	0.40	0.26	0.01	0.00	0.00	0.00	0.06	0.44	0.31	1.77
HSP82	0.15	0.14	0.31	0.29	0.13	0.18	0.20	0.12	0.12	0.21	0.32	1.96
HSP104	0.13	0.14	0.34	0.47	0.16	0.22	0.24	0.21	0.18	0.40	0.63	3.61
SSB2	0.13	0.09	0.03	0.05	0.10	0.07	0.04	0.02	0.14	0.04	0.04	0.42
ADH2	0.12	0.01	0.35	0.40	0.00	0.00	0.00	0.00	0.06	0.34	0.40	3.28
ACT1	0.12	0.10	0.10	0.12	0.11	0.10	0.08	0.04	0.13	0.10	0.11	1.04
HXT7	0.10	0.10	0.22	0.20	0.10	0.18	0.17	0.10	0.47	0.20	0.17	1.95
CIT1	0.09	0.04	0.29	0.30	0.05	0.06	0.04	0.06	0.14	0.29	0.26	3.47
CIT2	0.06	0.02	0.06	0.10	0.20	0.09	0.08	0.06	0.02	0.08	0.16	1.68
EFT1	0.04	0.01	0.22	0.16	0.11	0.08	0.10	0.07	0.03	0.15	0.16	3.46
CYC1	0.04	0.03	0.06	0.06	0.04	0.03	0.02	0.01	0.10	0.09	0.18	1.38
SPI1	0.01	0.00	0.02	0.03	0.04	0.05	0.07	0.04	0.02	0.02	0.03	2.87
HSP30	0.01	0.00	0.02	0.03	0.02	0.02	0.06	0.05	0.01	0.06	0.15	4.35
GAL1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.27	0.50	0.36	0.16

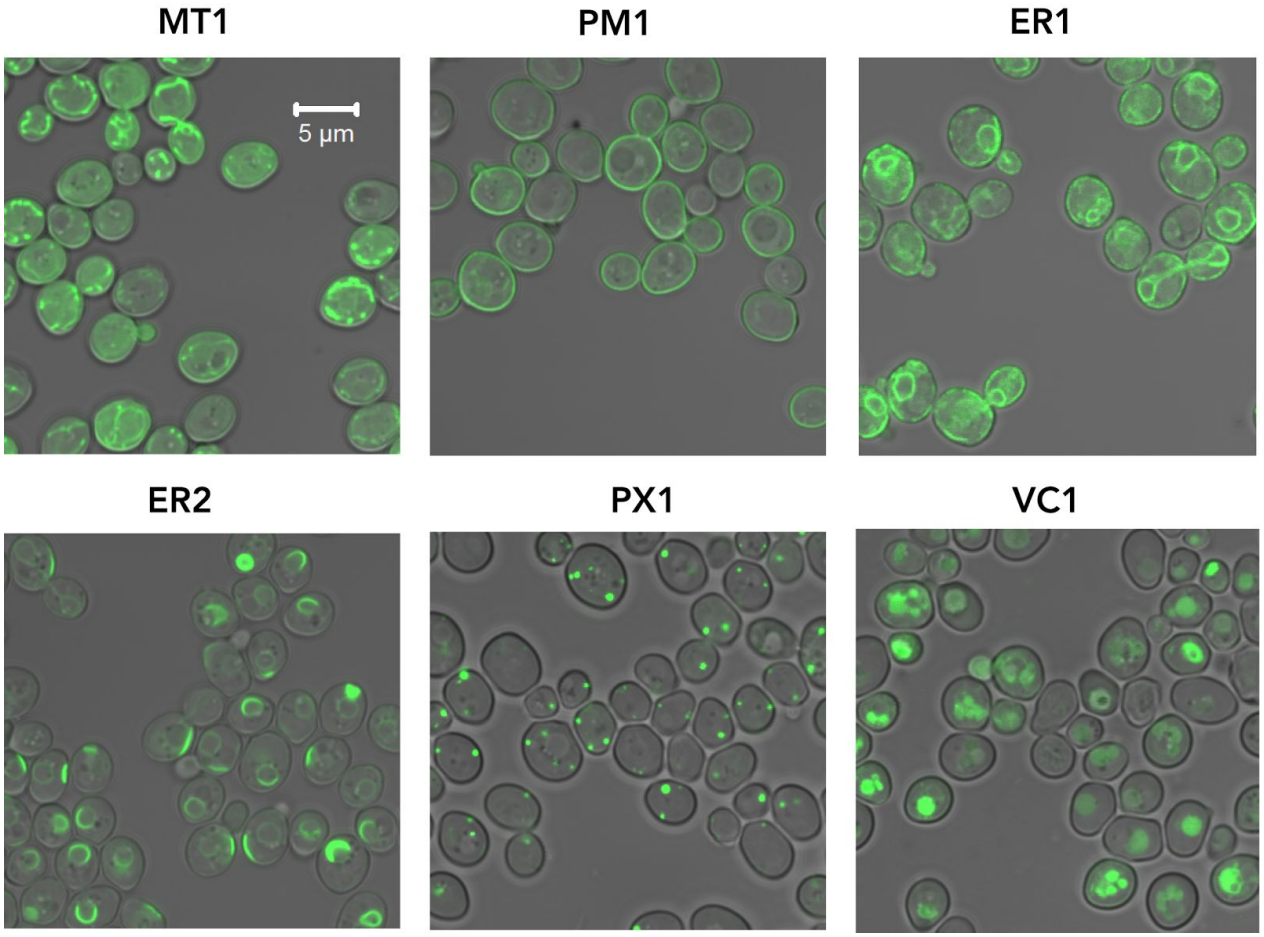
Terminators

We do not have data for terminators. However you can specify any *S. cerevisiae* gene by common name and CASdesigner will use its terminator, defined as the 250 bp downstream the named gene.

Protein Tags

We have several localization tags, MBP solubility tags, Ubiquitin-based stability tags, and GFP tags. When asked whether you want a tag, for the tags in the list below, simply write the name, e.g., “MBP”. For a new tag, type the name and DNA sequence separated by a comma. Be sure your tag sequence starts with a start codon and ends with a stop codon, and CASdesigner will do the rest. N-terminal tags will be attached via a flexible linker encoding “GGGGGG”, and C-terminal tags “GSGSGS”. If you don’t want a protein tag, leave the field empy.

Name	*Short description*
MBP	Solubility tag
GFP	Green fluorescent protein
PX1	Peroxisome lumen localization based on *PEX8*
mCherry	Red fluorescent protein
MT1	Mitochondria lumen localization based on *COX4*
ERG20	Isoprenoid production and possibly solubility or channeling
UbiX	Protein stability or destability tag
ER1	Endoplasmic reticulum lumen localization based on *CNE1*
ER2	Endoplasmic reticulum cytoplasmic face localization based on *CYB5*
PM1	Plasma membrane localization based on *SNC1*
NES1	Nuclear export tag
VC1	Vacuole cytoplasmic face localization based on *NYV1*

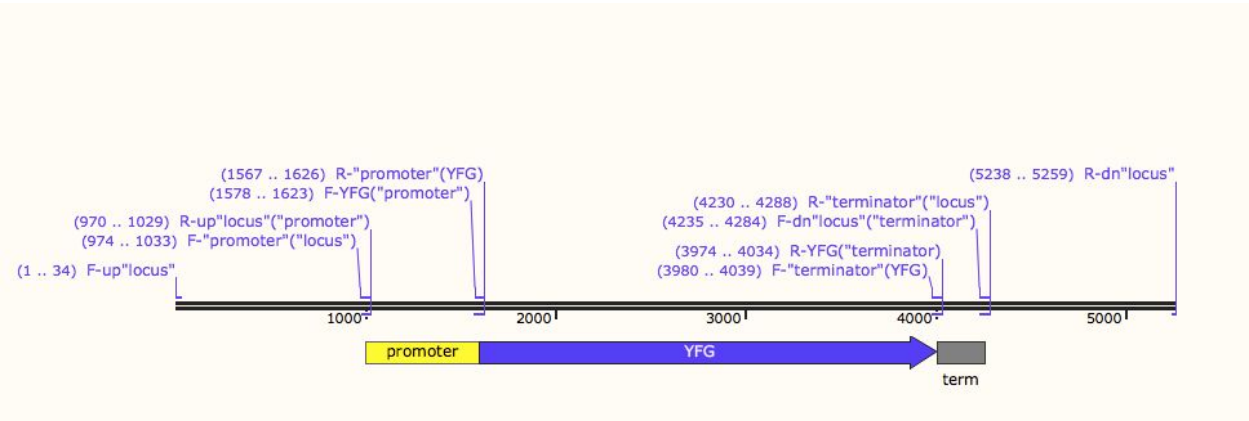


Cassette construction

The markerless cassette will contain your genetic edit flanked by 1-kb homology regions. In the case of a deletion, the donor DNA is just these homology regions fused so as to delete the protein-coding sequence from start to stop codon.

A “standard cassette” consists of a 600-bp promoter, your protein-coding sequence, and a 250-bp terminator. The promoter, terminator sequences are always the same size and can be PCRd from genomic DNA. CASdesigner creates primer sequences with an annealing temperature of 57˚C (to the template). Except for terminal primers, these sequences also include 5’ overhangs providing 30-60 bp of inter-fragment homology. These fragments can be assembled by homologous recombination inside the cell, Gibson assembly, or PCR sewing. We’ve had good success transforming up to ~4 fragments into one site.

Our primer nomenclature is described in Figure 1. Generally, F is used for the forward primer, and R for the reverse. The letter is followed by the feature the primer anneals to, and then, in parentheses, the feature the overhang confers homology to. As an example, F-YFG(TDH3ps) is the forward primer annealing to YFG, and conferring homology to TDH3ps. A PCR reaction amplifying YFG with the primers F-YFG(TDH3ps) and R-YFG(ADH1ts) results in a DNA fragment with 5’ homology to the TDH3 promoter, and 3’ homology to the ADH1 terminator. The total melting temperature and length of overhang vary for each primer. All primers are <60 nt.



Feedback

To report bugs, inquire about collaborations, and provide any feedback, please e-mail casdesigner@lbl.gov and/or leodespaux@gmail.com, or create a support ticket directly via github.