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, 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.

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.

Good luck,
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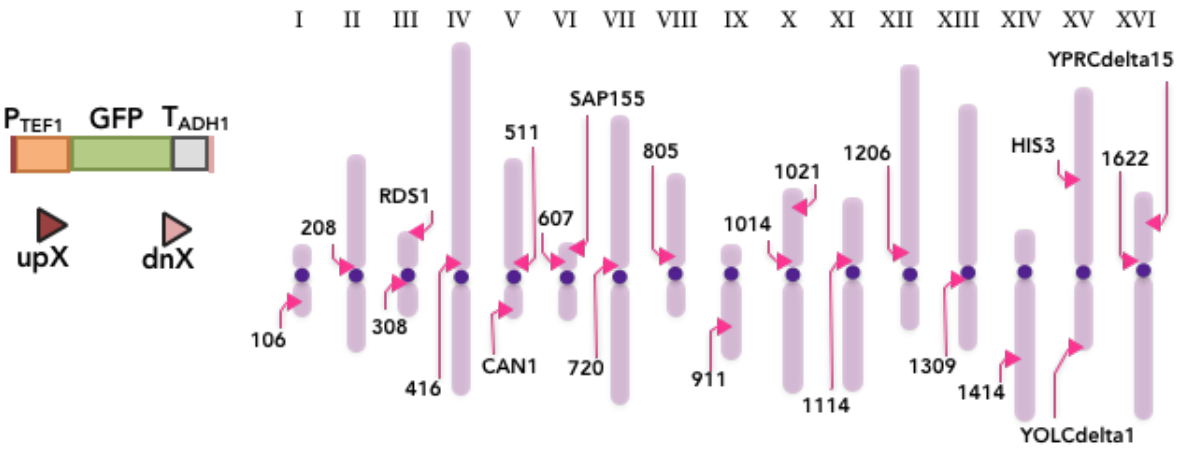
CASdesigner manual
Integration Loci
Promoters
Terminators
Protein Tags
Cassette construction

Integration Loci

We have characterized 23 integration loci. The integration deletes the 20-nt guide sequence and the subsequent 14 bp. Here 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 registry.



	YPD		CSM		Int
	8hr	24hr	8hr	24hr	Eff
208a	1.00	0.27	0.67	0.47	0.99
1622b	0.80	0.17	0.61	0.23	1.00
YPRCD15c	0.79	0.18	0.66	0.34	0.87
308a	0.78	0.23	0.57	0.40	0.98
1021b	0.78	0.16	0.50	0.29	0.72
911b	0.77	0.15	0.60	0.32	1.00
1014a	0.74	0.16	0.56	0.31	0.42
HIS3b	0.71	0.13	0.54	0.30	0.98
YOLCd1b	0.70	0.10	0.50	0.39	0.33
416d	0.70	0.28	0.54	0.30	0.99
1309a	0.69	0.17	0.54	0.22	0.99
SAP155b	0.62	0.15	0.66	0.29	0.83
805a	0.60	0.20	0.51	0.33	0.01
CAN1y	0.59	0.23	0.63	0.35	0.40
1206a	0.58	0.15	0.52	0.26	0.01
SAP155c	0.57	0.21	0.56	0.29	0.51
106a	0.54	0.13	0.39	0.22	1.00
607c	0.53	0.18	0.78	0.23	0.02
1114a	0.52	0.12	0.51	0.26	0.46
720a	0.52	0.19	0.56	0.28	0.98
1414a	0.47	0.10	0.75	0.27	0.82
RDS1a	0.46	0.17	0.42	0.23	0.16
511b	0.33	0.17	0.45	0.25	0.96

Promoters

We have characterized 37 promoters. Here are data for a GFP expressed from a fixed locus (1021b) tested in different media and time points. You can also use promoters of genes we did not test. In all cases, CASdesigner includes as the promoter the 600 bp upstream the named 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 name and CASdesigner will use that 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. These will be added to CASdesigner soon.

Cassette construction

The marker-less cassette will contain your genetic edit flanked by 1kb homology regions. In the case of a deletion, the donor DNA is just these homology regions fused so as to delete the CDS. A “standard cassette” consists of a 600 bp promoter, your CDS, and a 250 bp terminator. The promoter and terminator sequences are always the same size and can be PCRd from genomic DNA. CASdesigner creates primer sequences with a melting temperature of 57˚C for the amplification of the fragments. The DNA fragments resulting from PCR reactions have terminal homology to each other and can assemble by homologous recombination inside the cell, or PCR sewing. The primer nomenclature is described in Figure 1. Generally, F is used for the forward primer and R for the reverse primer. 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 TDH3p. An exemplary PCR reaction of YFG with the primers F-YFG(TDH3ps) and R-YFg(ADH1ts) would result in a DNA fragment that has 5’ homology to the TDH3 promoter and 3’ homology to the ADH1 terminator. The melting temperature and lengths of overhangs vary for each primer.