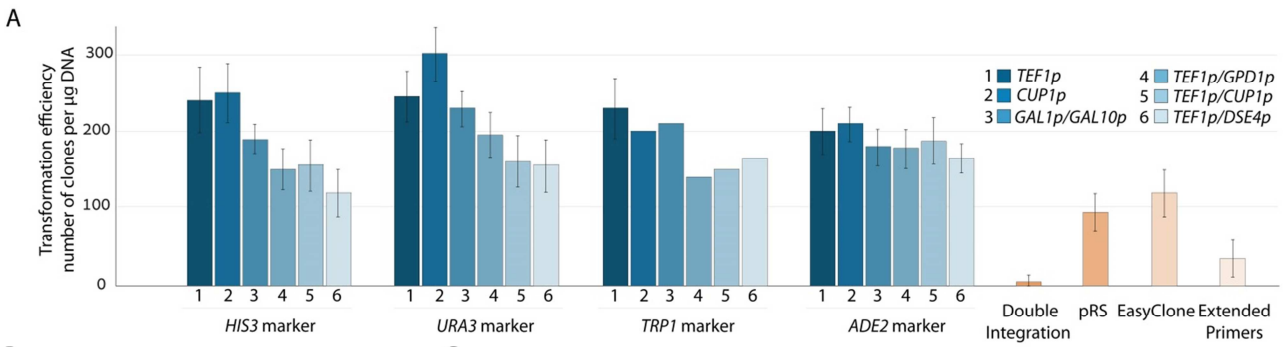
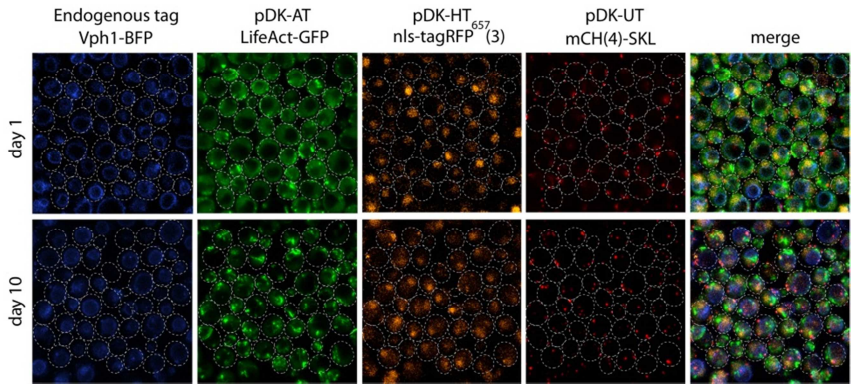
A



B

plasmid	correct integration	stability	plasmid	correct integration	stability
pDK-HT	100	100	pDK-TT	100	100
pDK-HC	100	100	pDK-TC	100	100
pDK-HGG	100	100	pDK-TGG	100	100
pDK-HTG	100	100	pDK-TTG	100	100
pDK-HTC	100	100	pDK-TTC	100	100
pDK-HTD	100	100	pDK-TTD	100	100
pDK-UT	100	100	pDK-AT	95	100
pDK-UC	100	100	pDK-AC	95	100
pDK-UGG	100	100	pDK-AGG	100	100
pDK-UTG	100	100	pDK-ATG	95	100
pDK-UTC	100	100	pDK-ATC	95	100
pDK-UTD	100	100	pDK-ATD	100	100

C



Supplemental FIGURE 1: Testing the pDK vector series. (A) Comparison of integration efficiency of pDK plasmids (24), double integration (using pDK-HT and pDK-UT), EasyClone plasmid pCfB2513, pRS series (pRS303, pRS304, pRS306), and extended primers (integration of BFP-HPH fragment with 45bp overhangs, see methods for details). Transformation efficiency is expressed as a number of transformants per µg DNA, for $5*10^7$ cells per transformation, error bars represent standard deviation. **(B)** Fidelity of integration was evaluated by PCR from 20 clones, and stability of integration was evaluated by fluorescence intensity of GFP reporter after 10 days of growing cells in non-selective rich medium. **(C)** Stability of multiple plasmids carrying the same promoter and terminator was evaluated by GFP fluorescence after 10 days of growing in non-selective medium.

Supplemental TABLE 1. Oligonucleotides used for generating the fragment for integration.

	Primers for integration	
Locus	**Forward primer sequence**	**Reverse primer sequence**
HIS3 (for pDK-HT, pDK-HC, pDK-HGG, pDK-HTG, pDK-HTC, pDK-HTD)	5′-gcgggattgctctcg-3′	5′-agtcttcagtggtgtgatg-3′
URA3 (for pDK-UT, pDK-UC, pDK-UGG, pDK-UTG, pDK-UTC, pDK-UTD)	5′-tacttcttctgccgcc-3′	5′-acaaaggaacctagaggc-3′
TRP1 (for pDK-TT, pDK-TC, pDK-TGG, pDK-TTG, pDK-TTC, pDK-TTD)	5′-cgtgtttcgtaatcaacc-3′	5′-ccaaccaagtatttcgg-3′
ADE2 (for pDK-AT, pDK-AC, pDK-AGG, pDK-ATG, pDK-ATC, pdK-ATD))	5′-gatggaagaggtaacttcg-3′	5′-gtatgccaaagtcctcg-3′

Supplemental TABLE 2. Oligonucleotides used for plasmid construction.

Primer	Sequence	Primer	Sequence
HIS3F1	5′-gactaagcttcgttttaagagcttggtg-3′	**GALAAF**	5′-gctcaatgcagaagaattgctatacctgagaaagcaac-3′
HIS3R1	5′-gactacatgtagtcttcagtggtgtg-3′	**GALTTF**	5′-ctatgcggtgtgagaattgctatacctgagaaagcaac-3′
HIS3F2	5′-gacttcccggagcgggattgctctc-3′	**CUPTEFR**	5′-catgcctgcaggtcgacagtatagcgaccagc-3′
HIS3R2	5′-gactgaattcccgtcgagttcaagag-3′	**CUPTEFHHF**	5′-gaactcgacgggaattgattgtactgagagtgcac-3′
URA3F1	5′-gatcgaattcccataccacagctttc-3′	**CUPTEFUUF**	5′-gctgtggtatgggaattgattgtactgagagtgcac-3′
URA3R1	5′-gatctcccggatacttcttctgccgc-3′	**CUPTEFAAF**	5′-ggcggctcaatgcagaagaattgattgtactgagagtg-3′
URA3F2	5′-gatcacatgtacaaaggaacctagagg-3′	**CUPTEFTTF**	5′-ctatgcggtgtgagaattattgtactgagagtgcac-3′
URA3R2	5′-gatcaagctttcacaccgcataggg-3′	**CUPbF**	5′-gatcaccggtggctgatatcttagcctt-3′
TRP1F1	5′-gatcaagcttgagagtgcaccataaacg-3′	**CUPbR**	5′-gatcgaattcgatgacttctatatgatattgc-3′
TRP1R1	5′-gatcacatgtccaaccaagtatttcgg-3′	**GPDbF**	5′-gatcaccggttcattatcaatactgccatttc-3′
TRP1F2	5′-tcccggacgtgtttcgtaatcaacc-3′	**GPDbR**	5′-gatcgaattctttgtttgtttatgtgtgttt-3′
TRP1R2	5′-gatcgaattctcacaccgcataggc-3′	**DSEbF**	5′-gatcaccggtaaaaaacctccccatcaaa-3′
ADE2F1	5′-gatcaagcttcttttgatgcggaattgac-3′	**DSEbR**	5′-gatcgaattcagctgtttttttgttttct-3′
ADE2R1	5′-gatcacatgtgtatgccaaagtcctcg-3′	**TEFbF**	5′-gatcaccggtgacatggaggcccag-3′
ADE2F2	5′-gatctcccggagatggaagaggtaacttcg-3′	**TEFbR**	5′-gatcggatccggttgtttatgttcggatg-3′
ADE2R2	5′-gatcgaattcttctgcattgagccgc-3′	**GFPxR**	5′- gatccccgggttacttgtacagctcgtcc-3′
TEF1PF	5′-gatcaccggtcatatggacatggaggcccag-3′	**GFPbF**	5′- gatcGGATCCatggtgagcaagggcgag-3′
TEF1PR	5′-gatcgaattcggttgtttatgttcggatg-3′	**GFPsF**	5′-gactGAGCTCatggtgagcaagggcgag-3′
CUP1PF	5′-gatccatatgggctgatatcttagccttg-3′	**CHeF**	5′-gatcggatccgtgagcaagggcgag-3′
CUP1PF	5′-gatcgaattcgattgattgattgtacag-3′	**CHnR**	5′-gatcgctagcttacttgtacagctcgtcc-3′
TEF1TF	5′-gatcgtcgacactgacaataaaaagattc-3′	**delINPF**	5-gtttgtttttacttacttgtgaaacgtttgttgattaacttaatacgctagggtaacaggg-3′
TEF1TR	5′-gatcaagcttcagtatagcgaccag-3′	**delINPR**	5′-ttaaagtgtaattagttatttcaaagtacatattaaaatatattagatgaattcgagctcg-3′
GALR	5′-atgcctgcaggtcgacttcgagcgtcccaa-3′	**eVPHF**	5′-gacatggaagtcgctgttgctagtgcaagctcttccgcttcagcggtg acggtgctggttta-3′
GALHHF	5′-gaactcgacgggaattgctatacctgagaaagc-3′	**eVPHR**	5′-aatgaagtacttaaatgtttcgctttttttaaaagtcctcaaaattcga tgaattcgagctcg-3′
GALUUF	5′-ctgtggtatgggaattgctatacctgagaaagcaac-3′		