

**S2 Table. *E. coli* plasmids used in this study.**

<b>Plasmid</b>	<b>Description</b>	<b>Marker</b>	<b>Reference</b>
pDML20	pFA6a-linker(5xGA)-msfGFP-T <sub>ADH1</sub> -kanMX6	Amp; G418	This study
pDML61	pFA6a-linker(5xGA)-msfGFP-T <sub>ADH1</sub> - <i>C.g. HIS3</i>	Amp; <i>HIS3</i>	This study
pDML63	pFA6a-linker(5xGA)-mNeonGreen (non-yeast codon-optimized)-T <sub>ADH1</sub> - <i>C.g. HIS3</i>	Amp; <i>HIS3</i>	This study
pDML99	pFA6a-linker(5xGA)-mNeonGreen-T <sub>ADH1</sub> - <i>C.g. HIS3</i>	Amp; <i>HIS3</i>	This study
pDML112	pFA6- <i>HO</i> -P <sub>TDH3</sub> -mCherry -T <sub>ADH1</sub> -NAT- <i>HO</i>	Amp; NAT	This study
pDML145	pNH604-P <sub>TDH3</sub> -mNeonGreen-T <sub>ADH1</sub>	Amp; <i>TRP1</i>	This study
pDML152	pFA6a-linker(5xGA)-mNeonGreen-T <sub>ADH1</sub> - <i>C.g. TRP1</i>	Amp; <i>TRP1</i>	This study
pDML166	pNH604-P <sub>EF1<math>\alpha</math></sub> -mNeonGreen-T <sub>ADH1</sub>	Amp; <i>TRP1</i>	This study
pDML190	pFA6a-mNeonGreen <sub>(1-177)</sub> - <i>K.l. URA3</i> -P <sub>EF1<math>\alpha</math></sub> -M-mNeonGreen <sub>(60-236)</sub> -linker(5xGA)	Amp; <i>URA3</i>	This study
pDML193	pFA6a-mCherry <sub>(1-177)</sub> - <i>K.l. URA3</i> -P <sub>EF1<math>\alpha</math></sub> -M-mCherry <sub>(60-236)</sub> -linker(5xGA)	Amp; <i>URA3</i>	This study
pDML200	pFA6a-GSG-mNeonGreen <sub>(3-177)</sub> - <i>K.l. URA3</i> -mNeonGreen <sub>(60-236)</sub> -GSG	Amp; <i>URA3</i>	This study
pDML219	pFA6a-linker(5xGA)-mNeonGreen <sub>(5-177)</sub> - <i>K.l. URA3</i> -mNeonGreen <sub>(60-236)</sub> -Stop	Amp; <i>URA3</i>	This study
pDML222	pFA6a-mNeonGreen <sub>(1-177)</sub> - <i>K.l. URA3</i> -P <sub>EF1<math>\alpha</math></sub> -mNeonGreen <sub>(non-yeast,1-59)</sub> -mNeonGreen <sub>(60-236)</sub> -linker(5xGA)	Amp; <i>URA3</i>	This study
pDML223	pFA6a-linker(5xGA)-mNeonGreen <sub>(5-177)</sub> -mNeonGreen <sub>(non-yeast, 178-236)</sub> - <i>K.l. URA3</i> -mNeonGreen <sub>(60-236)</sub> -Stop	Amp; <i>URA3</i>	This study
pNH603	Single-copy <i>his3</i> integration vector with <i>C.g. HIS3</i> marker	Amp; <i>HIS3</i>	[1]
pNH604	Single-copy <i>trp1</i> integration vector with <i>C.g. TRP1</i> marker	Amp; <i>TRP1</i>	[1]
pUG72	Plasmid with loxP-flanked <i>K.l. URA3</i> marker	Amp; <i>URA3</i>	[2]
pDH149	pFA6a-AfeI-linker-NmGFPmut3 <sub>(2-158)</sub> - <i>ura4</i> -mGFPmut3-BclI	Amp; <i>ura4</i>	This study
pDH165	pFA6a-AfeI-linker-NmGFPmut3 <sub>(2-158)</sub> -kanMX6-HSV1tk-mGFPmut3-BclI	Amp; kanMX6 HSV1tk	This study
pDH193	pFA6a-AfeI-linker-NmGFPmut3 <sub>(2-158)</sub> - <i>ura4</i> -mGFPmut3-HindIII	Amp; <i>ura4</i>	This study

## References

1. Chau AH, Walter JM, Gerardin J, Tang C, Lim WA. Designing synthetic regulatory networks capable of self-organizing cell polarization. *Cell*. 2012;151: 320–332. doi:10.1016/j.cell.2012.08.040
2. Gueldener U, Heinisch J, Koehler GJ, Voss D, Hegemann JH. A second set of loxP marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Res*. 2002;30: e23.