

# Rapid Assembly of Multi-Gene Constructs using Modular Golden Gate Cloning

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jove.com/video/61993

## Materials

Name	Company	Catalog Number	Comments
0.5 mm Glass beads	RPI research products	9831	For lysing yeast cells
Bacto Agar	BD & Company	214010	Component of the yeast complete synthetic medium (CSM)
Bacto Peptone	BD& Company	211677	Component of the yeast extract peptone dextrose medium (YPD)
Bsal-HFv2	New England Biolabs	R3733S	a highly efficient version of Bsal restriction enzyme
Carbenicillin	Fisher Bioreagents	4800-94-6	Antibiotic for screening at the transcription unit level
Chloramphenicol	Fisher Bioreagents	56-75-7	Antibiotic for screening at the entry vector level
CSM-His	Sunrise Sciences	1006-010	Amino acid supplement of the yeast complete synthetic medium (CSM)
Dextrose	Fisher Chemical	D16-500	Carbon source of the yeast complete synthetic medium (CSM)
Difco Yeast Nitrogen Base w/o Amino Acids	BD & Company	291940	Nitrogen source of the yeast complete synthetic medium (CSM)
dNTP mix	Promega	U1515	dNTPs for PCR
Esp3I	New England Biolabs	R0734S	a highly efficient isoschizomer of BsmBI
Frozen-EZ Yeast Transformation II Kit	Zymo Research	T2001	For yeast transformation
Hexanes	Fisher Chemical	H302-1	For carotenoid extraction from yeast cells
Kanamycin	Fisher Bioreagents	25389-94-0	Antibiotic for screening at the multigene plasmid level
LB Agar, Miller	Fisher Bioreagents	BP1425-2	Lysogenic agar medium for <i>E. coli</i> culturing
LB Broth, Miller	Fisher Bioreagents	BP1426-2	Lysogenic liquid medium for <i>E. coli</i> culturing
Lycopene	Cayman chemicals	NC1142173	For lycopene quantification
MoClo YTK	Addgene	1000000061	Depositing Lab: John Deuber

Monarch Plasmid Miniprep Kit	New England Biolabs	T1010L	For plasmid purification from <i>E. coli</i>
Nanodrop Spectrophotometer	Thermo Scientific	ND2000c	For measuring accurate DNA concentrations
NotI-HF	New England Biolabs	R3189S	Restriction enzyme for integrative multigene plasmid linearization
Nourseothricin Sulphate	Goldbio	N-500-100	Antibiotic Selection marker for the pCAS plasmid used in this study
Phusion HF reaction Buffer (5X)	New England Biolabs	B0518S	Buffer for PCR using Phusion polymerase
Phusion High Fidelity DNA Polymerase	New England Biolabs	M0530S	High fidelity polymerase for all the PCR reactions
pLM494	Addgene	100539	Plasmid used to amplify crtI, crtYB and crtE used in this study
Quartz Cuvette	Thermo Electron	10050801	For quantifying carotenoids
T4 ligase	New England Biolabs	M0202S	Ligase for Golden Gate cloning
Thermocycler	BIO-RAD	1851148	For performing all the PCR and cloning reactions
Tissue Homogenizer	Bullet Blender	Model: BBX24	For homogenization of yeast cells
UV-Vis Spectrophotometer	Thermo Scientific	Genesys 150	For quantifying carotenoids
Yeast Extract	Fisher Bioreagents	BP1422-500	Component of the yeast extract peptone dextrose medium (YPD)
$\beta$ -carotene	Alfa Aesar	AAH6010603	For $\beta$ -carotene quantification