LEU2
tLEU2
pTEF
NatMX
tTEF
Z3EV
pLEU2
tCYC1
HDV ribozyme
gRNA
Hh ribozyme
polyA
pTAG
8741 bp
GFP
pZ3EV
pAmpR
AmpR
ColE1 ori
pACT1
PCR
PCR
3014 bp
GFP
pLEU2
tLEU2
LEU2
tCYC1
Gene of interest
natural
3'UTR &
terminator
3014 bp
GFP
pLEU2
tLEU2
LEU2
tCYC1
Gene of interest
GFP
tADH1
pTEF
HIS3
tTEF
pZ3EV
AmpR
pAmpR
GFP
ColE1 ori
polyA
Hh ribozime
gRNA
HDV ribozime
pZ3EV
7019 bp
tCYC1
pTEF
pACT1
NatMX
tTEF
Z3EV
PCR
5157 bp
pACT1
Z3EV
NatMX
tCYC1
GFP
pZ3EV
tTEF
pTEF
TRP1
chr IV
SwaI
NP..
tTEF
KanMX
pTEF
pAmpR
..R2
pCYC1m
mCherry
AmpR
pACT1
pdCAS9int
11799 bp
ColE1 ori
tCYC1
VP64
dCAS9
SwaI
digestion
11799 bp
tTEF
pTEF
tCYC1
pCYC1m
NP..
KanMX
AmpR
dCAS9
pACT1
mCherry
..R2
pAmpR
ColE1 ori
VP64
NPR2
chr V