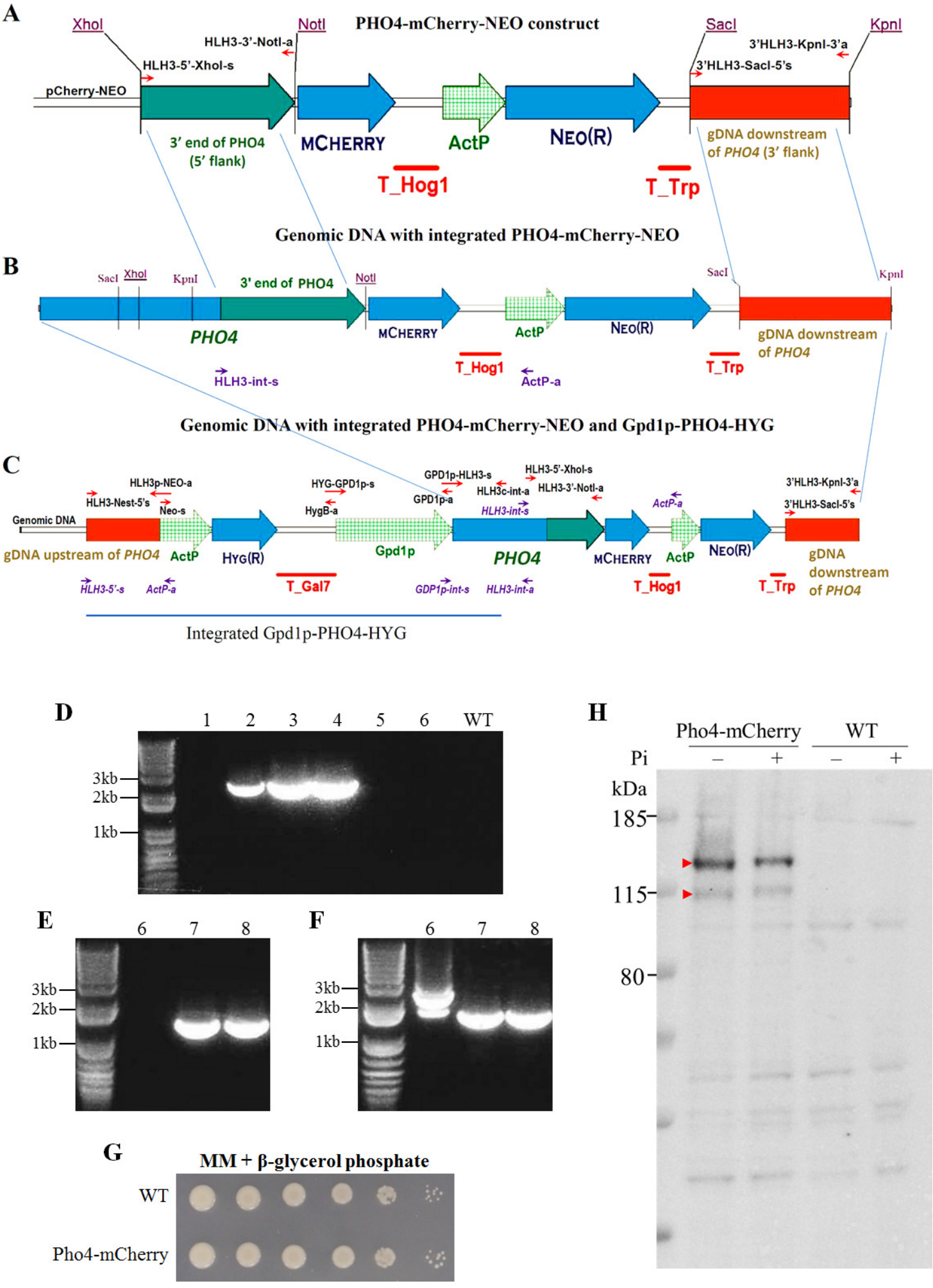
A
PHO4-mCherry-NEO construct
XhoI
NotI
SacI
KpnI
HLH3-3'-NotI-a
HLH3-5'-XhoI-s
3'HLH3-KpnI-3'a
3'HLH3-SacI-5's
pCherry-NEO
3' end of PHO4
(5' flank)
MCHERRY
ActP
NEO(R)
gDNA downstream
of PHO4 (3' flank)
T_Hog1
T_Trp
Genomic DNA with integrated PHO4-mCherry-NEO
B
SacI
XhoI
KpnI
3' end of PHO4
NotI
SacI
KpnI
PHO4
MCHERRY
ActP
NEO(R)
gDNA downstream
of PHO4
HLH3-int-s
T_Hog1
ActP-a
T_Trp
Genomic DNA with integrated PHO4-mCherry-NEO and Gpd1p-PHO4-HYG
C
HLH3p-NEO-a
HLH3-Nest-5's
Neo-s
HYG-GPD1p-s
HygB-a
GPD1p-HLH3-s
GPD1p-a
HLH3c-int-a
HLH3-5'-XhoI-s
HLH3-3'-NotI-a
HLH3-int-s
ActP-a
3'HLH3-KpnI-3'a
3'HLH3-SacI-5's
Genomic DNA
gDNA upstream of PHO4
ActP
HYG(R)
Gpd1p
PHO4
MCHERRY
ActP
NEO(R)
gDNA
downstream
of PHO4
HLH3-5'-s
ActP-a
T_Gal7
GDP1p-int-s
HLH3-int-a
T_Hog1
T_Trp
Integrated Gpd1p-PHO4-HYG
D
1 2 3 4 5 6 WT
3kb
2kb
1kb
E
6 7 8
3kb
2kb
1kb
F
6 7 8
3kb
2kb
1kb
G
MM + β-glycerol phosphate
WT
Pho4-mCherry
H
Pho4-mCherry
WT
Pi
– + – +
kDa
185
115
80