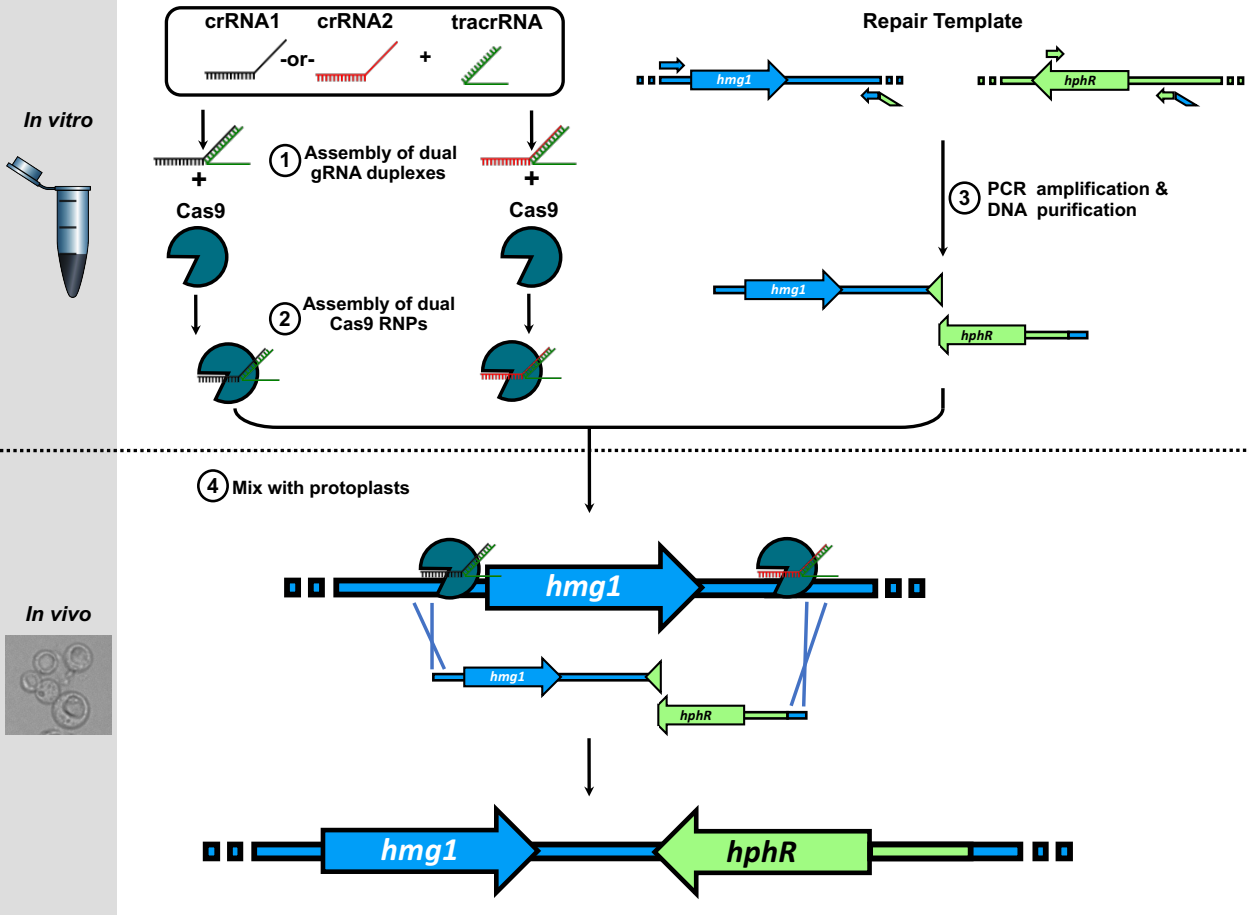
In vitro
crRNA1
crRNA2
tracrRNA
-or-
+
Repair Template
hmg1
hphR
1 Assembly of dual gRNA duplexes
+
+
Cas9
Cas9
2 Assembly of dual Cas9 RNPs
3 PCR amplification & DNA purification
hmg1
hphR
4 Mix with protoplasts
In vivo
hmg1
hmg1
hphR
hmg1
hphR