

PCR mix	
Component	Volume
H ₂ O	303.2 µl
5x Phusion HF buffer	80.0 µl
Fwd primer 100 µM	2.0 µl (final concentration 0.5 µM)
Rev primer 100 µM	2.0 µl (final concentration 0.5 µM)
Plasmid (100 ng/µl)	0.8 µl (final concentration 0.2 ng/µl)
Phusion DNA polymerase	4.0 µl
dNTPs 10 mM	8.0 µl
TOTAL	400.0 µl split into 8 x 50-µl reactions

PCR program – EGFP		
98°	– 2 min	x35 cycles
98°	– 30 sec	
60°	– 30 sec	
72°	– 45 sec	
72°	– 10 min	
4°	– ∞	

PCR program - wrmScarlet		
98°	– 30 sec	x35 cycles
98°	– 10 sec	
51°	– 15 sec	
72°	– 15 sec	
72°	– 10 min	
4°	– ∞	

PCR program - mCherry		
98°	– 2 min	x35 cycles
98°	– 30 sec	
67°	– 30 sec	
72°	– 45 sec	
72°	– 10 min	
4°	– ∞	

PCR program – 2xTY1::EGFP::3xFLAG		
98°	– 2 min	x35 cycles
98°	– 30 sec	
60°	– 30 sec	
72°	– 45 sec	
72°	– 10 min	
4°	– ∞	

Figure S2. Reagents and conditions for generation of universal PCR product repair templates for Nested CRISPR Step 2