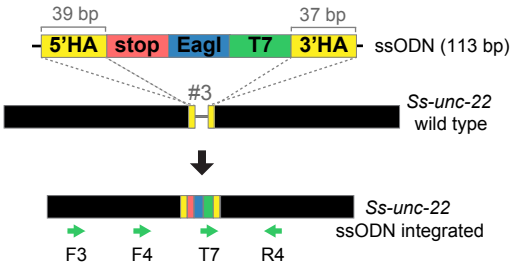
A

***Ss-unc-22* ssODN homology-directed repair**



T7 x R4	ssODN integration	356 bp
F3 x R4	region around *Ss-unc-22* site #3	960 bp
F4 x R4	region for EagI digest *Ss-unc-22* site #3	660 bp

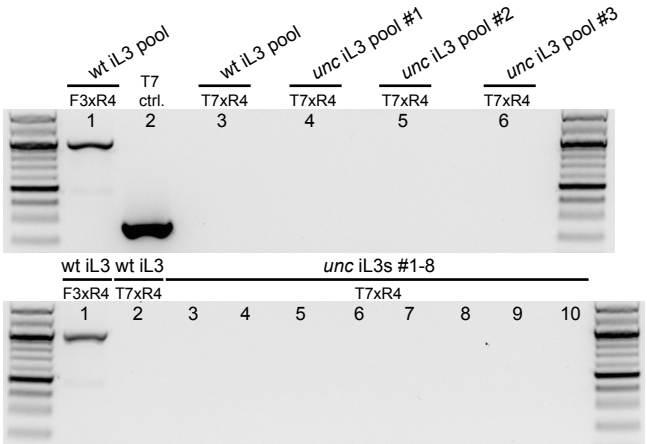
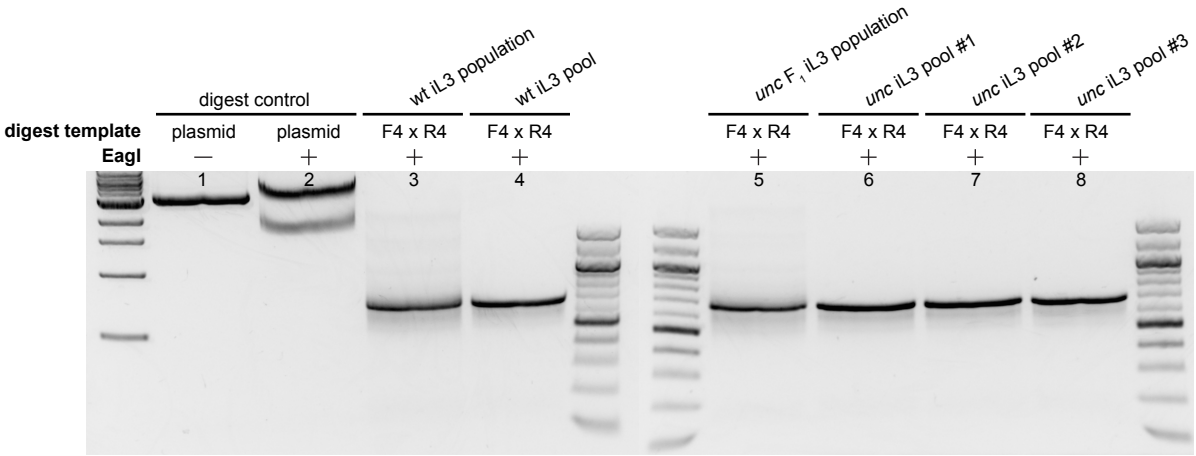
B

PAM

CATTTATTGCTAAAAATCCATTTGATCCACCAGGT**CCA**CTAACTAACTAA
CGGCCGTAATACGACTCACTATAGGGCCTGATTGTACTACTTTTAGTGA
AGATAGTATTATAG

homology arms
EagI site
stop codons (all reading frames)
T7 forward primer

C



D