

A

Single-stranded dDNA enables edits, but **Target** is still disrupted

Uncontrolled mutations disrupt **Target** to maintain cell viability

gDNA: Feature, Target

RGN: Spacer, Scaffold

Restriction

gDNA: Feature, Target, get

HDR

gDNA: Feature, *Target

NHEJ

gDNA: Feature, *Target

HDR

gDNA: Feature, *Target

NHEJ

gDNA: Feature, *Target

B

Double-stranded dDNA cannot produce intended edits, and **Target** is still disrupted

gDNA: Feature, Target

RGN: Spacer, Scaffold

Restriction

gDNA: Feature, Target, get

HDR

gDNA: Feature, *Target

NHEJ

gDNA: Feature, *Target

HDR

gDNA: Feature, *Target

NHEJ

gDNA: Feature, *Target

Double-stranded dDNA cannot produce intended edits, and **Target** is still disrupted

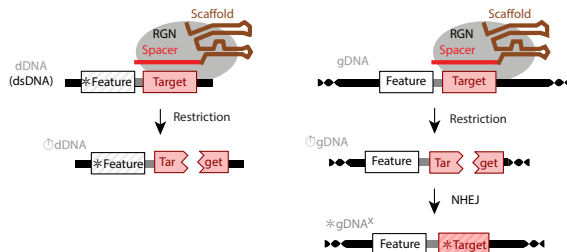


Figure S2

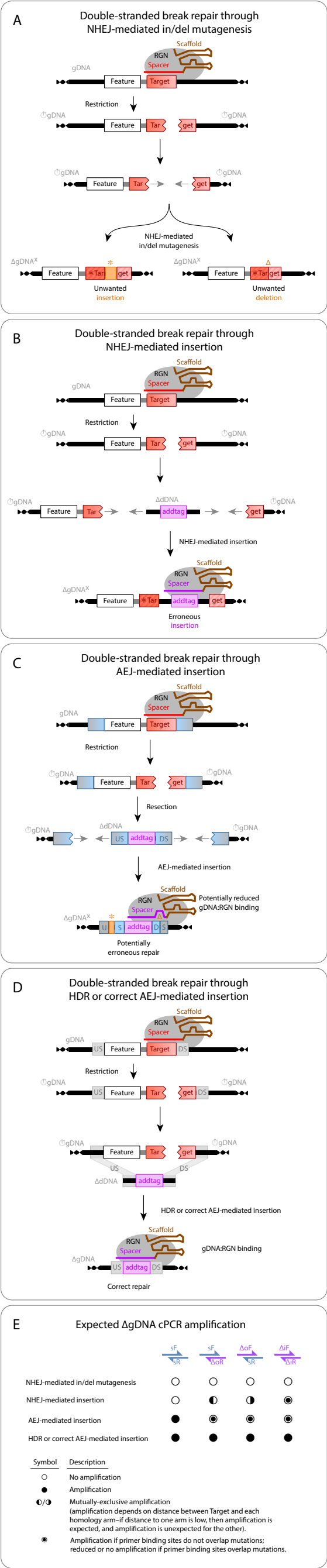


Figure S3

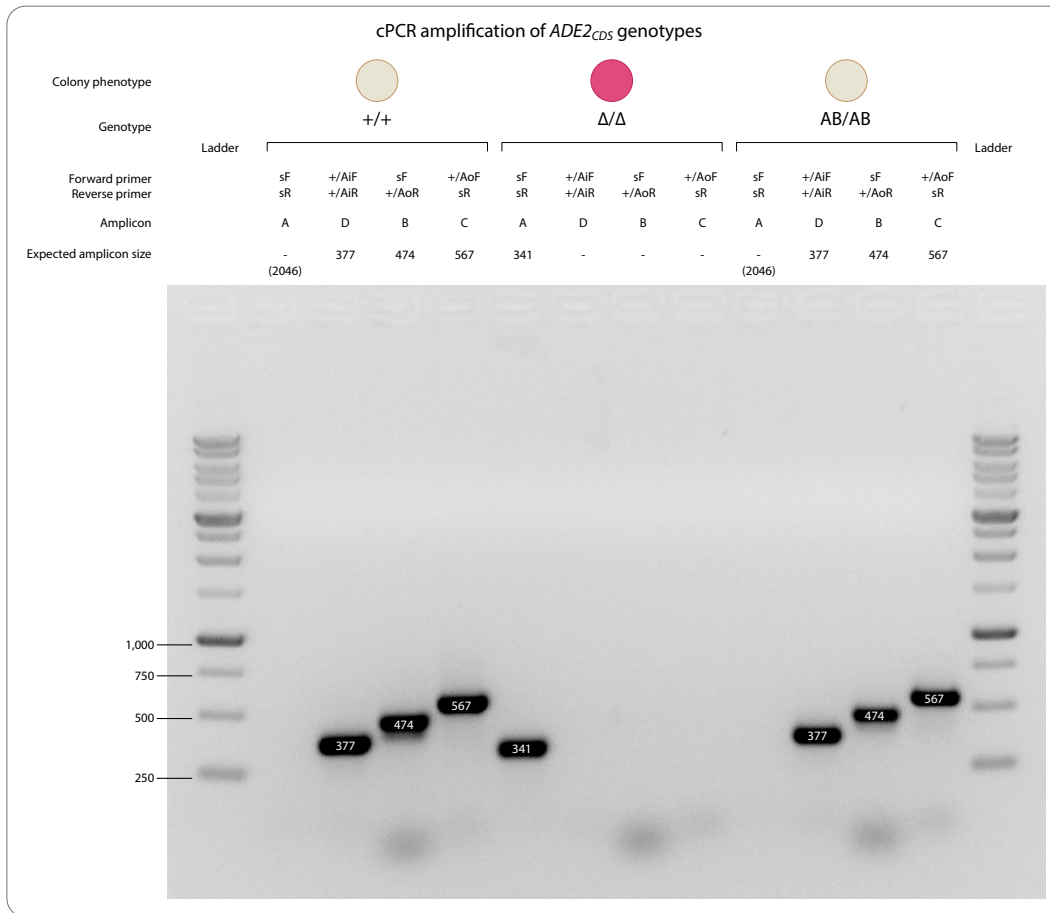


Figure S4

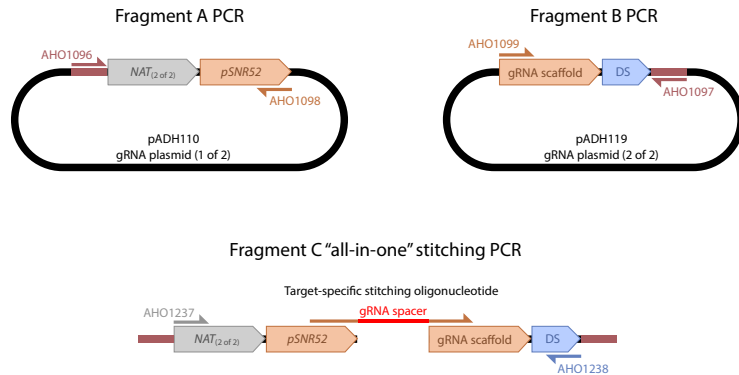
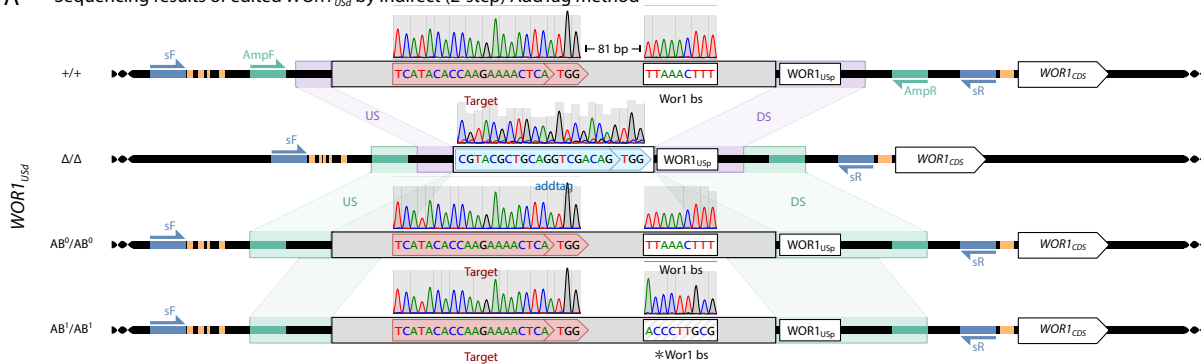


Figure S5

A Sequencing results of edited *WOR1_{Usd}* by indirect (2-step) AddTag method



B Sequencing results of edited *WOR1_{Usd}* by indirect (2-step) AddTag method

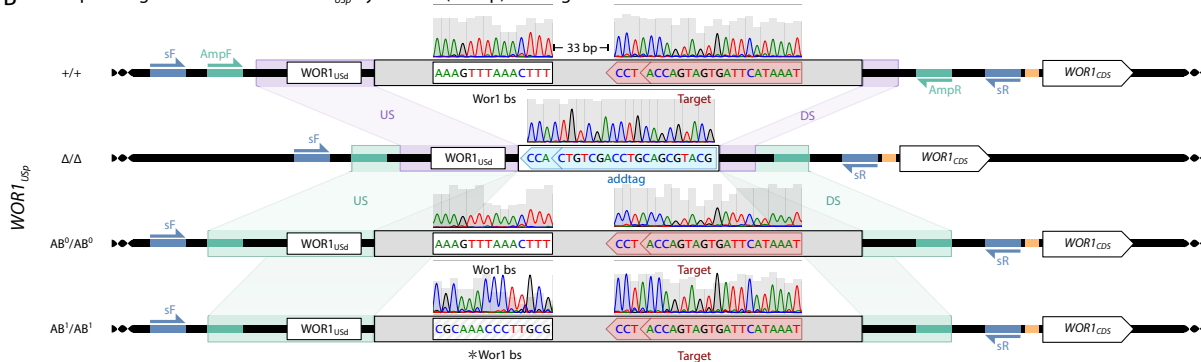


Figure S6

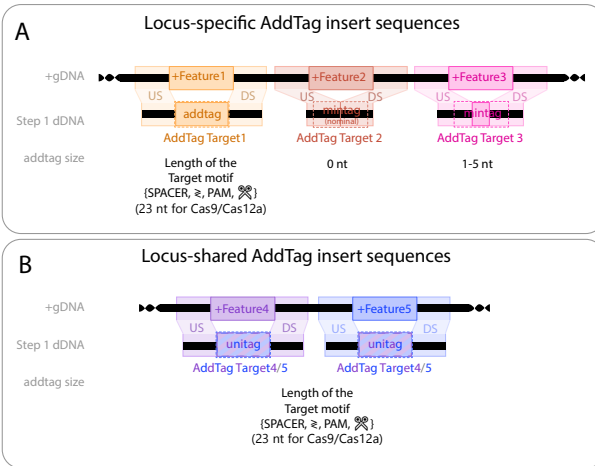


Figure S7

A priori weight functions for on-target Algorithm scores

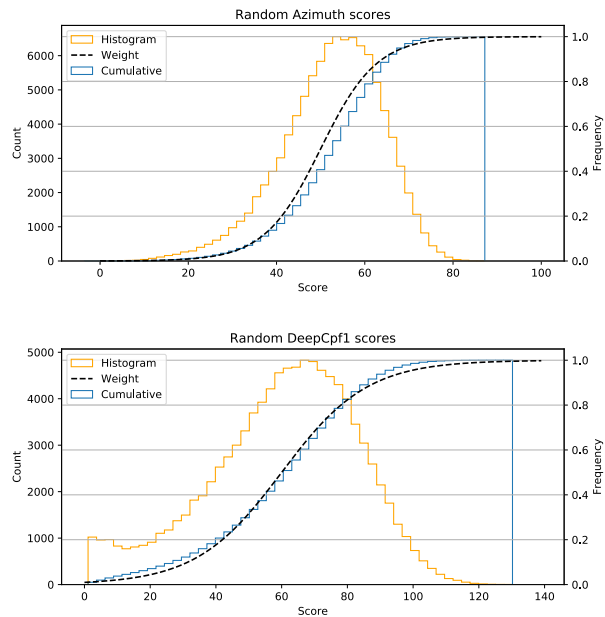


Figure S8

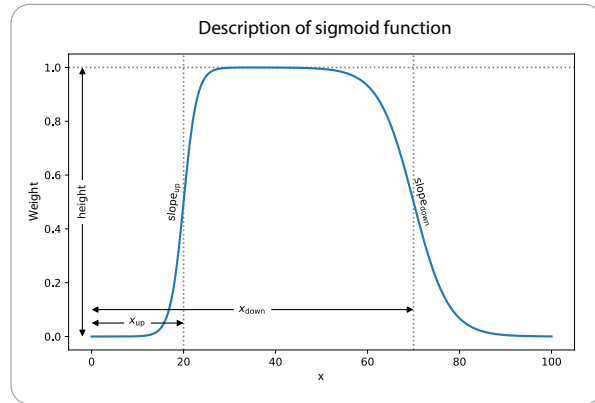


Figure S9

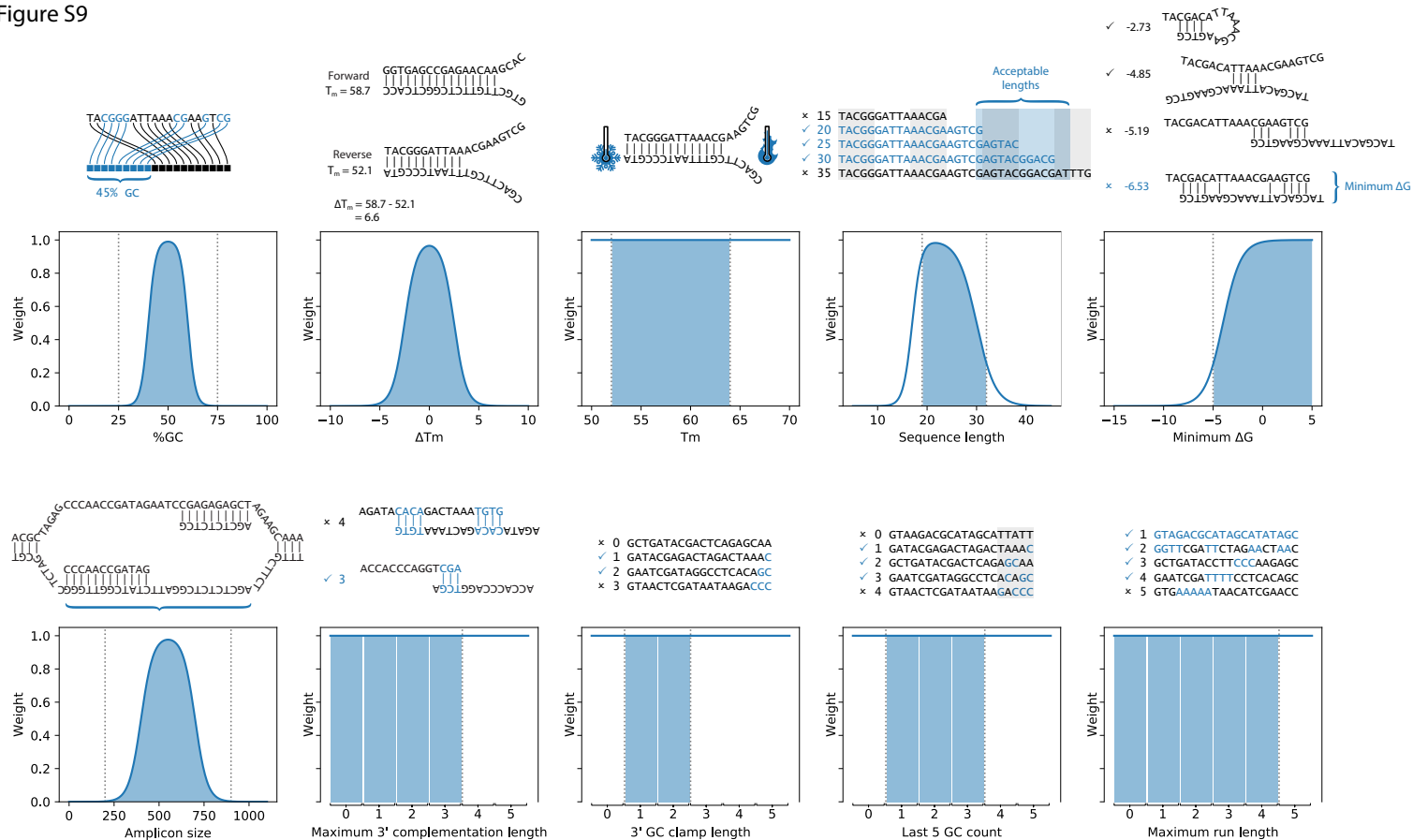
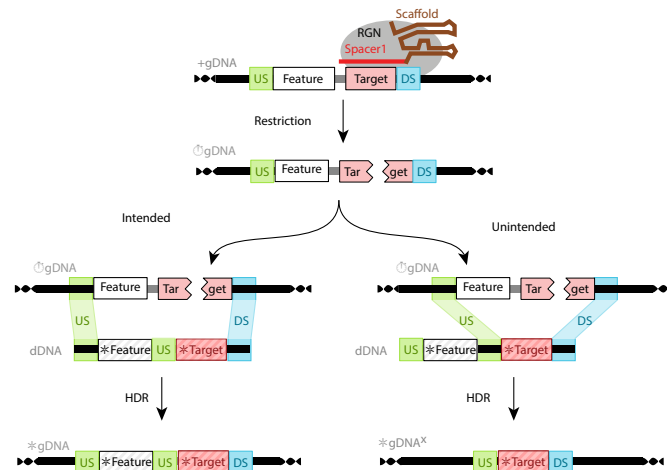
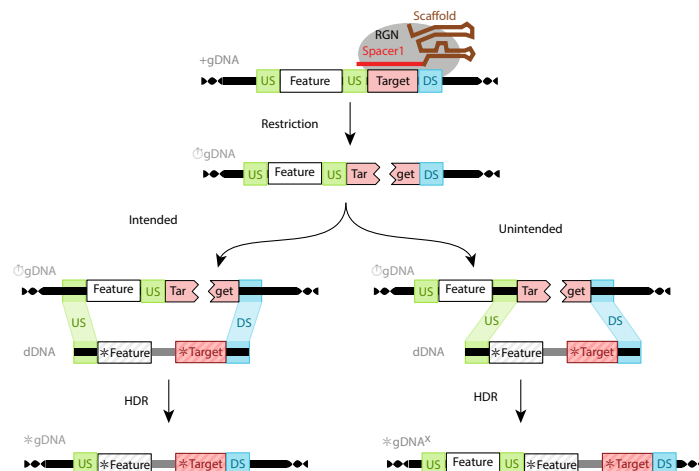


Figure S10

A Excessive homology within dDNA reduces efficiency of intended dDNA incorporation



B Excessive homology within gDNA reduces efficiency of intended dDNA incorporation



C Excessive homology in long dDNA reduces efficiency of combinatorial direct (1-step) traditional editing

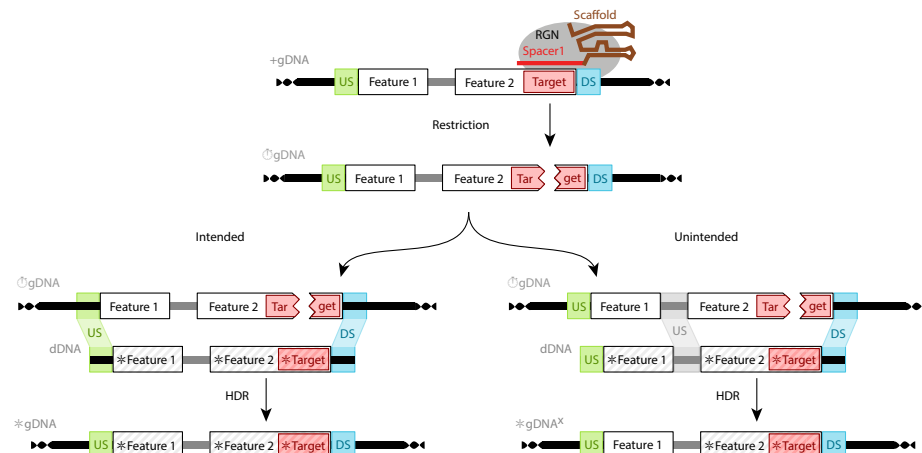


Figure S11

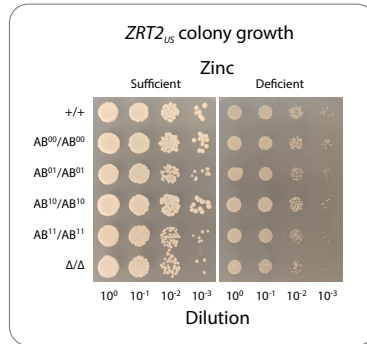


Figure S12

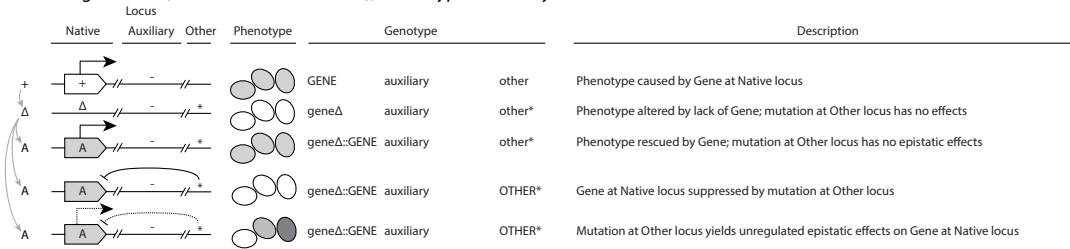
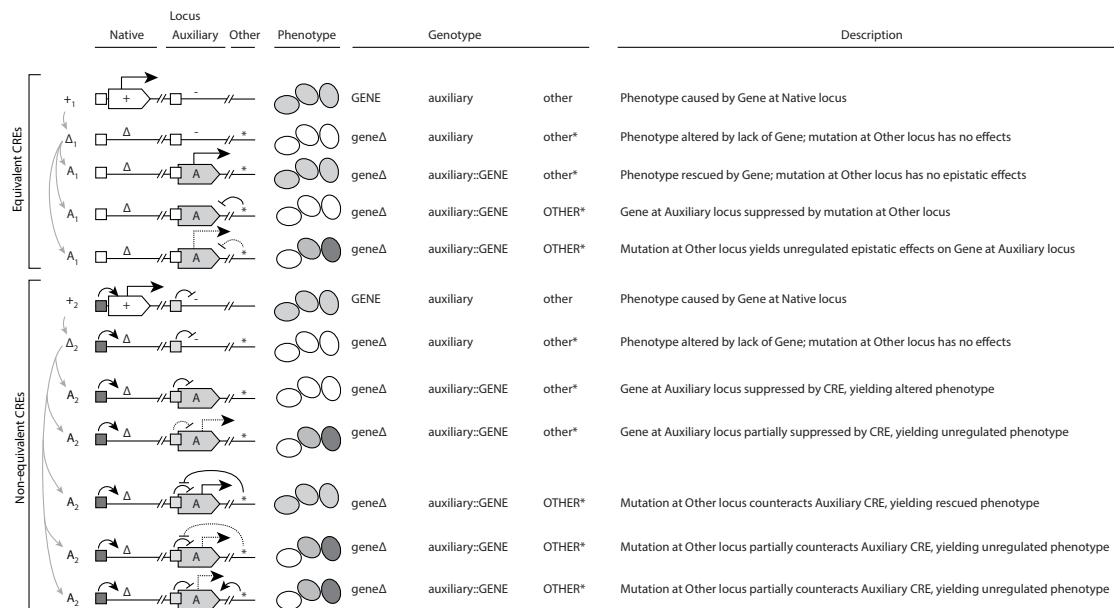
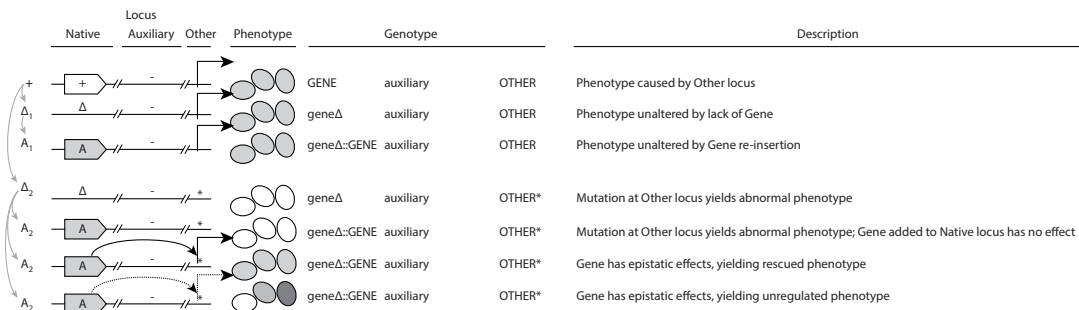
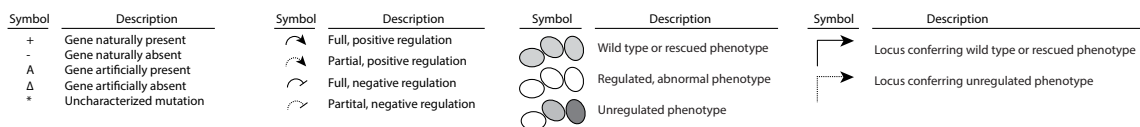
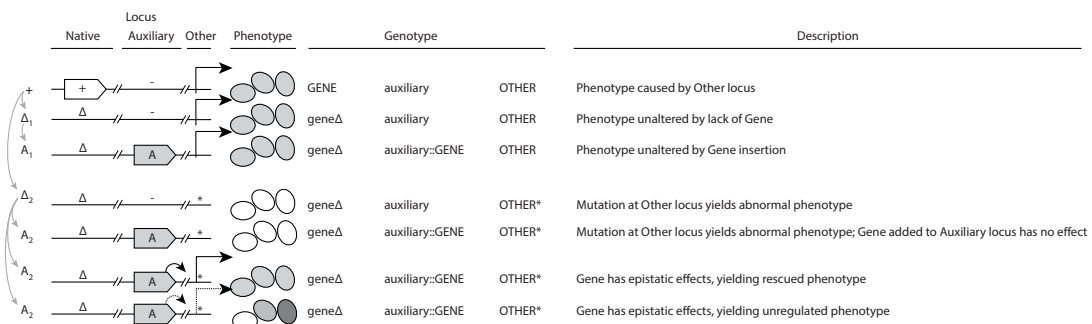
A AddTag method (add-back at Native locus); Phenotype caused by Gene**B Traditional method (add-back at Auxiliary locus); Phenotype caused by Gene****C AddTag method (add-back at Native locus); Phenotype caused by Other locus****D Traditional method (add-back at Auxiliary locus); Phenotype caused by Other locus**

Figure S13

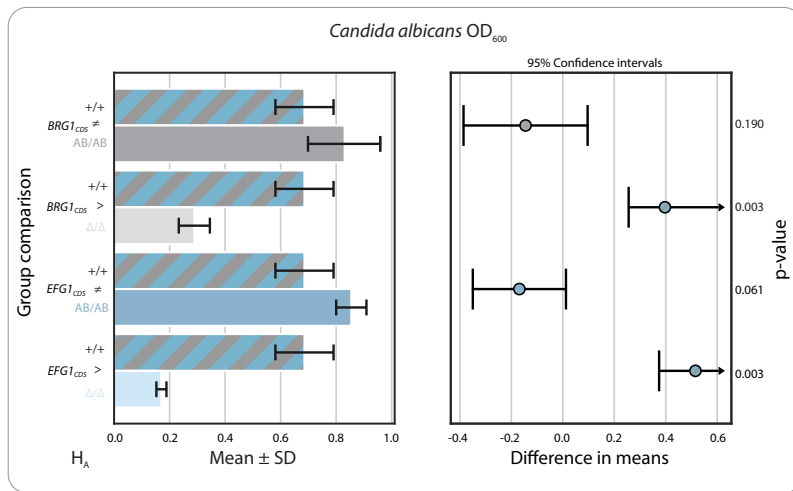


Figure S14

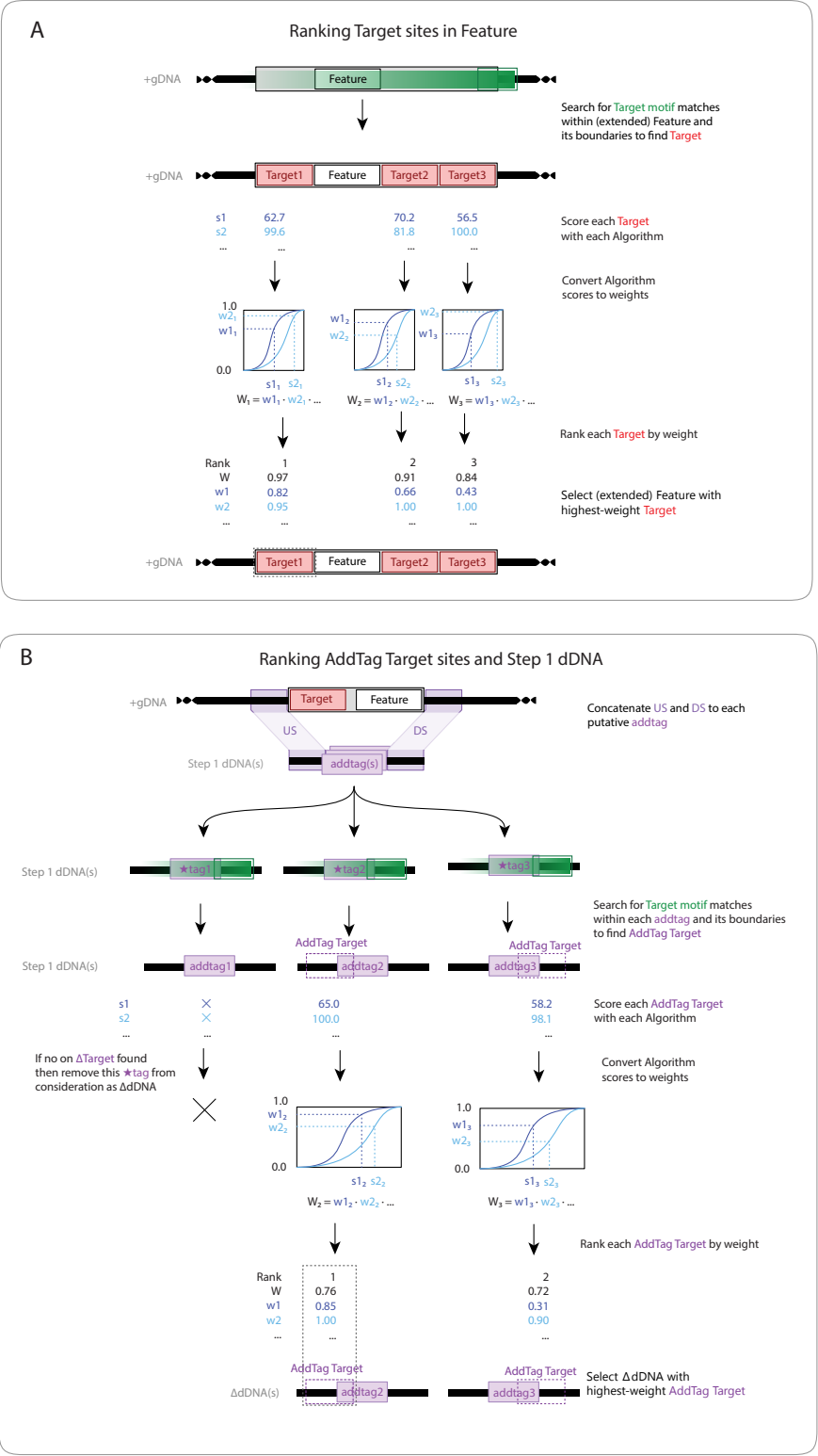


Figure S15

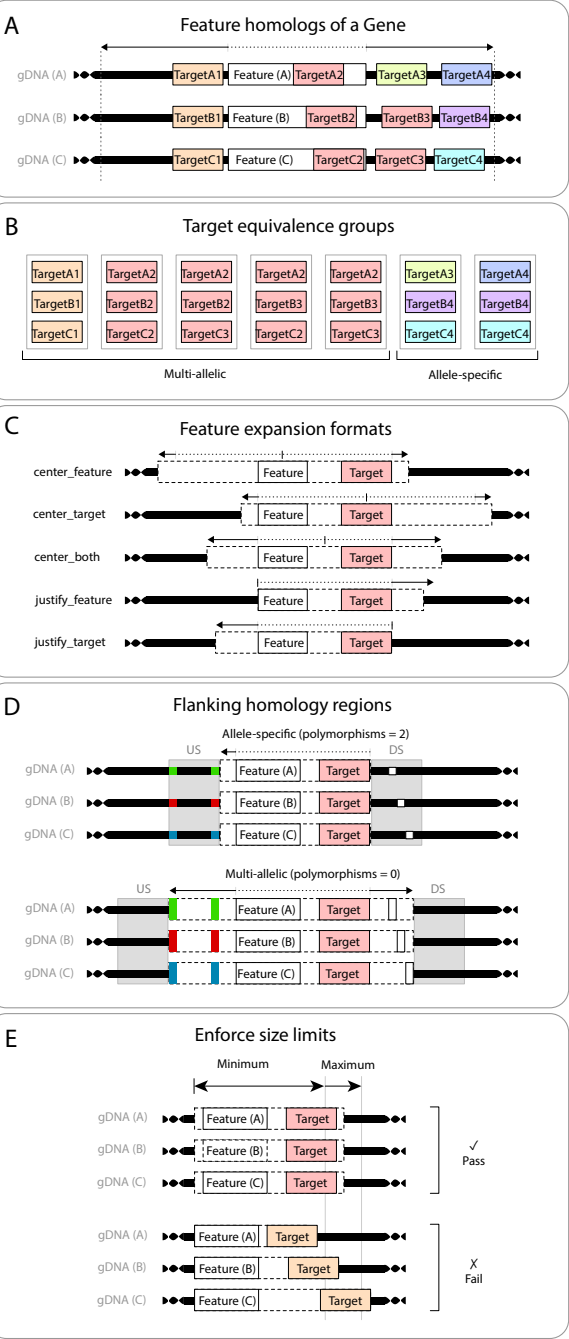


Figure S16

