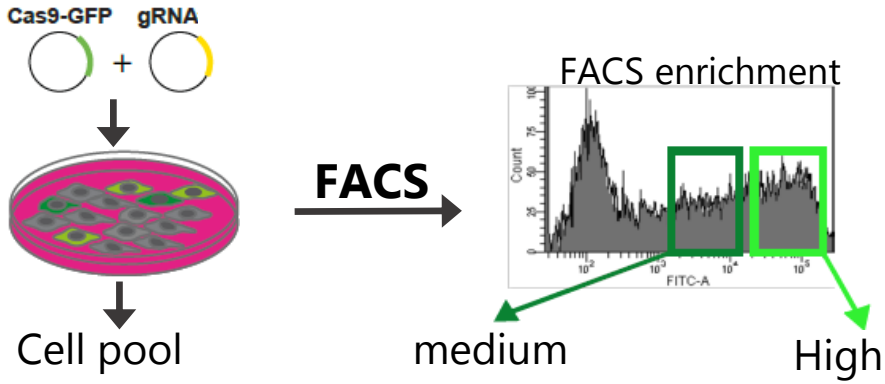
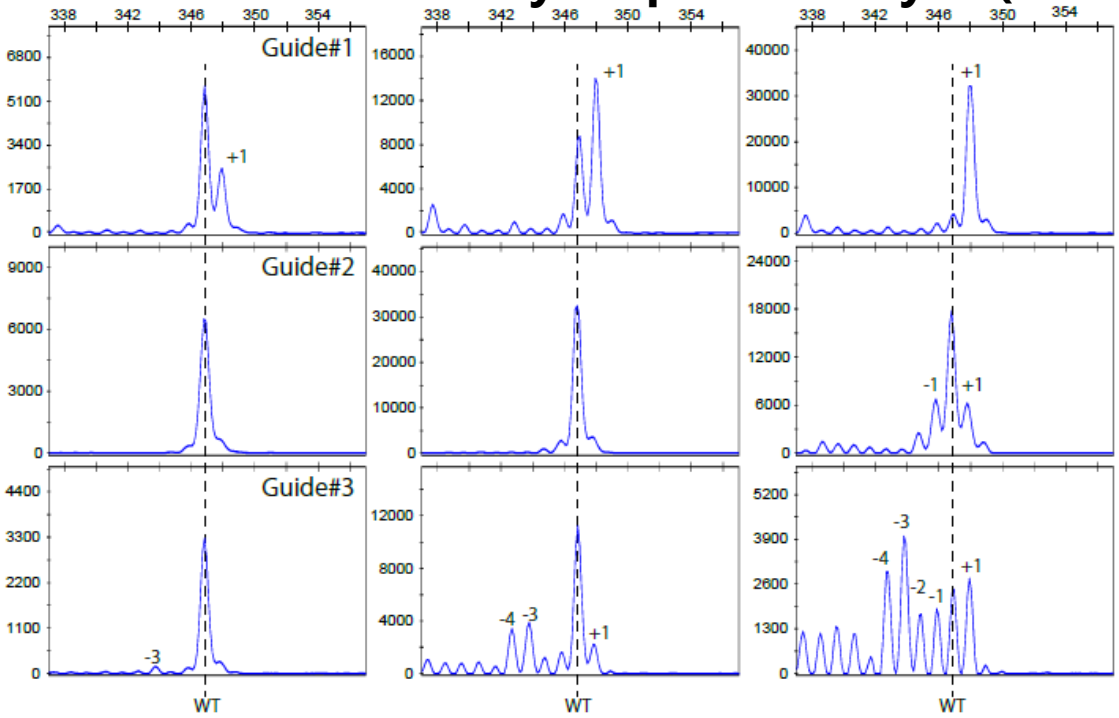
gRNA validation by IDAA

Transfection with web-tool/algorithm gRNA designs

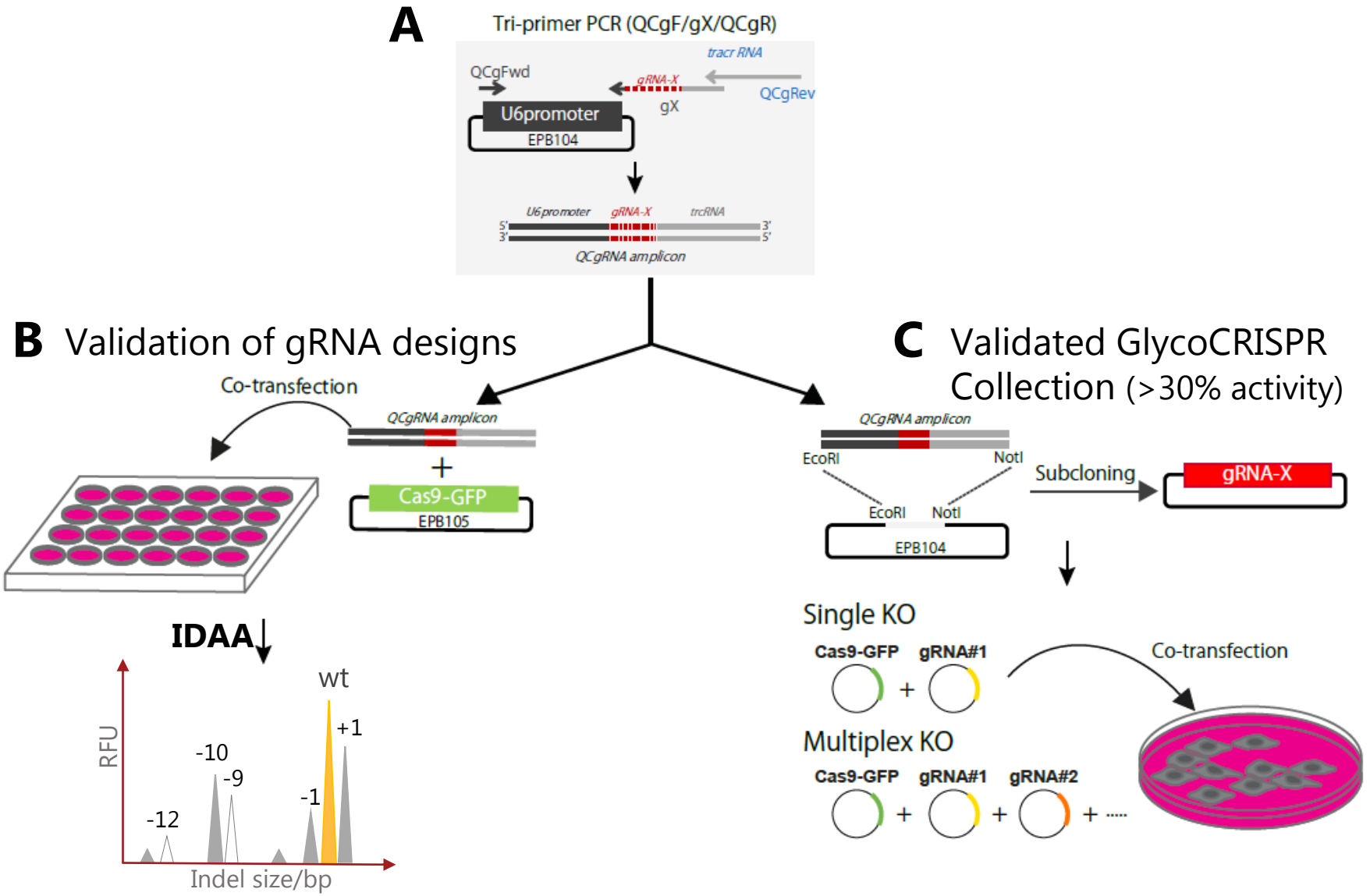


Indel Detection by Amplicon Analysis (IDAA)



GlycoCRISPR collection gRNAs were designed as described in (Y.Narimatsu et al., Glycobiology, 2018) using Deskgen https://www.deskgen.com/landing/) algorithm and experimentally validated by previously published methods (L.Lonowski et al., Nat Protoc, 2017). Figure shows 3 gRNA design examples targeting *GCNT6* exon1 (#1-#3), transfected into HEK293 and IDAA (Z.Yang et al., NAR, 2015) indel profiled 2 days post transfection for; 1) the "cell pool", 2) FACS enriched "medium" GFP positive population or 3) "high" GFP positive population. WT alleles are indicated by dotted line and detected indel sizes in bp shown above peaks.

Workflow for establishment of the GlycoCRISPR collection



The GlycoCRISPR collection gRNAs are based on experimentally validated gRNA designs with proven indel formation activity >30%. **A.** 1-7 Deskgen derived gRNA designs for all human glycogenes were experimentally validated by QCgRNA tri-primer amplicon gRNA validation procedure previously published (L.Lonowski et al., Nat Protoc, 2017). **B.** HEK293 transfected gRNA amplicons and Cas9 were indel profiled by IDAA (Z.Yang et al., NAR, 2015) and designs with total indel induction activity >30% selected for plasmid insertion. **C.** Plasmid cloned validated gRNAs ensure >30% cutting effiecincy and thus, are ideally suited for single or multiplex gene KO experiments.