Adenine Base Editing of Gamma Globin Gene Promoters Shows no Detectable Off-Target RNA or DNA Editing

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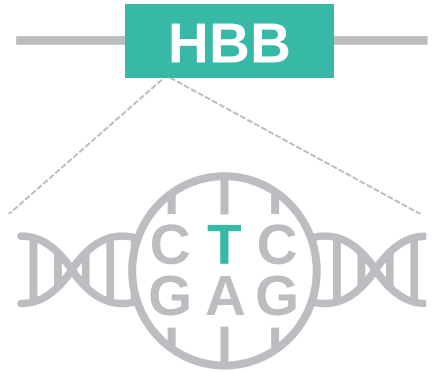
Disclosure

- I and all authors are Beam employees and shareholders

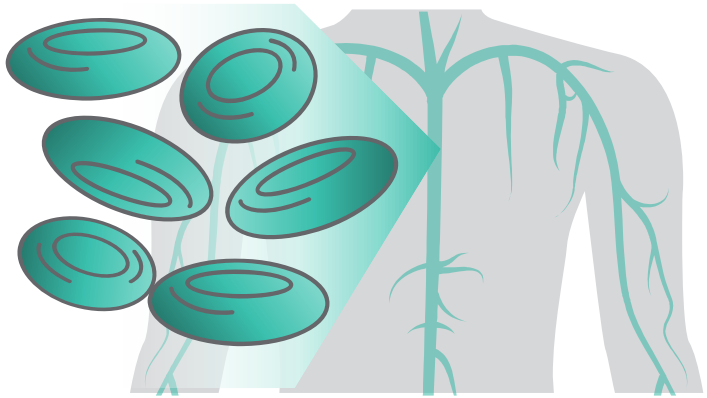
Sickle Cell Disease (SCD)

β-globin gene

Adult β-globin gene



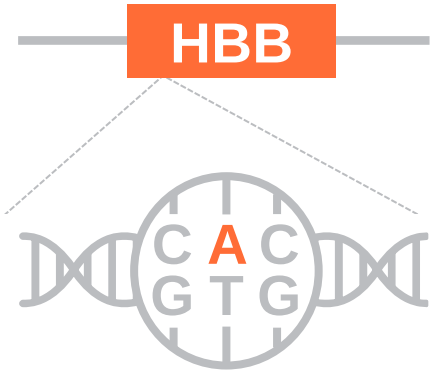
Glutamic acid at 6th amino acid (HbA)



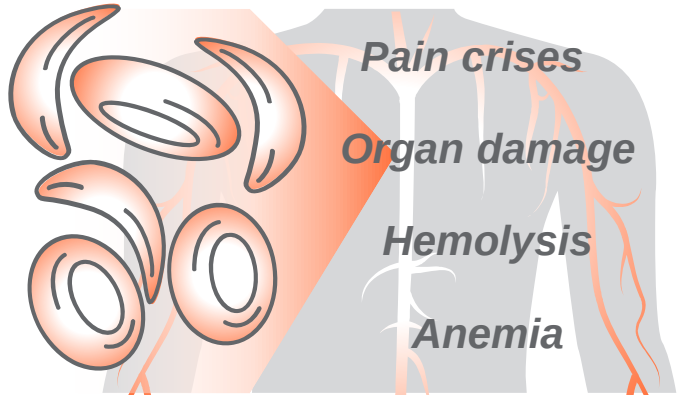
Normal red blood cells

T-to-A mutation causes sickling

Sickle β-globin gene



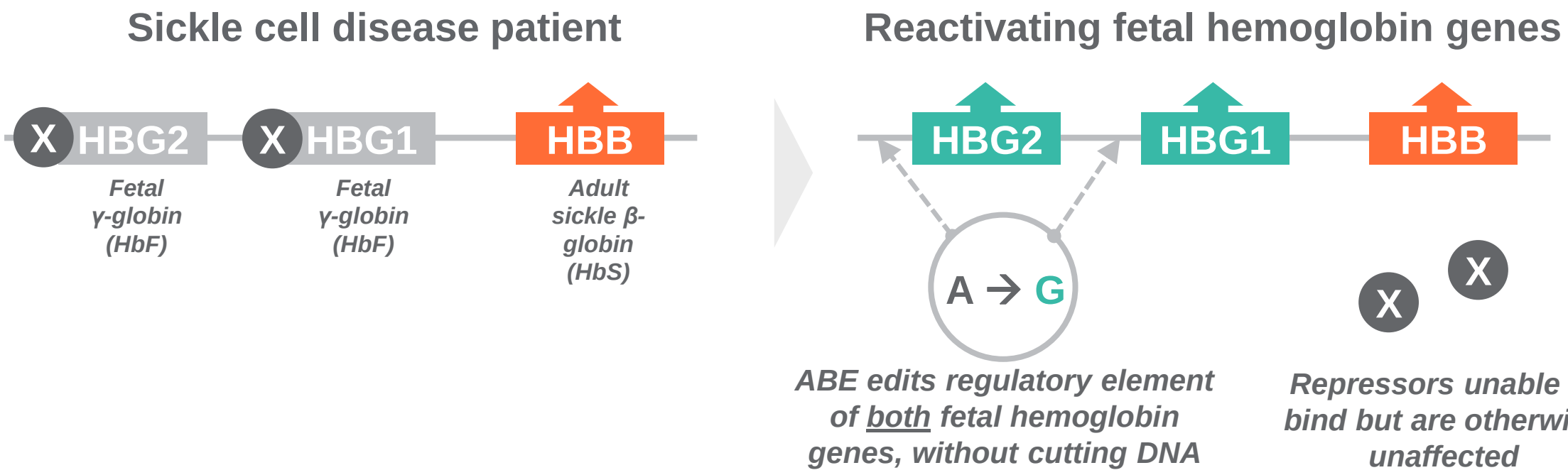
Valine at 6th amino acid (HbS)



Sickling red blood cells

Approximately 100,000 sickle cell disease patients in the US

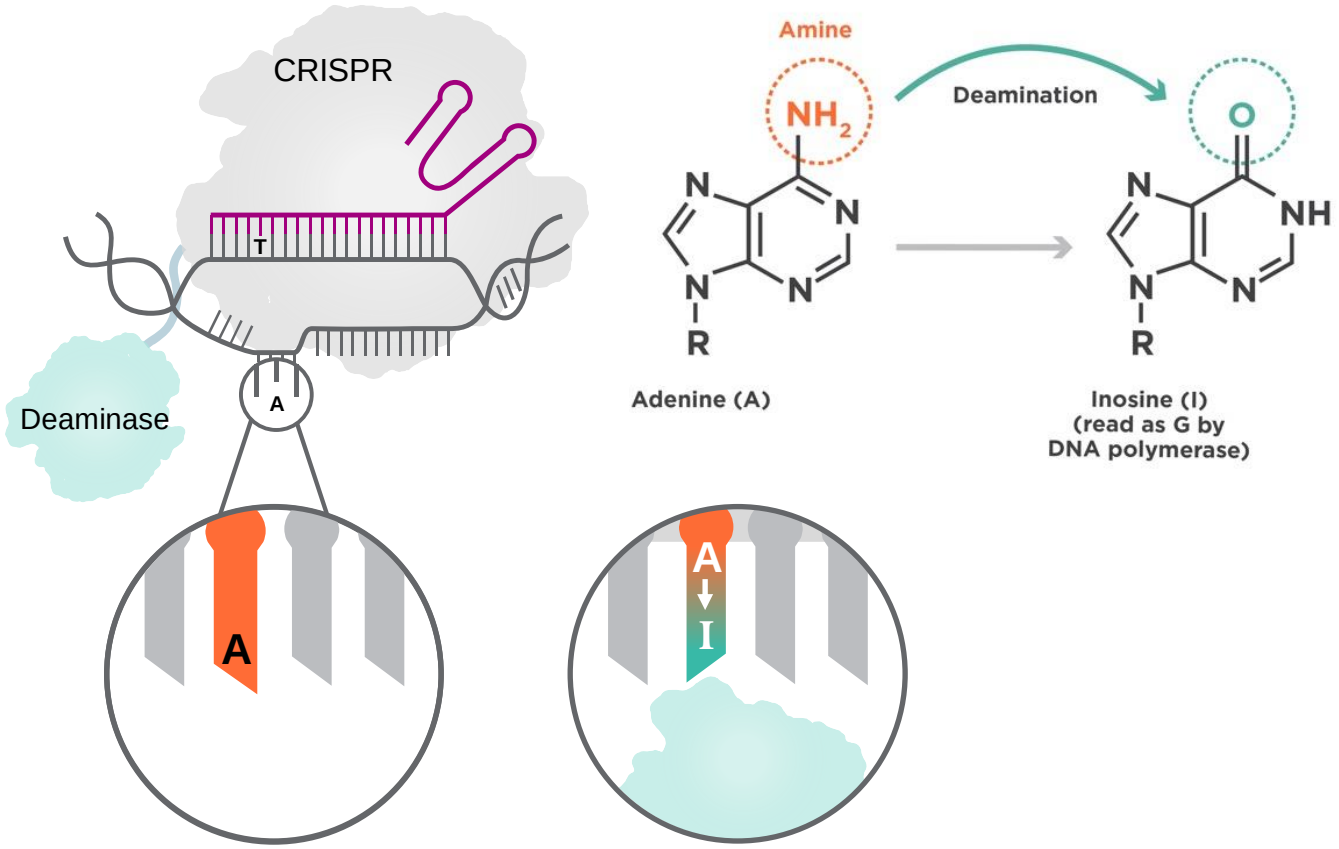
BEAM-101: Recreating Hereditary Persistence Of Fetal Hemoglobin (HPFH) With Base Editing



- Naturally-occurring base changes cause Hereditary Persistence of Fetal Hemoglobin (HPFH), which protects patients from SCD/B-Thal
- Base editors can reproduce these changes, leading to elevated levels of fetal hemoglobin
- Higher fetal hemoglobin likely to correlate with further reductions in disease symptoms

Mussallam, et al. 2012. *Blood.*

Adenine Base Editing Technology



- Adenine Base Editor (ABE) comprises a deaminase enzyme fused to catalytically impaired CRISPR protein.
- Guide RNA (gRNA) directs the ABE to a target genomic DNA sequence and exposes the editing window.
- Deaminase chemically converts target adenine (A) to inosine (I) via deamination.
- Two types of off-target events possible that we must characterize: guide-dependent and guide-independent

Gaudelli NM *et al*., *Nature* (2017); Gaudelli NM *et al*., *Nature Biotechnology* (2020).

Strategy of Off-Target Editing Assessment

Guide-dependent

In-silico Analysis
- 42,804 potential sites identified

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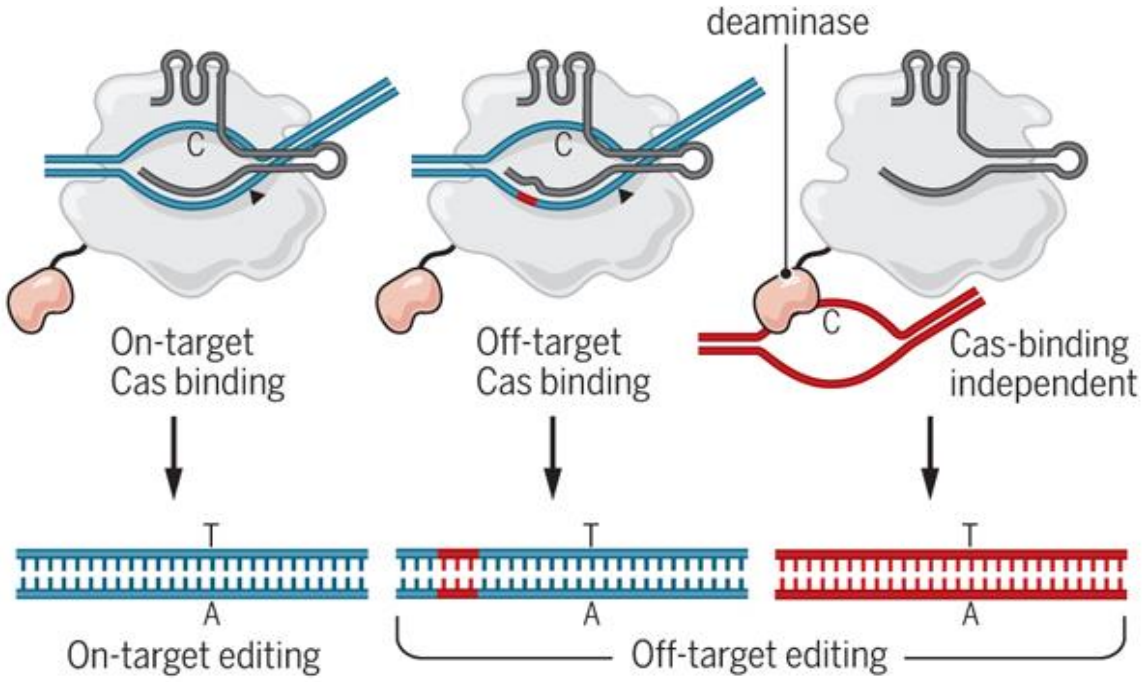
ONE-Seq
- 3,773 sites prioritized from in-silico analysis
- 143 sites selected for *in vivo* testing in cells

Guide-dependent Guide-independent



Kempton & Qi, Science, 2019

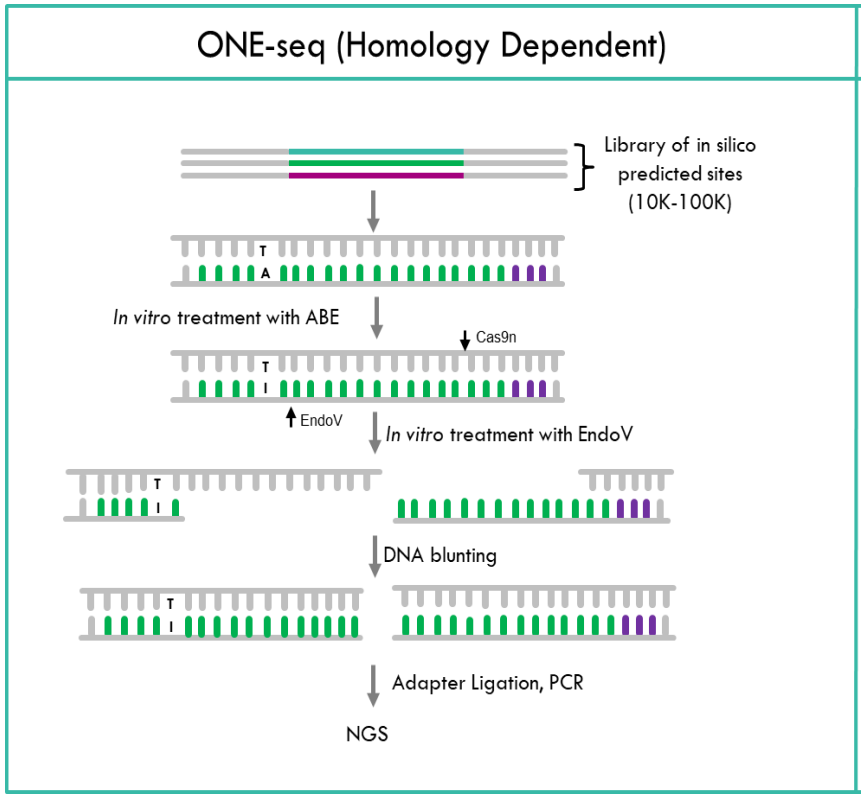
Guide-independent

Spurious RNA deamination
- mRNAseq
- 96h post electroporation

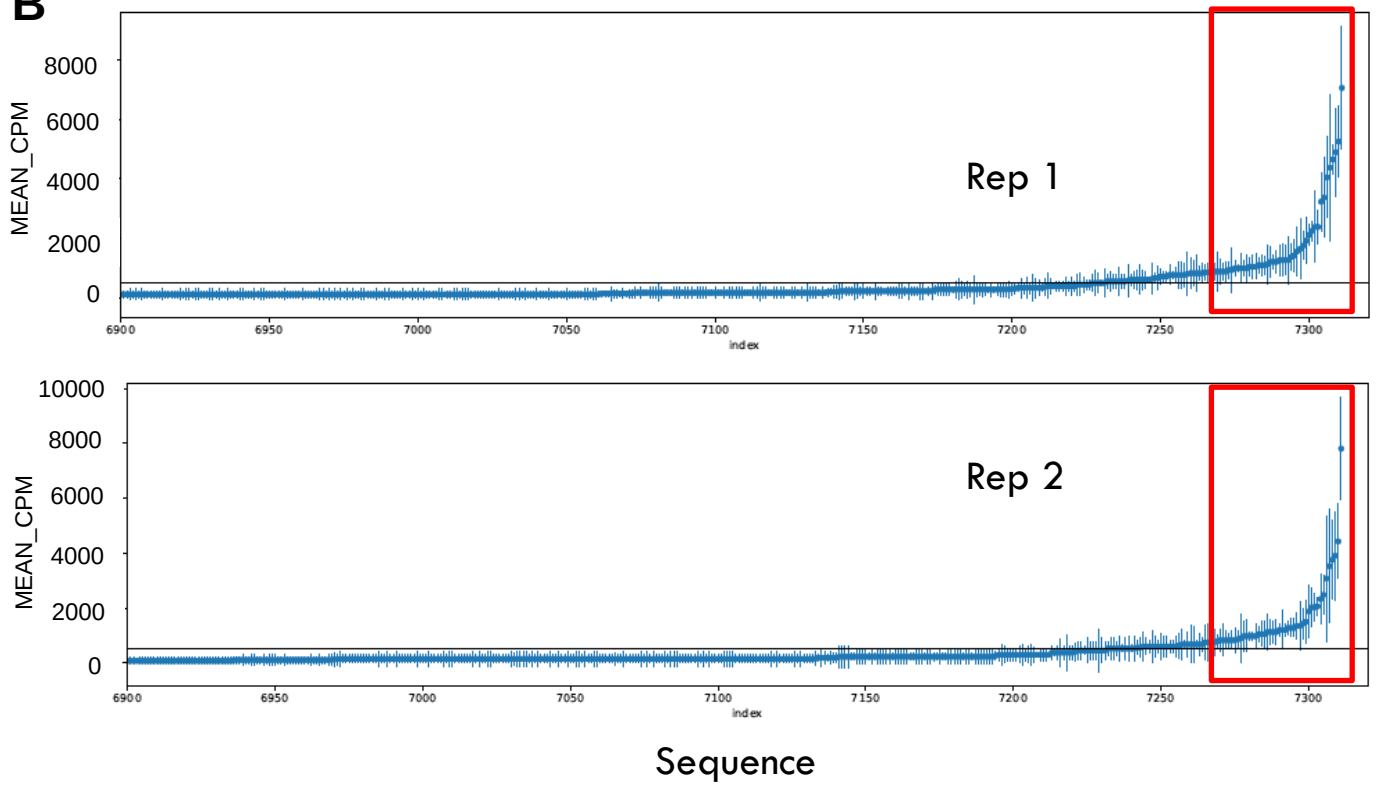
Spurious DNA deamination
- CFU colonies from edited CD34+ HSPCs
- Whole genome sequencing

Guide-Dependent Off-target Assessment Shows No Significant Off-targets

A

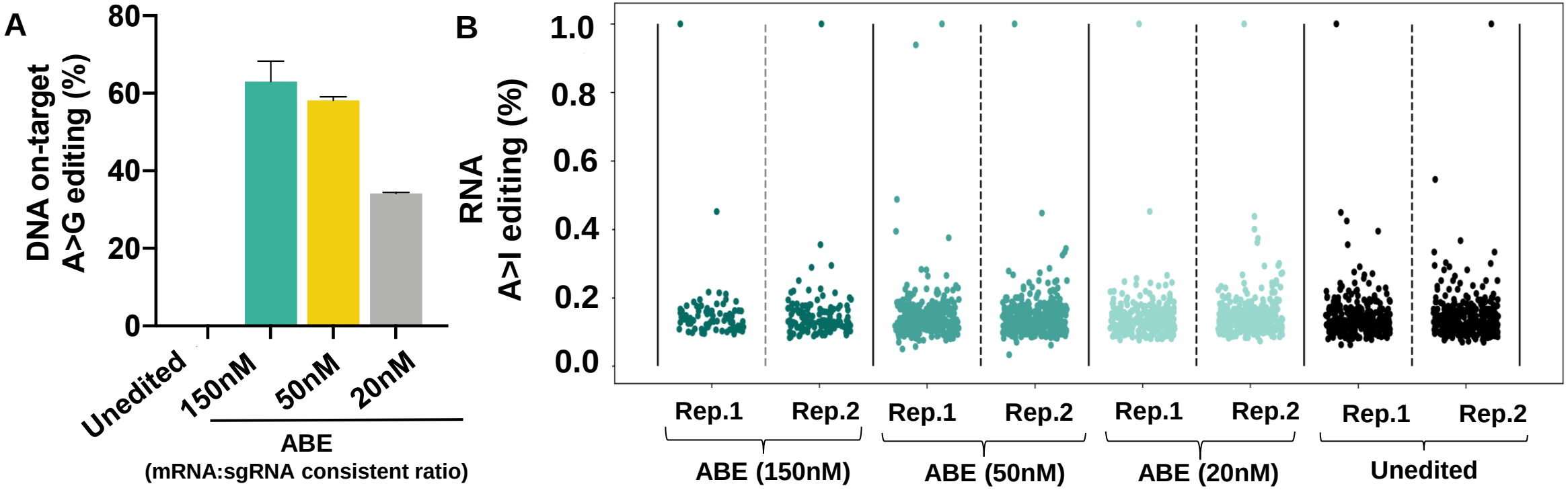


B



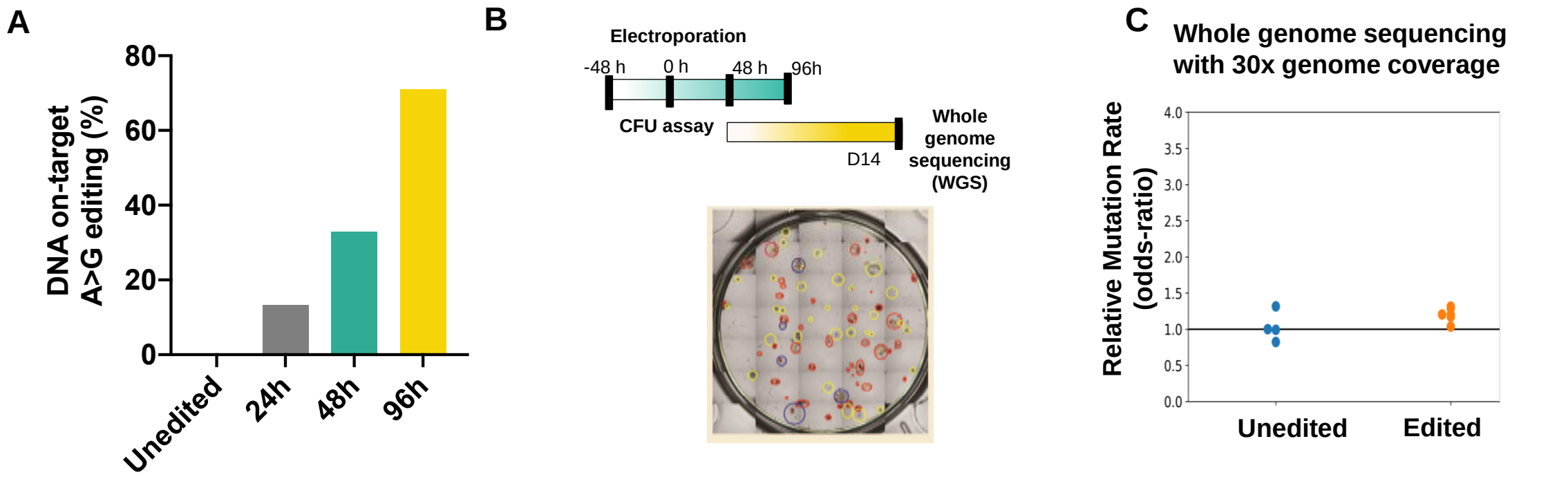
- 143 sites selected for testing in cells using rhAmpSeq
- 90% of potential sites have >10k reads sequencing coverage
- Only on-target editing observed

Guide-Independent RNA Editing Shows No Difference Between Edited And Unedited Groups



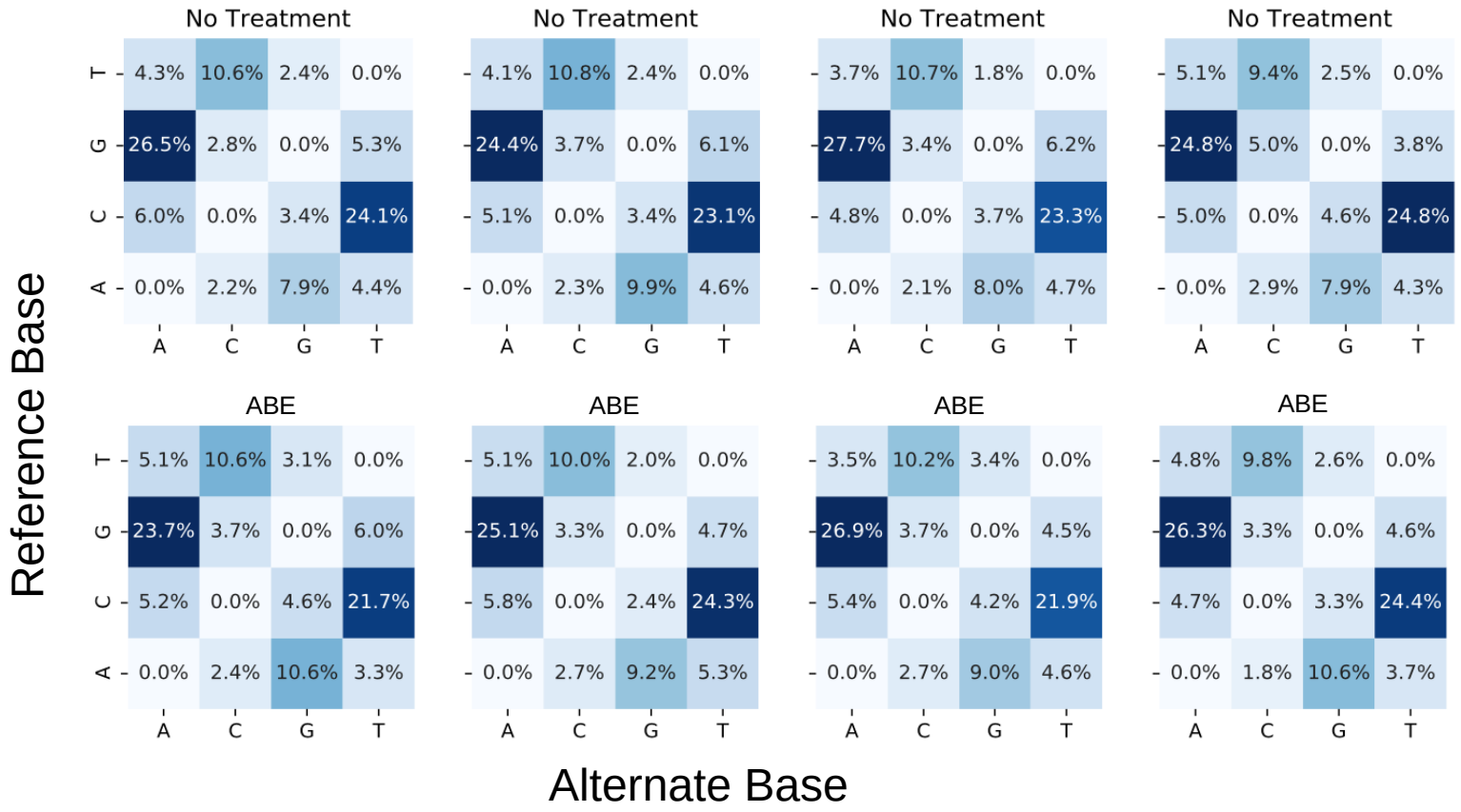
- A sgRNA targeting -200 region of *HBG1/2* promoter was used to investigate the guide independent RNA deamination.
- On-target DNA editing is elevated in the edited groups in a dose dependent fashion.
- RNA A-to-I editing rate is similar between edited groups and unedited group.

Guide-Independent DNA Editing Shows No Significant Enrichment Of A > G Mutations



- On-target editing was >70% on day 4 after electroporation.
- Individual BFU-E clones were picked at 14 days after seeding for genomic DNA isolation and WGS.
- Mutations from ABE edited cells compared to unedited controls was not significant.

Guide-Independent DNA Editing Shows No Significar Enrichment of A > G Mutations In CD34+ HSPCs



- WGS studies show mutational classification of all somatic mutations observed genome wide in single clones.
- No significant difference between edited and unedited groups.

Key Takeaways

✓ No guide-dependent DNA off-target editing

- Comprehensive determination of all the potential off-target editing using ONE-seq
- Further validation of actual editing in CD34+ HSPCs edited at supra-saturating dose revealed no off-target DNA editing in ABE edited cells

✓ No guide-independent RNA off-target editing

- Whole transcriptome sequencing and somatic variant calling revealed no guide-independent off-target RNA editing at supra-saturating doses

✓ No elevated genome-wide guide-independent DNA deamination

- WGS in a clonal population of ABE edited cells shows no significant fold change of A-to-G mutations compared to unedited controls

Thank you!

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