One Genome or Thousands of Genomes? Population genomics and off-target risks

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Regulators are asking for off-target risks across a representative sample of genomes

Human Gene Therapy Products Incorporating Human Genome Editing

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Draft Guidance for Industry

B. Assessment of Activity

Effects of genetic variation on editing activity across the target population.

C. Assessment of Safety

The analysis should be performed using the target human cell type(s) from multiple donors.

Why that matters clinically?

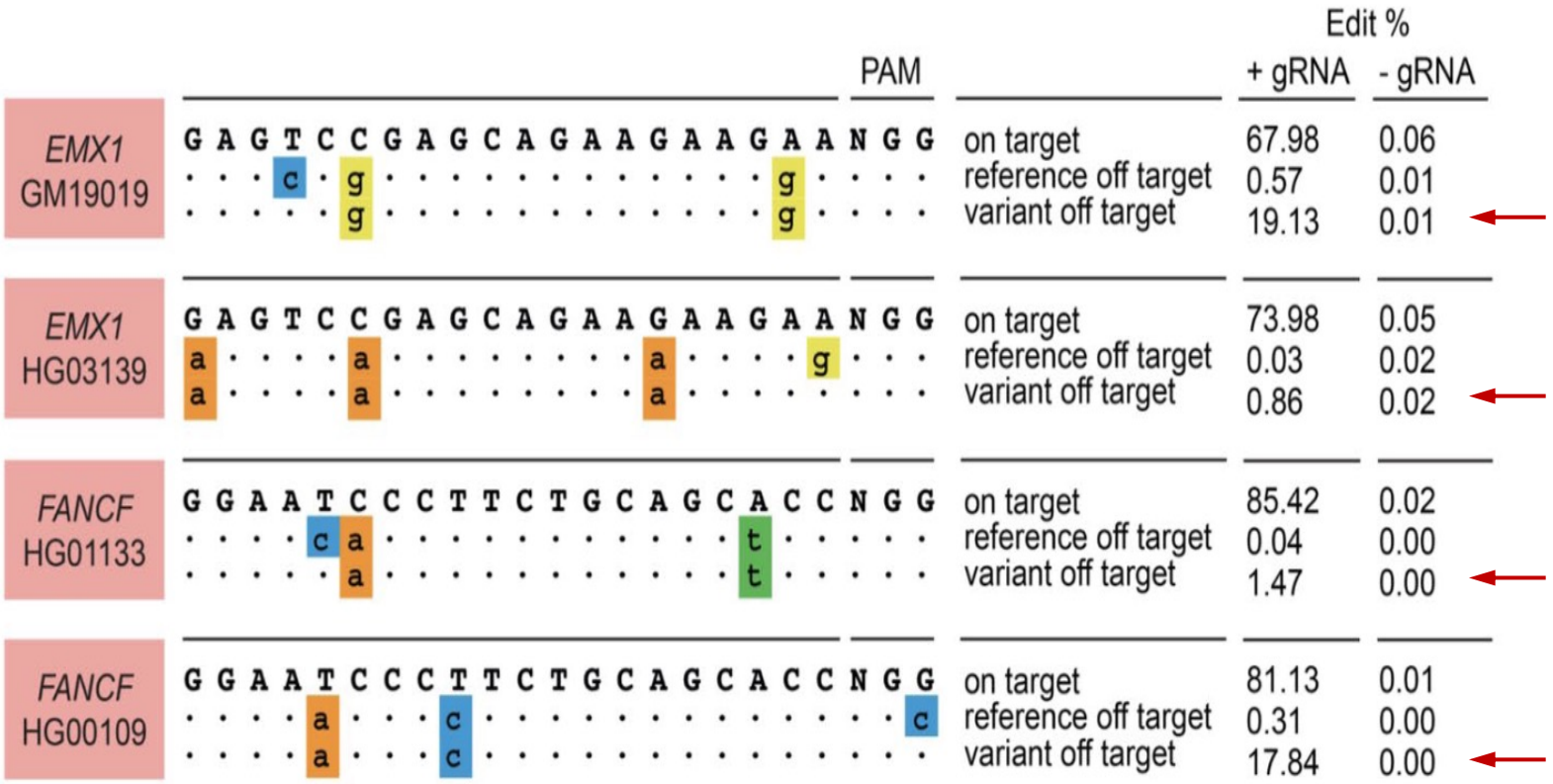

Figure 1: Petri K., Kim D., et. al. Global-scale CRISPR gene editor specificity profiling by ONE-seq identifies population-specific, variant off-target effects. bioRxiv 2021.04.05.438458; doi: https://doi.org/10.1101/2021.04.05.438458

Genomic differences aka genomic variants increase off-target editing frequency, as assessed in human cells

Genomic variants arise through three primary means

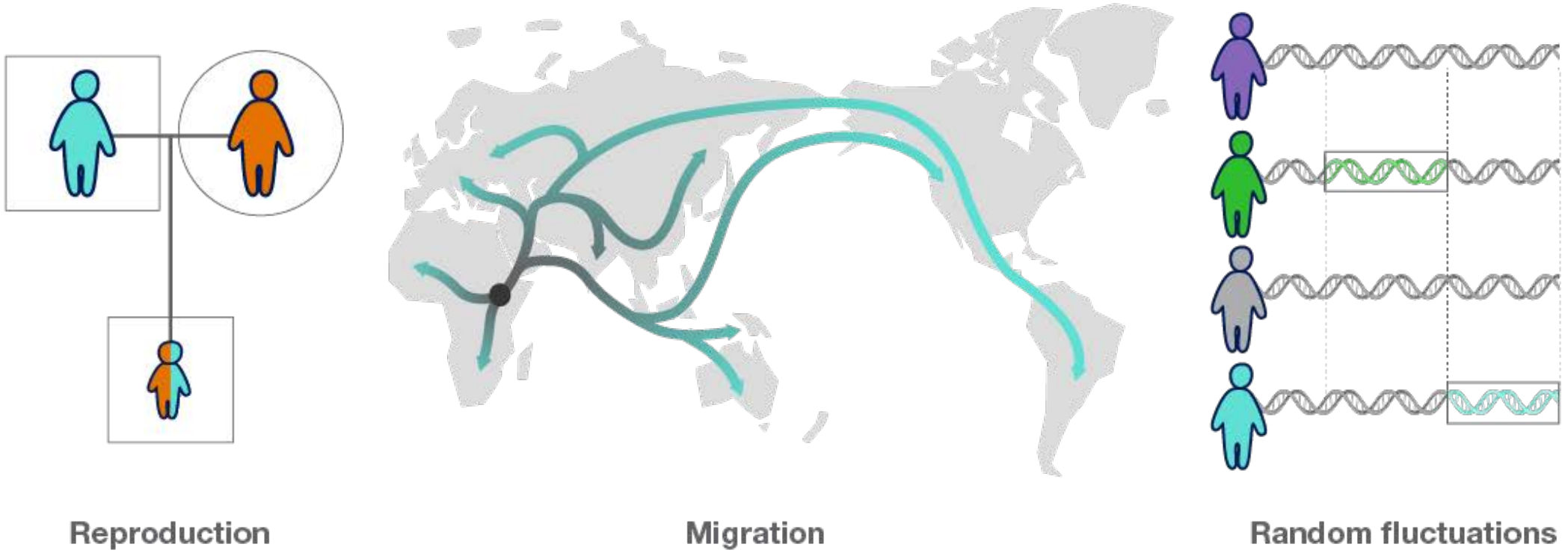

Figure 2: Courtesy of the National Human Genome Research Institute (NHGRI)

While some genomic variants are shared across populations many are not

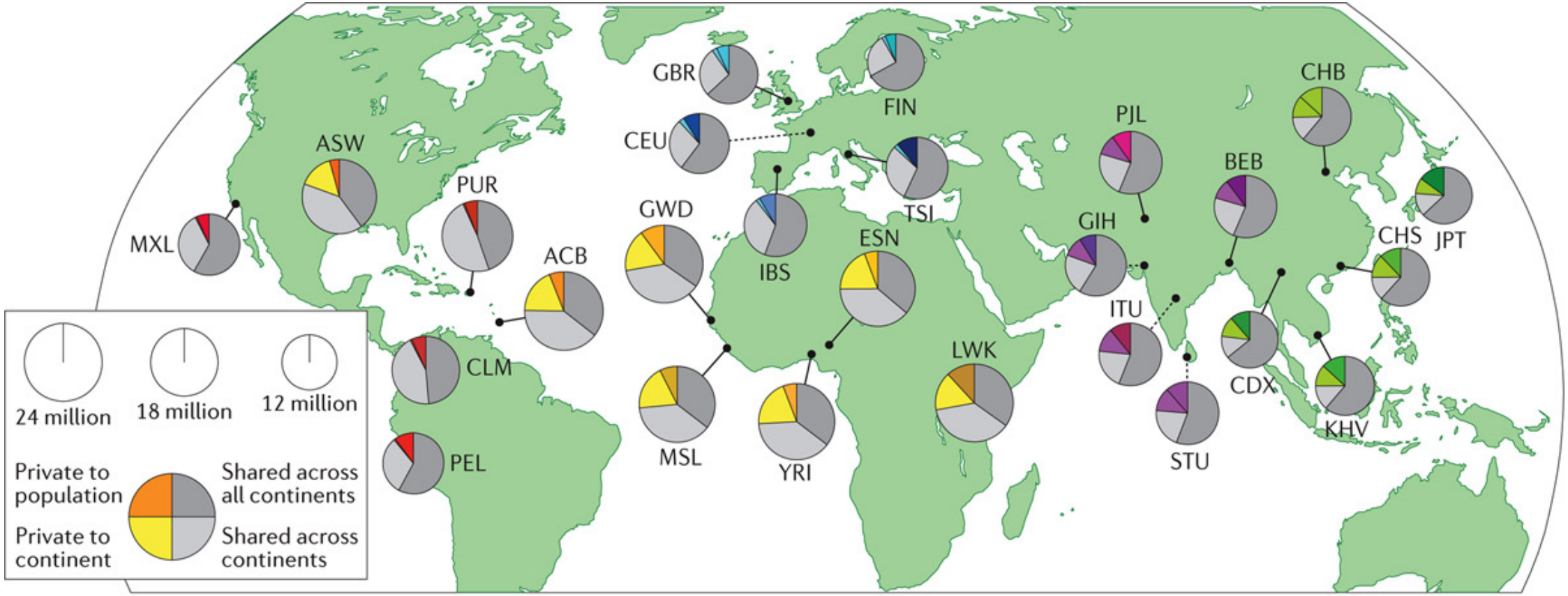

Figure 3: Hindorff, L., Bonham, V., Brody, L. et al. Prioritizing diversity in human genomics research. Nat Rev Genet 19, 175–185 (2018). https://doi.org/10.1038/nrg.2017.89

Available genomic data may not adequately represent the intent to treat population

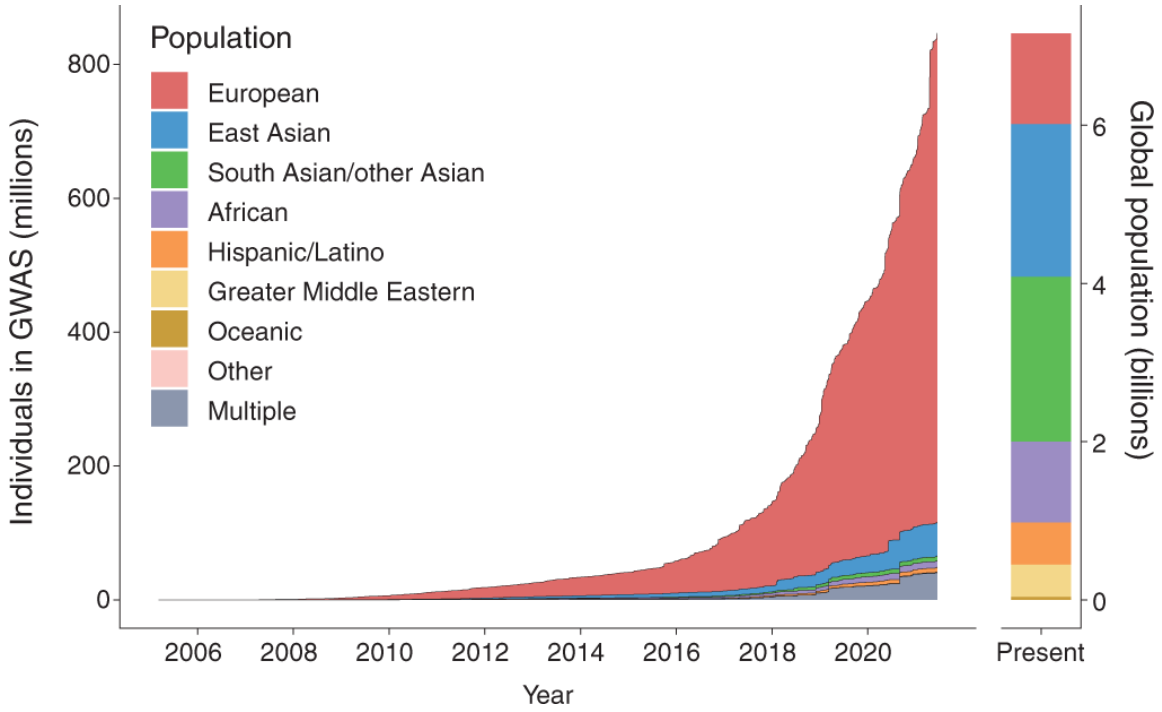

Figure 4: Fatumo, S., Chikowore, T., Choudhury, A. et al. A roadmap to increase diversity in genomic studies. Nat Med 28, 243–250 (2022). https://doi.org/10.1038/s41591-021-01672-4

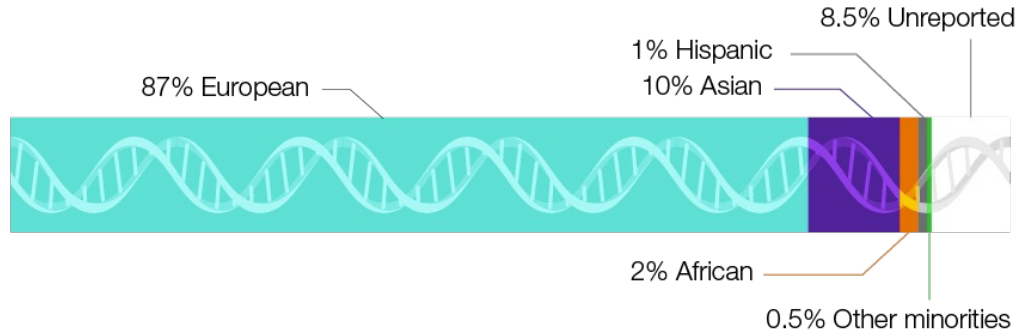

Figure 5: Courtesy of the National Human Genome Research Institute (NHGRI)

Consider the example of Sickle Cell Anemia mismatch of genomic data compared to the target population

Countries that regularly collect genomic data for their population

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Incidence of sickle cell anemia births

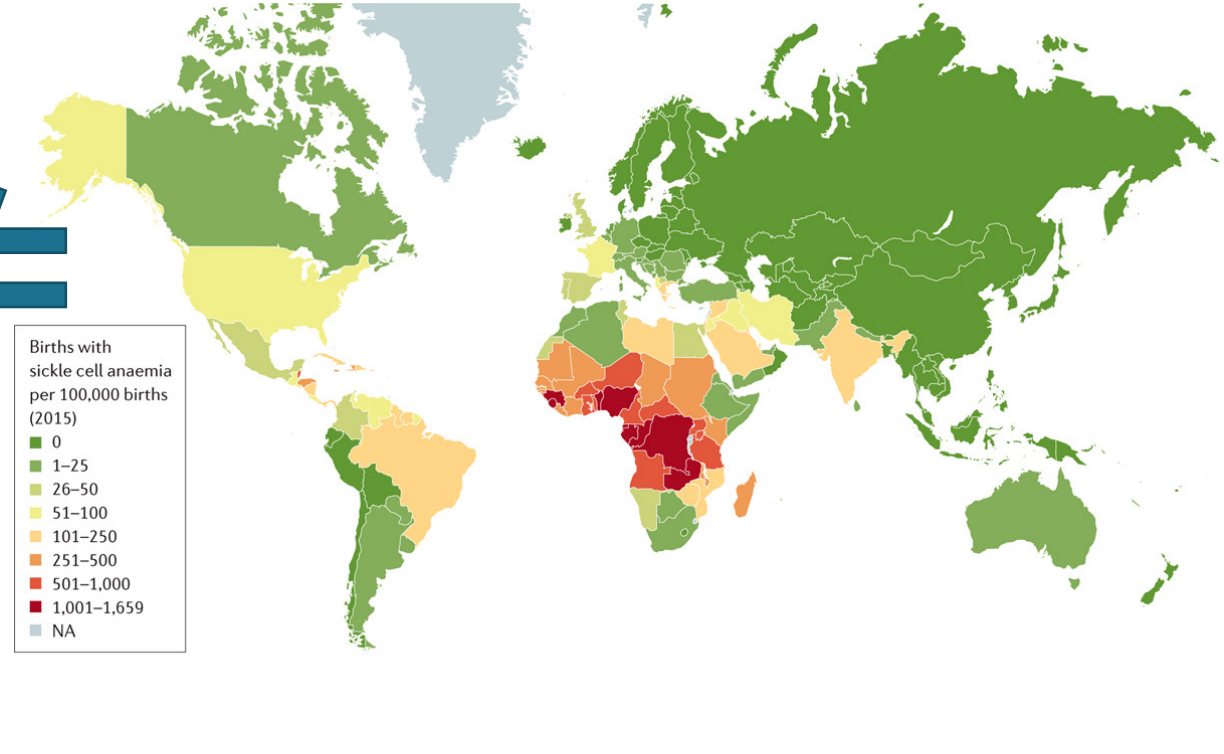


Figure 6: Kovanda, A., Zimani, A.N. & Peterlin, B. How to design a national genomic project—a systematic review of active projects. Hum Genomics 15, 20 (2021). https://doi.org/10.1186/s40246-021-00315-6

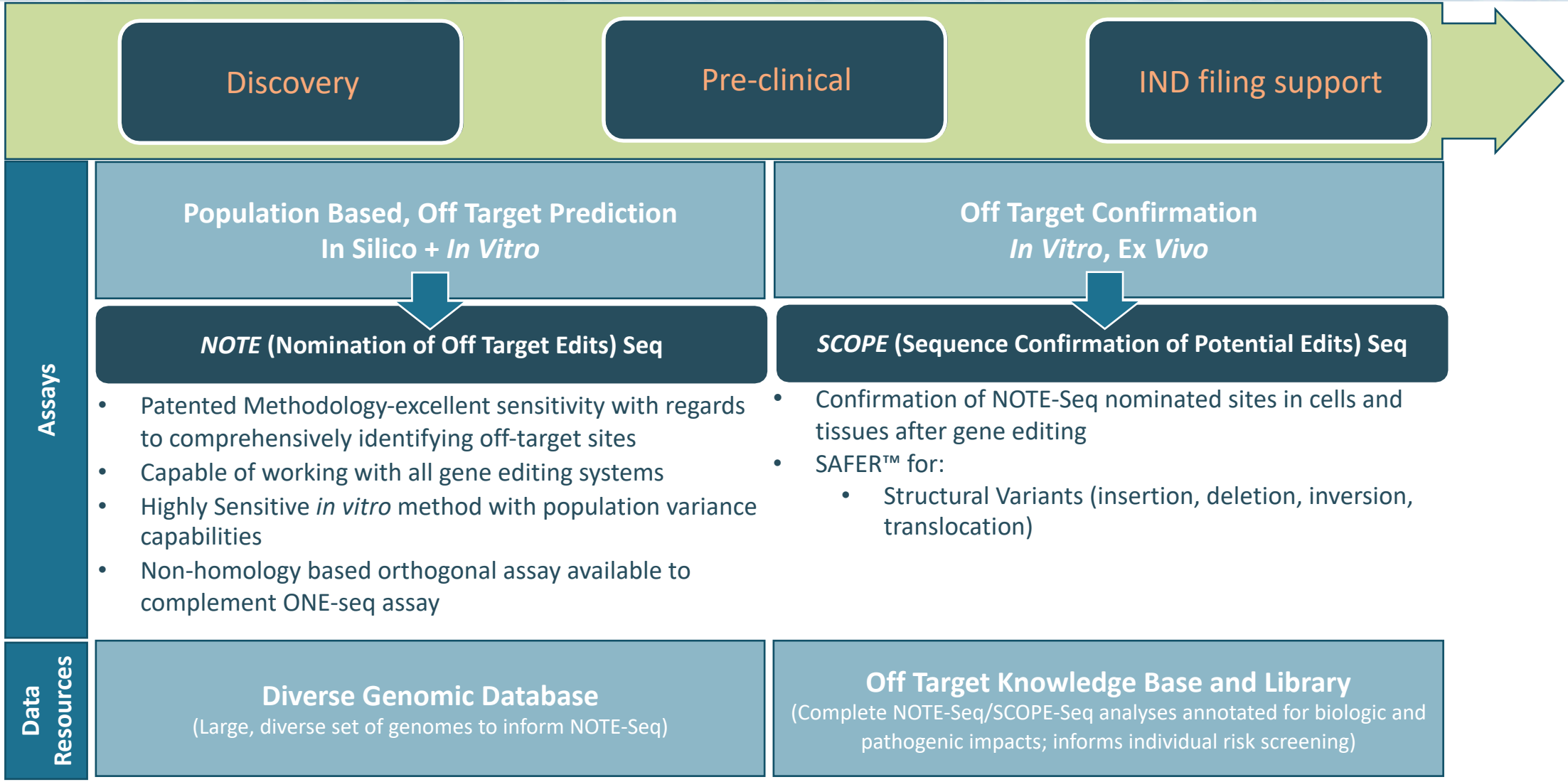
Figure 7: Kato, G. J. et al. (2018) Sickle cell disease Nat. Rev. Dis. Primers doi:10.1038/nrdp.2018.10

Highlights two simultaneous problems that should be addressed prior to a clinical trial

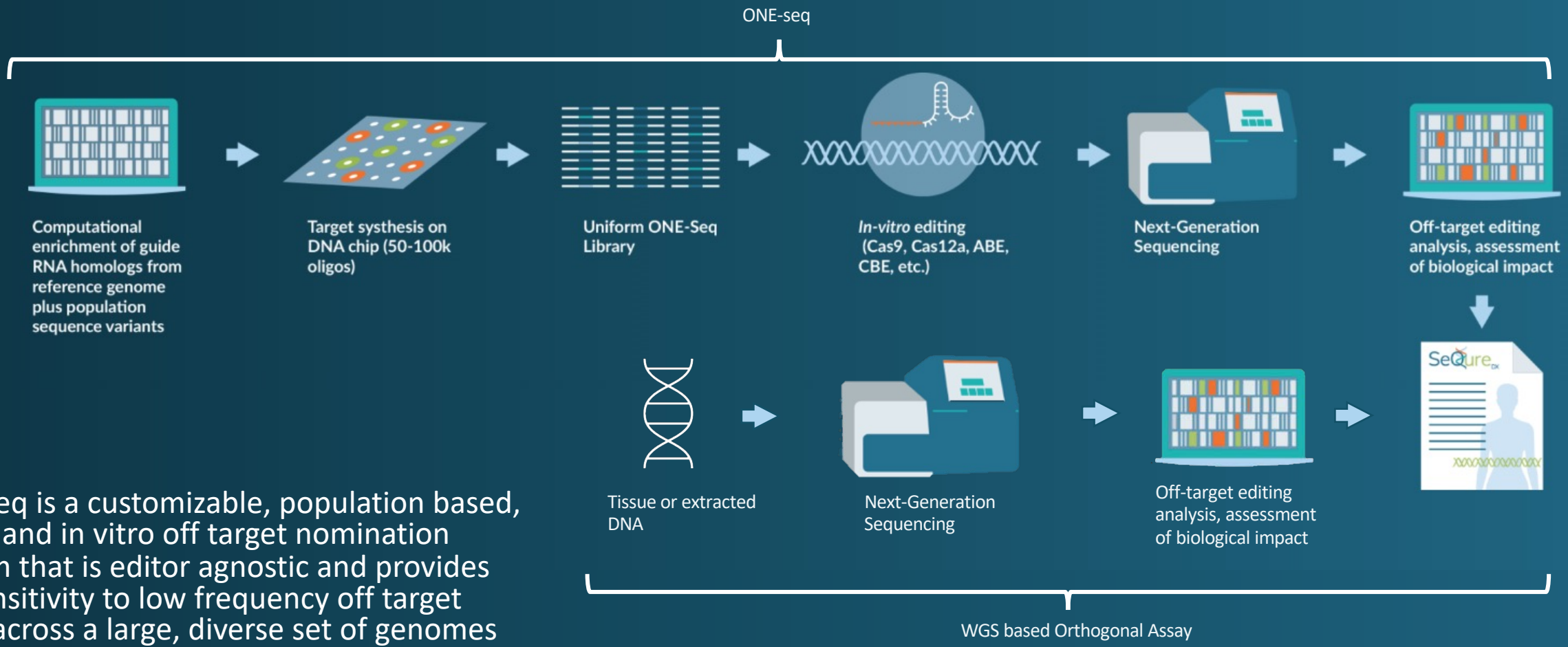
1. How to incorporate genomic diversity in pre-clinical safety evaluation of off-target editing risks

2. How to ensure the profile of genomic data mirrors the intent to treat population for the therapy

An approach to address both genomic diversity problems for off-target risk assessments

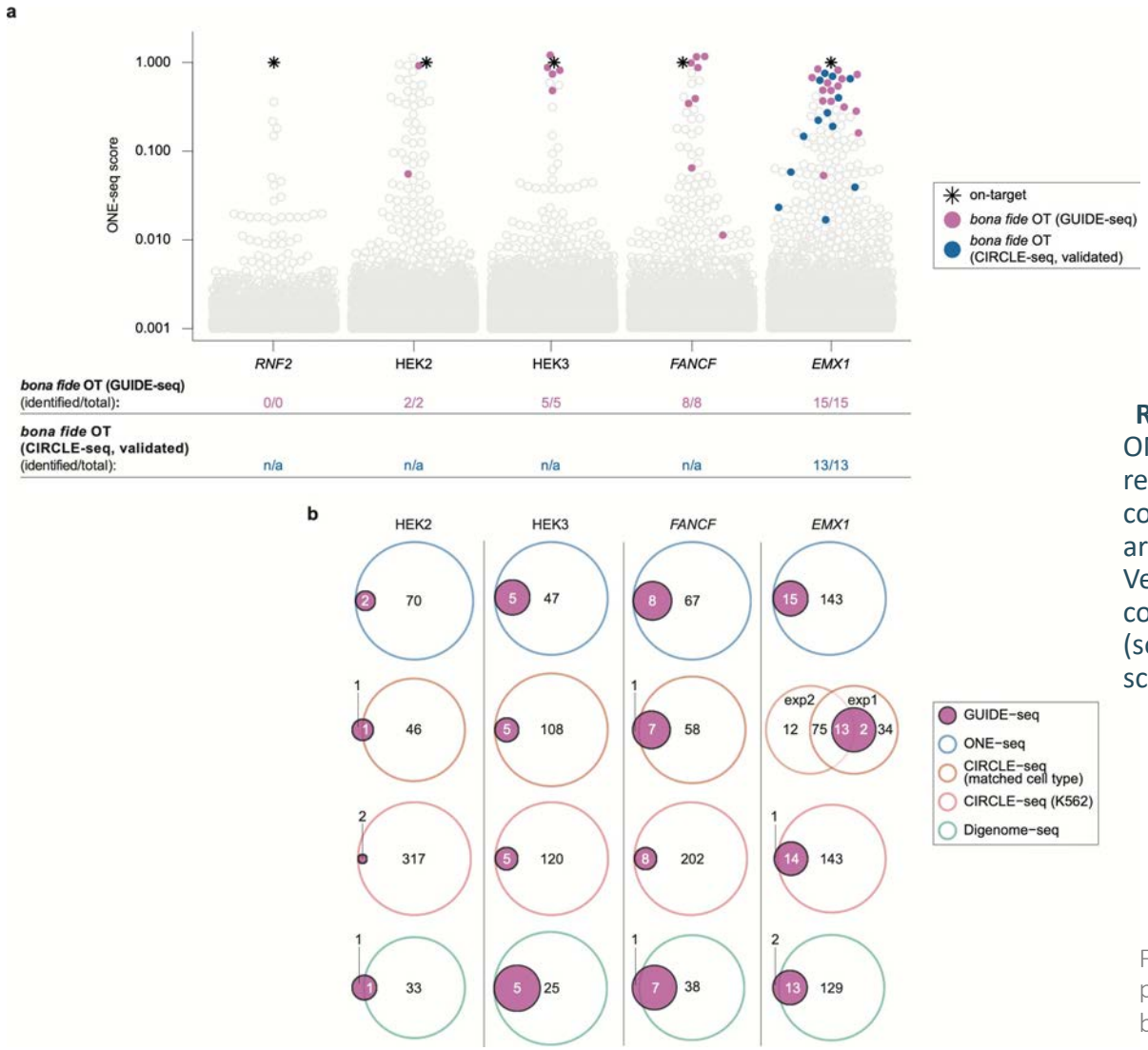


NOTE-Seq uses multiple methods to comprehensively assess off-target risks



NOTE-Seq is a customizable, population based, in silico and in vitro off target nomination platform that is editor agnostic and provides high sensitivity to low frequency off target events across a large, diverse set of genomes

NOTE-Seq (ONE-seq) population informed off-target analysis with SpCas9 nucleases versus single genome assays



Results of ONE-seq off-target analysis with SpCas9 nucleases a, Swarm plots showing ONE-seq nuclease scores for five previously analyzed SpCas9 gRNAs. Each circle represents an individual ONE-seq library member. Colored circles represent previously confirmed *bona fide* off-target sites. Sites with ONE-seq nuclease scores below 0.001 are not shown. n/a, no validation performed in previously published CIRCLE-seq study. **b,** Venn diagrams comparing abilities of ONE-seq, CIRCLE-seq, and Digenome-seq (open colored circles) to nominate *bona fide* off-target sites previously validated by GUIDE-seq (solid purple circles). All sites considered as validated by ONE-seq had ONE-seq nuclease scores >0.01.

Figure 8: Petri K., Kim D., et. al. Global-scale CRISPR gene editor specificity profiling by ONE-seq identifies population-specific, variant off-target effects. bioRxiv 2021.04.05.438458; doi: https://doi.org/10.1101/2021.04.05.438458

Questions

Send Additional Questions to:

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