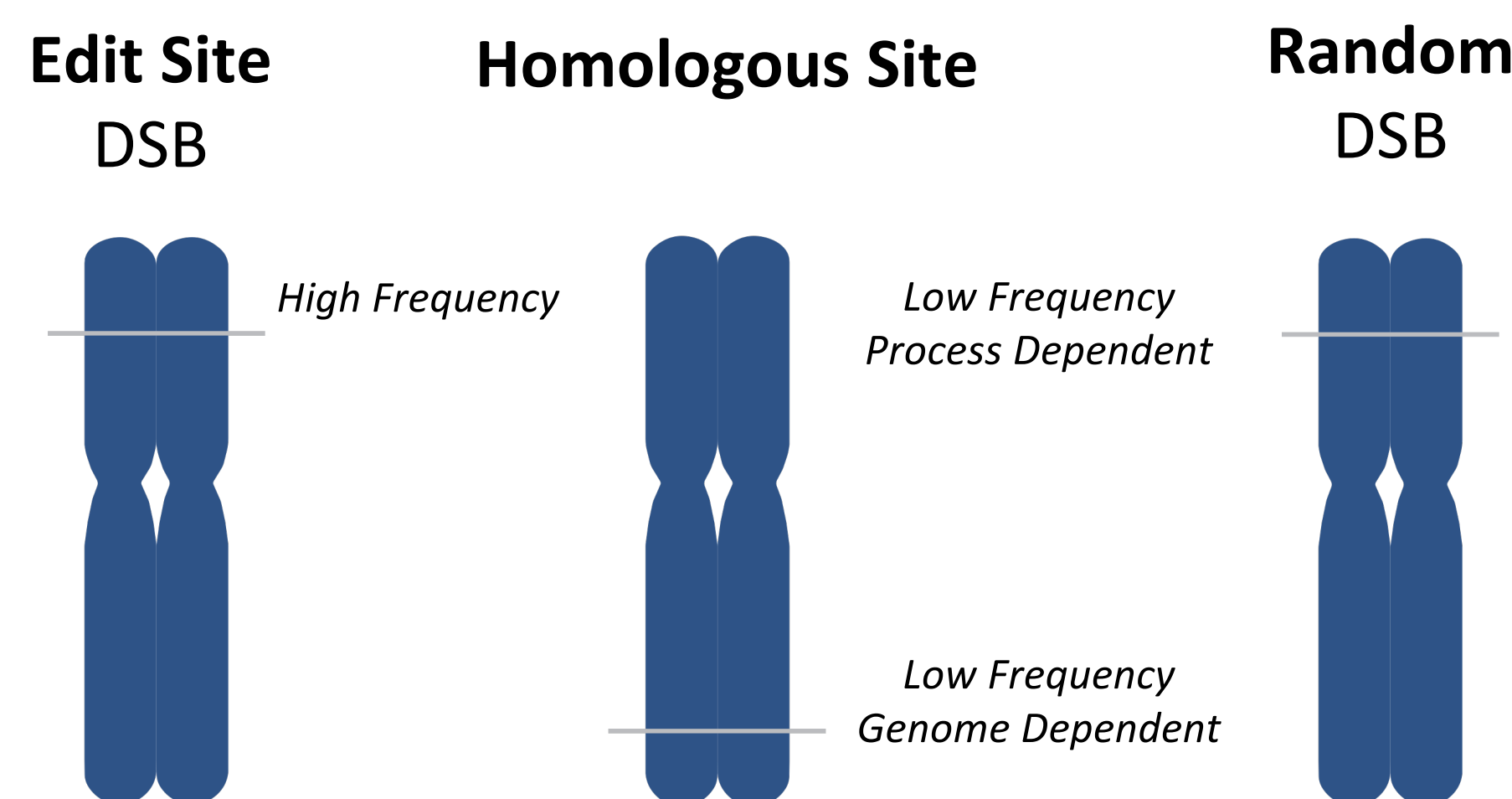# Cytogenetics-based Tools for Process Characterization, Optimization and Control of CRISPR/Cas9 (or any other) Gene Editing

Erin Robinson[1], Molishree Joshi[2], Stephen Hughes[1], Gretchen Pratt[1], David Sebesta[1], Christopher Tompkins[1]

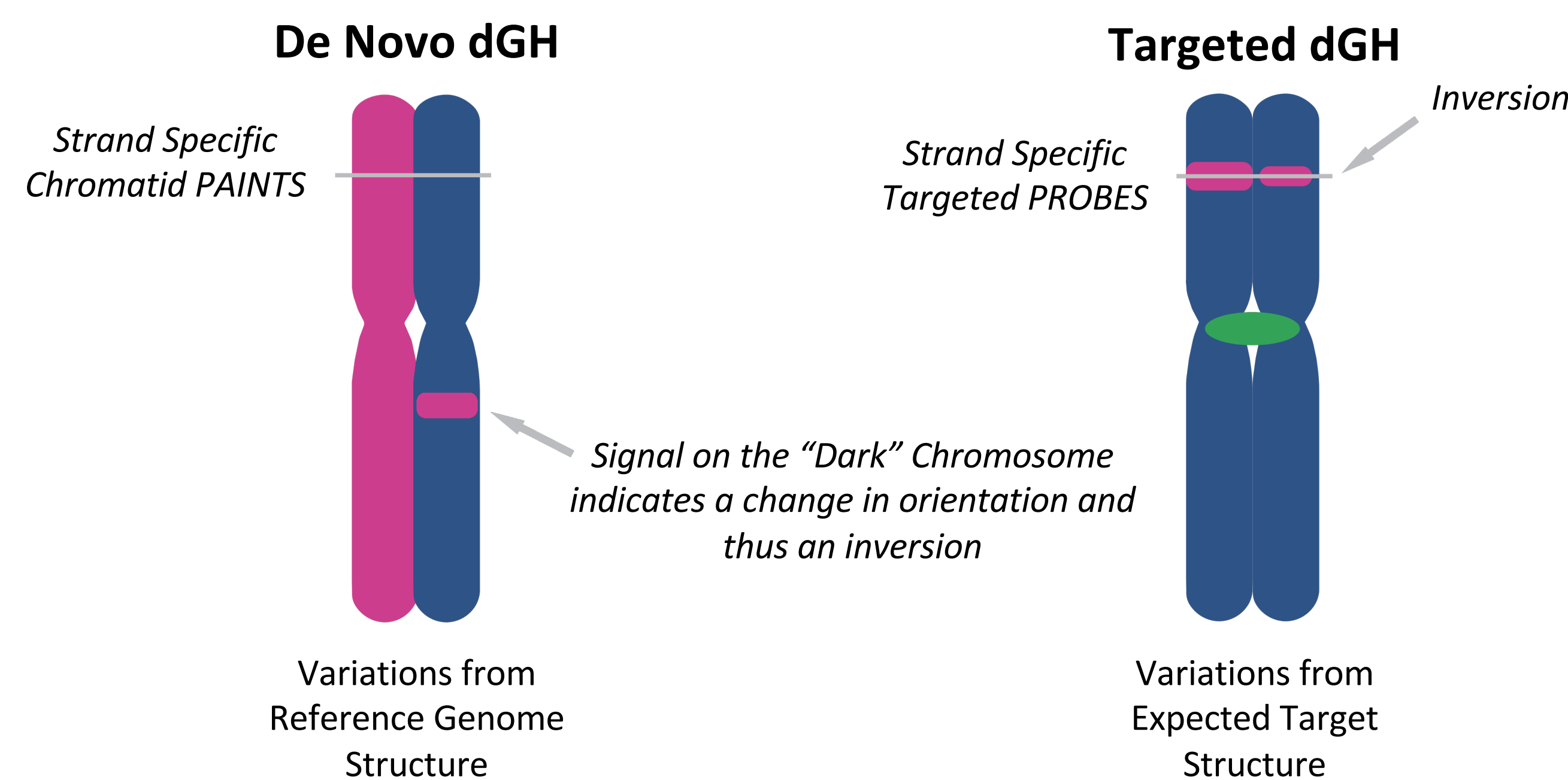1. KromaTiD, Inc. 2. University of Colorado, Functional Genomics Facility

dGH for Gene Editing

Structural variation resulting from DNA mis-repair occurs regardless of the editing method used. Directional Genomic Hybridization or dGH can discover and track pre-existing structural variation, as well as structural genomic changes resulting from faulty repair of double strand breaks (DSB) at the edit site, between the edit site and other locations, and variation at random locations in the genome.

Directional Genomic Hybridization (dGH), developed originally as a sensitive biodosimeter, is uniquely suited to characterize the complex variation associated with editing.
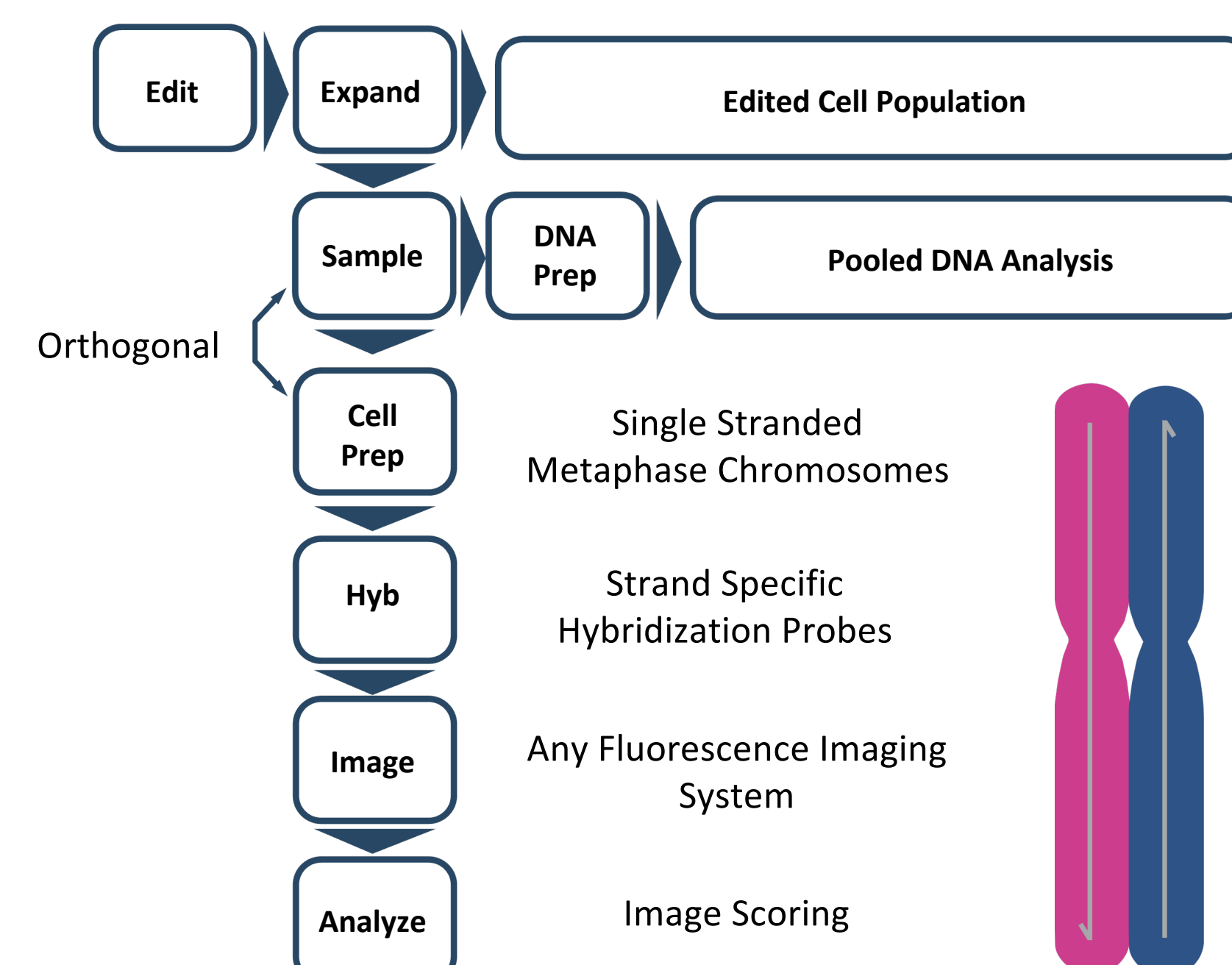
Edit Site DSB — *High Frequency*

Homologous Site — *Low Frequency Process Dependent* / *Low Frequency Genome Dependent*

Random DSB

Single Cell Analysis Whole Genome

De Novo dGH
Strand Specific Chromatid PAINTS
Variations from Reference Genome Structure

Targeted dGH
Strand Specific Targeted PROBES
Inversion
Signal on the "Dark" Chromosome indicates a change in orientation and thus an inversion
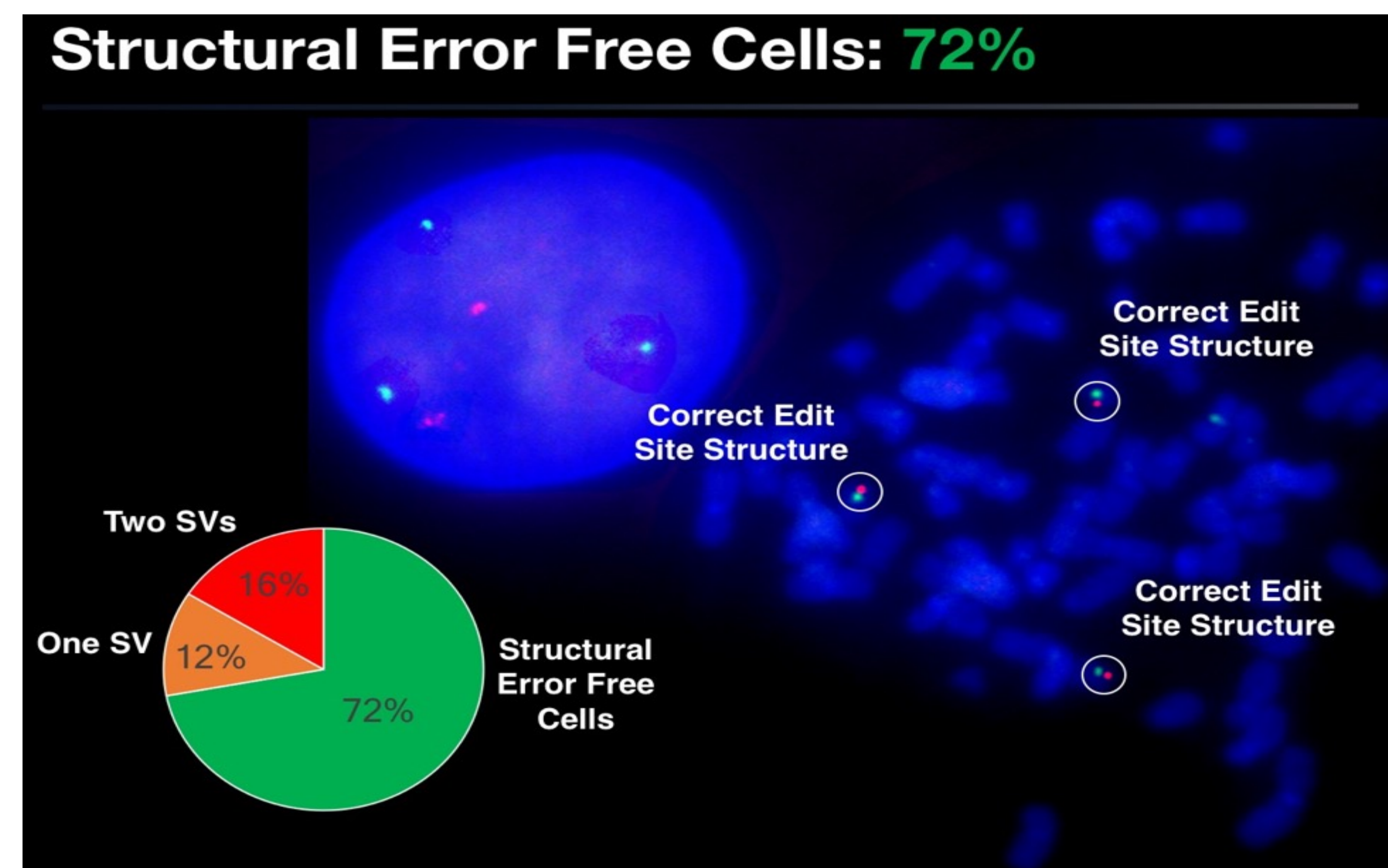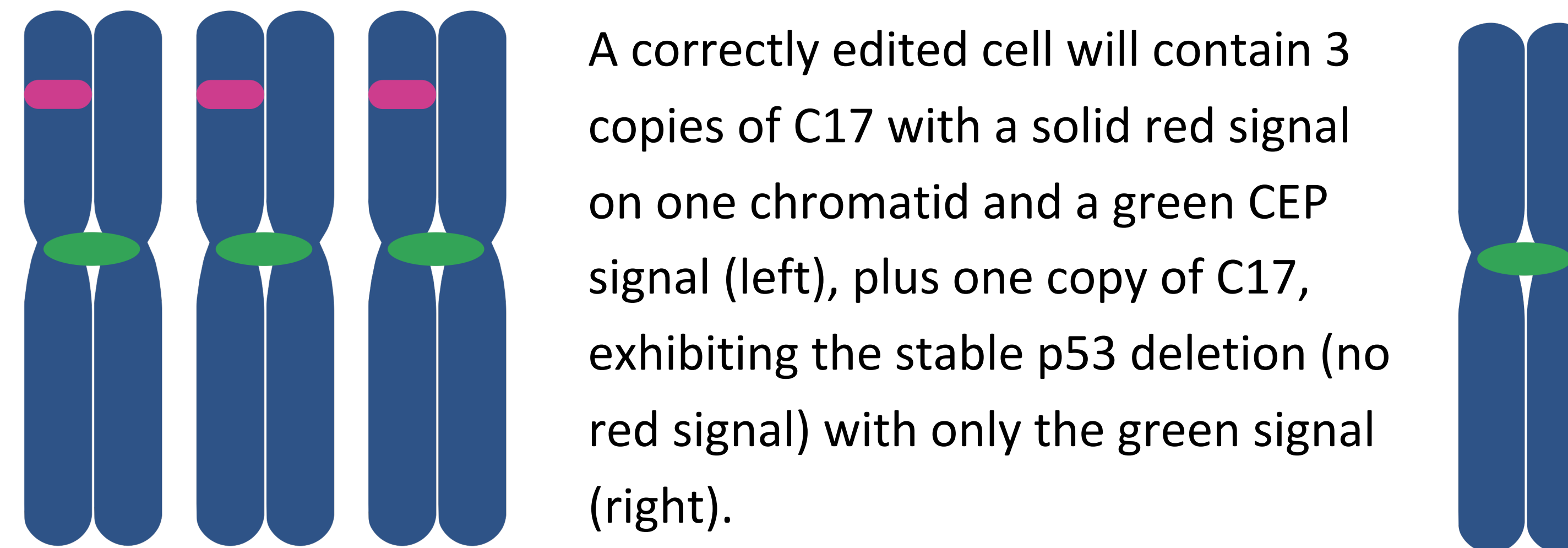Variations from Expected Target Structure

dGH Integrates with Gene Editing Workflows:

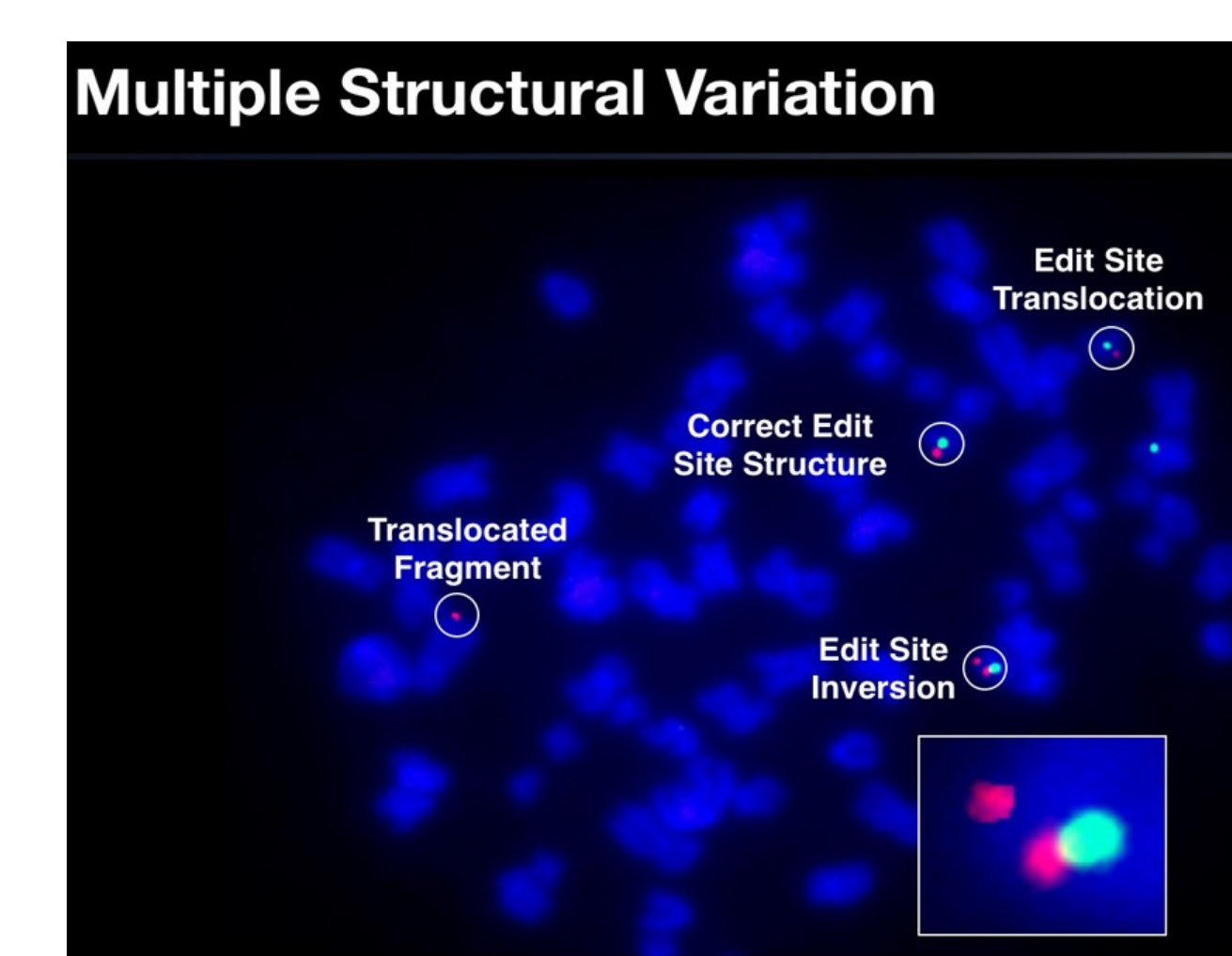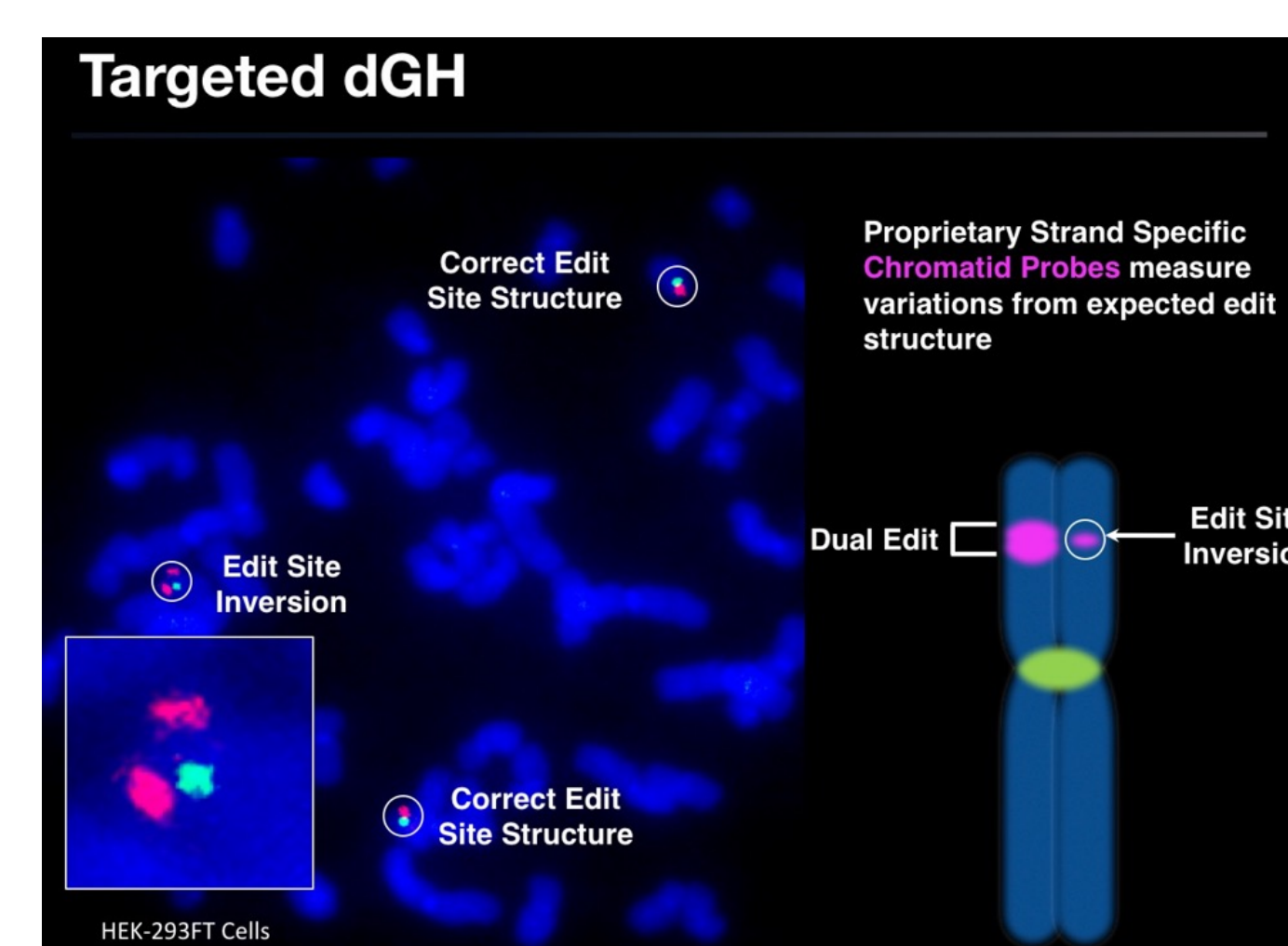- dGH is Orthogonal to Sequencing and other techniques relying on pooled DNA
- dGH can measure baseline rearrangement in subjects and cells

Edit → Expand → Edited Cell Population
Sample → DNA Prep → Pooled DNA Analysis
Orthogonal
Cell Prep → Single Stranded Metaphase Chromosomes
Hyb → Strand Specific Hybridization Probes
Image → Any Fluorescence Imaging System
Analyze → Image Scoring

Structural Variants

A correctly edited cell will contain 3 copies of C17 with a solid red signal on one chromatid and a green CEP signal (left), plus one copy of C17, exhibiting the stable p53 deletion (no red signal) with only the green signal (right).

Structural Error Free Cells: 72%

Correct Edit Site Structure

Two SVs 16%
One SV 12%
72% Structural Error Free Cells

HEK293FT cells were transfected with CRISPR-Cas9 and guide RNAs for two different sites in the p53 gene, then edited cells were enriched via flow cytometry. HEK293FT has four copies of C17—one with a stable p53 deletion which is not edited—leading to a minimum of three double-strand breaks that must be repaired correctly to generate an error free edited cell (above).
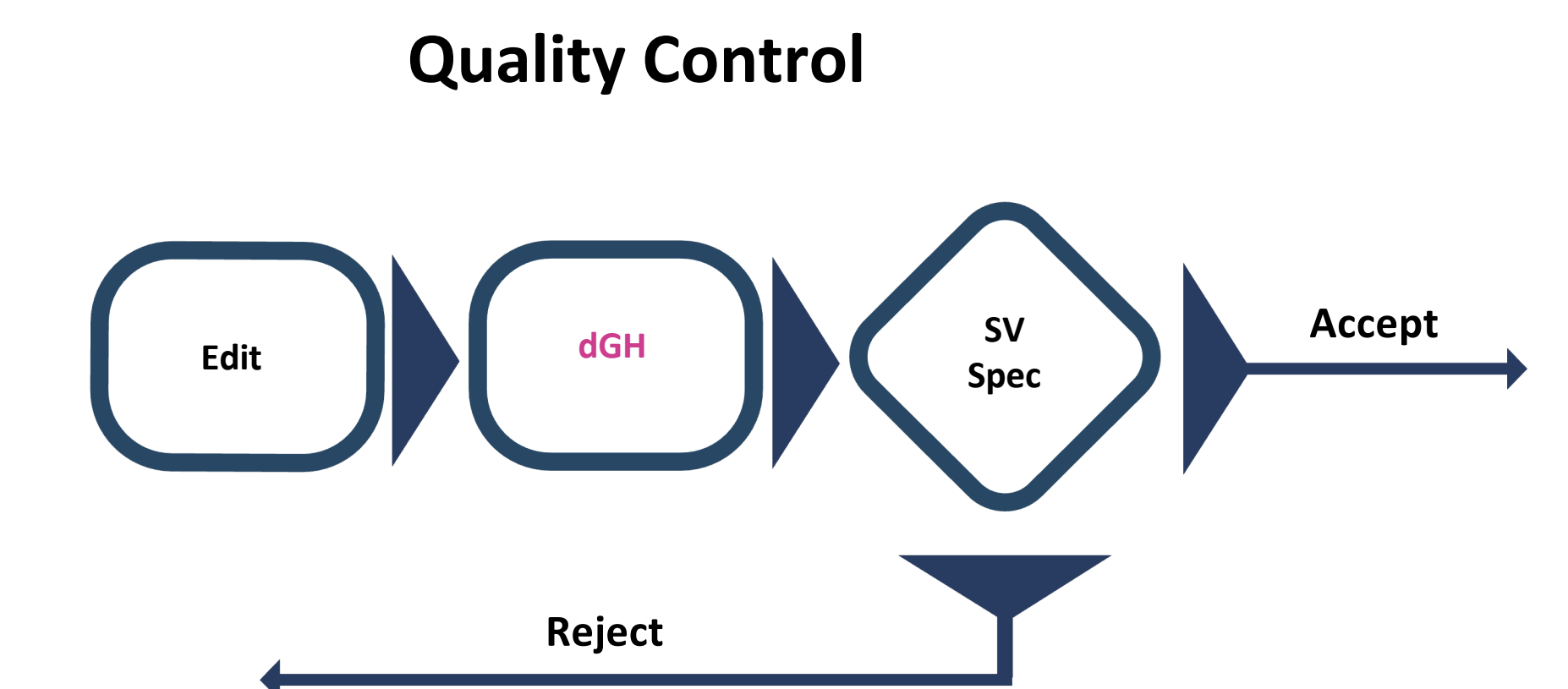
Targeted dGH
Correct Edit Site Structure
Proprietary Strand Specific Chromatid Probes measure variations from expected edit structure
Edit Site Inversion
Dual Edit — Edit Site Inversion
Correct Edit Site Structure
HEK-293FT Cells

Multiple Structural Variation
Edit Site Translocation
Correct Edit Site Structure
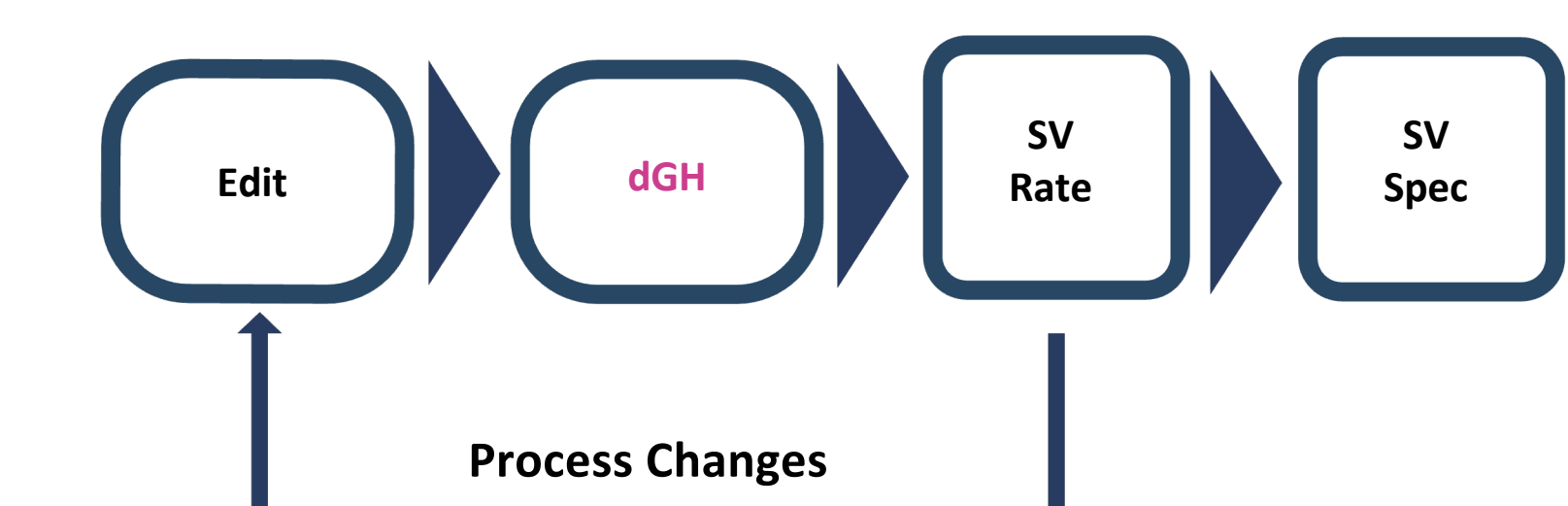Translocated Fragment
Edit Site Inversion

Optimization and Control

dGH analysis provides useful aggregate parameters to qualitatively and quantitatively characterize edited cell populations.

Quality Control
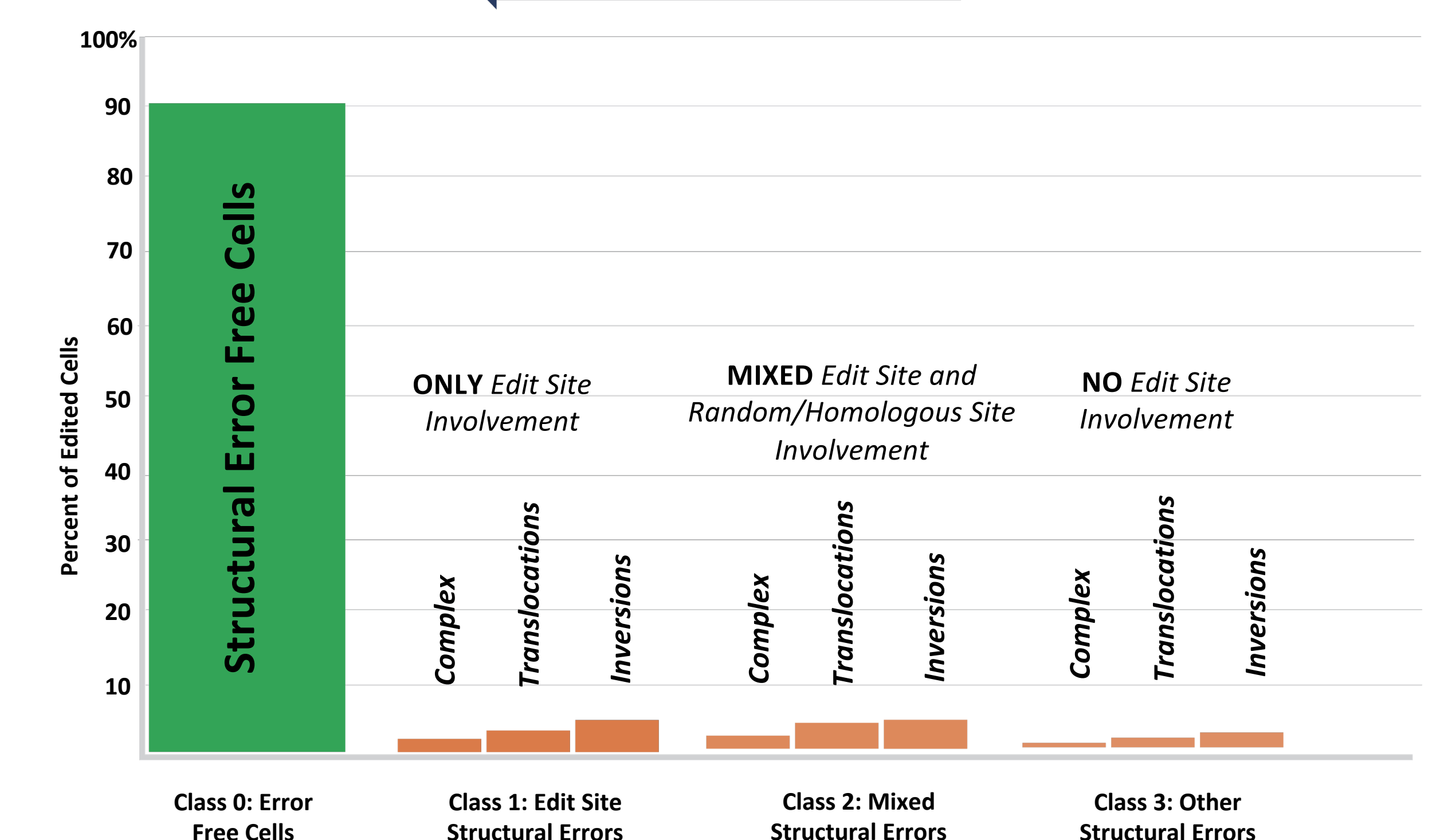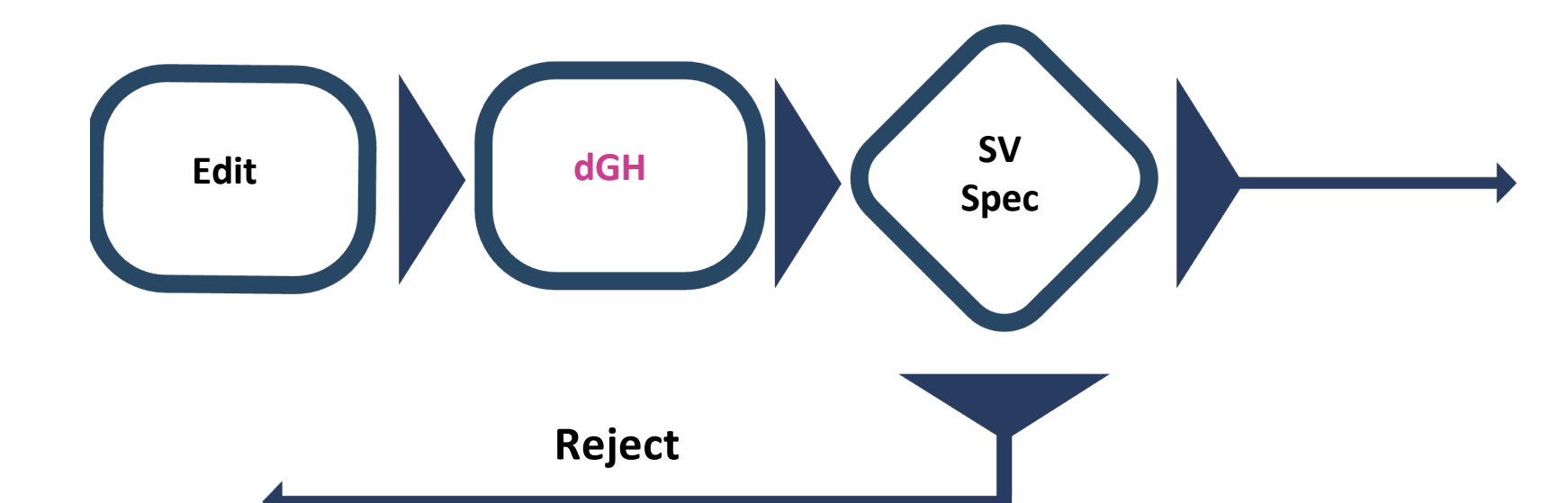
Edit → dGH → SV Spec → Accept
Reject

dGH can be used as a functional test for raw material quality, as a method to optimize gene editing process variables and is a necessary final quality control measurement for *ex vivo* edited cells

Optimization

Edit → dGH → SV Rate → SV Spec
Process Changes

Material Qualification

Edit → dGH → SV Spec
Reject

Percent of Edited Cells

Structural Error Free Cells

ONLY *Edit Site Involvement*
Complex / Translocations / Inversions

MIXED *Edit Site and Random/Homologous Site Involvement*
Complex / Translocations / Inversions

NO *Edit Site Involvement*
Complex / Translocations / Inversions

Class 0: Error Free Cells
Class 1: Edit Site Structural Errors
Class 2: Mixed Structural Errors
Class 3: Other Structural Errors

*Possible edit structures depend on number and location of edits

Cytogenetics-based Tools for Process Characterization, Optimization and Control of CRISPR/Cas9 (or any other) Gene Editing
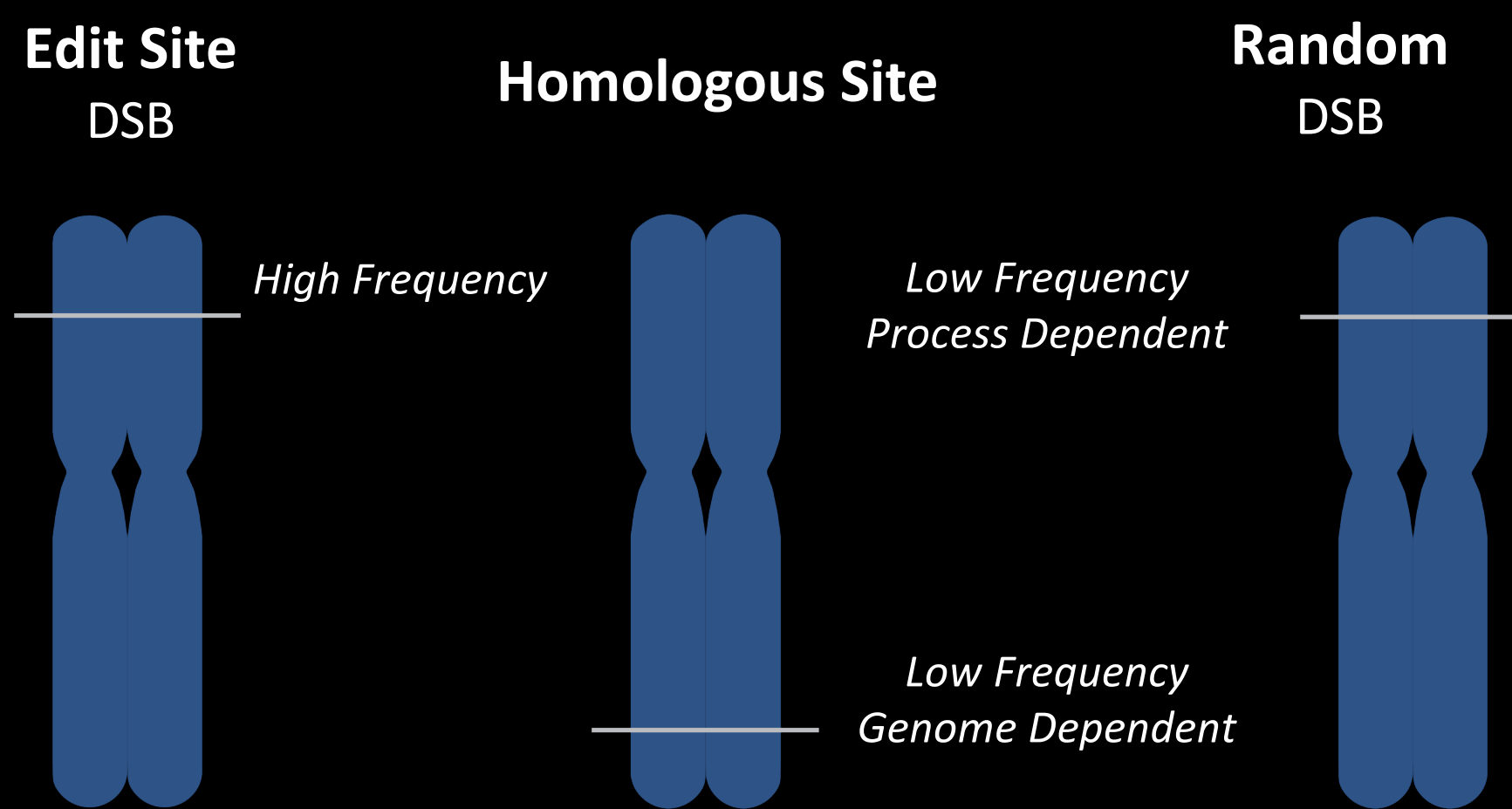
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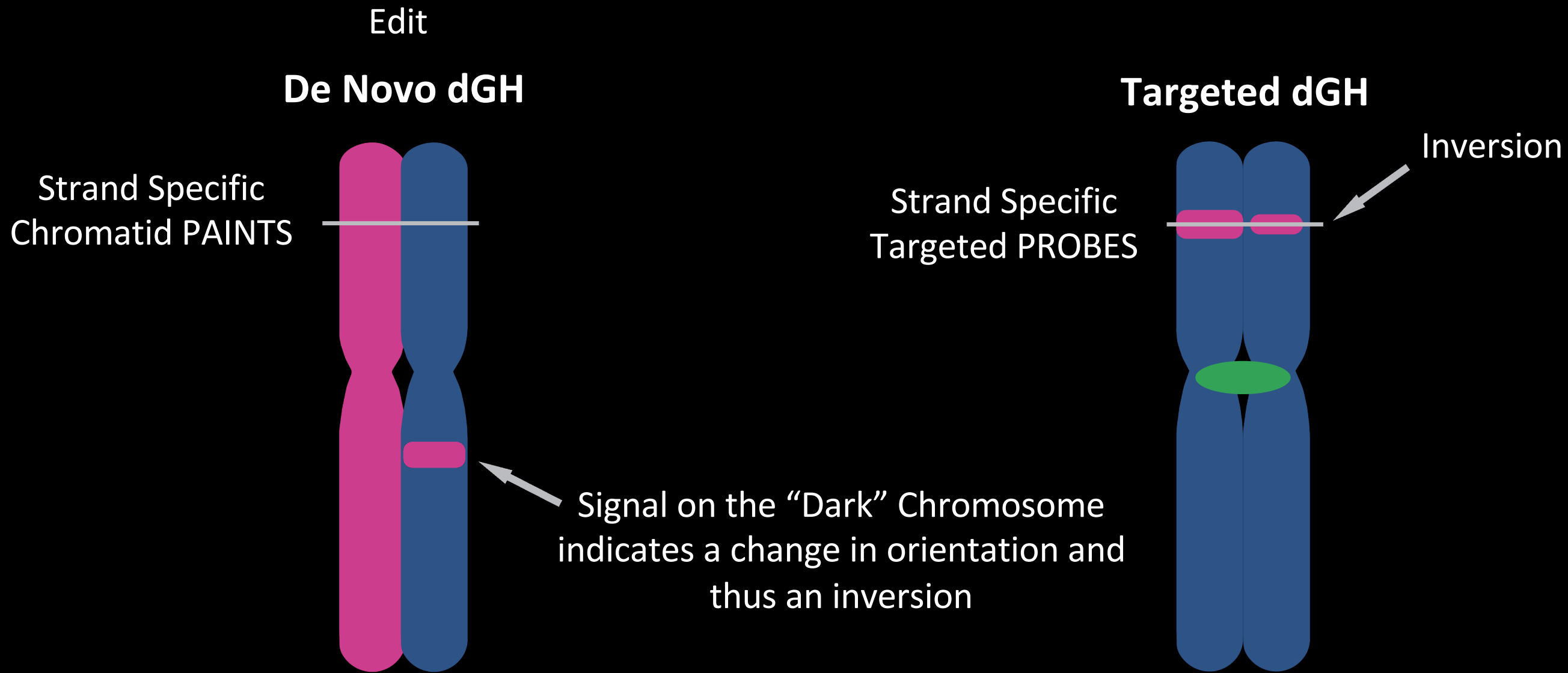
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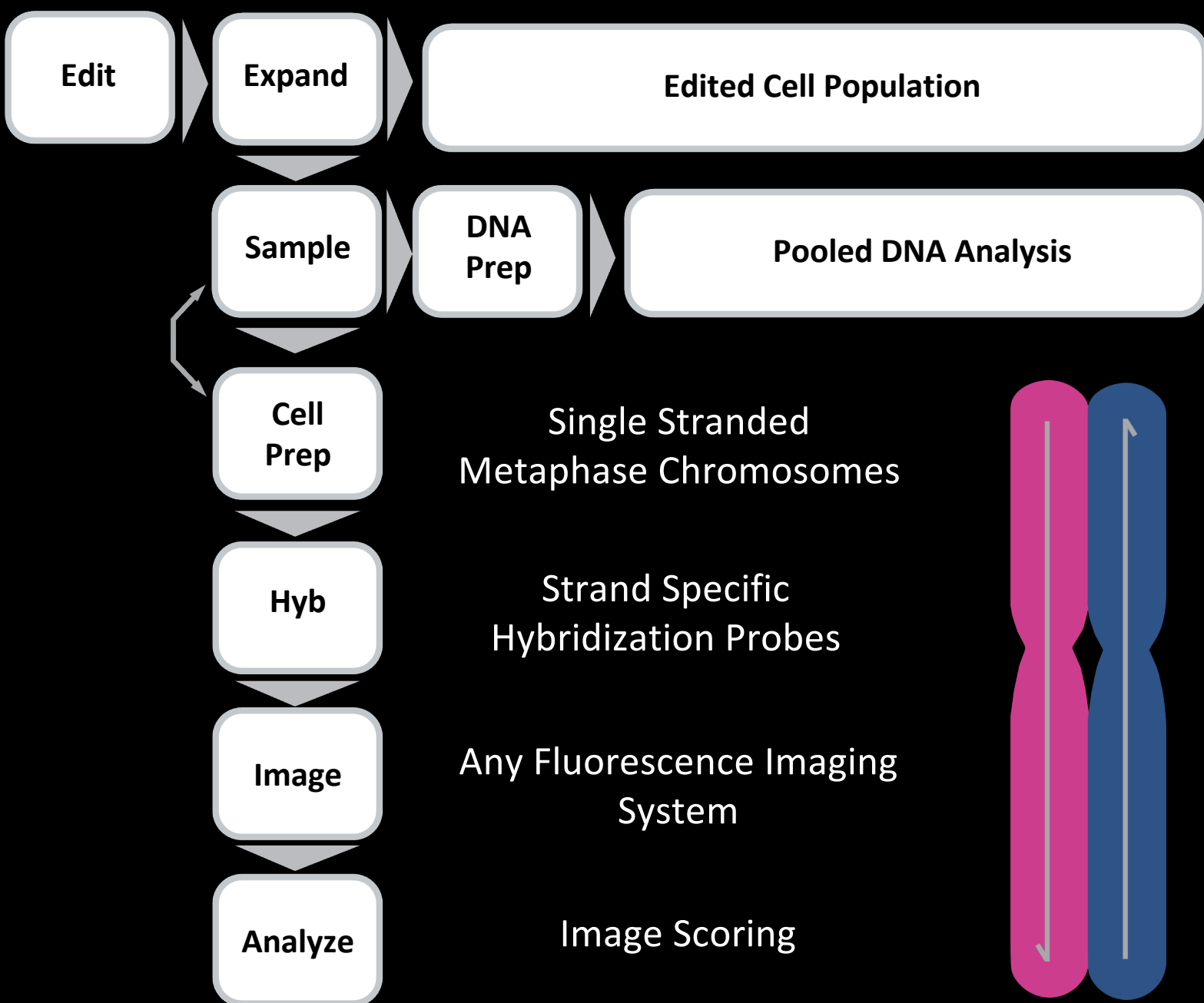
Edit Site
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High Frequency

Low Frequency
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Single Cell Analysis Whole Genome

Edit
De Novo dGH

Targeted dGH

Strand Specific
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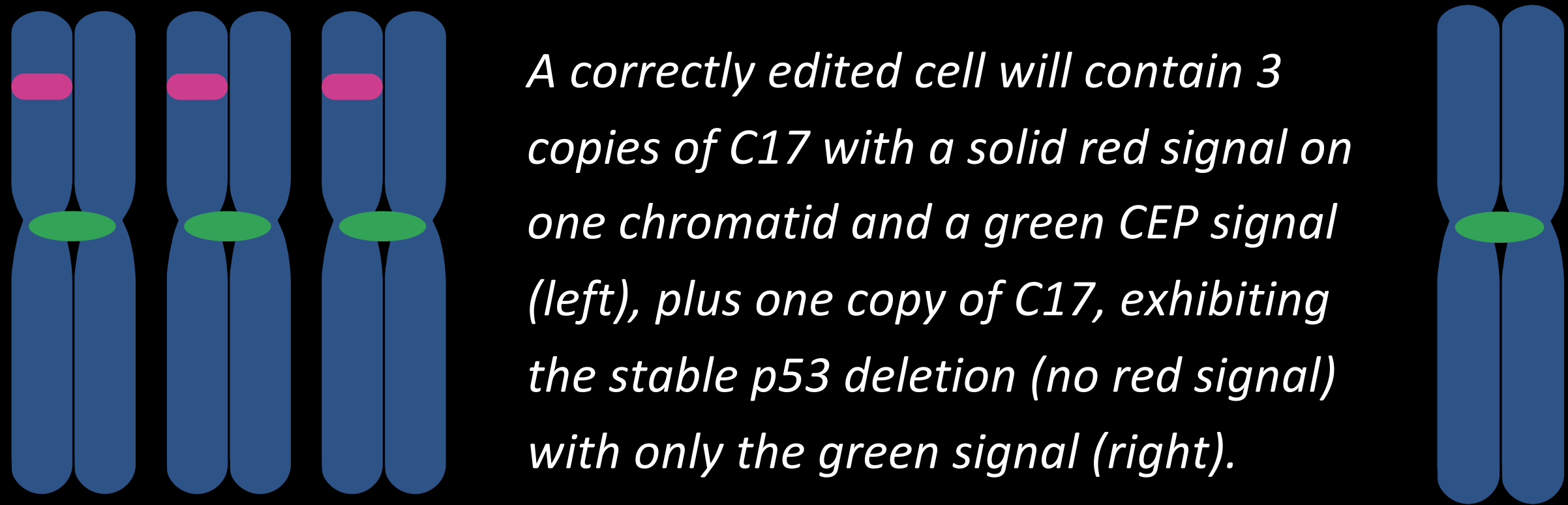
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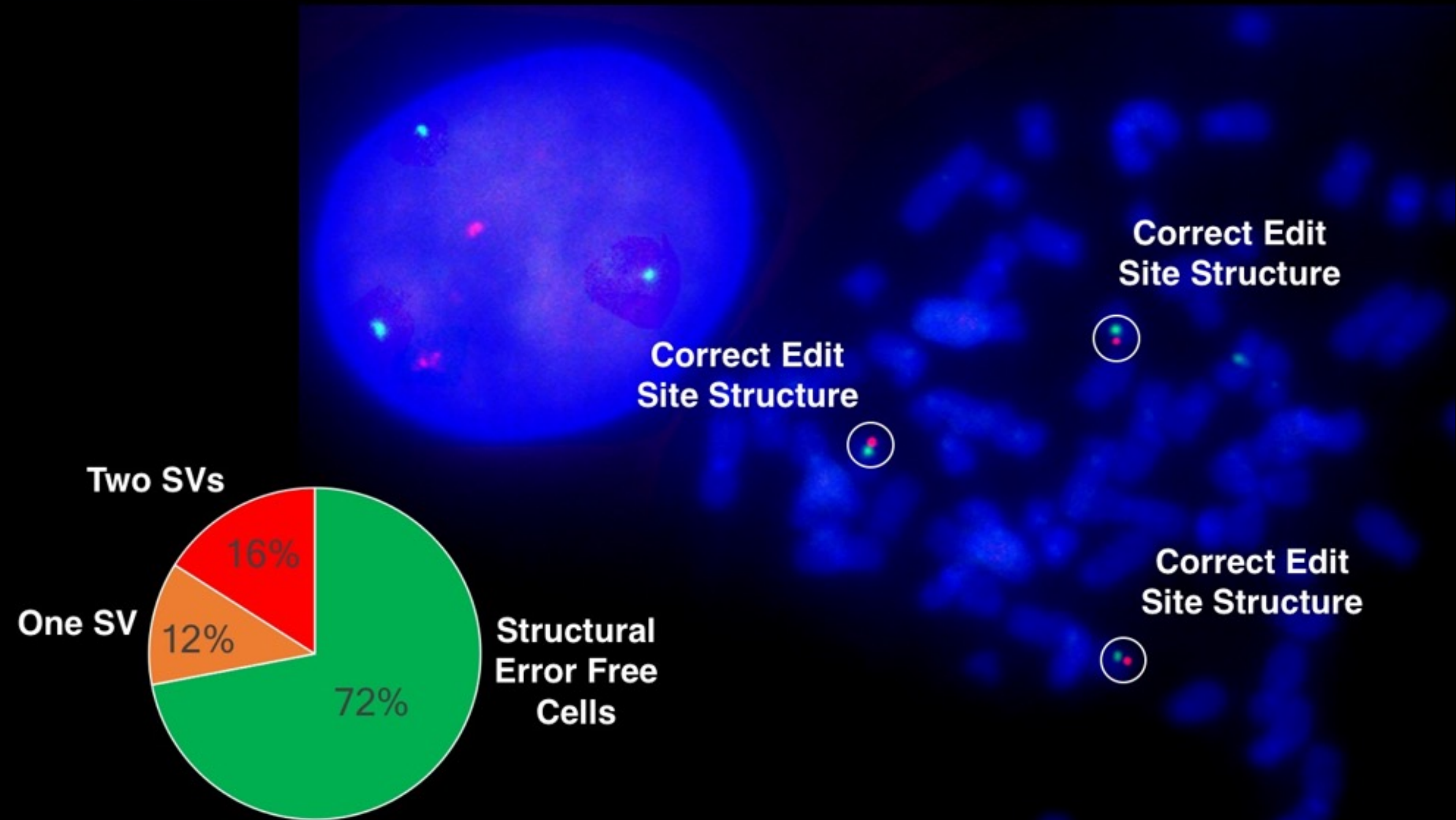- dGH can measure baseline rearrangement in subjects and cells

Edit → Expand → Edited Cell Population

Sample → DNA Prep → Pooled DNA Analysis

Cell Prep

Hyb — Single Stranded Metaphase Chromosomes

— Strand Specific Hybridization Probes

Image — Any Fluorescence Imaging System

Analyze — Image Scoring

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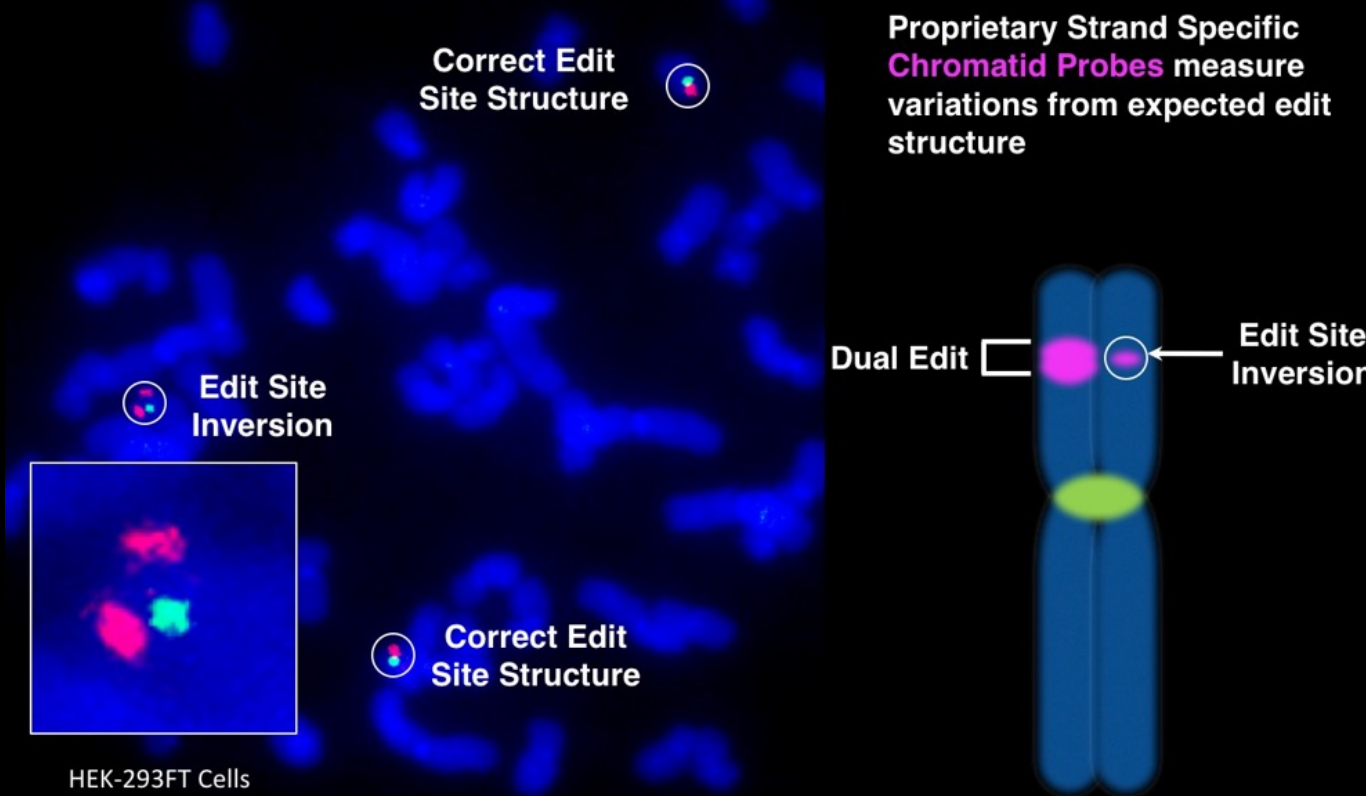
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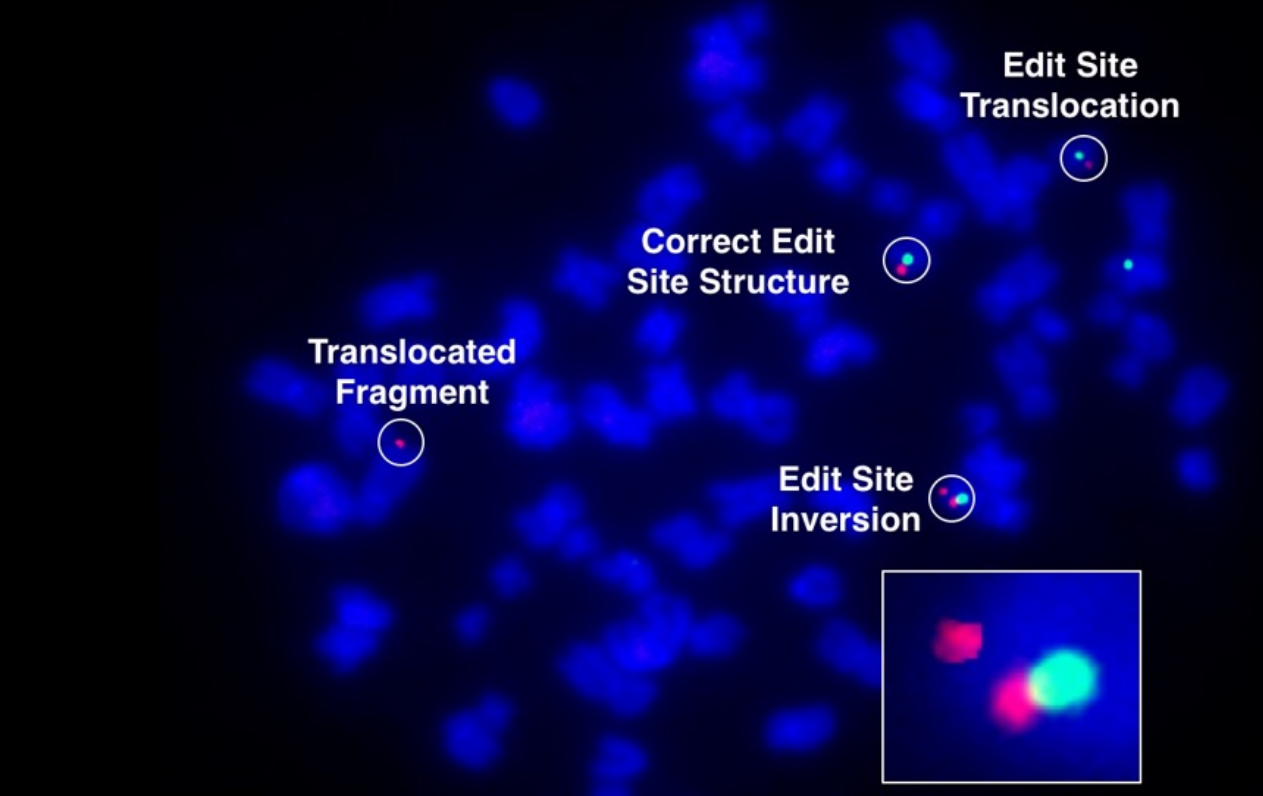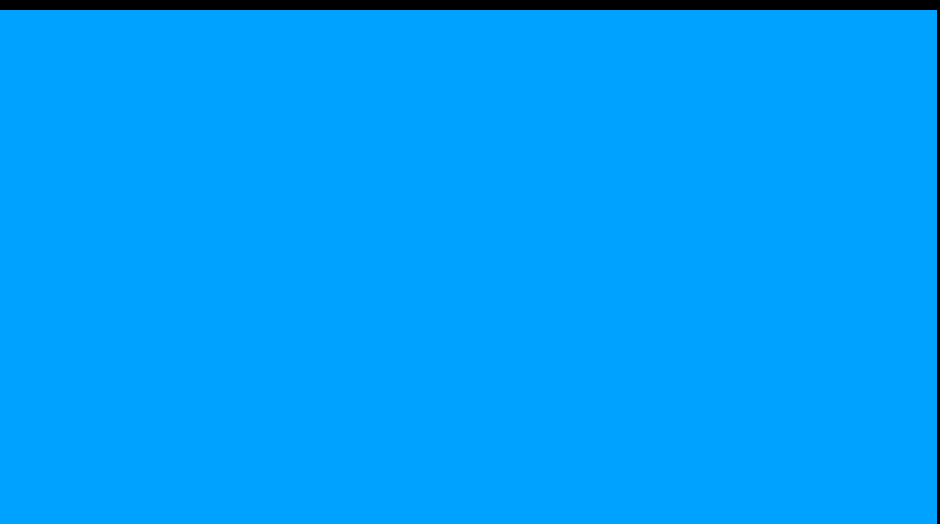
Structural Error Free Cells: 72%

Correct Edit Site Structure

Correct Edit Site Structure

Correct Edit Site Structure

Correct Edit Site Structure

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One SV 12%
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Targeted dGH

Correct Edit Site Structure

Proprietary Strand Specific Chromatid Probes measure variations from expected edit structure

Edit Site Inversion

Dual Edit — Edit Site Inversion

Correct Edit Site Structure

HEK-293FT Cells

Multiple Structural Variation

Edit Site Translocation

Correct Edit Site Structure
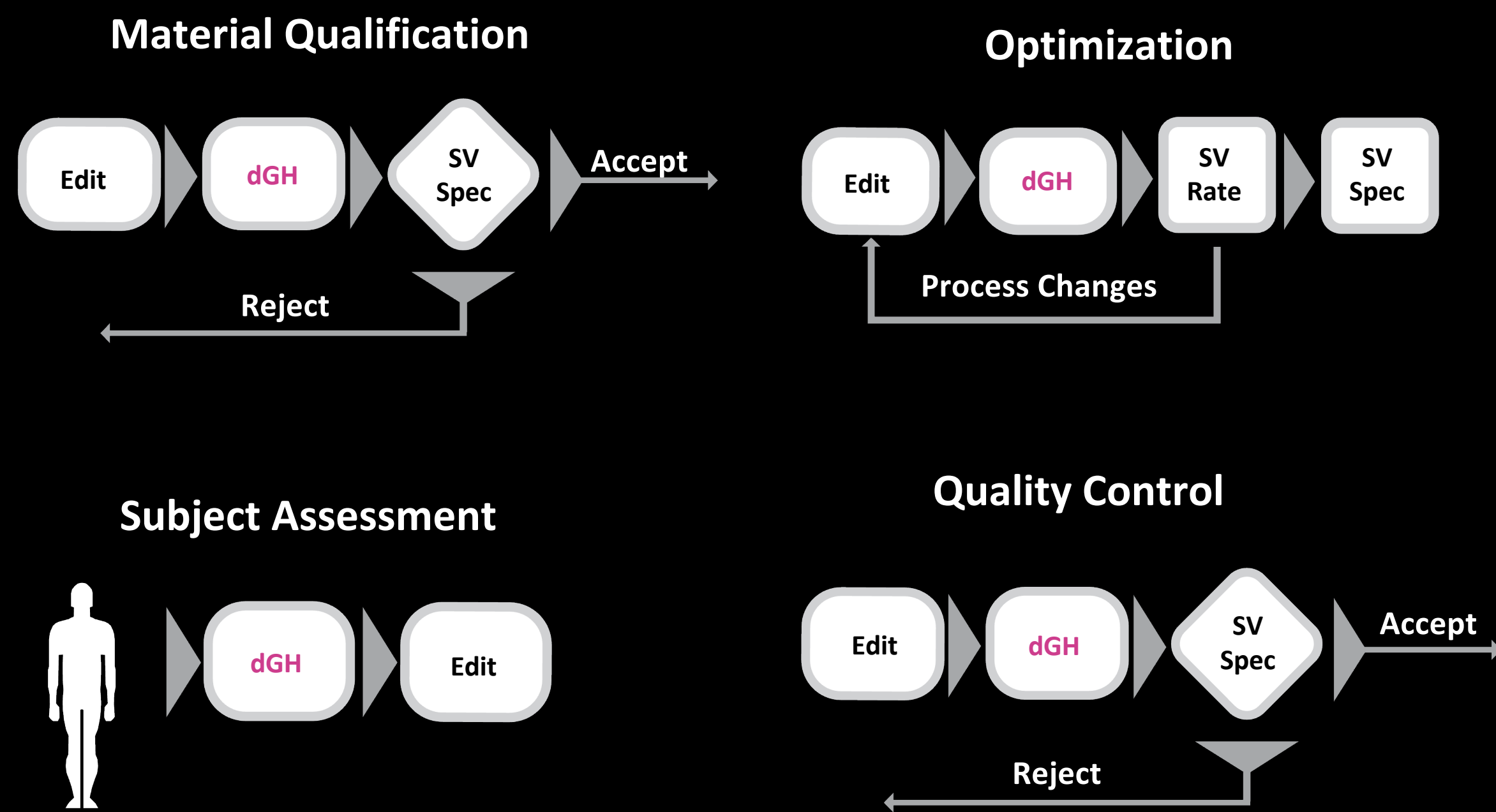
Translocated Fragment

Edit Site Inversion

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Material Qualification

Edit → dGH → SV Spec → Accept
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Optimization

Edit → dGH → SV Rate → SV Spec
Process Changes

Subject Assessment

→ dGH → Edit

Quality Control

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Percent of Edited Cells

Structural Error Free Cells

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Complex
Translocations
Inversions

Complex
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Inversions

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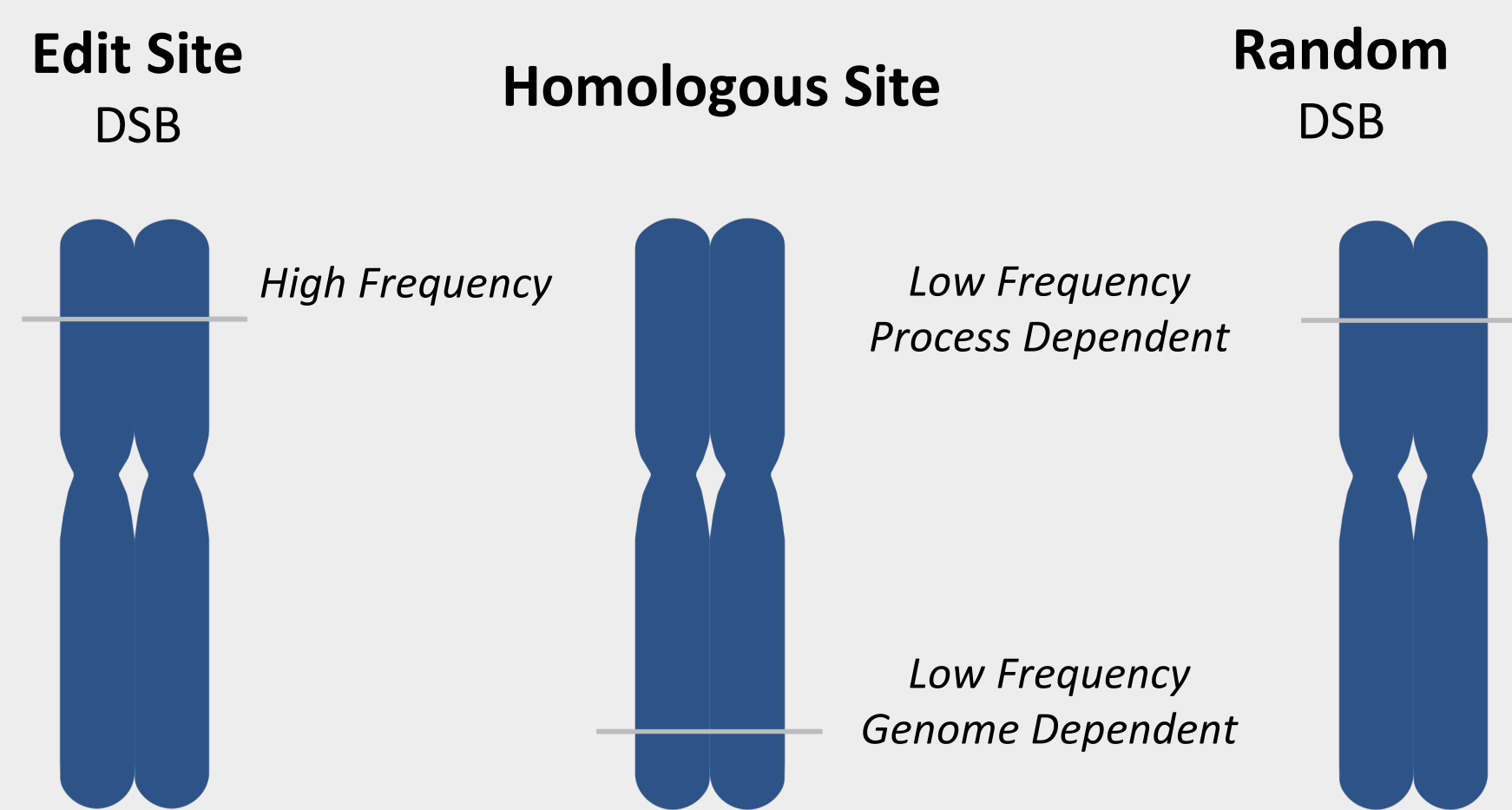
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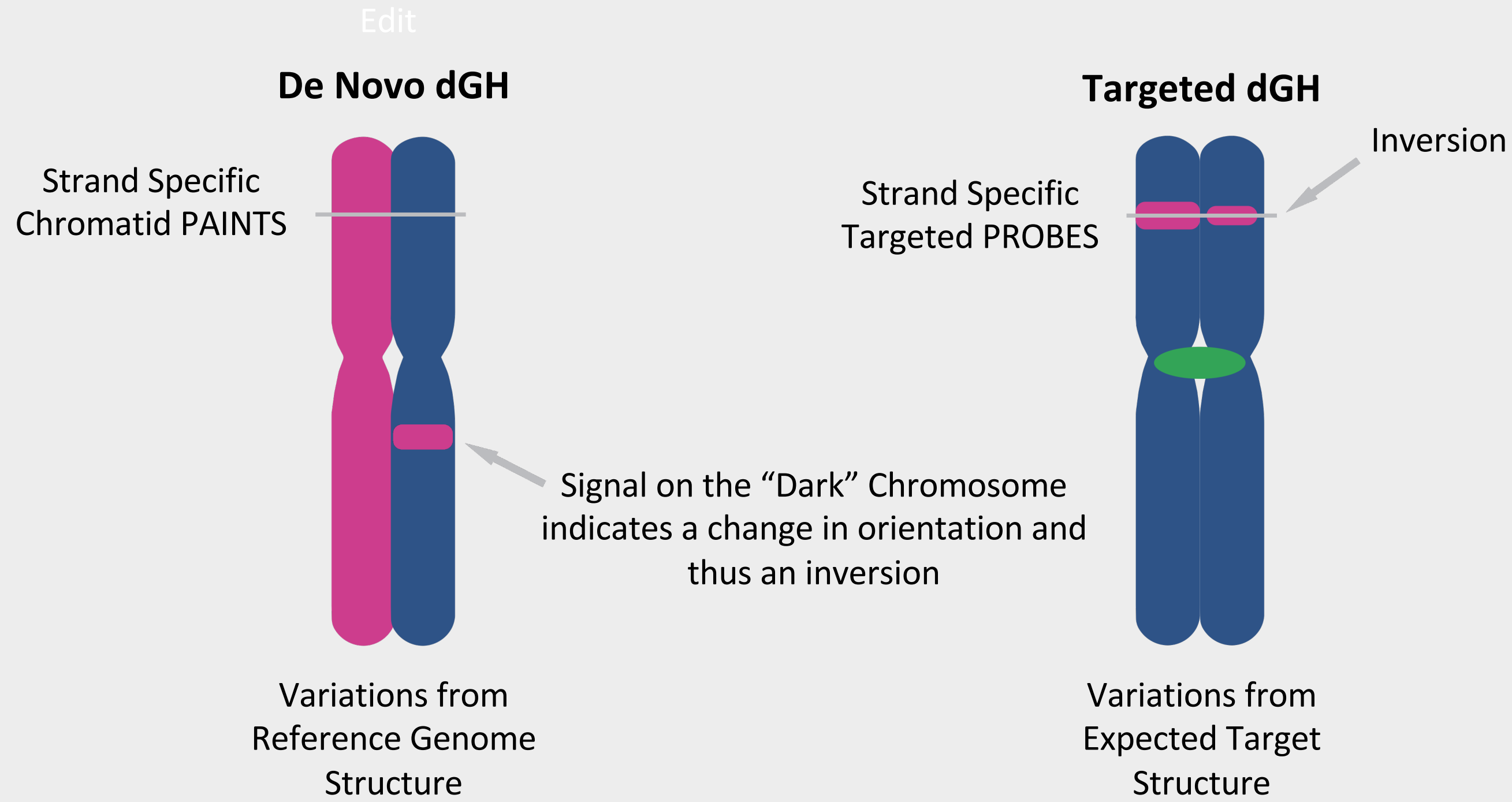
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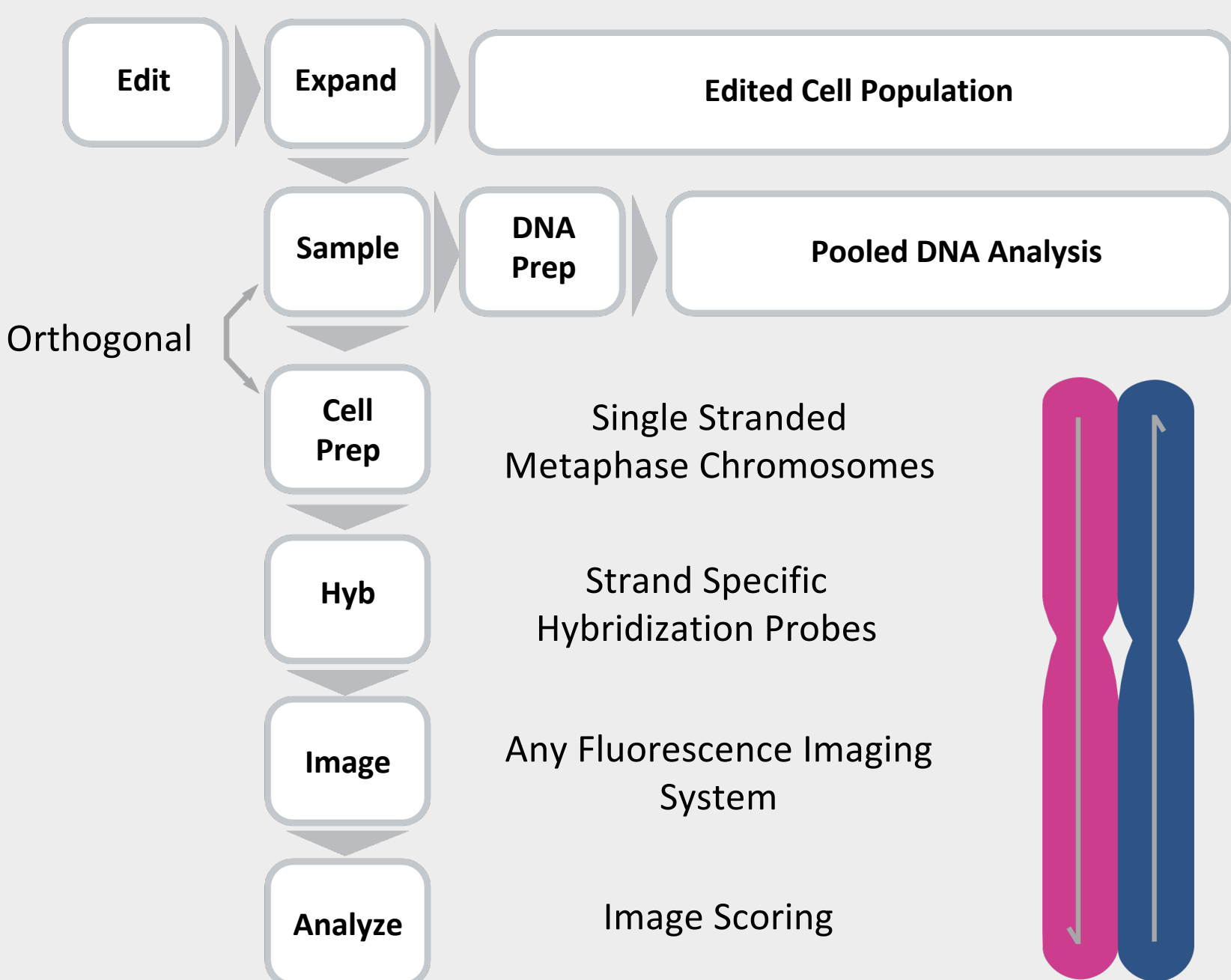
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Variations from Reference Genome Structure

Targeted dGH
Strand Specific Targeted PROBES
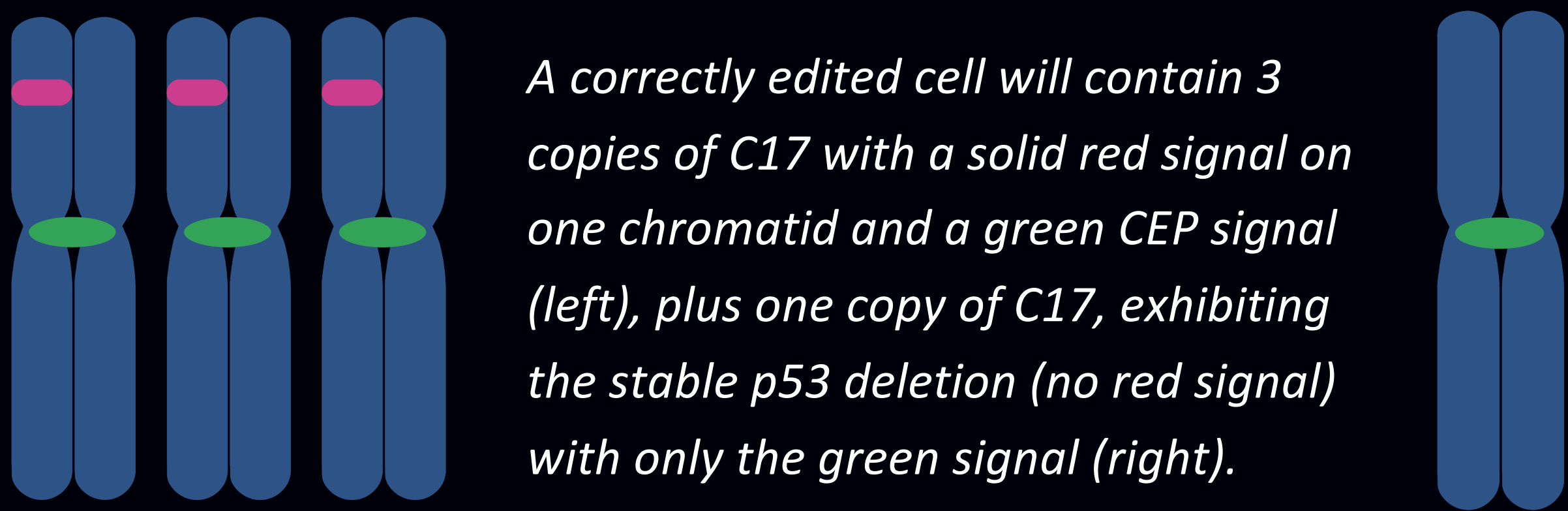Inversion
Variations from Expected Target Structure

dGH Integrates with Gene Editing Workflows:

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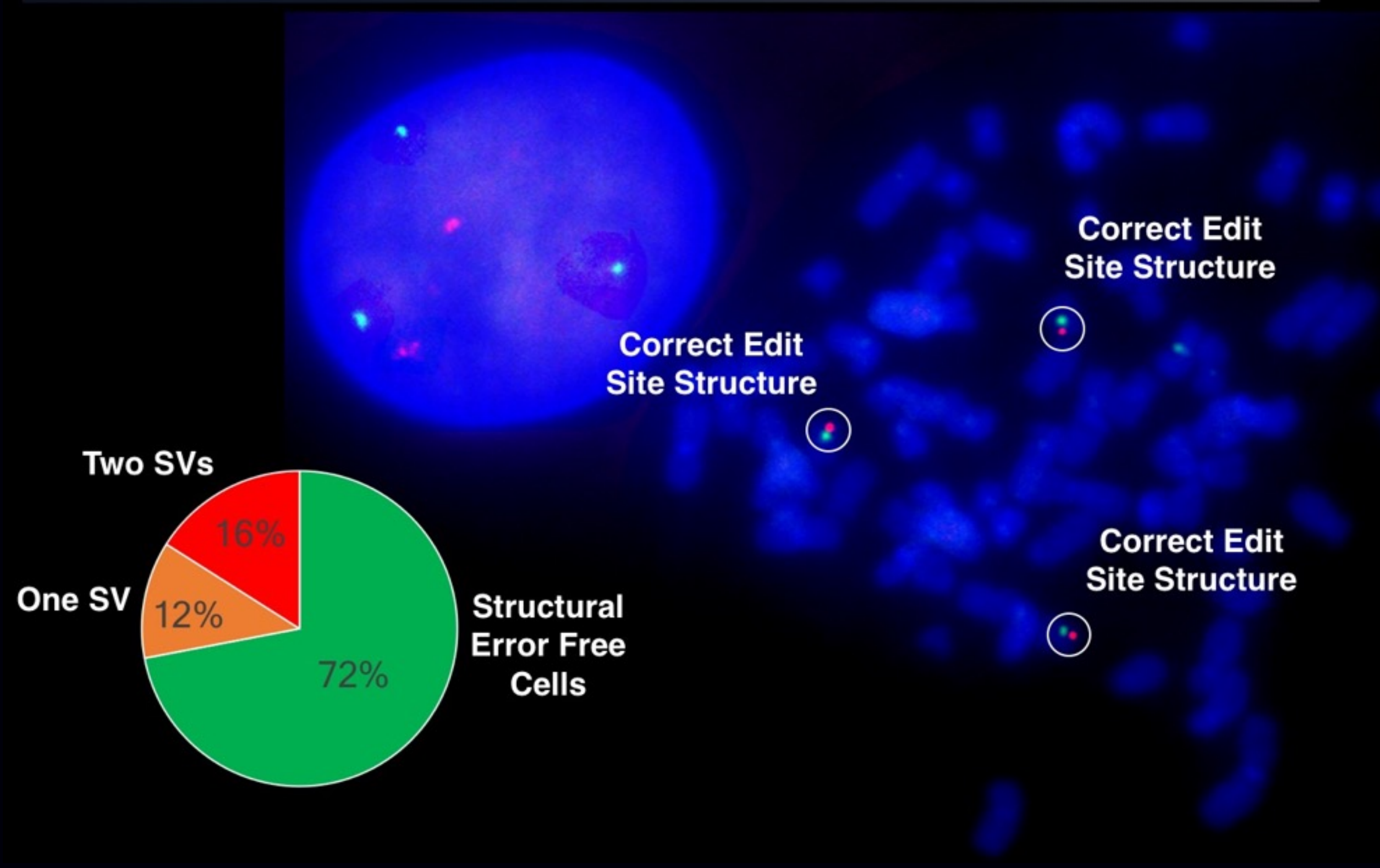- dGH can measure baseline rearrangement in subjects and cells

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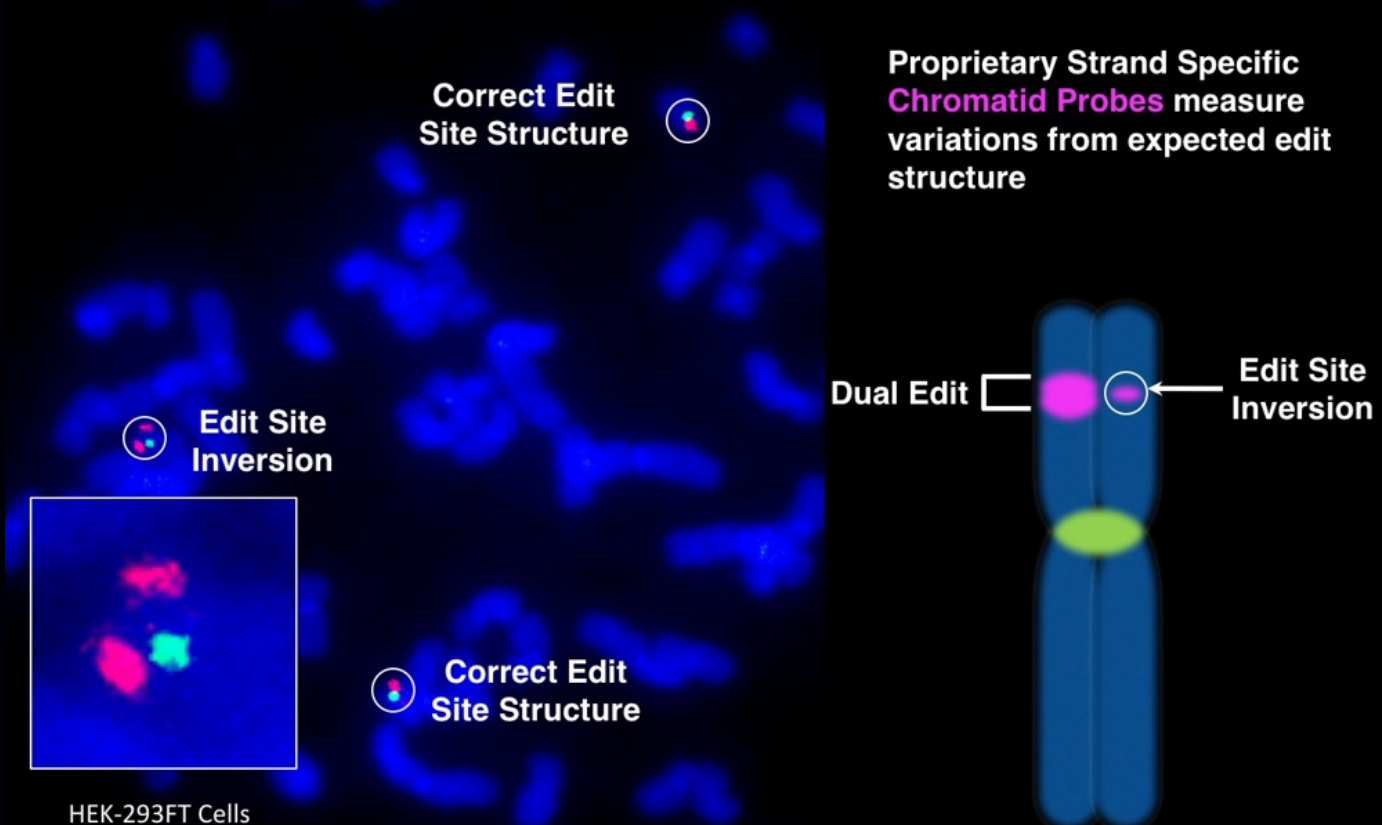
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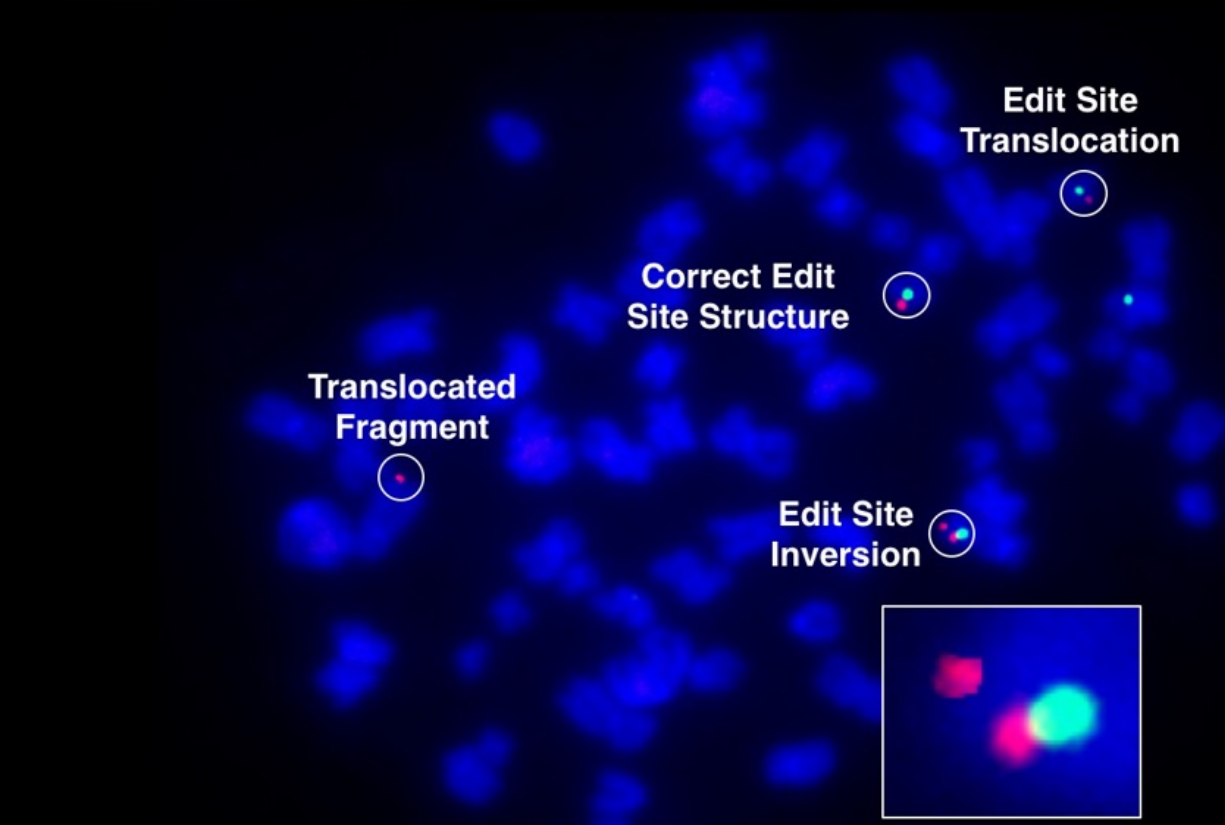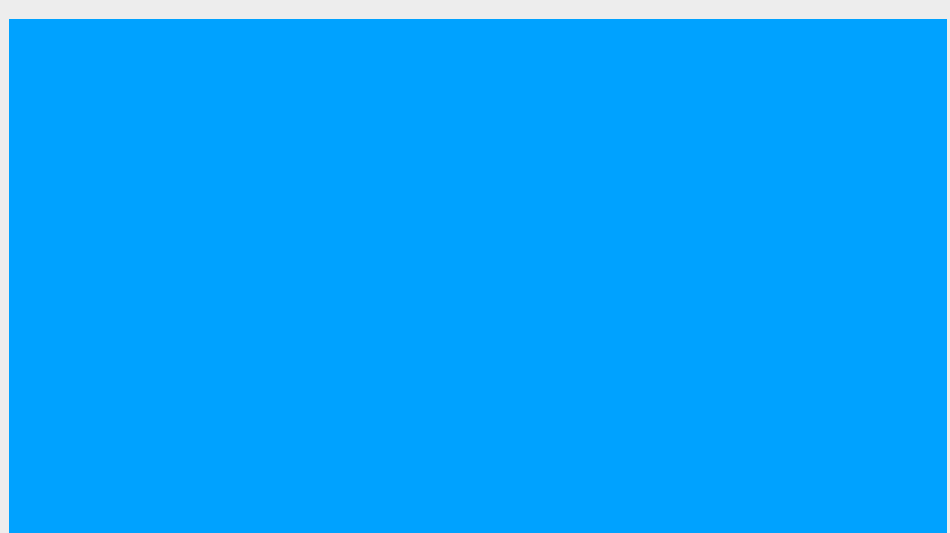
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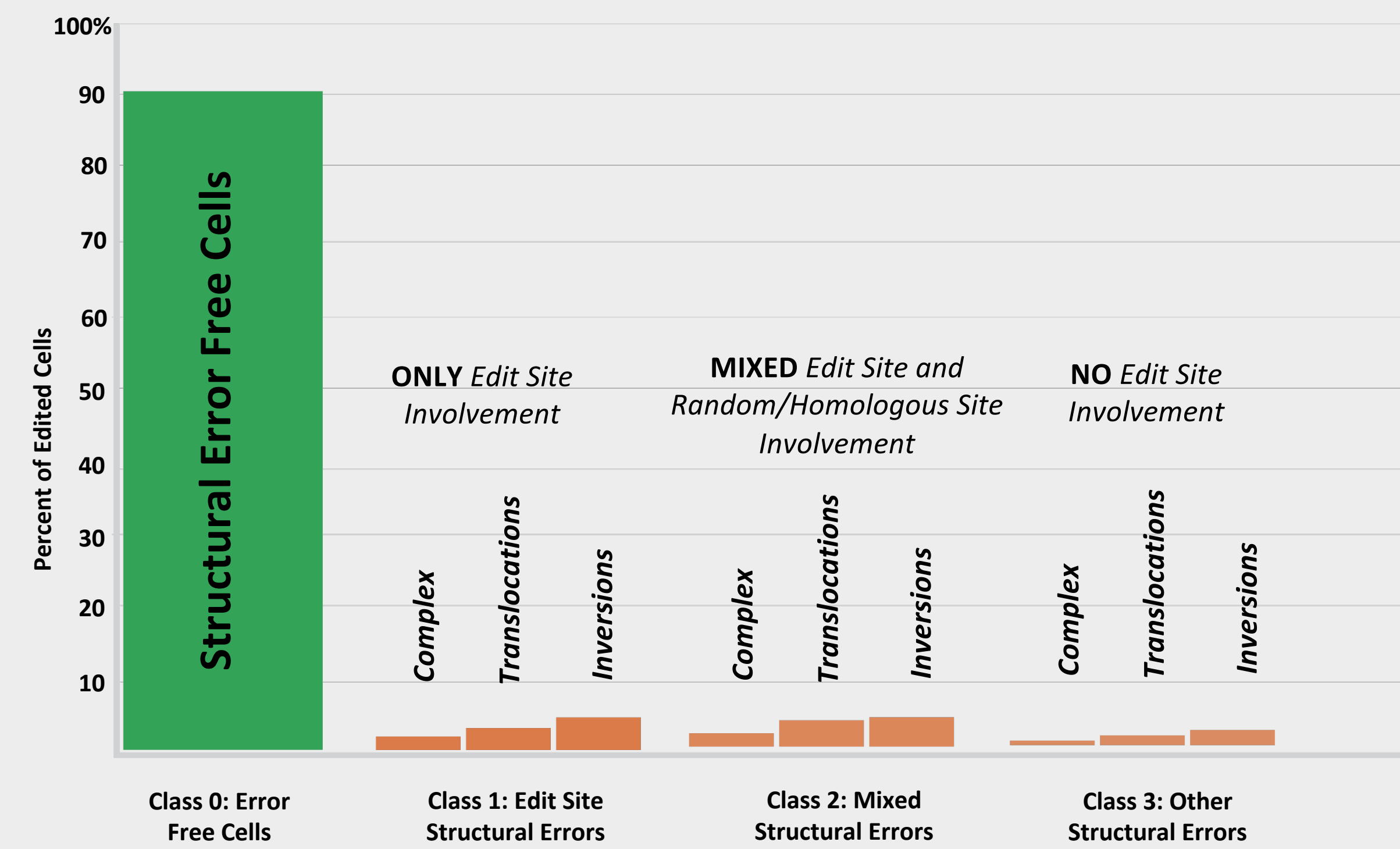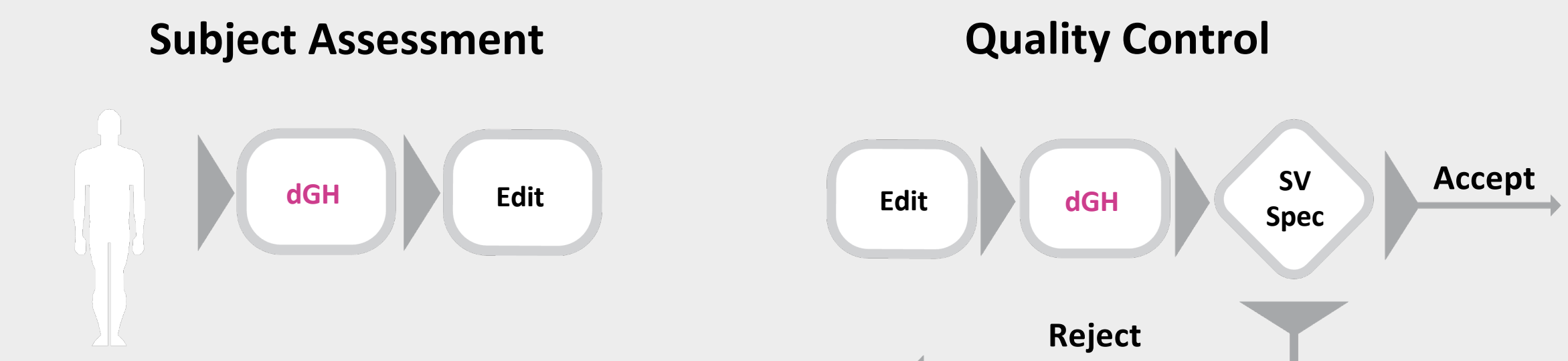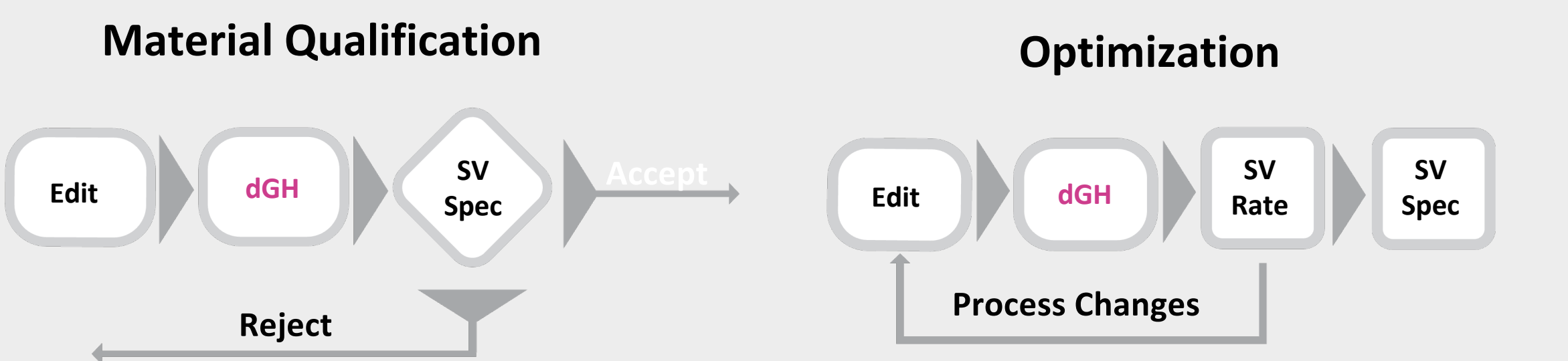Translocated Fragment
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Optimization
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Percent of Edited Cells (Y-axis: 10–100%)

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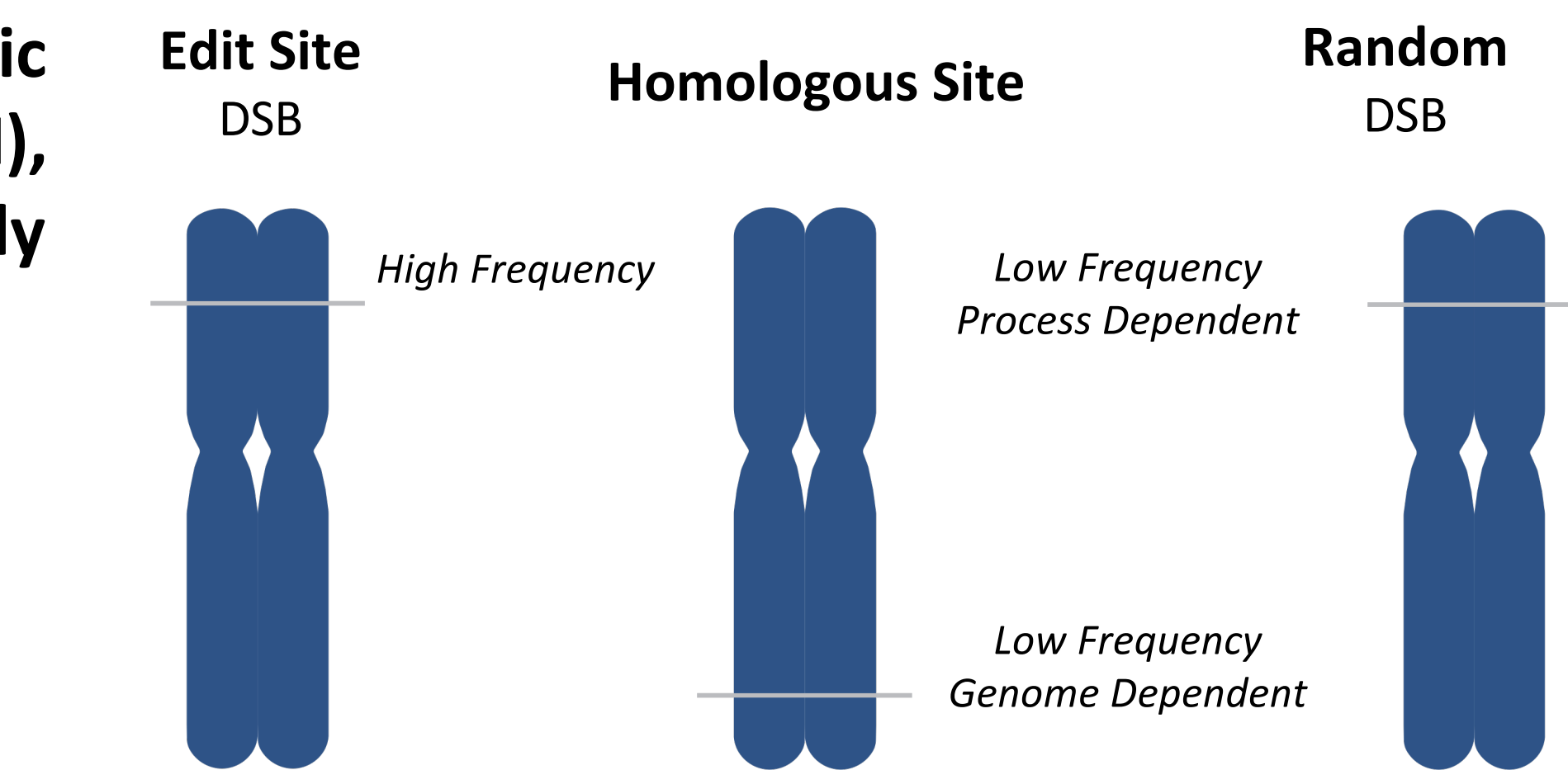
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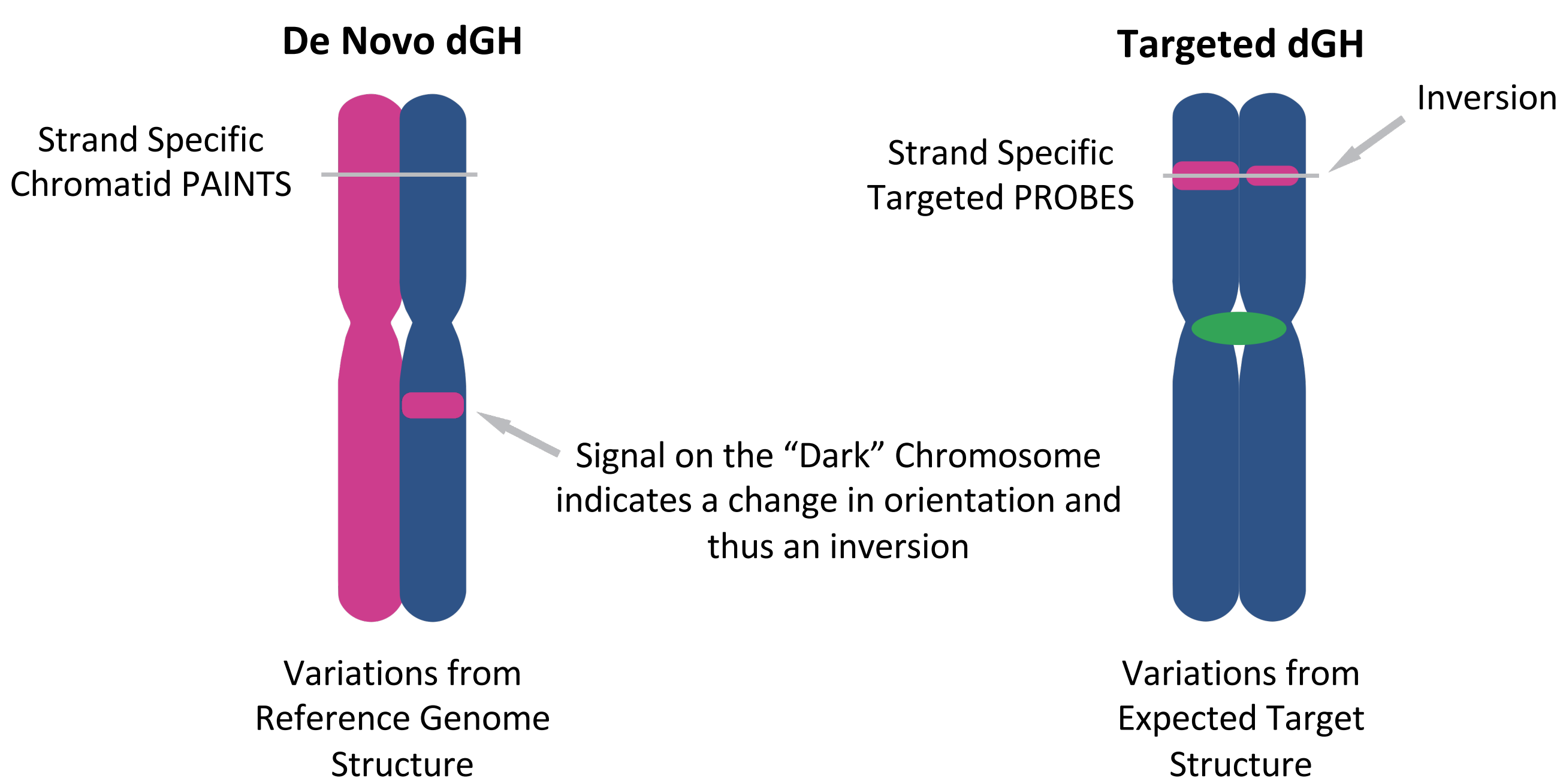
KromaTiD

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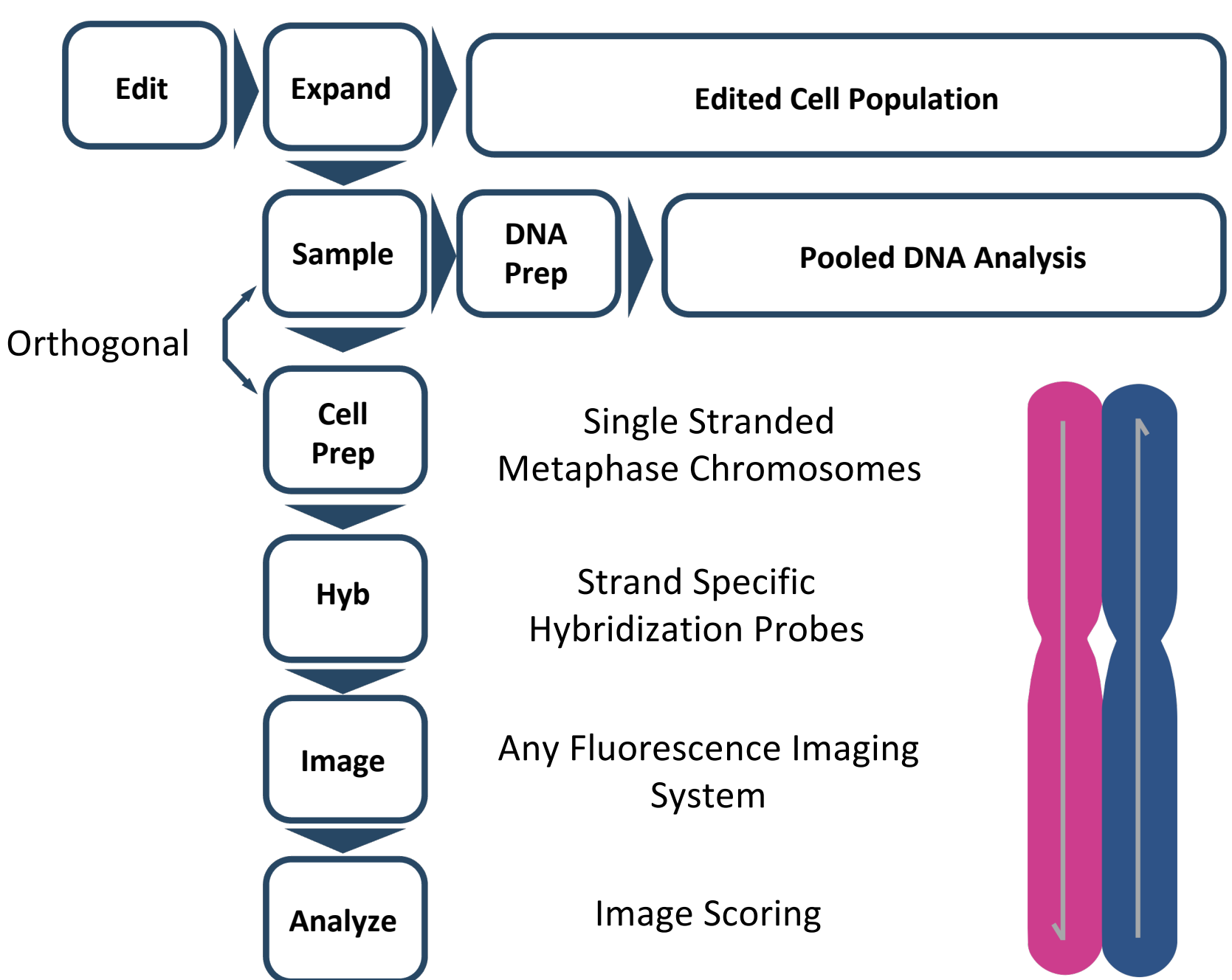

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Homologous Site

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Variations from
Reference Genome
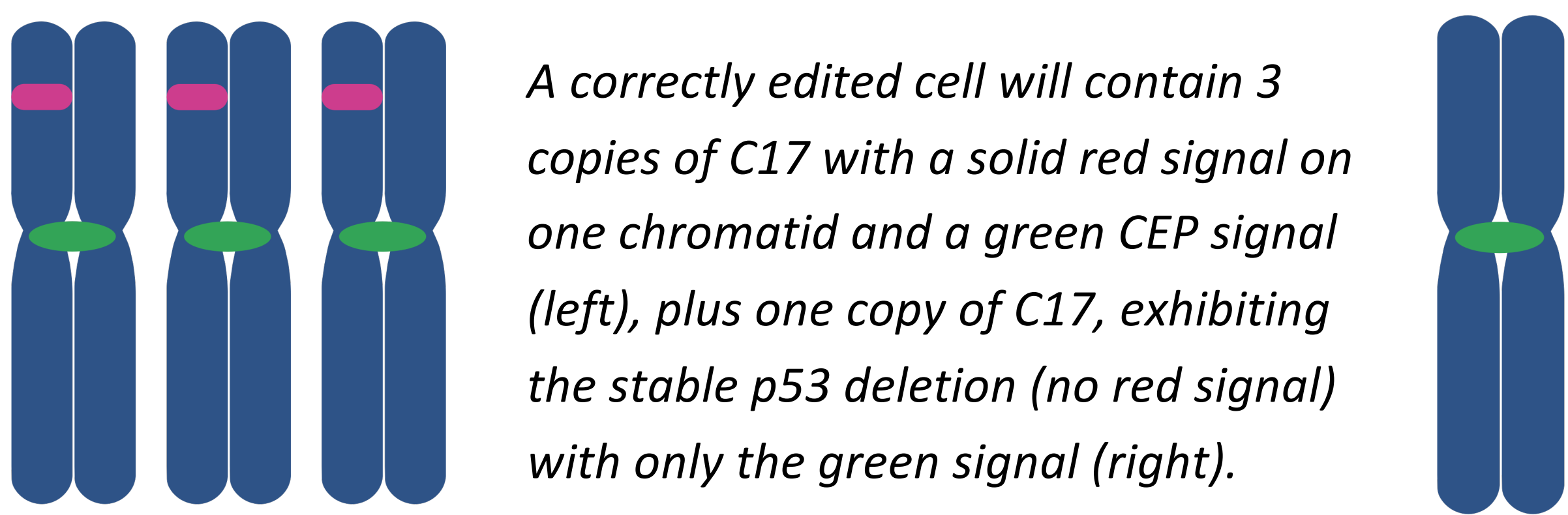Structure

Variations from
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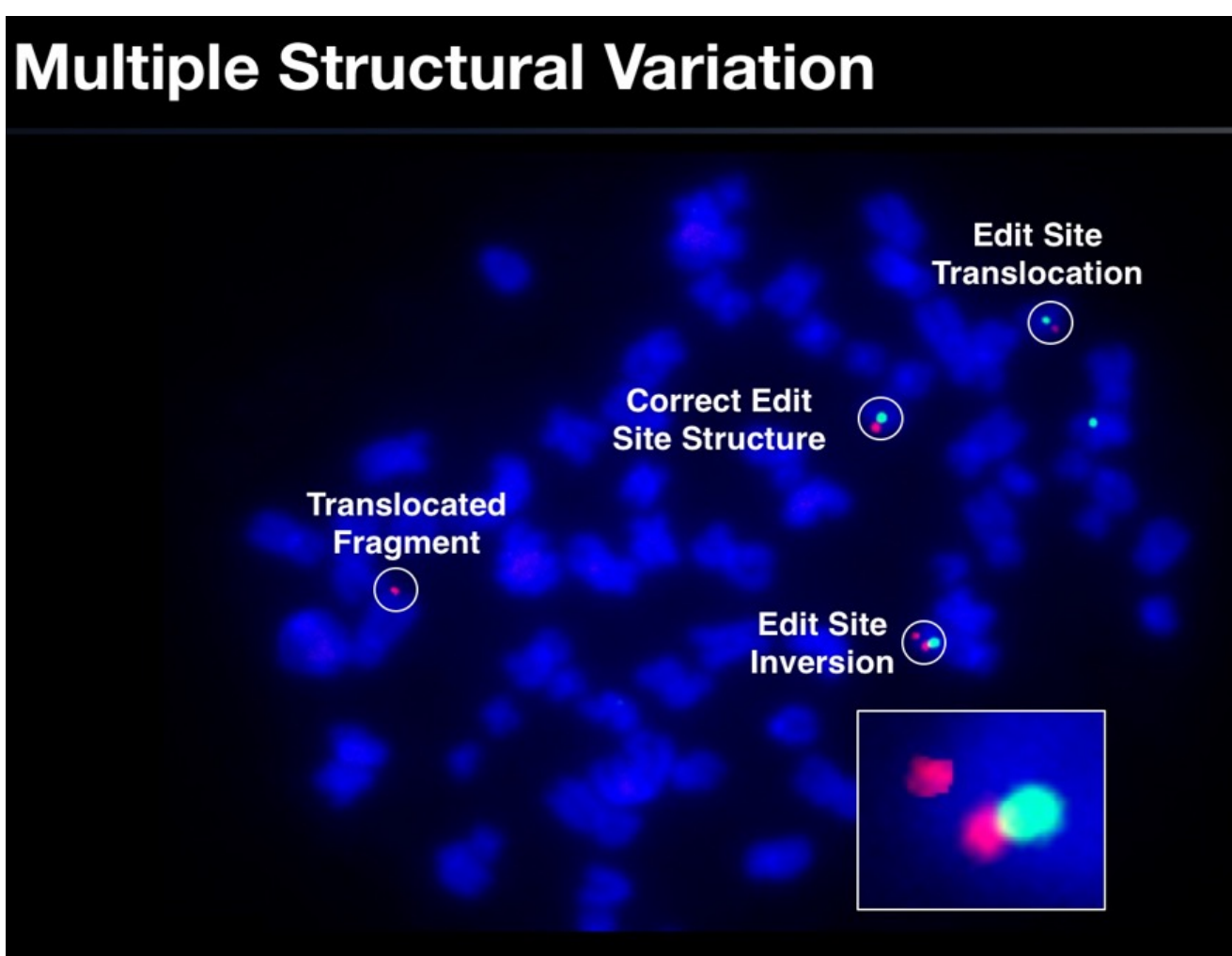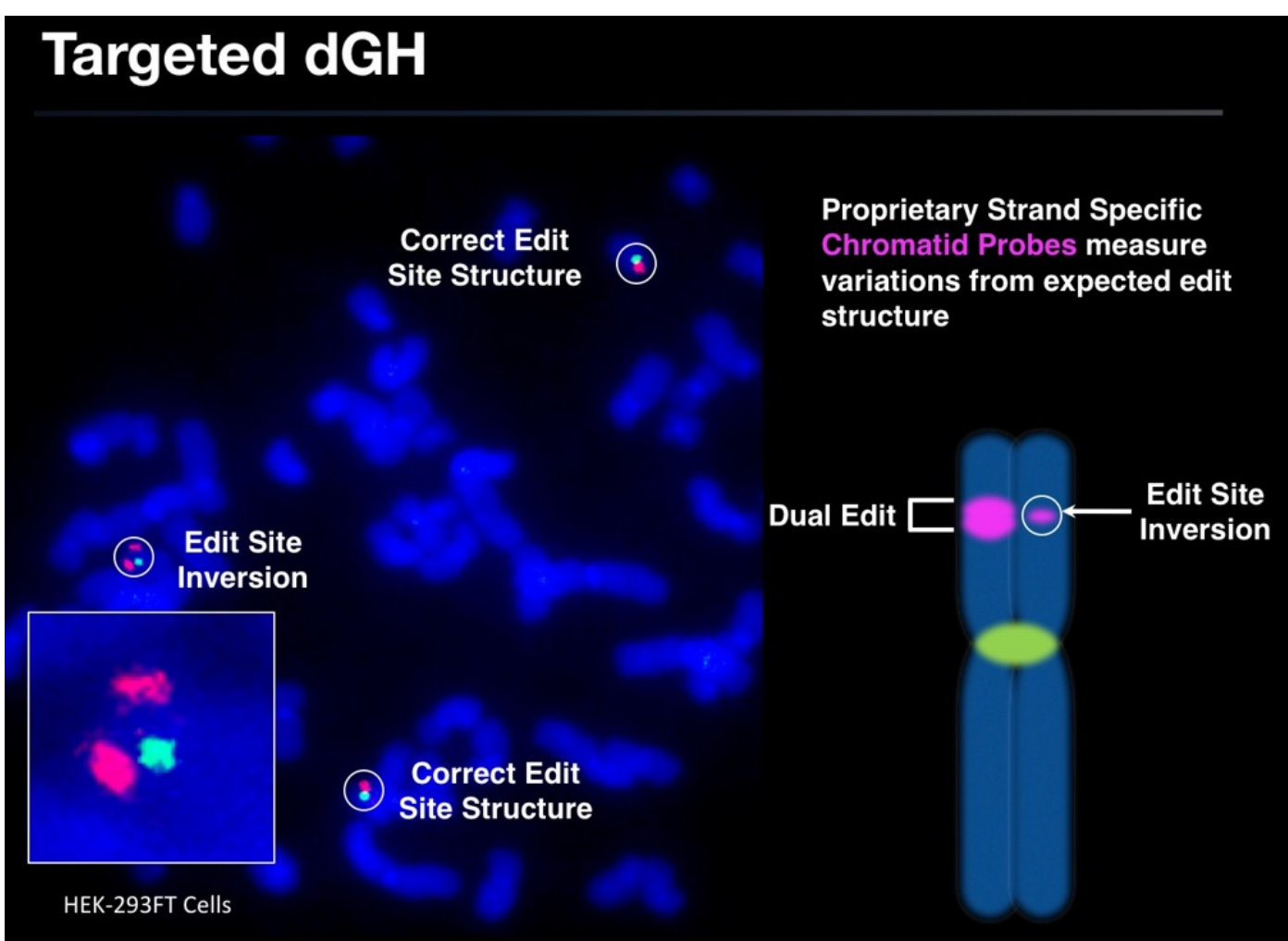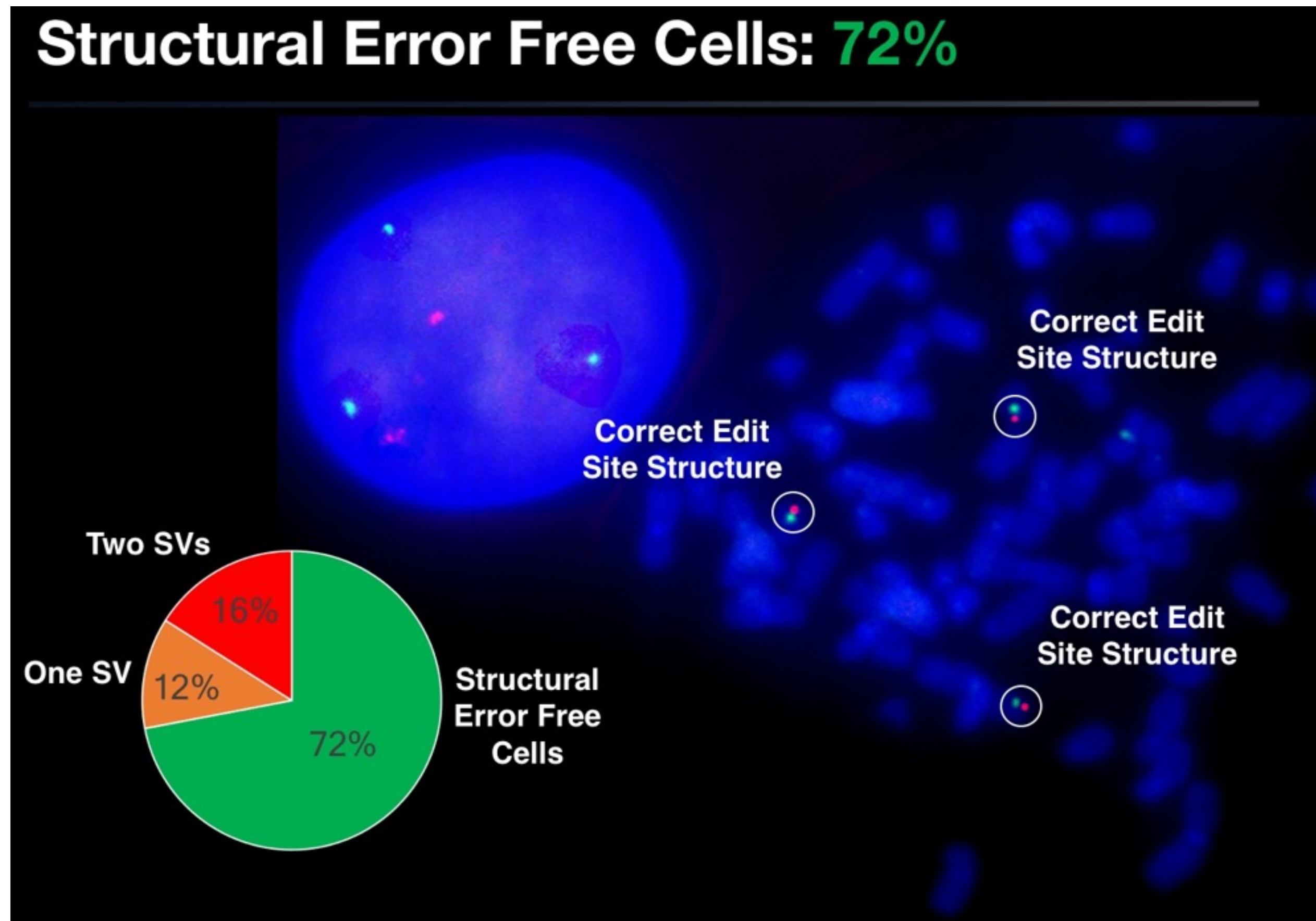
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Proprietary Strand Specific **Chromatid Probes** measure variations from expected edit structure

Edit Site Inversion

Dual Edit — Edit Site Inversion

Correct Edit Site Structure

HEK-293FT Cells

Multiple Structural Variation

Edit Site Translocation

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