dGH in-Site™
ASSAY SERVICES

KromaTiD
Direct, Definitive Genomics

KromaTiD

Direct, Definitive Genomics

Who We Are

A team of expert scientists providing unparalleled genomics tools, services and support.

Your partner for

- Biomarker discovery
- Genotoxicity studies
- Assessment of gene editing-associated errors
- Plasmid manufacturing

Our Products

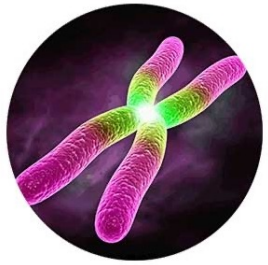
- Patented directional Genomic Hybridization™ (dGH™) technology
- An extensive collection (>700) of chromosome probes and paints
- Improve the sensitivity and specificity of your FISH assays

Our Services

- FISH assays utilizing our patented Pinpoint FISH™ and dGH™ technology
- Plasmid manufacturing (RUO, pre-GMP & cGMP)
- G-banded karyotyping
- Cell culture

What We Provide

Products



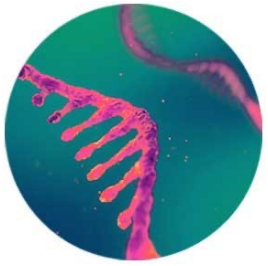
Centromere Probes

Subtelomere Probes

Oncology Probes

Pinpoint FISH™ Kits

Whole Chromosome Paints

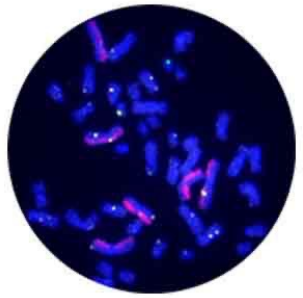


dGH Cell Prep Kit

Services

Cell Culture Services

G-banding Services



dGH in-Site™ Targeted Assays

dGH SCREEN™ Unbiased Assays

Pinpoint FISH™ Assay Services

Plasmid Manufacturing Services

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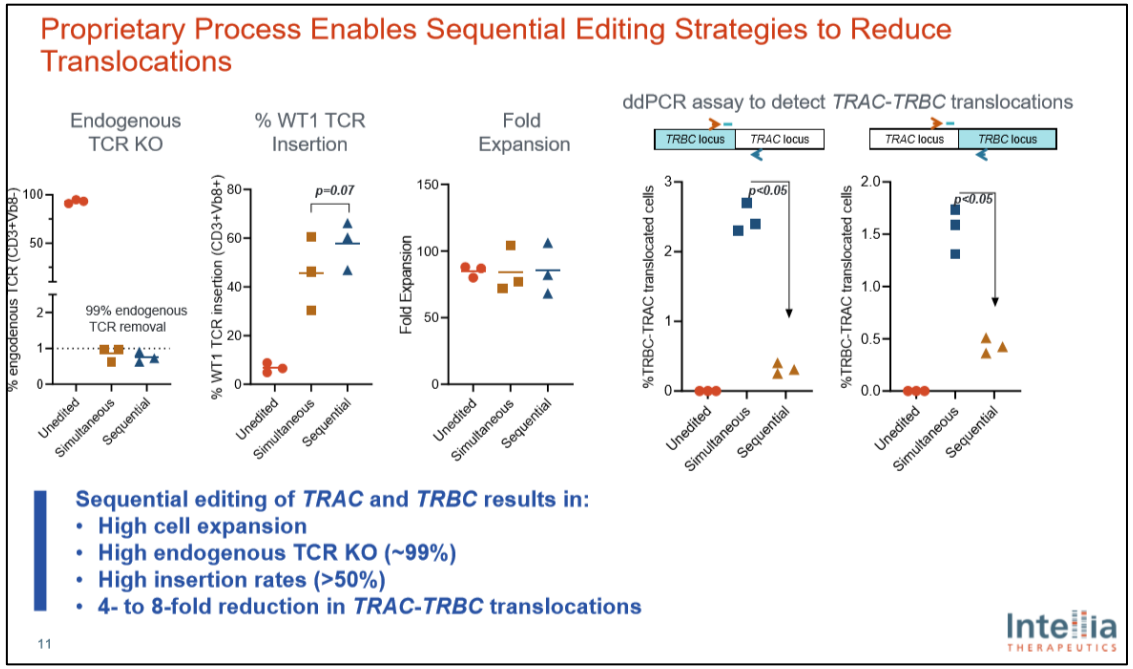
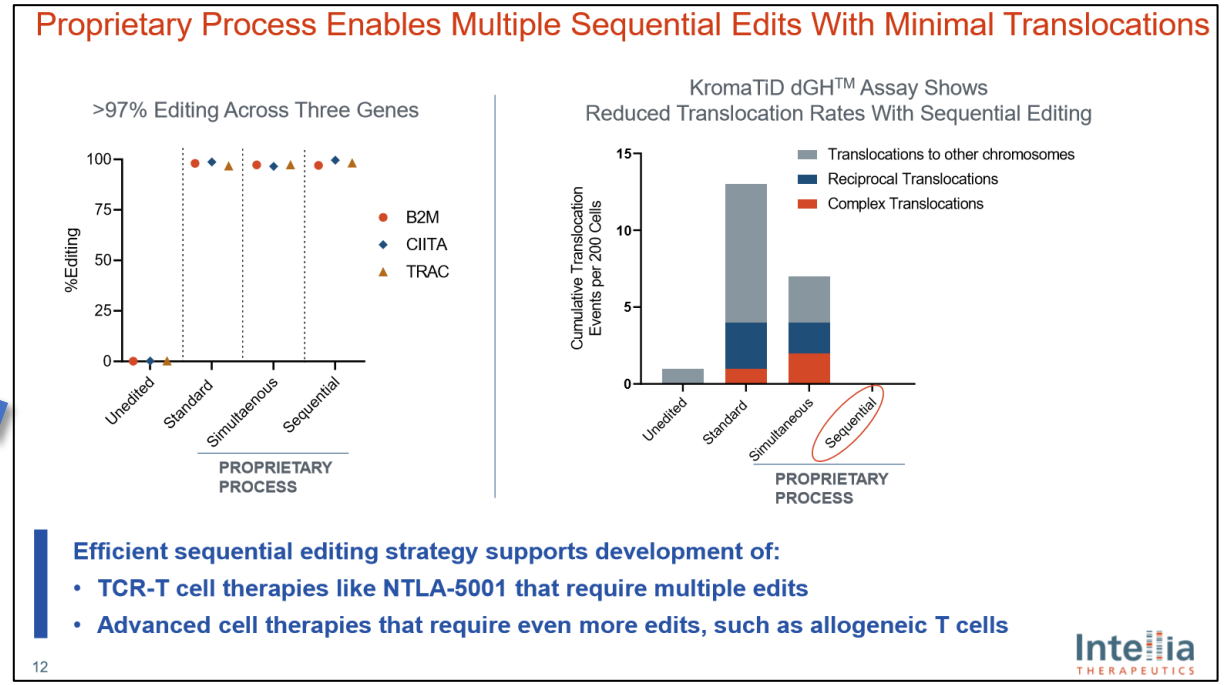
dGH™ Provides CRISPR Therapy Client* Direct Evidence of Process Optimization



ddPCR only detects translocations between edit sites…
No measurements of edit-random site TLs or other heterogeneous rearrangements

PCR data shows the three editing approaches look the same

KromaTiD dGH™ shows clear differences and more comprehensively captures process-associated structural changes



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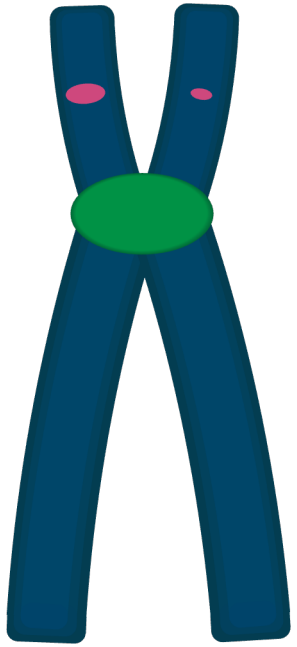
*Presented during **ASGCT 2020** by Dr. Aaron Prodeus, Sr Scientist, T-Cell Engineering, Intellia Therapeutics

directional Genomic Hybridization™

De novo dGH

Direct measurement of **structural variations** in many **single cells**

- De novo, targeted and mixed formats
- Complete characterization of simple, heterogenous and/or complex structural variations
- Single cell analysis retains the full cellular and chromosomal context
- Definitive measurement of structural variations using image data without bioinformatic interpretation
- High specificity method for variation of <4 KB or larger and <0.5% prevalence



Targeted dGH

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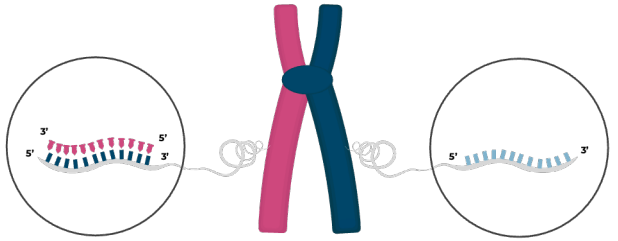
dGH™ Overview

Robust cryptic inversion discovery, detection and diagnosis

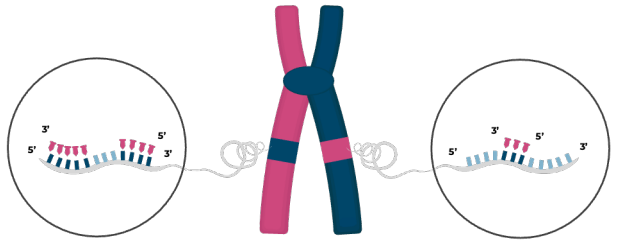
- Simple fluorescent imaging method without proprietary capital equipment
- dGH detects location, sequence, and **orientation** in a single, simple test

dGH detects most classes of DNA rearrangements in parallel to inversions

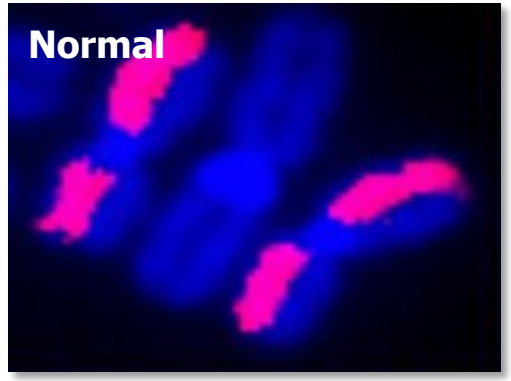
Specific designs for deletions and CNVs

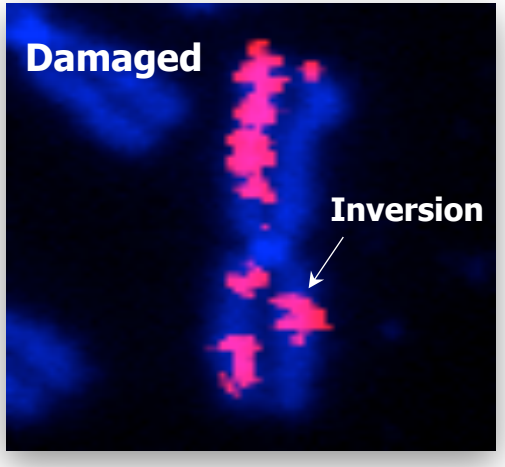


Normal dGH painted chromosome



Painted dGH chromosome with inversion





dGH analysis of Chromosome 3

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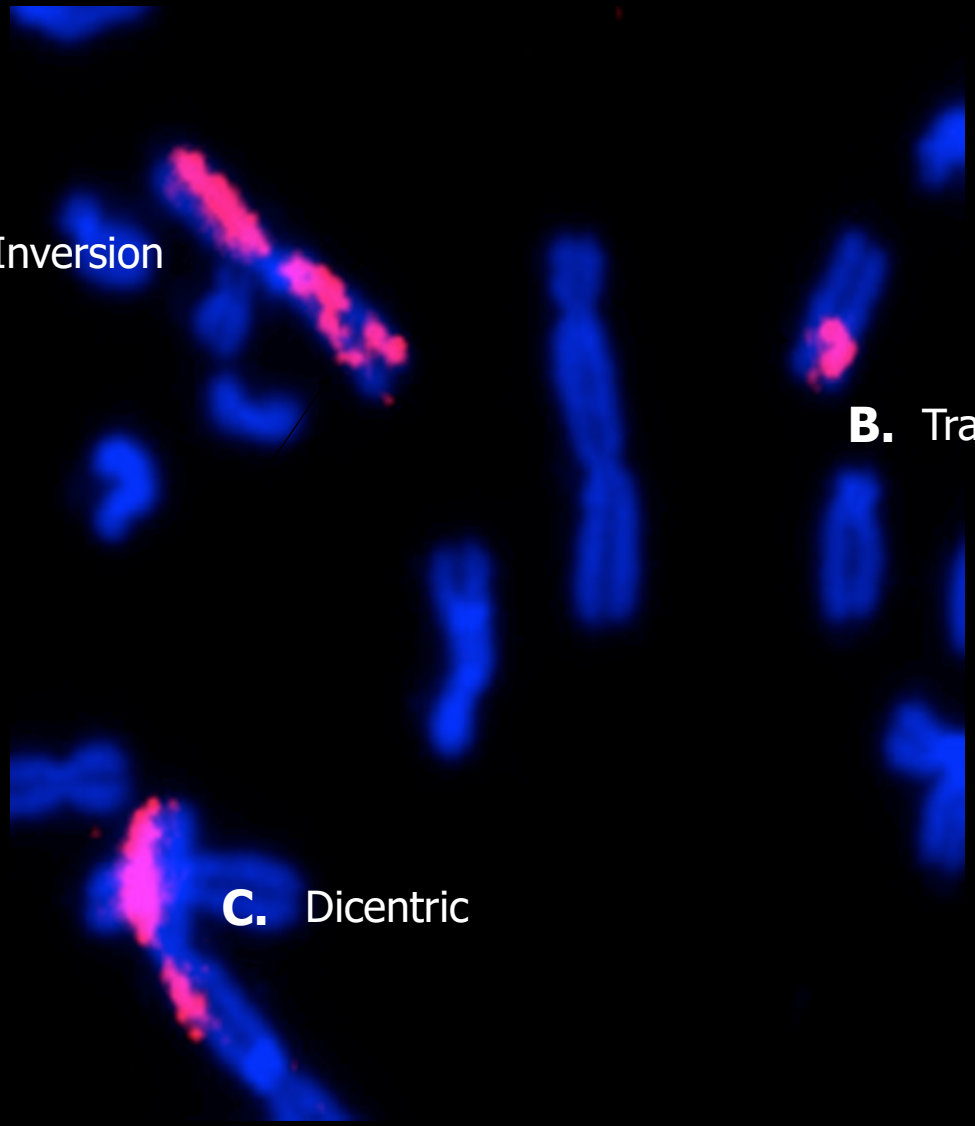
Broad Rearrangement Detection in a Single Assay



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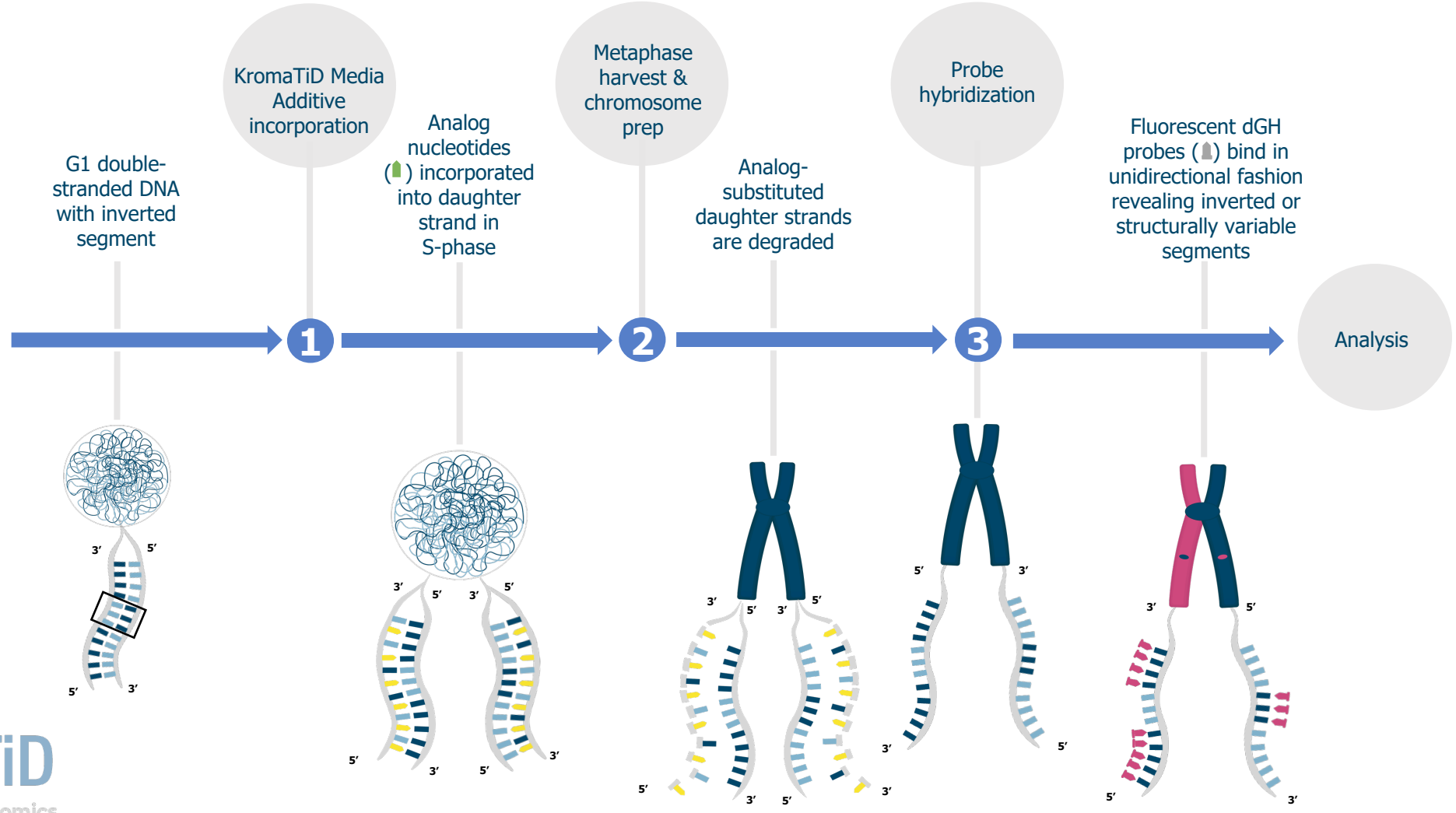
dGH™ Process Flow



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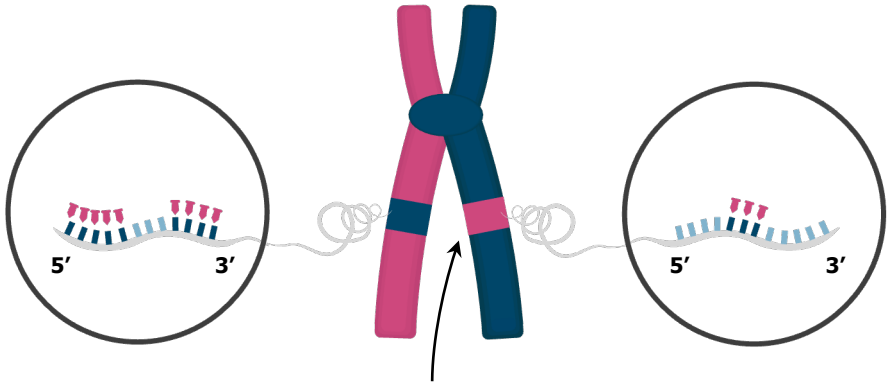
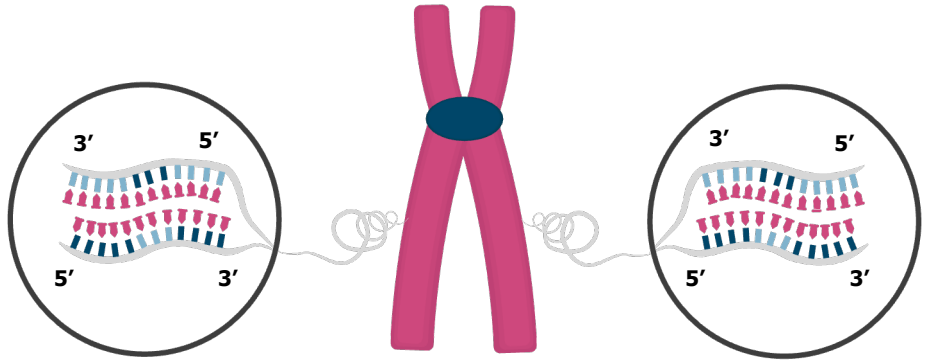
Single Cell dGH™ is not Metaphase FISH

dGH



Inversion visualized as a result of unidirectional dGH probe binding

Metaphase FISH



Inversion cannot be visualized in standard metaphase FISH spread

dGH is distinguished from standard FISH by both the chromosome prep and probe design.

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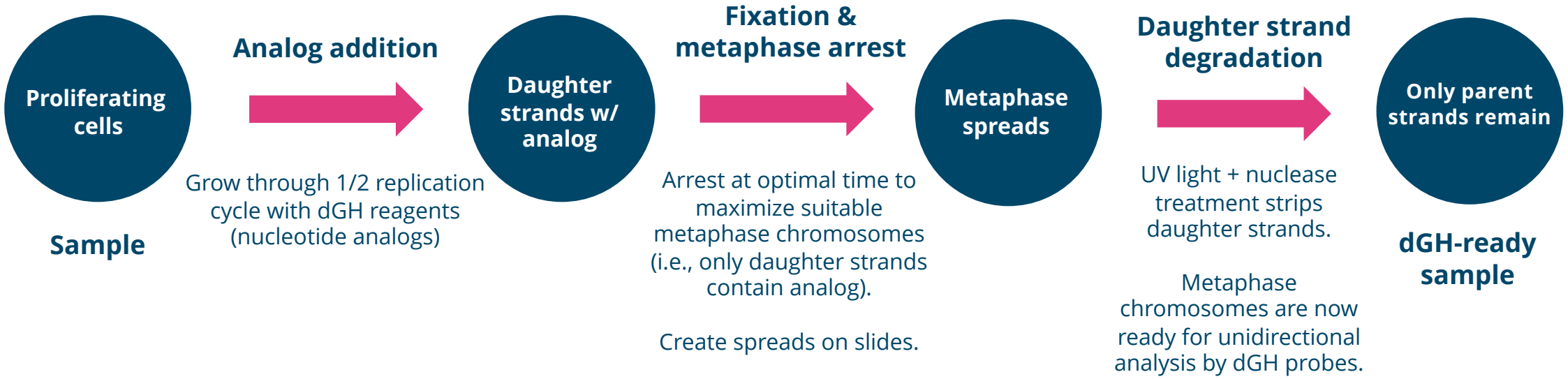
dGH™ Sample Prep:
Specially Prepared Chromosomes for Unidirectional Analysis



All or part of dGH sample prep can be carried out by client using the dGH™ Cell Prep Kit.
KromaTiD works closely with client to ensure right-first-time execution of sample prep.

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dGH™ Process Flow: Why it Works

Customer provides targets and samples

1. Probe and assay design

- Oligonucleotide-based probes bind only unique sequence target of interest.
- Non-specific, background binding is eliminated.
- Unidirectional: complementary to only one strand

2. Sample prep

- Measurement quality begins with an optimized sample prep.

Critical steps:

(1) Analog incorp.
(2) Arrest timing to maximize metaphase chromosomes for analysis
(3) Controlled application to slides for optimal spread morphology

3. Hybridize

- Unidirectional probes, designed to target a *single strand* of a reference genome.
- Hybridized to specially prepared metaphase spreads

4. Scanning

- ASI fluorescent scanner platform.
- Scalable, highly automated production of thousands of images per sample

Note: Many CLIA certified labs use ASI scanners

5. AI and human scoring

- AI-driven sorting of images into “normal/not-normal” for standard assay designs. Human scoring requirements for more nuanced read-outs and R&D-stage projects.
- AWS or other cloud-based storage of processed/ annotated images

6. Analysis & reporting

- R&D report with quantitative outcomes and sample images

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Sample Images from a CRISPR/Cas9- Gene Therapy Approach

Considerations

- Track small targeted regions (presence/absence of signal) – dGH in-Site LOD is ~3kb
  - In the following experiment a10 Kb transgene was inserted.
- This assay design includes
  - an on-target bracketing probe (green)
  - an insert region probe (yellow)
  - a concurrently run dosimetry assay of 3 off-target, large chromosome paints (pink)
- Data provided for
  - Structural on-target editing efficiency
  - Off-target incorporation of the insert sequence
  - Background rates of rearrangements
- Proper controls are critical to segregating effects of the edit from background repair/rearrangement activity.

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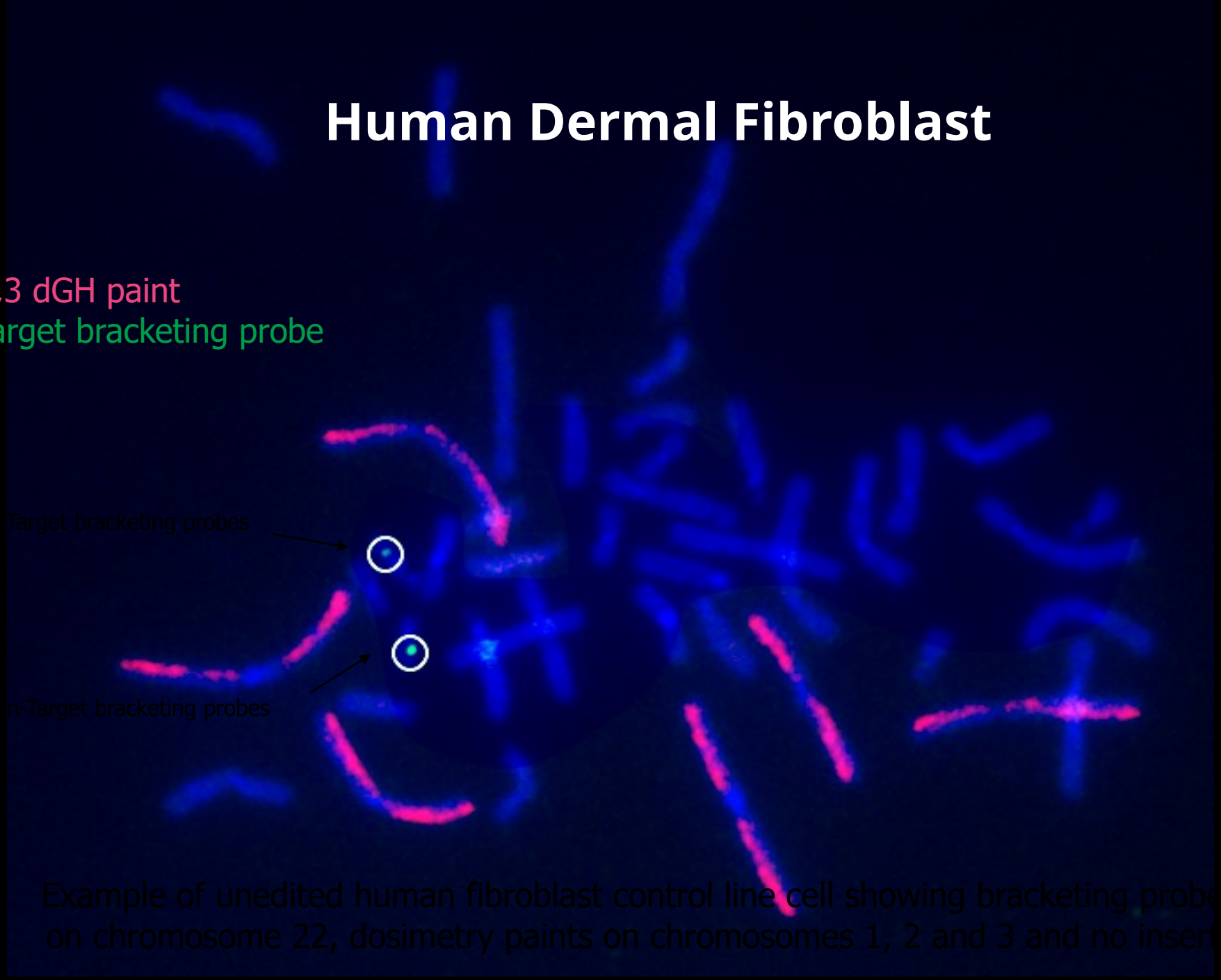
Human Dermal Fibroblast

Pink: Chr1,2,3 dGH paint
Green: On-target bracketing probe



Example of unedited human fibroblast control line cell showing bracketing probe
on chromosome 22, dosimetry paints on chromosomes 1, 2 and 3 and no insert

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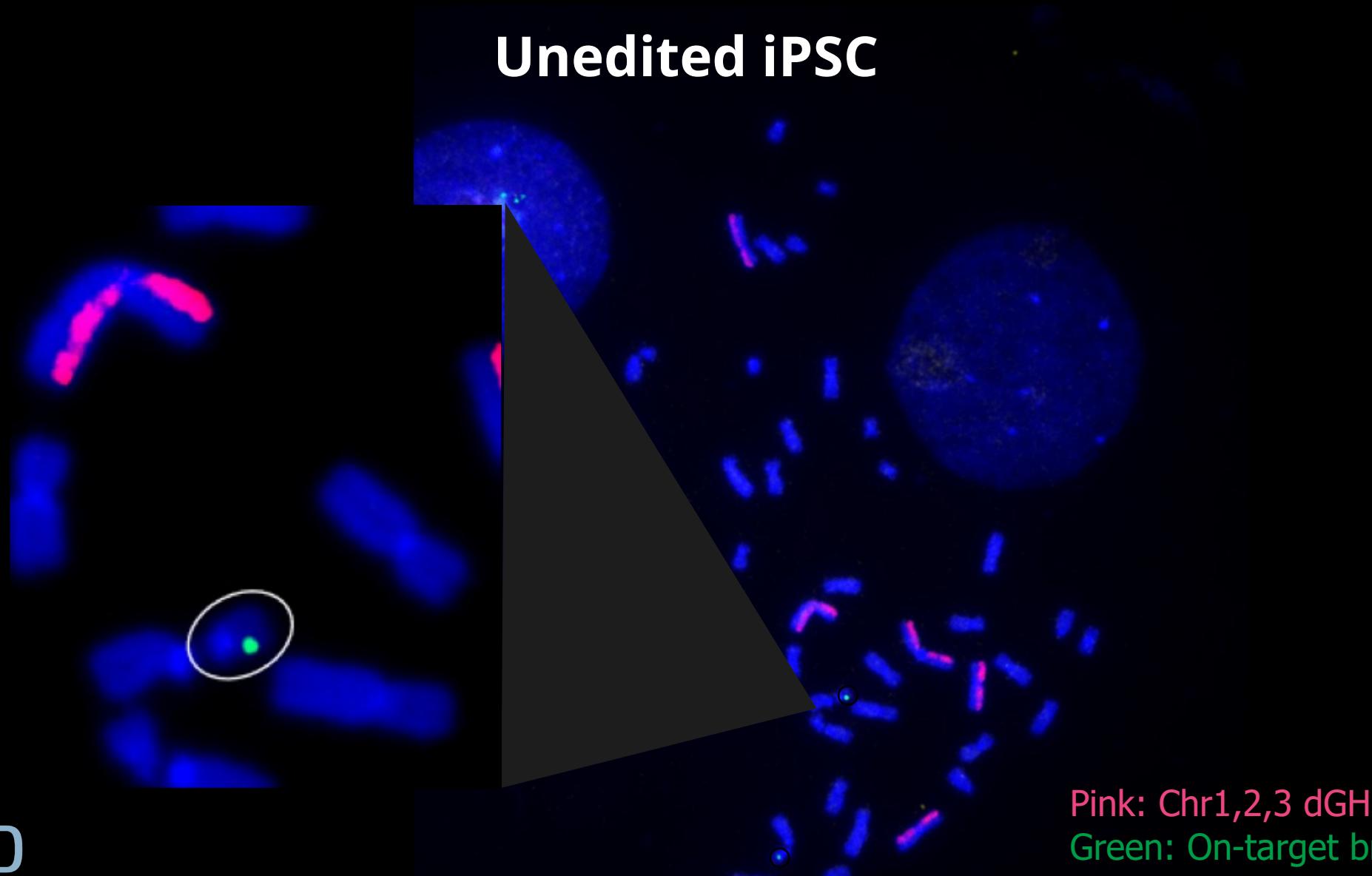
Unedited iPSC



Pink: Chr1,2,3 dGH paint
Green: On-target bracketing probe

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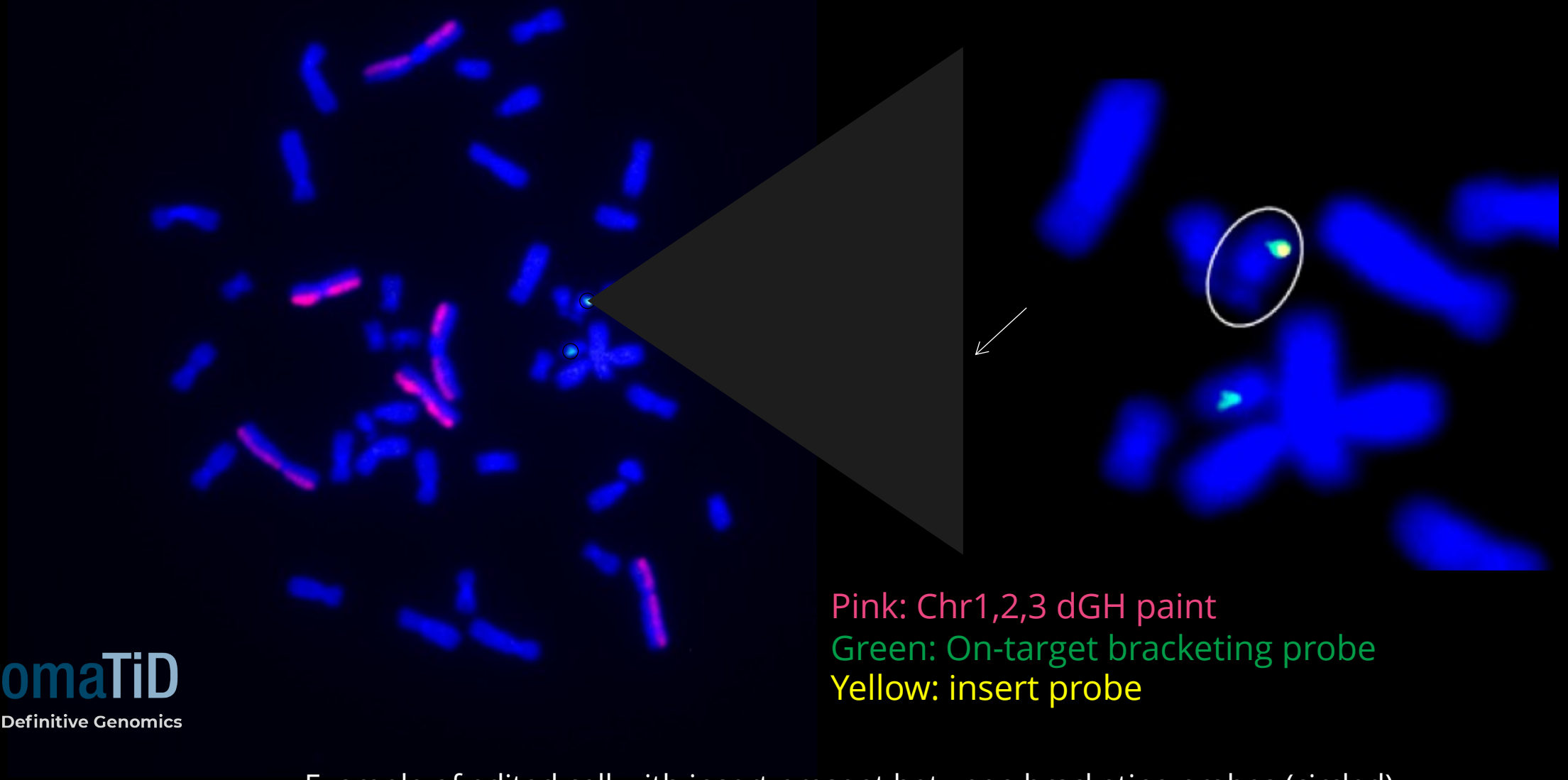
Edited iPSC: CRISPR with 10 Kb Insert Present in 1 Homolog



Pink: Chr1,2,3 dGH paint
Green: On-target bracketing probe
Yellow: insert probe

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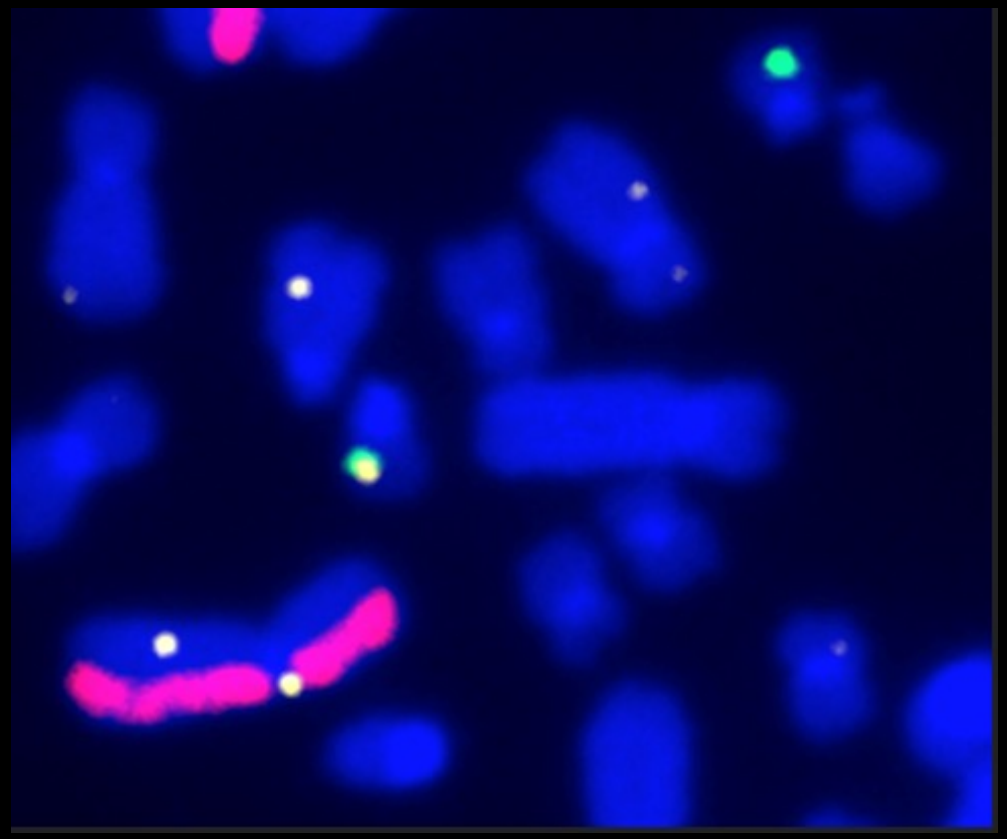
Example of edited cell with insert present between bracketing probes (circled)

10 Kb Inserts in iPSC



Study Design:

- **Measurements:**
  1. Insert Number & Location
  2. Structural variation of target insertion site
  3. Random structural variation
- **Assay:** de Novo dGH on Off-Target Chromosomes PLUS Insert Probe PLUS Bracketing Probes

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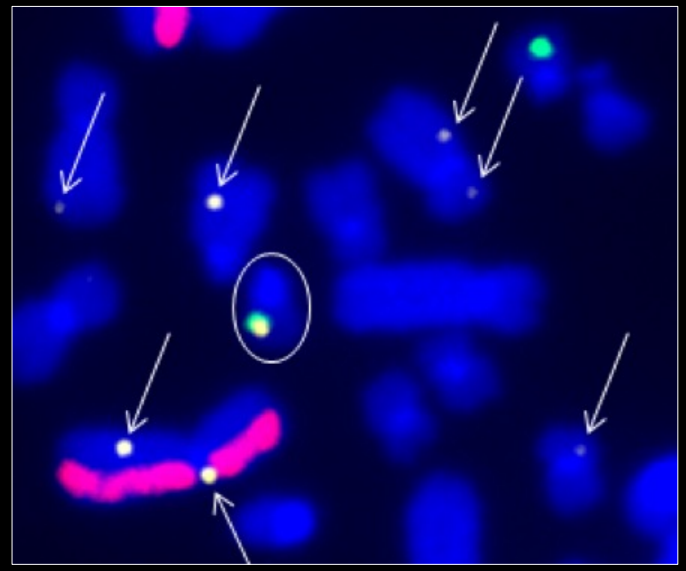
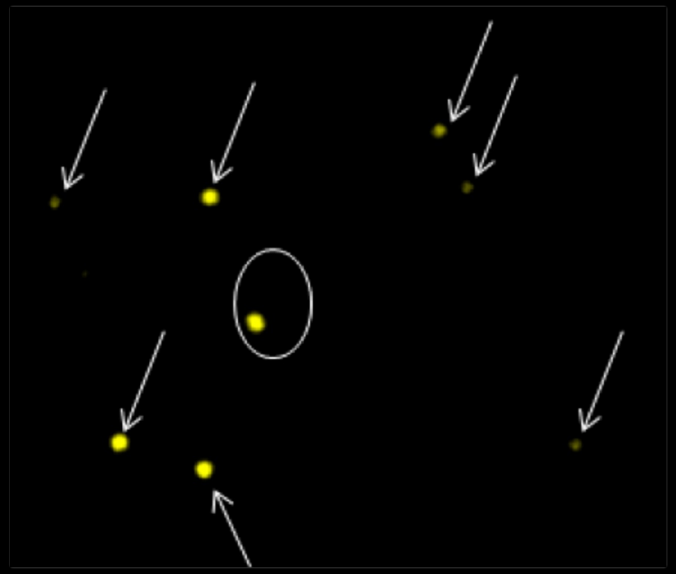
Results

- High prevalence of off-target insertion events in addition to on-target insertions
- Random and Insert Site structural variations observed

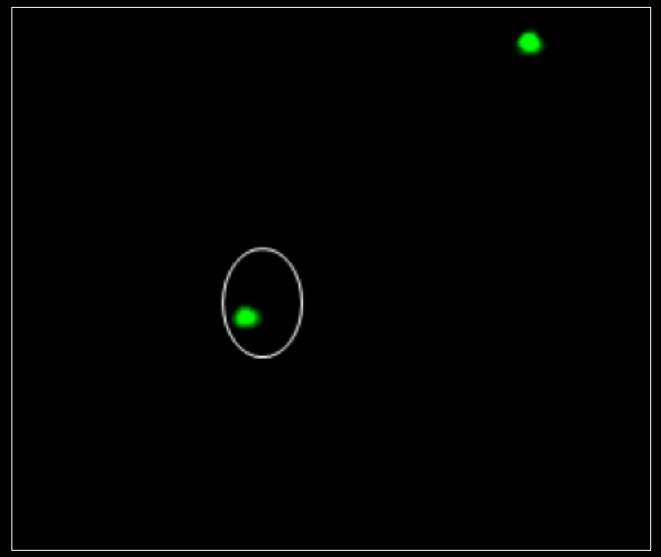
10 Kb Inserts in iPSC: Same Region, 3 Different Color Channels



Fluorescence channels overlay. Insert and bracketing probes both visible on one copy of target chromosome and off-target inserts visible in multiple chromosomes



Yellow fluorescence channel, on-target insertion visible on one homolog (circled) and multiple off-target sites throughout genome



Green fluorescence channel, bracketing probes visible on both homologs of target chromosome. Circled green probe signal shows insertion (as seen from yellow channel) while un-circled does not

Same cell with images broken out by fluorescence channel to make the presence of insert more visible. Arrows indicate off-target insertion events.

dGH in-Site™ Services Pricing

SKU	Product Description	List Price
DGH-001	dGH in-Site™ Adherent/Suspension Culture Development: Thaw, recovery, and harvest optimization	$1,250.00
DGH-002	dGH in-Site™ Standard Probe Production: Design and verification of standard probes	$1,155.00
DGH-003	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 200 cells counted per sample	$8,163.75
DGH-004	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 200 cells counted per sample	$6,029.10
DGH-005	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 200 cells counted per sample	$4,874.10
DGH-006	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 1 probe assay, 200 cells counted per sample	$3,719.10
DGH-007	dGH in-Site™ Custom Probe Production: Design and verification of custom probes	$6,250.00
DGH-008	dGH in-Site™ Adherent/Suspension Metaphase Prep and Harvest	$1,250.00
DGH-009	dGH in-Site™ T-Cell Culture Development: Thaw, recovery, and harvest optimization	$1,500.00
DGH-010	dGH in-Site™ IPSC Cell Culture Development: Thaw, recovery, and harvest optimization	$1,625.00
DGH-011	dGH in-Site™ Whole Blood Culture Development: Thaw, recovery, and harvest optimization	$1,125.00
DGH-012	in-Site T Cells Metaphase Prep and Harvest	$1,500.00
DGH-013	in-Site IPSC Metaphase Prep and Harvest	$1,625.00
DGH-014	in-Site Whole Blood Metaphase Prep and Harvest	$1,125.00
DGH-015	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 20 cells counted per sample	$1,224.56
DGH-016	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 50 cells counted per sample	$2,551.17
DGH-017	dGH in-Site™ Execution and Analysis: Imaging and scoring for 4 probe assay, 20 cells counted per sample with two quadrants per slide	$795.97
DGH-018	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 50 cells counted per sample with two quadrants per slide	$1,658.26
DGH-019	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 20 cells counted per sample with four quadrants per slide	$517.38
DGH-020	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 50 cells counted per sample with four quadrants per slide	$1,077.87
DGH-021	dGH in-Site™ Whole Blood Metaphase Prep - Elongated	$1,687.50
DGH-022	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 100 cells counted per sample	$3,738.04
DGH-023	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 50 cells counted per sample	$1,869.02
DGH-024	dGH in-Site™ NK Cells Metaphase Prep and Harvest	$1,500.00
DGH-025	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 1 probe assay, 100 cells counted per sample	$1,007.50
DGH-026	dGH in-Site™ Calibration per sample	$2,062.50
DGH-027	dGH in-Site™ Assay Execution and Analysis: Imaging and Scoring for 1 probe assay, 50 cells per sample.	$1,875.00
DGH-028	dGH in-Site™ QC one timepoint test per sample	$625.00
DGH-029	dGH in-Site™ NK Cell Culture Development: Thaw, recovery, and harvest optimization	$1,500.00
DGH-030	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 1 probe assay, 20 cells counted per sample	$1,218.75
DGH-031	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 1 probe assay, 500 cells counted per sample	$5,552.93
DGH-032	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 1 probe assay, 600 cells counted per sample	$7,496.46
DGH-033	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 20 cells counted per sample	$1,340.63
DGH-034	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 50 cells counted per sample	$2,010.94
DGH-035	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 100 cells counted per sample	$3,016.41
DGH-036	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 500 cells counted per sample	$6,108.22
DGH-037	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 2 probe assay, 600 cells counted per sample	$8,246.10
DGH-038	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 20 cells counted per sample	$1,474.69
DGH-039	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 500 cells counted per sample	$6,719.04
DGH-040	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 3 probe assay, 600 cells counted per sample	$9,070.71
DGH-041	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 100 cells counted per sample	$3,649.85
DGH-042	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 500 cells counted per sample	$7,390.95
DGH-043	dGH in-Site™ Assay Execution and Analysis: Imaging and scoring for 4 probe assay, 600 cells counted per sample	$9,977.78

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dGH in-Site™ Pricing
Assay Execution and Analysis Breakdown: Imaging and Scoring

Cells counted per sample

Probes per assay	20	50	100	200	500	600
1	DGH-030 $1219	DGH-027 $1828	DGH-025 $2742	DGH-006 $4113	DGH-031 $5553	DGH-032 $7497
2	DGH-033 $1341	DGH-034 $2011	DGH-035 $3016	DGH-005 $4525	DGH-036 $6108	DGH-037 $8246
3	DGH-038 $1475	DGH-023 $2212	DGH-022 $3318	DGH-004 $4977	DGH-039 $6719	DGH-040 $9070
4	DGH-015 $1622	DGH-016 $2433	DGH-041 $3650	DGH-003 $5475	DGH-042 $7391	DGH-043 $9978

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Working with KromaTiD is Simple

Example workflow with KromaTiD running in-Site™ or alternative assays on engineered lines in-house.

KromaTiD is committed to **collaborative excellence** through dedicated project management and **expert technical analysis**.

- Customer
- KromaTiD
- KromaTiD & Customer

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Why You Win With Us

Collaboration: The trusted structural genomics partner for leading gene therapy innovators

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Performance: Gold standard products for the measurement of genomic structure and structural variation

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Scalability: End to end process automation**,** high-throughput analysis, AI meta-analysis

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Excellence: Experienced team of 20 operating today in a world class, 11,000 square foot genomics facility

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Proprietary: Issued patents, broadened applications, trade secret methods, proprietary bioinformatics

Thank you!

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For Research Use Only. Not for use in diagnostic procedures.

to inquire and for orders
visit: kromatid.com
or contact: sales@kromatid.com