



dGH™ : The Next Generation of Metaphase FISH Techniques



June 29, 2023

Presentation by

Christopher Tompkins, Chief Technology Officer
Ivan Perez, Technical Applications Scientist

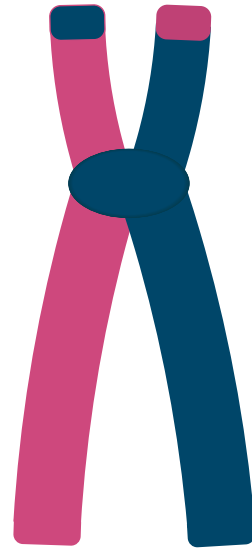
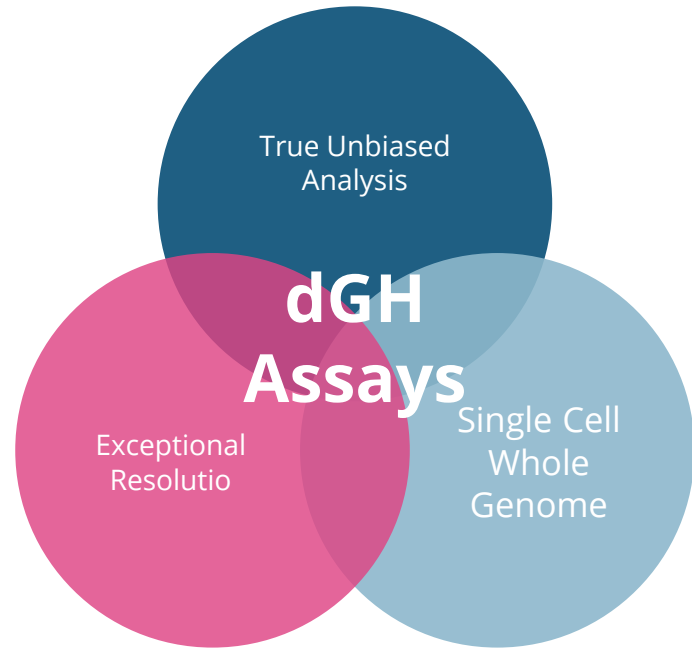


KromaTiD

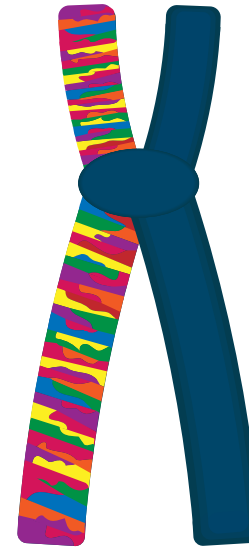
Direct, Definitive Genomics



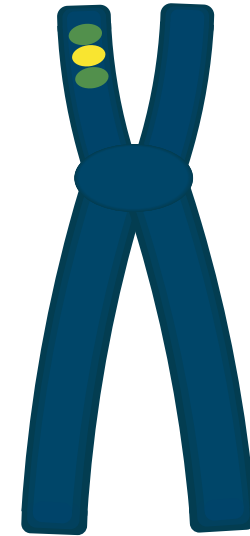
Directional Genomic Hybridization



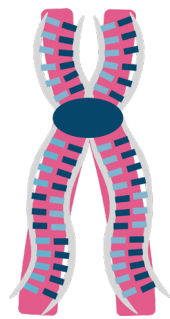
dGH SCREEN



dGH DSCVR

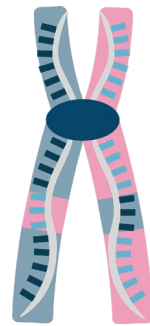


dGH In-Site



FISH

dGH is **NOT** Metaphase FISH:

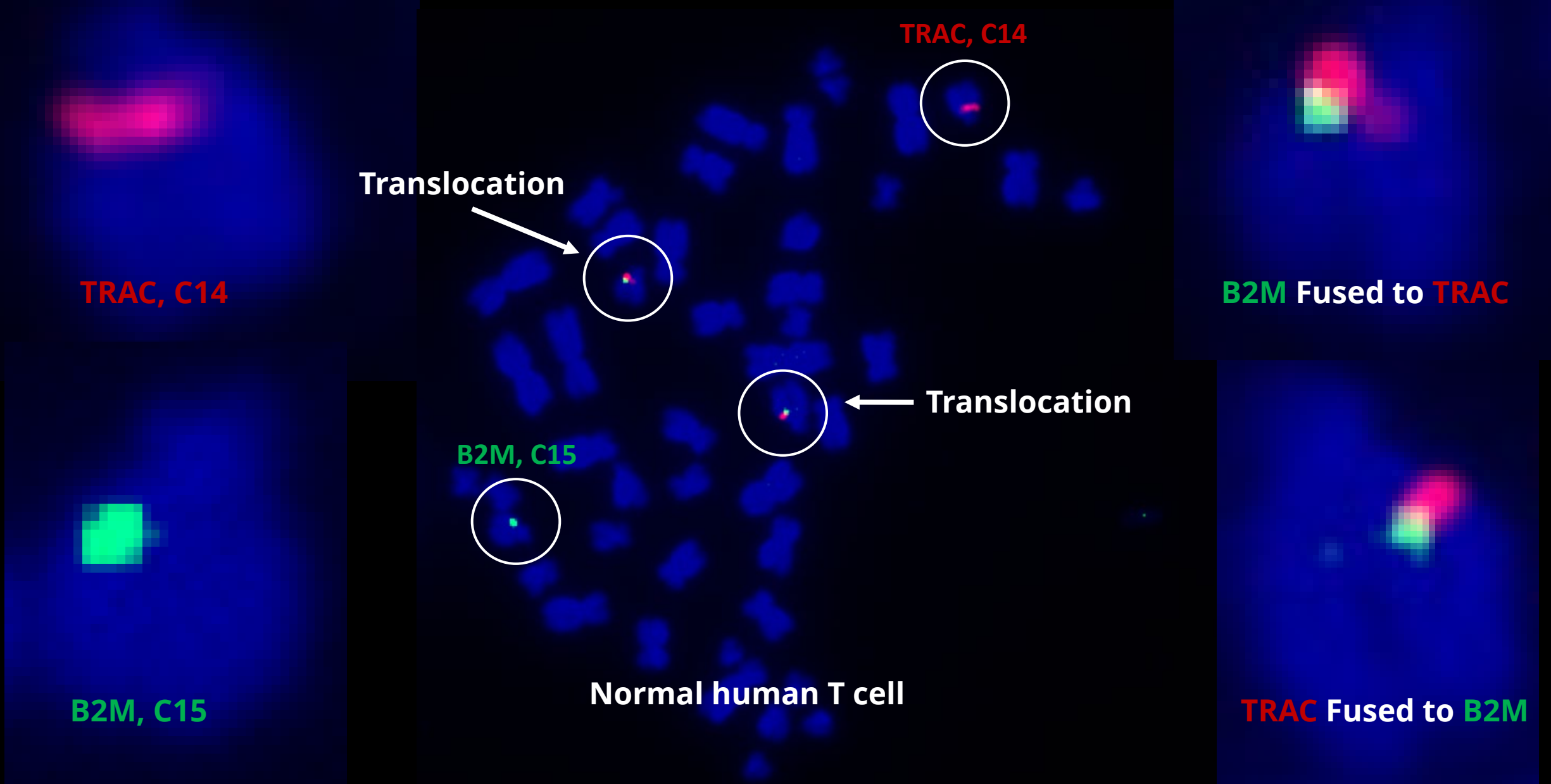


dGH

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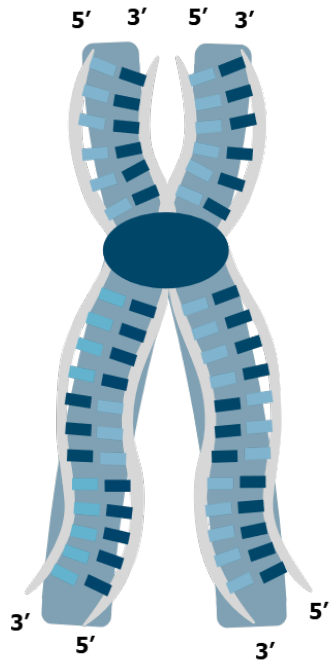
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Balanced Reciprocal Translocation



dGH™ is Chromatid Painting (not Metaphase FISH)

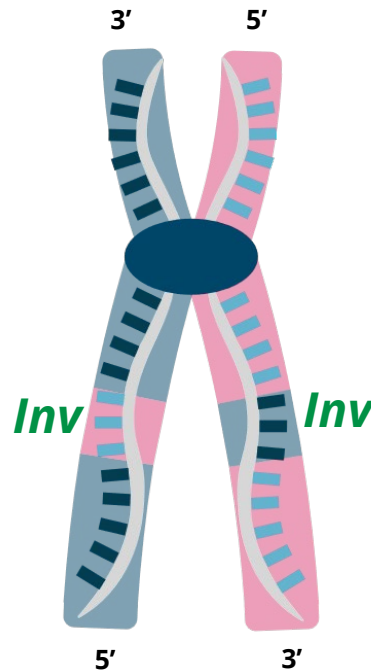
Blue = DAPI Staining of Chromosome Structure



Double Stranded Metaphase Chromatid

Pink = Fluorescently Labeled Hybridization Probes

Daughter Strand Stripping
→
Single Stranded Hybridization Probe



Analyte: Single Stranded dGH Chromatid

DNA Orientation from Image Data

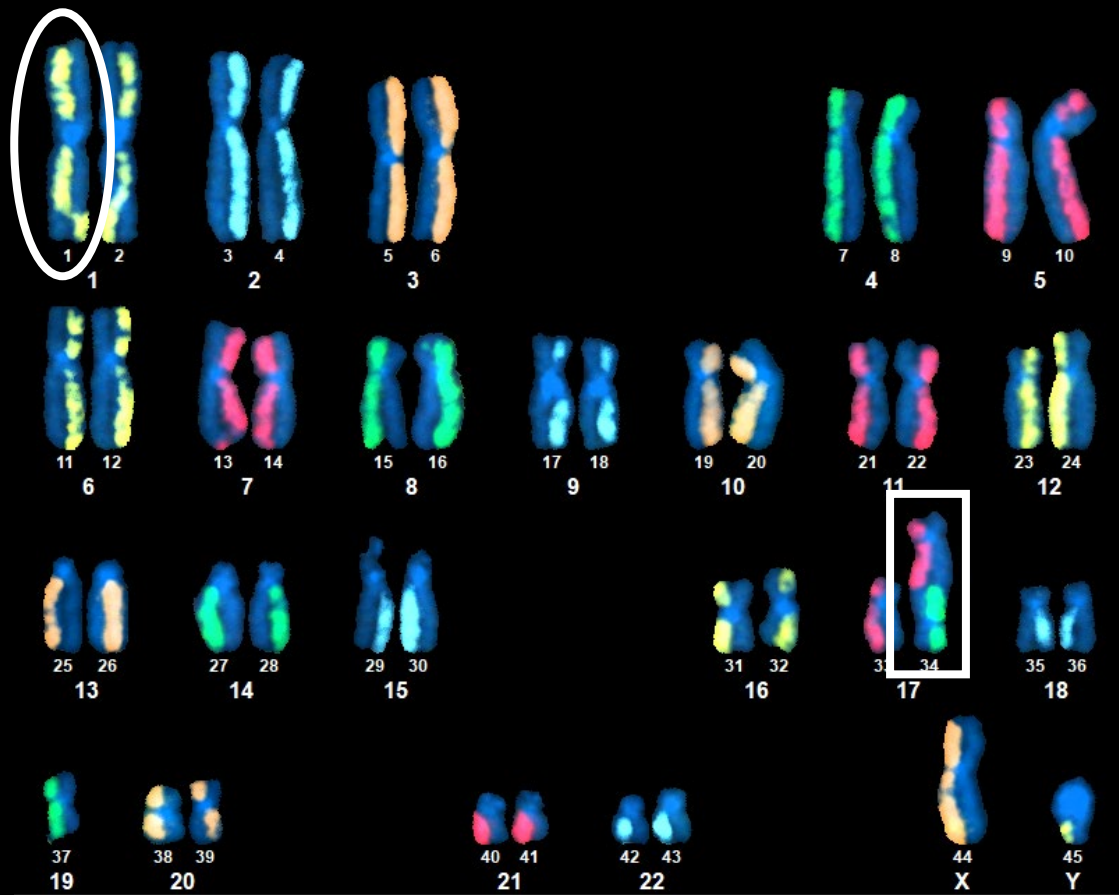
dGH chromosomes contain 2 strands of oppositely oriented, parental DNA only—NO daughter strands

Single-stranded probes are designed to target *only* the Watson strand:

Signal appears on one sister chromatid only.

Signal from inverted targets appear on the opposite sister chromatid

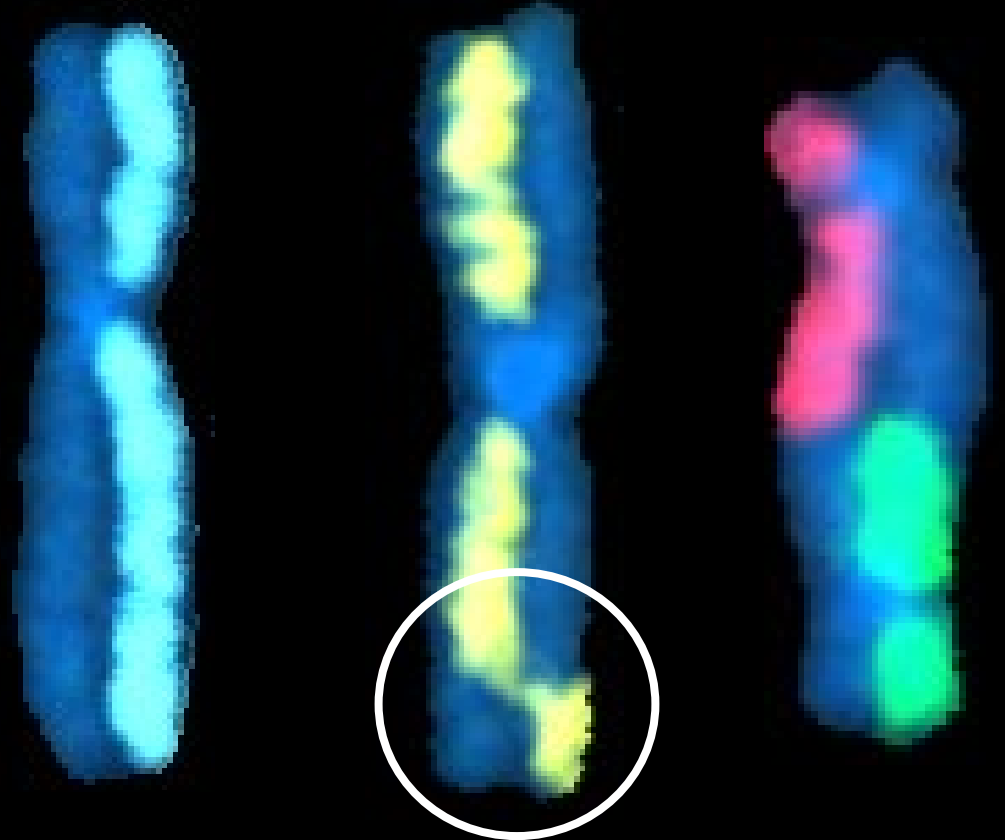
dGH is a Single Cell map of Genomic Structure*



Reference or "Normal
Genome Structure

C1 Telomeric
Inversion

Translocation
C19 to C17



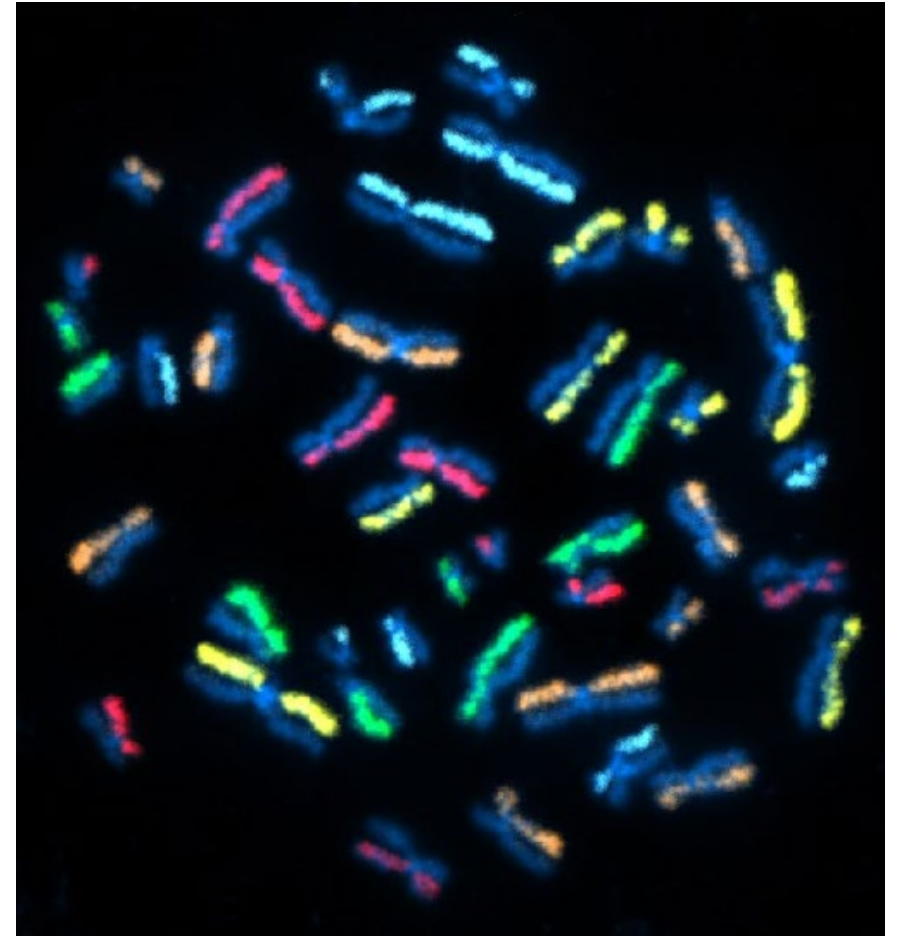
*including DNA damage and structural variation

Only with dGH can you directly measure the orientation
of a genomic target in single cells



Key Points

1. Traditional FISH workflow
2. dGH workflow
3. Key differences (process and data)
4. Closer look at dGH probes
5. Closer look at dGH samples
6. Imaging method and equipment

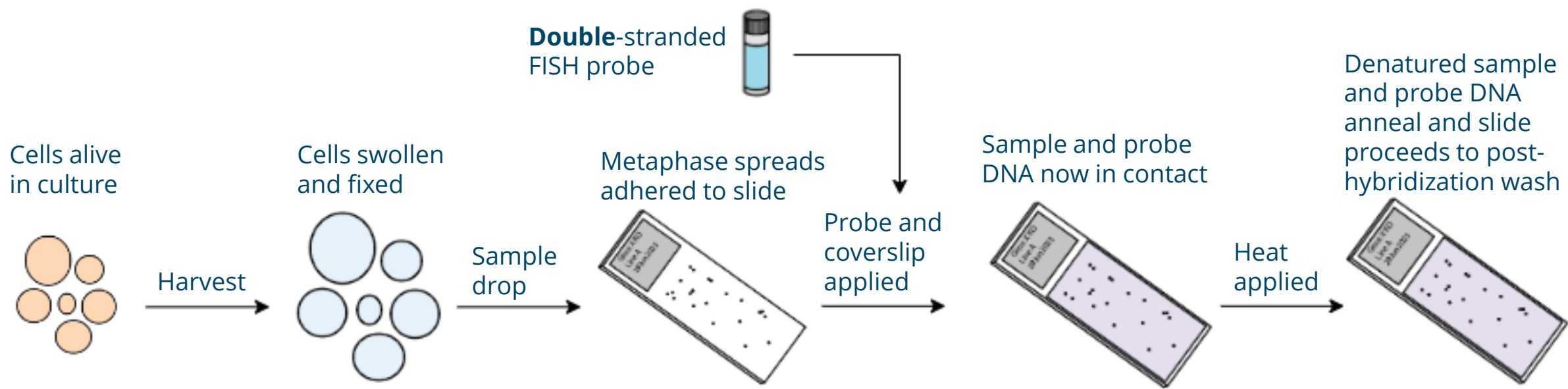


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Traditional Fluorescence In Situ Hybridization (FISH)

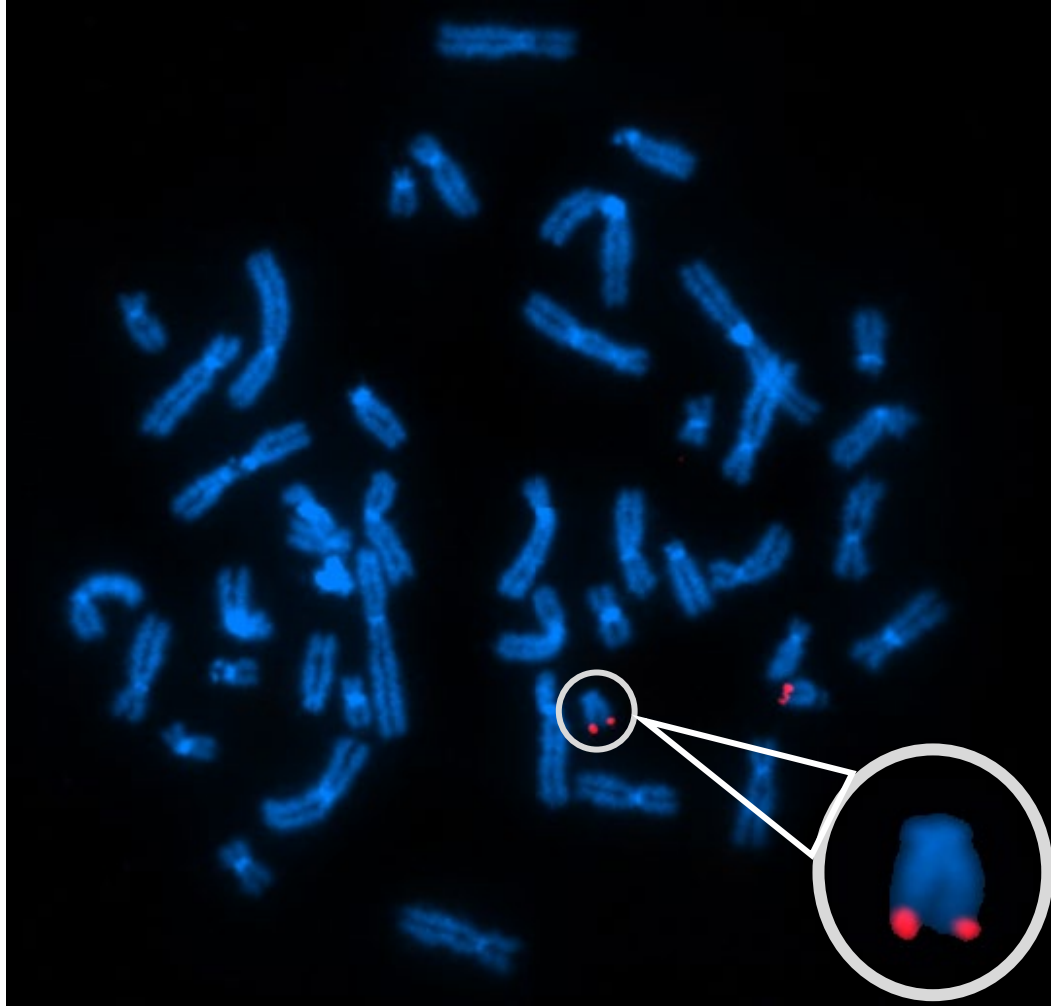


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Example FISH Image



Example of FISH hybridization outcome.

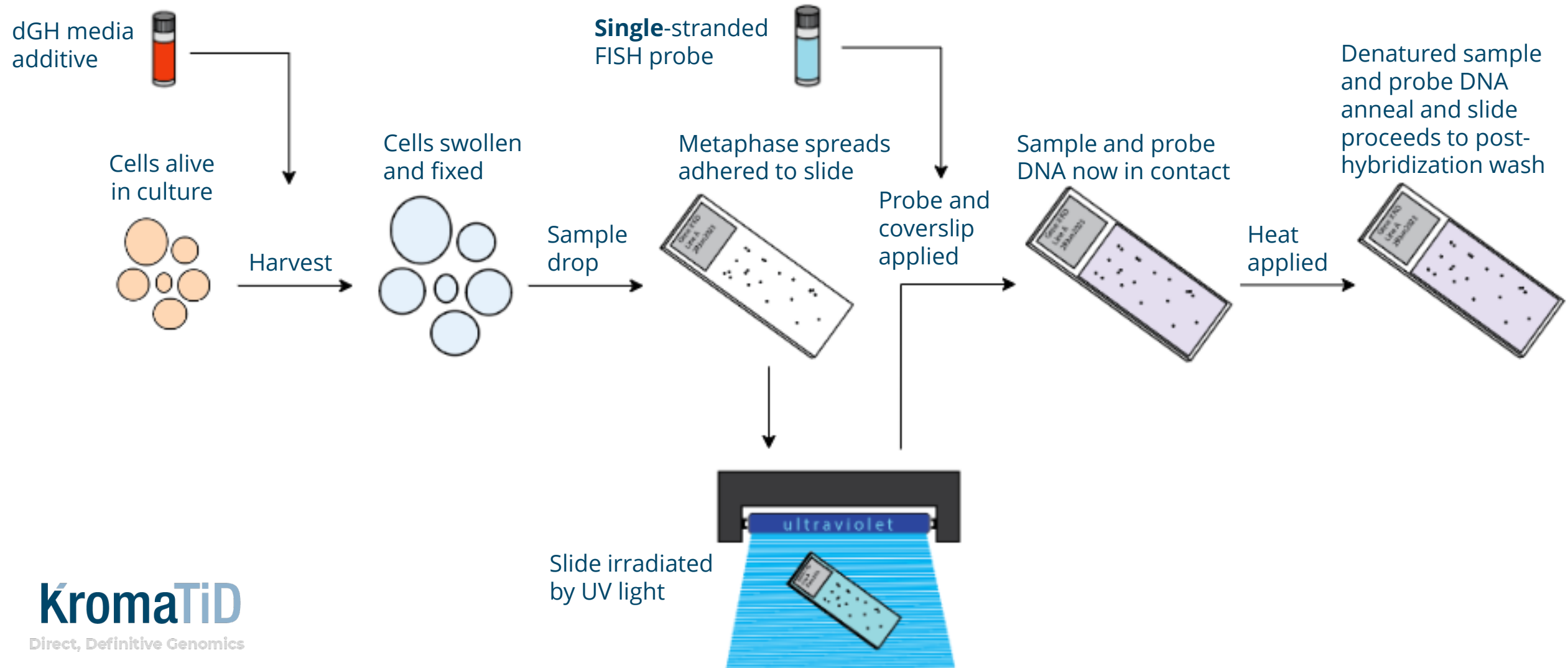
Fluorescence is seen in the subtelomeric region of the q-arm of chromosome 21.

The corresponding target site on each of the two chromatids fluoresces with its own signal.

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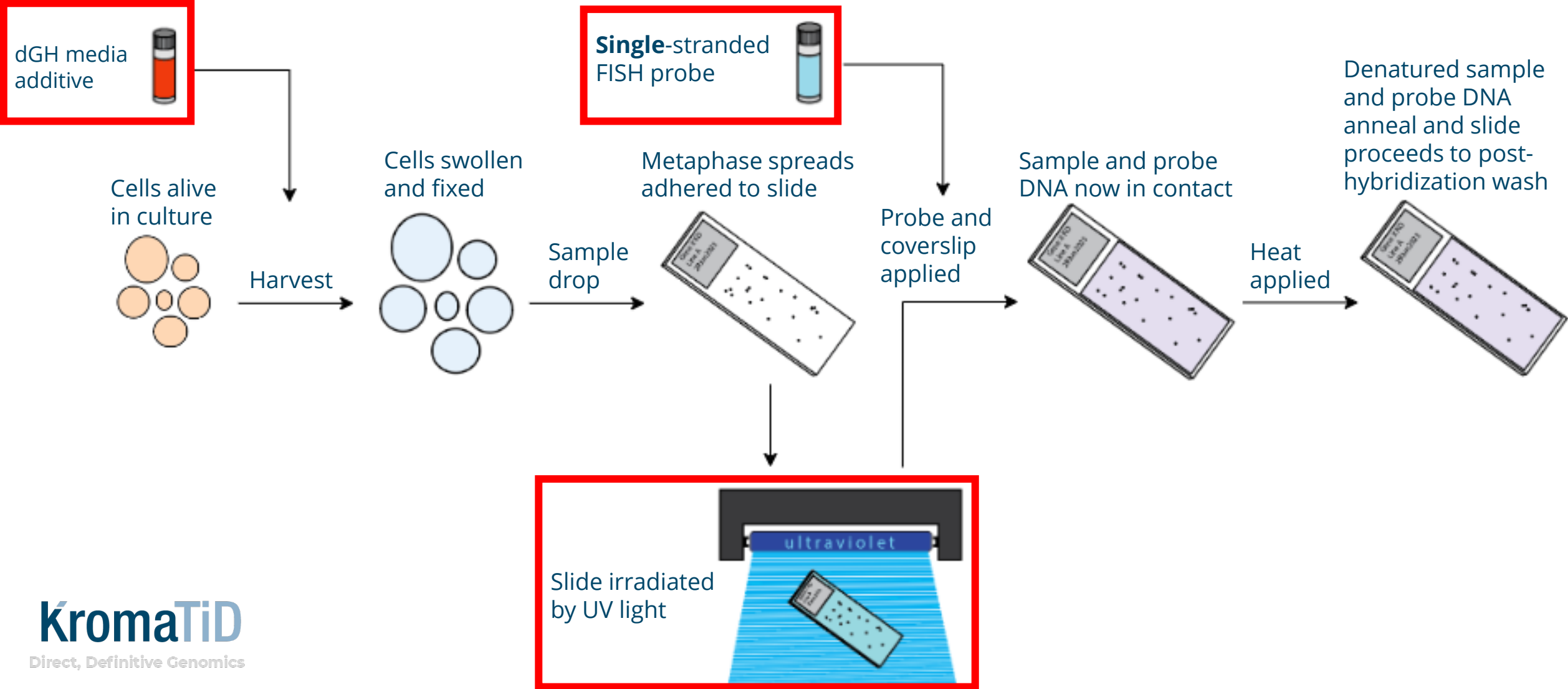
directional Genomic Hybridization (dGH™)



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directional Genomic Hybridization (dGH™)

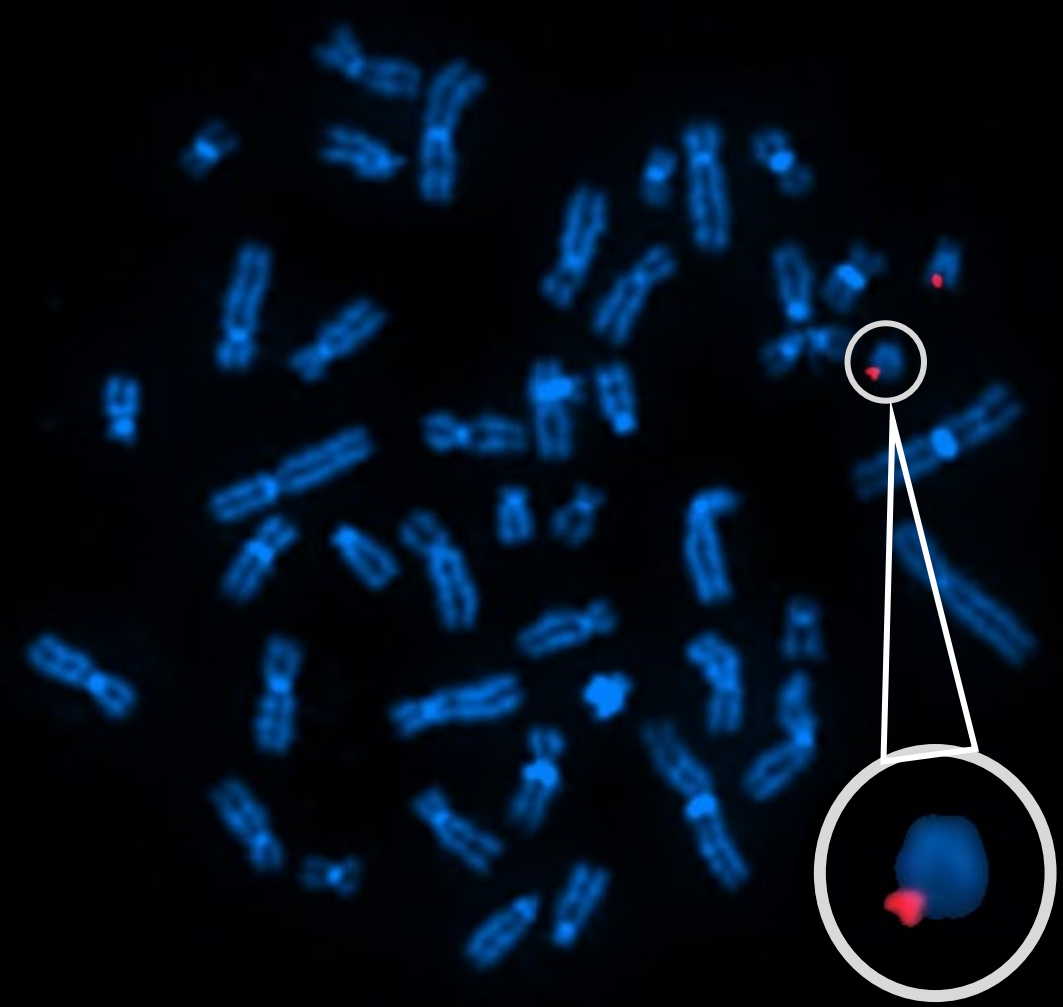


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Example dGH Image



Example of dGH hybridization outcome.

Fluorescence is seen in the same subtelomeric region of chromosome 21.

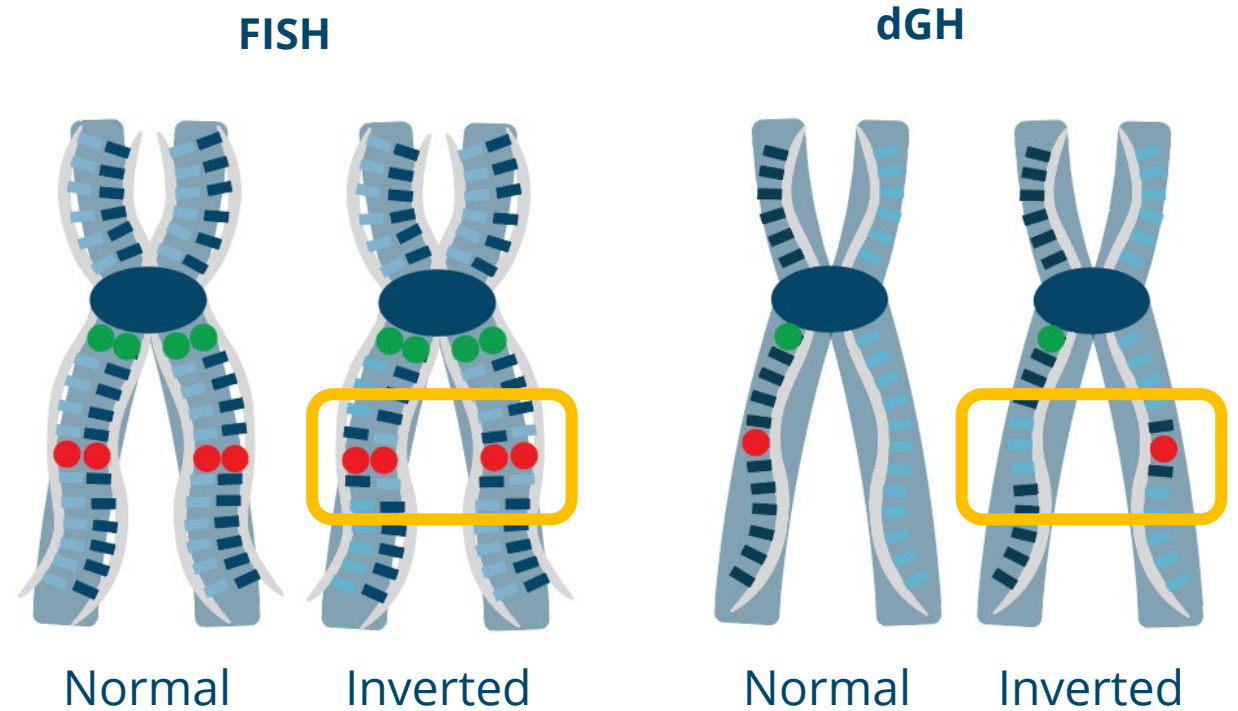
Now the chromosome has only one target site. Only one of the chromatids has DNA complementary to the probe sequences.

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Key Differences in Process and Outcome

1. Cells cultured with dGH media additive
2. dGH probes are **single-stranded**
3. dGH samples are **single-stranded**

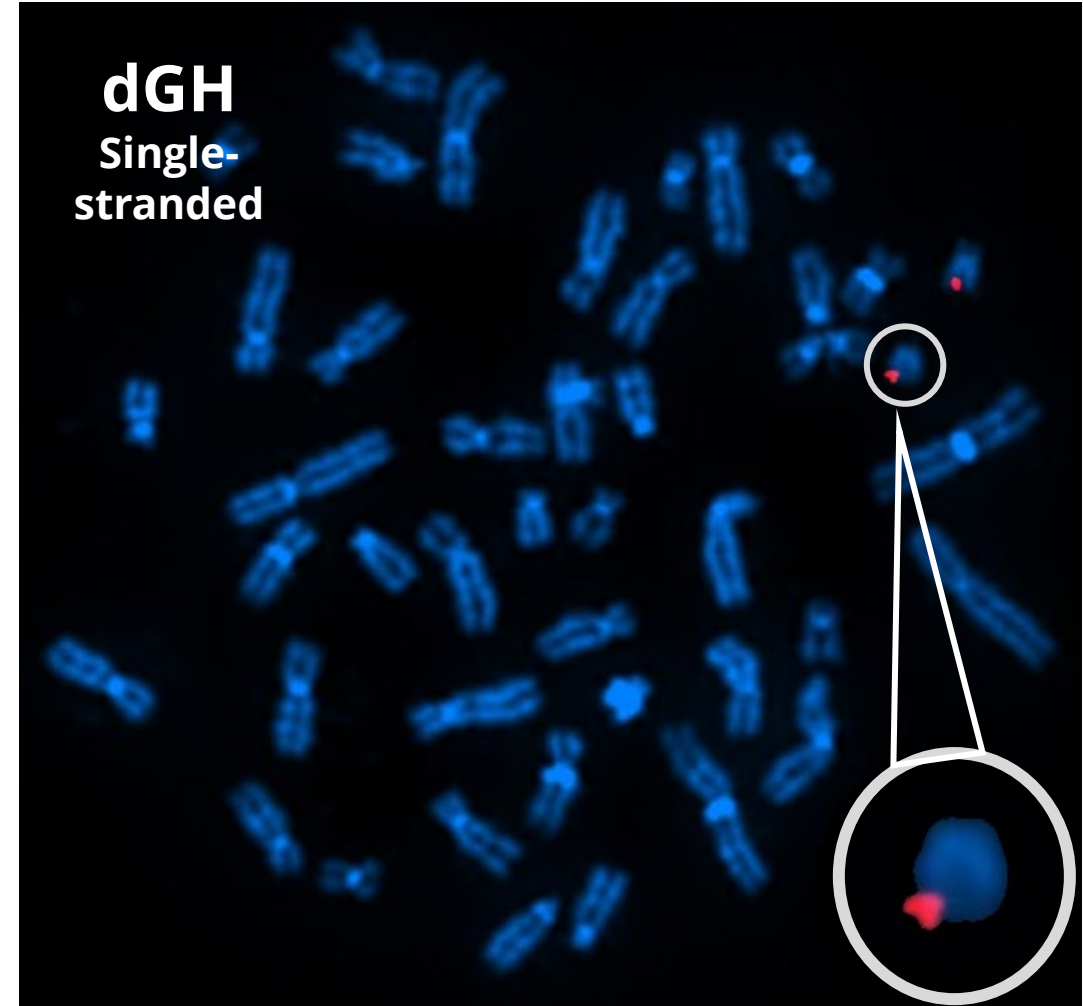
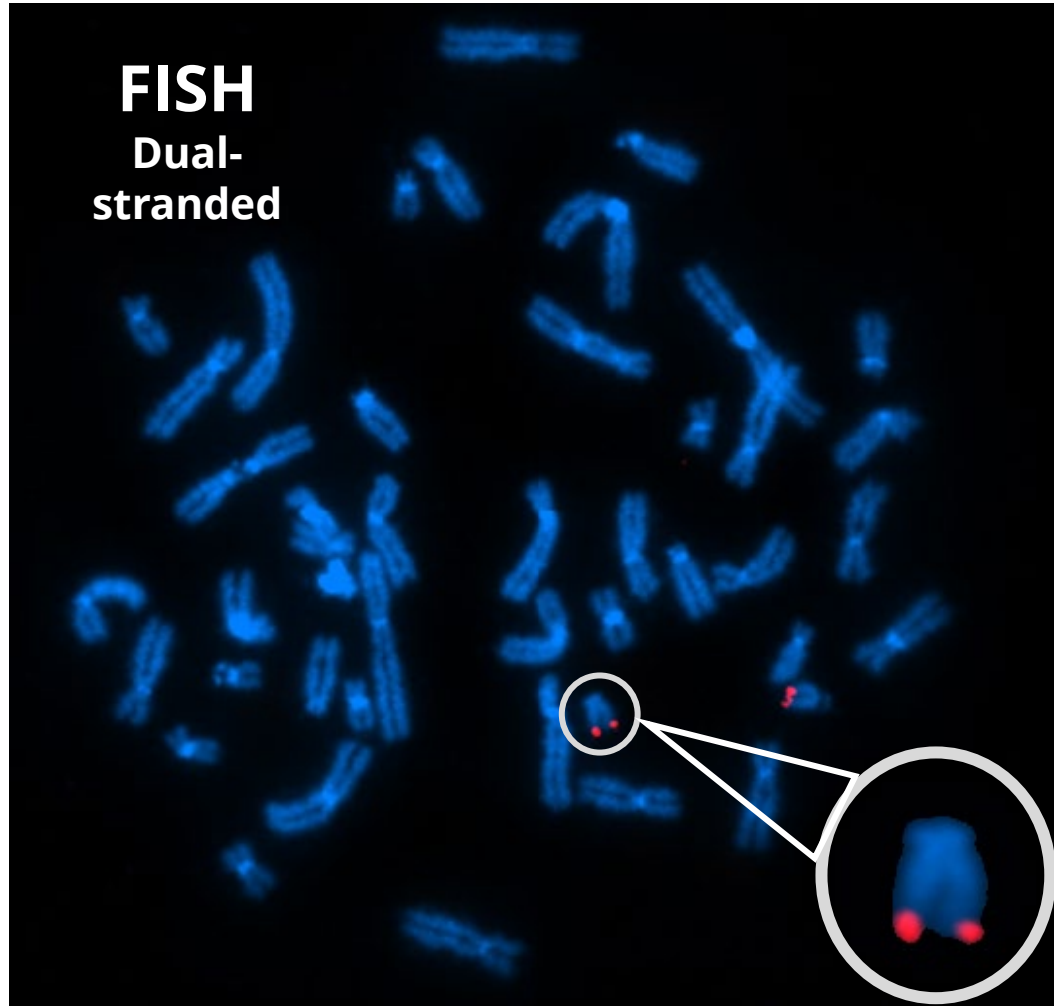


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FISH and dGH Side-by-Side



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dGH Probe Attributes



ATACGACAAGCACTGCACCTGCAAAGCTTTTGTGCAGAGGAGTAGAATGCCGTAGAGTGTTTTCGGCATGGAATGTATGCTCTGTAGACATAAGTT

dGH: Single-stranded and unidirectional.

dGH probes are complementary to only one of the two remaining strands.

TCATCCGATCTCACAAAAGCCAAAGCCGT

TATGCTGTTCAAAGCTTTTGTGCAGAGGAGTAGAATGCCGTAGAGTGTTTTCGGCATGGAATGCTGTAGACATAAAGCACTGCACCTGCAATCAA

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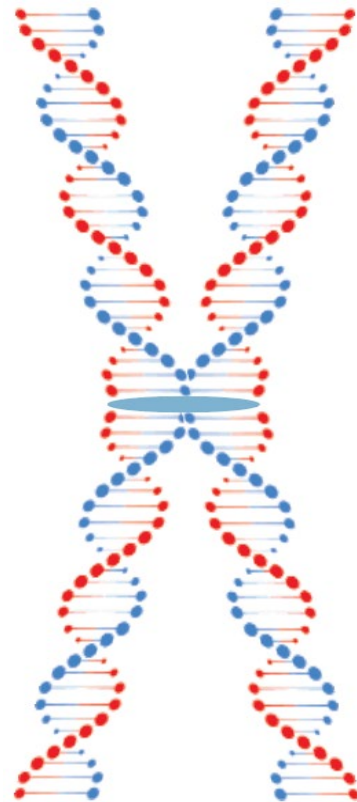
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dGH Sample Attributes

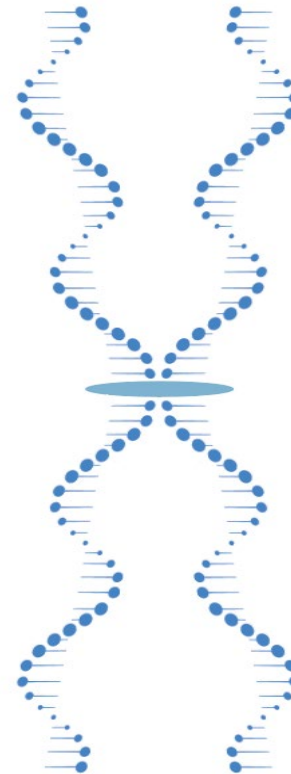
dGH sample DNA before irradiation.

The strand composed of red beads represents the daughter-strands.

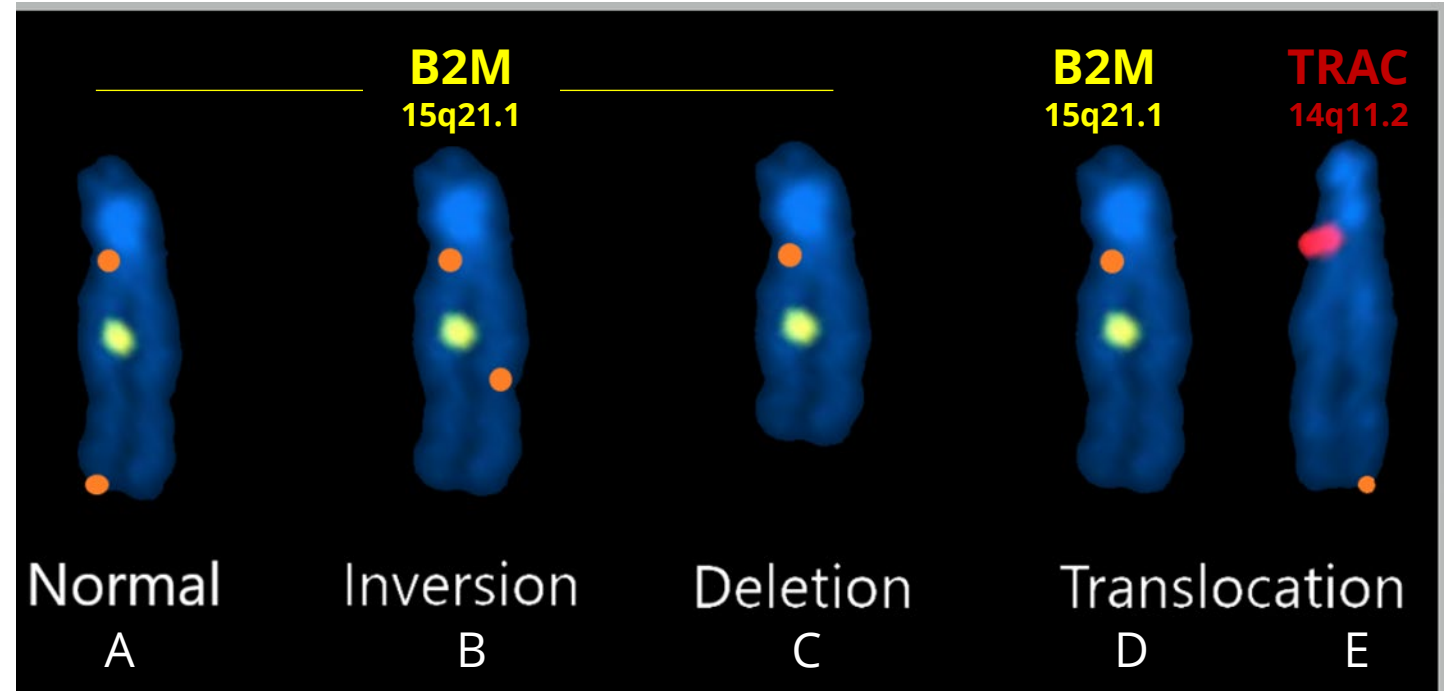
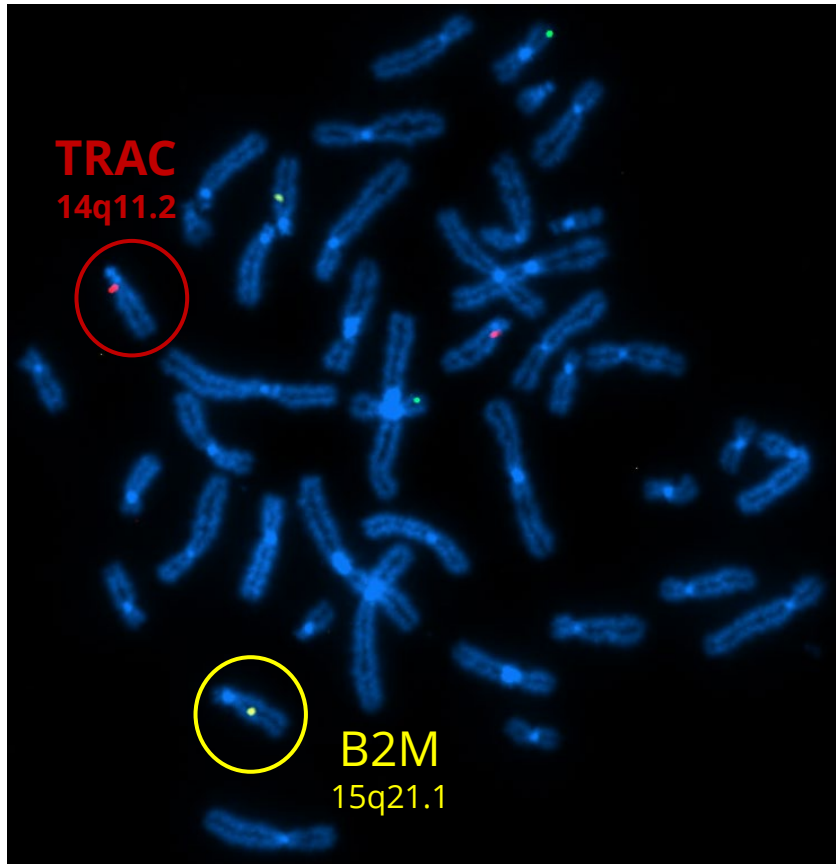


DNA after UV and Exonuclease.

The red strand has been removed, leaving only one parent-strand for each chromatid.



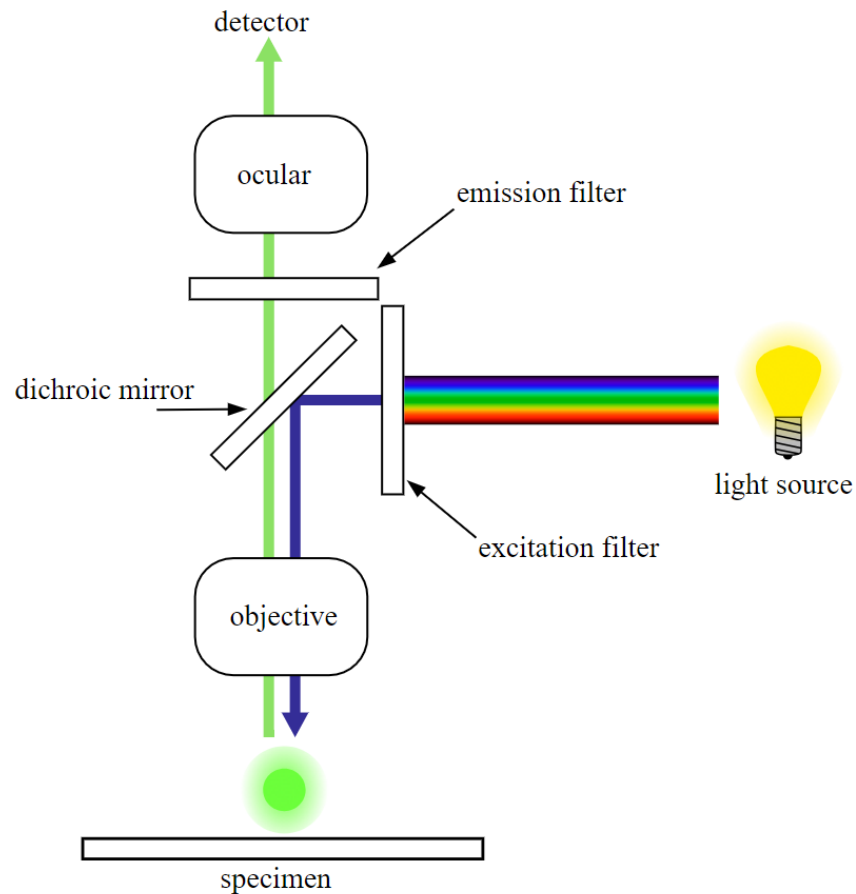
What dGH Can Reveal



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Microscope Requirements to Run dGH™ Assays in Your Lab



Light Source: Two options

- Broad spectrum white light
- Multiple LED and/or laser sources.

Filter Cubes:

- Filters with Excitation/Emission wavelength values corresponding to the fluorophore(s) of the probes to be used.

Objective Lens:

- 60X to 100X magnification
- Oil immersion
- High Numerical Aperture (NA)
- NA of 1.4 is recommended

Camera: Monochrome CMOS or sCMOS

Image: By derivative work: Henry Mühlpfordt (talk)Fluoreszenzmikroskopie_2008-09-28.svg:

Key Takeaway: The Power of Single-Strand Analysis

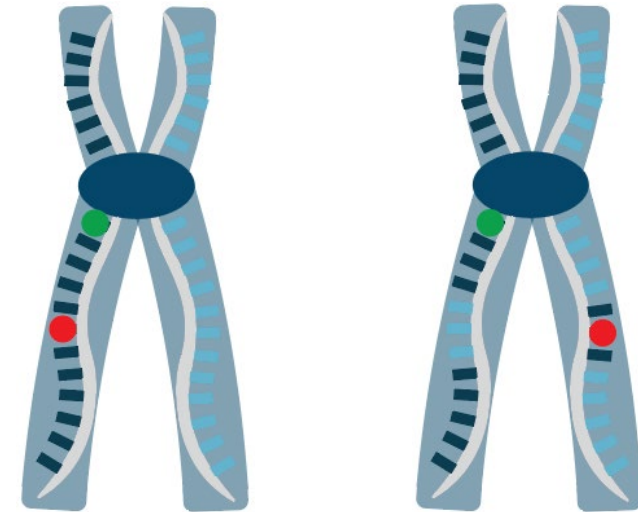
A **single dGH assay** provides confirmation and identification for genomic targets

Sequence, Location and Orientation

dGH sample DNA is **single-stranded**.

dGH probes are also **single-stranded**.

This enables dGH assays to provide genomic information that neither metaphase nor interphase FISH can.



Normal

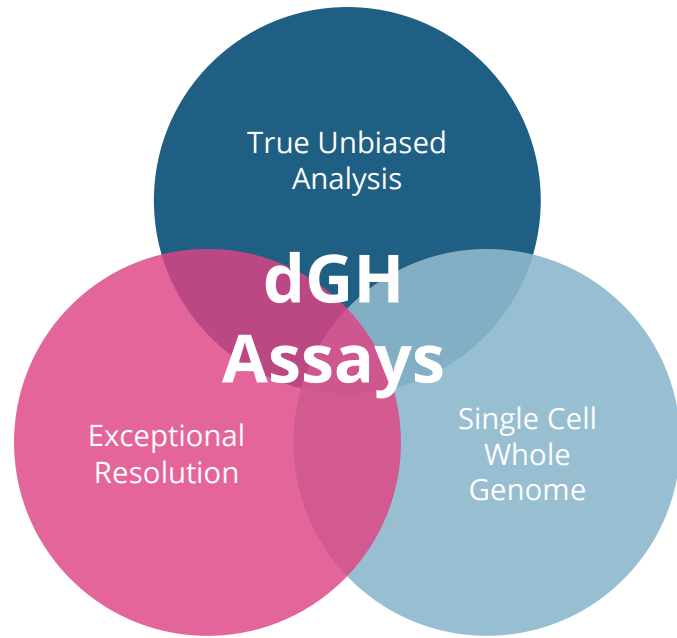
Inverted

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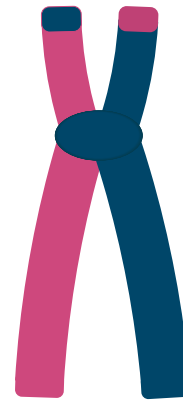
Direct, Definitive Genomics



KromaTiD Products and Services



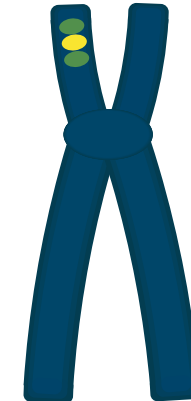
directional Genomic Hybridization (dGH™)



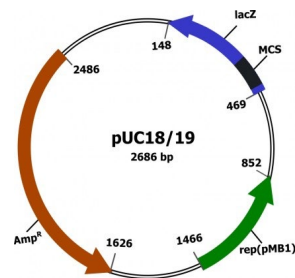
dGH SCREEN



dGH DSCVR



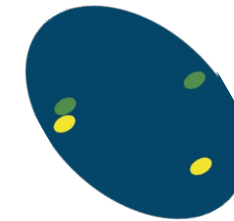
dGH In-Site



Plasmid Services



G-Banding
Orthogonal



Pinpoint FISH
Non-Dividing Cells

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Thank You! Q&A



Christopher Tompkins, Chief Technology Officer
ctompkins@kromatid.com

Ivan Perez, Technical Applications Scientist
iperez@kromatid.com