## See What You've Been Missing with dGH in-Site™ DNA Probes





### Identify the Genomic Information Often Missed by NGS and FISH.

dGH in-Site<sup>™</sup> assays produce powerful, single-cell datasets.

#### dGH in-Site assays provide:

- Direct visualization of all classes of genomic structural variation, including orientation
- Resolution of targets as small as 2 kb
- Exceptional signal-to-noise ratio
- Genomic information unobtainable by either metaphase or interphase FISH





### directional Genomic Hybridization (dGH<sup>™</sup>) Process

**Blue** = DAPI Staining of Chromosome Structure



Daughter strand stripping Hybridization with single-stranded probe



**Pink** = Fluorescently Labeled

**Hybridization Probes** 

Double Stranded Metaphase Chromatid

**KromaTiD** 

**Direct. Definitive Genomics** 



Analyte: Single Stranded dGH Chromatid

#### DNA Orientation Determined 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* one strand:

Signal appears on one chromatid <u>only.</u>

An inversion at a target locus causes fluorescence on the opposite sister chromatid.

Williams, E., & Bailey, S. (2009). Chromosome Orientation Fluorescence In Situ Hybridization (CO-FISH)

### **Uncover the Biology Hidden in FISH**

- Cells are cultured with dGH analog nucleotide media additive and Colcemid<sup>®</sup>
- 2. dGH <u>probes</u> are **single-stranded**
- 3. dGH <u>samples</u> are **single-stranded**
- 4. An inversion hidden in FISH is revealed with dGH in-Site

 Normal
 Inverted

**FISH** 





Normal

Inverted



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### **Pinpoint FISH and dGH in-Site™ Comparison**





**KromaTiD** Direct, Definitive Genomics Side-by-side comparison of Pinpoint FISH and dGH in-Site probes hybridized to the subtelomeric region of the q-arm of chromosome 21

### **What Single-Strand Analysis Can Reveal**



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**Fig 1.** dGH<sup>™</sup> in-Site TRAC 14q11.2 (red), B2M 15q21.1 (yellow) and CIITA 16p13.13 (green) probes in GM12753 control lymphoblastoid cell line.



**Fig 2:** Simulated hybridization of orange-labeled subcentromere and subtelomere dGH in-Site probes on chromosome 15 that is normal (A), inverted (B), deleted (C) and translocated (D) with chromosome 14 (E).

### Example: 3-plex dGH in-Site™ Subtelomere Assay





#### Multiplexed dGH in-Site assay results.

dGH<sup>™</sup> in-Site subtelomere probes for chromosomes Yp (yellow), Xp (green), and 21q (red) in GM12753 control lymphoblastoid cell line.



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#### **Example: Balanced Reciprocal Translocation**



#### **Example: Unbalanced Reciprocal Translocation**



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### Build and Execute Your Own dGH in-Site<sup>™</sup> Assays

**Design** your in-Site assay by choosing from our extensive list of subcentromere, subtelomere and gene-specific probes, or by having our Kromatid specialists create a custom probe to your specifications.

**Prepare cell samples** using the <u>dGH Cell Preparation</u> and <u>Metaphase Slide Preparation</u> protocols to obtain single-stranded metaphase spreads.

**Hybridize** your set of dGH in-Site single-stranded probes to your chromosome spreads with the <u>dGH</u> <u>Probe Hybridization Protocol</u> for the highest-quality results.

View and analyze your outcomes using standard fluorescence microscopy hardware.





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### Build and Execute Your dGH in-Site<sup>™</sup> Assays

#### Subcentromere and Subtelomere dGH Probes

Fluorophore	Size	Price
Atto550/Spectrum Orange	10 Tests	\$195.00
TexRed	10 Tests	\$195.00
6-FAM/Spectrum Green	10 Tests	\$195.00
Atto643/647/Cy5	10 Tests	\$195.00

Find the complete lists of dGH in-Site probes and pricing on our website:

<u>36 Subcentromere dGH in-Site Probes</u> <u>42 Subtelomere dGH in-Site Probes</u>

#### CAR Gene-Specific dGH in-Site Probes

Product	Fluorophore	Size	Price
dGH™ Probe B2M (15q21.1)	6-FAM/Spectrum Green	10 Tests	\$800.00
dGH™ Probe TRAC (14q11.2)	TexRed	10 Tests	\$800.00
dGH™ Probe CIITA (16p13.13)	ATTO550/Spectrum Orange	10 Tests	\$800.00
dGH™ Probe PDCD1 (2q37.3 )	ATTO550/Spectrum Orange	10 Tests	\$800.00
dGH in-Site™ Endogenous CD19	ATTO643/647/Cy5	10 Tests	\$800.00
dGH in-Site™ Custom Probe	Consult with Technical Support	10 Tests	\$6,250.00
<u>dGH in-Site™ CAR-T Probe Kit</u>	B2M 6-FAM/Spectrum Green; TRAC TexRed	10 Tests	\$1,500.00

KromaTiD specialists can help you:

- Combine our catalog probes into a multiplexed assay uniquely designed for you.
- Design a custom probe to any genomic locus, transgene or other inserted sequence.
- Provide image and data analysis support.



### **Recommended Microscope Configuration for dGH™ Assays**



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:

### **KromaTiD Products and Services**

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<u>www.kromatid.com</u>

### dGH<sup>™</sup> Assays Detect all Classes of Structural Variants

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**Direct, Definitive Genomics** 

# **Thank You!**

Contact us today at <u>techsupport@kromatid.com</u> with questions or <u>sales@kromatid.com</u> to order.

