

The Advantage of Bioinformatics-Enabled Design & Synthesis of Pinpoint FISH™ Probes



- KromaTiD Overview
- Synthetic Oligo Probe Design and Manufacturing
- Pinpoint FISH™ Probes and Applications
- Customizing the Design Process
- Ordering Pinpoint FISH Probes

KromaTiD

Direct, Definitive Genomics

Presented by Ivan Perez | Technical Applications Scientist, KromaTiD

Overview of KromaTiD

KromaTiD is staffed by a committed team specializing in delivering top-tier genomic tools and services.

Located in Longmont, Colorado, our primary mission is to offer the tools and assistance required to propel the progress of genomic medicine and research.

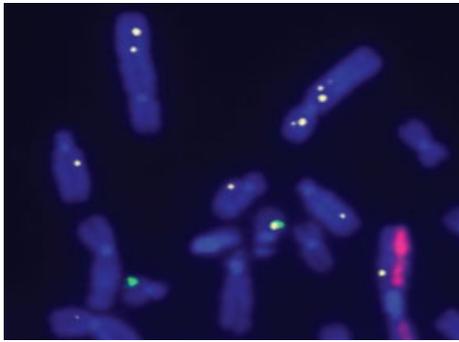


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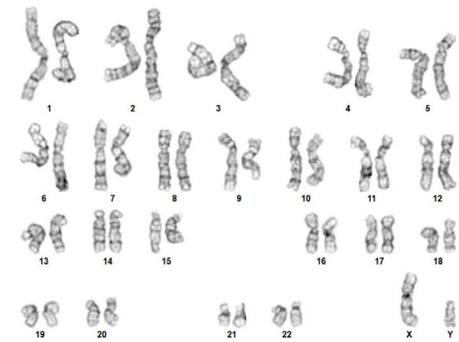
Direct, Definitive Genomics



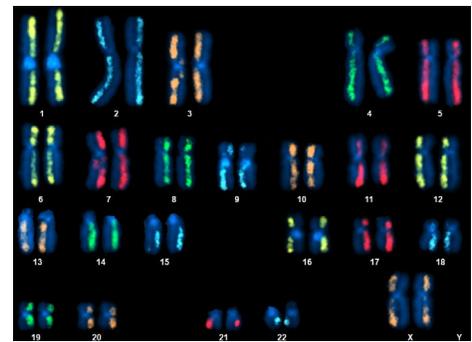
KromaTiD Offers Unique Products and Services



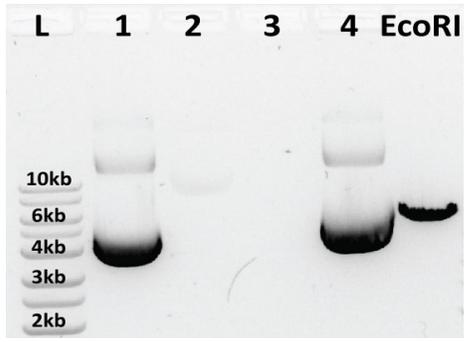
dGH in-Site™



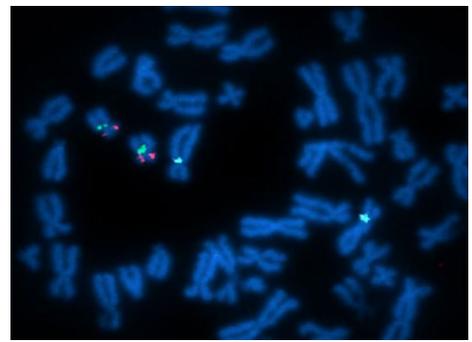
G-Banding



dGH SCREEN™



Plasmid Manufacturing



Pinpoint FISH™

KromaTiD provides a molecular toolkit which gives scientists unmatched insight into gene editing outcomes, indicators of genomic instability, and disease biomarkers.

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Human Genome Project and Bioinformatics Design



The HGP was completed in April 2003.

The genome can be mined for candidate sequences meeting key requirements:

- Target binding strength: GC%, T_m , length, ...
- Uniqueness: Alignment and masking algorithms
- Interactions: Screening for hairpin loops, dimers, ...

Probes can be designed against any published non-human genomes too.

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Streamlined Manufacturing Process: Synthesis and Labeling of Pinpoint FISH™ Probes



In Silico Design



Fluorescently Labeled Oligonucleotides

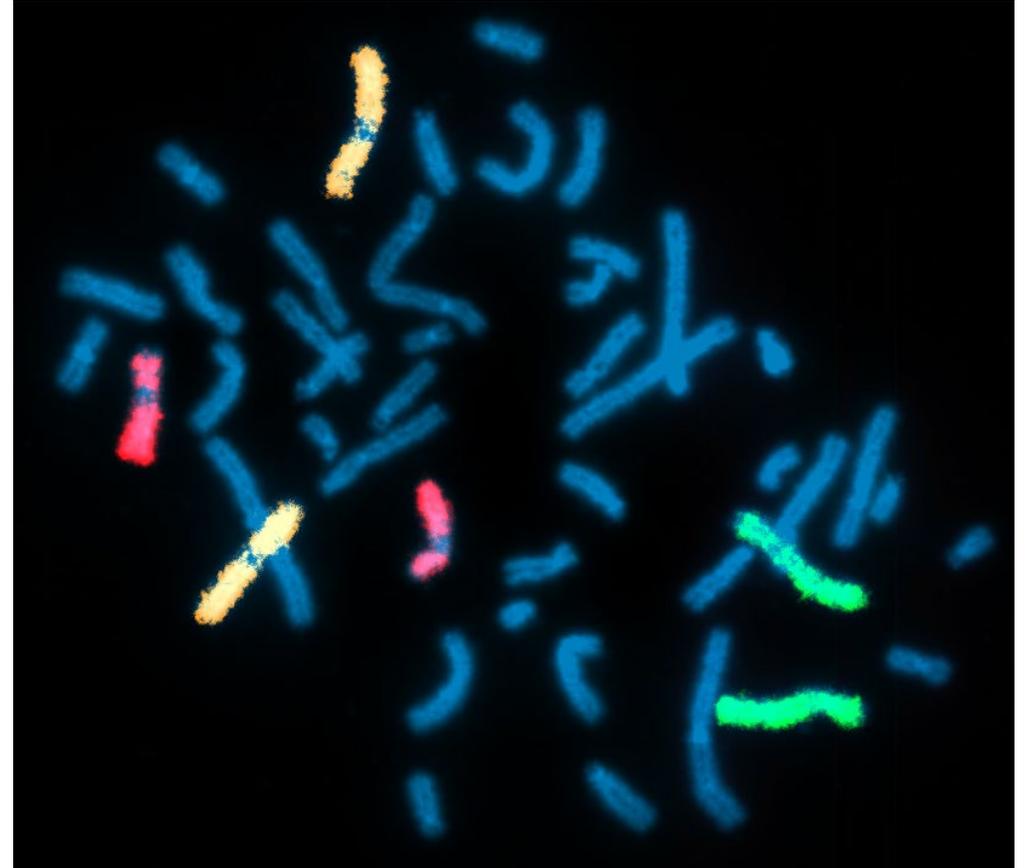
- Selected sequences are synthesized, amplified and fluorescently labeled.
- Reduced hands-on time during manufacturing lowers cost for the user.

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Benefits of Synthetic Oligonucleotide Pinpoint FISH™ Probes

- Superior Signal-to-Noise Ratio: Off-target binding eliminated algorithmically and confirmed through experimental QC testing post-manufacturing.
- High Resolution: High signal-to-noise ratio allows visualization of very small targets. (<5 kb)
- Reduced Cost: Manufacturing is more streamlined.
- Tailored Designs: Bioinformatic design customization can address unique research needs.



HD chromosome paints multiplex targeting chromosome 3 (orange), chromosome 4 (green), and chromosome 7 (red) in immortalized human leukocyte control cell line.

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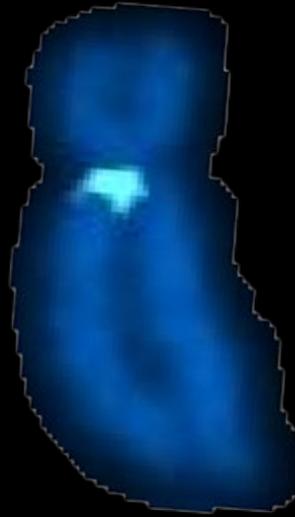
Subcentromeric Pinpoint FISH™ Probes

Subcentromere Probes

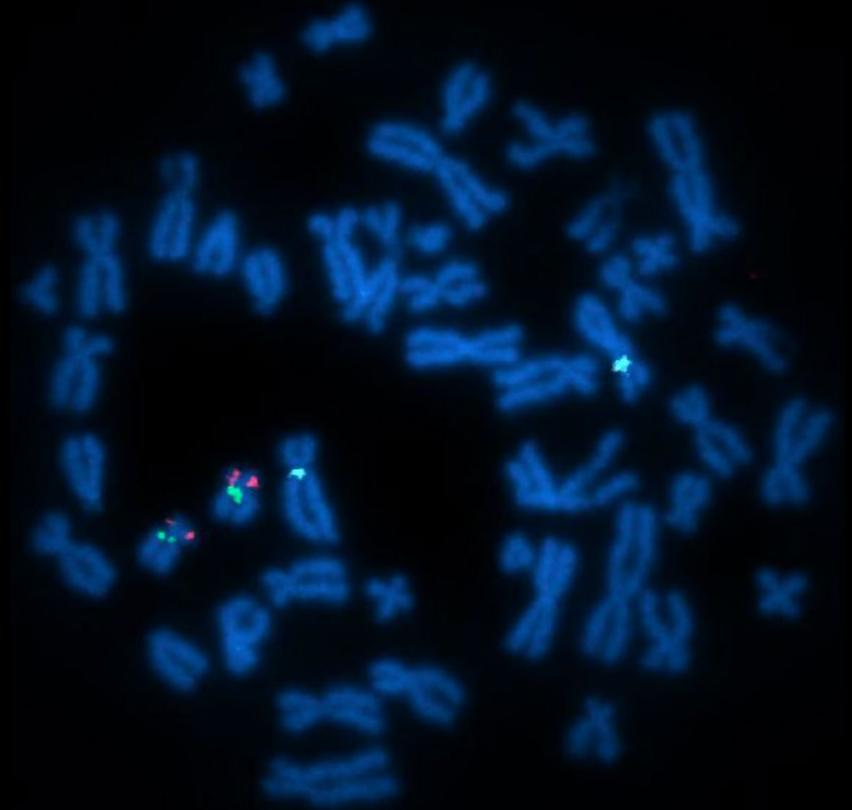
Probe category targeting arm-specific, pericentric genomic DNA

Applications

- Centromere enumeration
- Control signal in multiplex
- Detection of rearrangements at target locus
 - Translocations
 - Inversions
 - Complex rearrangements



Chr 5q
subcentromere

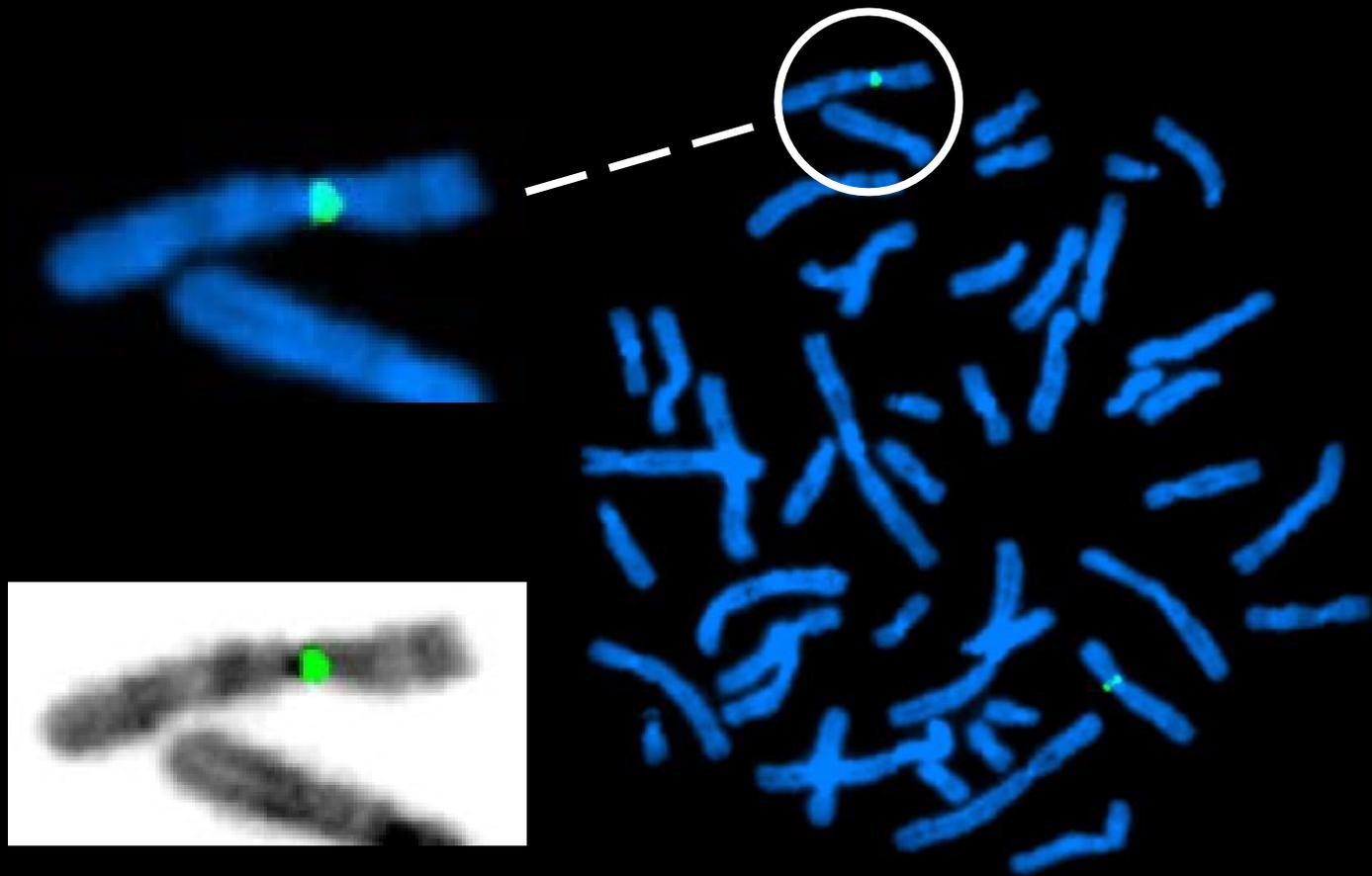


Chromosome 5q subcentromere probe (aqua) in multiplex hybridization with KromaTiD's TP53/CEP17 kit in human control metaphase spread.

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Chromosome 7p Subcentromere Probe



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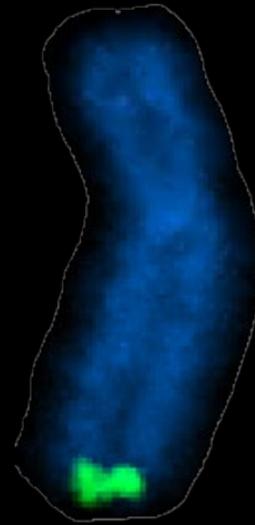
Subtelomeric Pinpoint FISH™ Probes

Subtelomere Probes:

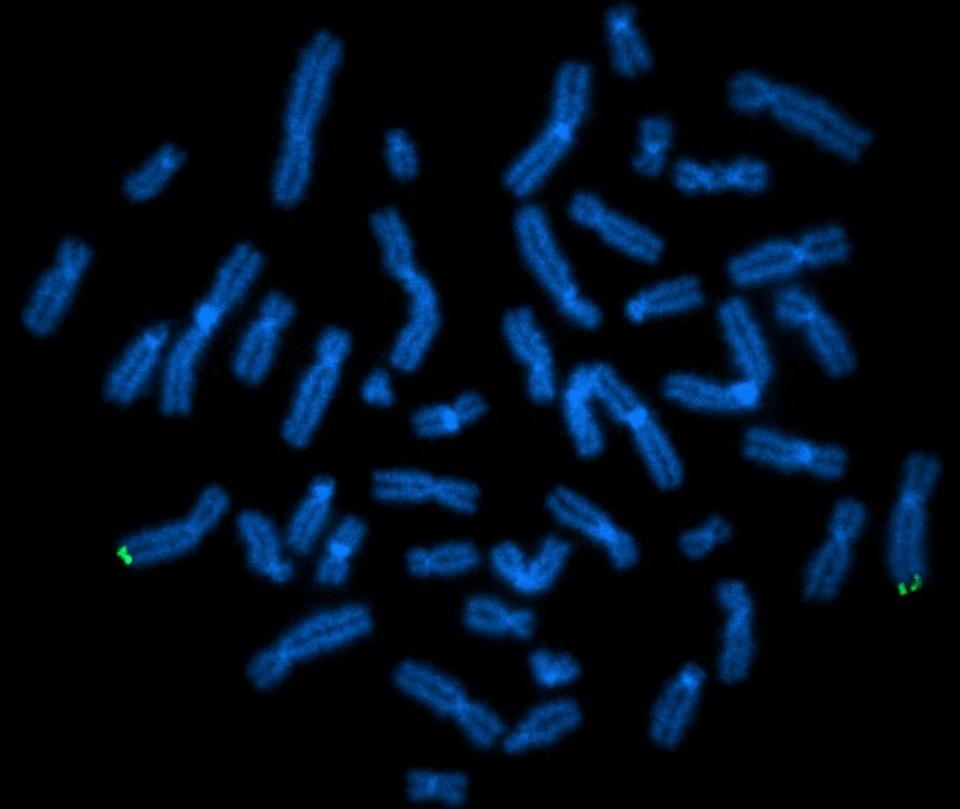
Probe category targeting arm-specific, peritelomeric genomic DNA

Applications:

- Chromosome enumeration
- Control signal in multiplex
- Detection of rearrangements at target locus
 - Any terminal deletion
 - Translocations
 - Complex rearrangements Generate arm-specific data



Chr 6q
subtelomere

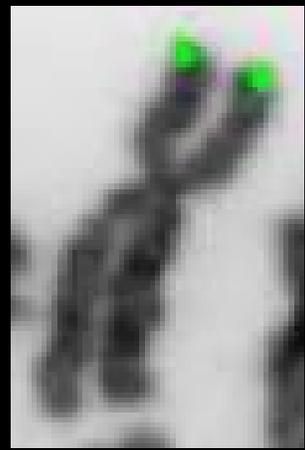
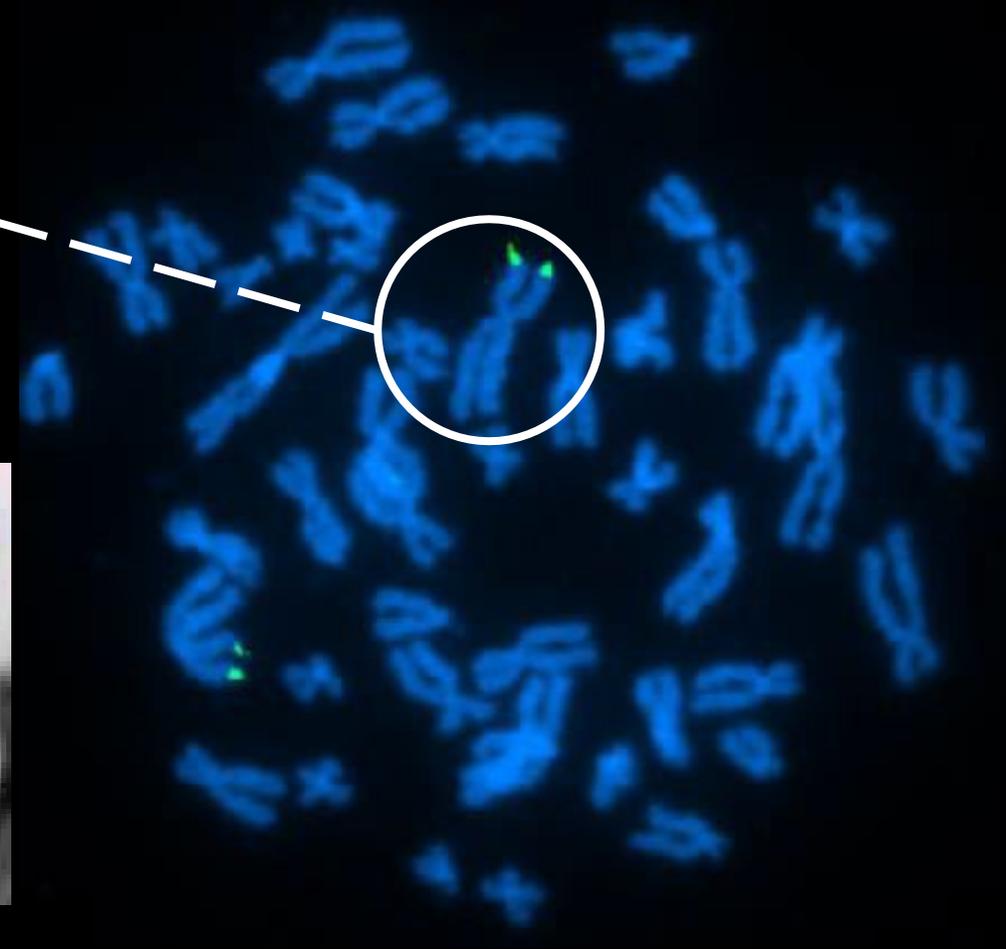


Chromosome 6q subtelomere probe (green) hybridized in human control metaphase spread.

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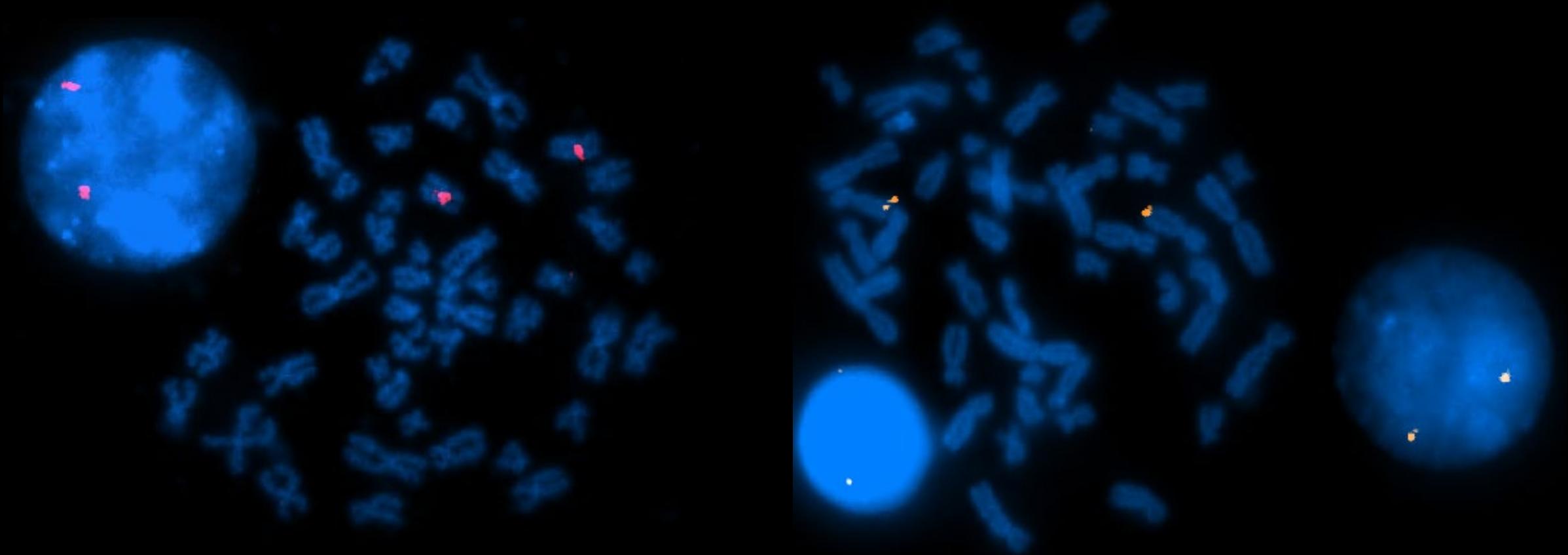
Subtelomeric Pinpoint FISH™ Probes



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Pinpoint FISH™ Metaphase-Interphase Examples



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(Left) Q-arm subcentromere probe for chromosome X labeled in red.
(Right) Q-arm subtelomere probe for chromosome 7 labeled in orange.

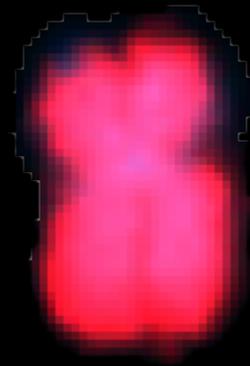
HD Whole Chromosome Paints

High-Density (HD) Paints

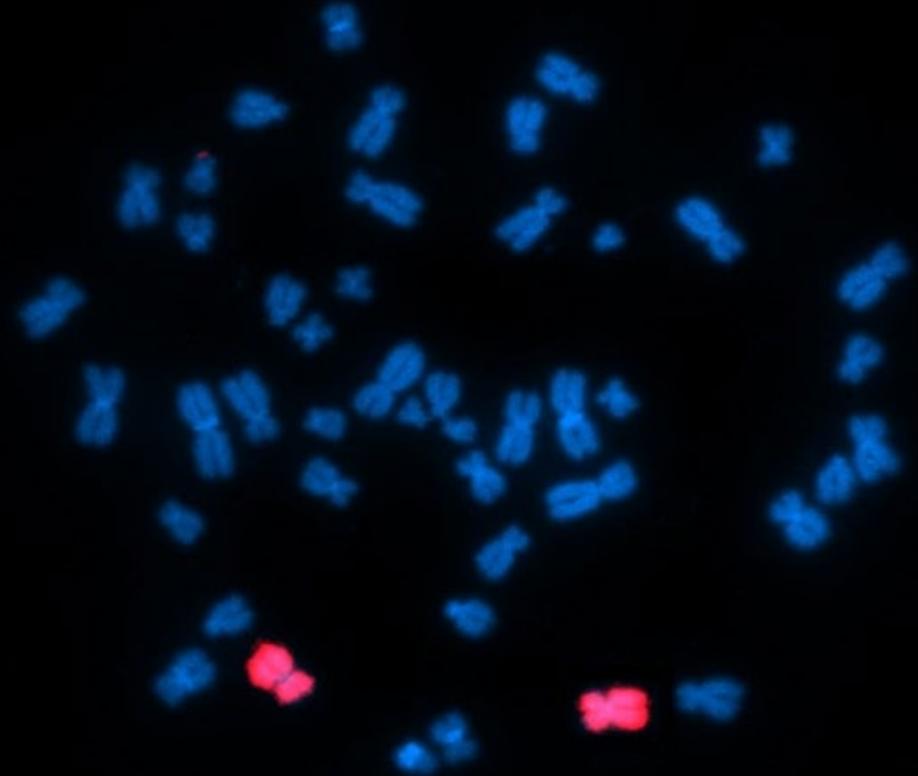
Probe category targeting all qualifying unique-sequence loci along the length of the chromosome, resulting in blanket fluorescence.

Applications

- For both interphase and metaphase studies
- Detection of aneuploidy
- Detection of polyploidy by multiplexing multiple paints at once
- Detection of structural rearrangements
- Spatial localization of unwound chromatin within nuclei



Chr 11
HD paint



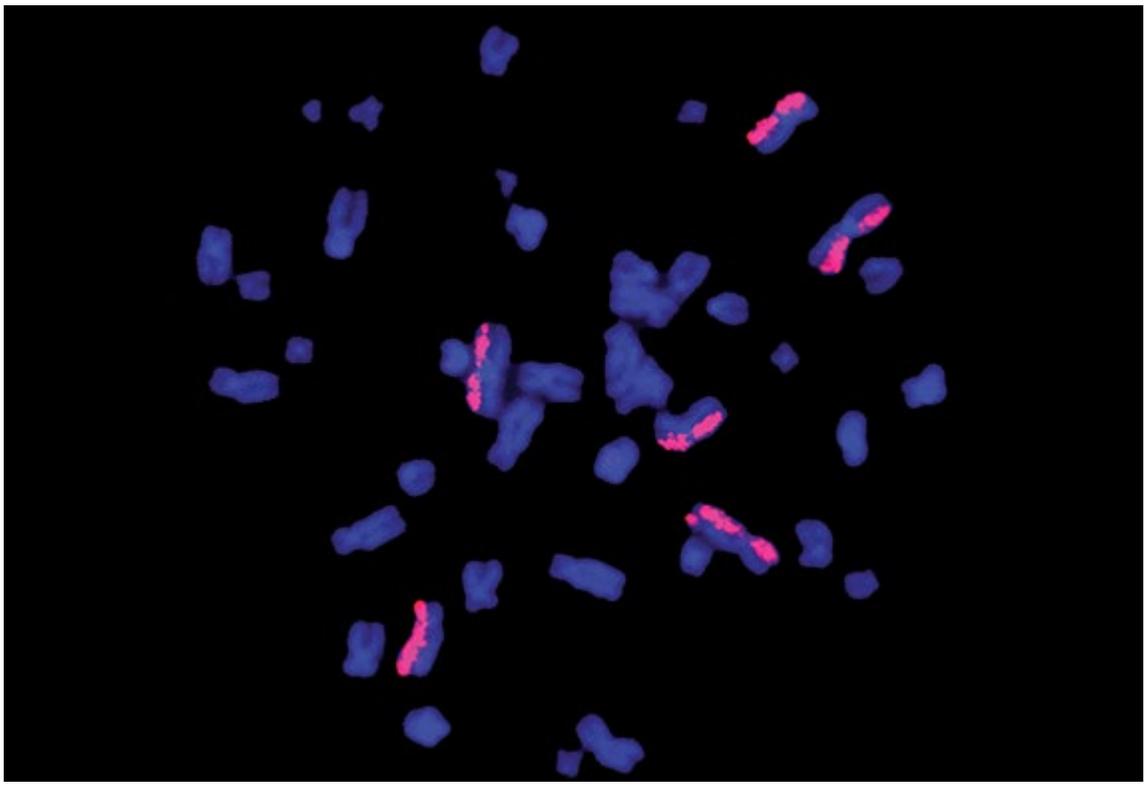
Chromosome 11 High-Density Paint (red) hybridized in human control metaphase spread.

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Use Case: NASA "Twin's Study" HD Chromosome Paints



KromaTiD work with NASA in the "Twins Study"

- Assay of DNA damage from radiation in Space.
- KromaTiD continues to work with NASA.



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Fluorescent Paints Spot DNA Damage from Radiation, Gene Editing. (2019). Retrieved from https://spinoff.nasa.gov/Spinoff2019/hm_3.html

Gene Target Probes

Featured: TP53/CEP17

Multiplex probe kit designed to target TP53 gene region and pericentromeric q-arm of chromosome 17.

Applications

- Detecting deletion of TP53 gene region
- Characterizing other rearrangement events involving the two target loci
 - Translocations
 - Inversions
 - Complex rearrangements



TP53 (red) and chr17q subcentromere (green) probes hybridized in human control metaphase spread.

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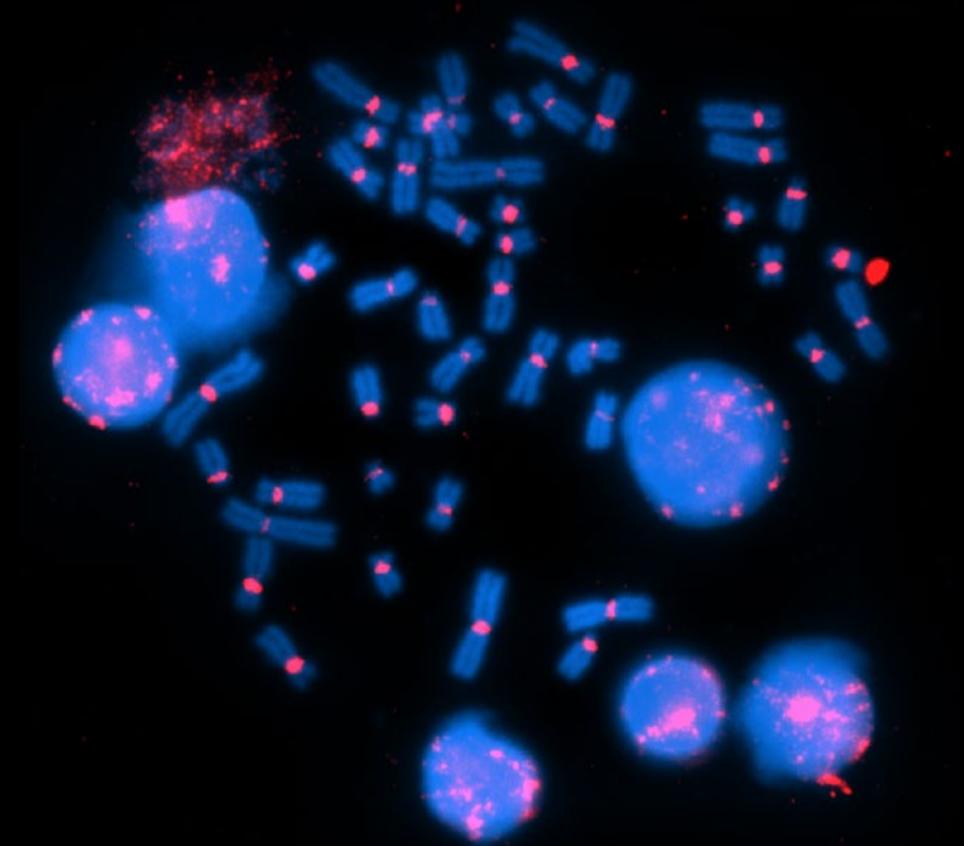
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Human PanCEP Probe

Probe design which targets repetitive DNA within all human centromeres at once.

Applications

- Assaying ploidy
- Detecting acentric and multi-centric chromosomes
- Qualification of spatial distribution of centromeres within nuclei



Human PanCEP probe (red) hybridized in human control metaphase spread and interphase nuclei.

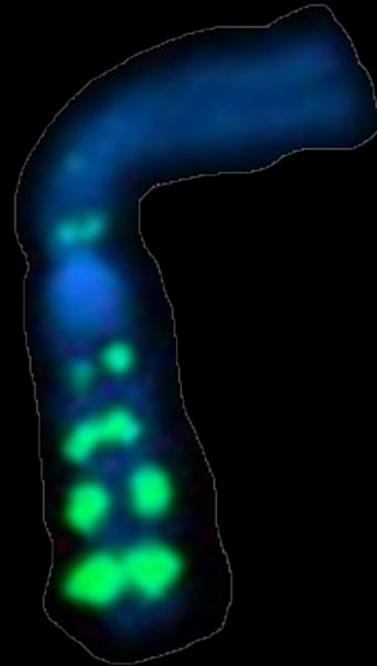
Custom Probe: Chromosome 1 Ladder

Chromosome 1 LOD Ladder

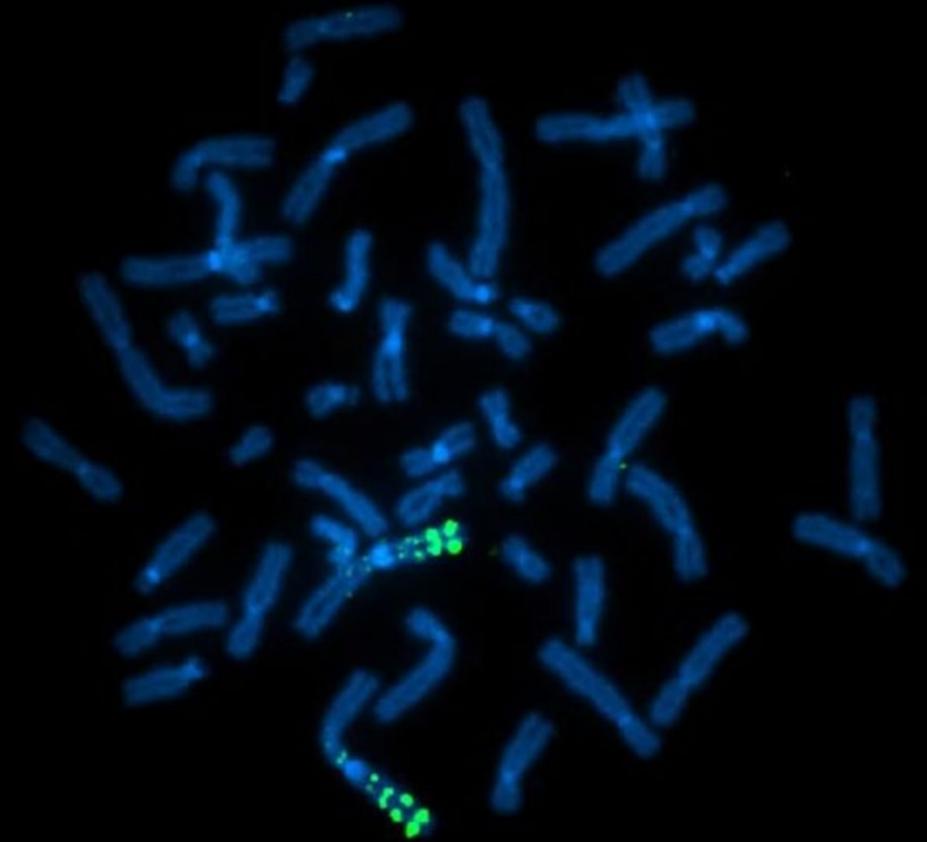
Evenly spaced loci targeted by probe sets comprised of progressively fewer probe molecules.

Applications

- Troubleshooting elements of FISH assay
 - Sample quality
 - Individual protocol steps
 - Reagent preparation
 - Imaging hardware problems



Chr 1
LOD ladder



Chromosome 1 LOD ladder (green) hybridized in human control metaphase spread.

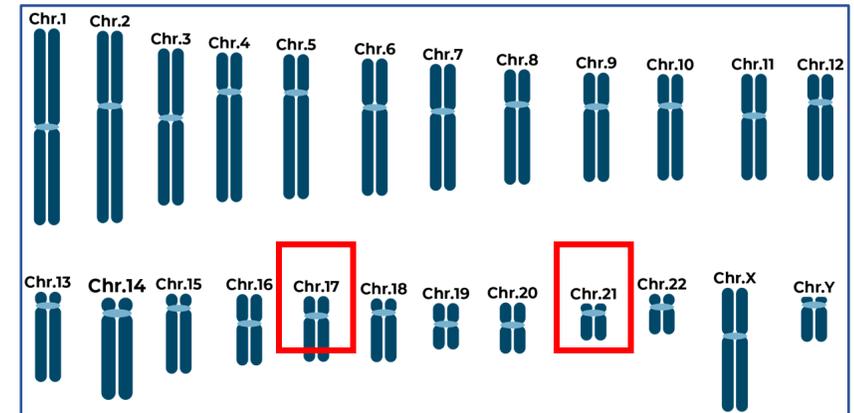
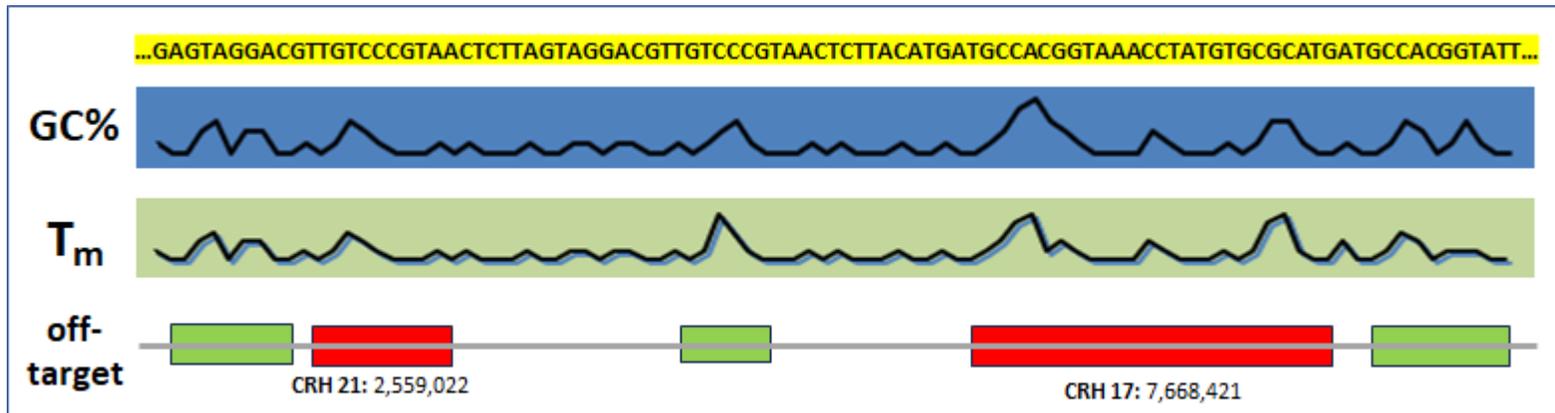
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Custom Probe Design Parameters

The design process itself can be customized to accommodate specific challenges.

- Control for sequences with calculated level of off-target affinity
- Use target-site probe density to threshold-out stray signals during imaging
- Accept off-target binding on known chromosome(s)
- Manually remove problem sequences in case-by-case fashion.



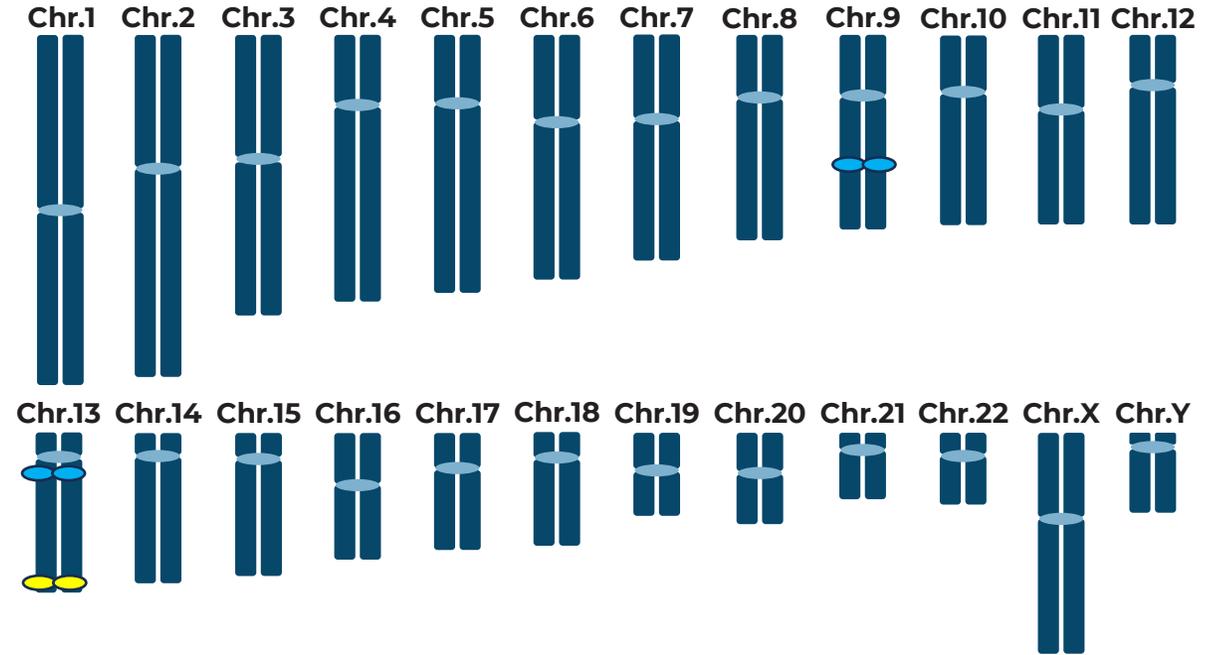
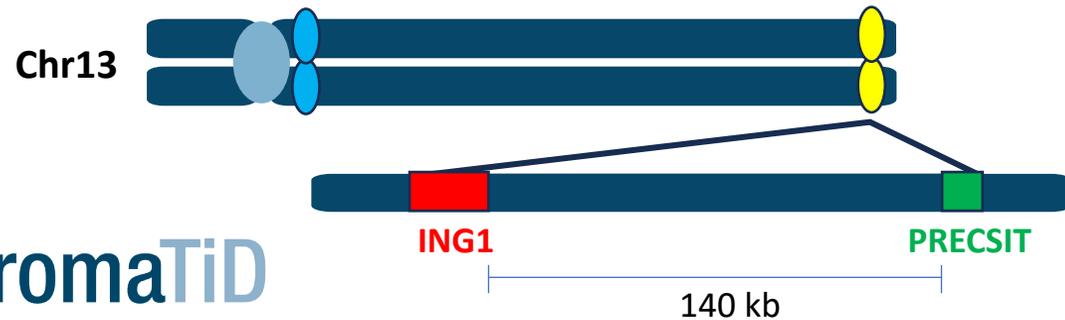
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Tailoring Designs around Assay Goals

Assay Customization Options

- Inclusion of companion marker probes
- Modulation of fluorescence intensity
- Arranging target loci strategically



Hypothetical Custom Chr13 Deletion Sub-Categorization Assay:

ING1 (10.7 kb)

PRECSIT (6.3 kb)

Chr13 Reference Probe

Homology Marker Probe

● chr13: 110,713,700-110,723,339

● chr13: 110,863,987-110,870,307

● chr13: 25,191,642-27,410,224

● chr9: 97,523,005-99,819,022

Pinpoint FISH™ Services



You and your team can have KromaTiD run your custom assay as an in-house service.

Beneficial for assay designs involving:

- Very small probes,
- Difficult sample types,
- Complex sample conditions.

Also helpful if you are:

- Lacking specialized equipment,
- Without in-house expertise,
- Desiring efficient turn-around time.

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Pinpoint FISH™ Services



Example workflow with KromaTiD running Pinpoint FISH or alternative assays in-house.

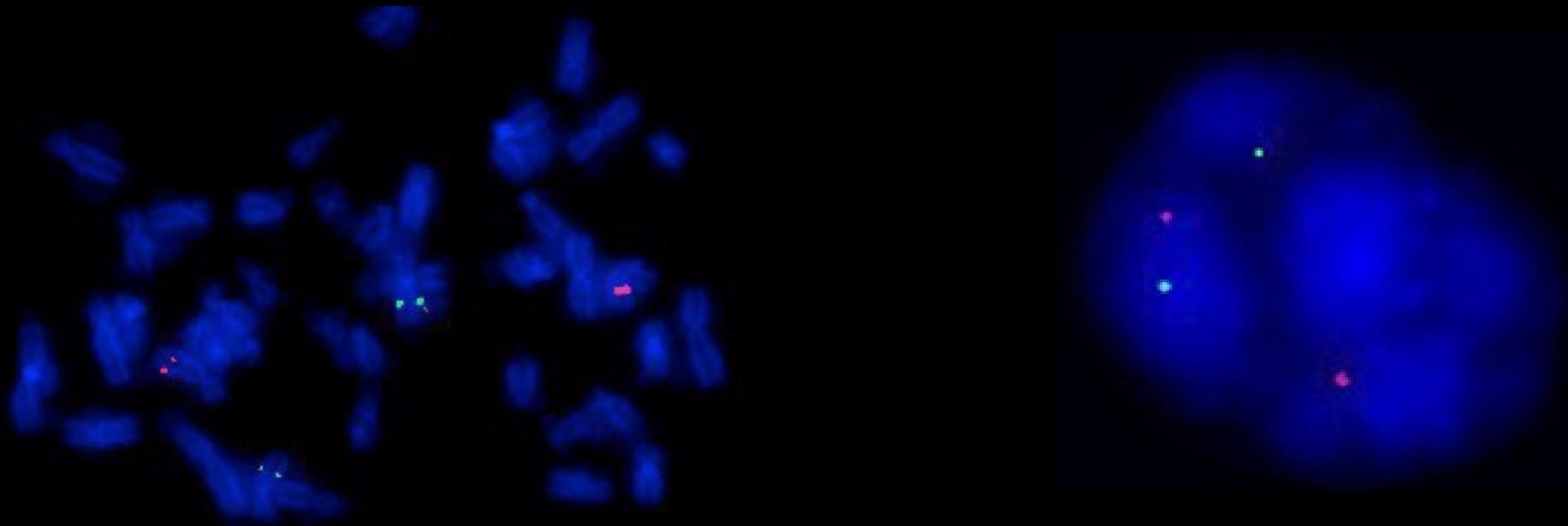
KromaTiD is committed to **collaborative excellence** through dedicated project management and **expert technical analysis**.

- Your role
- KromaTiD's role
- Collaborative

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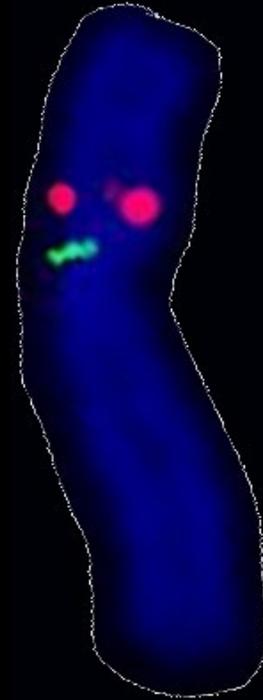
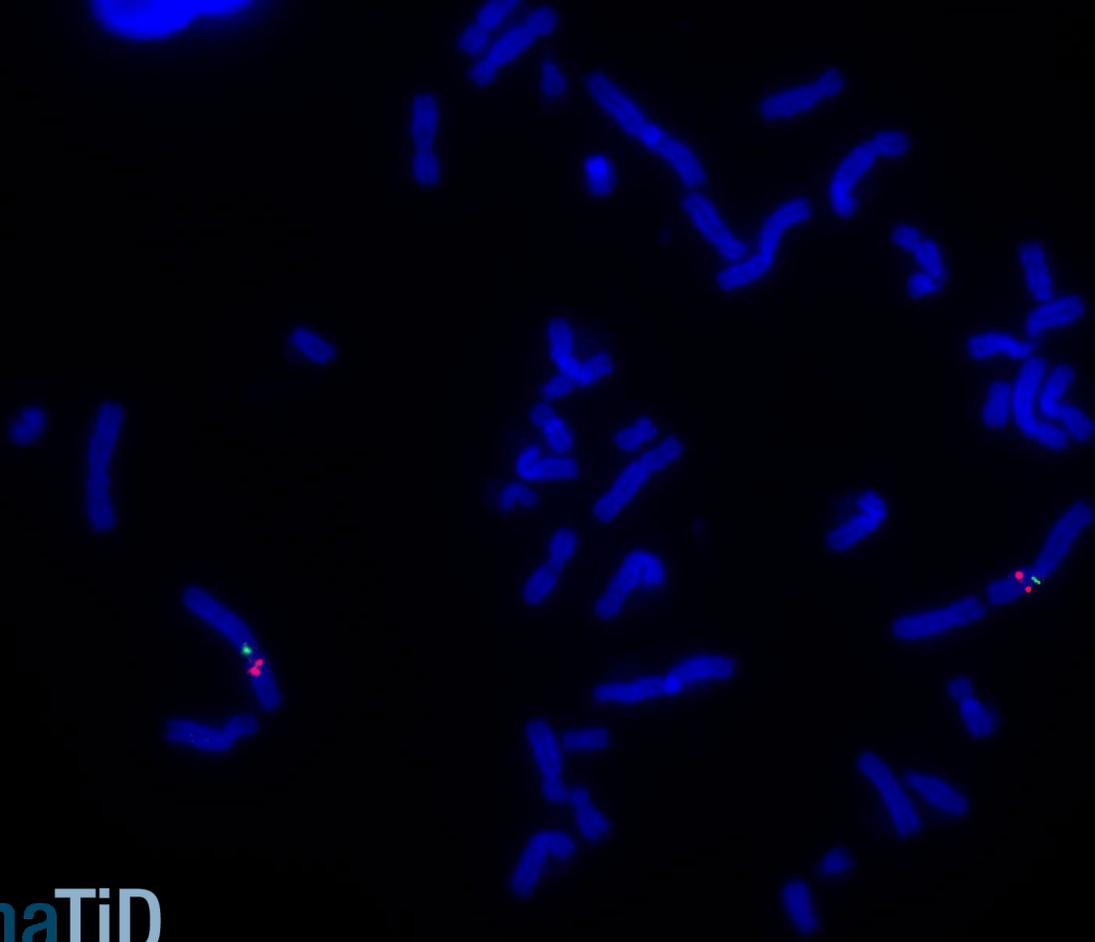
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Example Service Assay: CUX1 and KIF20A



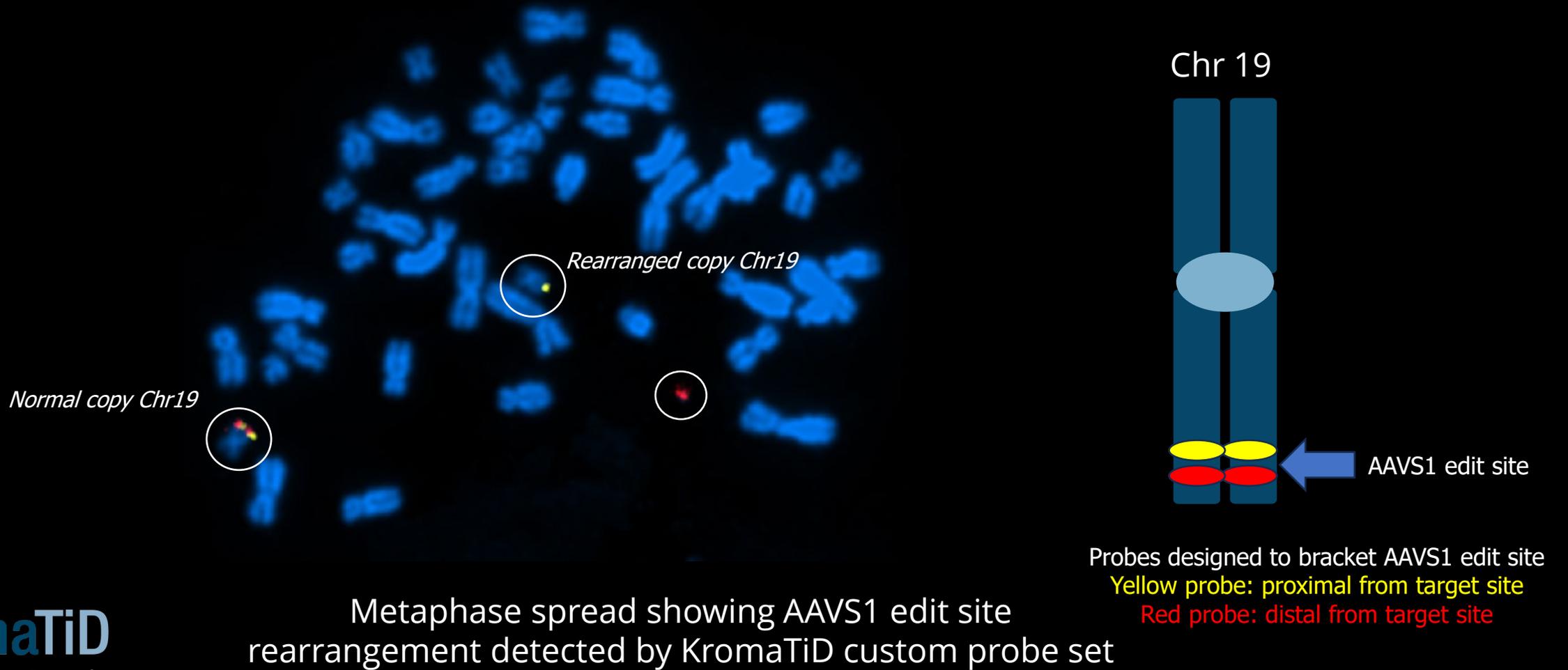
Metaphase spread and interphase nucleus hybridized with custom probes for CUX1 (green; 0.5 Megabases) and KIF20A (red; 8.6 kilobases)

Example Service Assay: DCTN1 Multiplex with Commercial Probe



KromaTiD custom probe for DCTN1
(red; 30 kilobases) and third-party
control probe (green; 170 kilobases)

Example Service Assay: CAR-T Structural Rearrangement Assay

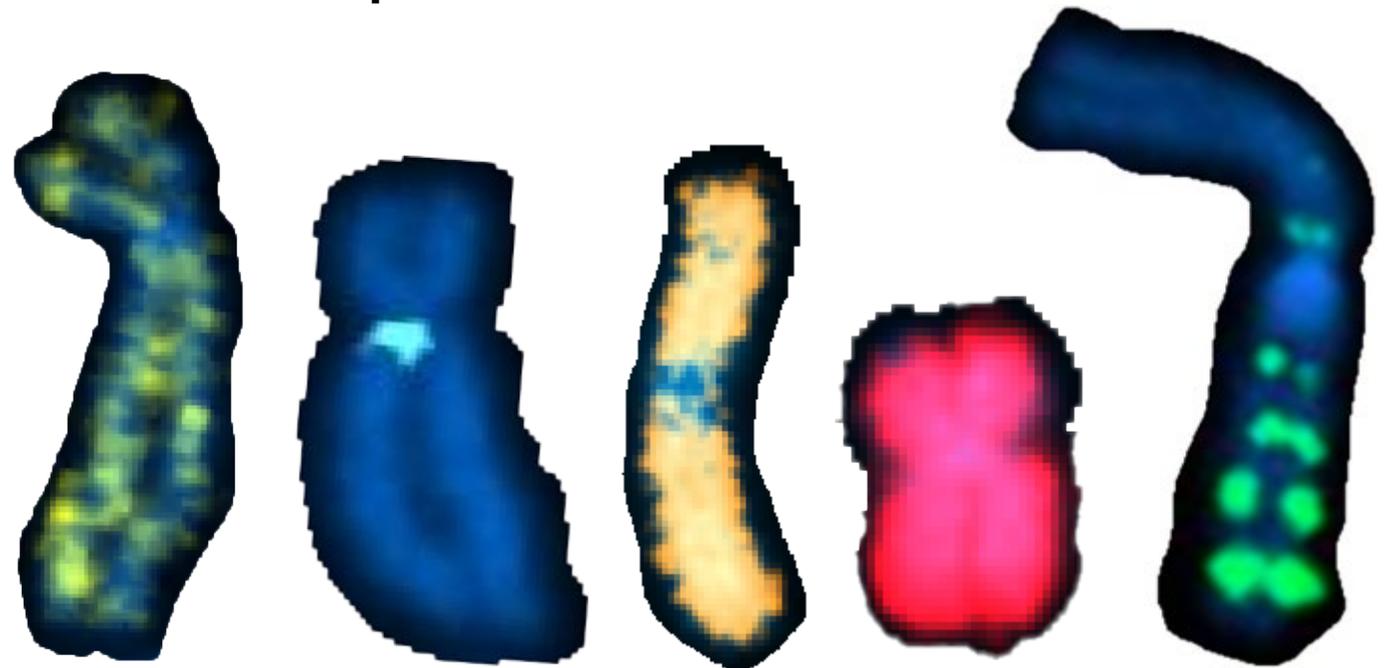


Standard Color Label Options and Custom Configuration

- **Five standard color labels**
- **Custom labeling available**

- **Probe concentrations: standard, custom or dehydrated**
- **Probes can be pre-combined**

Color Label	Excitation λ	Emission λ
Atto 425 (aqua)	436	485
6-FAM (green)	490	525
Atto 550 (orange)	555	576
Texas Red (red)	595	620
Atto 643 (far red)	643	669



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Ordering Pinpoint FISH™

We provide convenient ordering online of all our catalog probes.

Visit <https://kromatid.com> and click on FISH Probes from our menu.

- Credit Cards Accepted
- Purchase Orders Accepted

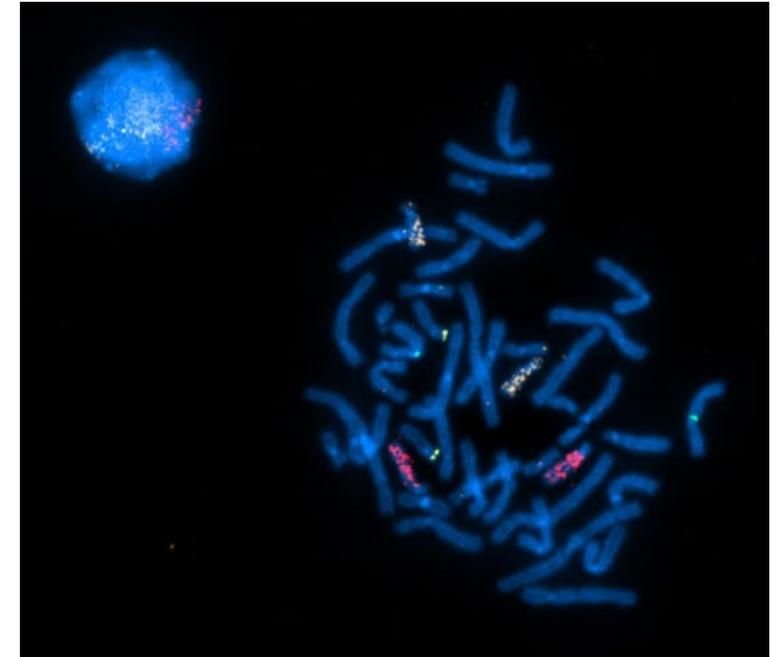
For custom services or probes, ordering can be done from any of the FISH probe pages on our website, by clicking the “Request a Quote” button.

REQUEST QUOTE

Your local sales representative can discuss your fluorescent labeling needs and any specific experimental requirements.

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TECHNICAL SUPPORT:
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EMAIL: TECHSUPPORT@KROMATID.COM

INQUIRIES AND ORDERS:
EMAIL: SALES@KROMATID.COM
WEB: KROMATID.COM

Ph: 720.815.2898
Fx: 720.815.2902

The background of the slide features a soft-focus, artistic representation of biological cells and DNA. In the center, a large, glowing cyan structure resembles a chromosome or a complex DNA molecule, with a red double helix running through its core. Surrounding this are various other cells in shades of purple, blue, and green, some containing internal organelles or smaller DNA structures. The overall aesthetic is clean and scientific.

Q & A

[Questions? Contact Us Today!](#)

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