

dGH in-Site™ CAR-T Kit Overview



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Direct, Definitive Genomics

dGH in-Site™ CAR-T Kit Overview

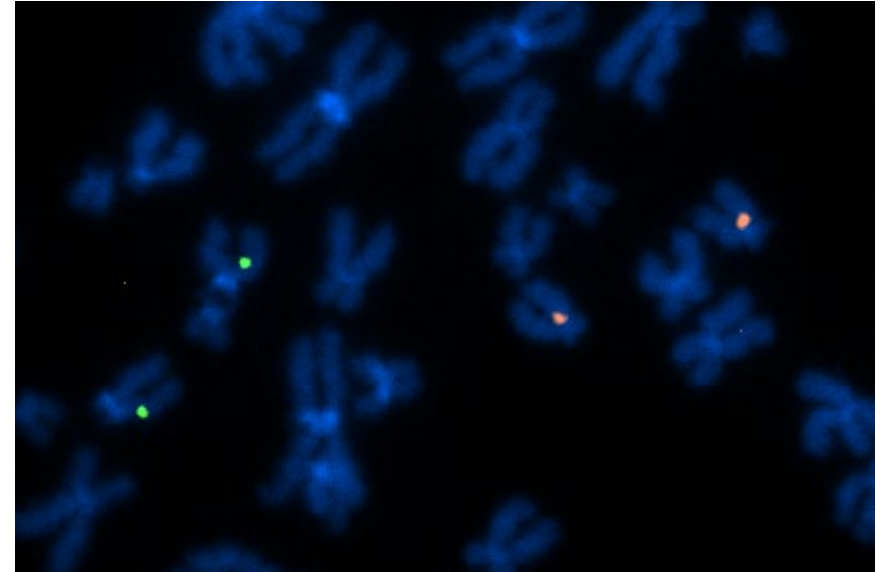
Track the sequence, location and orientation of your CAR-T genomic loci, in a single test.

Technical features

- Provides single-cell data
- Ultra-high resolution, as low as 2 kb
- Identification of structural variation in engineered T cell products
- Use with familiar cytogenetic equipment and workflows

Advantages

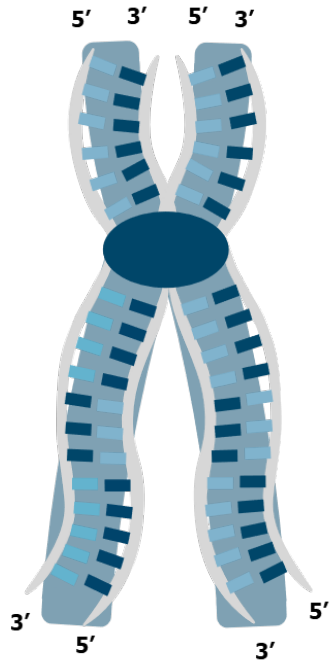
- Genomic information unobtainable by either metaphase or interphase FISH
- High signal-to-noise ratio boosts scoring confidence and efficiency
- Direct visualization of double strand misrepairs
- Orthogonal and complementary to sequencing and G-banding
- Expert support from KromaTiD scientists on sample processing and image analysis



dGH™ probes targeting TRAC (orange) and B2M (green) in control GM12753 lymphoblastoid cell line

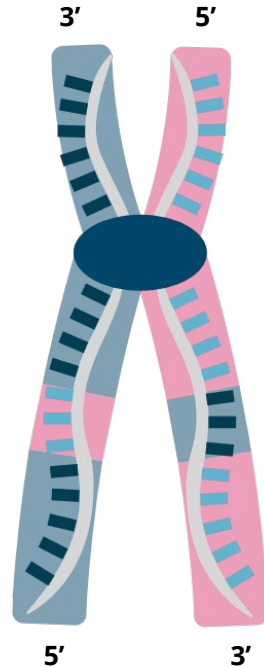
directional Genomic Hybridization (dGH™) Is Not FISH

Blue = DAPI Staining of Chromosome Structure



Double Stranded Metaphase Chromosome

Pink = Fluorescently Labeled Hybridization Probes



Single Stranded dGH Chromosome

Daughter strand stripping
→
Hybridization with single-stranded probe

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 only.

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

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

What Single-Strand dGH Analysis Can Reveal

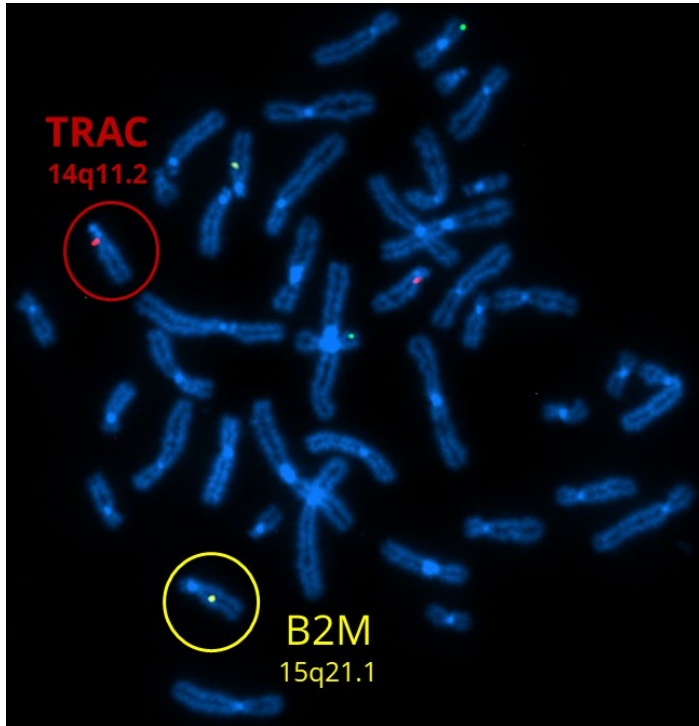


Fig 1. dGH™ in-Site TRAC 14q11.2 (red), B2M 15q21.1 (yellow) and CIITA 16p13.13 (green) probes in GM12753 control lymphoblastoid cell line.

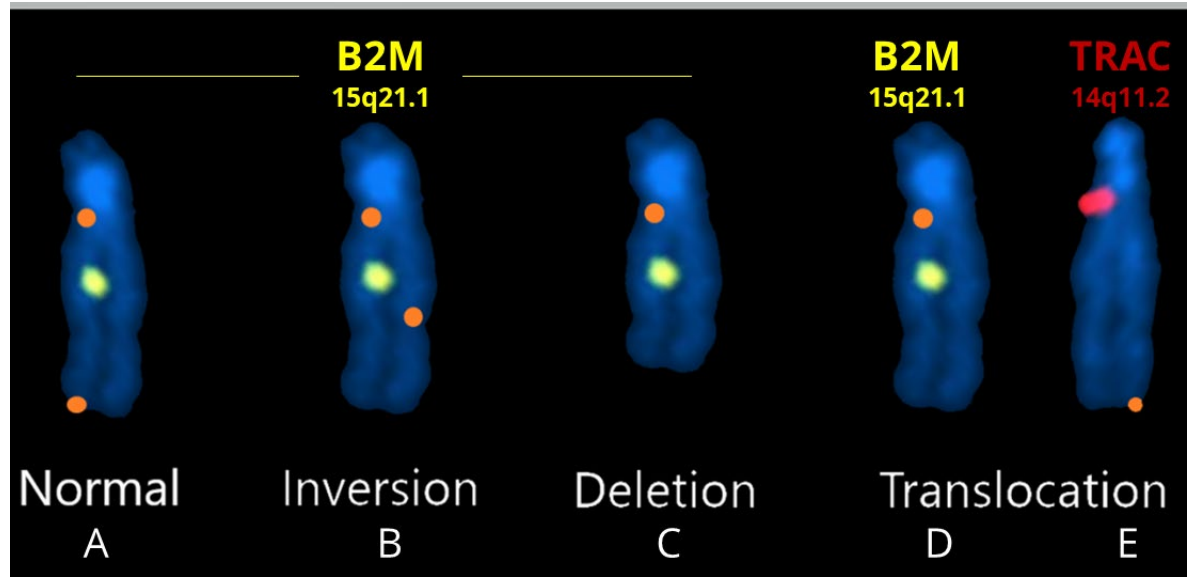


Fig 2: Simulated hybridization of orange-labeled subcentromere and subtelomere dGH in-Site probes on chromosome 15 that is normal (A), inverted (B), deleted (C) and translocated (D) with chromosome 14 (E).

dGH in-Site™ CAR-T Kit

Track the sequence, location and orientation of your CAR-T genomic loci in a single test.

The dGH CAR-T Kit provides the **most detailed and sensitive analysis** of **localized genome edits** available.

By adding the CAR-T Kit to your analytical suite you will get a truly comprehensive characterization of your engineered T cell products.

dGH in-Site™ CAR-T Kit

10 Assays \$1,500

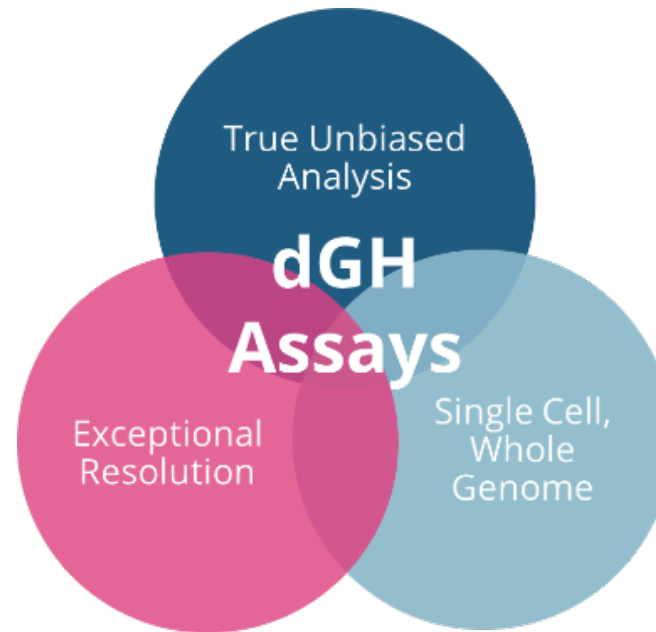
Contents

dGH Probe TRAC TexRed
dGH Probe B2M 6-FAM/Spectrum Green
dGH Hybridization Buffer



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dGH in-Site™ CAR-T Kit Bundle

10 Assays \$2,799

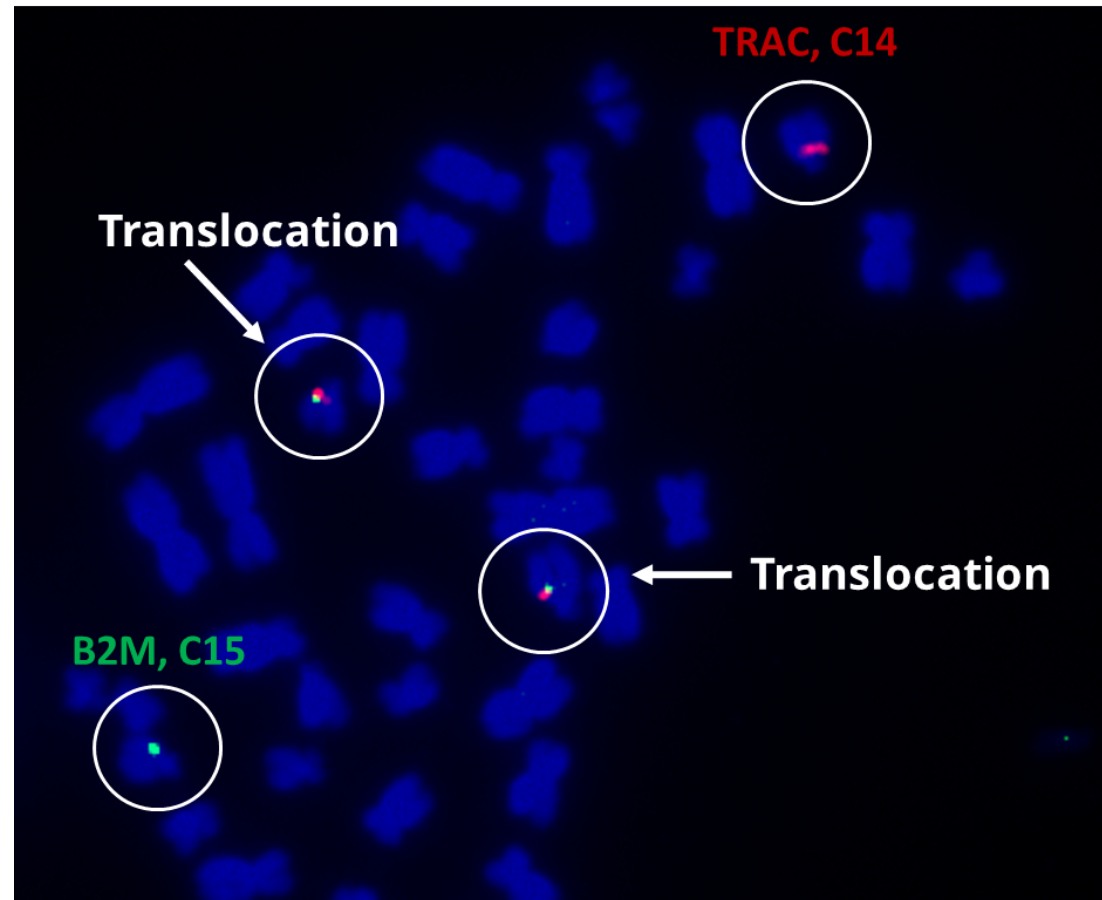
Contents

dGH Probe TRAC TexRed
dGH Probe B2M 6-FAM/Spectrum Green
dGH Hybridization Buffer
dGH Cell Prep Additive 250 µL
Demecolcine 2.5 ml
White Glove Tech Transfer Support

dGH at a Glance

Track the sequence, location and orientation of your CAR-T genomic loci in a single test.

- dGH single-stranded probes provide a high-resolution, localized view of CAR-T edits.
- Direct visualization of target orientation and balanced/unbalanced structural variants
- Abnormal signal patterns are easy to locate and visualize.
- Detects small inversions missed by FISH



Available dGH™ CAR-T Cell Probe Options



dGH in-Site™ probe for PDCD1 labeled with Atto550 in GM12753 control lymphoblastoid cell line.

dGH Probe	Cat. No.	Fluorophore	Excitation/Emission (nm)
B2M	DGHP-002-C	6-FAM / Spectrum Green	490 / 525
PDCD1 CIITA	DGHP-003-A DGHP-004-A	Atto550 / Spectrum Orange	555 / 576
TRAC	DGHP-001-B	Texas Red	595 / 620
CD19 (Endogenous)	DGHP-005-D	Atto643 / 647 / Cy5	643 / 669
Custom dGH in-Site™ Probe	DGH-007	Inquire	Inquire



Custom probe additions require customized specifications. Please email sales@kromatid.com



Build and Execute Your dGH in-Site™ Assay for CAR Cells

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	Inquire
<u>dGH in-Site™ CAR-T Probe Kit</u>	B2M 6-FAM/Spectrum Green; TRAC TexRed	10 Tests	\$1,500.00

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:

[36 Subcentromere dGH in-Site Probes](#)

[42 Subtelomere dGH in-Site Probes](#)

[dGH in-Site™ CAR Probes](#)

KromaTiD Application 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.

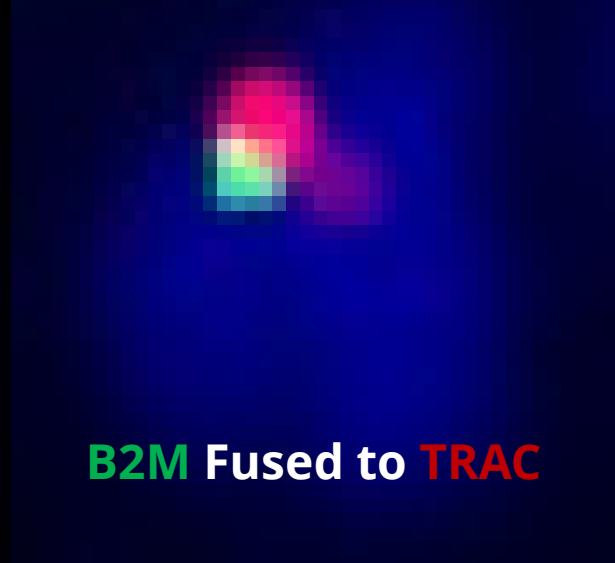
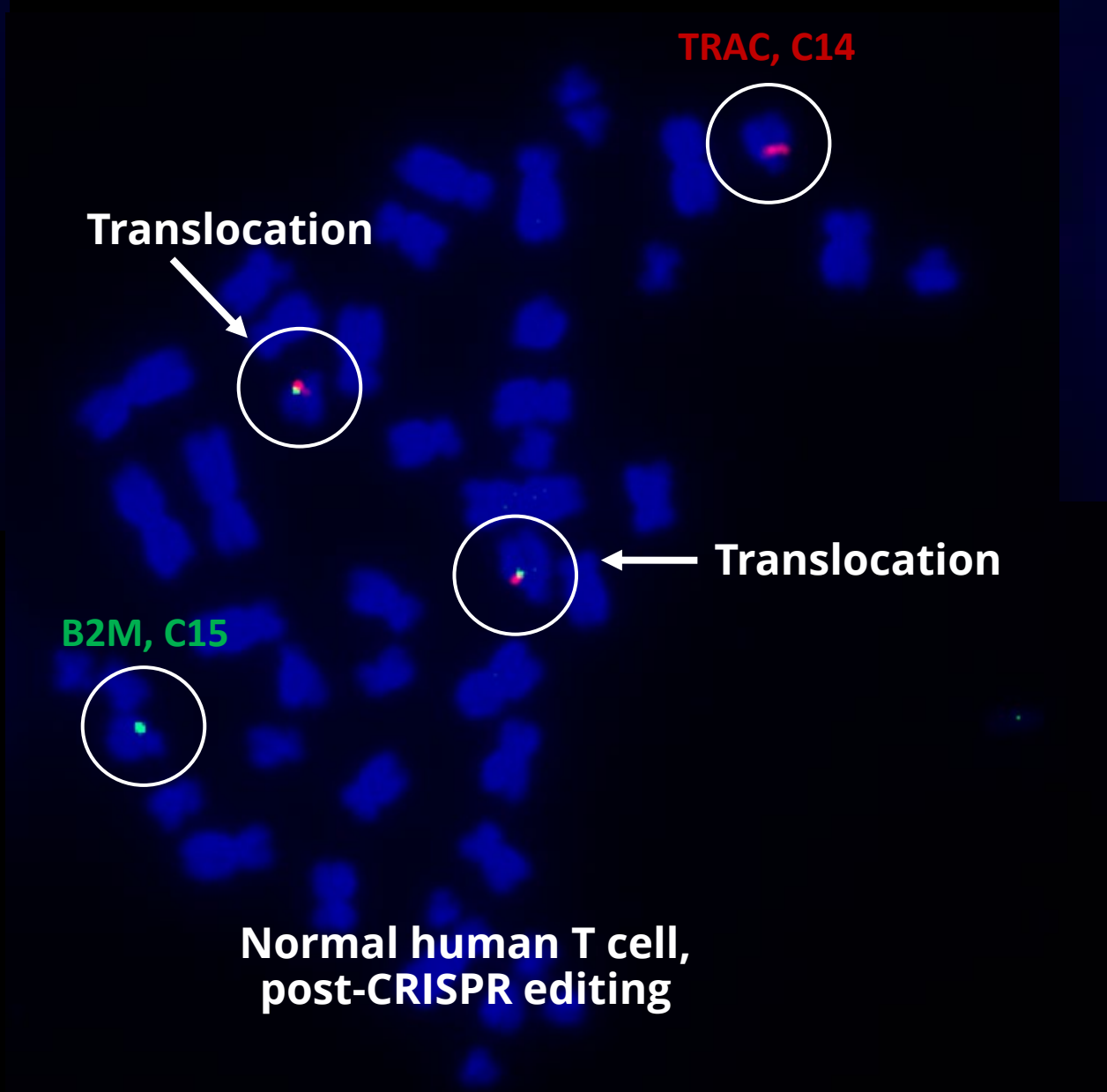
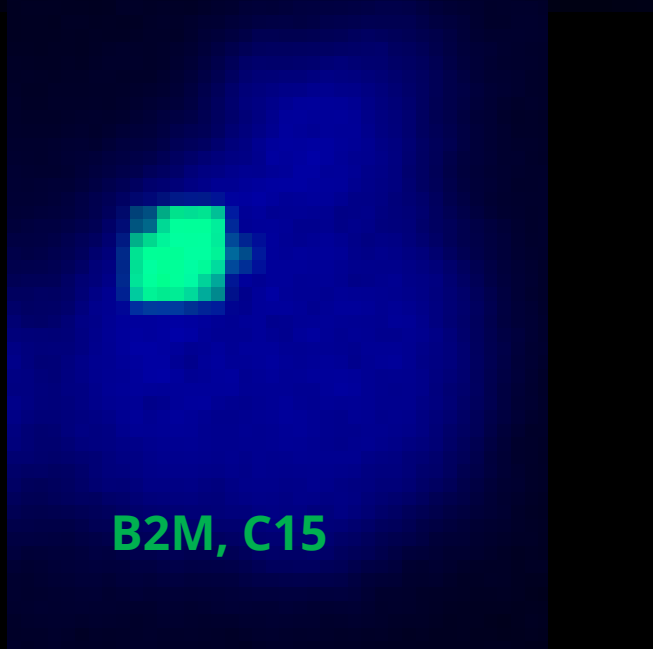
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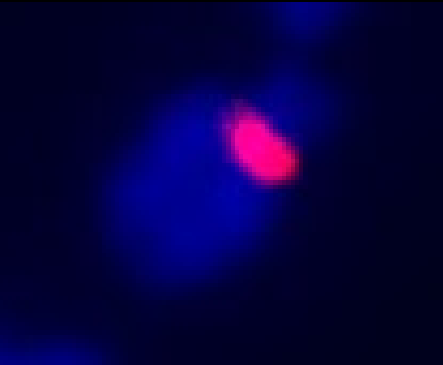
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Detection of TRAC and B2M Structural Variations In Normal Human T Cells after CRISPR Editing

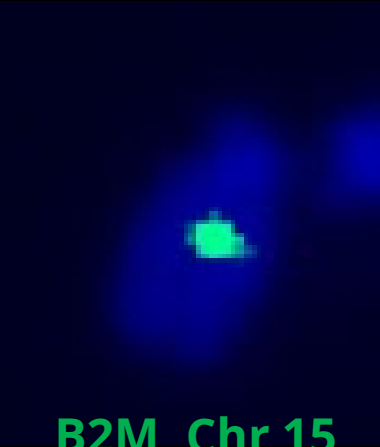
Example: Balanced Reciprocal Translocation



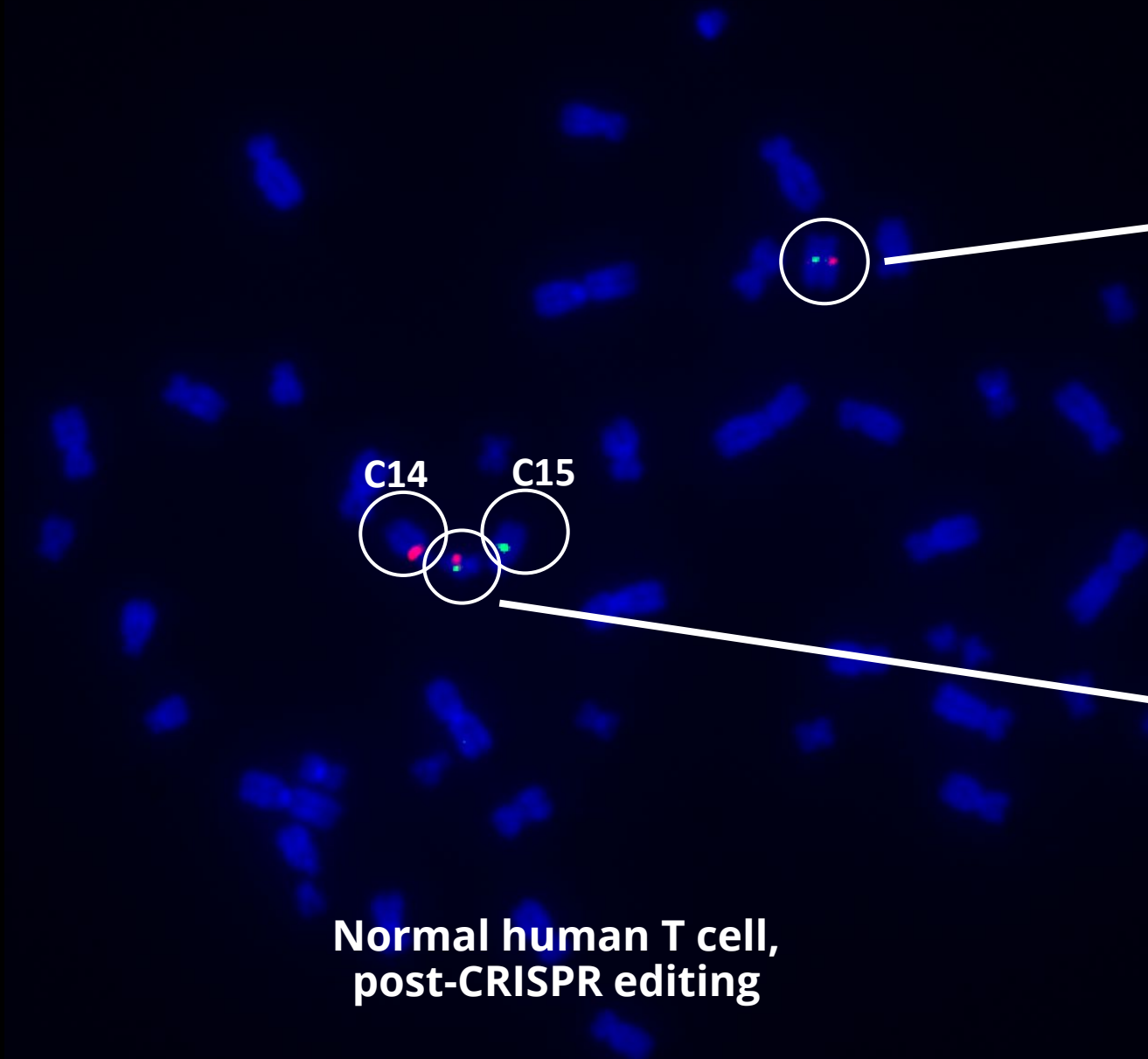
Example: Unbalanced Reciprocal Translocation



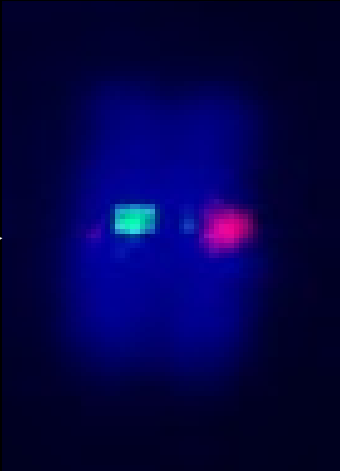
TRAC, Chr 14



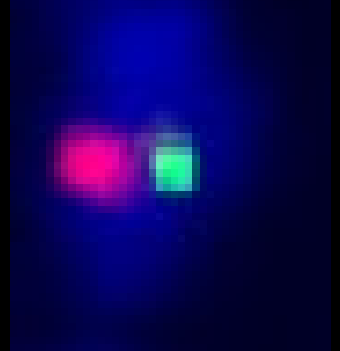
B2M, Chr 15



Normal human T cell,
post-CRISPR editing



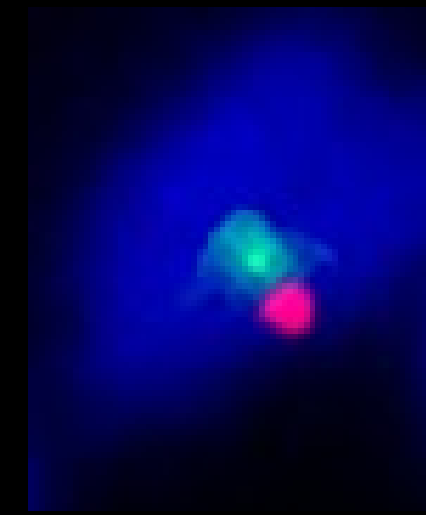
B2M Fused to TRAC
(Acentric)



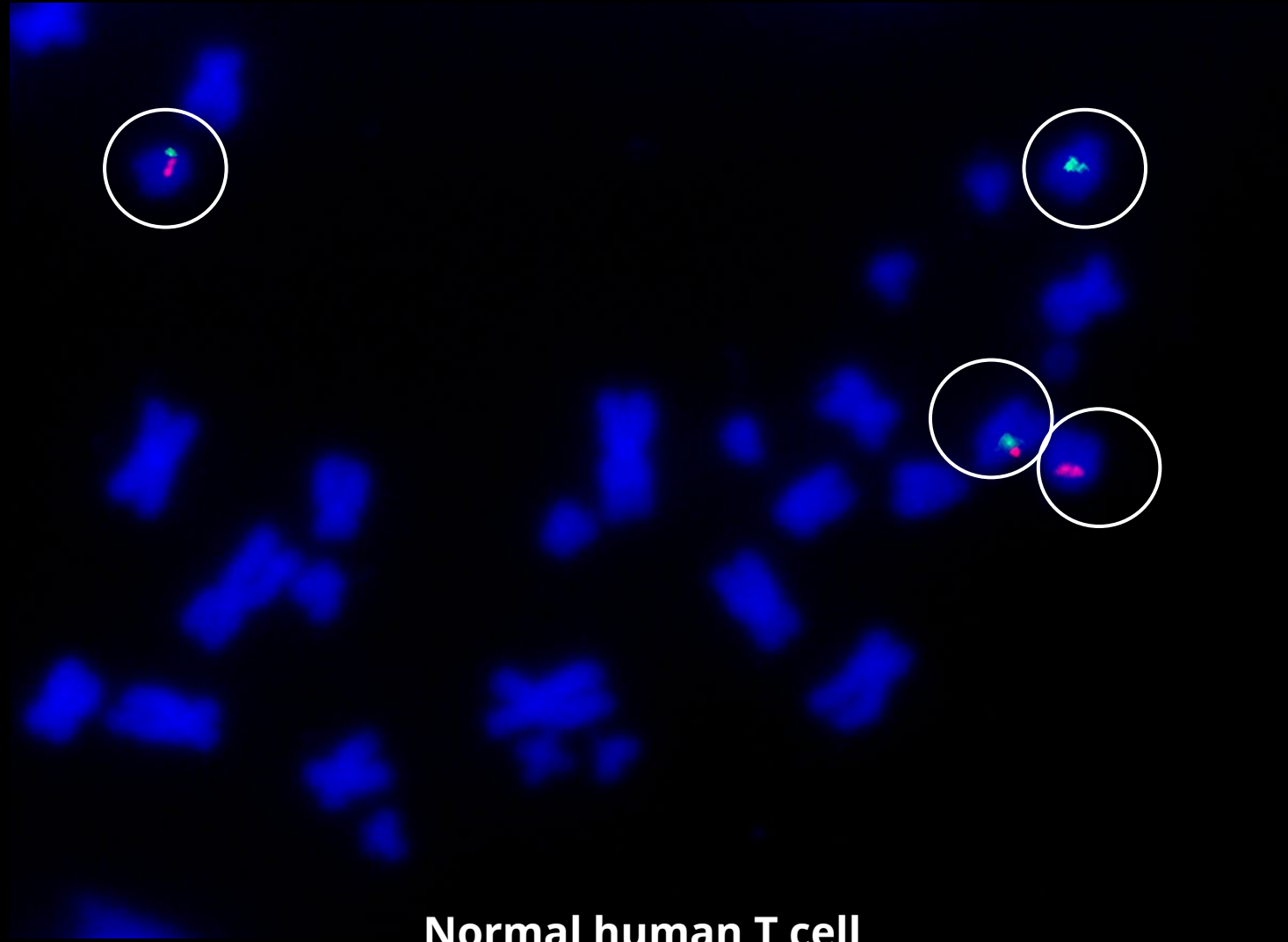
TRAC Fused to B2M
(Dicentric)

Multiple Structural Rearrangements in a Single Cell

B2M Fused to TRAC

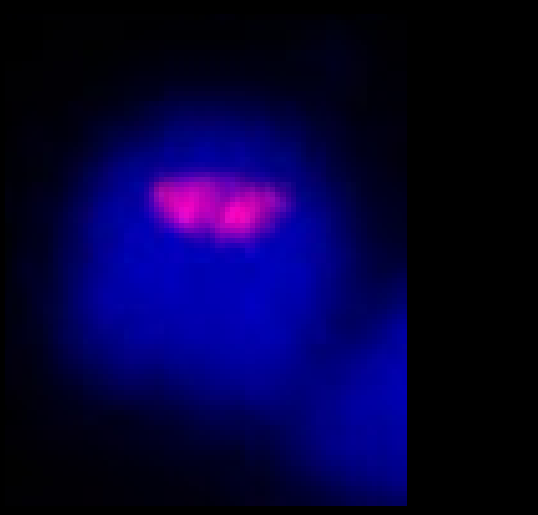


TRAC Fused to B2M

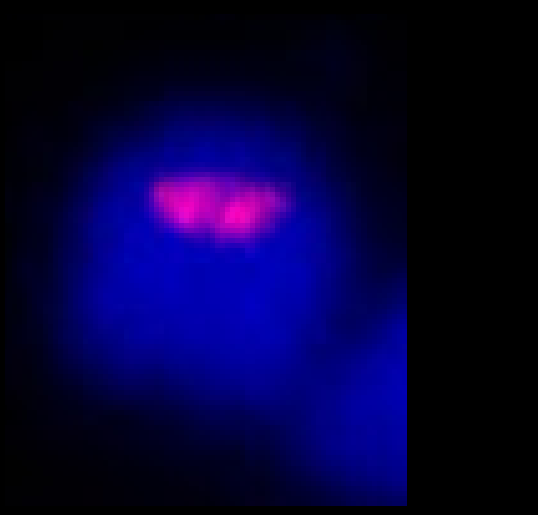


Normal human T cell,
post-CRISPR editing

B2M (inv/SCE); Chr 15

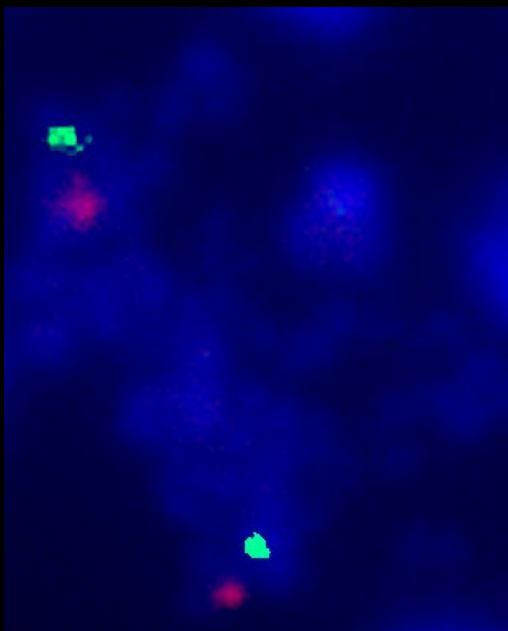


TRAC (inv/SCE); Chr 14

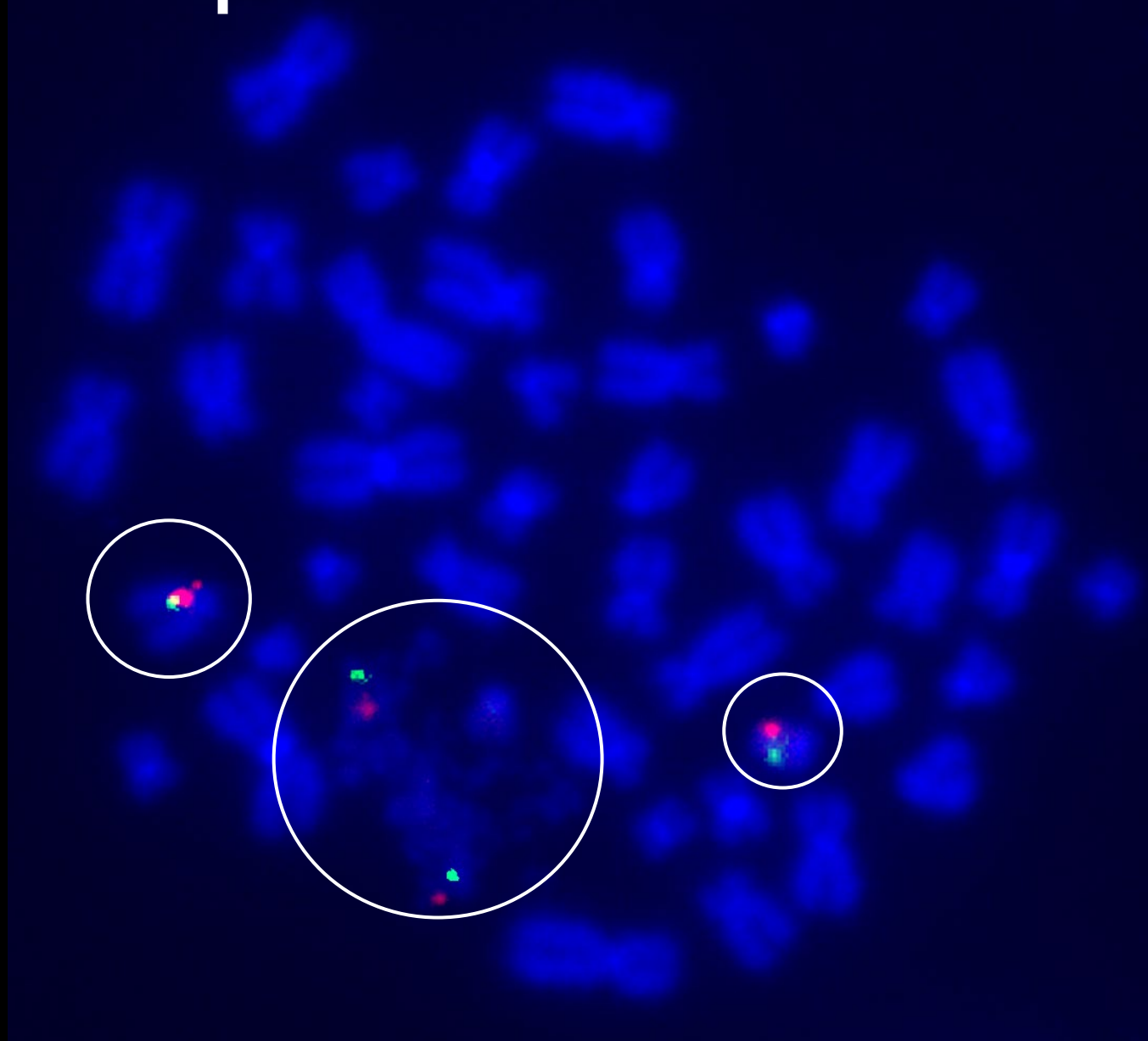


Chromothripsis of Translocated Chromosomes

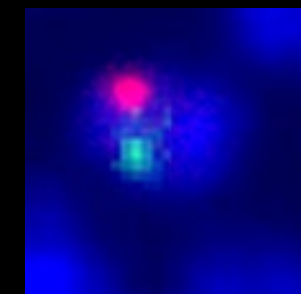
Cloud like presentation of C14 and C15 indicate shattered chromosomes



Chromothripsis of a pair of **TRAC/ B2M** fusion signals



B2M Fused to **TRAC**



TRAC Fused to **B2M**

Build and Execute Your Own dGH in-Site™ 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 [dGH Cell Preparation](#) and [Metaphase Slide Preparation](#) protocols to obtain single-stranded metaphase spreads.

Hybridize your set of dGH in-Site single-stranded probes to your chromosome spreads with the [dGH Probe Hybridization Protocol](#) for the highest-quality results.

View and analyze your outcomes using standard fluorescence microscopy hardware.

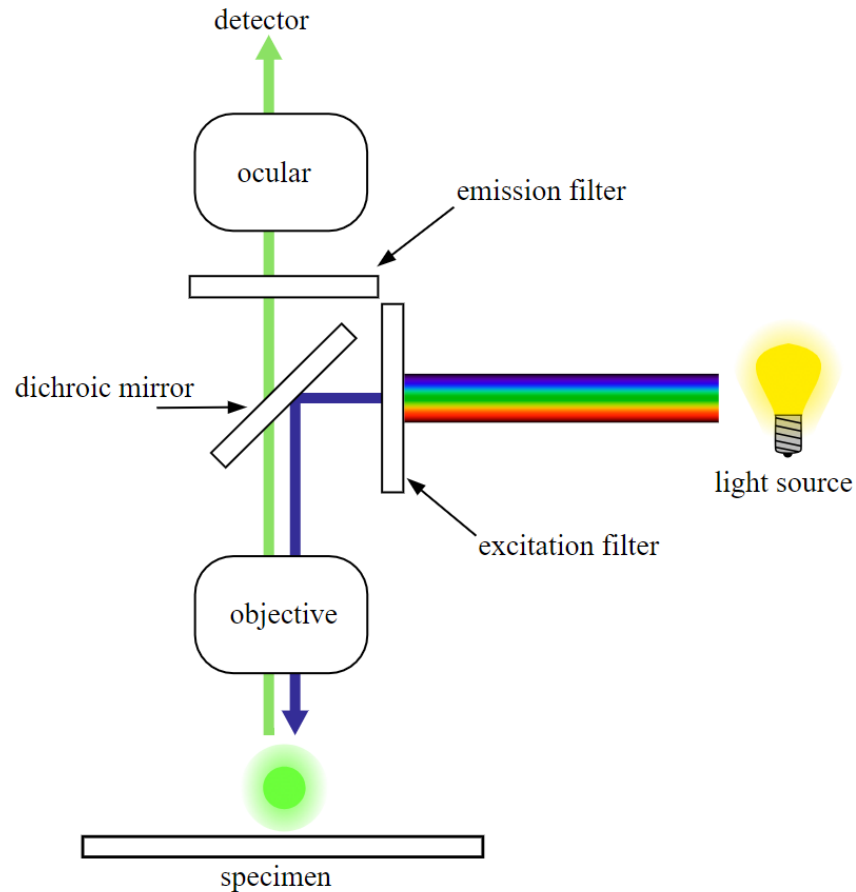


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Recommended Microscope Configuration for dGH™ Assays



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:

Tech Transfer Support for dGH in-Site Assays

KromaTiD offers expert technical assistance and consulting every step of the process.

Overall Assay Design

- Total spread count
- Regulatory considerations
- Custom probe design
- Specific loci to target

Slide Dropping and dGH Sample Preparation

- Cell pellet concentration
- Humidity and temperature
- UV exposure specifics

Image Analysis

- Scoring template
- Signal pattern interpretation
- Metaphase selection and morphology

Cell Culture and Harvest

- dGH Cell Prep Additive
- Colcemid™ and harvest time points

Slide Scanning

- Optics and hardware
- Illumination
- Light filter cubes

Data Management

- Working with the scoresheet
- Planning efficient data collection

dGH Hybridization


- Hybridization and multiplexing



Technical Support Information

Phone: 720-815-2901

Email: techsupport@kromatid.com

Website Chat : M-F 9:00am-5:00pm MST

Thank you!



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



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