KromaTiD

Direct, Definitive Genomics

Simultaneous Mapping of On / Off Target Structural Variants & Transgene Insertions

Presented By:
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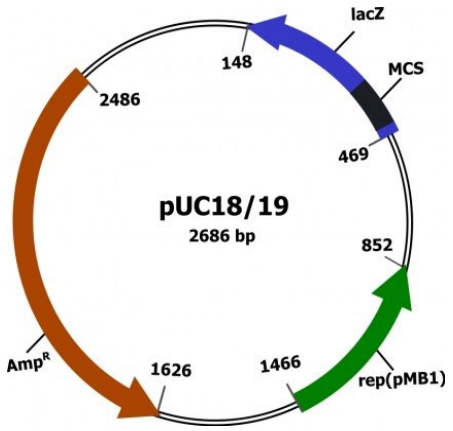
Erin Cross – Senior VP of Quality

March 9th, 2023

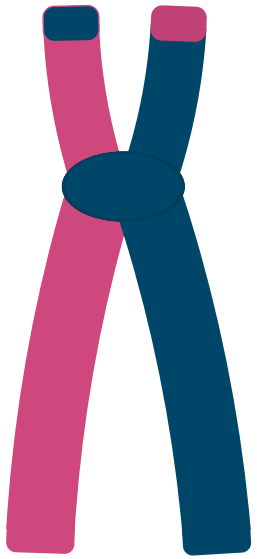
The Universe of KromaTiD Products and Services

Vectors

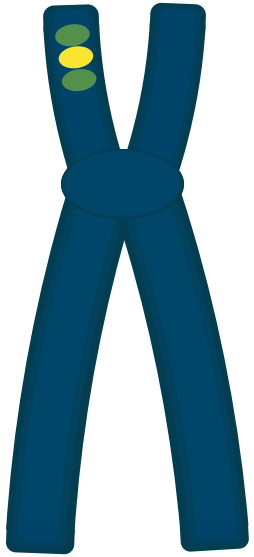


Plasmid Products

Single Cell Genomic Mapping



dGH SCREEN

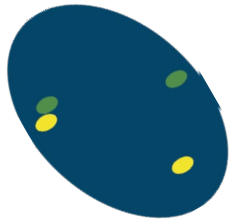


dGH In-Site

G-Band

Orthogonal

Non-Dividing Cells



Pinpoint FISH

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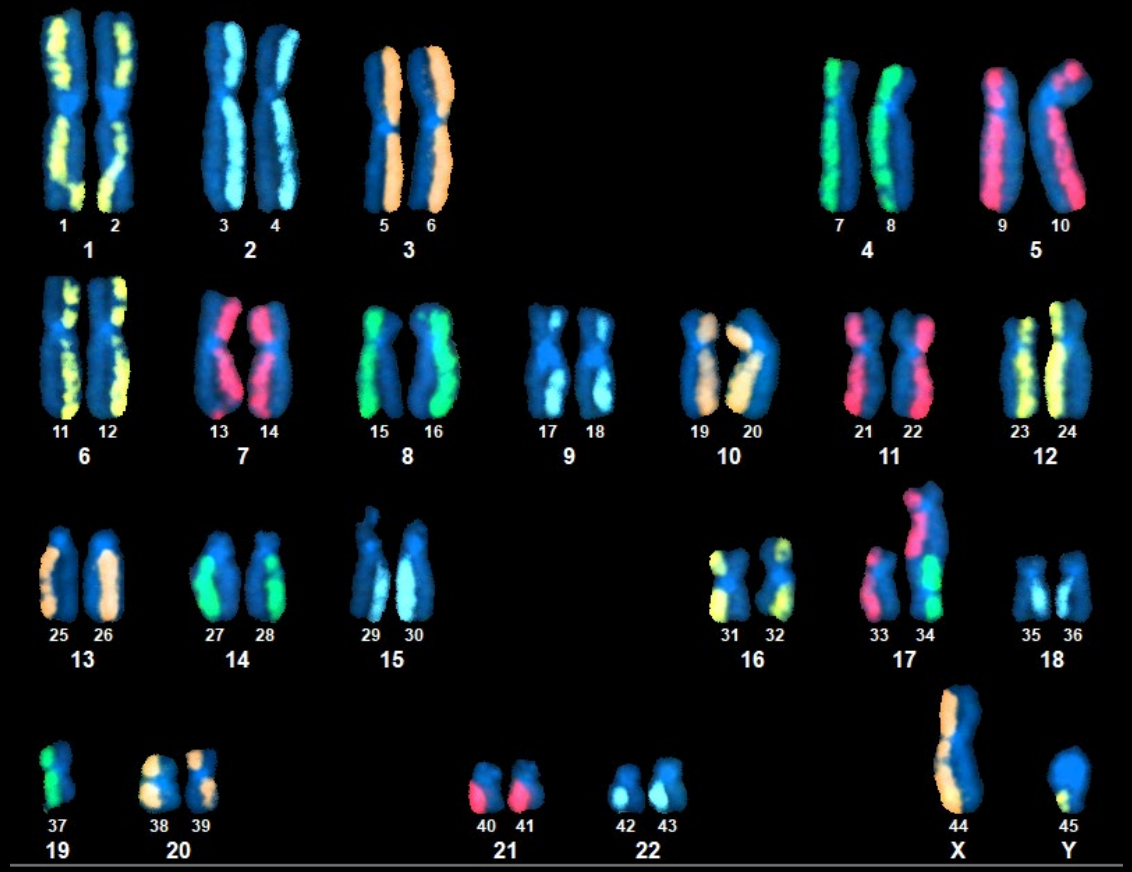
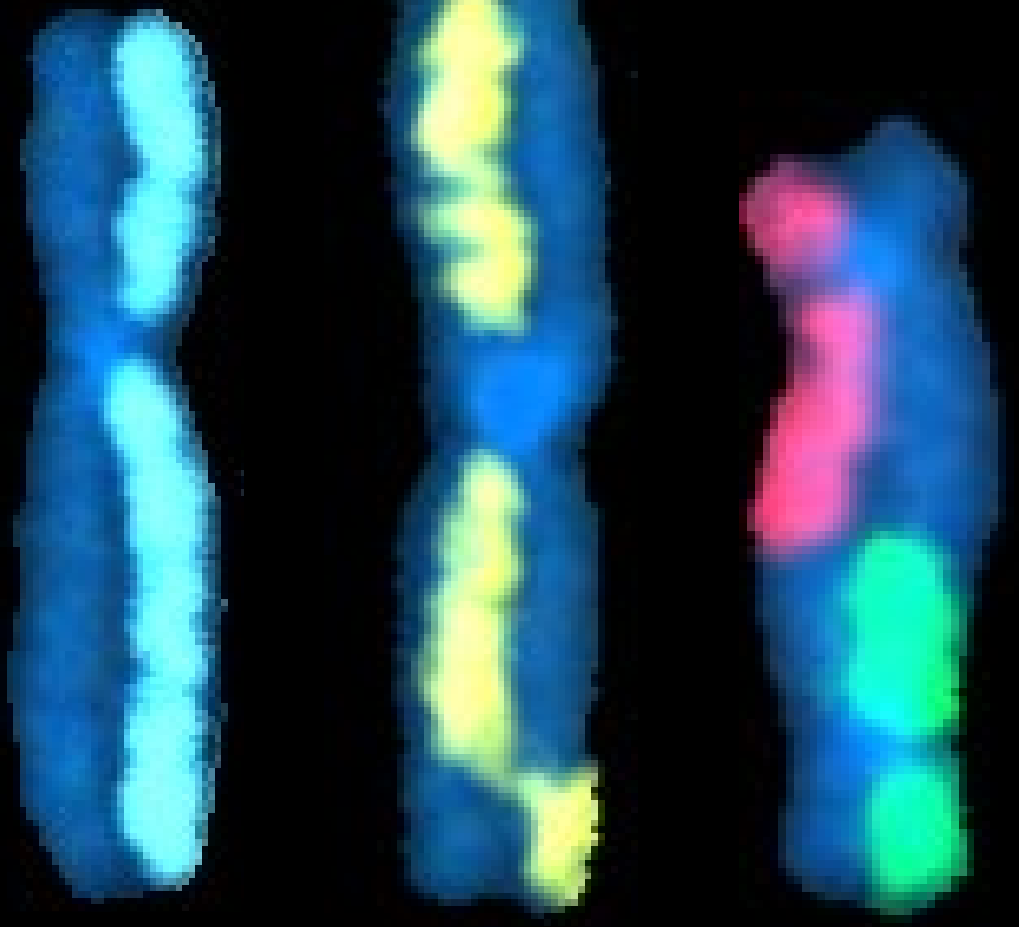
www.kromatid.com

The Power Of dGH



Whole Genome Structural Map



C2 Reference Genome Structure

C1 Telomeric Inversion

C19 to C17 Translocation

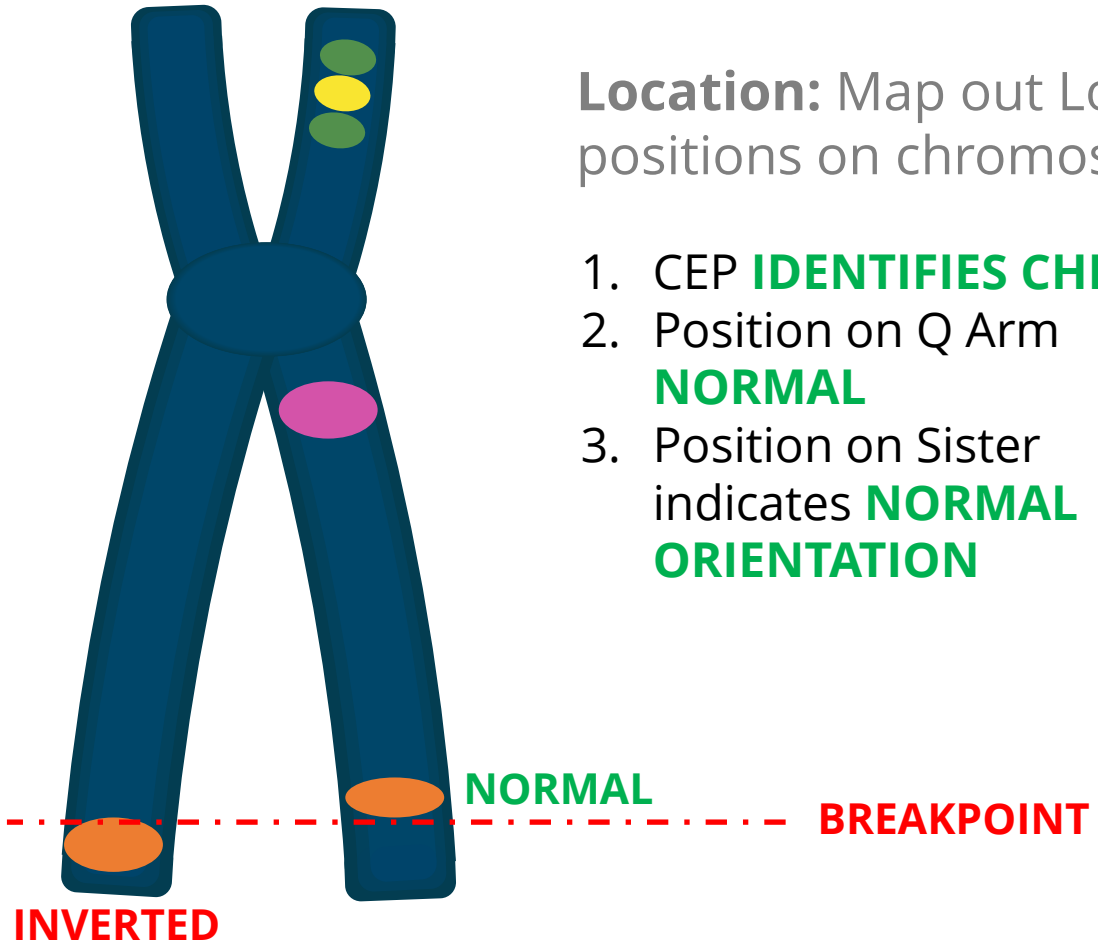
Sequence Location and Orientation From Image Data

Sequence: Identify Target & CNV by Probe Color

- LOCI Bracket
- Non-Genomic Insert
- CEP
- TEP

Orientation: Opposite Sister Chromatid Indicates Inverted Target

1. TEP Identifies **CHR11**
2. Split TEP Signal Indicates **BREAKPOINT** in Telomeric Region
3. Position on Opposite Sister Chromatid Indicates **INVERSION**



Location: Map out Loci positions on chromosome arms

1. CEP **IDENTIFIES CHR11**
2. Position on Q Arm **NORMAL**
3. Position on Sister indicates **NORMAL ORIENTATION**

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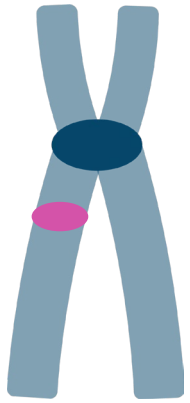
dGH In-Site for CAR-T

Verify the Structure of Important Loci

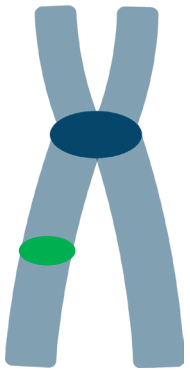
TRAC



TRAC MARKER PROBE

- Brackets Loci with 850 Kb – Texas Red
  - Inversions
  - Translocation
  - Chromosomal CN
  - TRAC Loci CN
  - On-Target Insert Verification

B2M



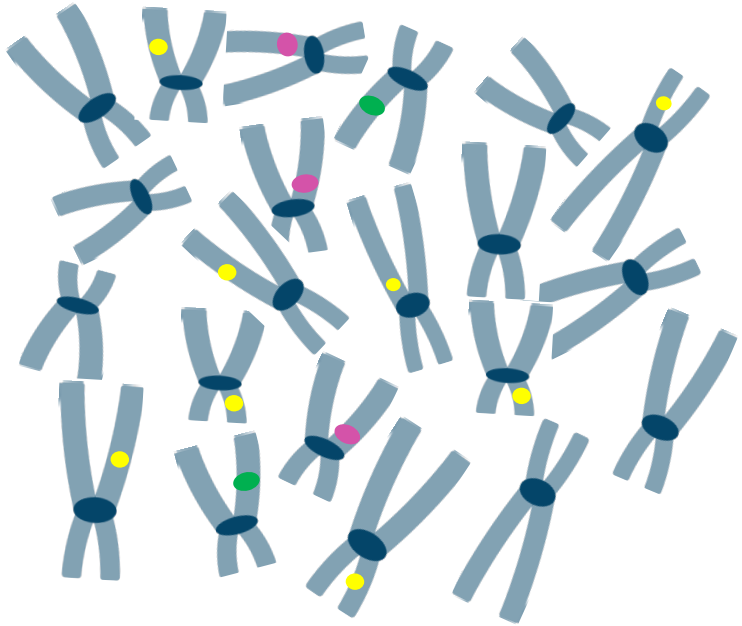
B2M MARKER PROBE

- Brackets Loci with 1.1 Mb – 6-FAM
  - Inversions
  - Translocation
  - Chromosomal CN
  - B2M Loci CN
  - On-Target Insert Verification

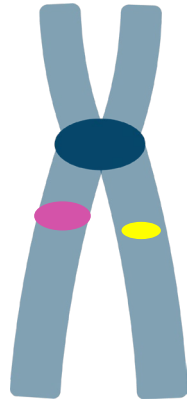
Measure & Locate CAR Insertions

CAR TRANSGENE PROBES

- Insertion Signals down to 2Kb – ATT0 643
  - On-Target CN
  - Off-Target CN
  - Inverted Inserts



On-Target



Inverted Insert

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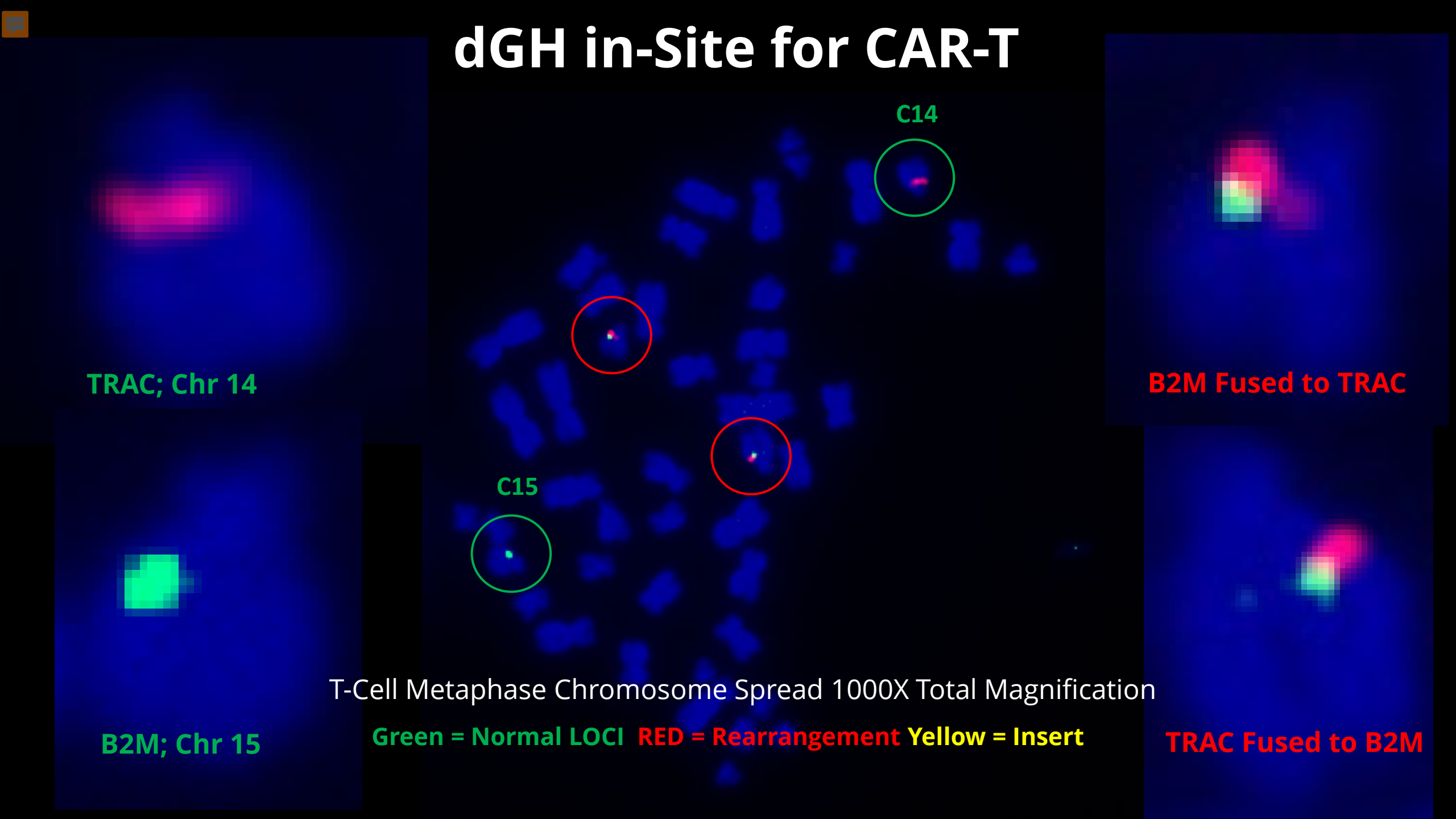
dGH in-Site for CAR-T

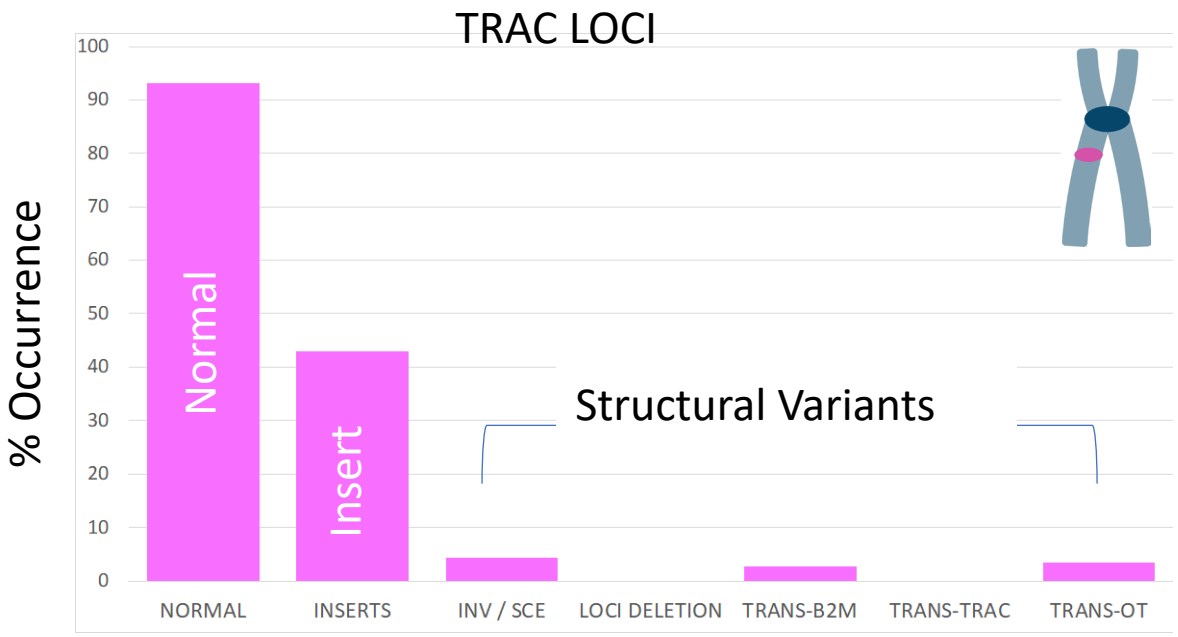


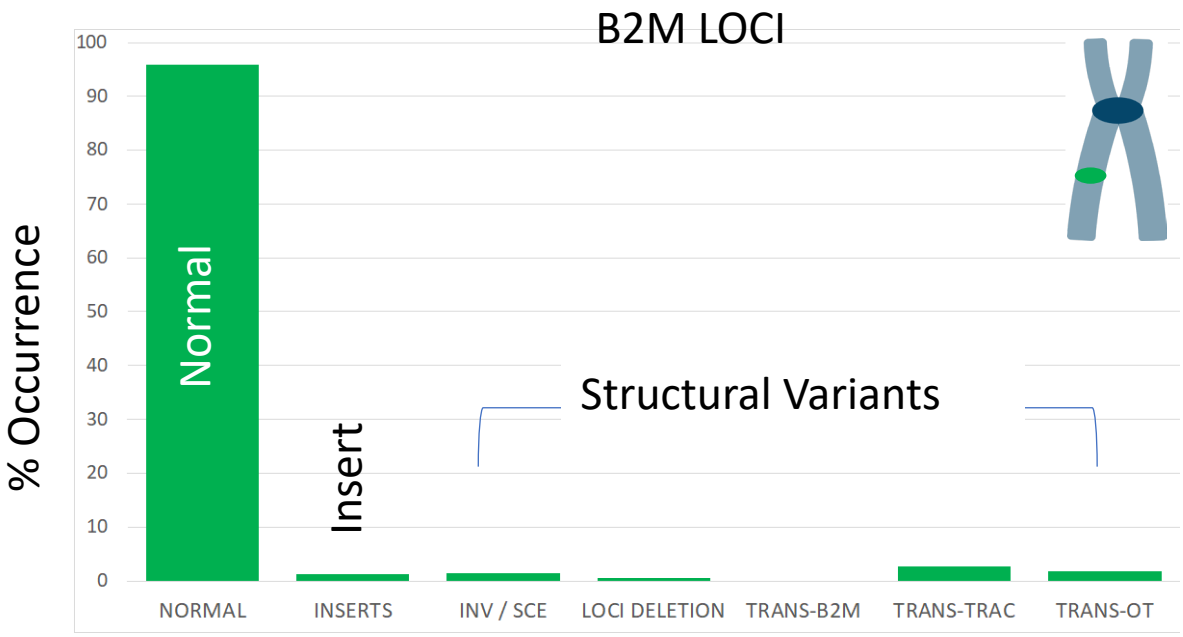
T-Cell Metaphase Chromosome Spread 1000X Total Magnification

Green = Normal LOCI RED = Rearrangement Yellow = Insert

Single Cell Measurement of Many Cells

Key Metric: **Lower Limit of Prevalence = f (**number of cells mapped**)**



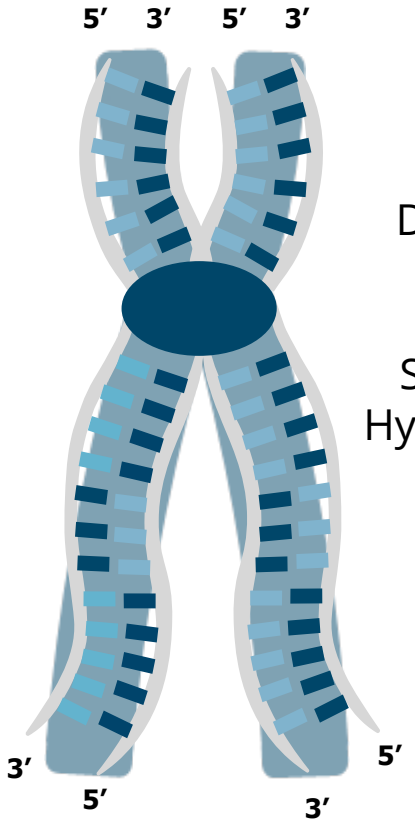


LOCI	NORMAL	INSERTS	INV / SCE	LOCI DELETION	T-B2M	T-TRAC	T-OFF TARGET
B2M	384	5	6	2		11	7
TRAC	373	172	18	0	11		14
Off-Target		0.86			1	2	400

200 Cells, 400 C14, 400 C15 Analyzed – Percentages Based on Normal Duplex Genome

dGH™ is Chromatid Painting (not Metaphase FISH)

Blue = DAPI Staining of Chromosome Structure

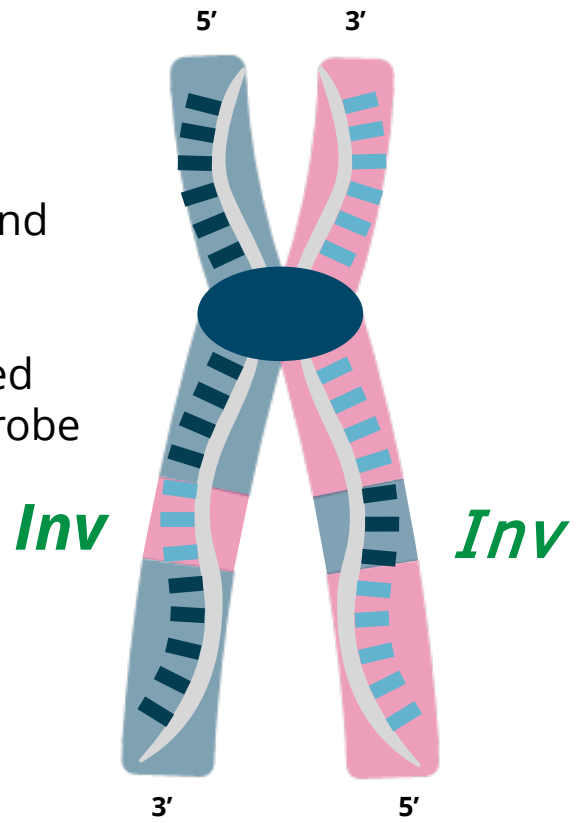


Double Stranded Metaphase Chromatid

Daughter Strand Stripping

Single Stranded Hybridization Probe

Pink = Fluorescently Labeled Hybridization Probes



Analyte: Single Stranded dGH Chromatid

DNA Orientation from Image Data

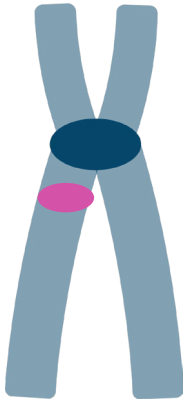
dGH chromosomes contain 2 strands of oppositely oriented, Parental DNA only—NO Daughter Strands

Single-stranded probes designed are to target *only* the Watson strand. **Signal appears on one sister chromatid only**

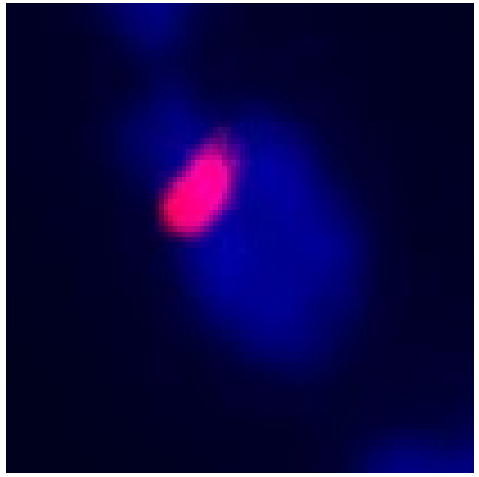
Signal from inverted targets appears on the opposite sister chromatid

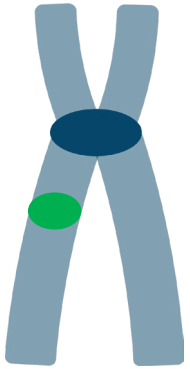
Williams, E., & Bailey, S. (2009). Chromosome Orientation Fluorescence In Situ Hybridization (CO-FISH)

Use Case 1: Tracking Structural Rearrangements

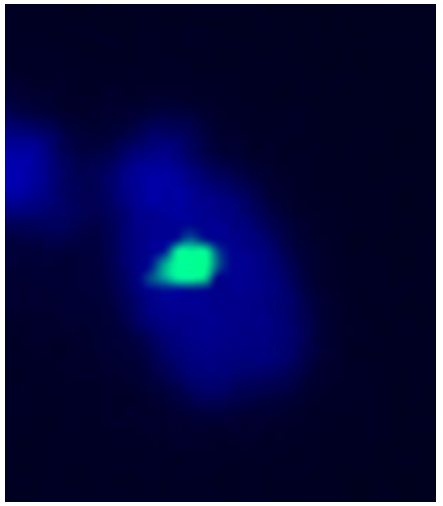


TRAC





B2M



TRAC MARKER PROBE

- Brackets Loci with 850 Kb – Pink
  - Inversions
  - Translocation
  - Chromosomal CN
  - TRAC Loci CN
  - On-Target Insert Verification

B2M MARKER PROBE

- Brackets Loci with 1.1 Mb – Green
  - Inversions
  - Translocation
  - Chromosomal CN
  - B2M Loci CN
  - On-Target Insert Verification

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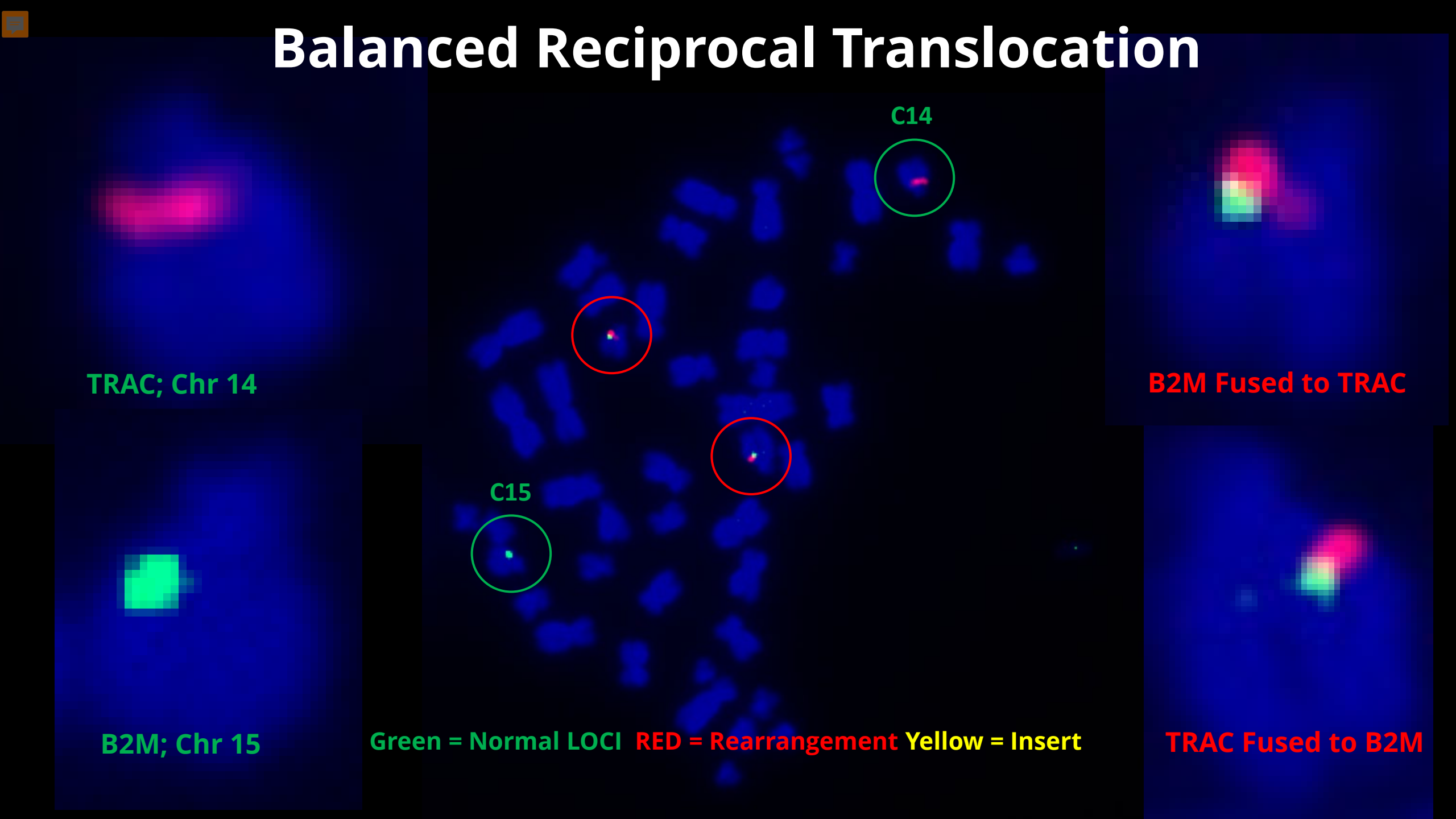
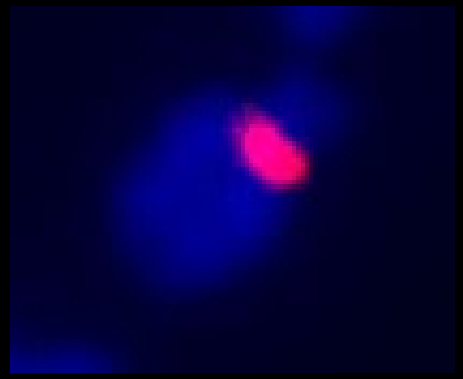
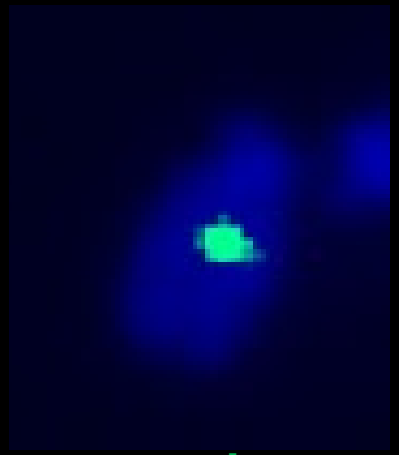
Balanced Reciprocal Translocation



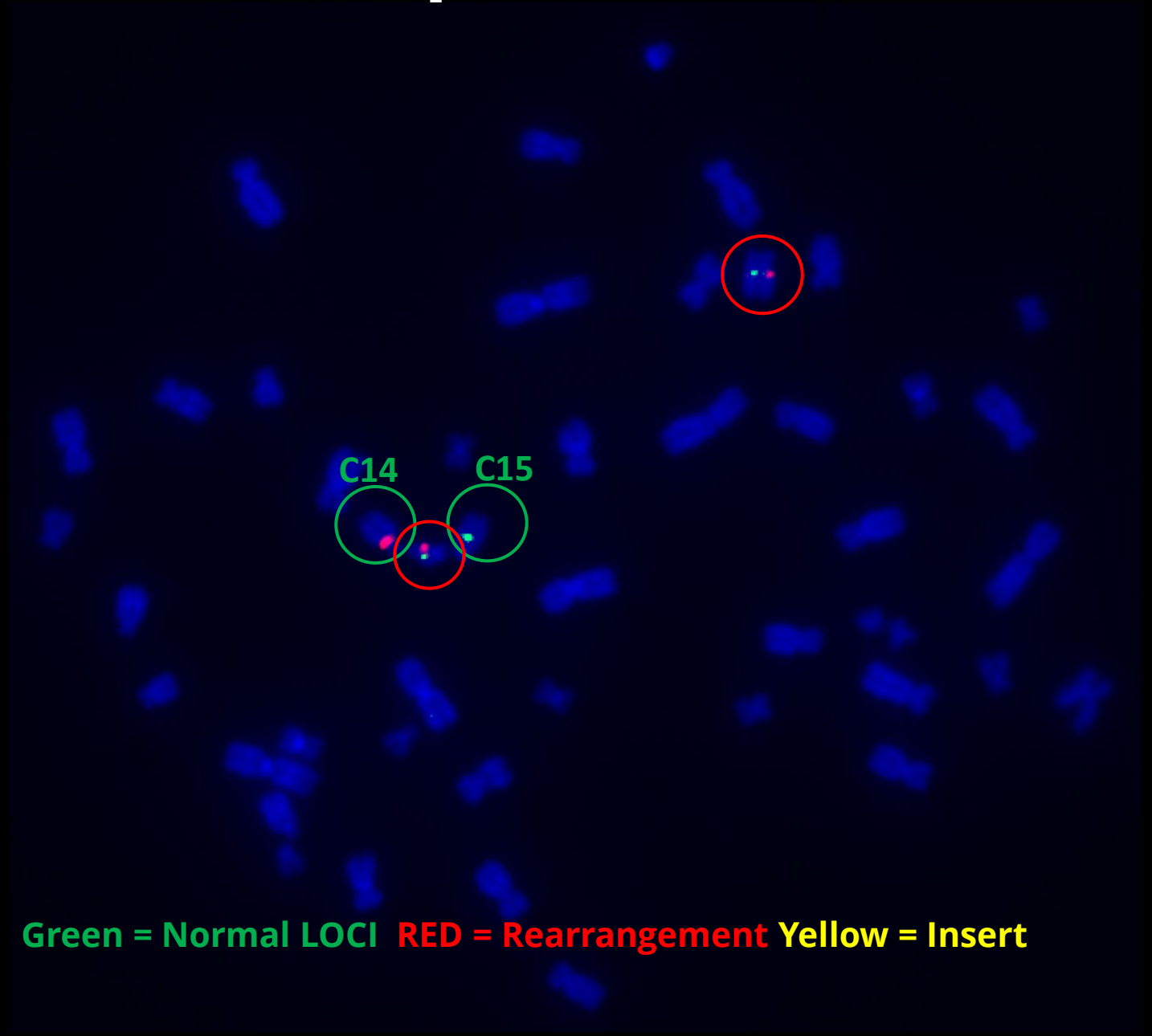
Unbalanced Reciprocal Translocation



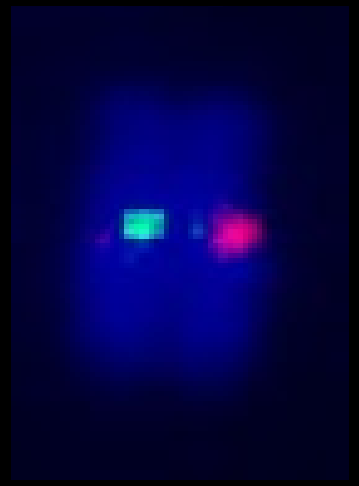
TRAC; Chr 14



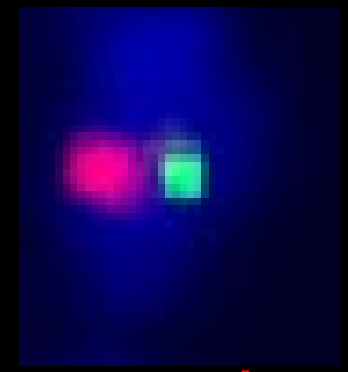
B2M; Chr 15



Green = Normal LOCI RED = Rearrangement Yellow = Insert

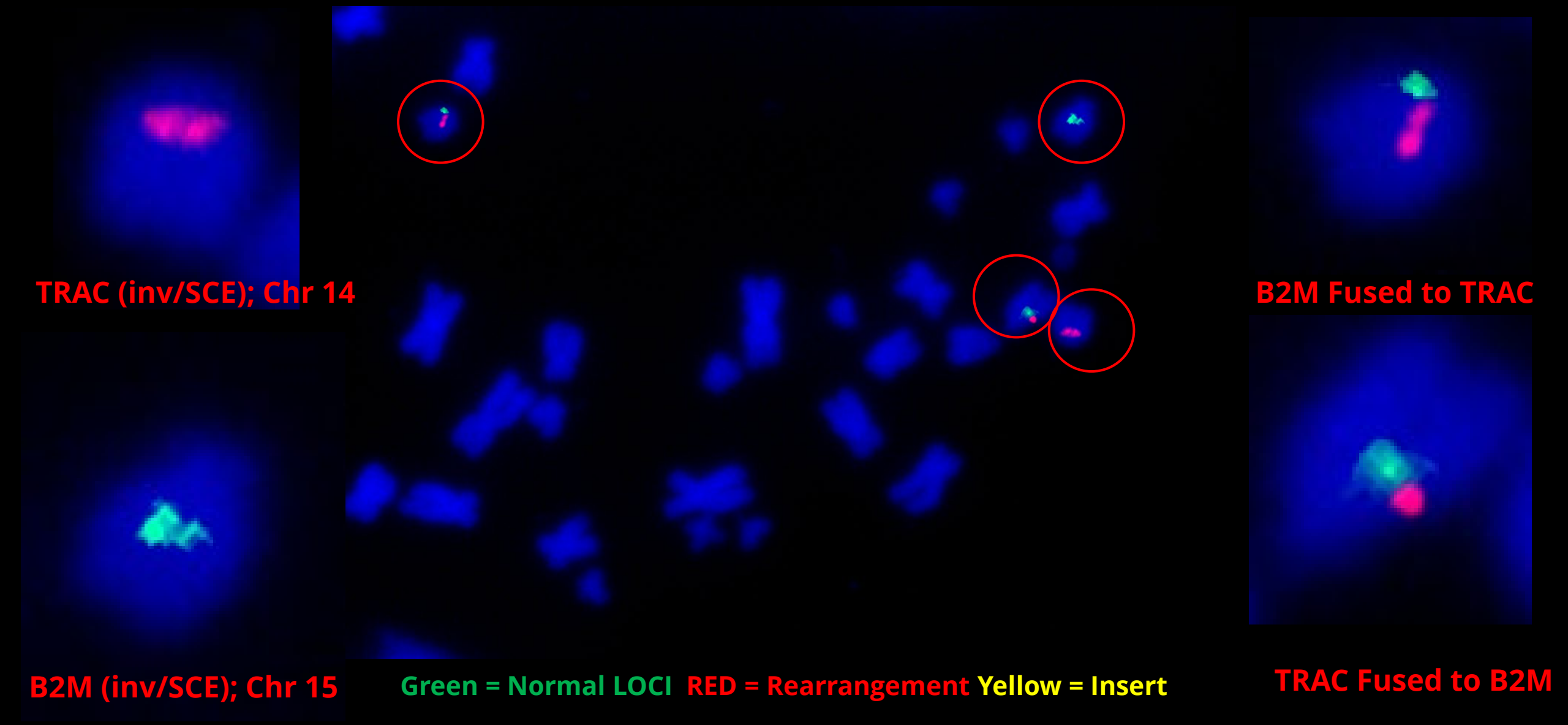


B2M Fused to TRAC (Acentric)



TRAC Fused to B2M (Dicentric)

Multiple Structural Rearrangements in a Single Cell



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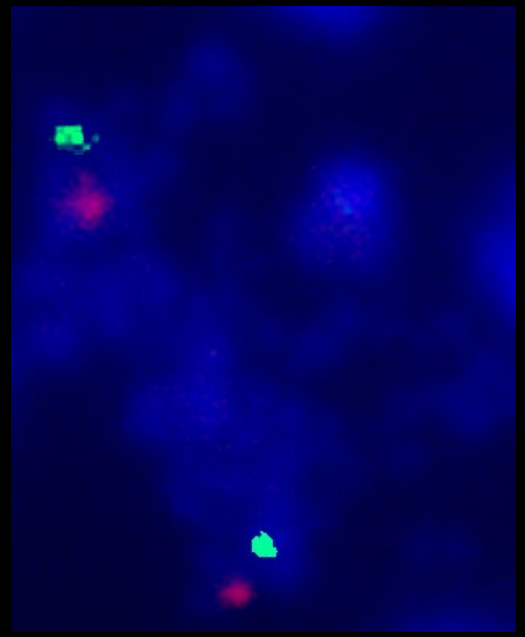
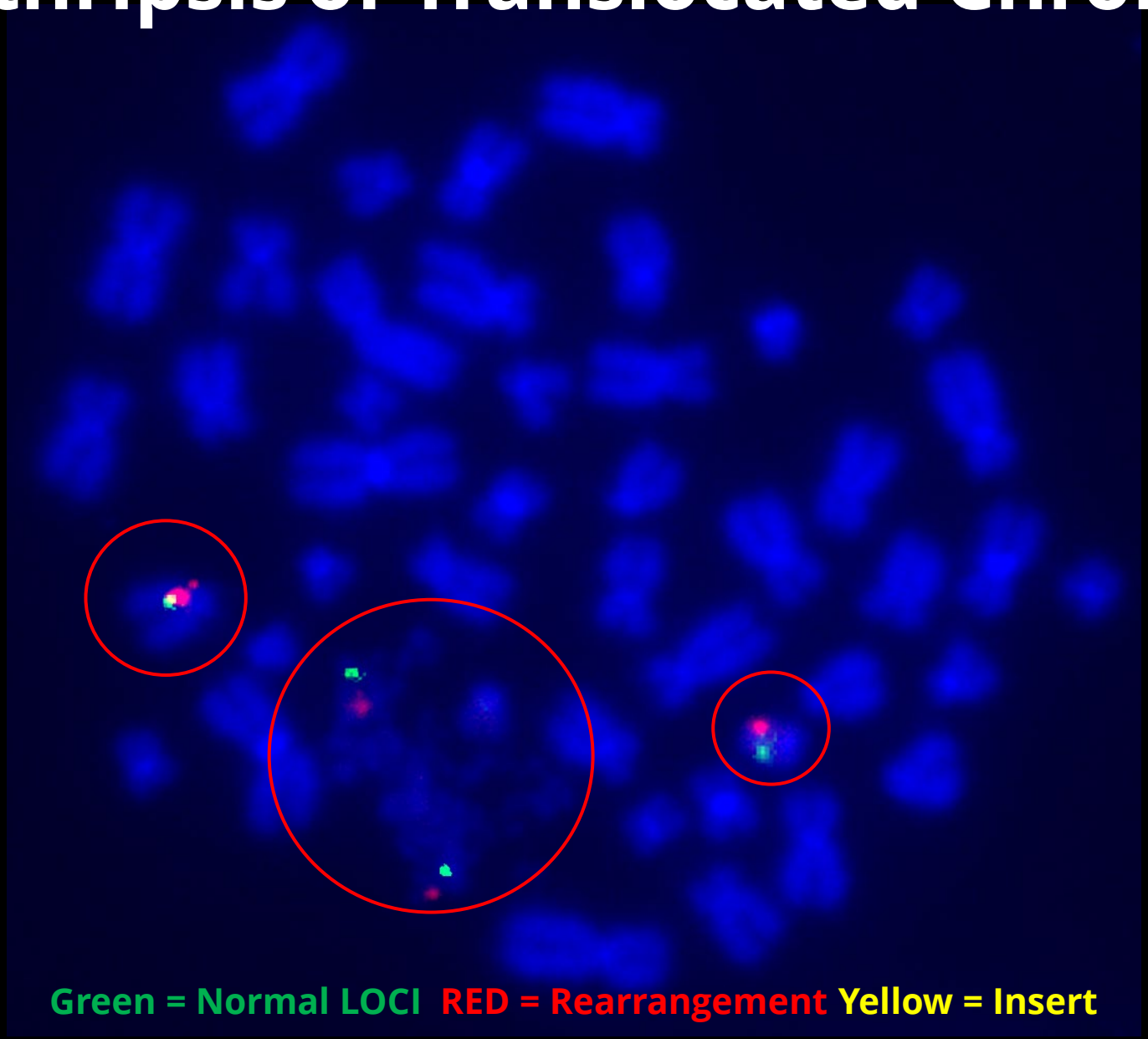
Chromothripsis of Translocated Chromosomes

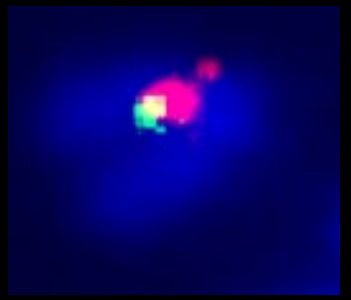
Cloud like presentation of C14 and C15 indicate shattered chromosomes



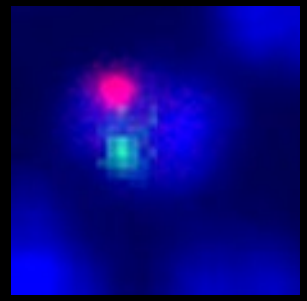
Chromothripsis of a pair of TRAC/ B2M fusion signals



Green = Normal LOCI RED = Rearrangement Yellow = Insert



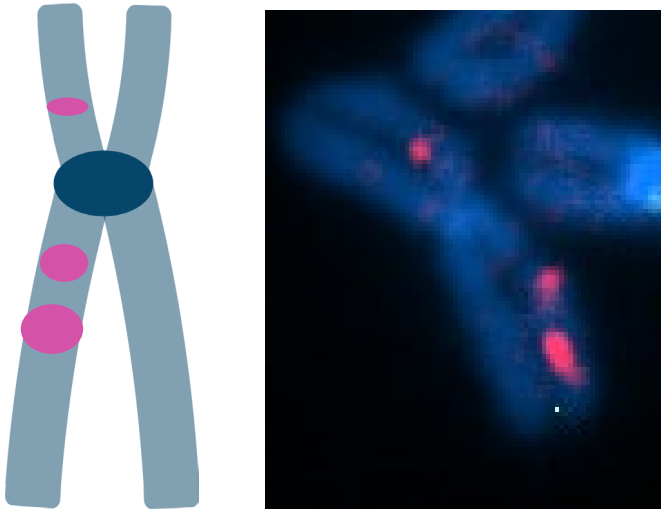
B2M Fused to TRAC



TRAC Fused to B2M

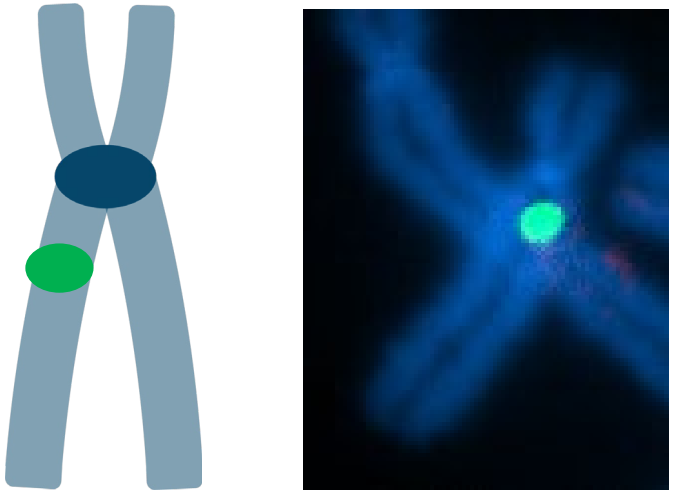
Leibowitz ML, et all. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat Genet. 2021 Jun;53(6):895-905.

Use Case 2: Random Transgene Insertions

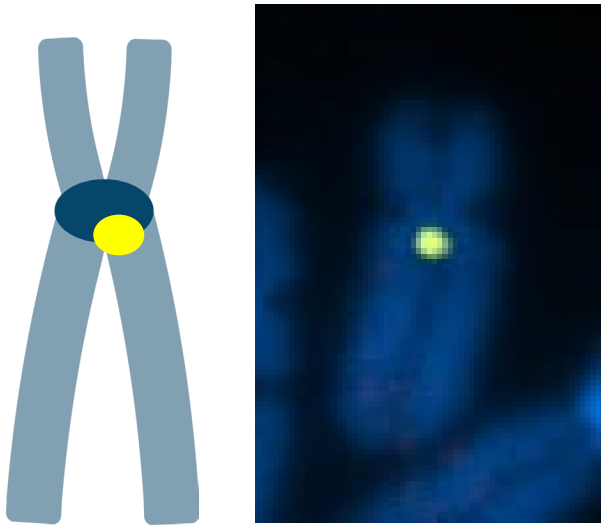


CHR 3 IN-SITE LADDER
Pink, 3 probe ladder for insert size estimation

- <1 copy
- 1 copy
- >1 copy



CHR 4 ALBUMIN GENE PROBE
Green, Housekeeping gene- serves as genome ploidy control probe



LENTI INSERT PROBE
Yellow, tracks transgene insertion across the genome

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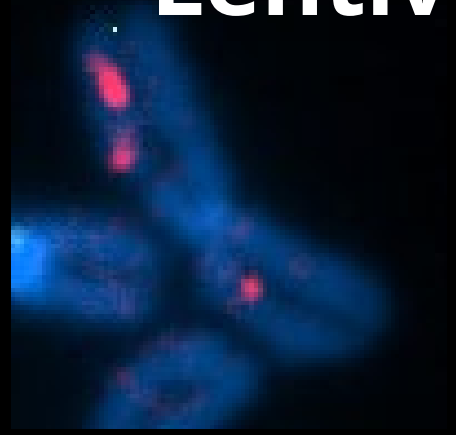
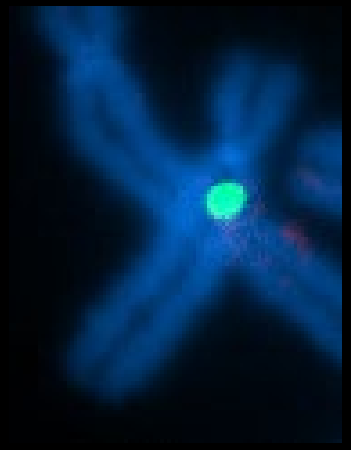
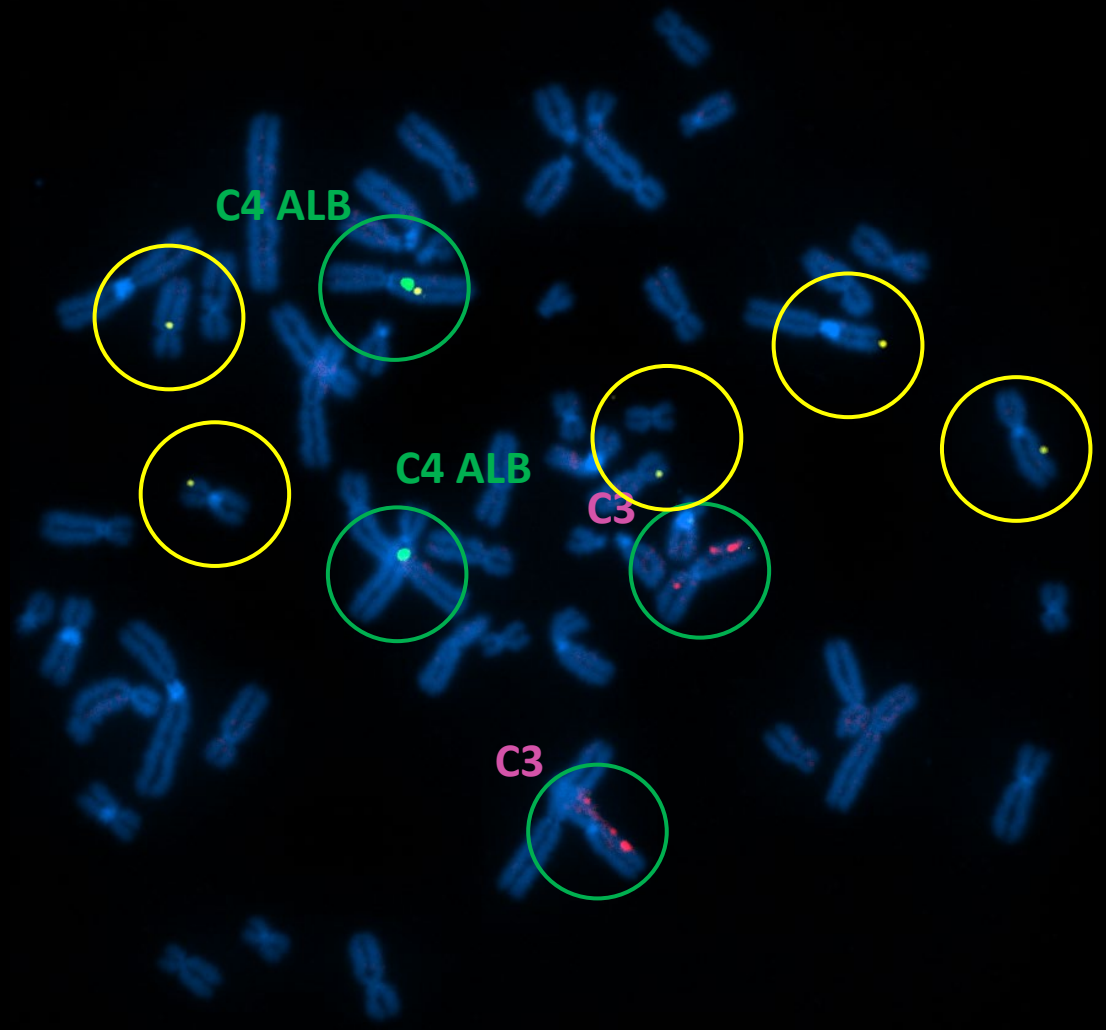
Lentiviral Transgene Insertions in HEK Cells



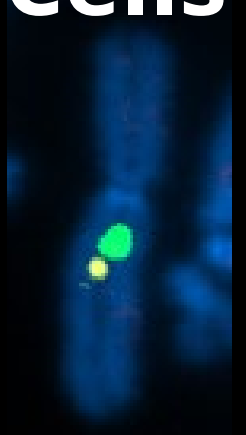
Chr3 in-Site Ladder for insert sizing



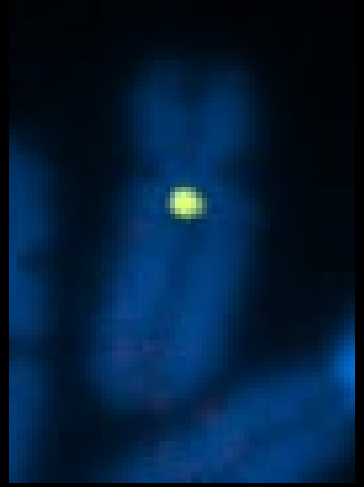
Chr4 ALB for ploidy assessment



Green = Normal LOCI RED = Rearrangement Yellow = Insert

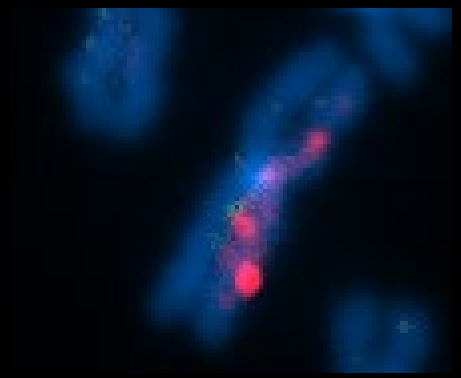


Insert on Chr4 near ALB

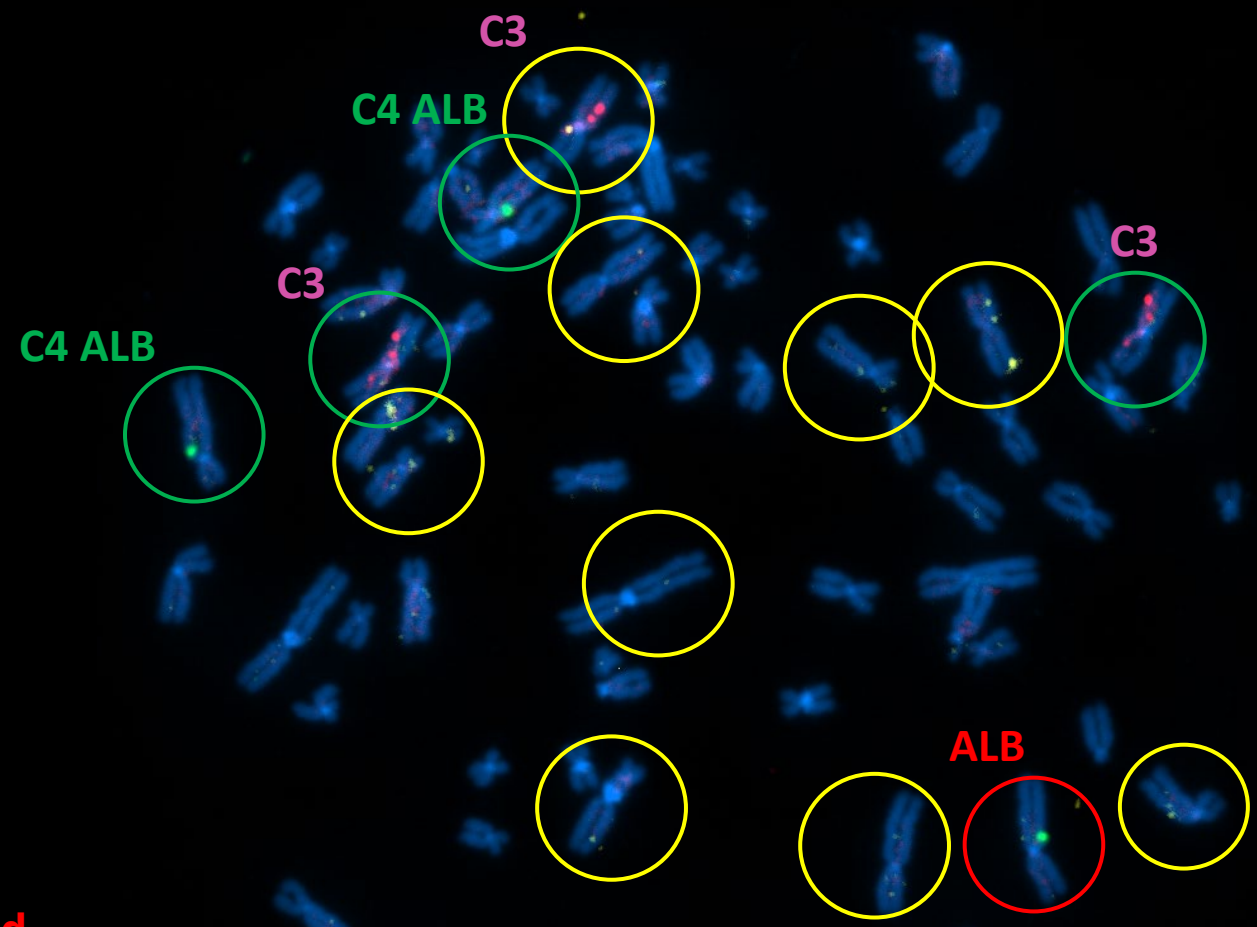


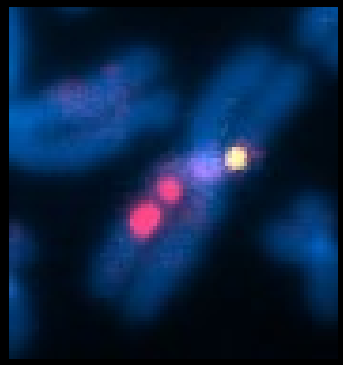
Random Insertion

Lentiviral Transgene Insertions in HEK Cells

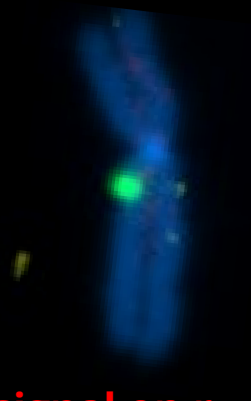


Chr3 in-Site Ladder for insert sizing

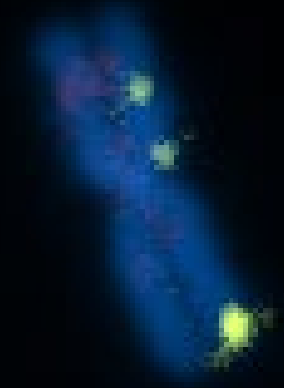




Insert on Chr3 in ladder region



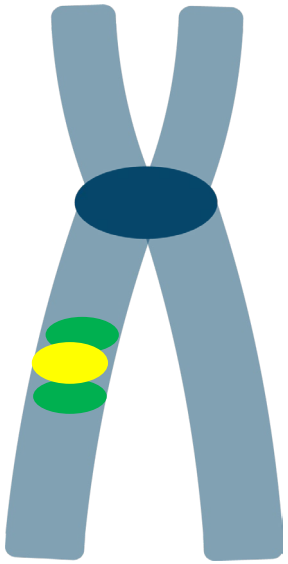
ALB signal on rearranged Chr 4



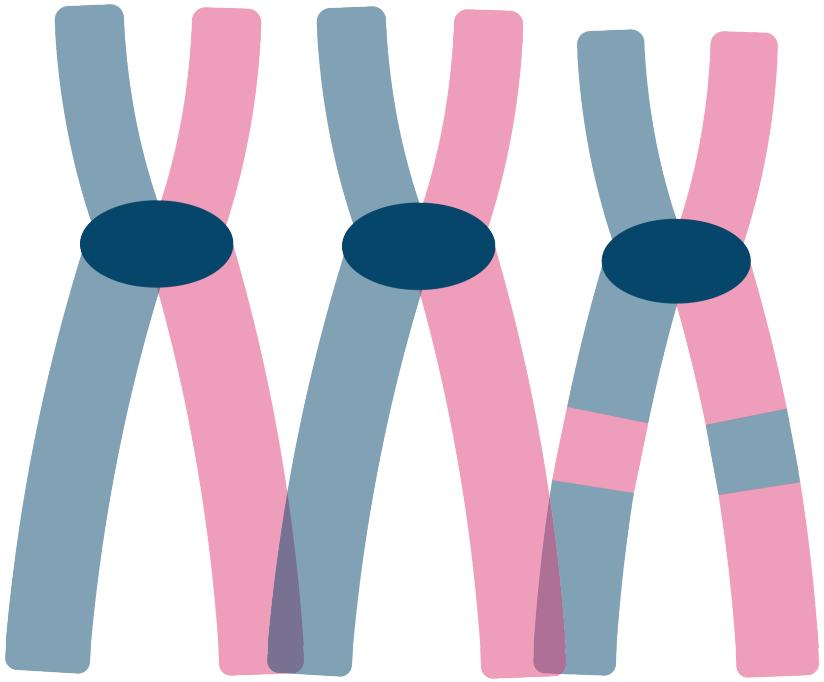
Variably sized inserts, random insertion

Green = Normal LOCI RED = Rearrangement Yellow = Insert

Use Case 3: Targeted & Random Insertions



LENTI INSERT PROBE
- Yellow
- Tracks transgene insertion across the genome

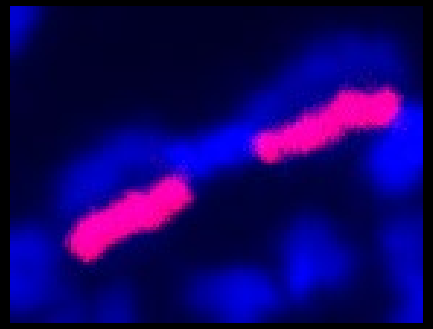


CHR 20 MARKER PROBE
Green- brackets loci with 1 Mb– Green
- Inversions
- Translocation
- Chromosomal CN
- Loci CN
- On-Target Insert Verification

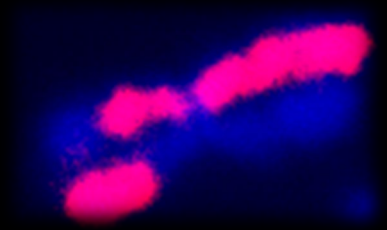
SINGLE-COLOR SCREEN ON CHRS 1, 2 & 3
Pink- Measures approximately 25% of the genome for
- Inversions
- Translocation
- Aneuploidy
- Chromosomal CN

Transgene Insertions in iPS Cells

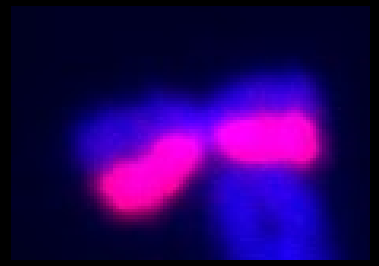
Pink = Screen Paint



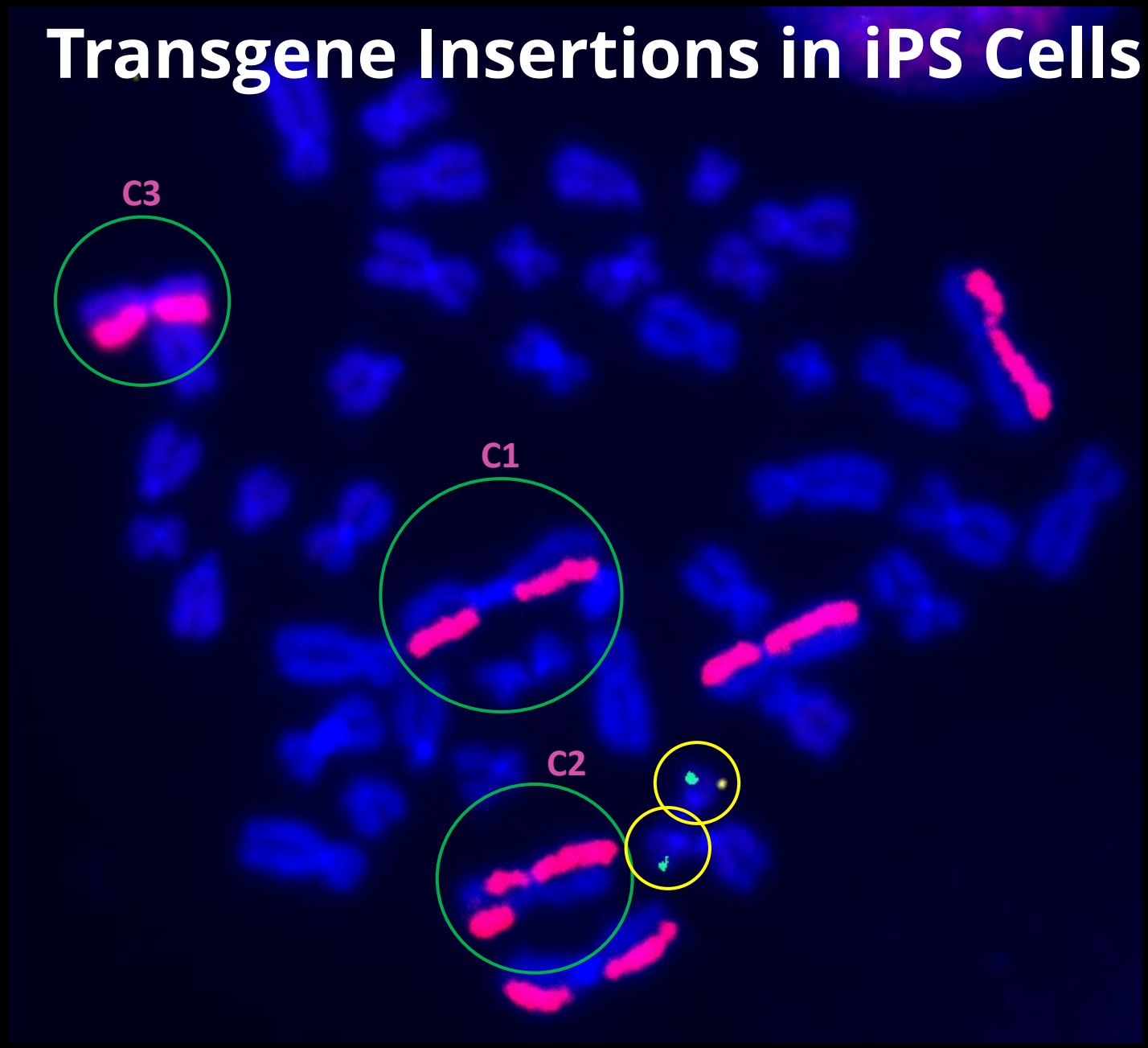
Chr1

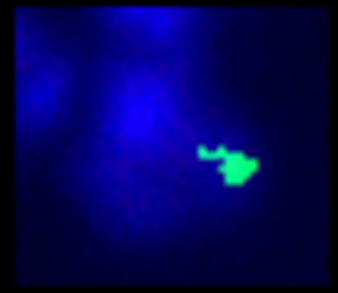


Chr2

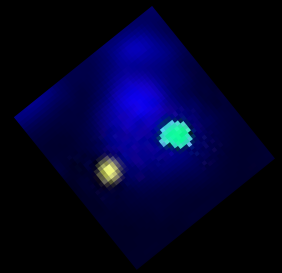


Chr3





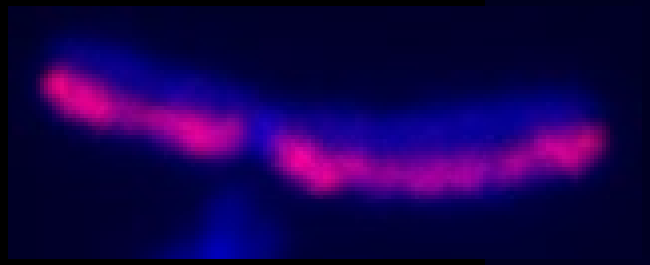
Chr 20 Target with no insert



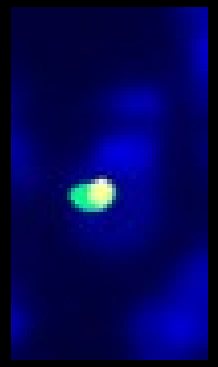
Yellow + Green = On -Target Insert

Green = Normal LOCI RED = Rearrangement Yellow = Insert

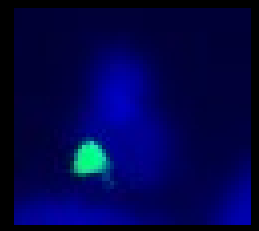
Transgene Insertions in iPS Cells



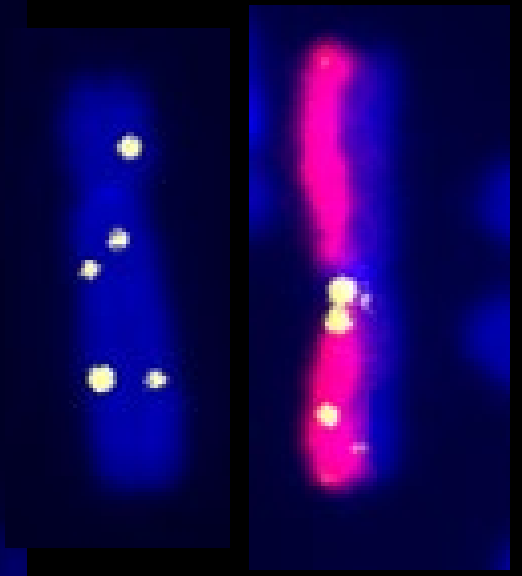
Screen Paint



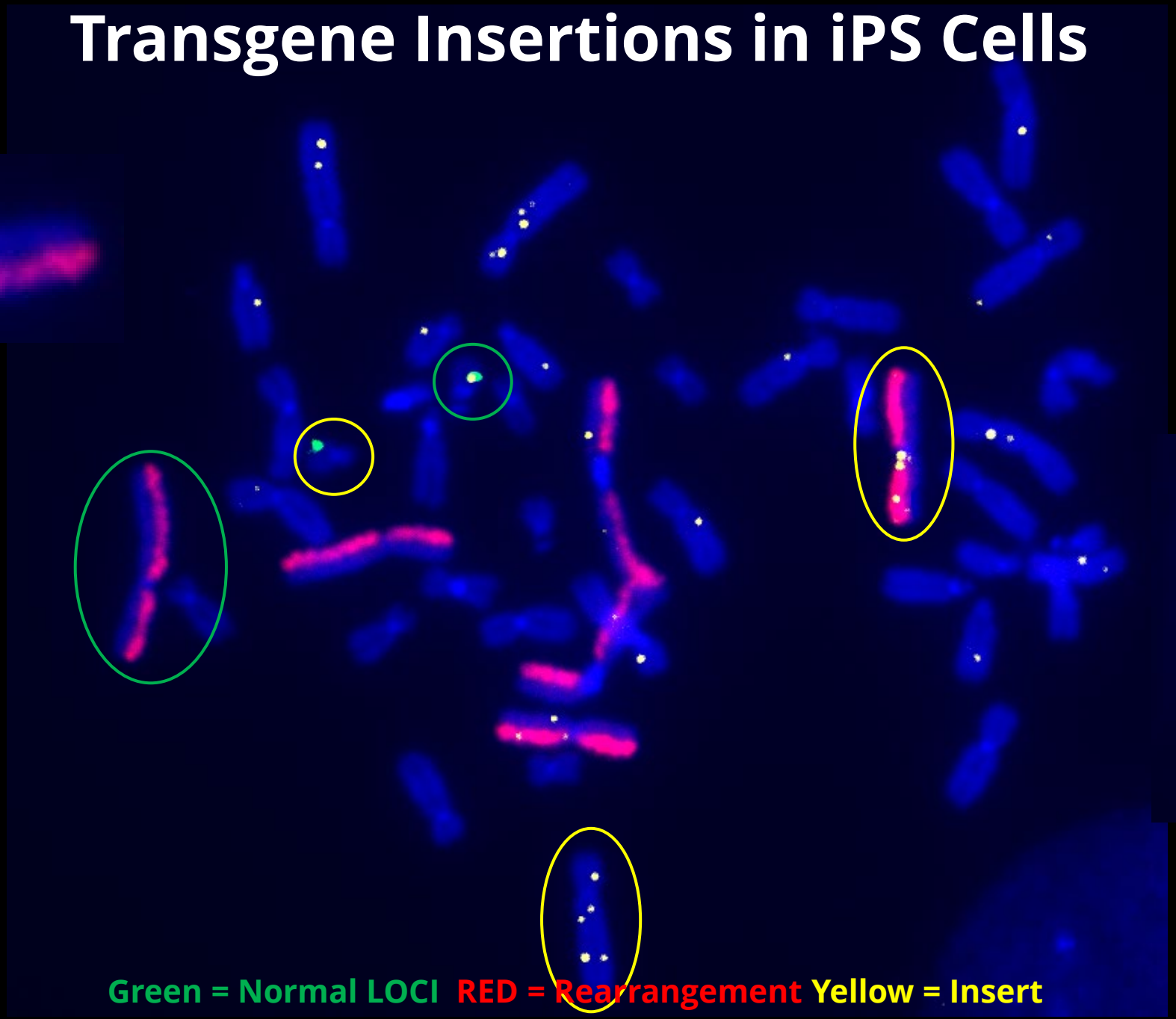
Yellow + Green =
On -Target Insert



Target site, no insert



Off-Target Inserts



Green = Normal LOCI RED = Rearrangement Yellow = Insert

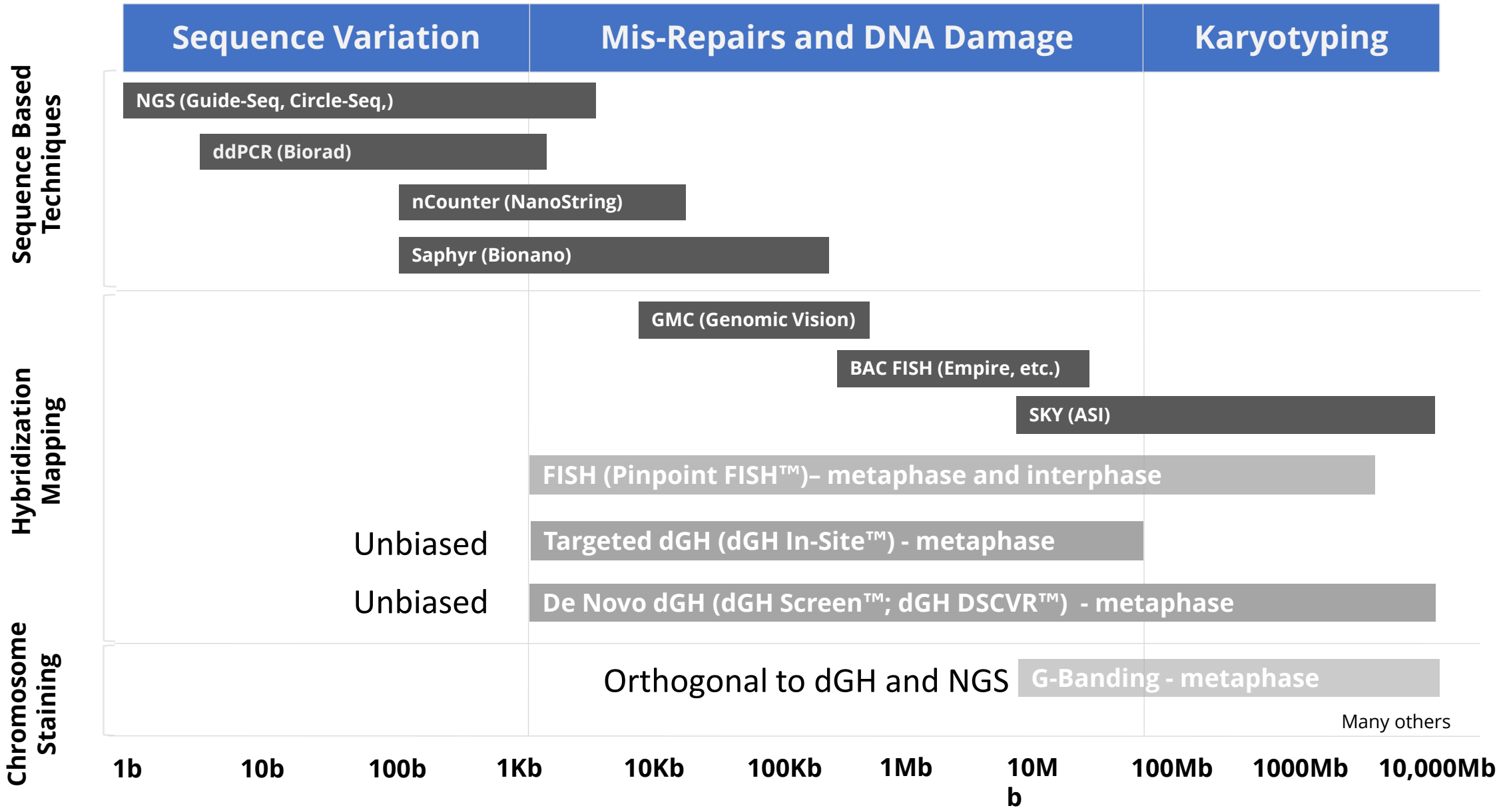
Transgene Insertions in iPS Cells

Average ICN per cell: 7.8

- On-target only: 2%
- On-target plus off-target: 14%
- Off-target only: 77%
- No Integrations: 7%

Green = Normal LOCI RED = Rearrangement Yellow = Insert

dGH is Part of a Comprehensive Analytical Package



dGH in-Site for CAR-T

Genome Engineering → **Edited Cell Cloning** → **Structural Analysis** → **Lead Selection** → **Pre/Clinical Dev**

Cellular Engineering
- Insertion Mapping
- Insertional Copy No.
- Edit Optimization
- Process Optimization
- Qualification

Single Cell Cloning
- Clone Selection
- Clone Qualification
- Clone Stability

Genome Mapping
- Normalcy
- Structural Variants
- Variation Rates
- DNA Damage
- Variation Discovery
- Variant Identification

Genotox
- Insertional Mutagenesis
- Clonal Outgrowth
- Chromothyrpsis
- Genomic Instability
- Karyotypic Abnormalities
- Model Animal Studies

Quality Control
- Batch Variability
- Cell Line Stability
- Subject Qualification
- Batch Release

CHROMOTHRYPSIS: Easily measurable in dGH in-Site, dGH Screen and g-banding

CLONAL OUTGROWTH: Measure using a dGH in-Site Time Course

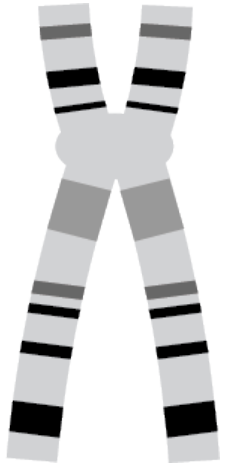
INSERTIONAL MUTAGENESIS: Mark high risk loci and track insertions with dGH in-Site

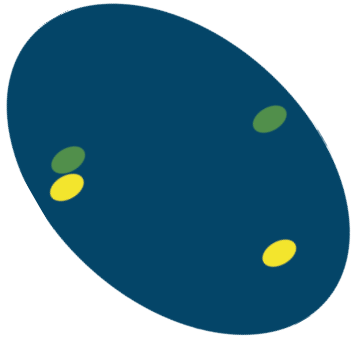
STRUCTURAL VARIATION: Single cell detection and mapping of variants with dGH in-Site

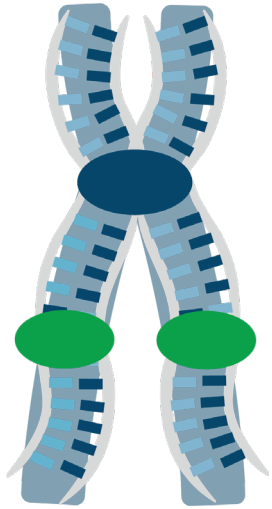
GENOMIC INSTABILITY: Early detection of instability with dGH SCREEN

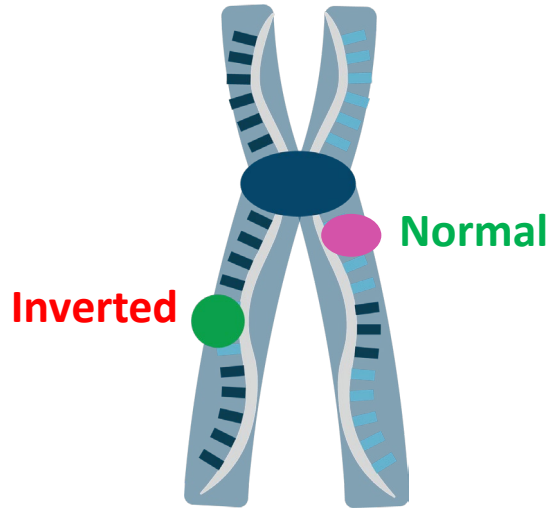
ANEUPLOIDY: Single cell detection with dGH SCREEN and g-banding

Compare & Contrast Single Cell Cytogenetic Methods









	G-Banding	FISH	Meta-FISH	dGH in-Site
Format	Non-Genomic Stain	Pseudo-Genomic	Pseudo-Genomic	True Genomic
Coverage	Whole Genome Only	Targets Only	Localized Genome Wide	Localized Genome Wide
Resolution	800 *BAND* resolution	0.5 Mb Resolution	0.5 Mb Resolution	5 Kb Resolution
Sample	Dividing Cells	Any Cells	Dividing Cells	Dividing Cells
Data	Bands	Targets CNV*	Target CNV + Location	TARGET CNV + LOCATION + ORIENTATION

*CNV = Copy Number Variation

KromaTiD

Direct, Definitive Genomics

Thank You!

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

Please Contact:

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Dan Vetter – Manager Business Development: **dvetter@kromatid.com**