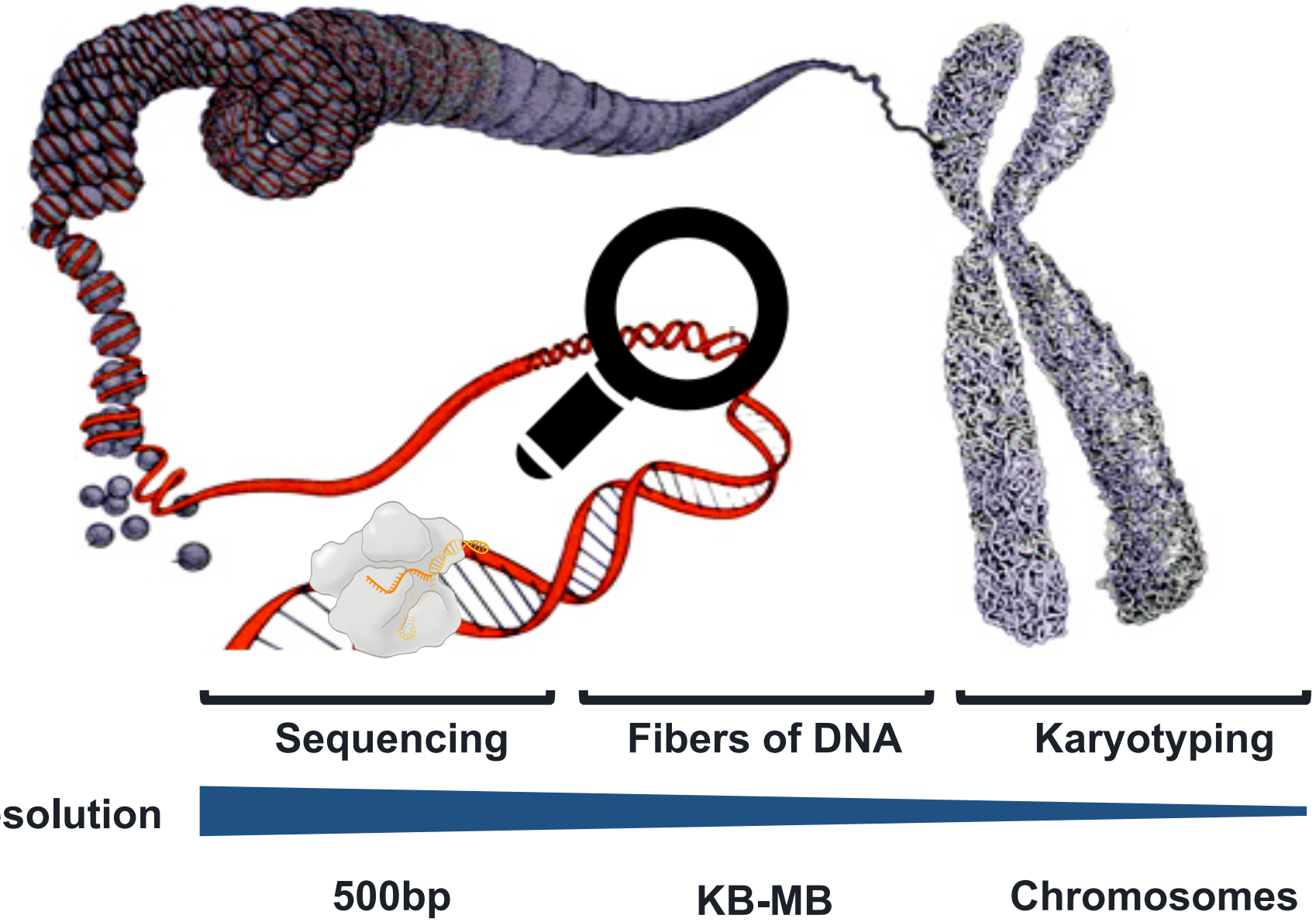
Characterization of Genomic Rearrangements in Response to CRISPR/Cas9-Induced Double-Stranded Breaks

Cecilia Cotta-Ramusino

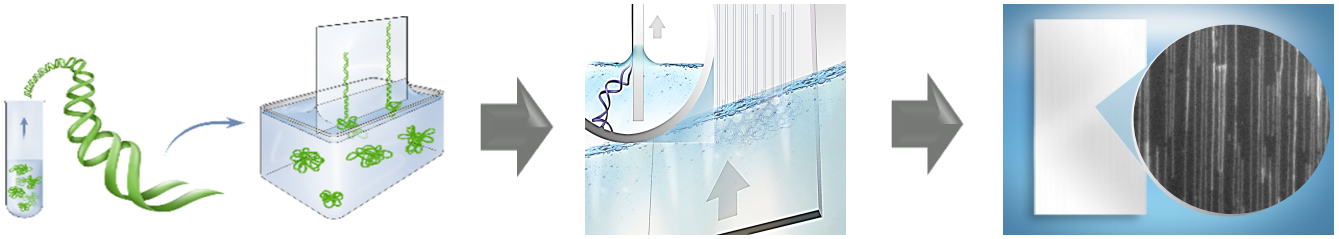
Are We Missing Rearrangements?



Single Fibers Analysis with Molecular Combing

Extraction & Molecular Combing

1. Stretching the genome for single molecule analysis



Vinyl Silane slide is immersed in the reservoir

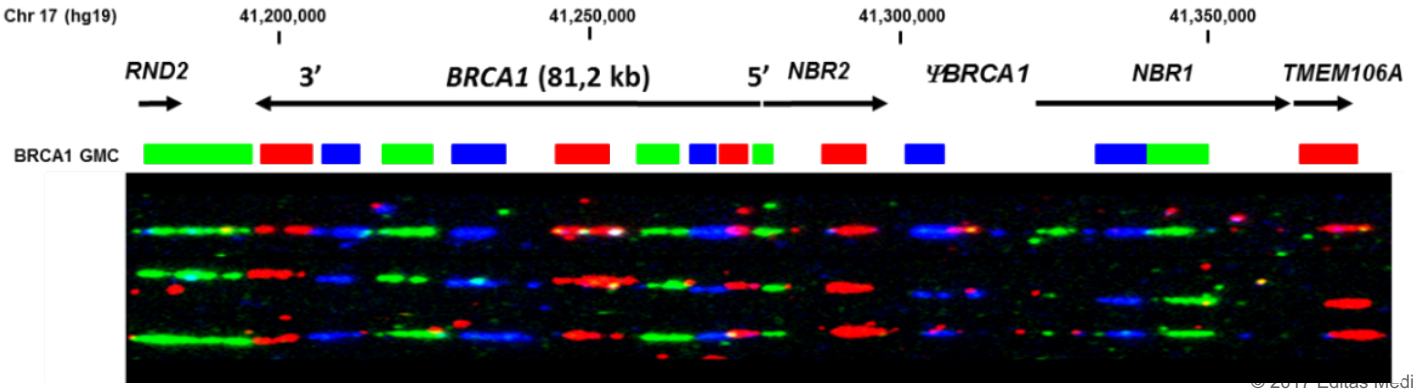
DNA attaches to the surface and is stretched in parallel strands

Hundreds of genome equivalents on one slide

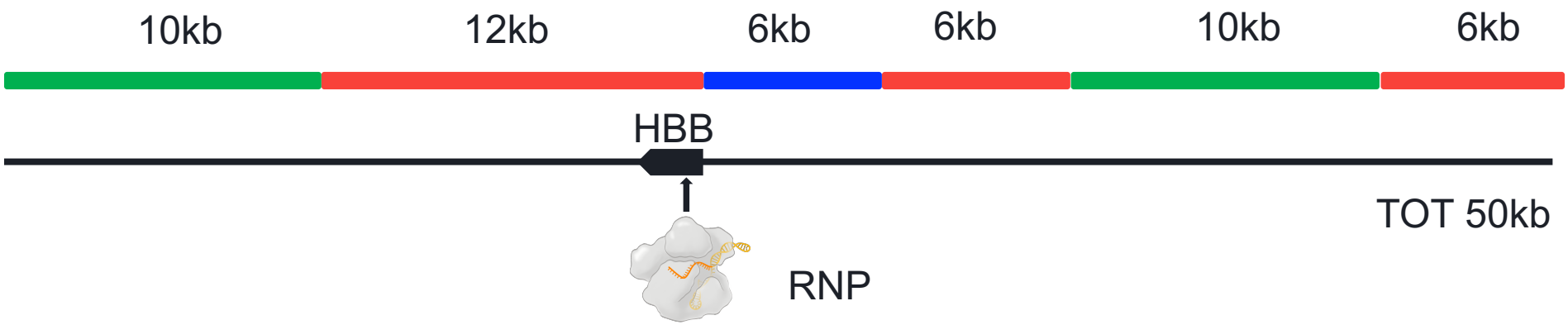
Genomic Morse Code (GMC)

2. Applying Genomic Morse Codes (GMC) to the genome

Combinations of fluorescent probes along the combed DNA molecules allows interrogation of several single fibers of DNA at your desired location (GMC up to 2MB)

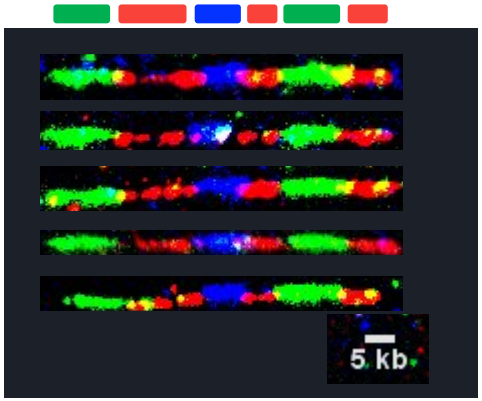


Single Fiber Analysis at HBB Locus



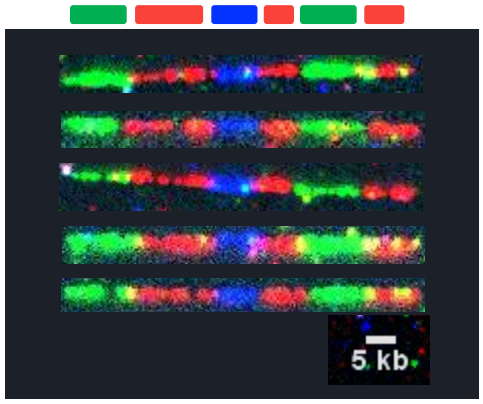
- 80% of modifications detected with NGS at the cut site in U2OS and T cells, 4 days after RNP

U2OS



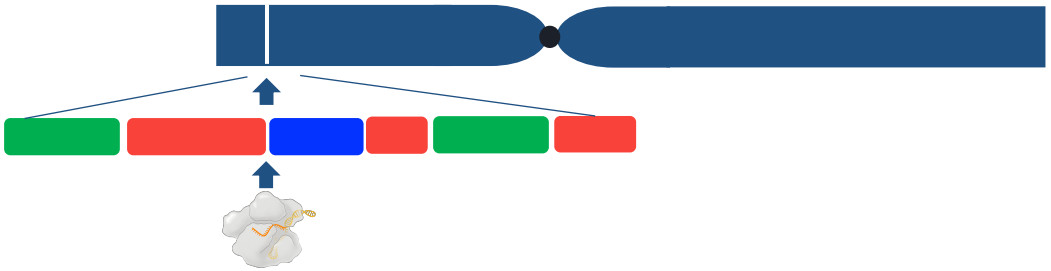
96.5% of canonical signal

T Cells

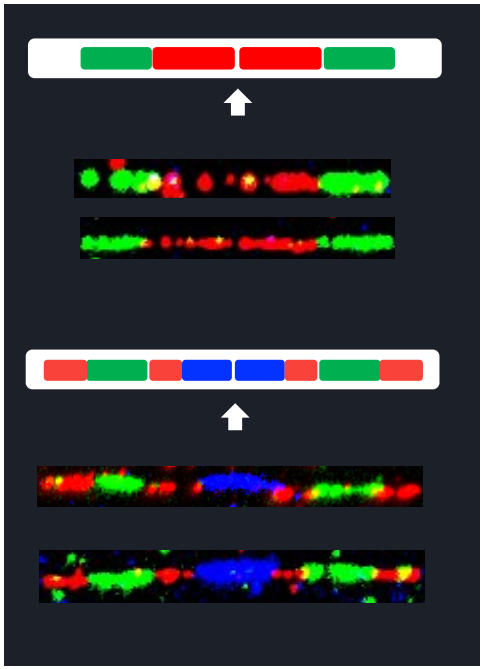


98.7 % of canonical signal

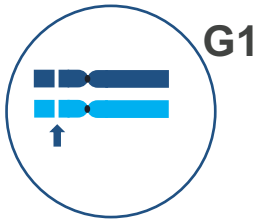
Non Canonical GMC Signal at the HBB

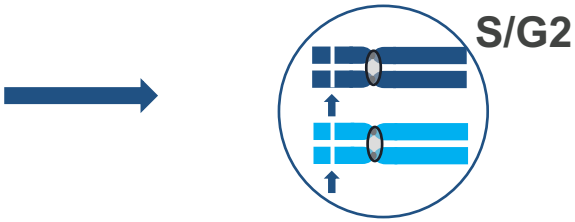


1. Symmetrical Signals



Unbalanced Translocations/Chromatid fusions



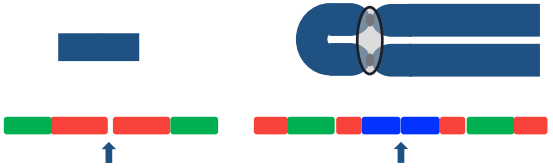


Balanced translocation
(homologous chromosomes)

Unbalanced translocation
(homologous chromosomes)

Sister chromatid fusion

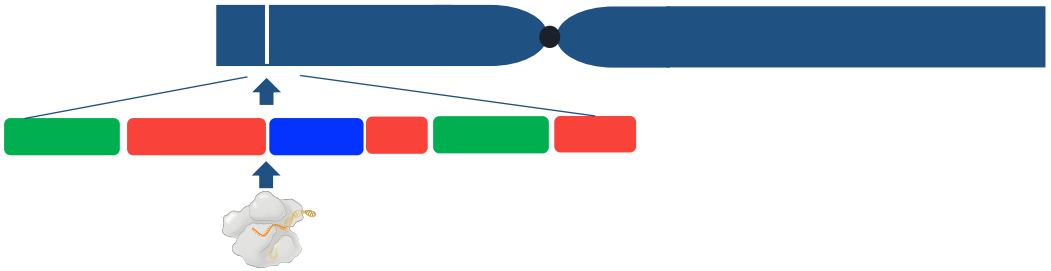
Canonical Signal



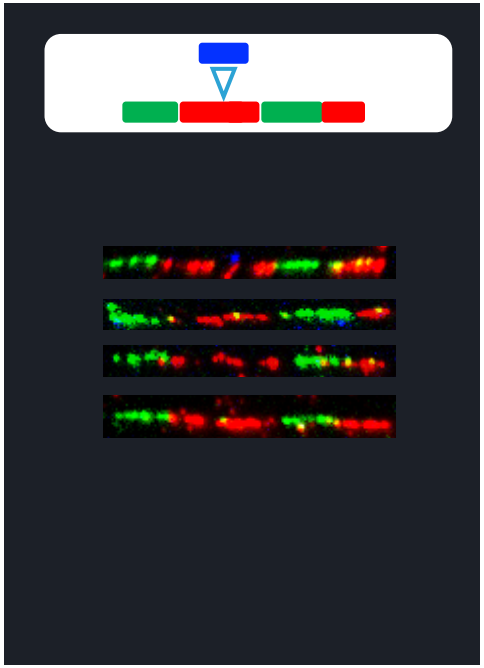
Acentric
Chromosome
Sister Fusion

Dicentric
Chromosome
Sister Fusion

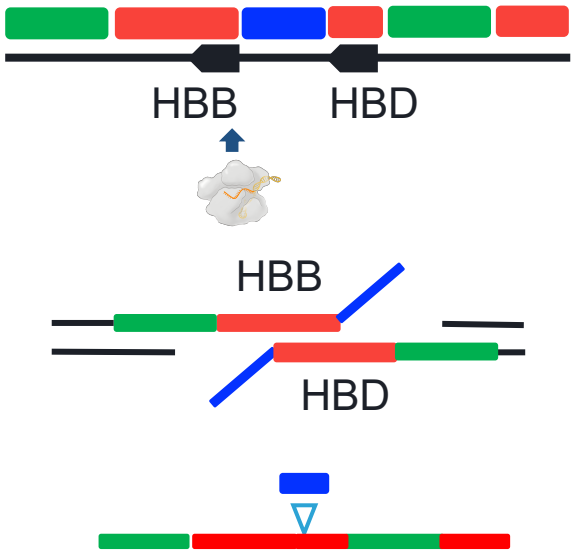
Non Canonical GMC Signal at the HBB



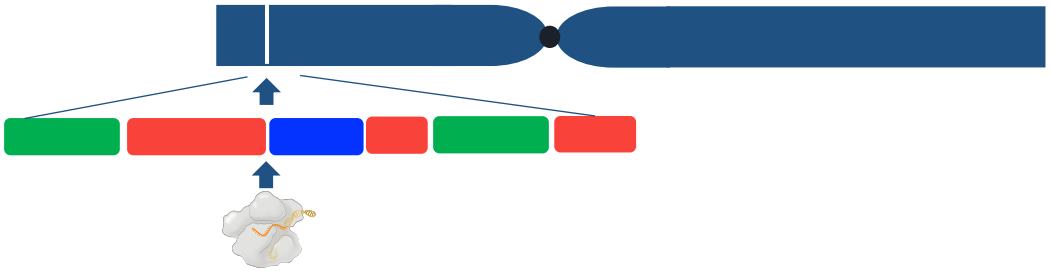
2. Loss of Blue Probe



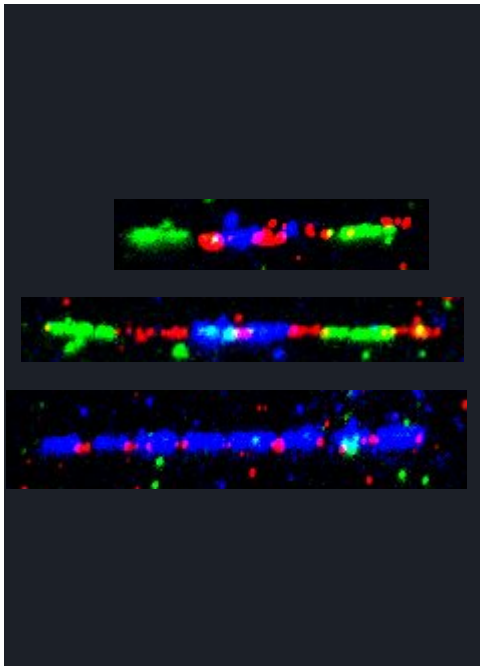
Single Strand Annealing



Non Canonical GMC Signal at the HBB



3. Other



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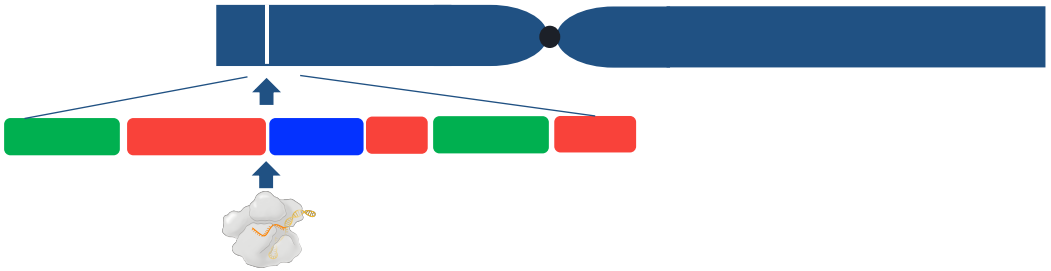
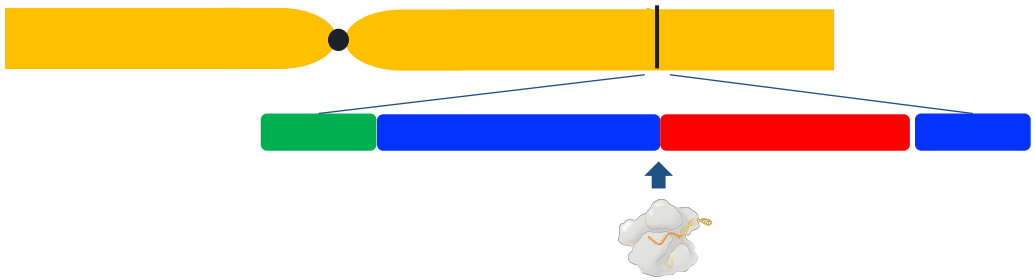
Non Canonical GMC Signal at the HBB

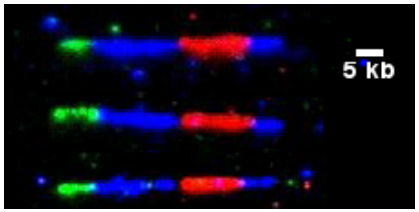


	# of Fiber Analyzed	Canonical Signals	1. Unbalanced Translocation/ Chromatid Fusion	2. Single Stand Annealing	3. Other
U2OS Cas9-wt	800	96.5 %	1.5%	0.5%	1.5%
T Cells Cas9-wt	1500	98.7 %	0.4%	0.3%	0.6%
T Cells dCas9	1500	100%	-	-	-

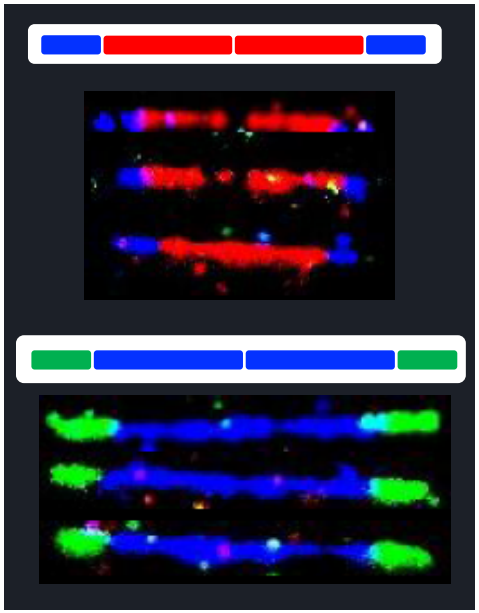
GMC Signal at Additional Locus



canonical signal

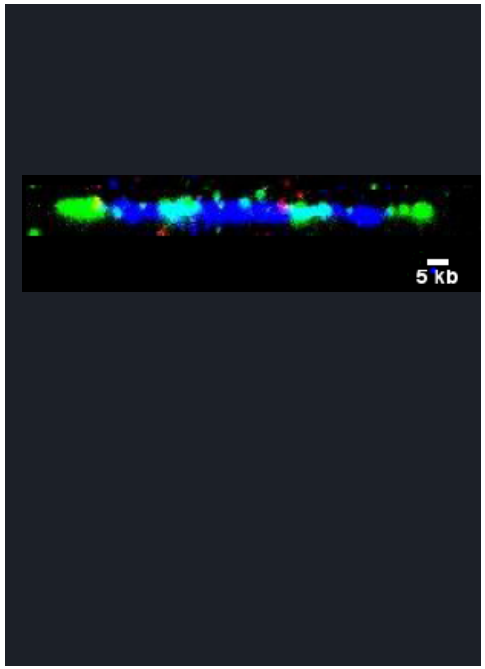


1. Unbalanced Translocations Chromatid fusions



2. Single Strand Annealing

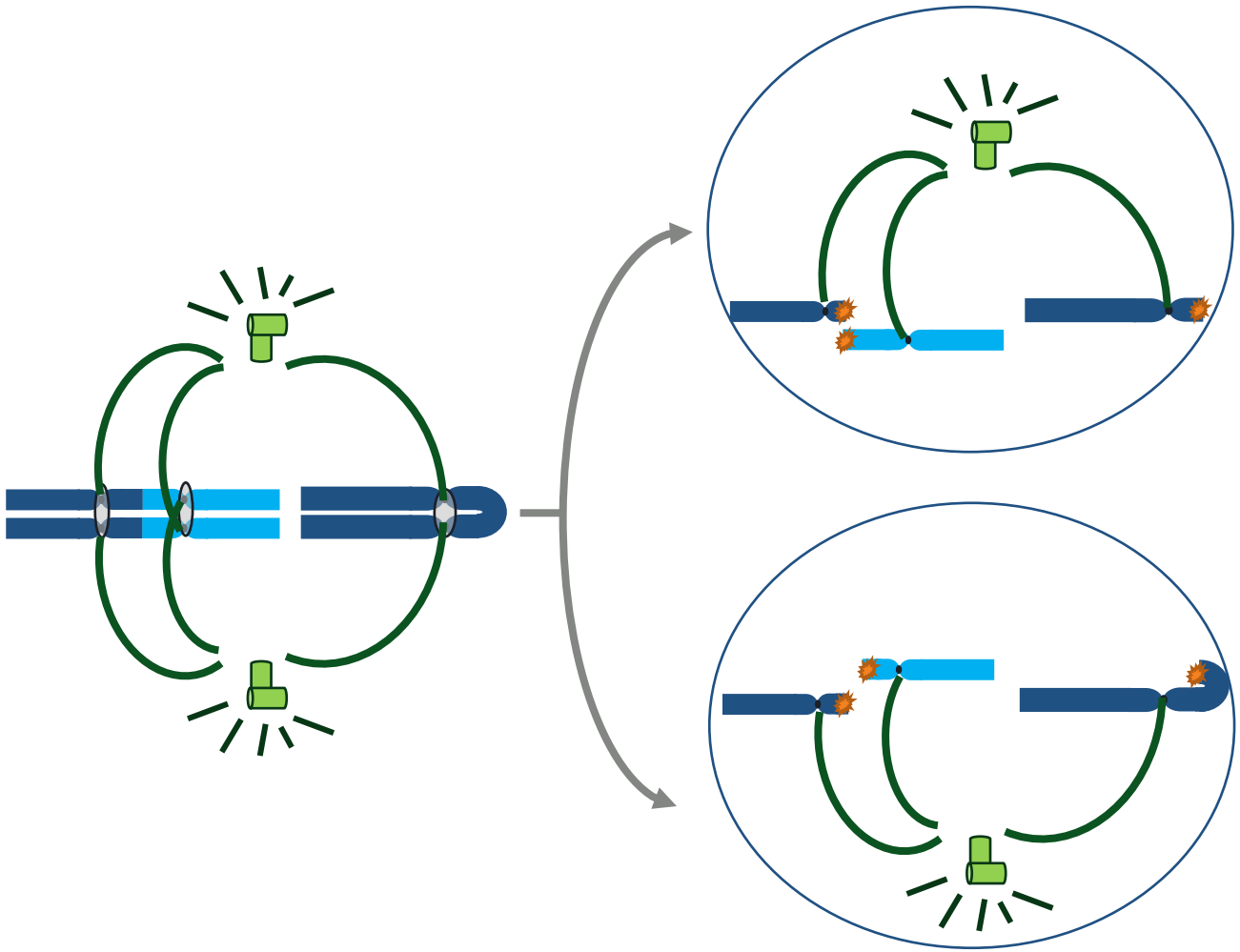
3. Other



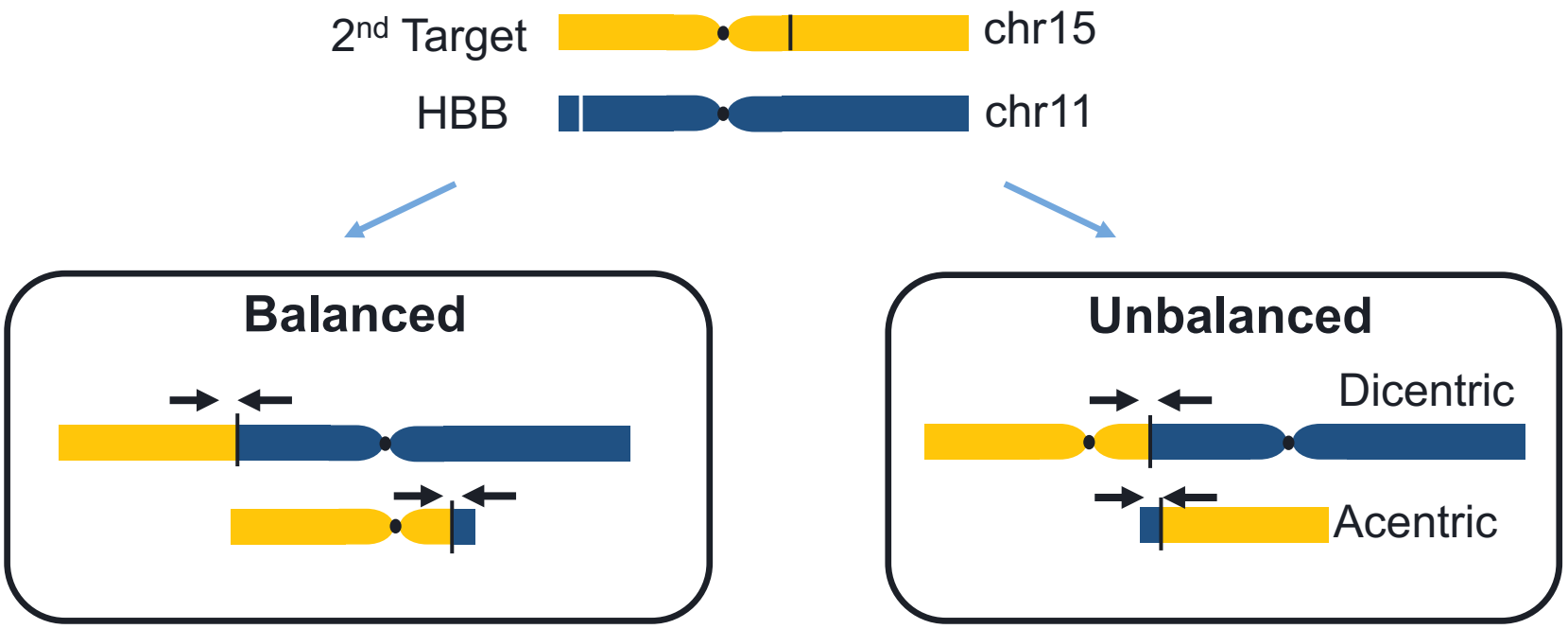
98% of canonical signal and 2 % of rearrangements, mostly unbalanced translocations

Fate of Unbalanced Translocations or Sister Chromatid Fusions

- **Loss of the acentric chromosome**
- **Dicentric chromosomes as well as fused sister chromatids can generate additional breakages -> breakage-fusion-bridge cycles**

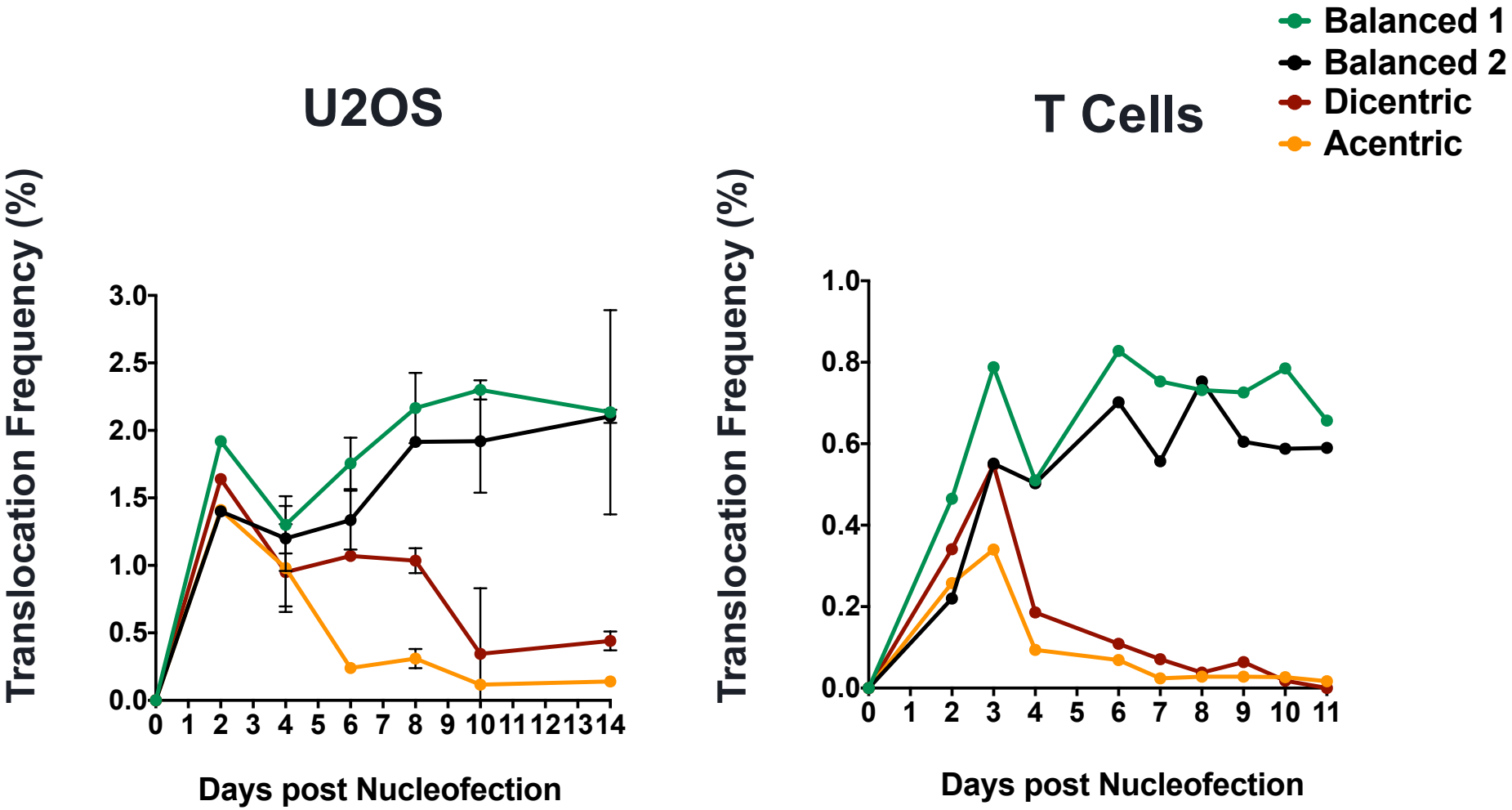


Modeling of Translocations



Translocation frequency measured by ddPCR

Unbalanced Translocation Do Not Persist Throughout Time

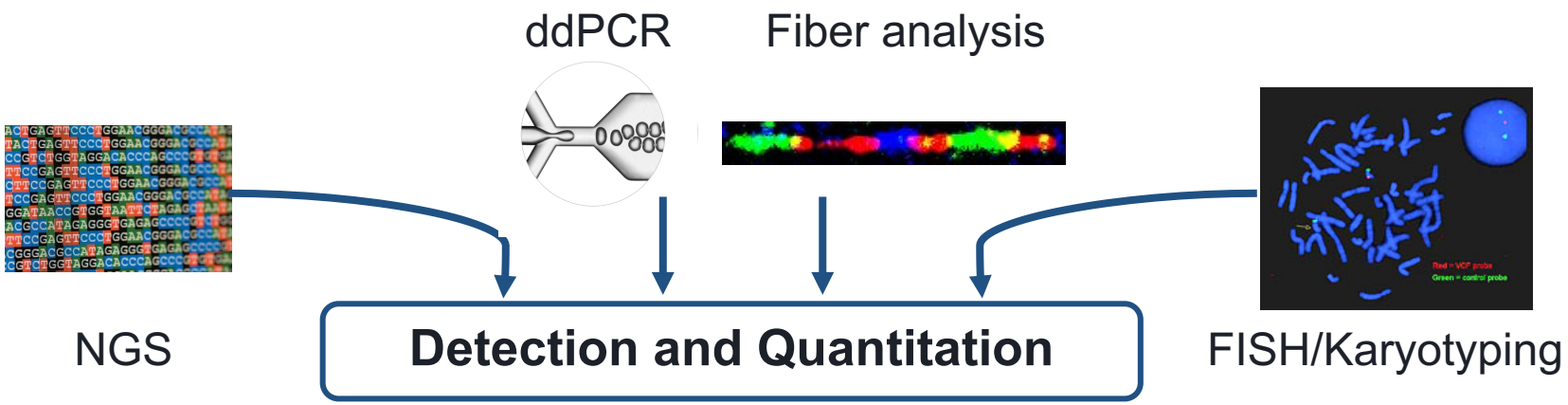


Unbalanced rearrangements are not maintained while balanced rearrangements persist

Conclusions

- **On-target cutting can lead to the formation of rearrangements, sister chromatid fusions, and translocations with the homologous chromosomes in various cell types**
- **These events are less stable and tend to be selectively lost with time/division**
- **Safety implications need to be investigated**

Overall Conclusions



Risk Assessment

Understand functional consequences of rearrangements

molecular signature ⟺ **phenotype**

Modulation

Explore and develop strategies with which rearrangement frequency can be minimized or specifically counter select

Thank you!

editas MEDICINE

GENOMIC VISION