Molecular Combing Technology:

digital and unbiased quantification of rearrangements resulting from targeted genome editing

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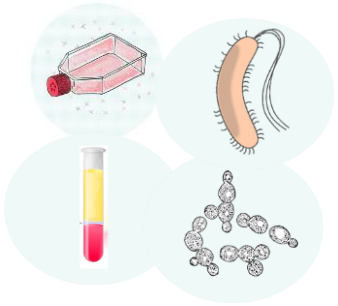
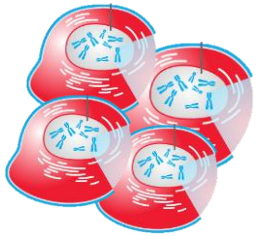
April 24th, 2018

NIST-FDA Genome Editing Workshop

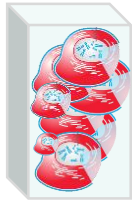
The Molecular Combing Technique



Cell harvesting

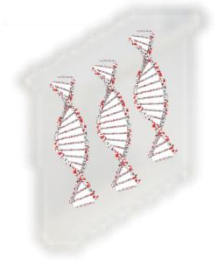


Embedding in agarose plugs to **protect** the DNA fibers

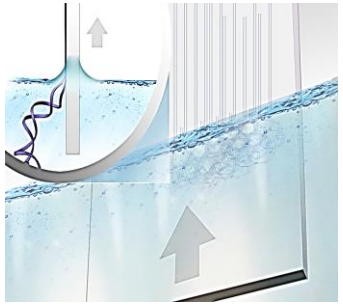


Enzymatic treatments and washes

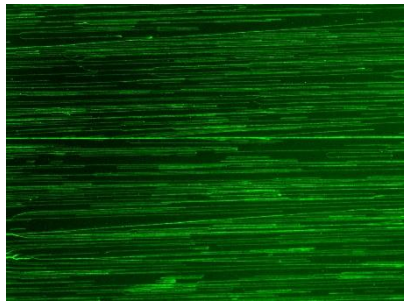
Extraction of **high molecular weight** DNA in solution



Combing at a **constant speed** of the intact DNA fibers on coated coverslip



Direct visualization of hundreds of genomes



⇒ **Direct visualization** and analysis of single DNA molecules, without amplification
⇒ **150-200 human genomes** stretched on each coverslip
⇒ **Accurate measurement** of distances with a constant stretching factor (1µm = 2kbp)

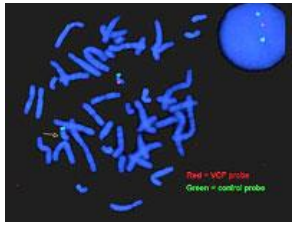
Molecular Combing: Range of Use

Designer Nuclease

Region of Interest

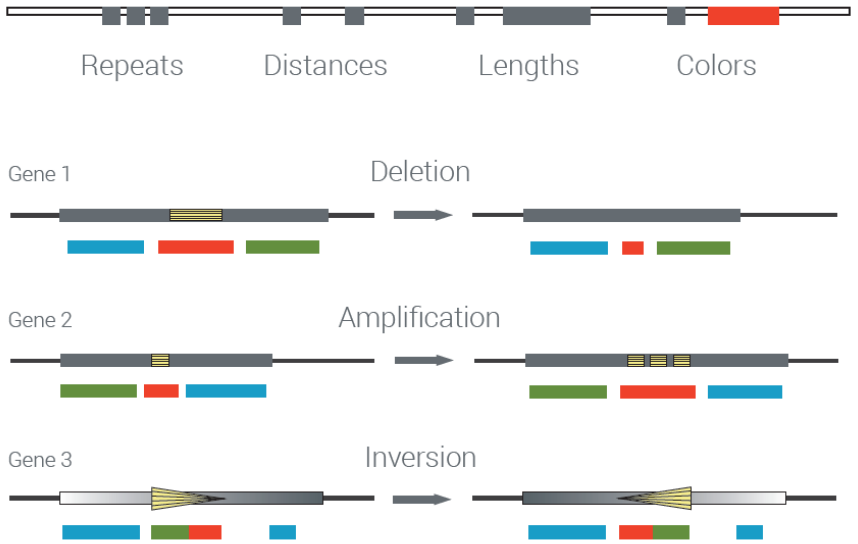
Detection and Quantitation of Modifications

Resolution

500bp	1 Kbp - 1Mbp	Chromosomes
NGS 	Molecular Combing 	
ddPCR 		
PCR-based methods	Fibers of DNA	FISH/Karyotyping

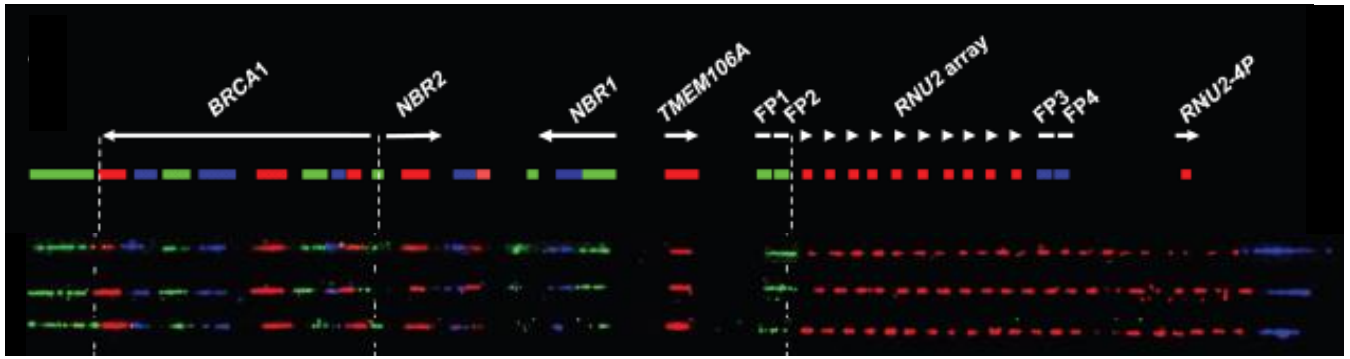
The Genomic Morse Code (GMC)

FiberProbes

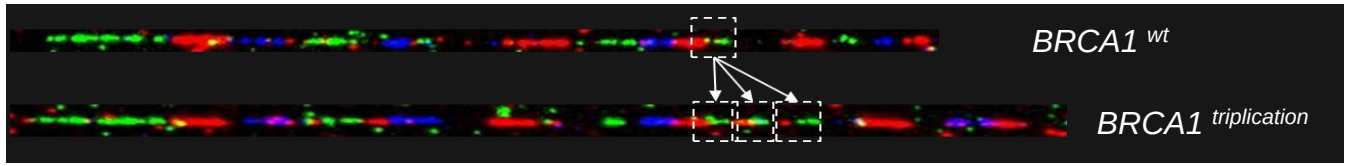


Changes in GMC pattern directly indicate structural variations with no ambiguity

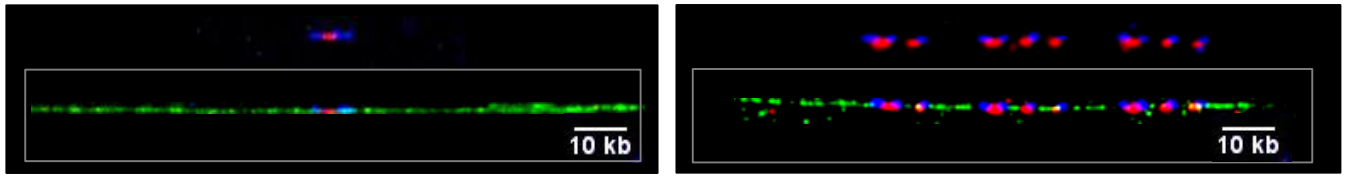
- Probes: 1 kb to 1000+ kb
- **Precision ~3 kb**
- Possible multiplexing



Sizing of RNU2 array CNV associated to BRCA1 gene



Triplication of 16kbp within the BRCA1 gene – ambiguous detection with CGH & MLPA

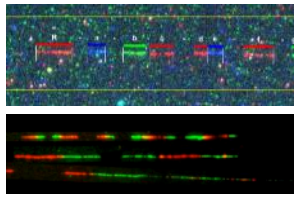
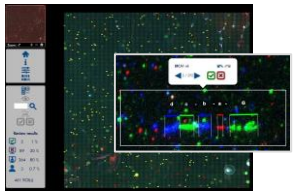


HPV16 genome integration (red and blue probes) into human host DNA (green line)

The FiberVision® Molecular Combing Platform

From DNA purification to Data Output

The platform offers a complete and flexible workflow

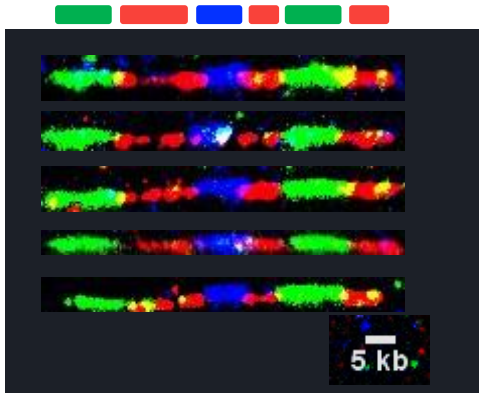
1	2	3	4	5
Extraction	**Combing**	**Hybridization & Detection**	**Image Acquisition**	**Analysis**
FiberPrep® DNA Extraction Kit	FiberComb® Molecular Combing System	FiberProbes® Genomic Morse Code	FiberVision® Automated Scanner	FiberStudio® Analysis Software
				

Quantitation of Non-Canonical Signals After Editing*

High frequency editing with a RNP (RiboNucleoProtein) in U2OS and T cells:

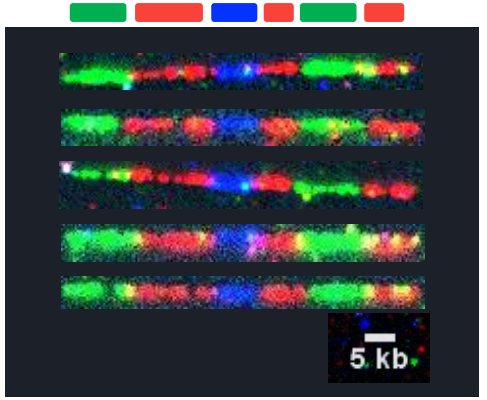
FiberCheck

U2OS



96.5% of canonical signal

T Cells



98.7 % of canonical signal

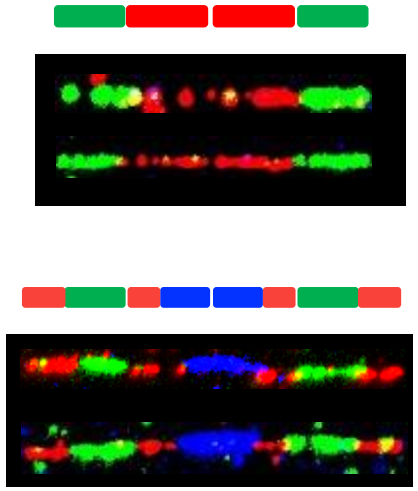
- Vast majority of signals are canonical, suggesting no large scale rearrangements
- Small percentage of signals are non-canonical

*Cecilia Cotta-Ramusino et al., CSHL Meeting: Genome Engineering: The CRISPR-Cas9 Revolution, July 21-23, 2017.

Quantitation of Non-Canonical Signals After Editing*

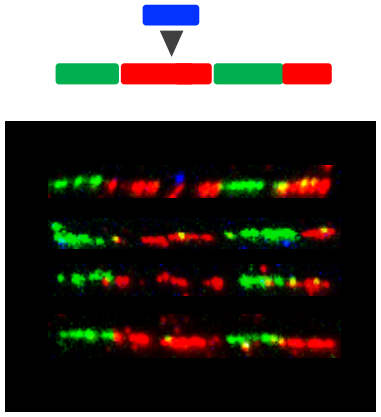
Canonical signal

Symmetrical: Same chromosome translocations



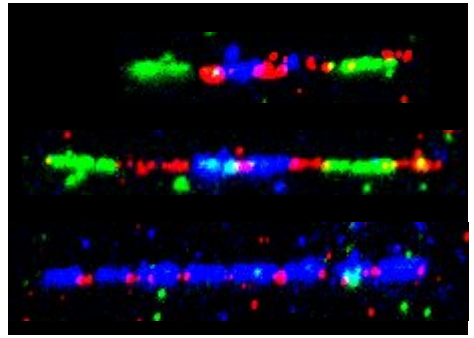
Challenging to detect by NGS/PCR-based strategy due to structural complexity

Loss of Blue Probe: Single Strand Annealing



Challenging to detect by NGS/PCR-based strategy due to size and unknown extent of rearrangement

Others: Unknown Mechanism

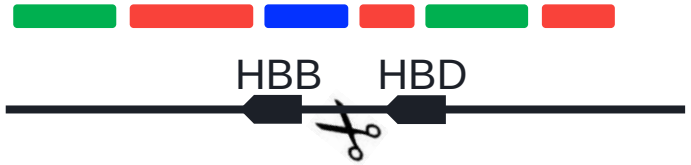


Not detectable by NGS/PCR-based strategy due to unexpected nature of events

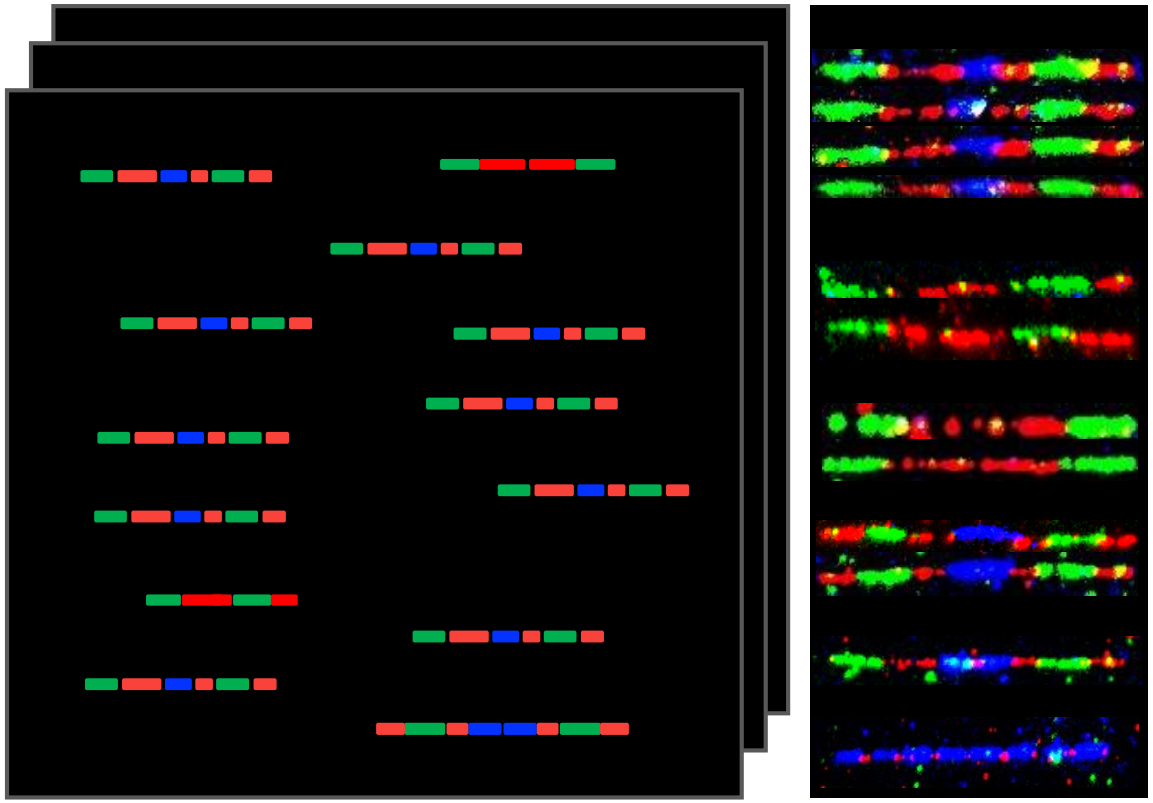
*Cecilia Cotta-Ramusino et al., CSHL Meeting: Genome Engineering: The CRISPR-Cas9 Revolution, July 21-23, 2017.

Quantitation of Non-Canonical Signals After Editing*

High frequency editing with RNP in T cells:
Comprehensive detection and quantitation of rearrangements



> 150x HG equivalents per slide
10 slides per sample



Classification	Frequency	Interpretation
...	98.7%	Canonical Signal
	0.3%	Single Strand Annealing
	0.2%	Same Chr Translocation
	0.2%	Same Chr Translocation
	0.4%	Unknown
	0.2%	Unknown

*Cecilia Cotta-Ramusino et al., CSHL Meeting: Genome Engineering: The CRISPR-Cas9 Revolution, July 21-23, 2017.

Concluding Remarks

Molecular Combing's Value for Specificity Measurements:

⇒ **Sensitive** : >150x coverage per slide; 1500x per sample (~0.25% sensitivity); can be increased
⇒ **Digital Quantitation** : single-molecule counting of ROI signals
⇒ **Unbiased by Complex Patterns & Translocations** : visual, direct detection

⇒ **Technical Advantages:**

- Multiple cell input types
- No amplification bias
- Highly complementary to NGS/PCR based assays

⇒ **Currently working with Genome Editing Biopharmas**
⇒ **Platform ready for translation in Process Development**
⇒ **Future potential as a QC assay in Manufacturing**