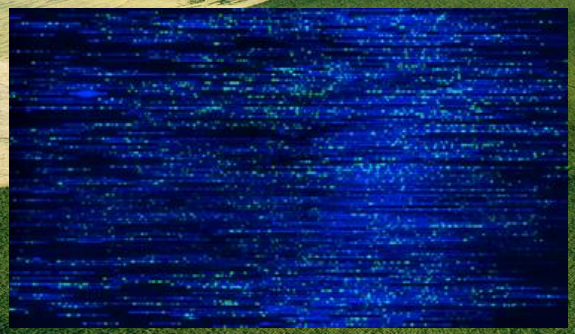
Toward a better understanding of plant genomes structure :

Combining NGS, optical mapping technology and CRISPR-CATCH approach



INRA SCIENCE & IMPACT

CNRGV

Hélène BERGES

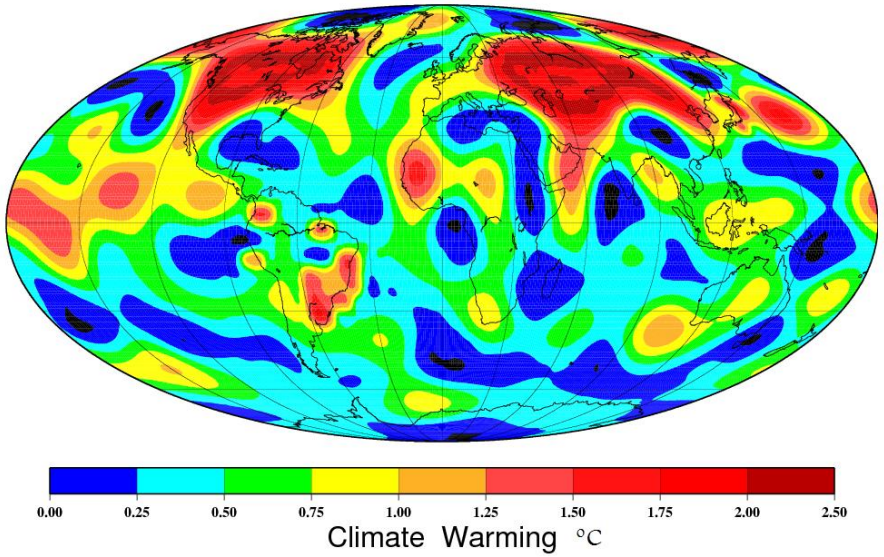
Director of the Plant Genomic Center

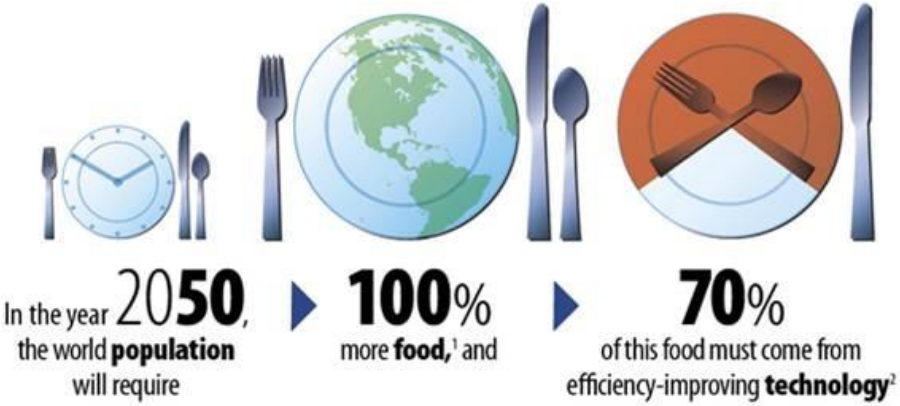

INRA
SCIENCE & IMPACT
PLANT GENOMIC CENTER
40 Plant species
406 Genomic libraries
20 Millions samples
283 Collaborators

Global warming effects, Population growth, Erosion of genetic progress, Consumer expectations

INNOVATION :

Genome's exploration is one of the strategic approaches for better understanding plant evolution and plant improvement and adaptation





The importance of a high quality reference genome sequence

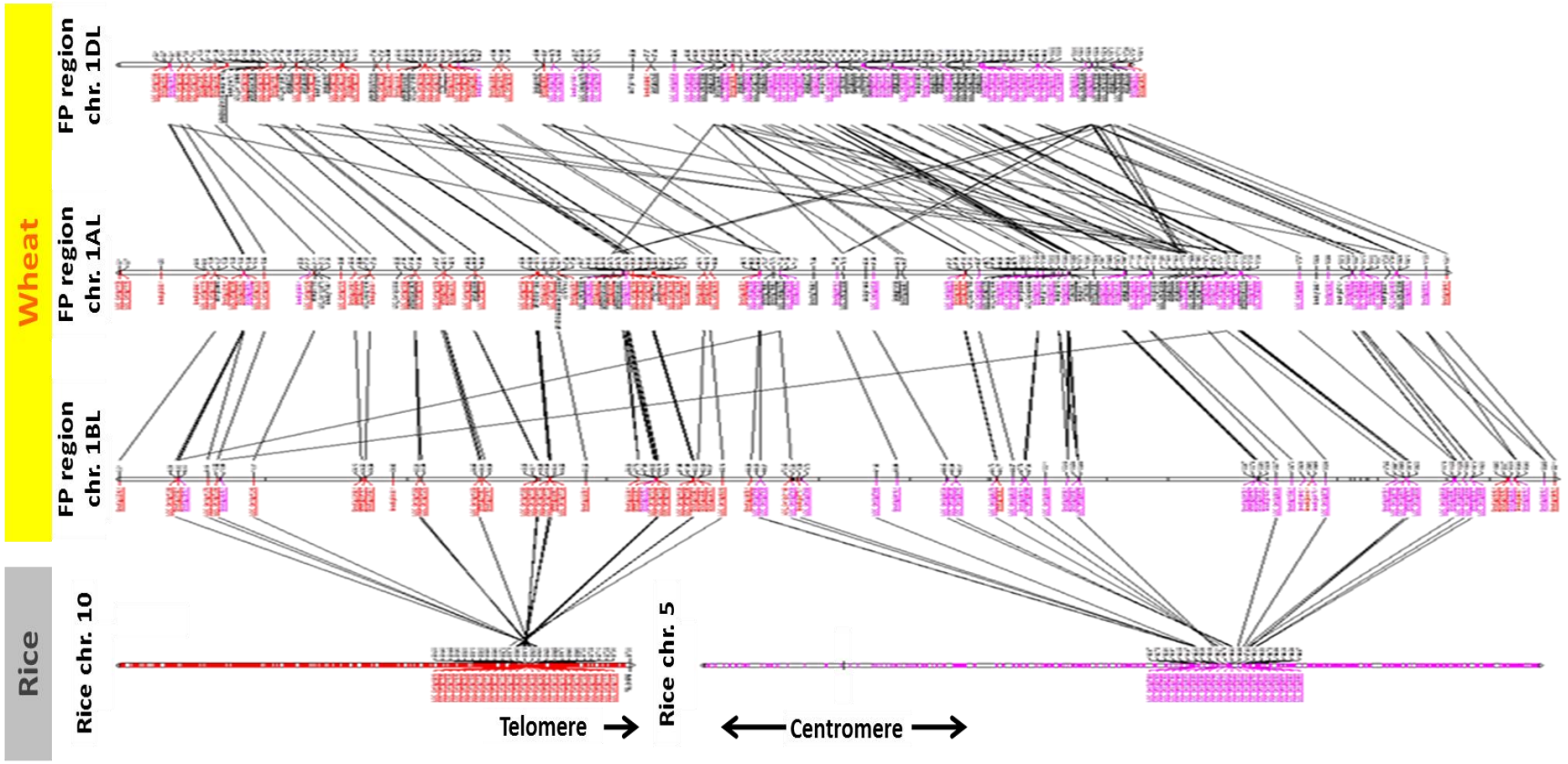
A. BELLEC

International Wheat Genome Sequencing Consortium

K. Eversole

Explore the genome at a large scale

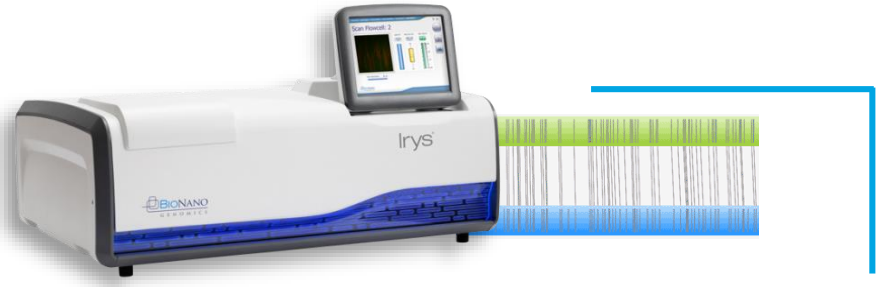
Project aiming at understanding plant genome evolution (Poster 40)



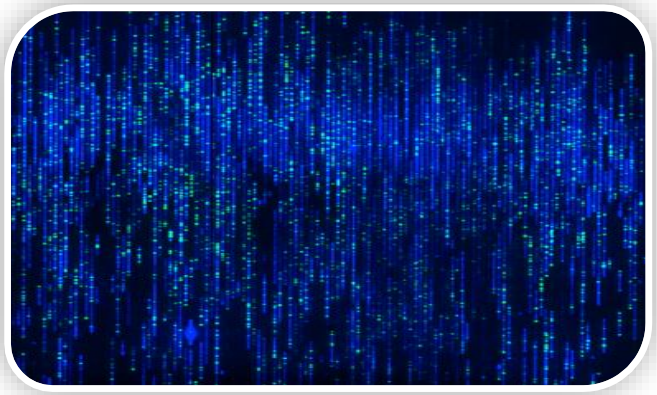
Structural and Evolutive Analysis of an Ancestral Chromosomes Fusion Point within the Hexaploid Wheat Genome

A way to improve the quality for reference genome sequence : the optical maps

The BioNanoIrys system



- Direct visualization of long DNA molecules (>100 kb) working on non-amplified native genomic DNA
- Real physical distance information
- Labelling of specific sites (nickases)
- Molecular barcodes assembled



Applications

Whole Genome Finishing
map = scaffolds

- Sequencing contigs converted *in silico* into molecular barcodes (highlighting the same sequence motifs)
- Sequencing based barcodes aligned to the BioNano maps

Targeting a specific genomic region / Comparison to a reference genome, looking for changes in the patterns:

- reveal insertion, deletion, inversion, translocation of genome segments

Improvement of the Sunflower Genome sequence

N. Langlade

- Species: *Helianthus annuus*
- 3.6 Gb
- 2n=34 chromosomes
- Genome sequence >100X PacBio (XRQ genotype)

# contigs	LEN Max	N50 BP	#>N50	MEDIAN	BP
12 318	3,35 Mb	524 kb	1 684	120 kb	2,93

=> 80% of the genome inside contigs

Gouzy et al., 2016

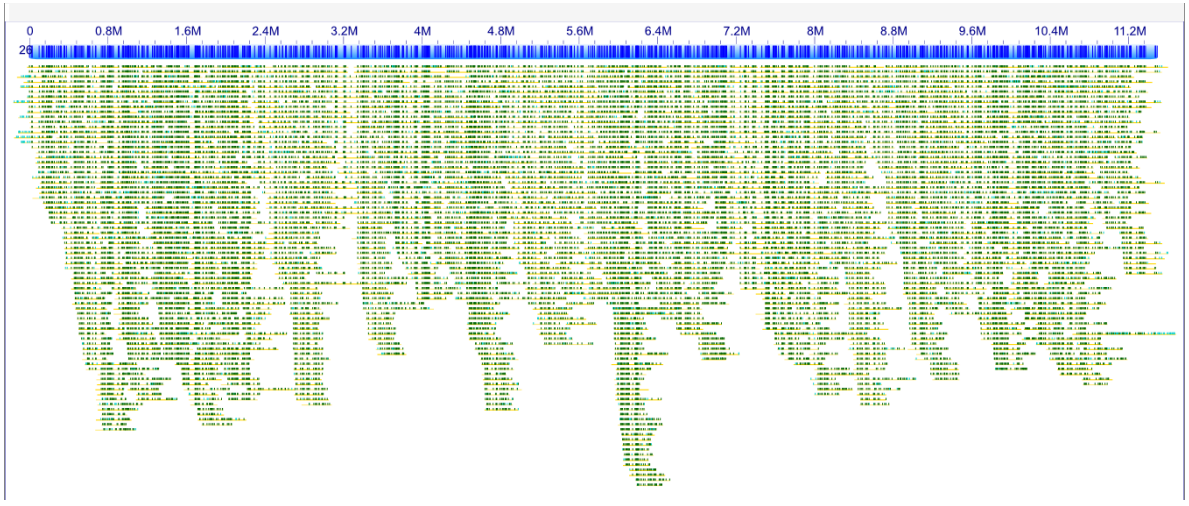
Two major repeats in the sunflower genome: 8 kb and 11.5 kb

Optical maps to improve the genome assembly

	BspQ1	Bsss1
	5′…GCTCTTCN…3′ 3′…CGAGAAGN…5′	5′…CACGAG…3′ 3′…GTGCTC…5′
Theoretical nb labels / 100kb	7,2	17,2
Real nb labels / 100kb	6,4	12,8
Raw data (Gb)	846 (235X)	845 (235X)
Filtered data >100kb (Gb)	635 (176X)	600 (167X)
Molecules N50 (kb)	206	187

2 nicking enzymes (BspQ1 & BssS1)

BioNano map



➢176X coverage, molecules from 150kb to 2,3Mb

The 2-steps hybrid scaffolding strategy improves significantly the resulting N50

	PacBio Assembly	BioNano BspQ1 Assembly	Hybrid scaffold BspQ1	BioNano BssS1 Assembly	Hybrid scaffold 2 Step
Count	12318	2228	1430	4287	1069
Median length (Mb)	0.120	0.999	1.442	0.551	1.914
N50 length (Mb)	0.524	1.979	2.87	0.968	4.166
Max length (Mb)	3.35				24.670
Total length (Mb)	2930	3191	2922	3112	2960
% genome	81%	88%	81%	86%	82%

More than 7 fold increase

Dedicated genomic tools to better understand the role of regions of interest

- Genetic map
- Physical map established on other genotypes
- Specific markers available in the region of interest

1. Optical map
2. BAC library from various genotypes
3. Sequence Capture

Sequencing (NGS)
Comparison

- Physical caracterisation of regions of interest (MTP)
- Isolation of the region of interest
- Identification of the region
- Comparison with reference map

Focusing on a genomic region of interest in Sunflower

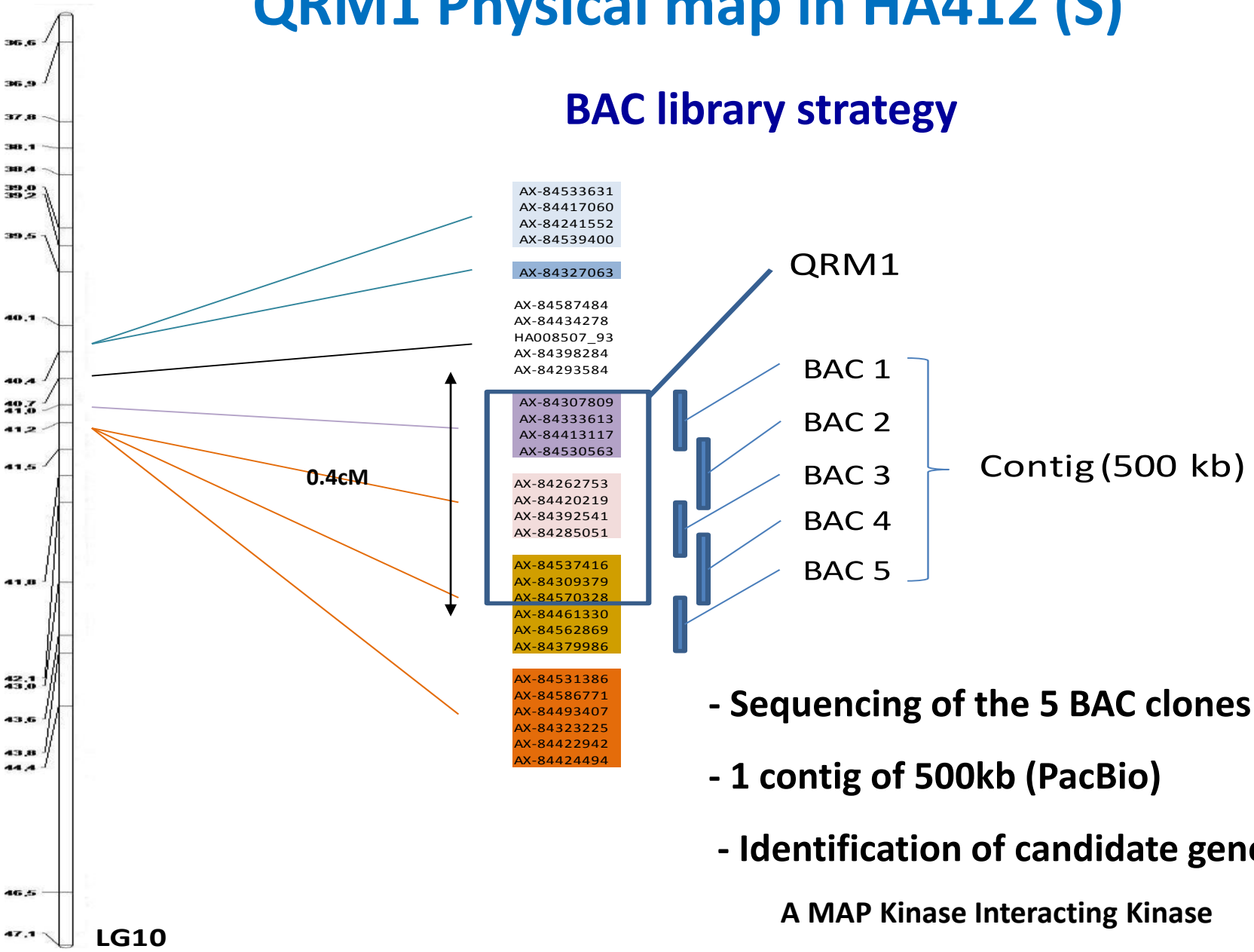
S. Munos

S. Vautrin

- QRM1 controls quantitative resistance to downy mildew
  Susceptible (HA412) /Resistant (XRQ)
- Establishment of a genetic map (0.4 cM window on LG10)
- Markers definition on the QMR1 locus
- XRQ : *in silico* analysis of the 2Mb sequence on chromosome 10 (based on 20 markers alignment) composed of 14 scaffolded Pacbio contigs separating by N gaps (10k missing nucleotides)

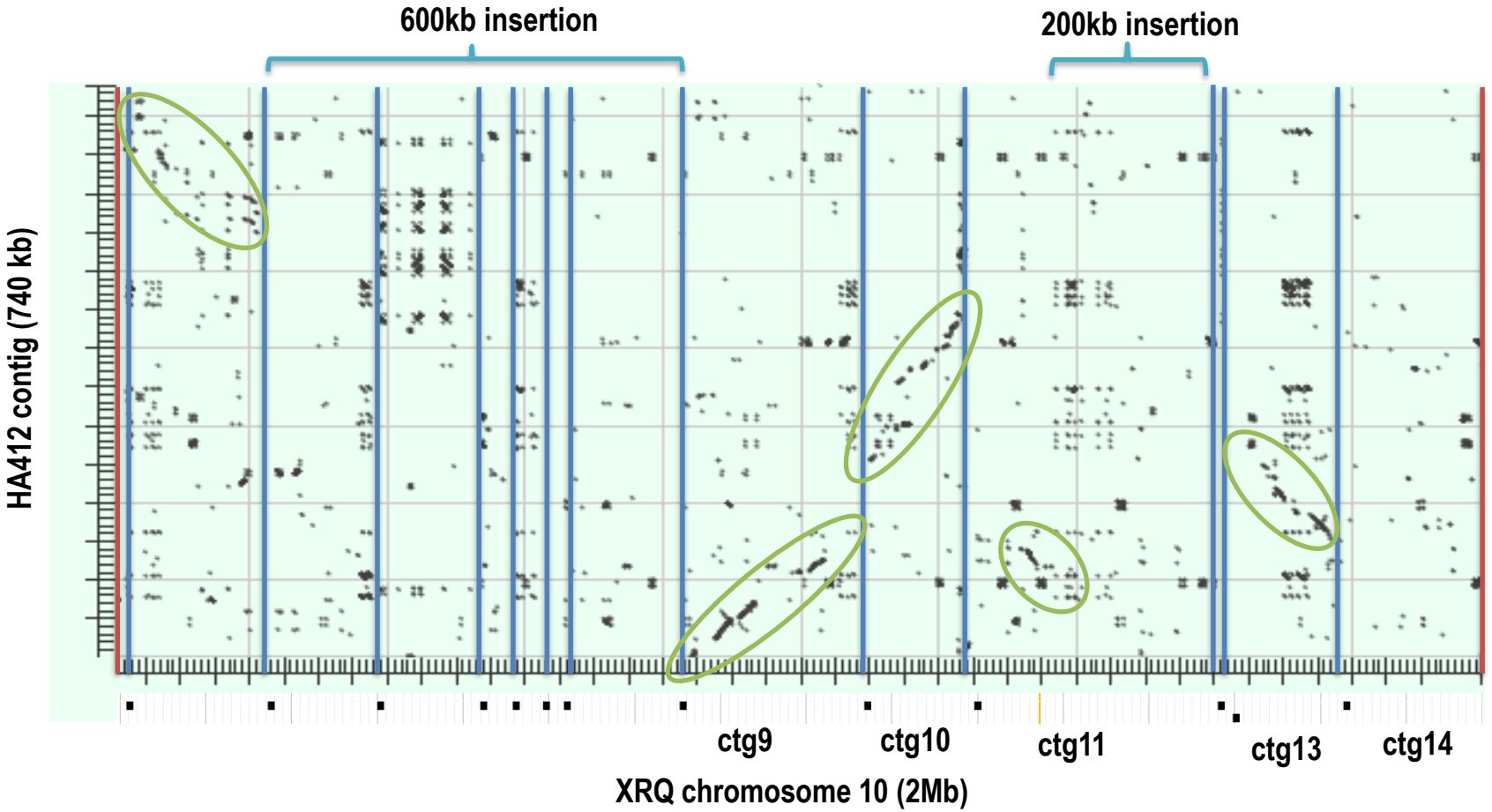
QRM1 Physical map in HA412 (S)

BAC library strategy



- **Sequencing of the 5 BAC clones (HA412)**
- **1 contig of 500kb (PacBio)**
- **Identification of candidate genes:**
  - **A MAP Kinase Interacting Kinase**
  - **A Proteinase inhibitor**

Comparison of the XRQ genome vs HA412 BAC clones



Low collinearity

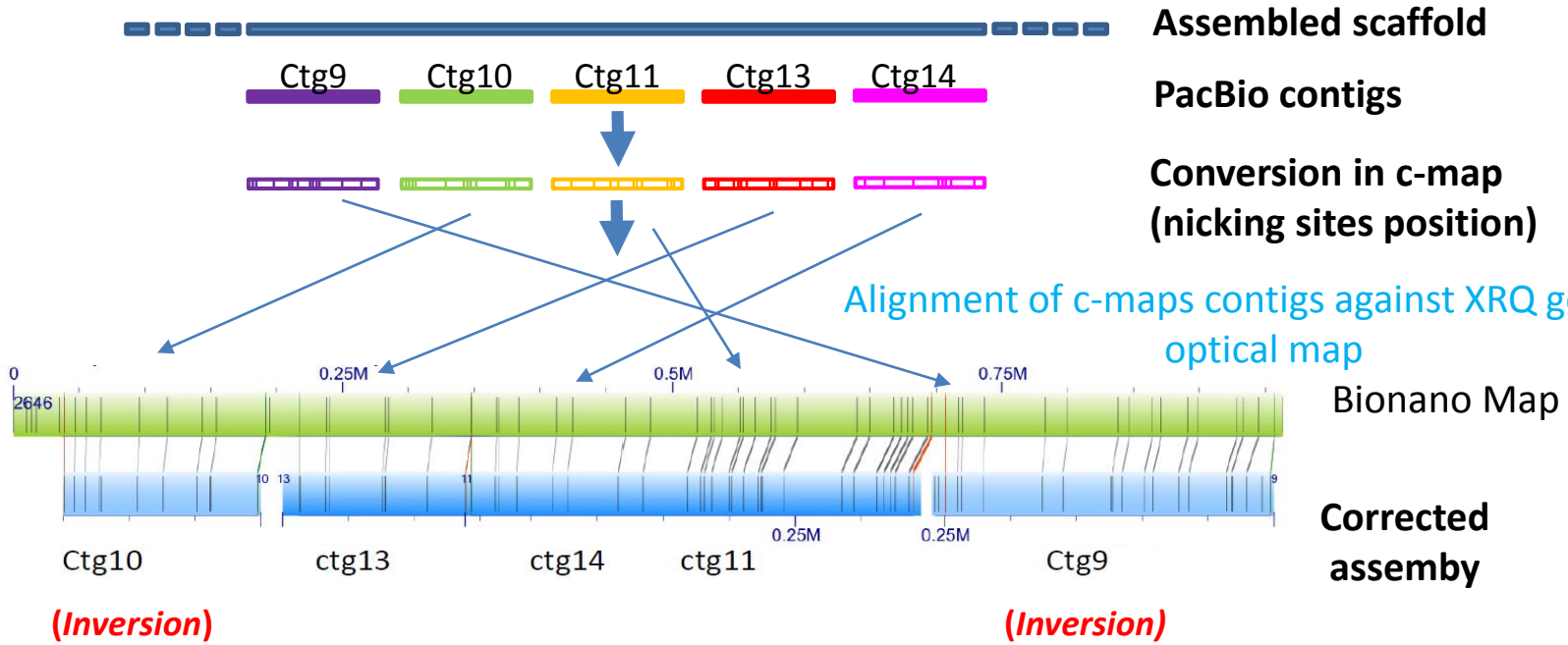
Fragmented alignment / orientation inconstancies :

Scaffolding errors OR true variability?

Optical maps to solve conflicts in the assembly

S. Cauet

Alignment of the contig against the BioNano assembly of XRQ genome



Assembled scaffold

PacBio contigs

Conversion in c-map (nicking sites position)

Alignment of c-maps contigs against XRQ genome optical map

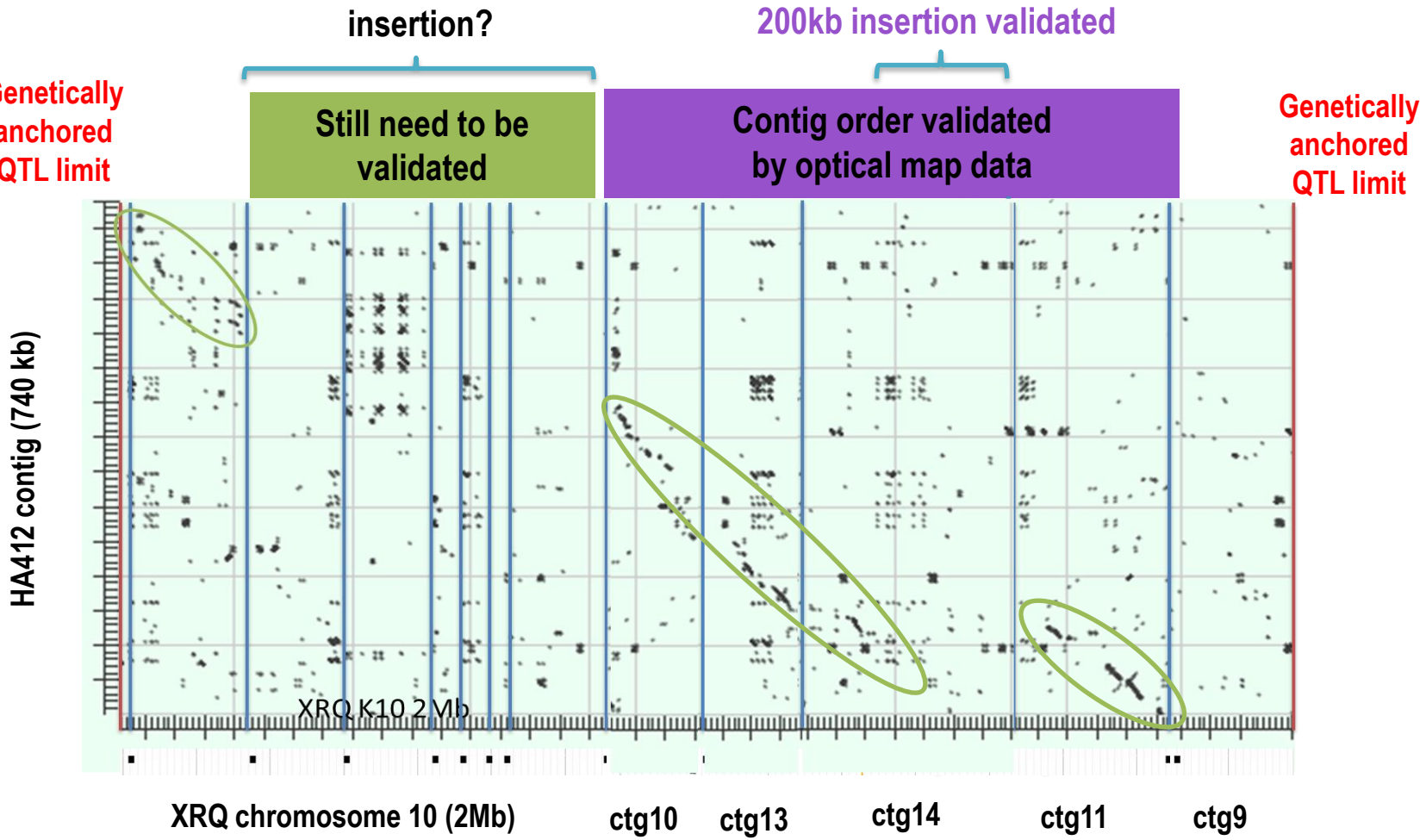
Bionano Map

Corrected assemby

On this targeted region, Optical Bionano map allowed:

- **to orientate some contigs**
- **to correct scaffolding of the PacBio contigs**

Comparison of the XRQ genome vs HA412 BAC clones



- Validation of the collinearity between XRQ and Ha412 sequences on QRM1 locus
- High variability observed: 2 major insertions of several hundreds of kb in XRQ
- Annotation of the 2 sequences and comparative analysis are under progress (9 candidates genes have been identified)

Genome assembly improvement will help linking genotype / phenotype

N. RODDE Poster41

Sunflower proves again to be a highly complex genome, showing very high diversity between genotypes

One reference genome is not enough!

Despite long reads sequencing, assembly (scaffolding) has to be checked when working on reference genomes

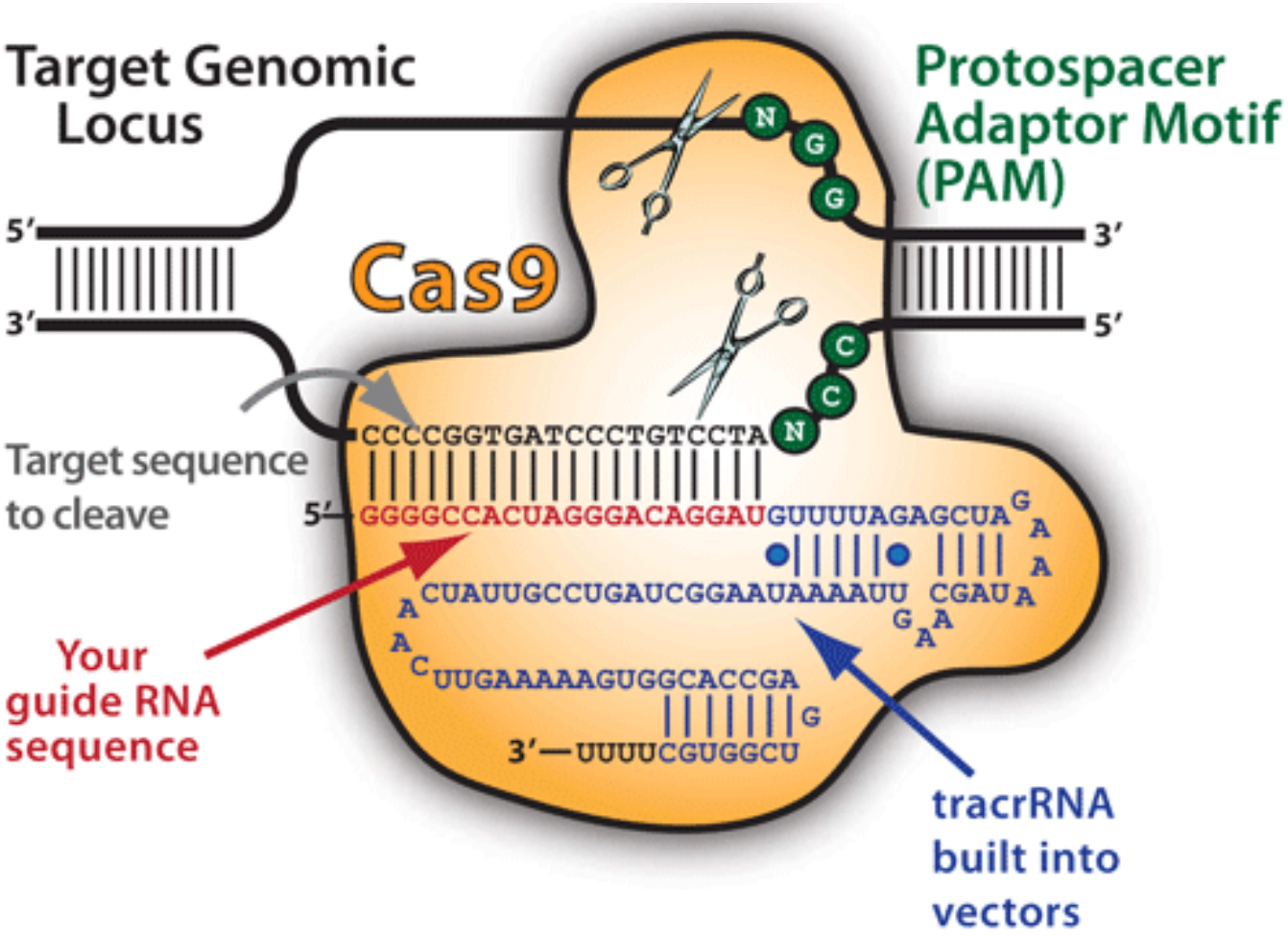
The optical map allowed to validate major rearrangements between the 2 genotypes

Proven interest of complementary approaches (NGS – optical map – BAC)

=> How to be more efficient in focusing on genomic regions?

Another way to target a genome segment

Based on the CRISPR-Cas9 technique

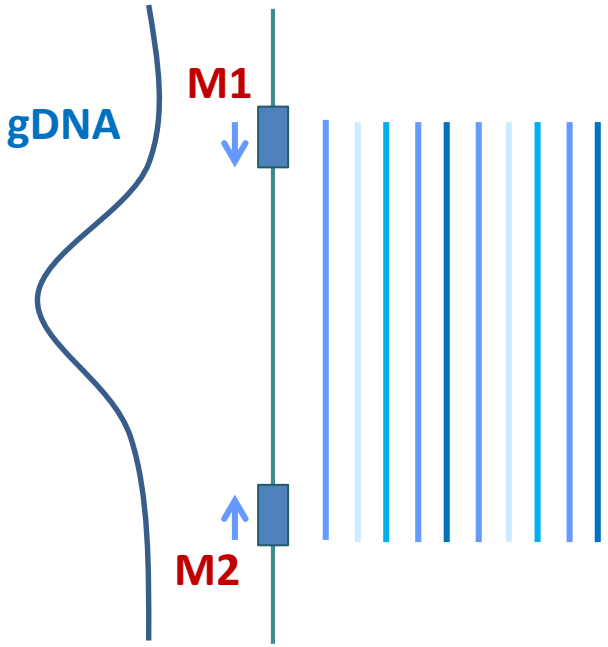


E. Charpentier / J. Doudna

CRISPR-CATCH: Cas9-Assisted Targeting of Chromosome segments

C. CHANTRY-DARMON

QTL identification



- Guide RNA to target a specific region in the genome (flanking markers of the QTL)
- Cas 9 : nuclease DNA to unzip and cut the DNA at the chosen locus

➢ From a region of several kb to Mb

ARTICLE

Received 11 Feb 2015 | Accepted 16 Jul 2015 | Published 1 Sep 2015 DOI: 10.1038/ncomms9101 OPEN

Cas9-Assisted Targeting of CHromosome segments CATCH enables one-step targeted cloning of large gene clusters

Wenjun Jiang[1], Xuejin Zhao[2], Tslil Gabrieli[3], Chunbo Lou[2], Yuval Ebenstein[3] & Ting F. Zhu[1]

PROTOCOL

Targeted isolation and cloning of 100-kb microbial genomic sequences by Cas9-assisted targeting of chromosome segments

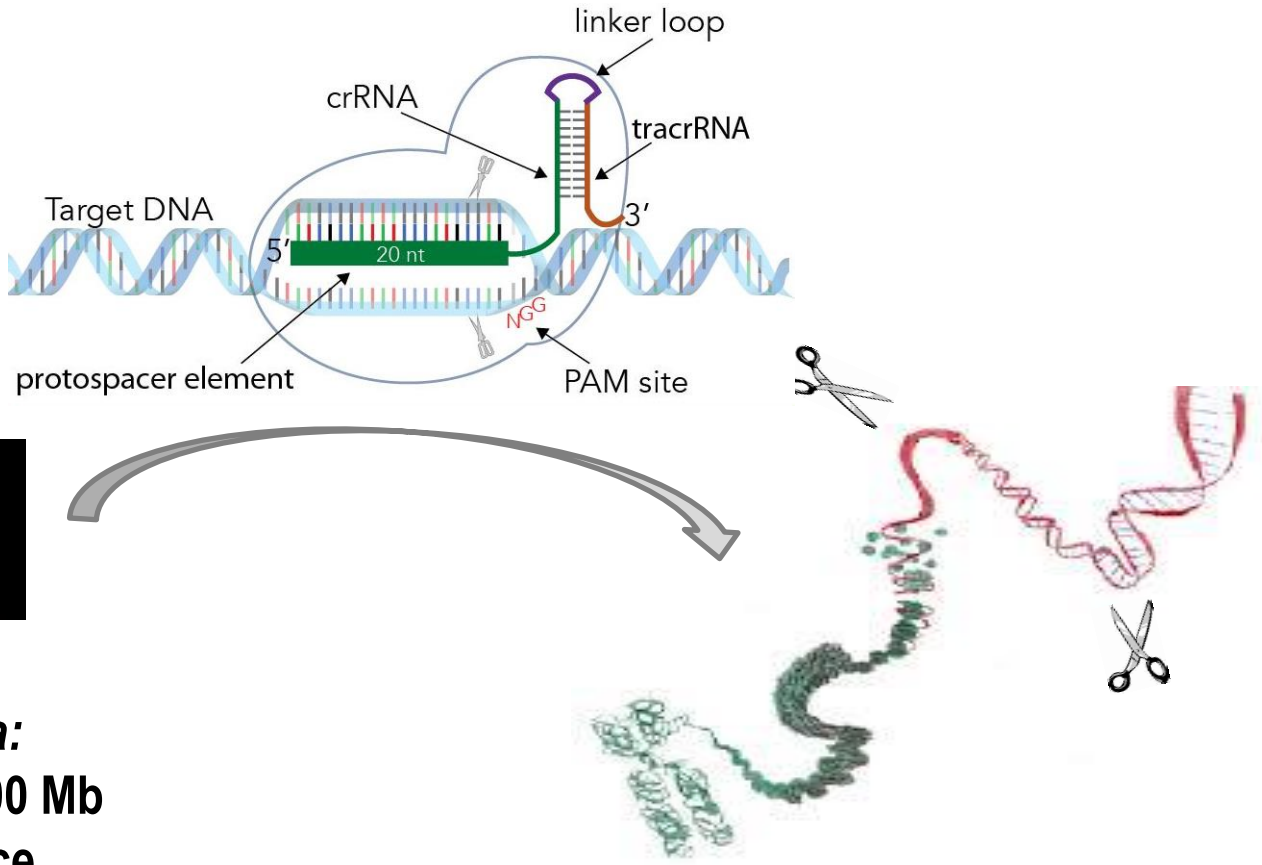
Wenjun Jiang & Ting F Zhu

School of Life Sciences, Center for Synthetic and Systems Biology, Ministry of Education Key Laboratory of Bioinformatics, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Tsinghua University, Beijing, China. Correspondence should be addressed to W.J. (jiangwj12@mails.tsinghua.edu.cn) or T.F.Z. (tzhu@biomed.tsinghua.edu.cn).

Published online 21 April 2016; doi:10.1038/nprot.2016.055

Targeting genomic regions with CRISPR-CATCH

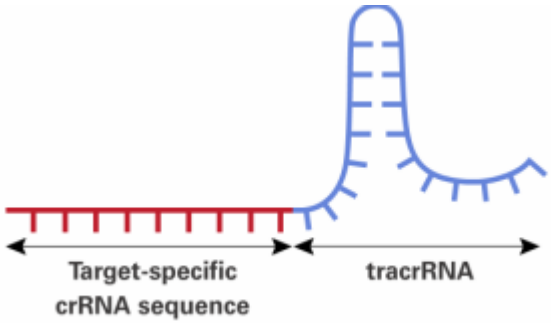
Pilot test on *Medicago truncatula*



Medicago truncatula:

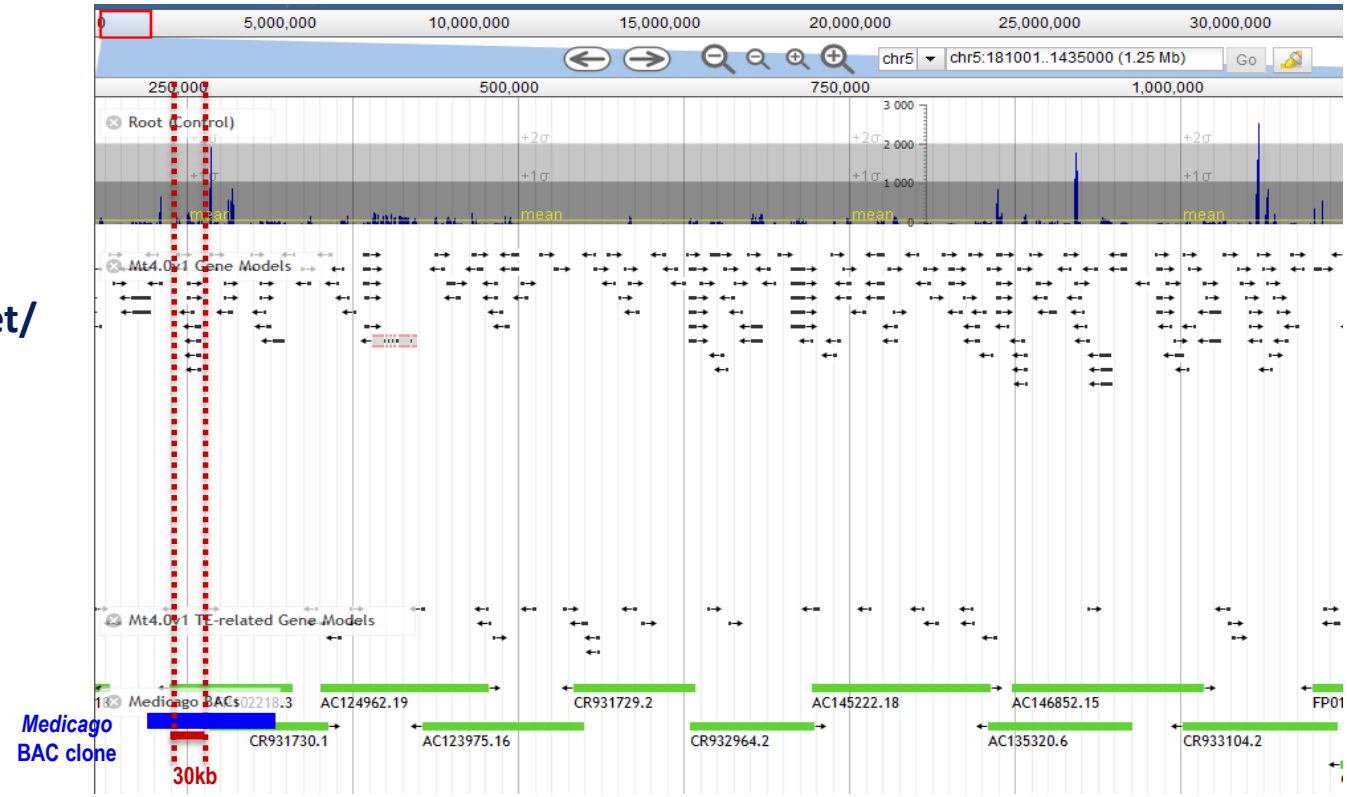
- **Small genome ~ 500 Mb**
- **Reference sequence**
- **BAC libraries**

Key step : single guide RNA design

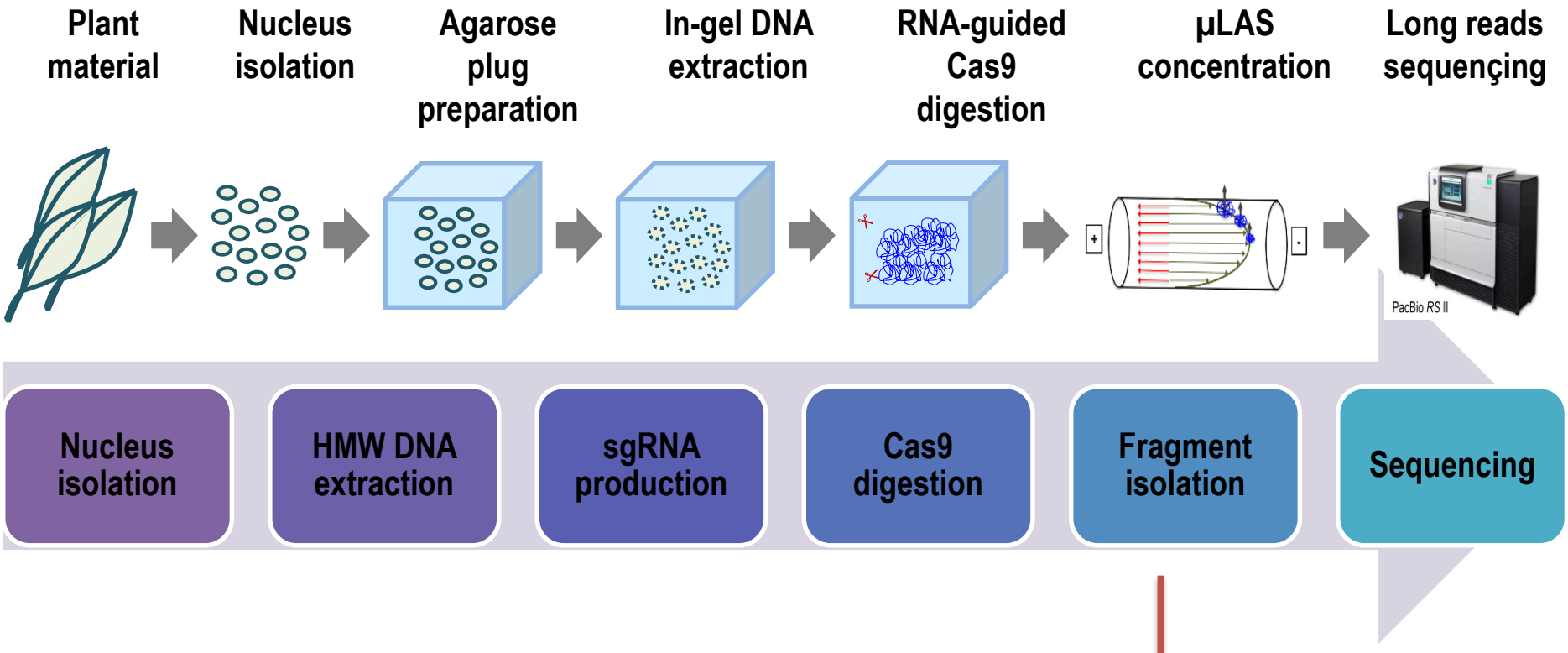


sgRNA contains a targeting sequence (crRNA sequence) and a Cas9 nuclease-recruiting sequence (tracrRNA).

http://crispor.tefor.net/



CRISPR-CATCH Workflow

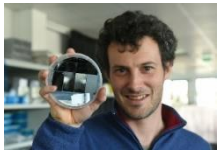


How to recover the fragment of interest?

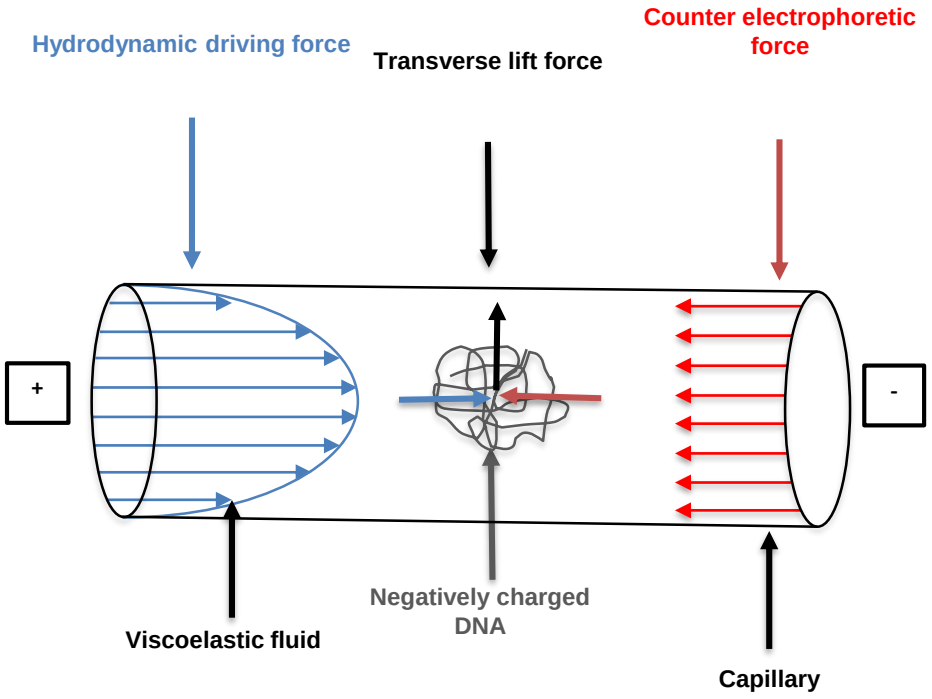
Developement of a new technology to enhance limit of DNA detection

µLAS: separation of large DNA fragments

Microfluidic device with controlled force to avoid DNA fragmentation



A. BANCAUD N. MILON



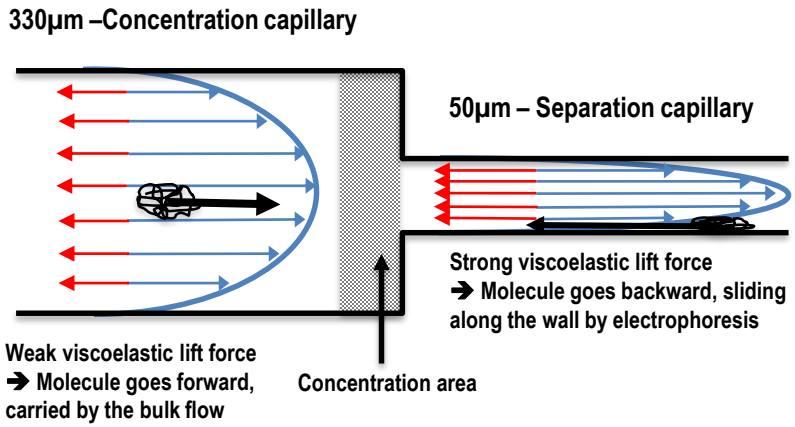
Counter-electrophoresis of DNA deforms fluid flow

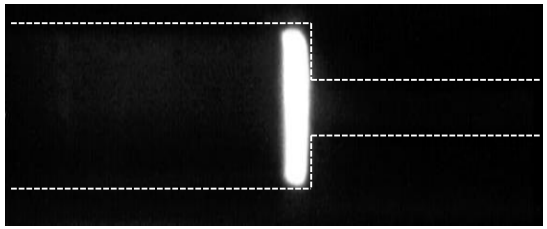
Force intensity depends on flow speed, shearing, electric field and DNA size

Larger molecules are sent closer to the capillary wall where the flow speed is lower leading to a size separation mechanism

Enhance the performance of DNA analysis based on a polymer with specific viscosity

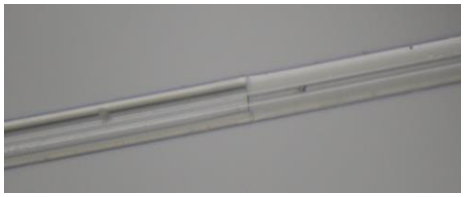
µLAS: Selective enrichment of large DNA fragments



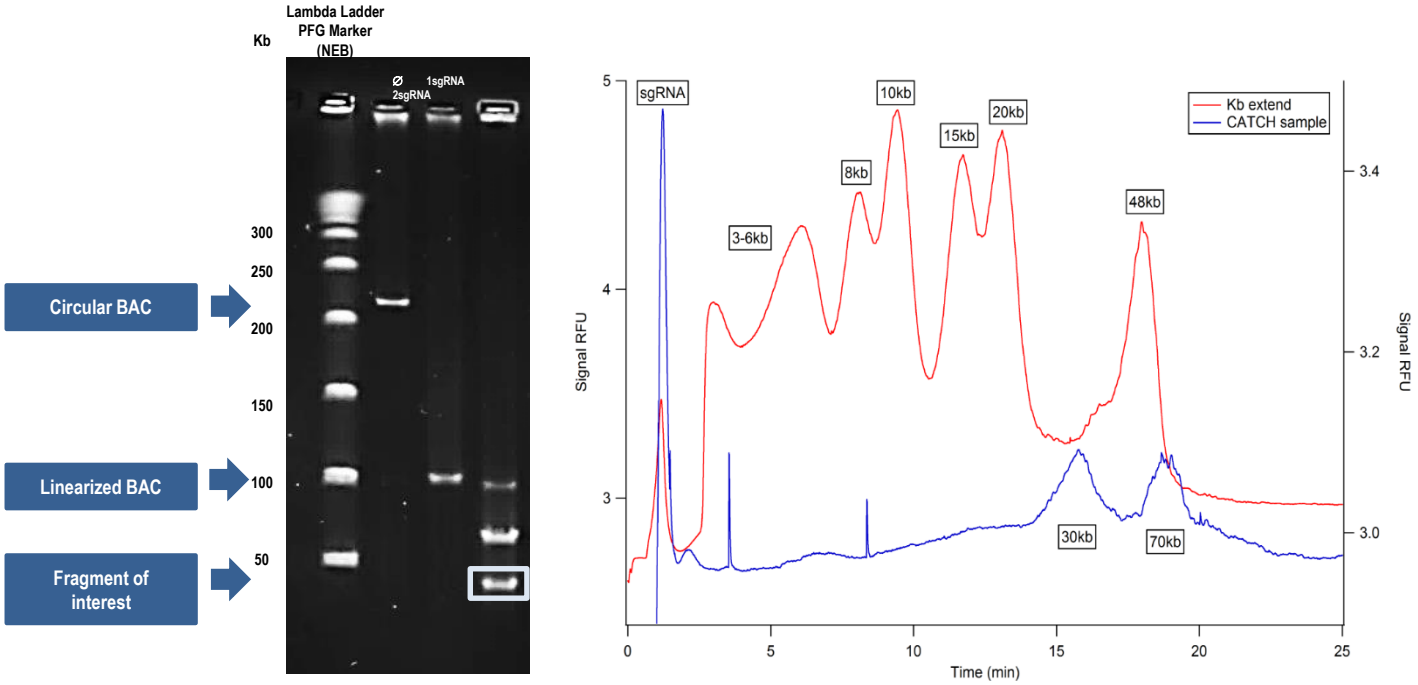


DNA concentration at capillary junction

- Selection and concentration of DNA fragments > 8kb
- Removal of any RNA traces, small fragments, uncharged molecules
- Sensitivity of few pg/mL



Proof of concept with a *Medicago* BAC containing the region of interest

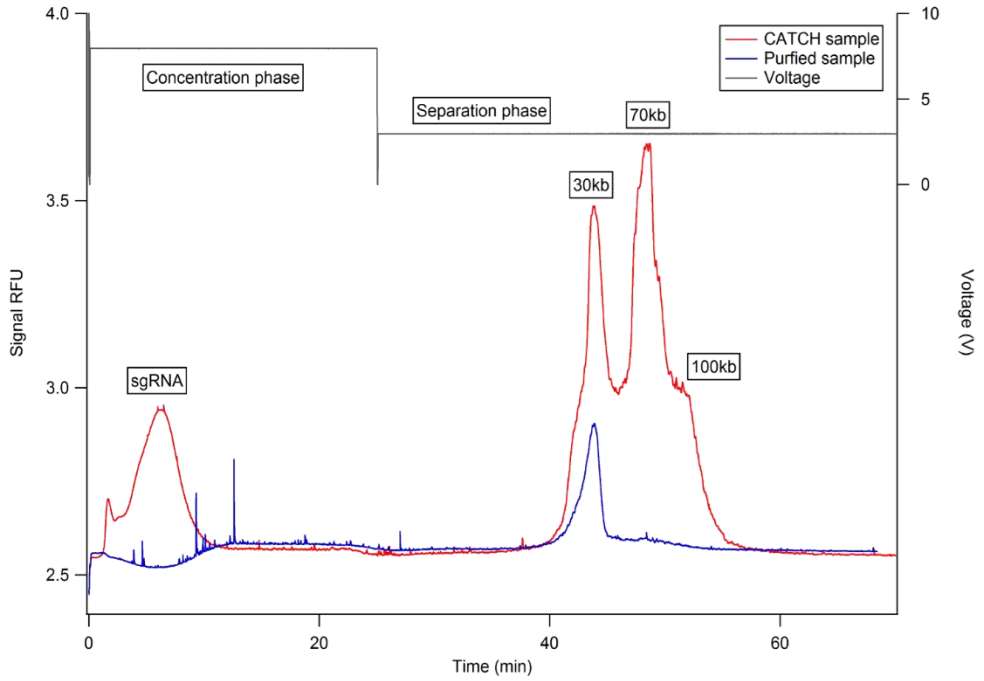


Separation → Amplification → Sequencing → Analyzis

Pulsed Field Gel Electrophoresis
µLAS electrophoresis capillary system

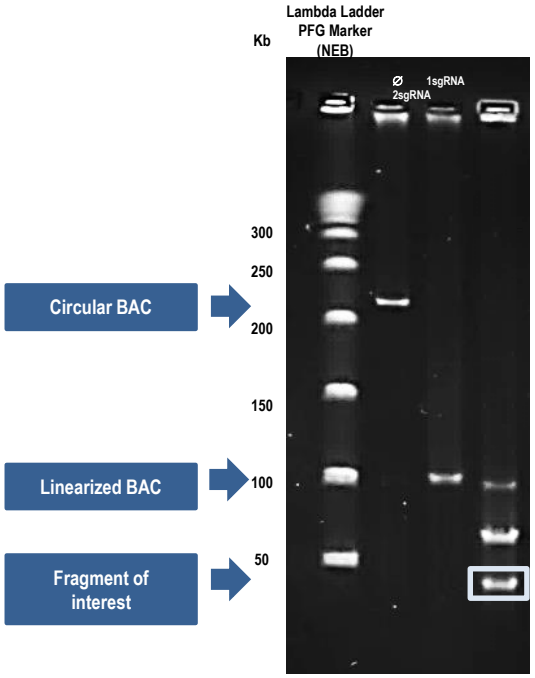
Proof of concept with a *Medicago* BAC containing the region of interest

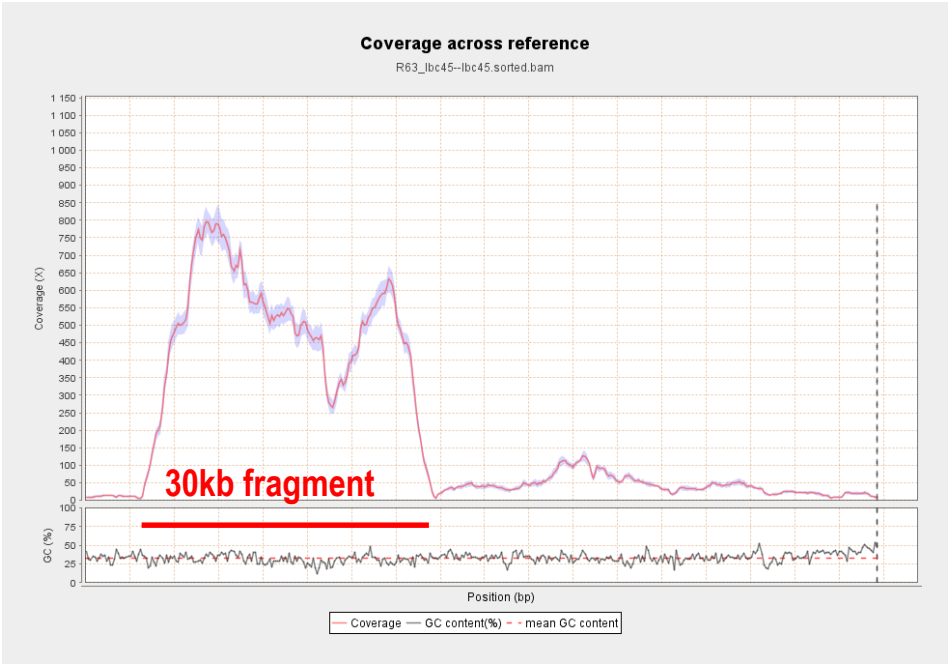
Isolation and enrichment of the fragment of interest



- sgRNA, 70kb and 100kb removal
- Collection of 25µL at 150pg/µL
- Collection yield of 65% for the 30kb fragment

Proof of concept with a *Medicago* BAC containing the region of interest



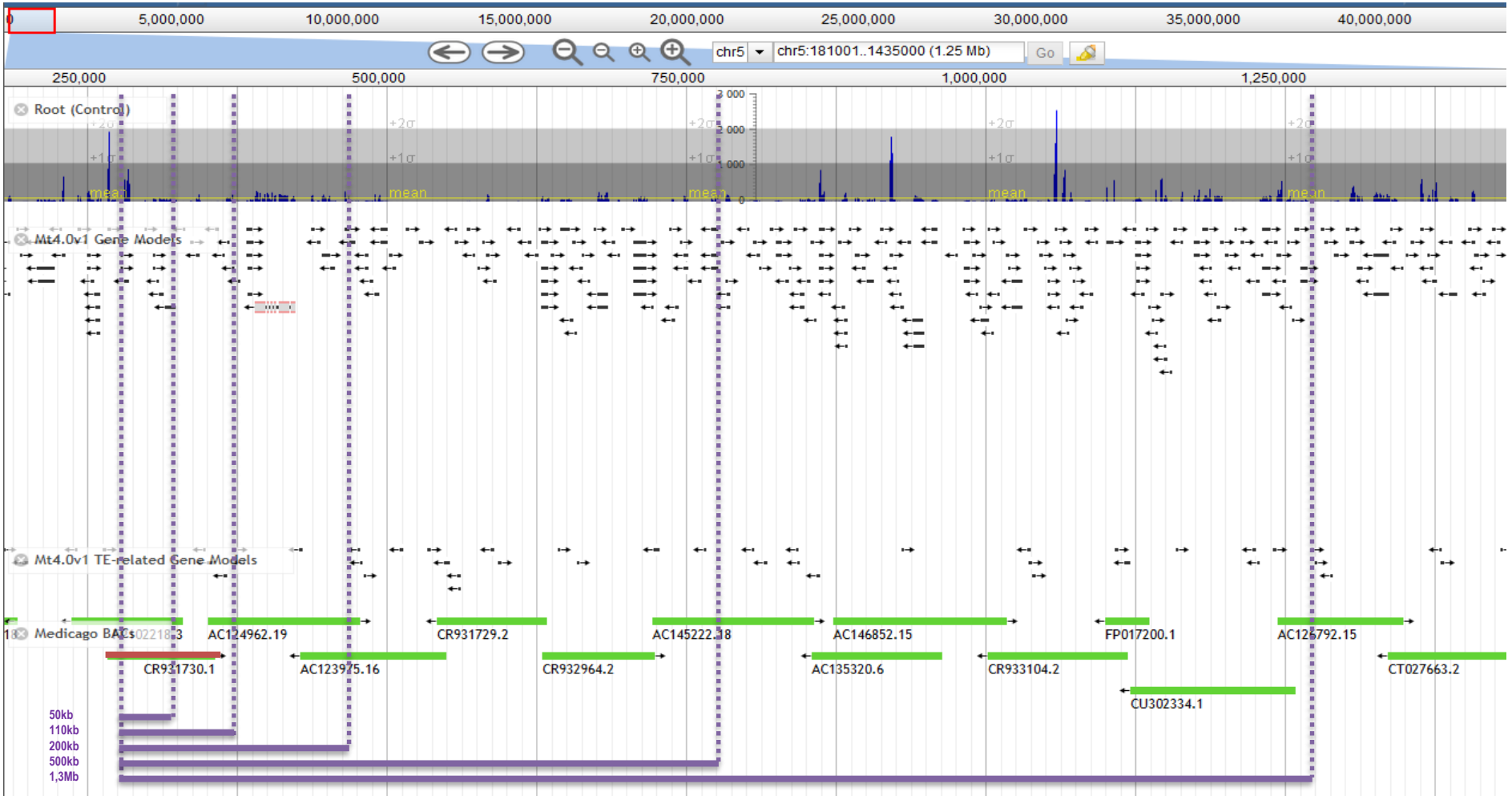


Separation → Amplification → Sequencing → Analyzis

Phi29 DNA amplification

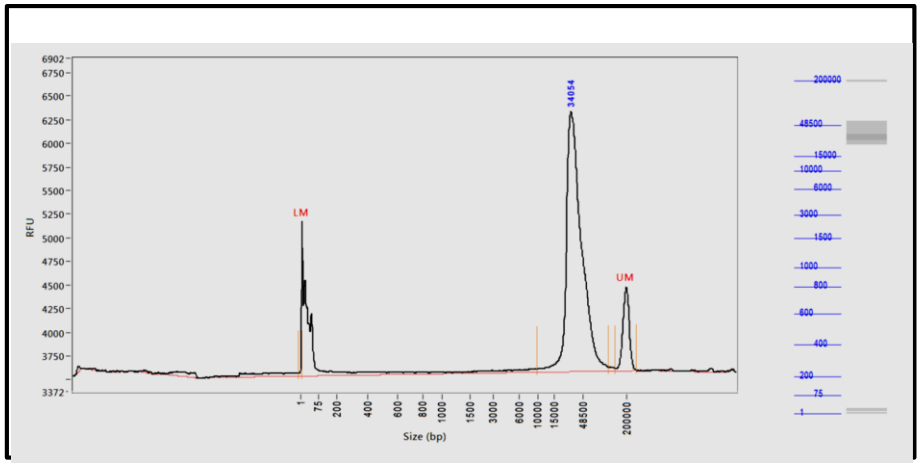
DNA sequencing

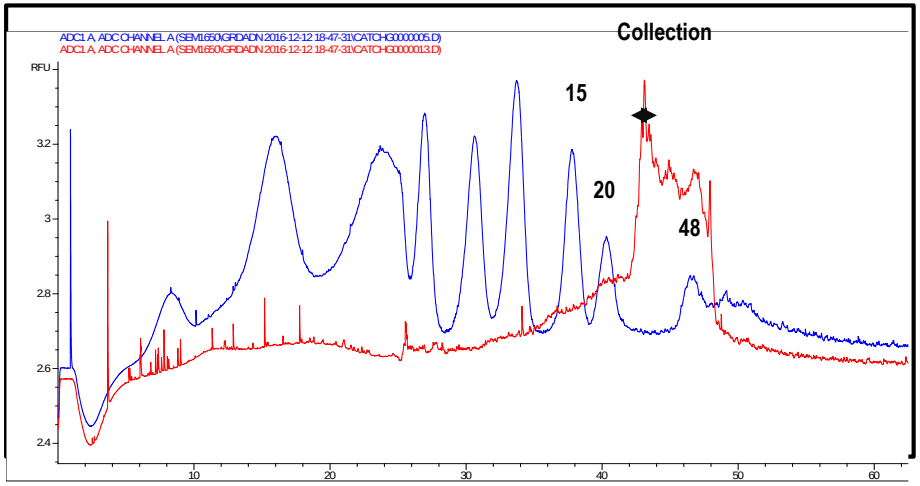
Transfer of the method on gDNA



	sgRNAB1	sgRNAB2	sgRNAB3	sgRNAB4	sgRNAB5	sgRNAB6
Position	270 000	320 000	370 000	470 000	770 000	1 270 000
Theo fragment size	0	50 000	100 000	200 000	500 000	1 000 000
Position	270 000	320 000	380 000	470 000	770 000	1 300 000
Real fragment size	0	50 000	110 000	200 000	500 000	1 030 000

Transfer of the method on gDNA





- Analyzis of the sequences under progress
- Improvement of the HMW DNA extraction
- Improvement of the Cas9 digestion
- Improvement of the fragment isolation
- Tests on larger fragments under progress

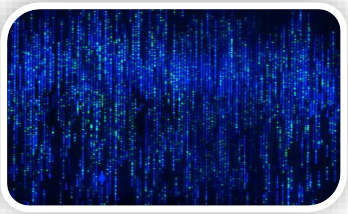
2-years postdoc position open!

http://cnrgv.toulouse.inra.fr/News/Come-working-with-us-on-a-very-exciting-project

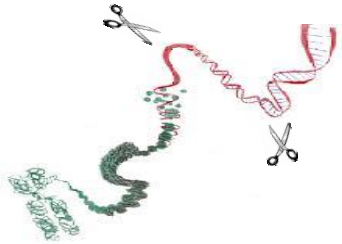
Genomics to help agriculture facing challenges

Integrated approaches to combine complementary technologies and tools

NGS
Ref sequence genomes



Optical maps



BAC library
Sequence Capture

Make the world get a grain!

Acknowledgements

INRA SCIENCE & IMPACT

CNRGV PLANT GENOMIC CENTER

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BIONANO GENOMICS

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International Wheat Genome Sequencing Consortium

Kellye Eversole

UNION EUROPÉENNE

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