Toward a better understanding of plant genomes: from a global organization to region of interest

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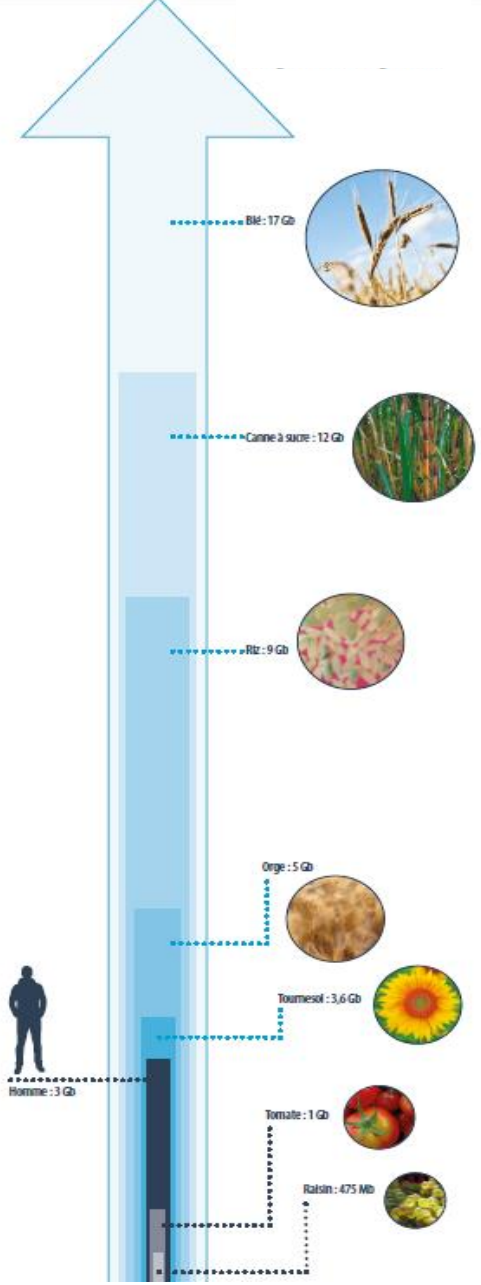
CNRGV
The French Plant Genomic Center

- Created in 2004 by INRA
- Depository of genomic libraries for the scientific community
  - BAC libraries
- A dedicated structure to assist plant genomic programs
  - Distribute the genomic resources at the international level
  - Provide high quality research material and efficient tools and services
  - Develop genomic projects in collaboration
  - Host scientists
  - Develop innovative solutions

ISO 9001:2008
Octobre 2005

The plant genomes complexity



- **Genomes size from 130Mb (*Arabidopsis thaliana*) to 150Gb (*Paris japonica*)**
- **High percentage of transposable elements (>80% in maize, barley, sunflower, wheat…)**
- **Polypoid genomes (bread wheat 6X, strawberry 8X…)**

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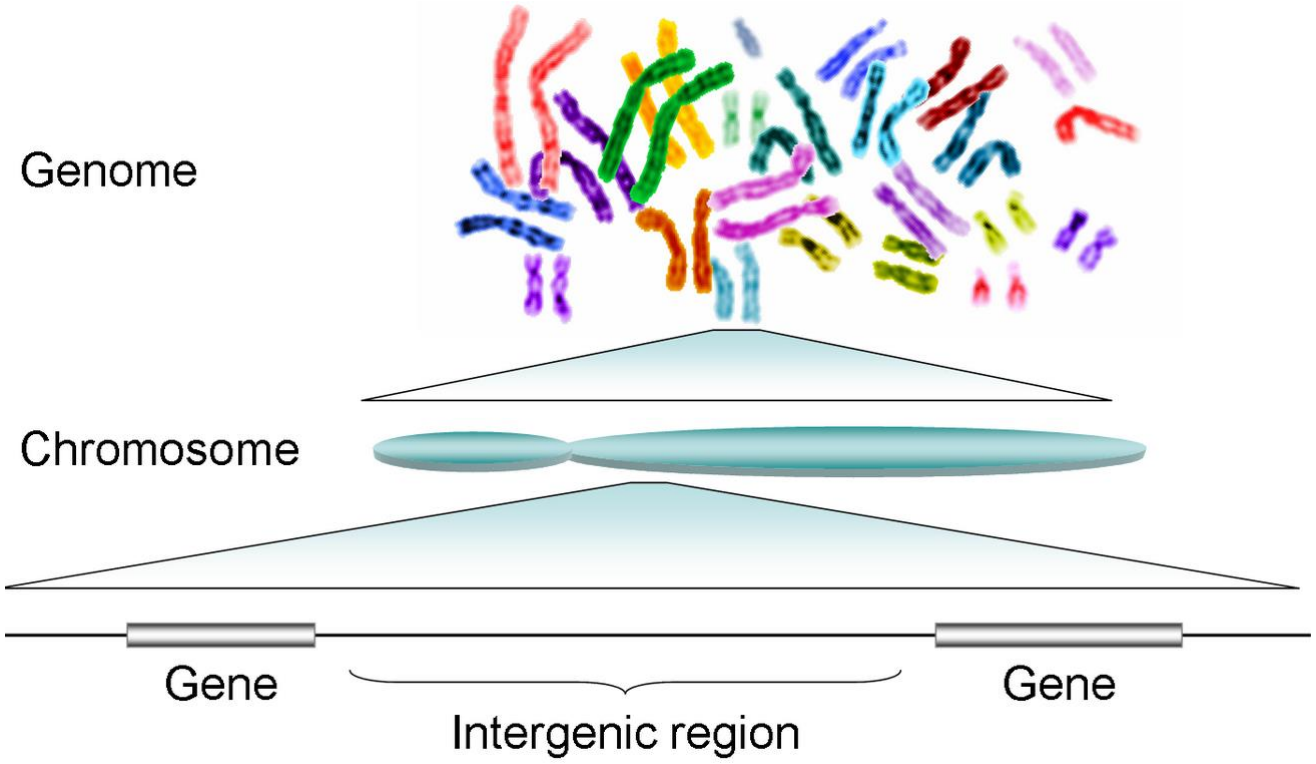
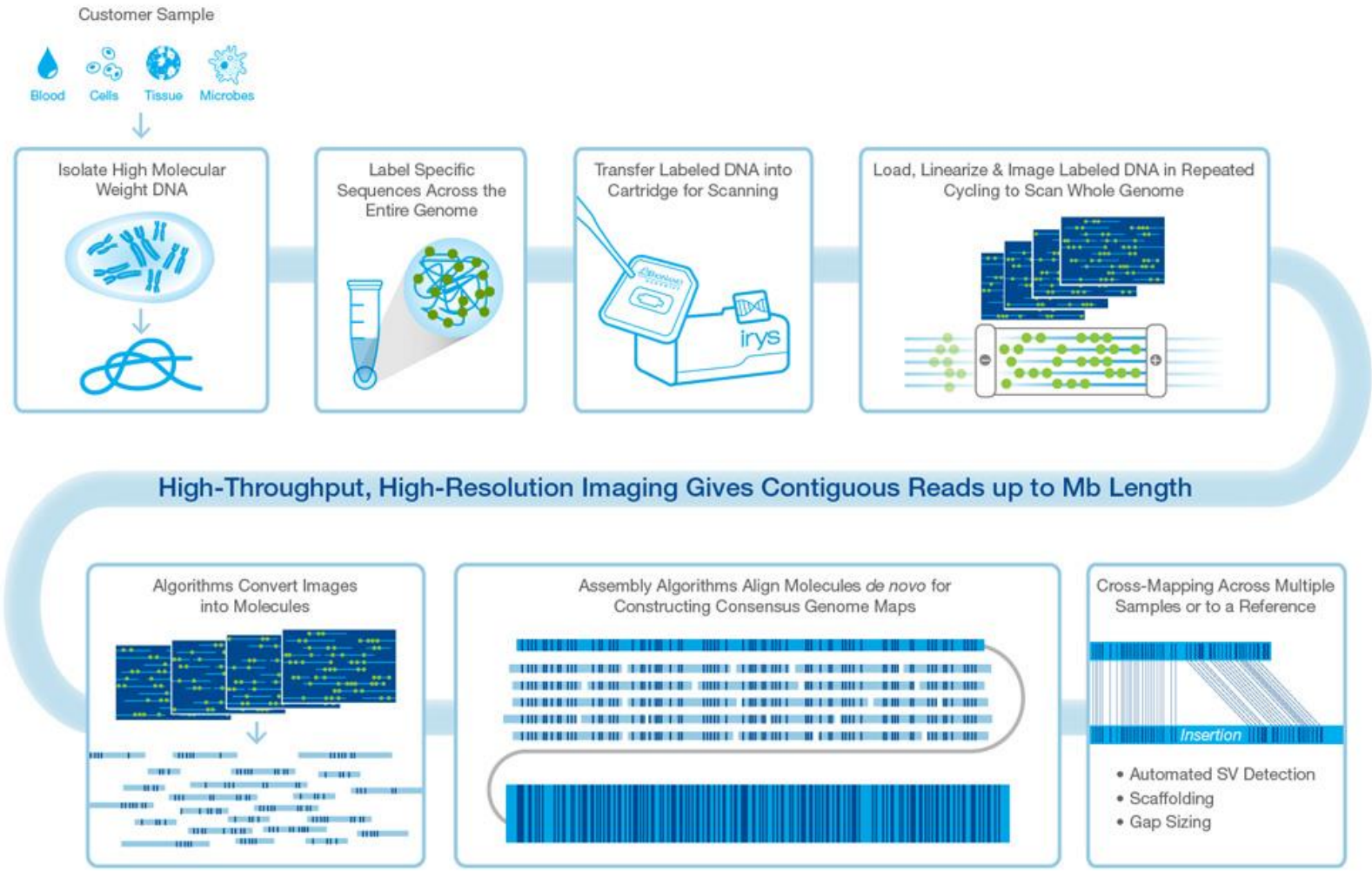
The challenge



- **Manage genome size and diversity**
- **Decrease genome complexity**
- **Target genomic region of interest**

PART I: A BETTER UNDERSTANDING OF GLOBAL GENOME ORGANIZATION

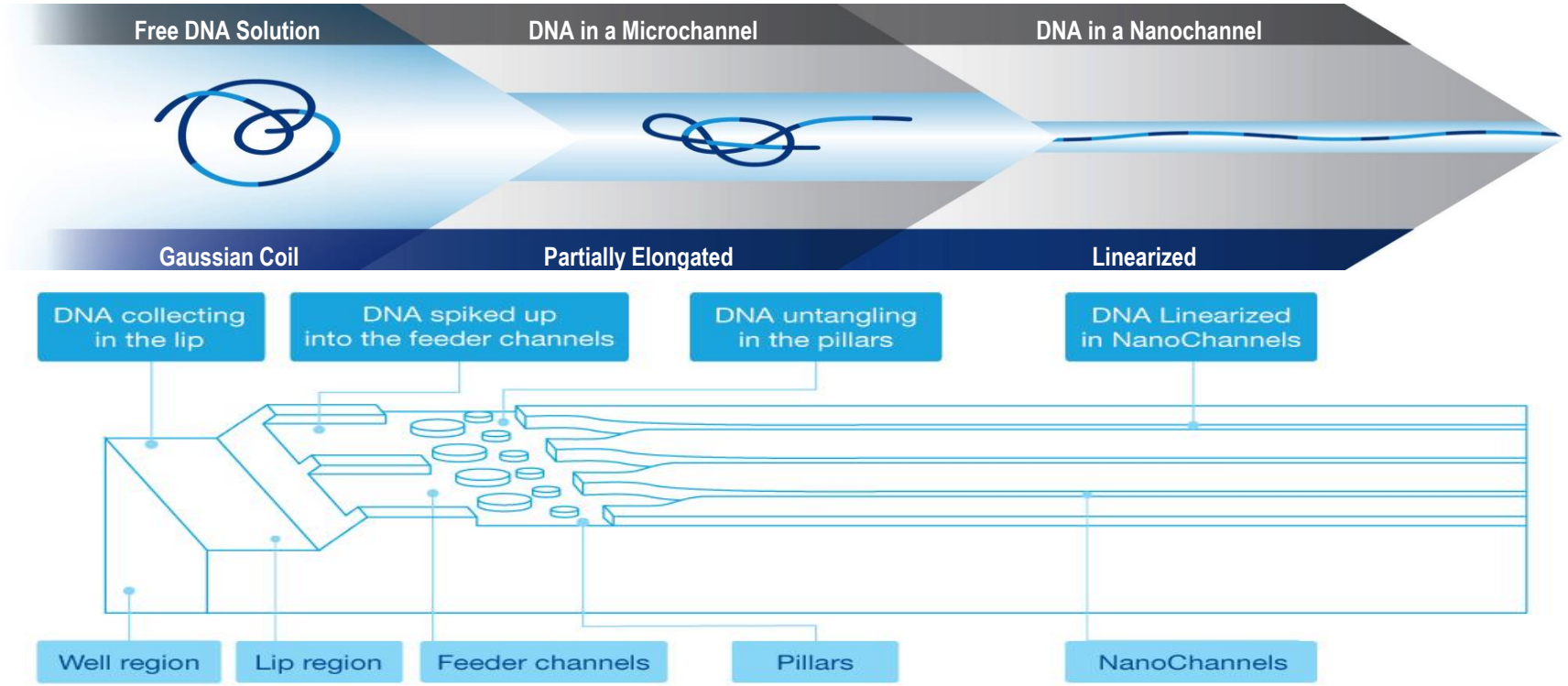
The BioNano Technology



➢ **50Gb data generated per flowcell (=> 100Gb / chip)**

BIONANO GENOMICS

HMW DNA molecules in nanochanels



➢ **HMW DNA molecules from 100kb to >2Mb**

BIONANO GENOMICS

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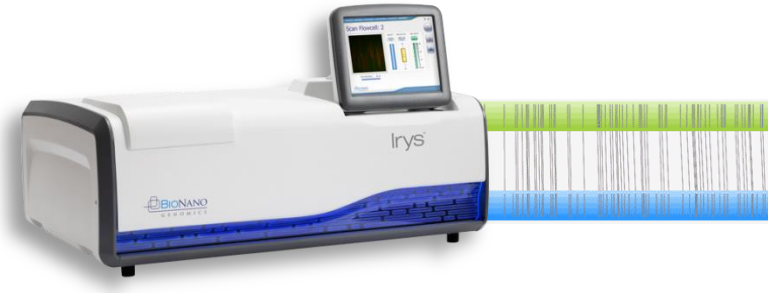
The Next Generation Mapping applications



For Genome Finishing, the maps serve as a scaffold:

- Sequencing contigs are converted in silico into molecular barcodes by highlighting the same sequence motifs
- These sequencing based barcodes are then aligned to the BioNano maps

For SV discovery/detection, compare to a reference or gold standard, looking for changes in the patterns:

- Shifts in barcode patterns reveal insertion (addition), deletion (subtraction), inversion (re-orientation, translocation of genome segments

BIONANO GENOMICS

Sunflower Genome Finishing

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

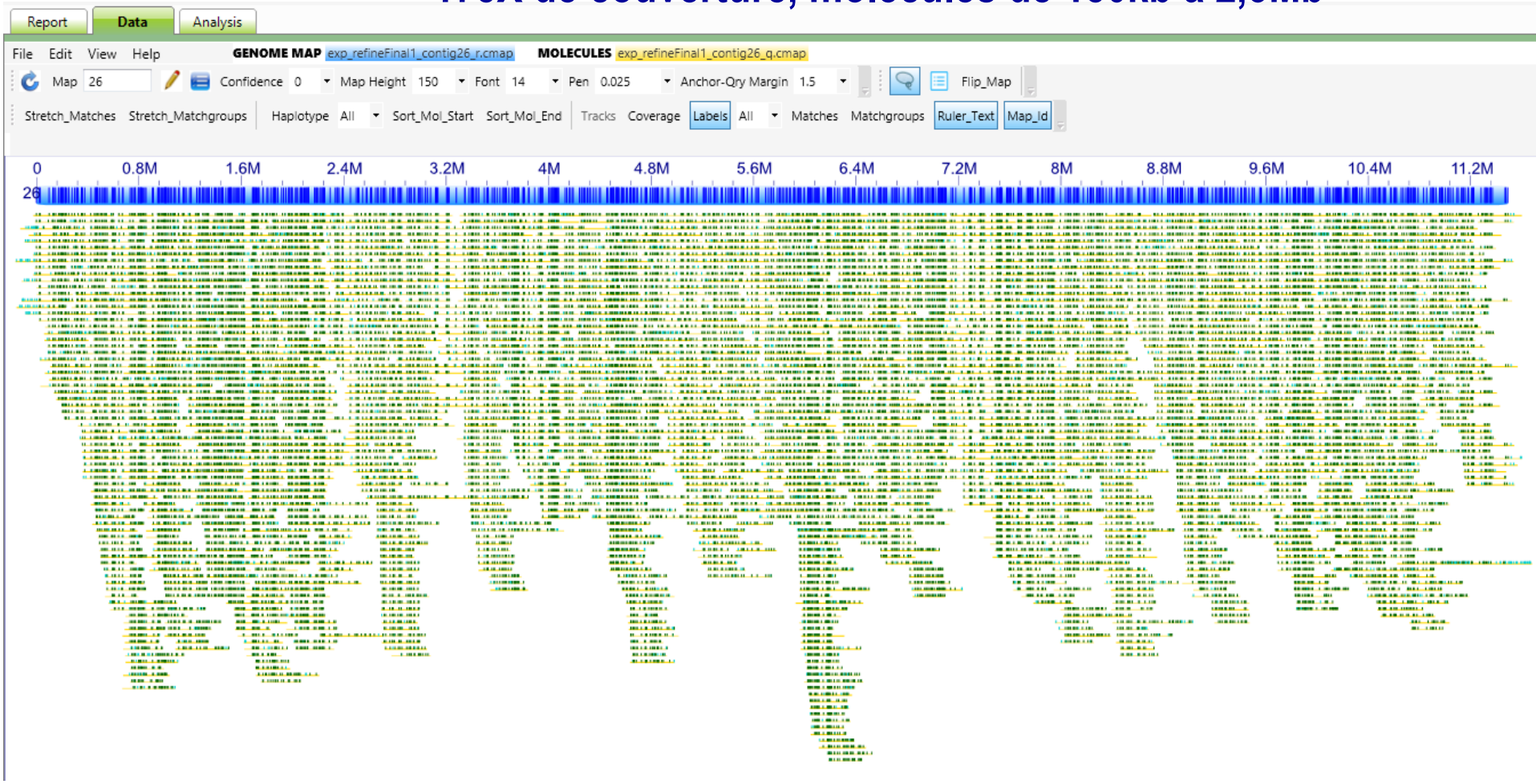
# contigs	LEN Max	N50 BP	#>N50	MEDIAN	BP
12 318	3,35 Mb	524 kb	1 684	120 kb	2,93

=> 80% of the genome inside contigs

Gouzy et al., 2016

Optical map of the sunflower genome

=> 175X de couverture, molecules de 150kb à 2,3Mb



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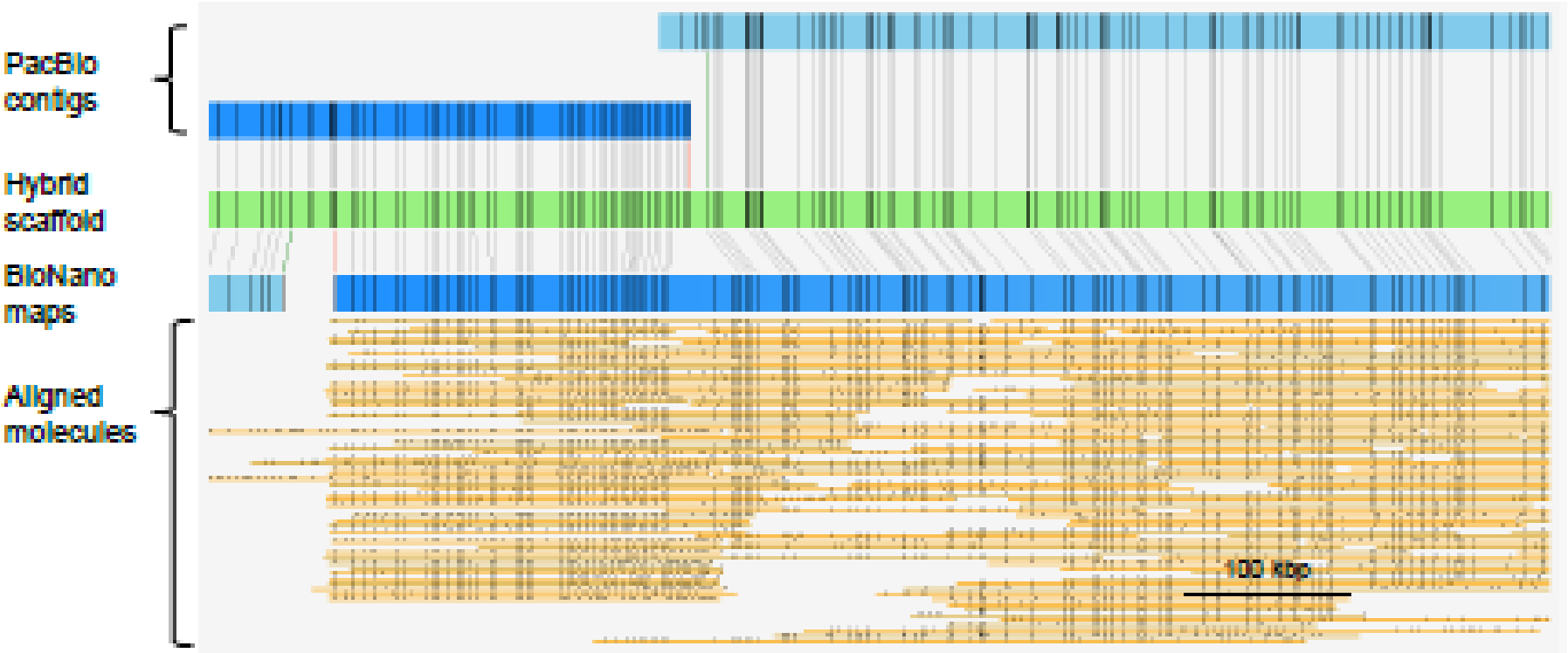
Hybrid scaffolding assembly



Sunflower genome enhancement

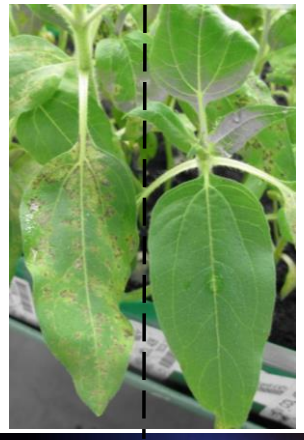
	PacBio Assembly	BioNano Assembly	Hybrid scaffold
Count	12318	2228	1430
Median length (Mb)	0.120	0.999	1.442
N50 length (Mb)	0.524	1.979	2.87
Max length (Mb)	3.35	11.49	17.45
Total length (Mb)	2930	3191	2922
% genome	81%	88%	81%

More than 5 fold increase

PART II: FOCUSING ON SPECIFIC REGIONS WITH NON-GRIDDED BAC LIBRARY

QRM1 QTL in Sunflower

Phenotypic analysis
(232 recombinant plants)
Susceptible | Resistant



- QTL controlling resistance to downy mildew
- Strong effect on LG10
- Explain 65% of the phenotypic variability
- 2 lines:
  - Susceptible (PSC8)
  - Resistant (XRQ)
- *In silico* physical mapping

=> reduction of the genetic gap to 0.4 cM

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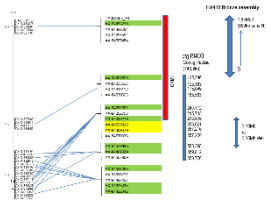
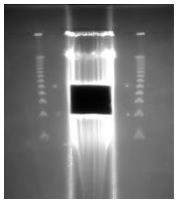
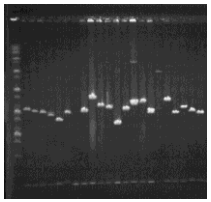
Non-gridded BAC library construction

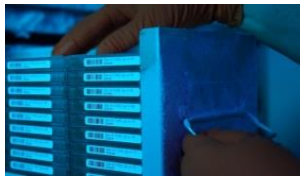
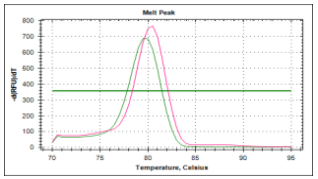



- Establishment of genetic map
- Markers definition on the QMR1 locus

- BAC library construction for PSC8
- Pooling of the transformants

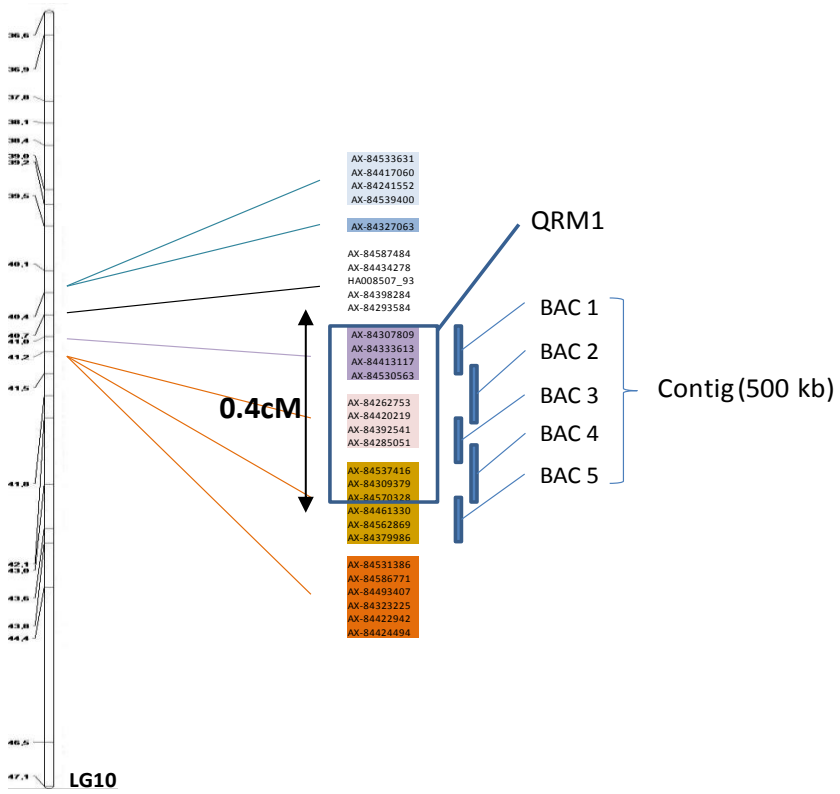
- BAC-Pool Sequencing
- 35 to 100 x coverage
- PacBio Technology :
  - ➢ MTP of BACs : 1 contig

- Screening of pools with specific markers
- Identification of positive BAC clones
- BAC clone characterization

Method derivated from Isidor et al., 2005

Map-based cloning of QRM1



- Sequencing of the 5 BAC clones
- 1 contig of 500kb
- Identification of 2 candidate genes:
  - A MAP Kinase Interacting Kinase
  - A Proteinase inhibitor

Laboratoire Interactions Plantes Micro-organismes

PART III: FOCUSING ON SPECIFIC REGION WITH SEQUENCE CAPTURE

nature COMMUNICATIONS

ARTICLE

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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

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

CATCH: Cas9-Assisted Targeting of Chromosome segments

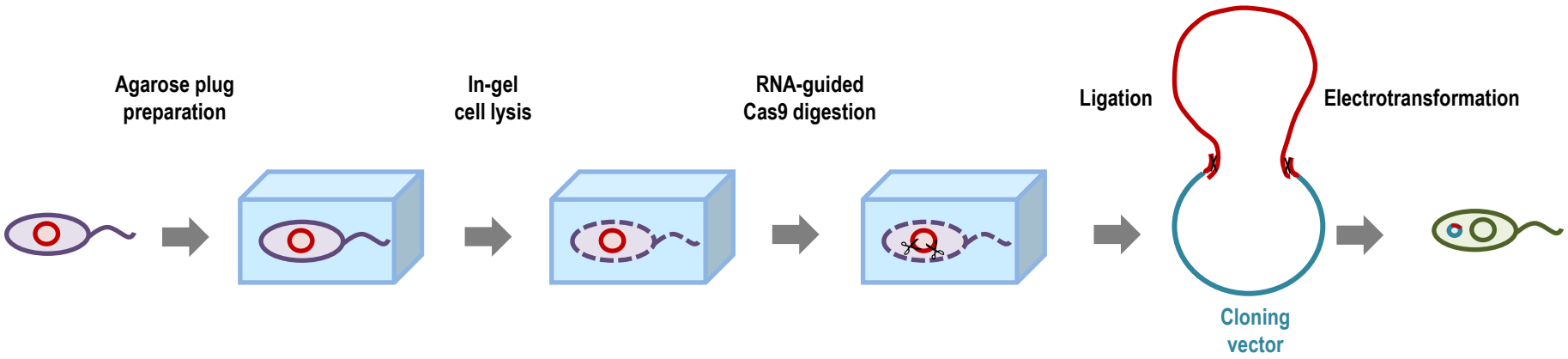
- One step targeting cloning of larges genes cluster:
  - Targeted
  - Fast
  - Specific

- Based on CRISPR/Cas9 technique

  => programmable molecular scissors

Jiang and Zhu, 2016

General workflow



HMW DNA extraction → Plug manipulation → sgRNA production → Cas9 digestion → Gibson assembly cloning → BAC clones validation

Jiang and Zhu, 2016

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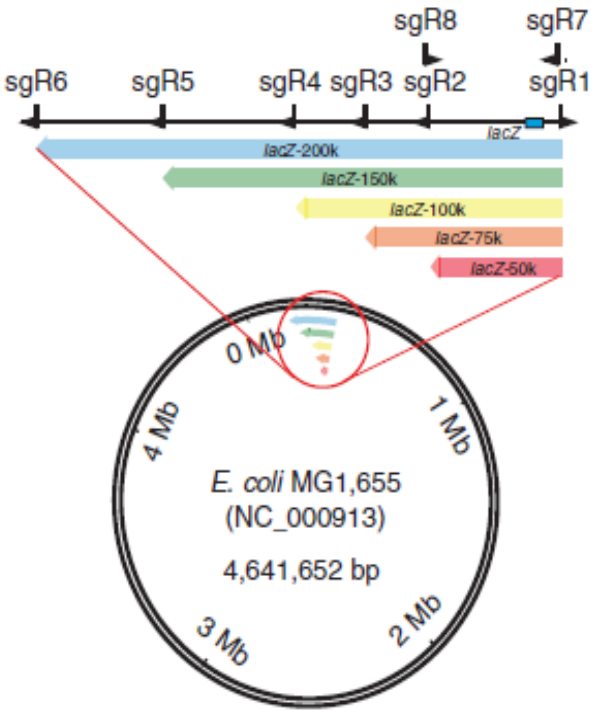
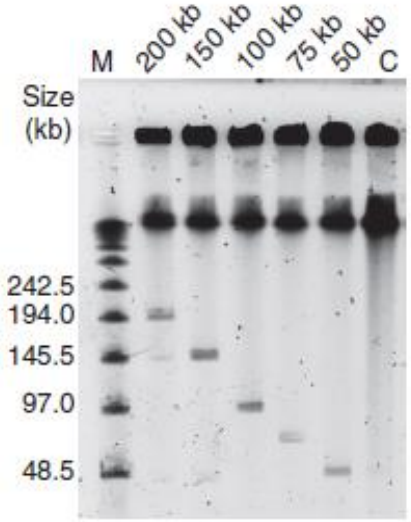
sgRNA design and results



- **Isolation of DNA fragment of several size : from 50 to 200kb**



- **In various bacteria**
- **Limitations in cloning efficiencies with long DNA fragments**

Jiang and Zhu, 2016

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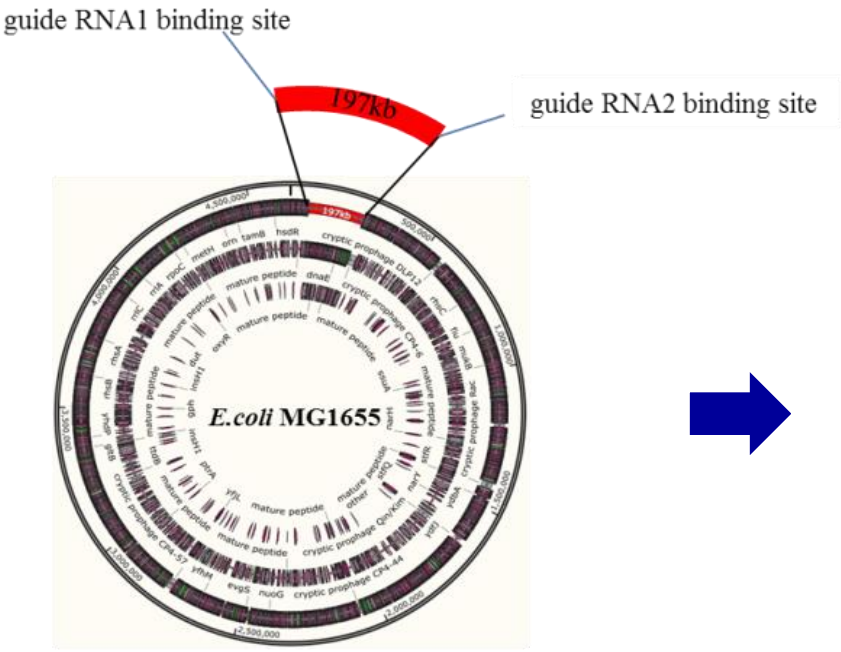
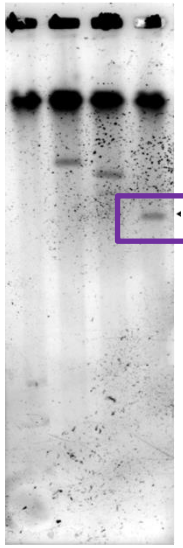
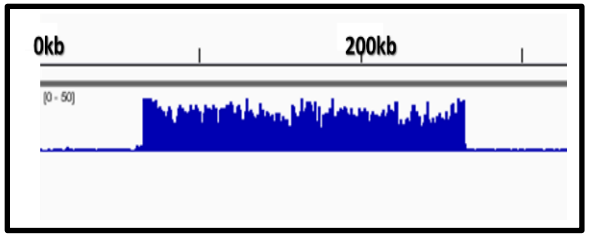
Direct sequencing





Illumina Hiseq2000 deep-sequencing

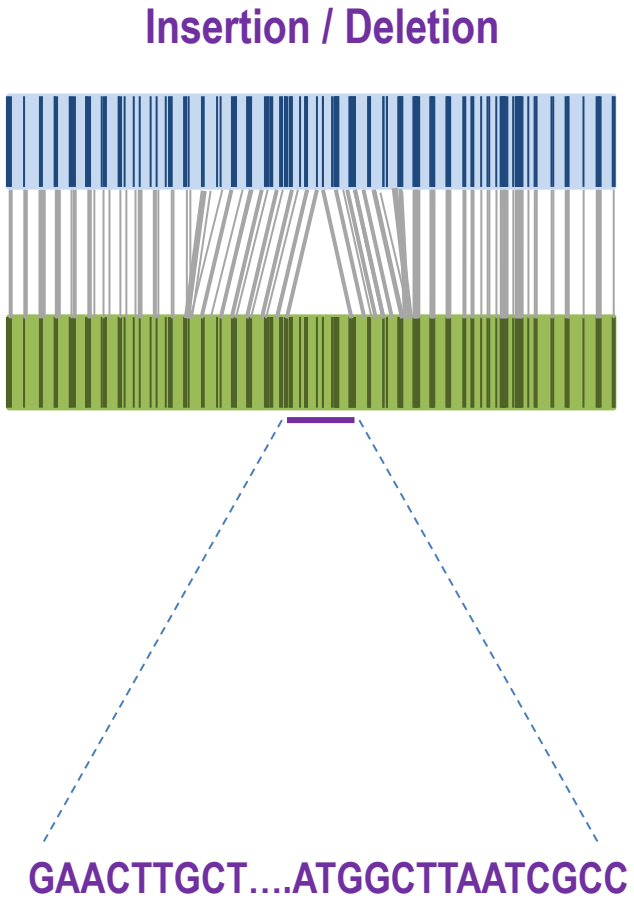


Tslil Gabrieli and Yuval Ebenstein, 2016

Choice of a model plant genome

- *Medicago truncatula*:
  - Small genome size ~ 500 Mb
  - Diploid
  - Complete genome sequence
  - BAC Minimal Tiling Path (MTP)
  - BAC libraries
  - Plants available at LIPM

I have a dream…



- The straightest way from phenotype to gene
- From the global to the specific
- Integration of the different tools

Acknowledgements

@CNRGV

http://cnrgv.toulouse.inra.fr/

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