Toward a better understanding of plant genomes structure: combining NGS and optical mapping technology to improve the sunflower assembly

Céline CHANTRY-DARMON

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CNRGV
The French Plant Genomic Center

- Created in 2004 by INRA
- 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

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Interactions with laboratories around the world

- More than 3 millions BAC clones distributed during the last 5 years

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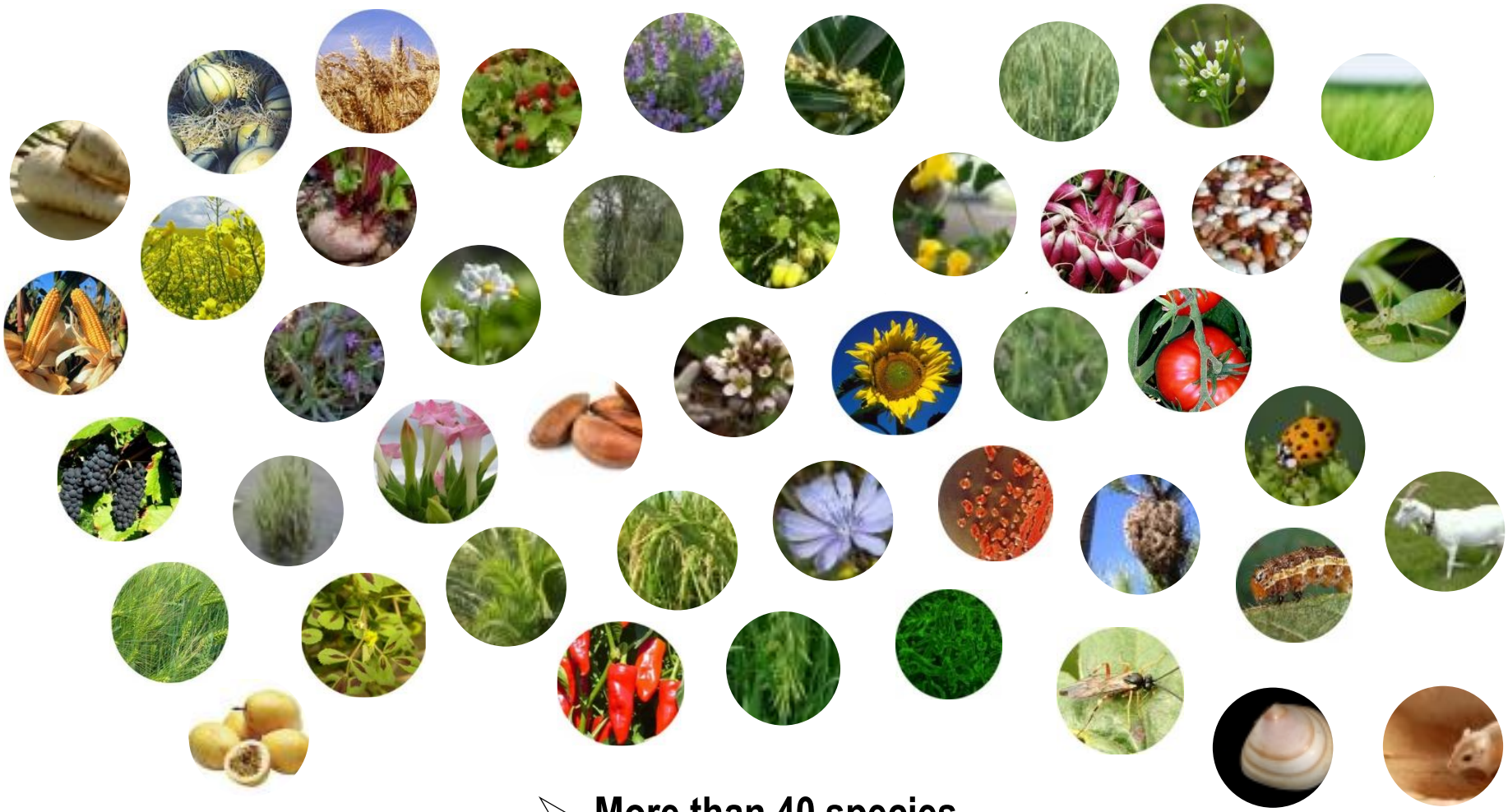
Plants project diversity



- More than 40 species

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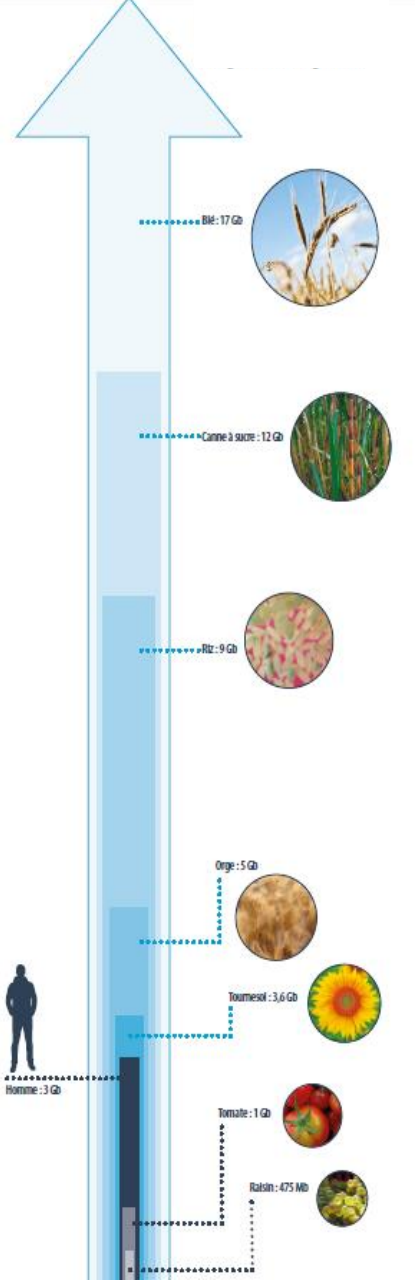
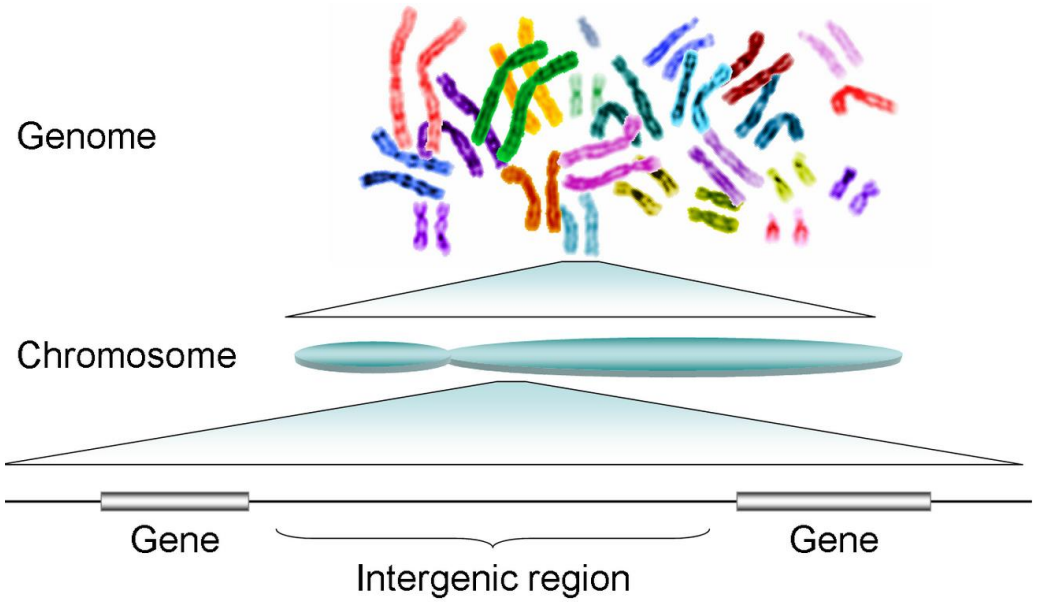
The goal for the Pant Genomic Center



- Large genome size
- Repeats elements
- Polyploidy



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

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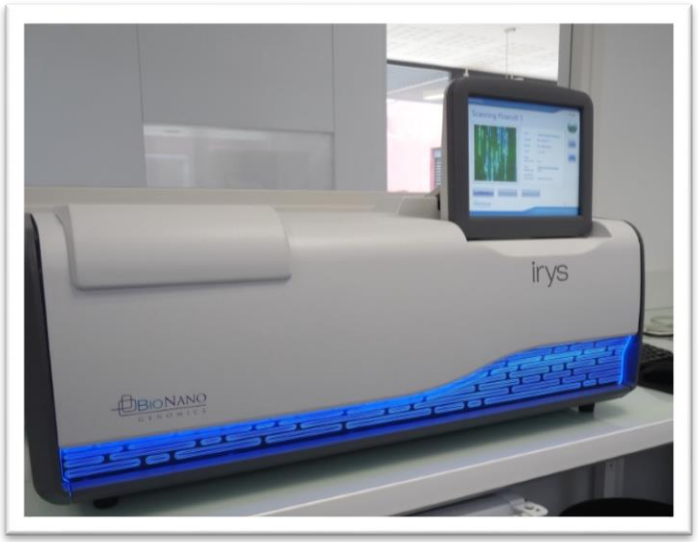
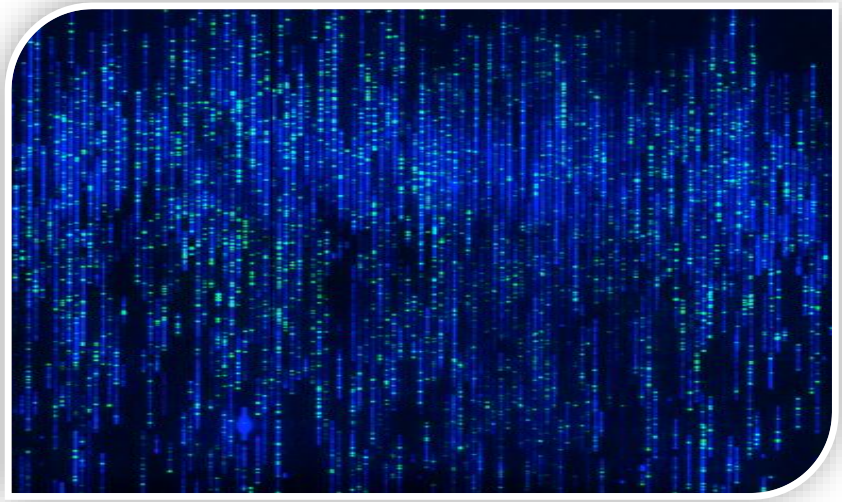
Focus on the optical mapping with BioNano

- The Bionano Irys system: a new tool to study complex genomes
- Advantages of BioNano optical mapping:
  - Direct visualization of long DNA molecules (>100 kb)
  - Provides real physical distance information





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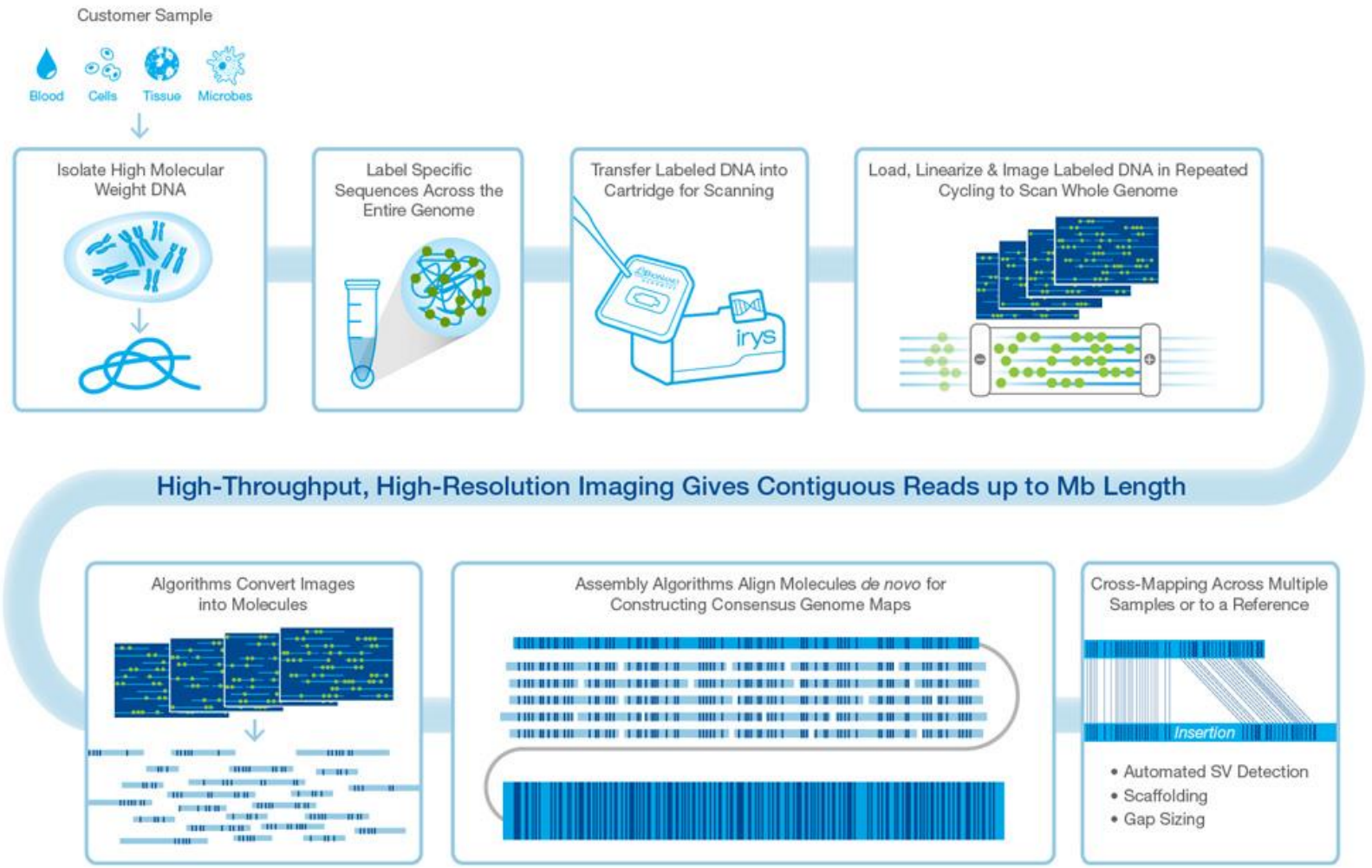
The Workflow



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

BIONANO GENOMICS

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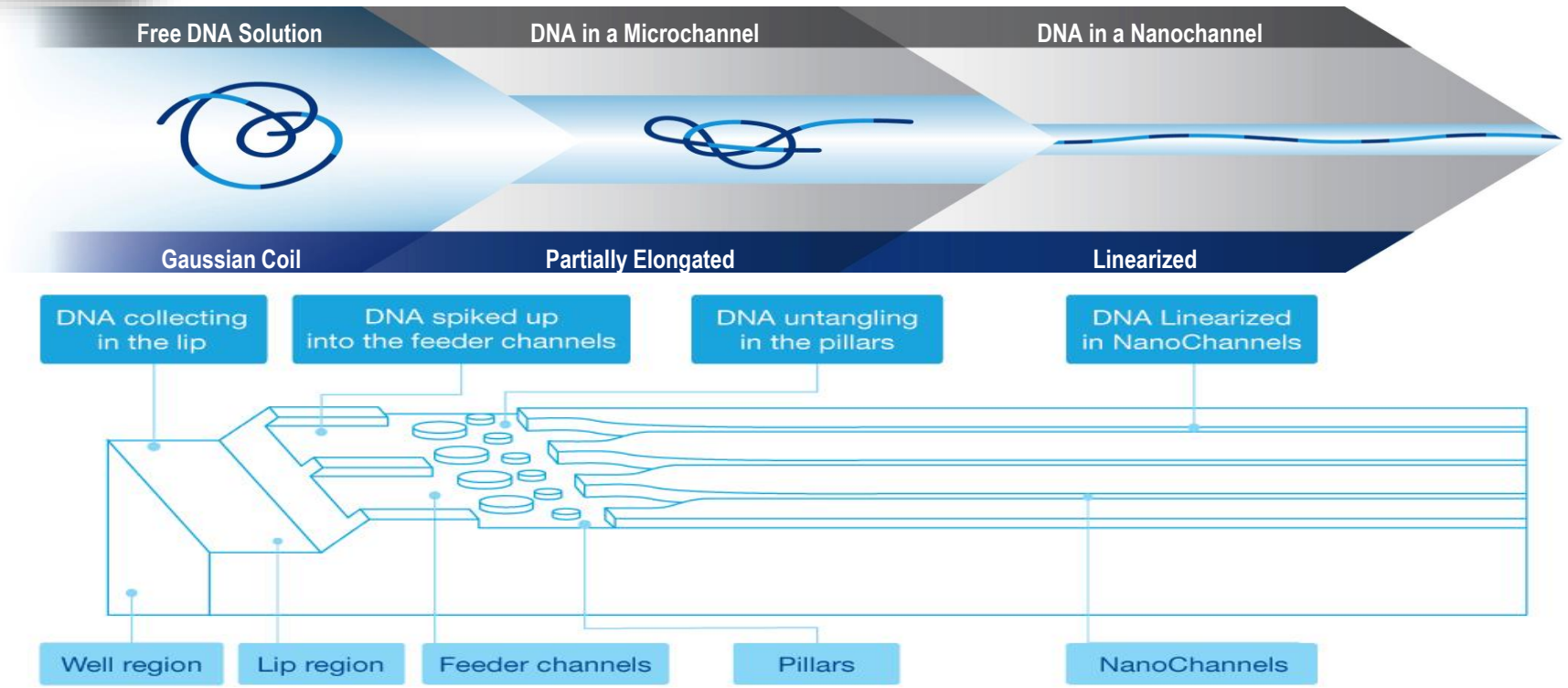
HMW DNA molecules in nanochanels



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

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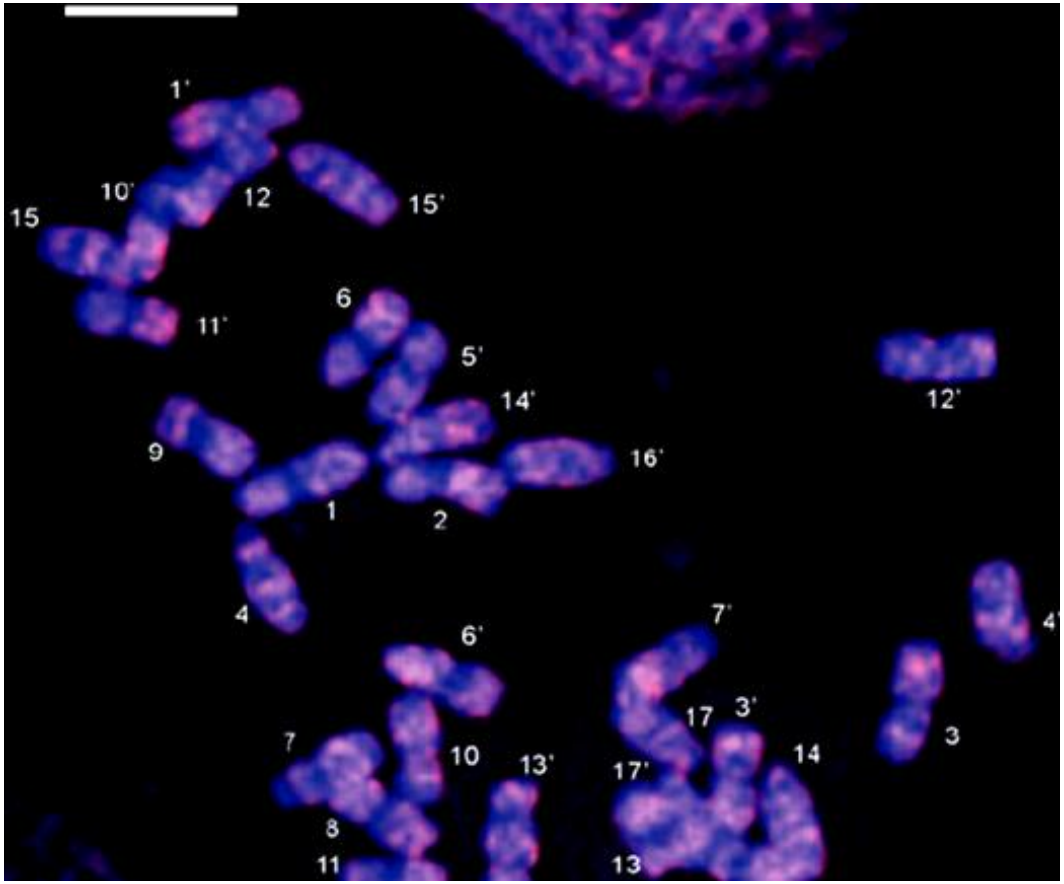
The Sunflower genome



- *Helianthus annuus*
- 3.6 Gb
- 2n=34 chromosomes

Cytological characterization of sunflower by in situ hybridization using homologous rDNA sequences and a BAC clone containing highly represented repetitive retrotransposon-like sequences

P. Talia, E. Greizerstein, C. Díaz Quijano, L. Peluffo, L. Fernández, P. Fernández, H.E. Hopp, N. Paniego, R.A. Heinz, and L. Poggio

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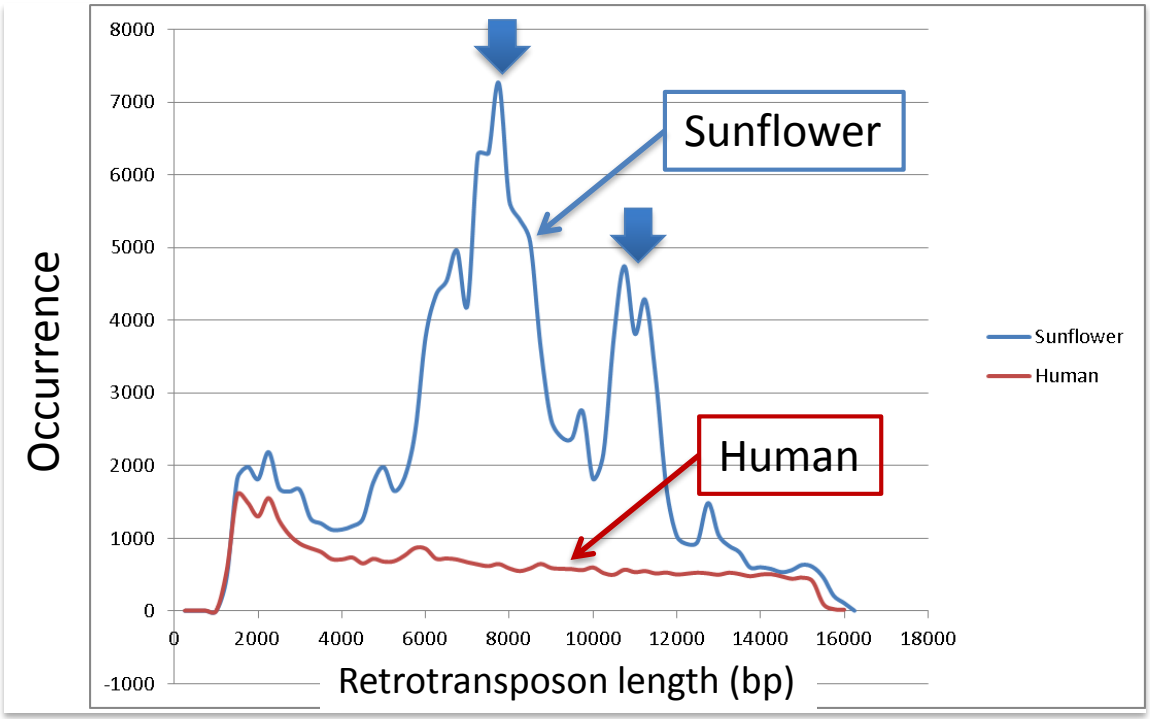
Sunflower genome contains long repeated sequences

Length distribution of LTR retrotransposons



LTRharvest (Ellinghaus *et al.* 2008, default parameters)

J. Gouzy

Repeats = 33% of the sunflower genome

Repeats = 8% of the Human genome

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

The repeats make the assembling very difficult

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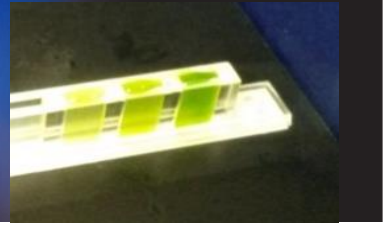
BioNano analyses



- HMW DNA Extraction of fresh young dark treated leaves
- 2 nicking enzymes (BspQ1 & BssS1)

	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

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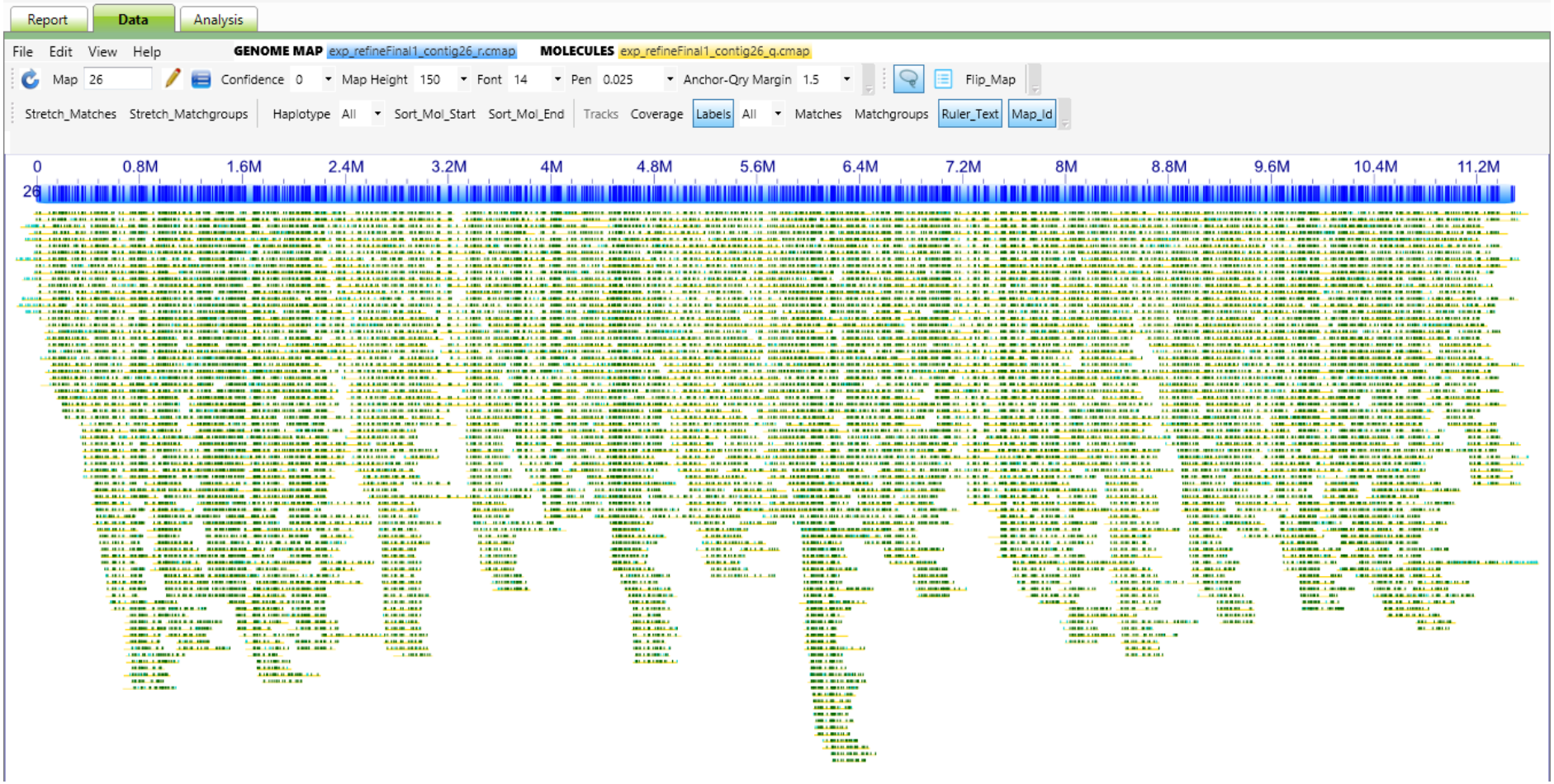
Example of a BioNano map



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

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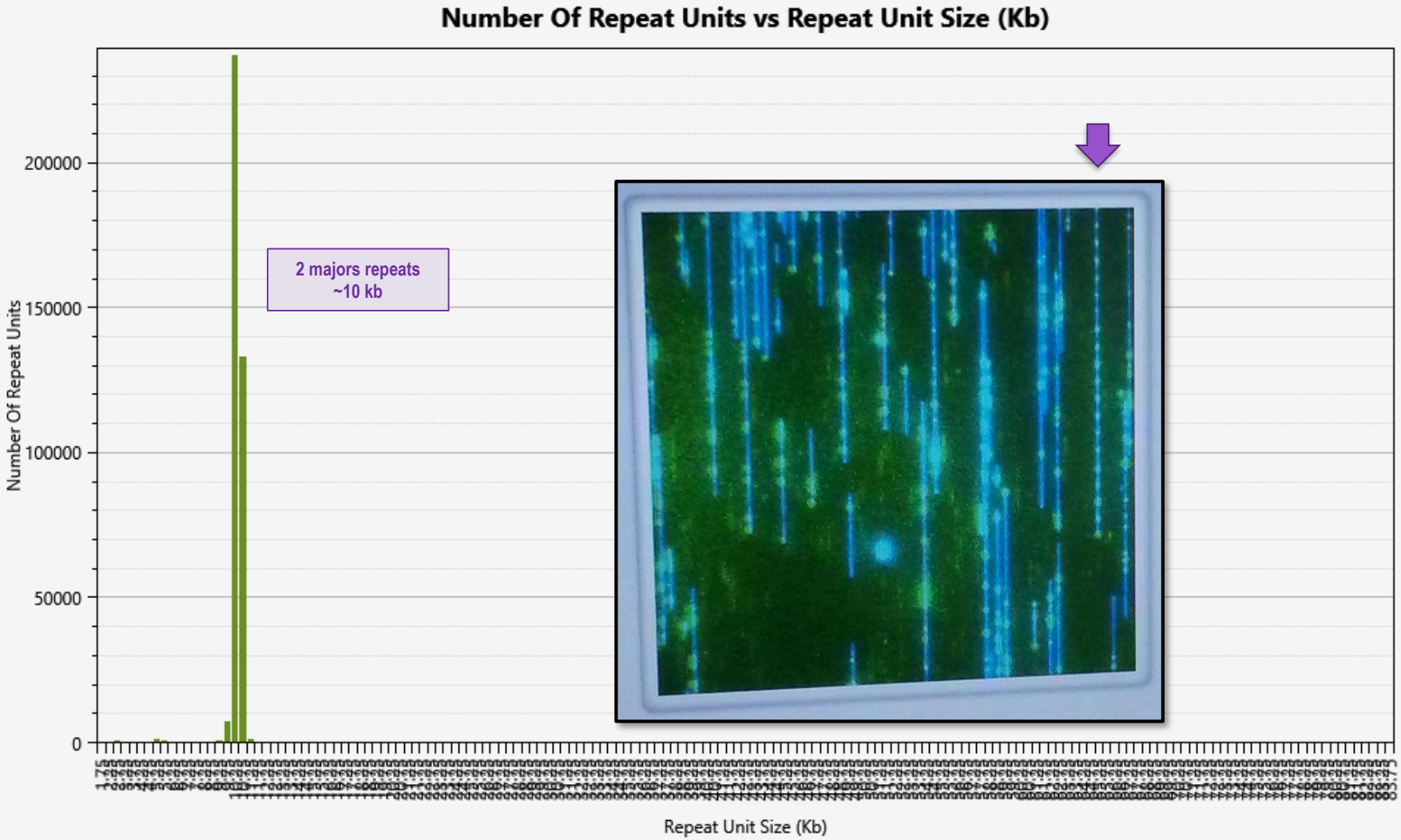
Visualization of the repeats



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BioNano Maps

	PacBio Assembly	BioNano BspQ1 Assembly	BioNano BssS1 Assembly
Count	12318	2228	4287
Median length (Mb)	0.120	0.999	0.551
N50 length (Mb)	0.524	1.979	0.968
Max length (Mb)	3.35	11.49	5.322
Total length (Mb)	2930	3191	3112
% genome coverage	81%	88%	86%

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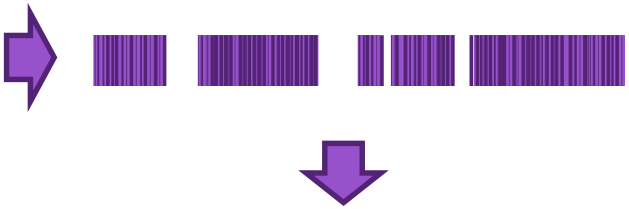
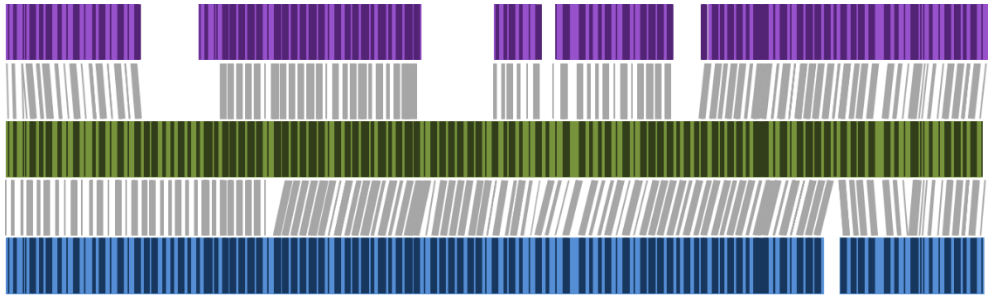
Hybrid Assembly

PacBio Assembly

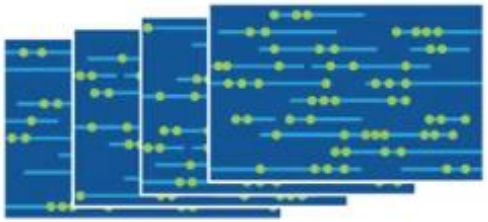


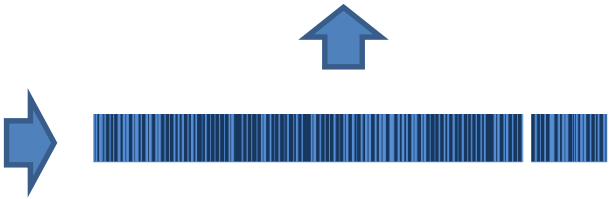
Nickase sites on NGS contigs



Hybrid scaffold

BioNano Mapping





Nickase event Fingerprint

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Sunflower Hybrid Assembly

	PacBio Assembly	BioNano BspQ1 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

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Sunflower genome enhancement

	PacBio Assembly	BioNano BspQ1 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%

Hybrid scaffold + not scaffolded PacBio contigs : 3611Mb

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2 Step Hybrid Assembly

	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	11.49	17.45	5.322	24.670
Total length (Mb)	2930	3191	2922	3112	2960
% genome	81%	88%	81%	86%	82%

More than 7 fold increase

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Conclusion

- This preliminary results are enhancing the sunflower genome
- More than 7 fold improvement of the N50 length
- 2-step hybrid scaffolding strategy improves significantly the resulting N50
- Now we must look to the conflicts between the NGS assembly and the BN assembly more in details

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Acknowledgements

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