A mosaic monoploid reference sequence for the highly complex genome of sugarcane

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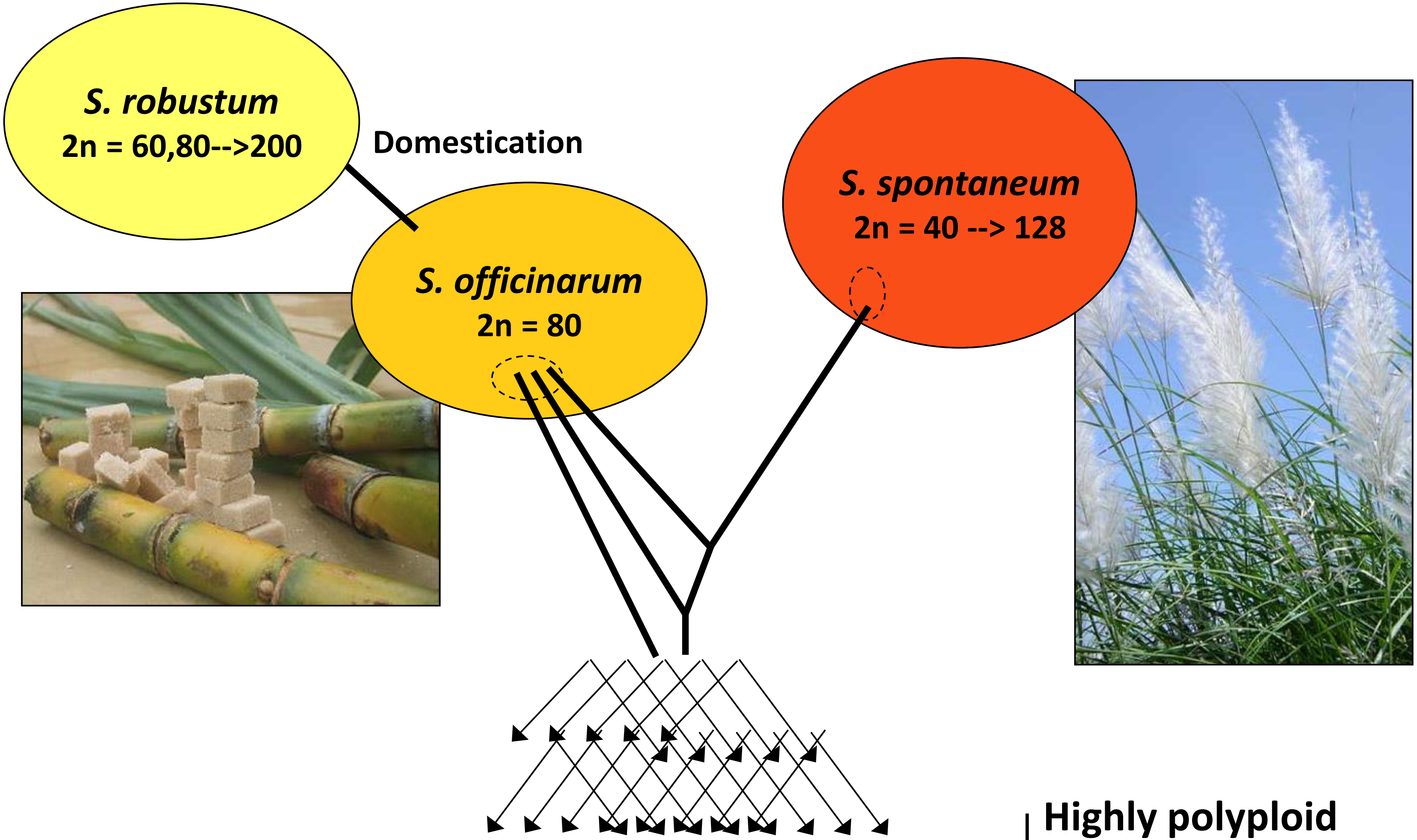
Modern sugarcane cultivars



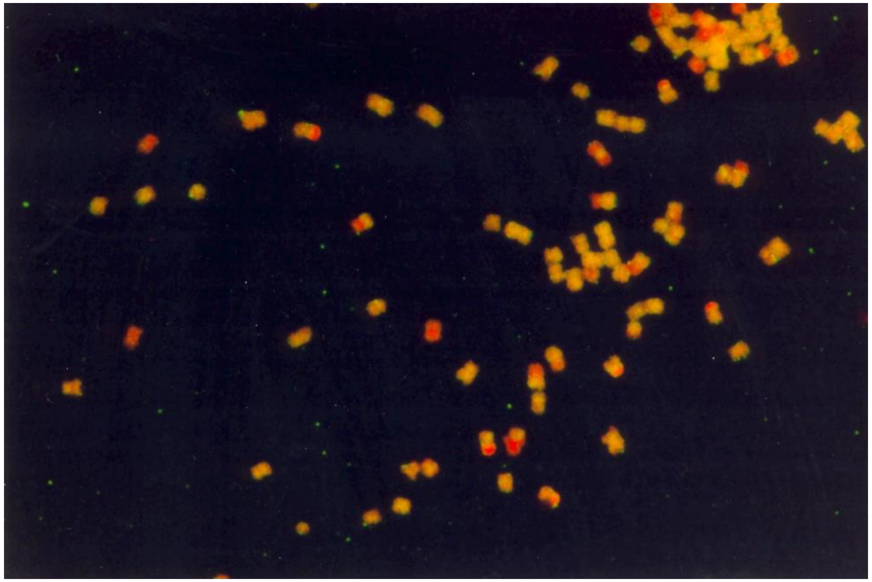
modern cultivars

2n ~ 110-130

Highly polyploid
Aneuploid
Interspecific

Global organization of the complex genome of sugarcane

Genomic in situ hybridization

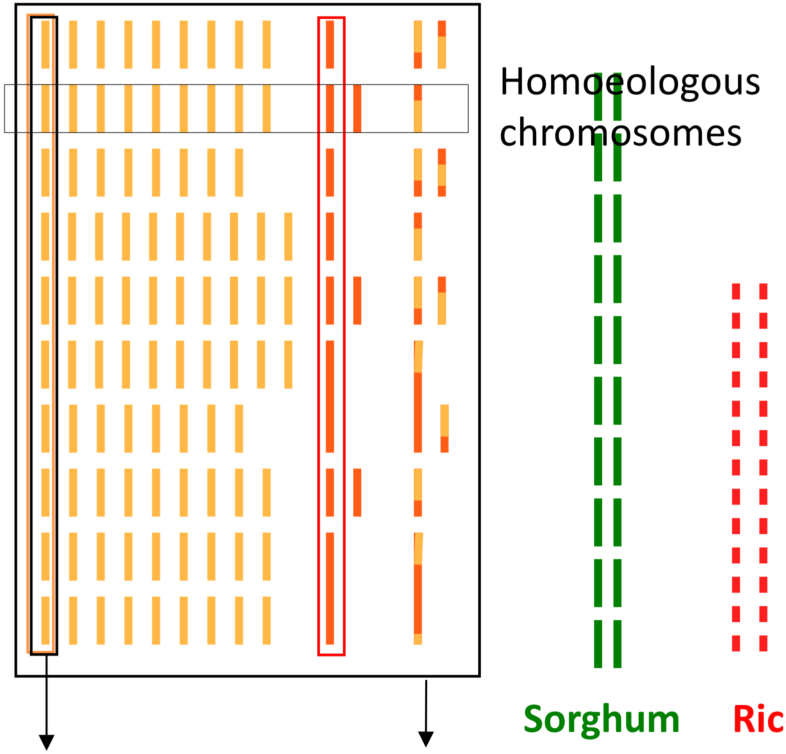


R570, 2n=ca 115

80 % S. officinarum

10% S. spontaneum

10% recombinants



S. spontaneum

S. officinarum

x=10

x=8

recombinants

Homoeologous chromosomes

Sorghum

750 Mb

Rice

400 Mb

Monoploid genome size: 930 Mb

Total genome size: 10 Gb

(photo and figure adapted from D'Hont et al, 1996; D'Hont et al, 1998; D'Hont 2005, Piperidis et al 2010)

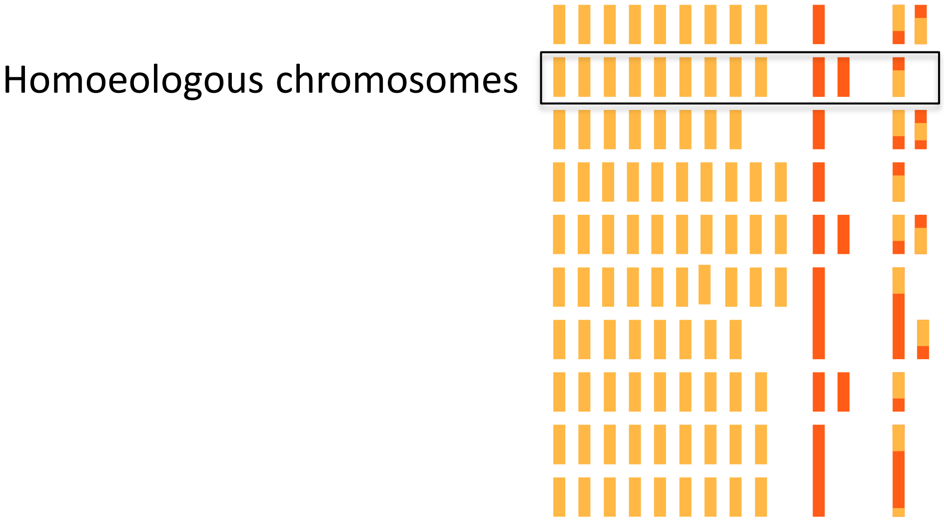
Sequencing sugarcane

The sugarcane genome poses challenges that have not been addressed in any prior sequencing project.

The main difficulties reside in

- its **high polyploidy**, with a complete set of homoeologous chromosomes predicted to range from **10 to 12 copies**

- its **high level of heterozygosity** which makes an assembly of the whole genome very challenging through a classical shotgun sequencing approach.



Sugarcane

Comparison of sugarcane homeologs and comparison with sorghum

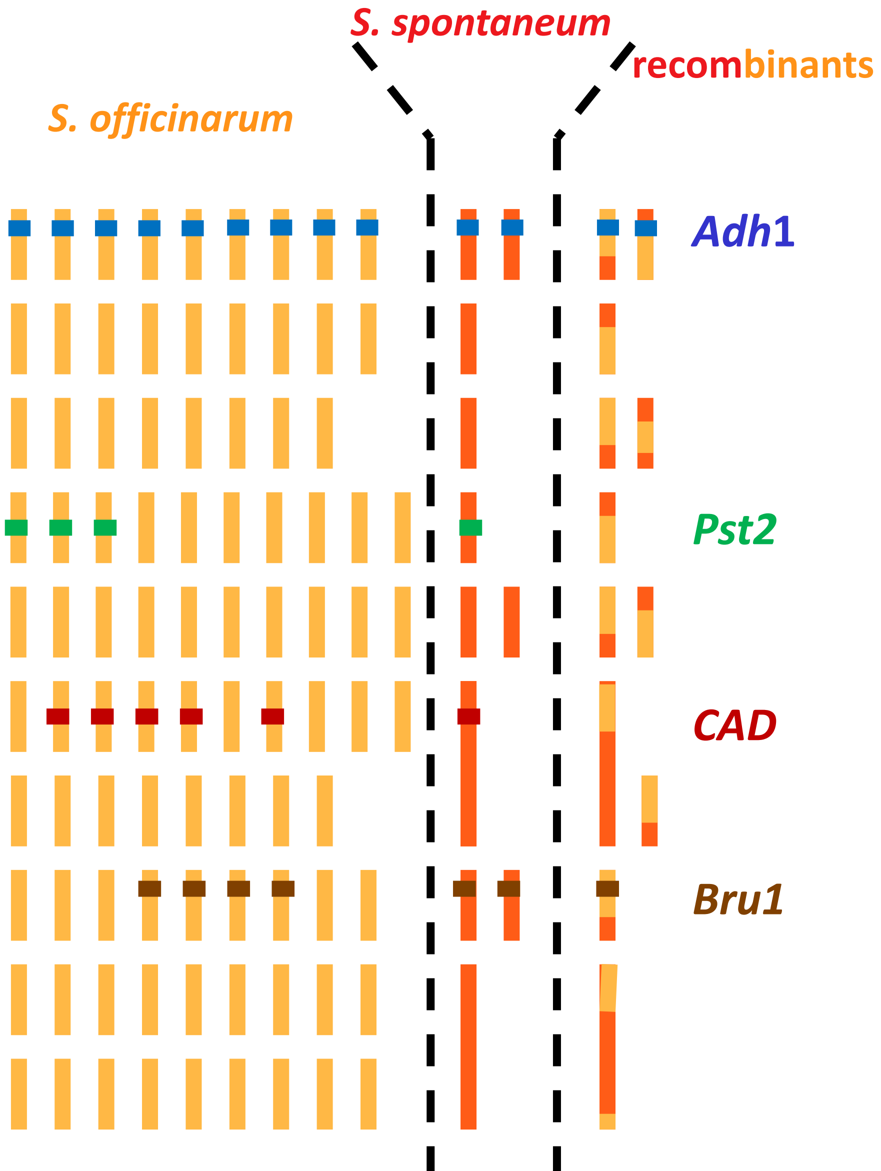
→ Comparison of sugarcane homoeologous regions

Thirteen hom(oe)ologous BAC clones corresponding to the ***Adh1*** region

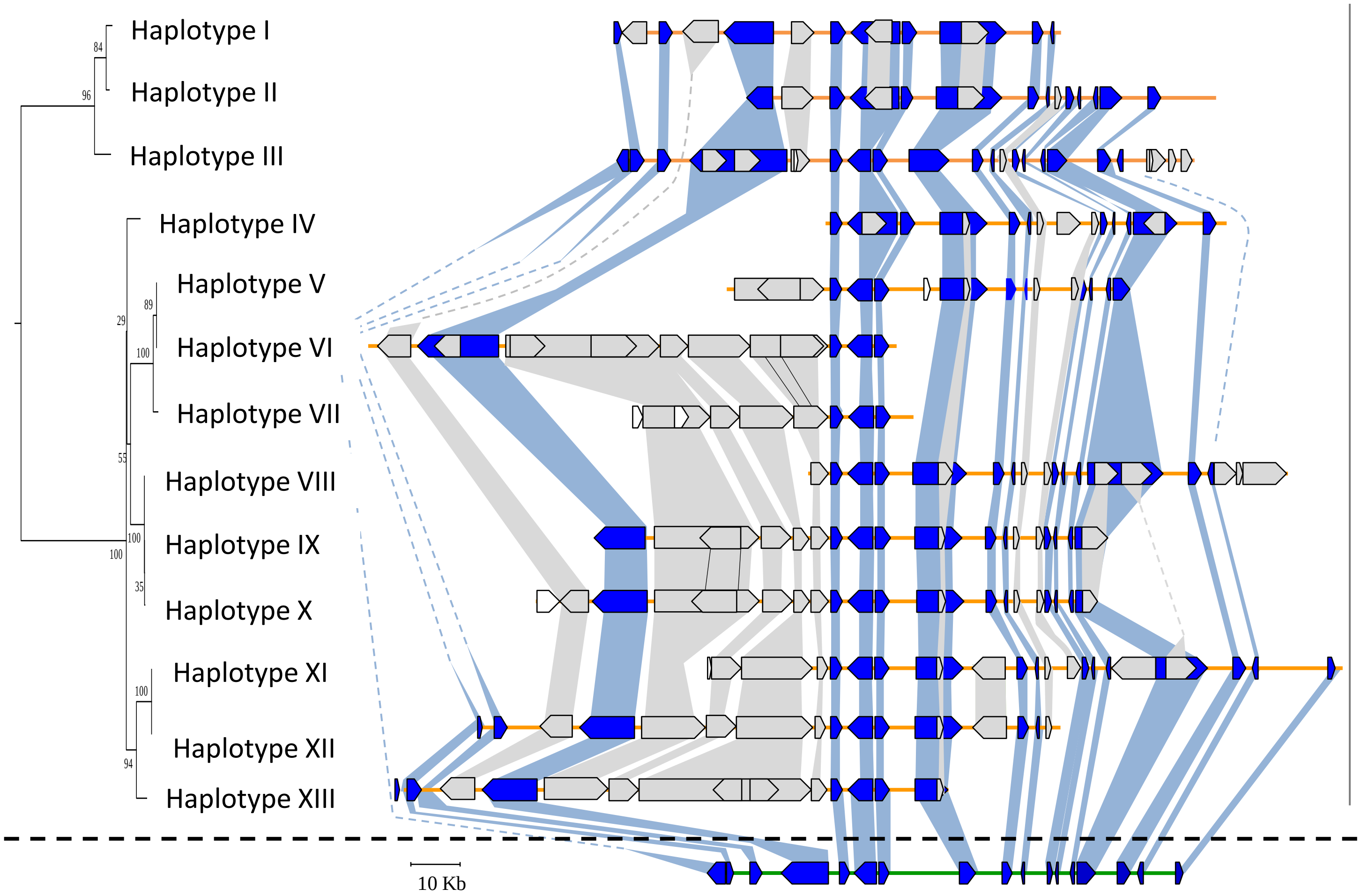
Four hom(oe)ologous BAC clones corresponding to the ***Pst2*** region

Six hom(oe)ologous BAC clones corresponding to the ***CAD*** region

Seven hom(oe)ologous BAC clones corresponding to the ***Bru1*** region



Comparison of sugarcane homeologs and comparison with sorghum



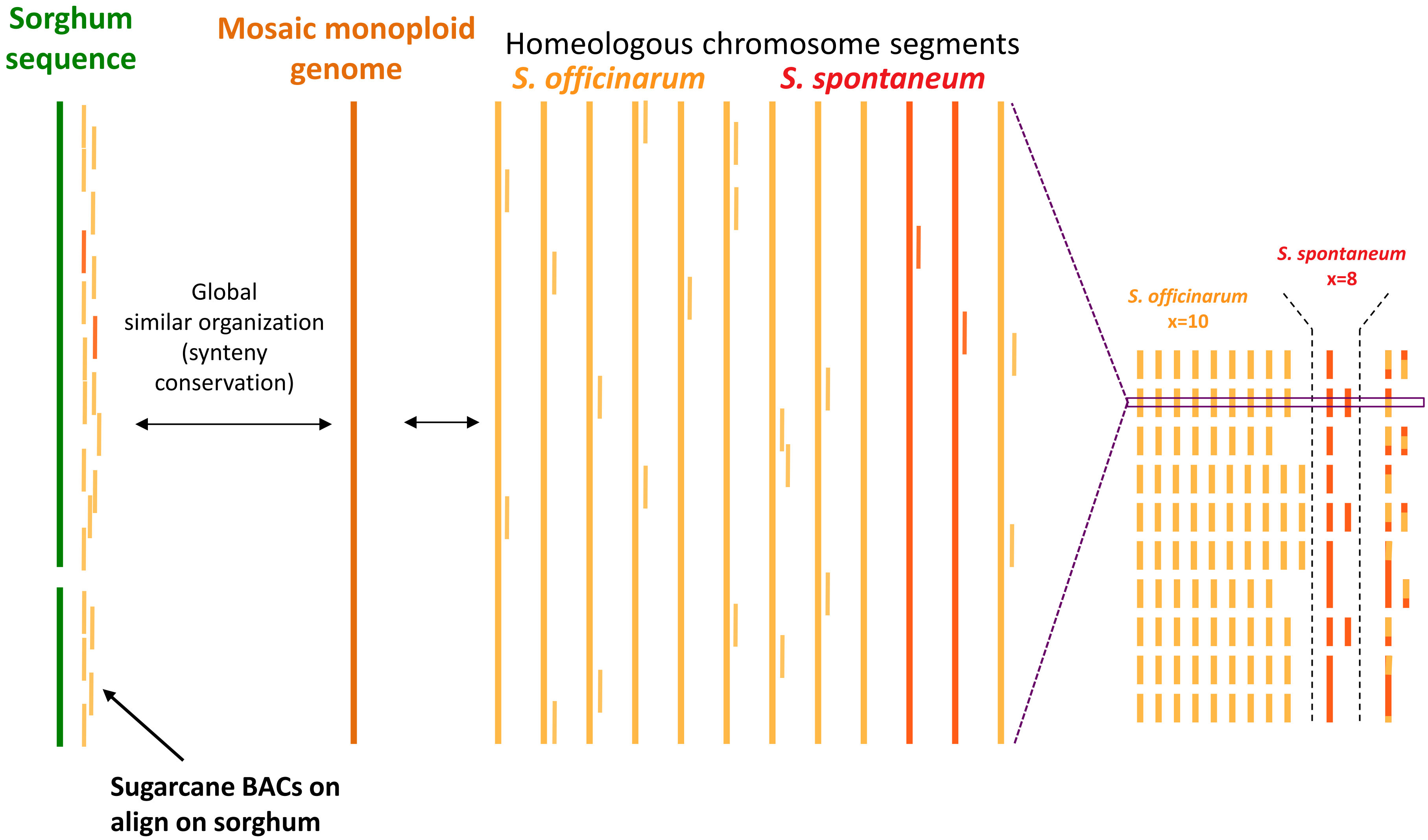
Sugarcane homoeologous BAC clones

Sorghum Orthologous region

- → Gene structure conservation among sugarcane homeologs
- → All hom(oe)o-alleles predicted functional
- → High colinearity with sorghum
- →Transposable elements variations

Garsmeur *et al*. New Phytologist 2010
unpublished data

The sugarcane sequencing strategy



Sorghum sequence

Mosaic monoploid genome

Homeologous chromosome segments

S. officinarum ***S. spontaneum***

Global similar organization (synteny conservation)

Sugarcane BACs on align on sorghum

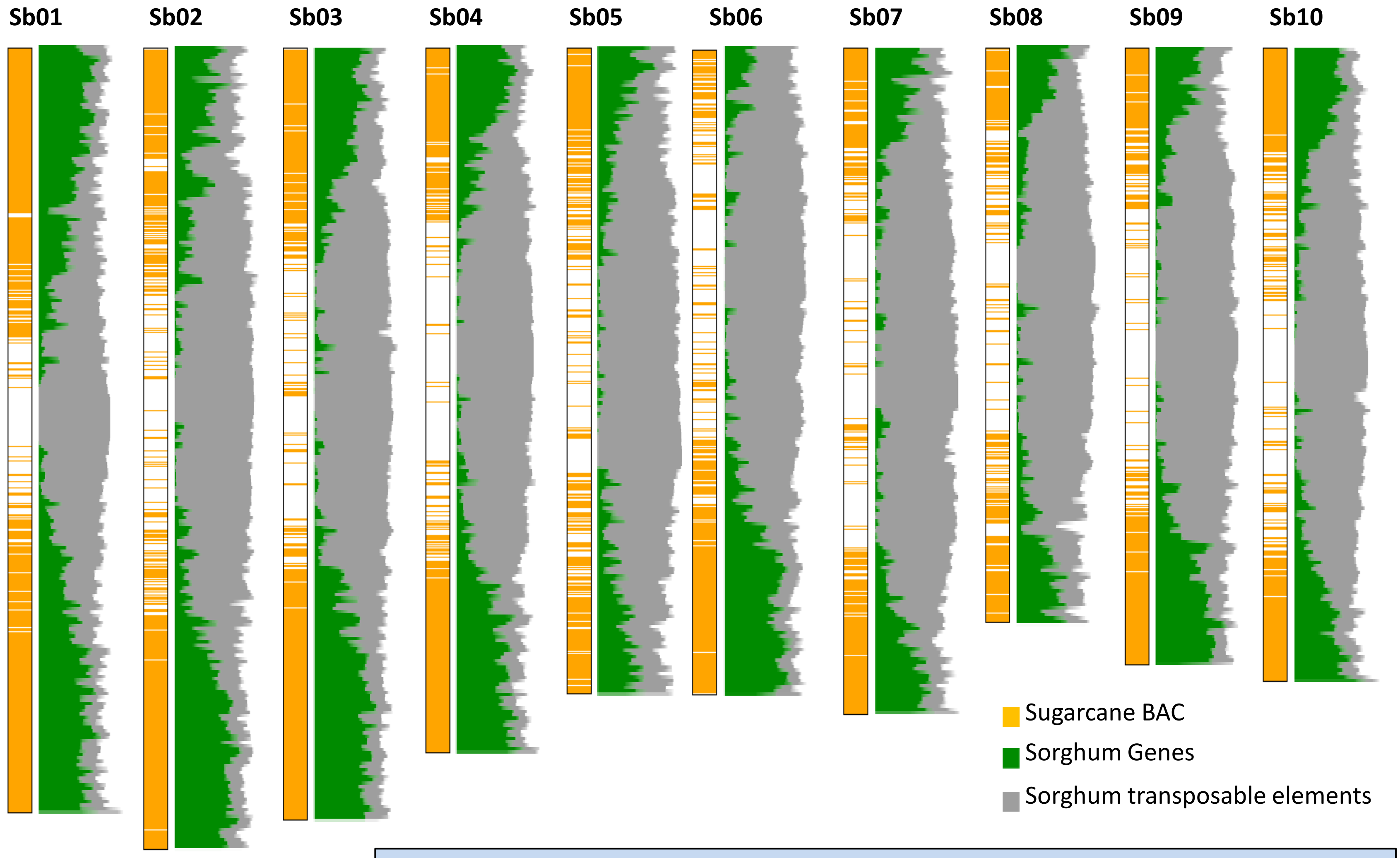
→ Sugarcane BAC sequences represent a mosaic of the monoploid genome

Sugarcane BAC selection using the sorghum genome sequence

→ **Whole Genome Profiling (WGP) technology**

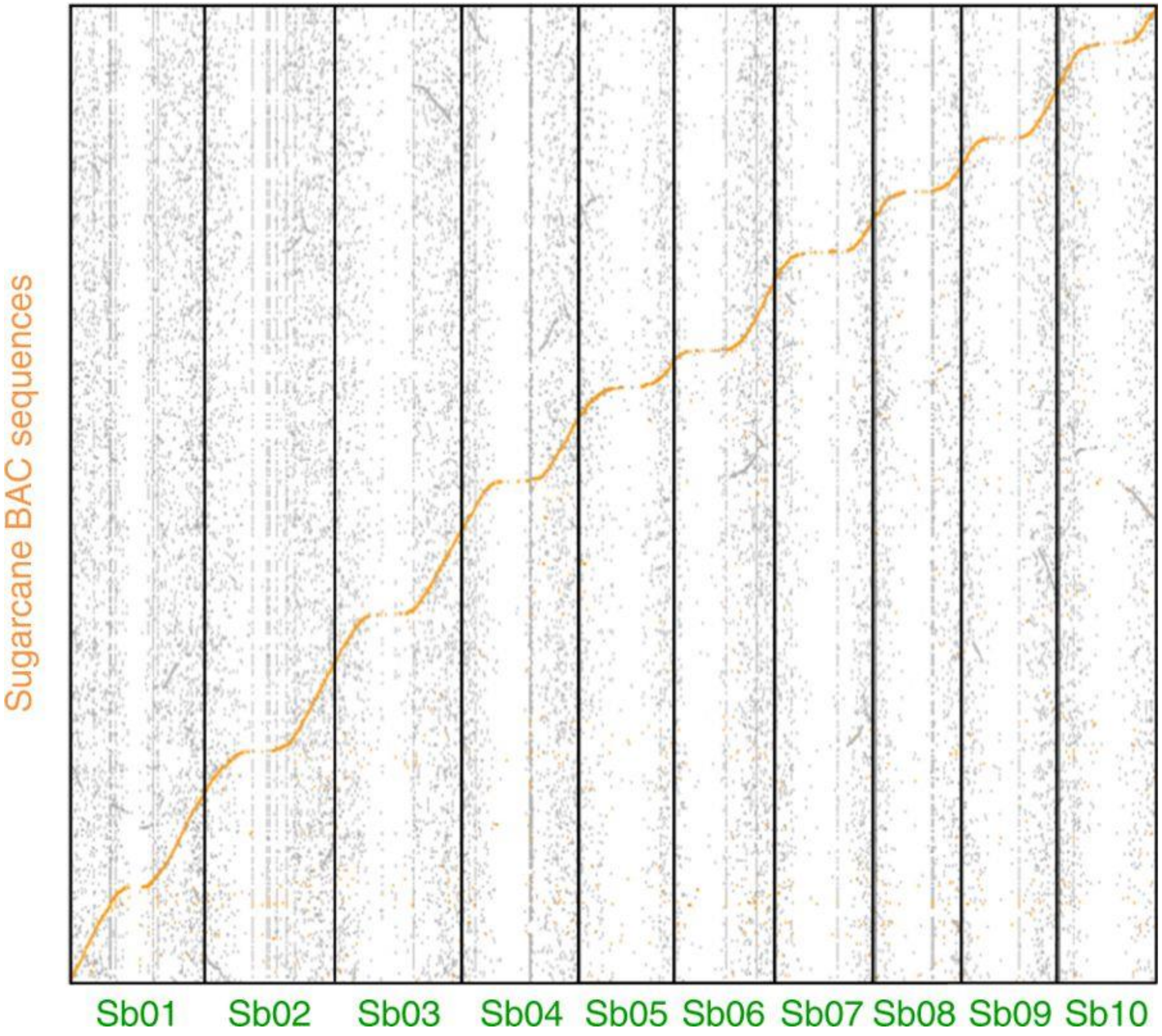
produce short illumina sequence tags from terminal ends of restricted BAC fragments

20,736 sugarcane BAC → 455,656 uniq WGP tags



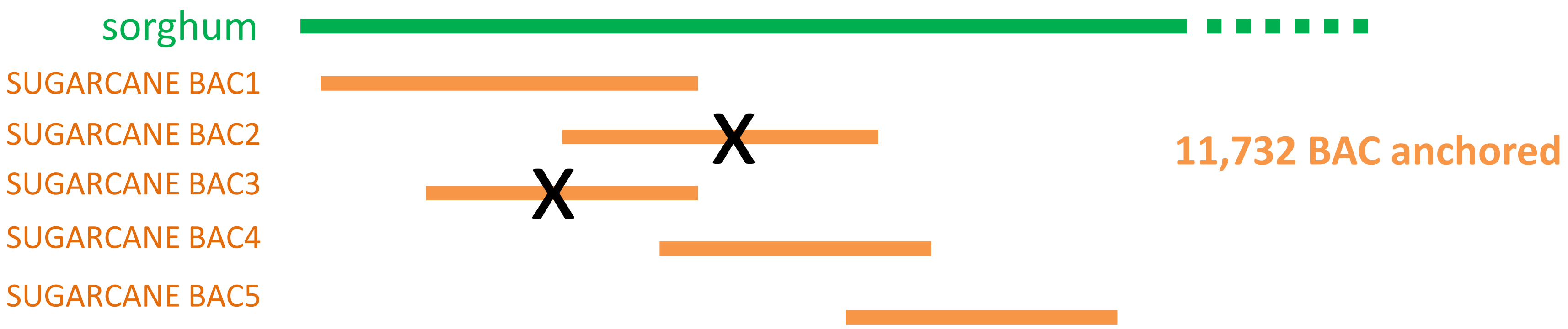
→ **11,732 R570 sugarcane BACs anchored onto the sorghum**

Coverage of the sorghum genome by sugarcane BAC sequences



Selection of a Minimum Tiling Path (MTP) of sugarcane BACs

= minimum set of BACs to be sequenced to obtain the best coverage of chromosomes



11,732 BAC anchored => Minimun Tiling Path 4,660 sugarcane BACs to be sequenced

Represent the number of BAC to be sequenced to cover the gene-rich part of a basic/monoploid sugarcane genome

Sequencing a Minimum Tiling Path (MTP) of R570 BAC

Aim = High quality sequence and BAC assembled in 1 contig

BAC sequenced using PacBio RSII technology and 100X depth coverage

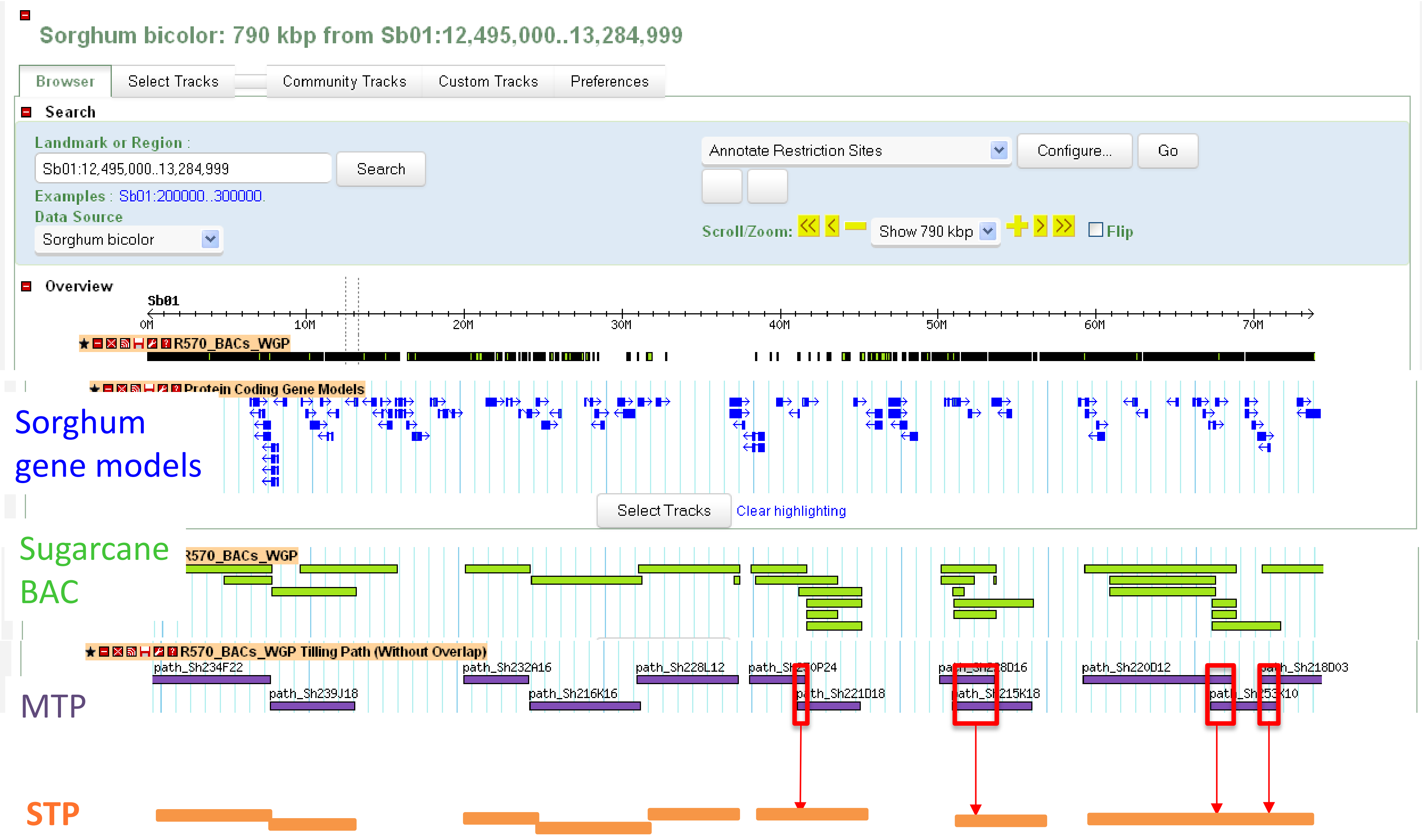
Single Molecule Real Time sequencing (SMRT) → produce long reads (several kb)
high 100X sequencing → High quality sequence

BAC sequenced through international collaboration

→ 4535 BAC sequenced
→ 97.5% of BAC assembled in <= 3 contigs

→ 531 Mb of high quality sequences
(0.02% of N)

Assembly of Single tiling path (STP) of sugarcane BACs



Sorghum bicolor: 790 kbp from Sb01:12,495,000..13,284,999

Landmark or Region : Sb01:12,495,000..13,284,999

Examples : Sb01:200000..300000.

Data Source: Sorghum bicolor

Annotate Restriction Sites

Scroll/Zoom: Show 790 kbp ☐ Flip

From MTP = 531 Mb → Assembly STP = 382 Mb

Global genome comparison with sorghum

→ Construction of a R570 SNP genetic map

Map constructed with 186 individuals from the self progeny of R570 genotyped with **DArTseq**

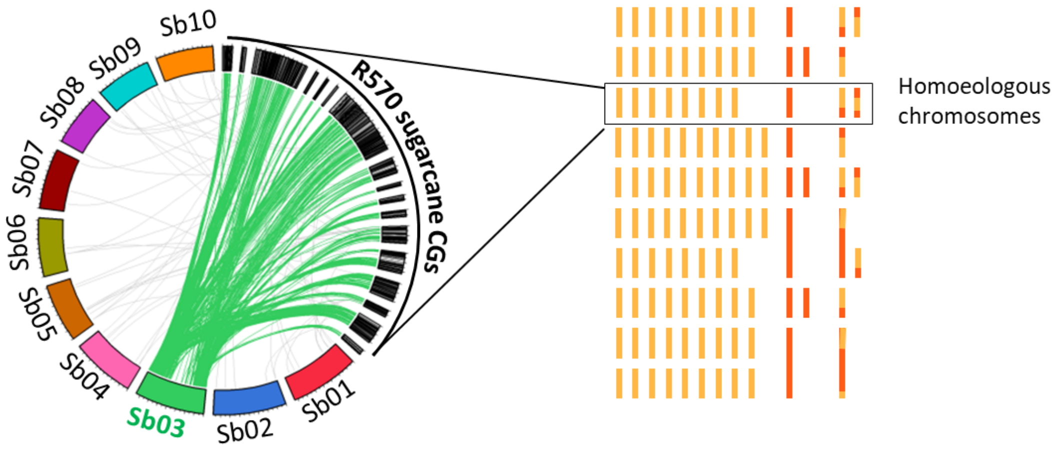
Data analyzed with in-house pipeline : **Process_reseq and vcf2pop**
Available at https://github.com/SouthGreenPlatform/VcfHunter/

→ 12,468 Single dose SNPs assembled in 132 Cosegregation Groups (CG)

Markers aligned onto the sorghum genome

→ 95 % of the CGs with most markers aligning on one sorghum chromosome

→ Allow assembling CGs in sugarcane Homoeology Groups (HG)

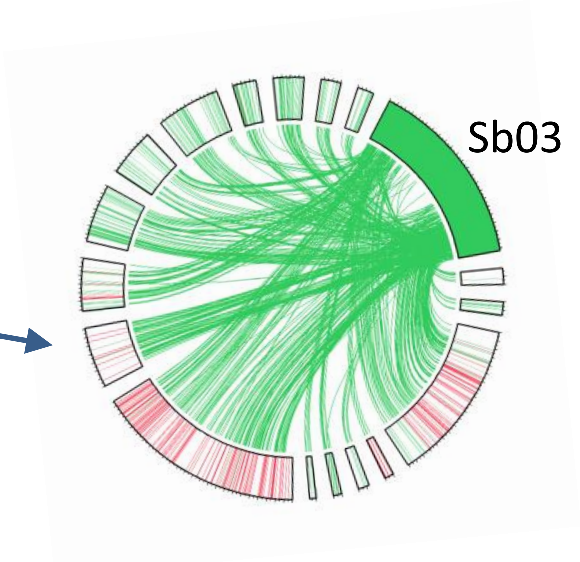


origin of R570 cultivar CGs

DArTseq Genotyping of a panel of 34 Saccharum accessions

-12 *S. officinarum*
- 9 S. robustum
- 13 S. spontaneum

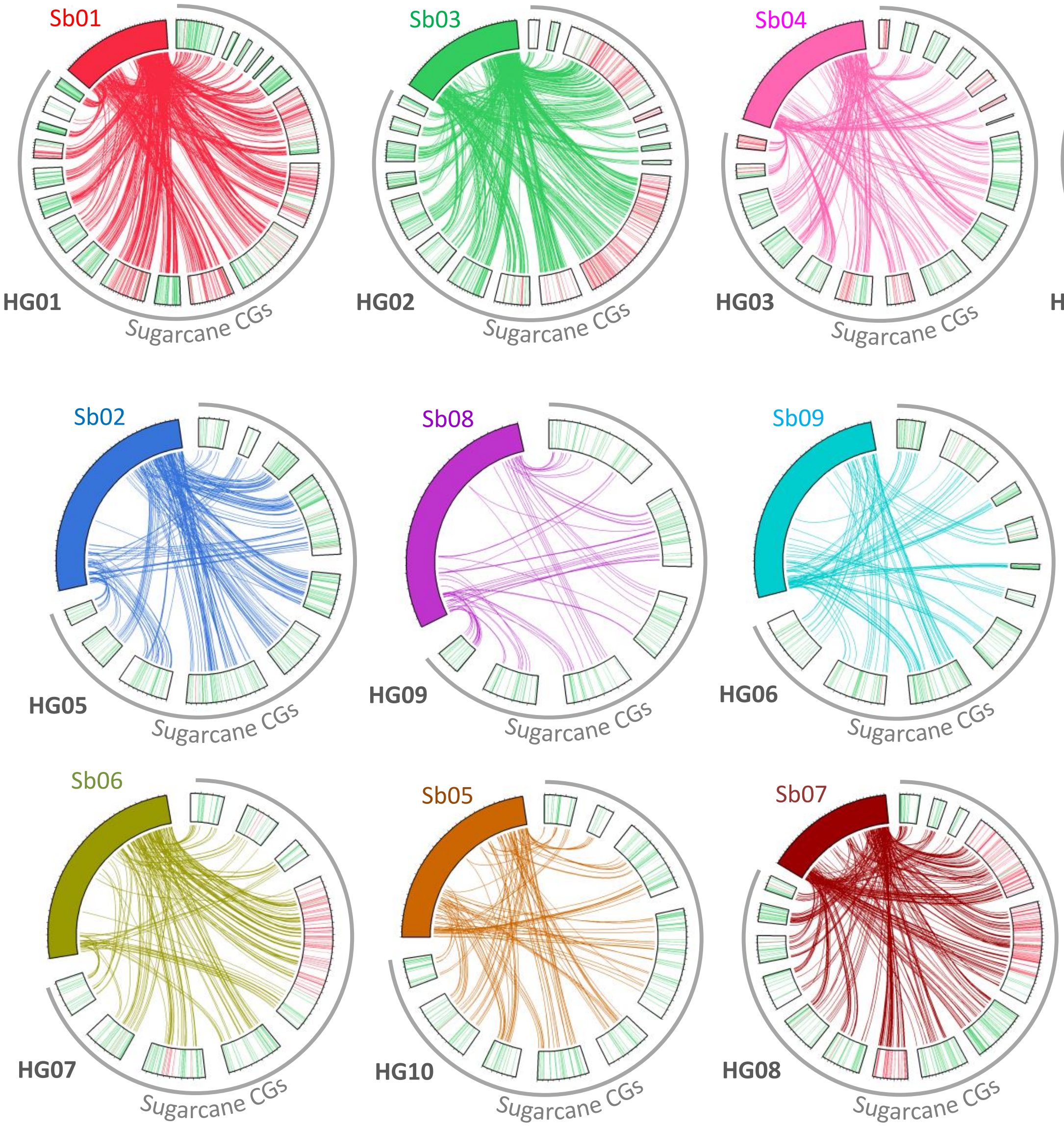
→ Identification diagnostic SNP



S. officinarum/robustum VS S. spontaneum

Global genome comparison with sorghum

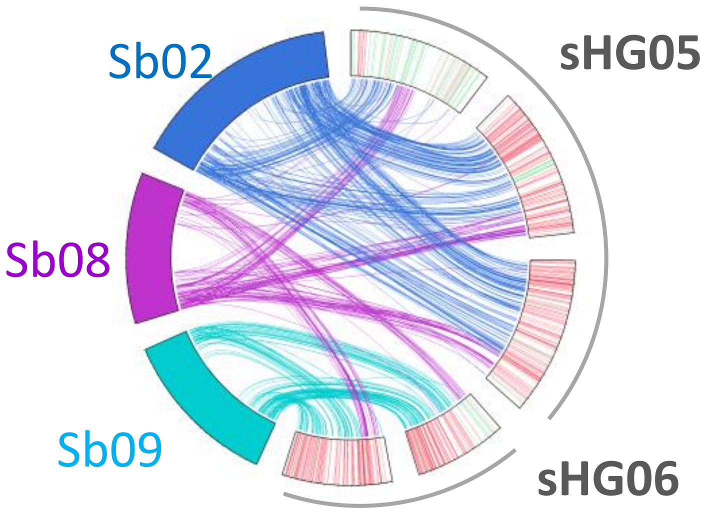
→ **125 CGs assembled in 10 homoeology groups aligning on one sorghum chromosome**

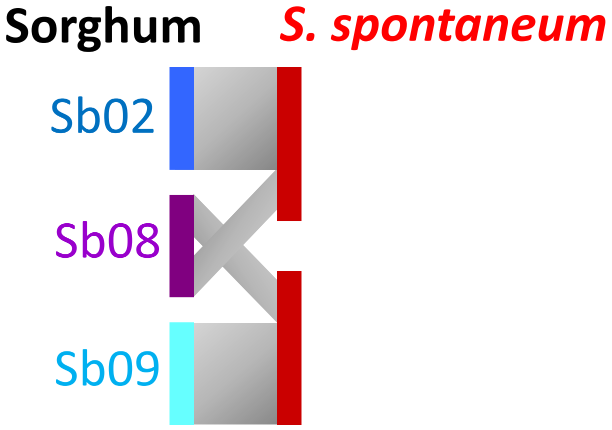


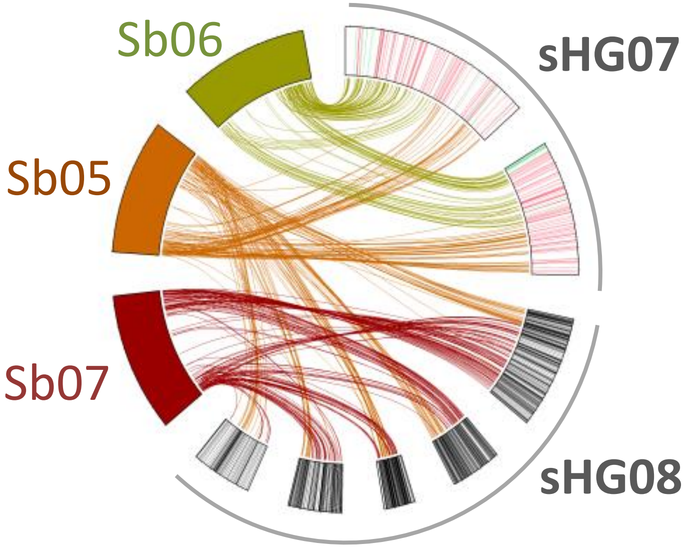
S. spontaneum
S. officinarum

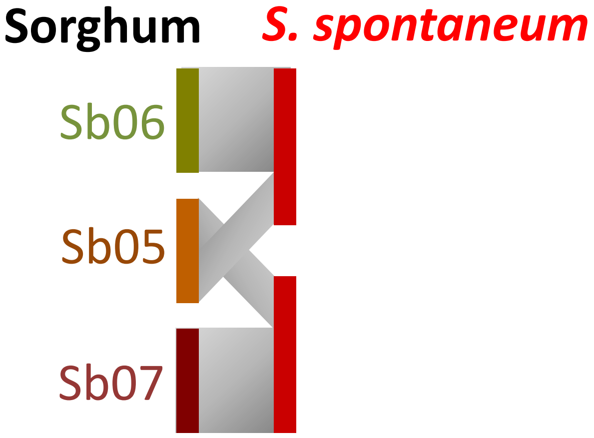
Global genome comparison with sorghum

→ **For a few CGs most markers aligning on two sorghum chromosomes**

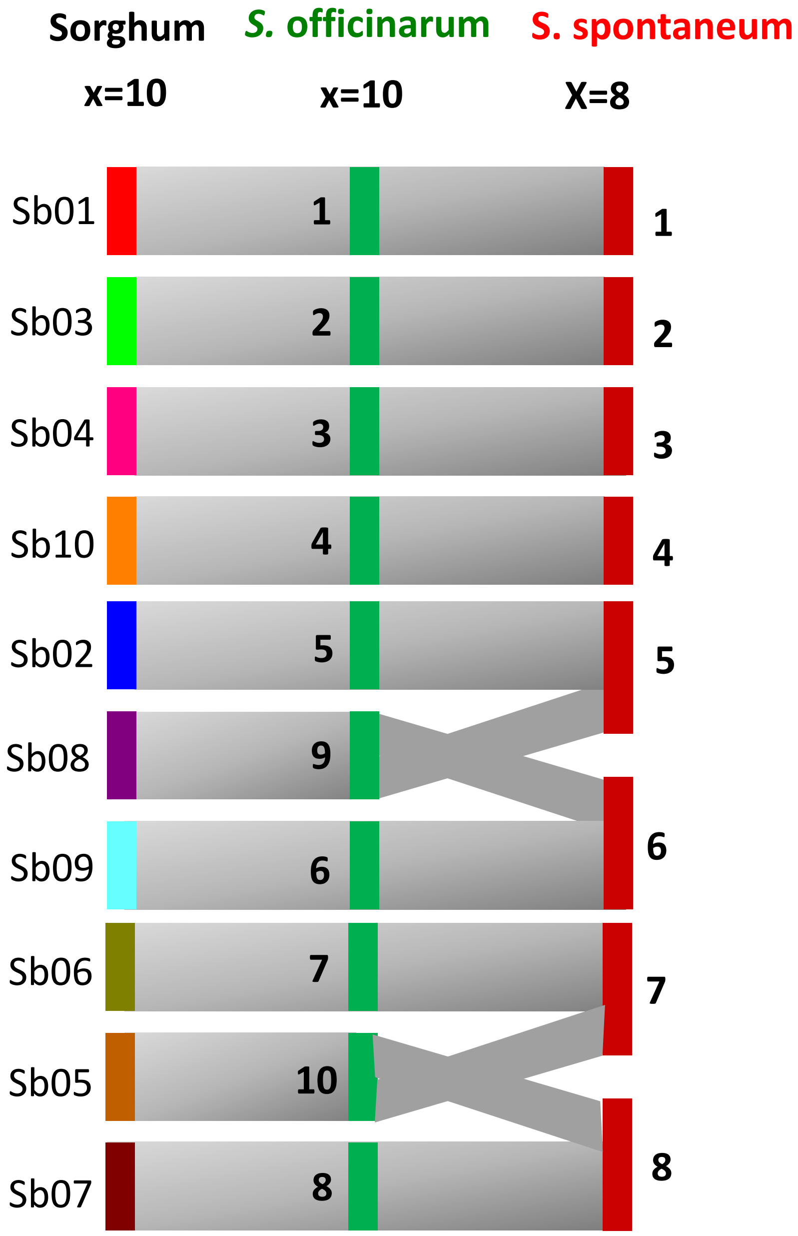








Global Sorghum and Sugarcane genomes comparison



Conclusions

☑ A high quality sequences of 382 Mb representing the gene rich fraction of the sugarcane monoploid genome

☑ 25,316 protein-coding gene models within their genomic sequence context providing access to regulatory elements for functional studies

Serve as high quality framework to help assemble the whole genome of sugarcane

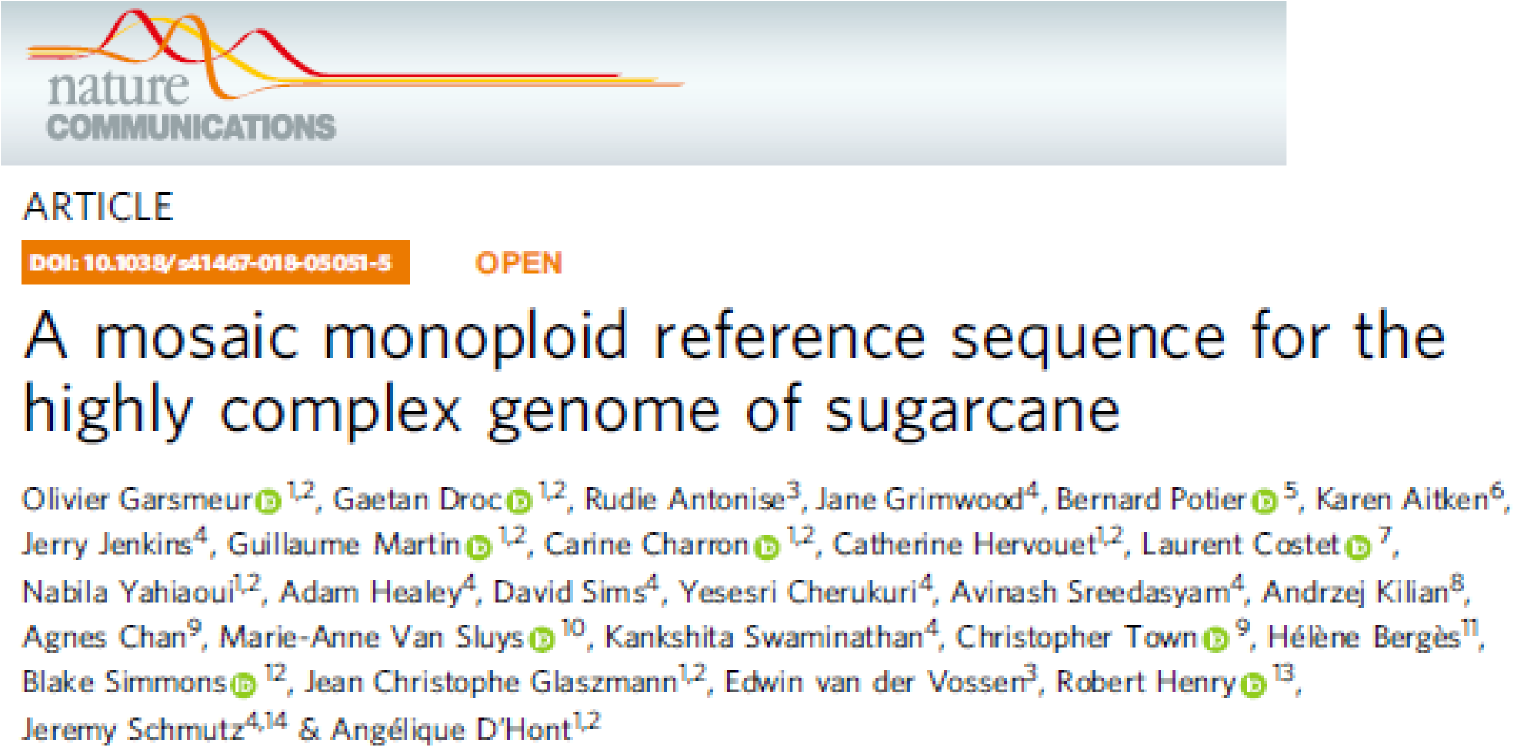
→ Project in progress in progress with JGI

☑ Confirm global colinarity between sugarcane and sorghum

☑ A few chromosome rearrangements between *Sorghum/S. officinarum and S. spontaneum*

→ These rearrangements corroborate and explain a variation of basic chromosome numbers in *S. officinarum* vs *S. spontaneum* with x= 10 and x=8, respectively

→ x=10 is recognized to be ancestral in the Saccharinae-Sorghinae, these rearrangements thus must have arisen in the *S. spontaneum* lineage after its divergence from the lineage of *S. officinarum*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http://sugarcane-genome.cirad.fr

Welcome to the Sugarcane Genome Hub

Genomic resources for sugarcane

An integrated web-based database providing centralized access to the sugarcane reference genome sequences and genomic resources.

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