

HIGH-RESOLUTION RECOMBINATION TO DISSECT THE G. longicalyx SEGMENT THAT CONFERS RESISTANCE TO RENIFORM NEMATODES

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Collaborations (so far):

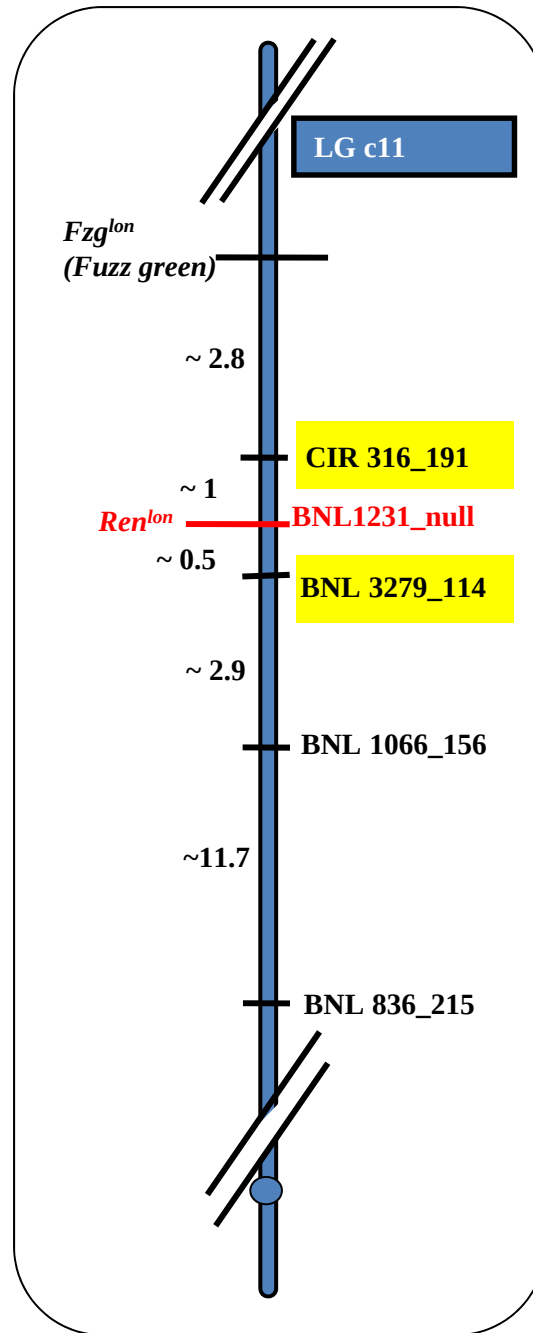
- Van Deynze and Ashrafi: seq'g, assemblies, informatics
- Ulloa & Roberts: BACs and BAC seqs
- Yu: RIL map and access to RILs
- ...

Slide 1

DS1

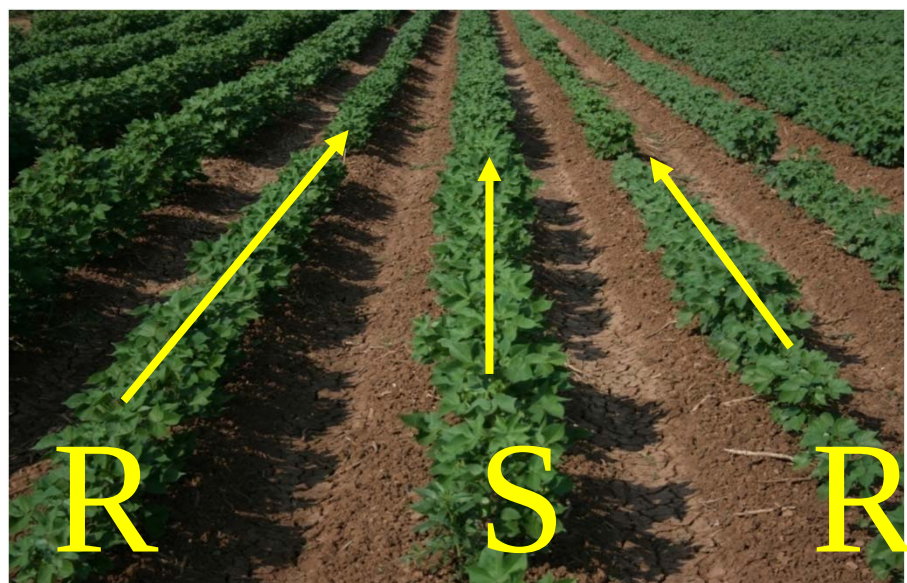
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Testing of LONREN in diverse environments quickly revealed susceptibility to field-specific “Stunting”

- Paired resistant (R) & susceptible (S) isolines in diverse fields revealed all R lines were susceptible to field-specific stunting.



Focus

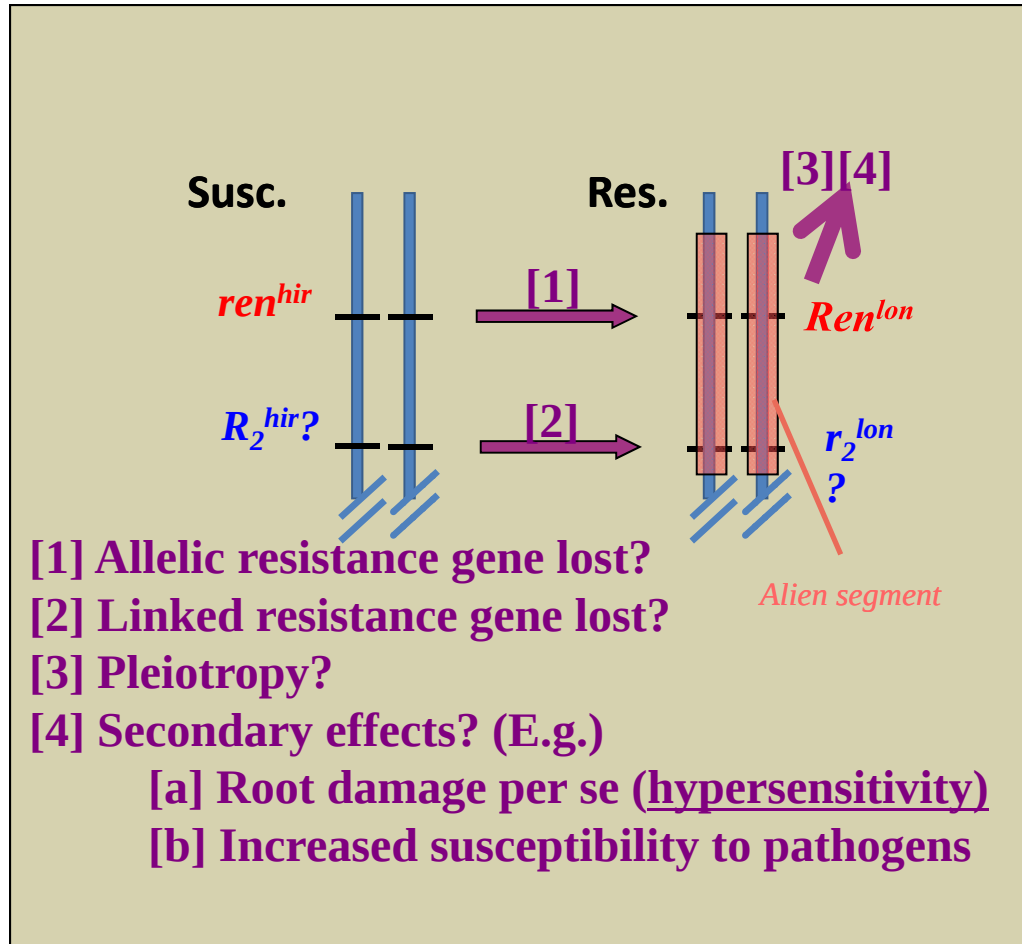
- Understand the “stunting”
- Determine if there is a genetic solution that we can exploit to make *Ren^{lon}* easier to use
- Create genomic and germplasm resources that will facilitate genetic manipulation of disease resistance genes involving
 - *G. longicalyx* chromosome segments in two locations
 - **c11 (A-genome) --- FOCUS FOR TODAY**
 - c21 (D-genome)
 - *G. barbadense* chromosome segments
 - c18
 - c21

Slide 5

DS2

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Hypotheses for Stunting



Since many genes important to resistances are known to be clustered. It is most likely the undesirable effects are due to linkage drag. In either case, high resolution recombination offers the simplest approach to investigate the cause for stunting.

A high resolution map is needed for this region.

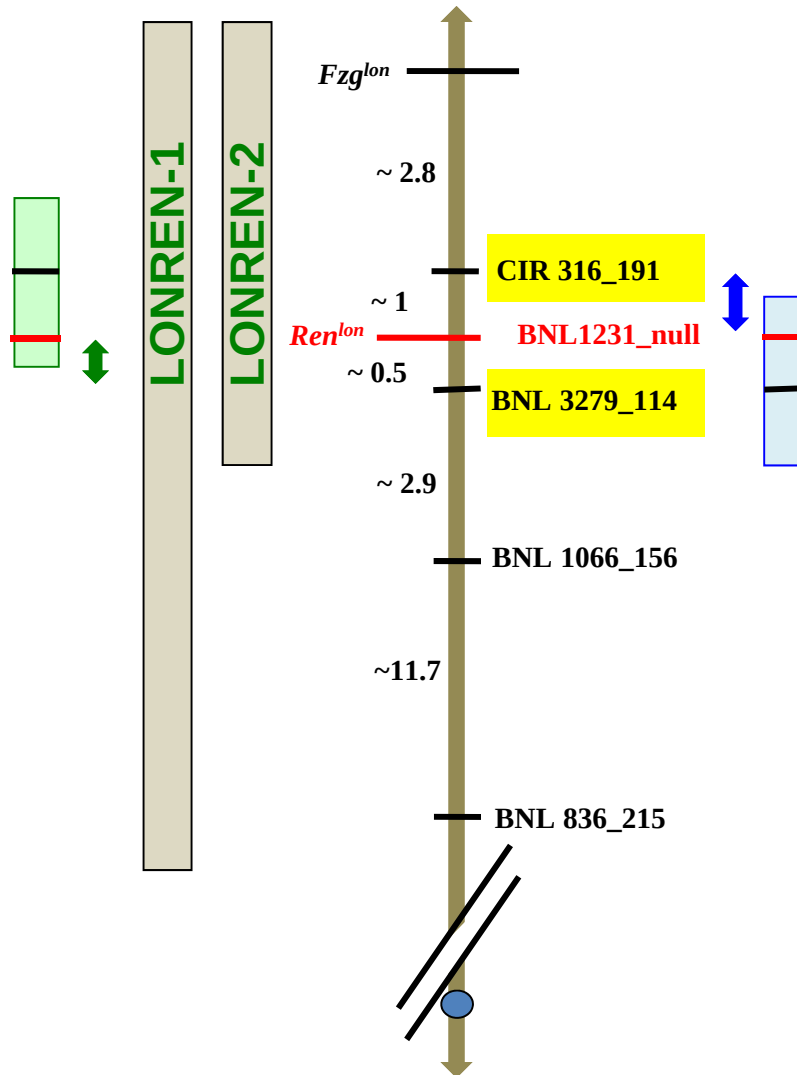
Main Objectives

- Highly resolve the *Ren^{lon}* and surrounding regions by high-resolution recombination and linkage mapping. (Extend to *Ren₂^{bar}*, *Ren₃^{bar}*(?), *Ren₄^{bar}*): *need recombinants, markers, and co-analysis of recombinants and markers*
- Determine if debilitating effects correlated to date with resistance are separable by recombination, i.e., due to linkage drag: if so, derive improved LONREN types with no or reduced stunting
- Obtain recombinants involving the two sources of resistance genes for phenotypic analysis (LONREN, BARBREN)

Preliminary Accumulation of Nearby Recombinants

3 Proximal Recombinants:

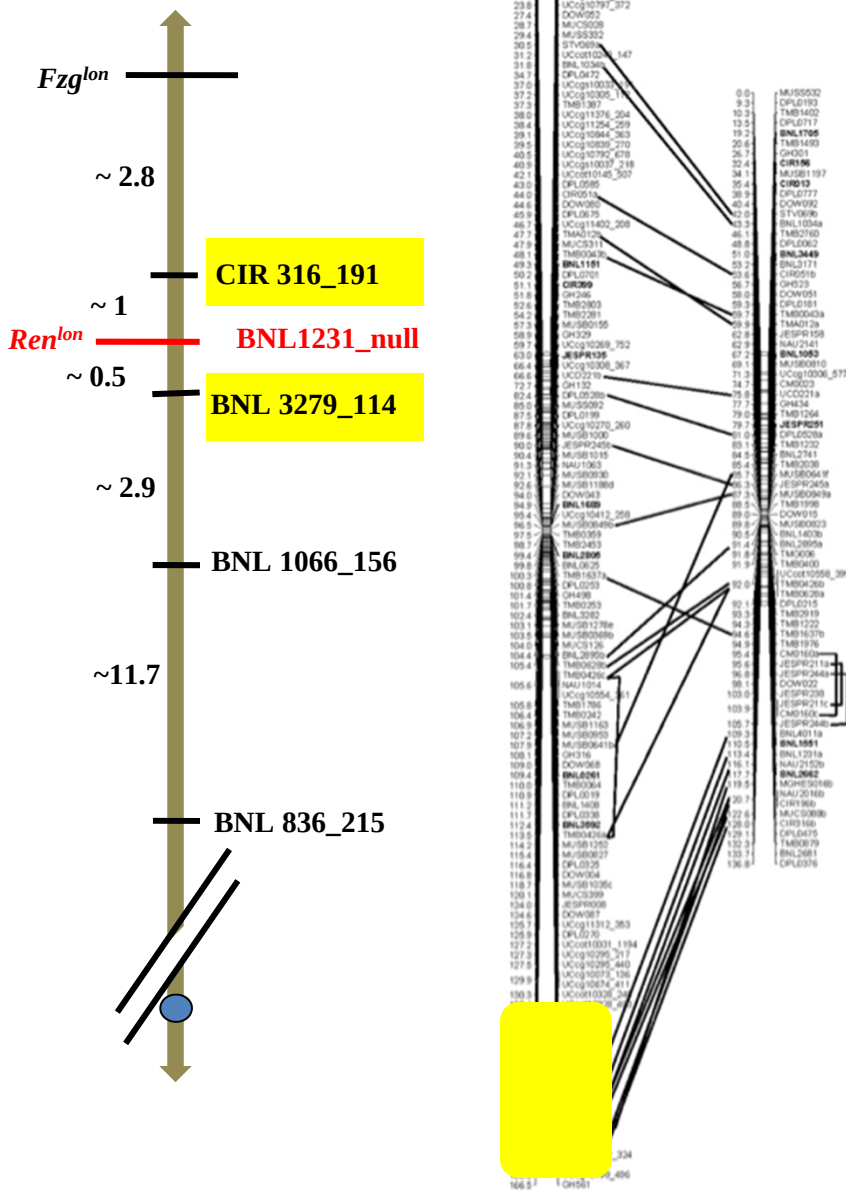
- 7117,
- 7118
- 7119



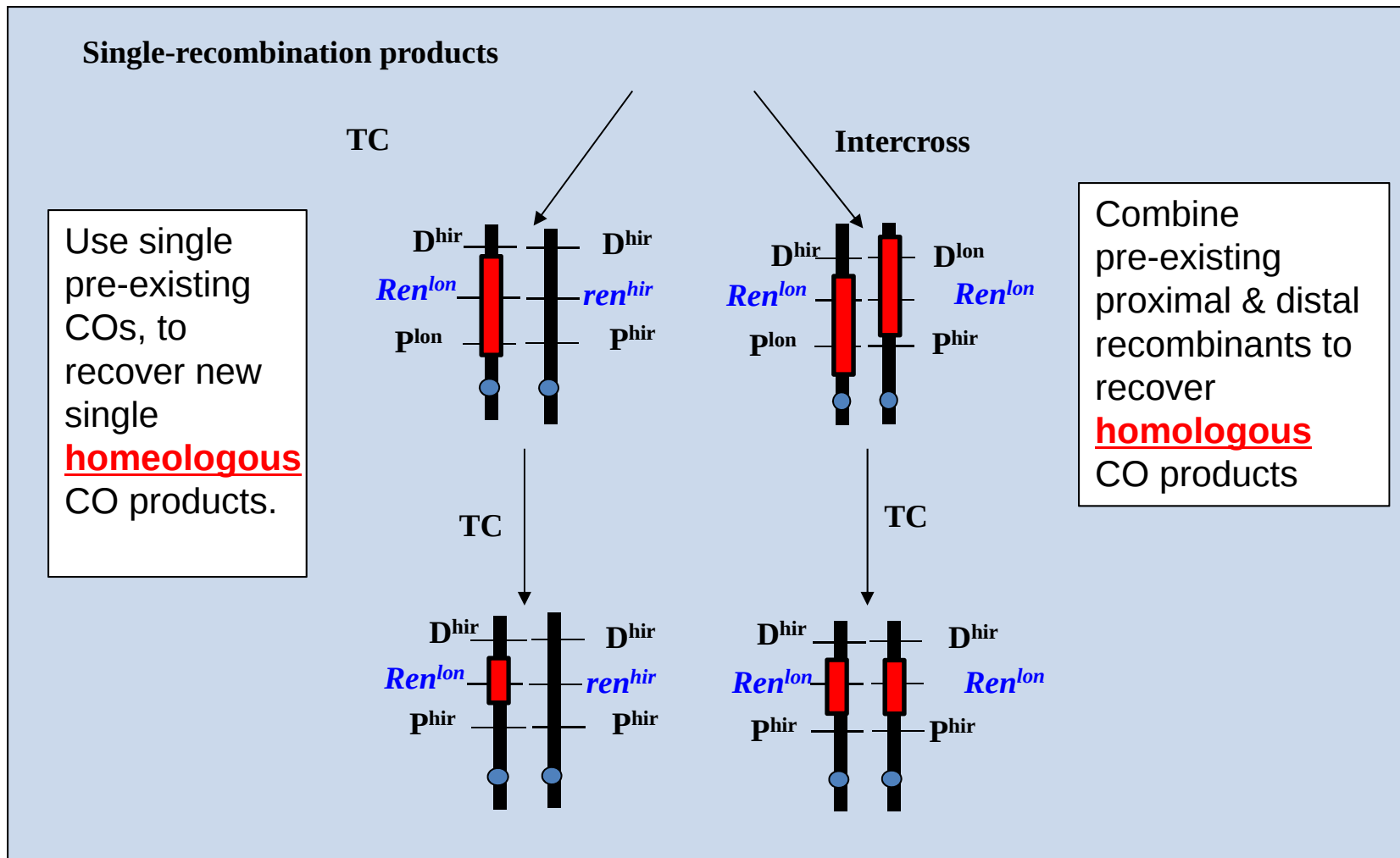
16 Distal Recombinants:

- 7109, 7110, 7111,
- 7112, 7113, 7114,
- 7115, 7116, 7121,
- 7122, 7123, 7124,
- 7125, 7126, 7127,
- 7128

Region of intense
interest.



Strategies for Segment Reduction via Genetic Recombination



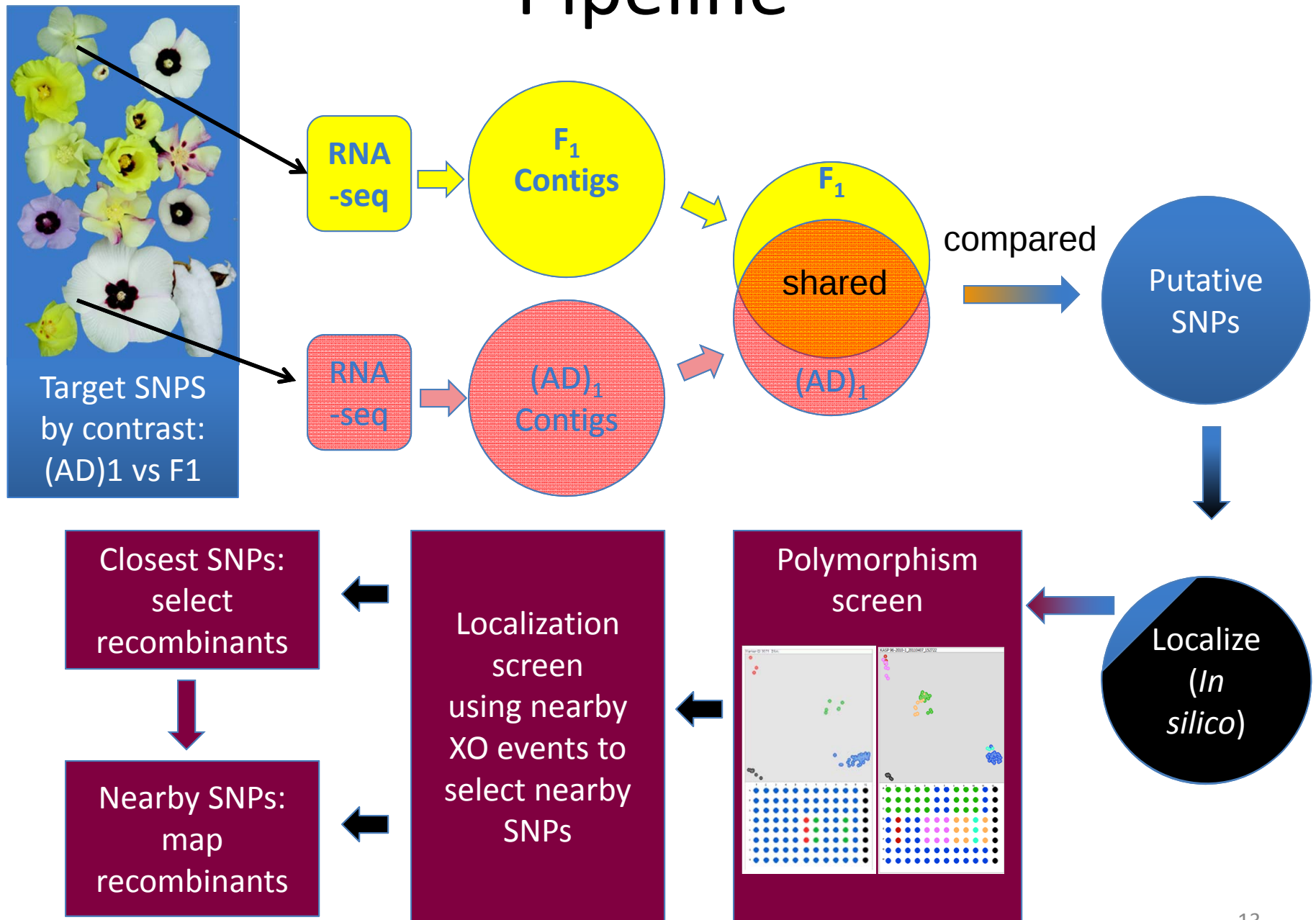
TC Seed to Reduce the Alien Segment

- New CO Events : >20,000 seed
 - Proximal
 - Distal
- Recombine previously identified proximal and distal events: >3000 TC seed

Marker Development

- SSRs – harvesting a few from the literature
- SNPs --- primary focus

“Pipeline”

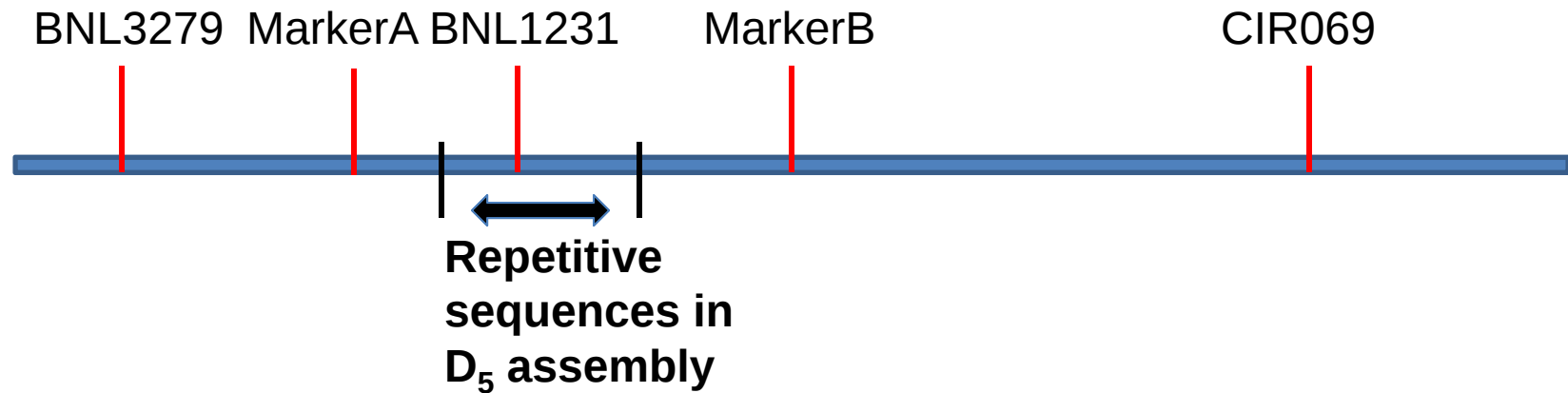


Identifying Gh-Gl SNPs in *Ren^{lon}* region

- ❖ RNA-seq: identified ~70,000 Gl-Gh SNPs
- ❖ ~90% convertible to assays
- ❖ Identified subset in regions of interest using the *G.raimondii* D5 genome sequence assembly (<http://www.phytozome.net/cotton.php>)

Gh-GI SNPs localization

Scaffold_7

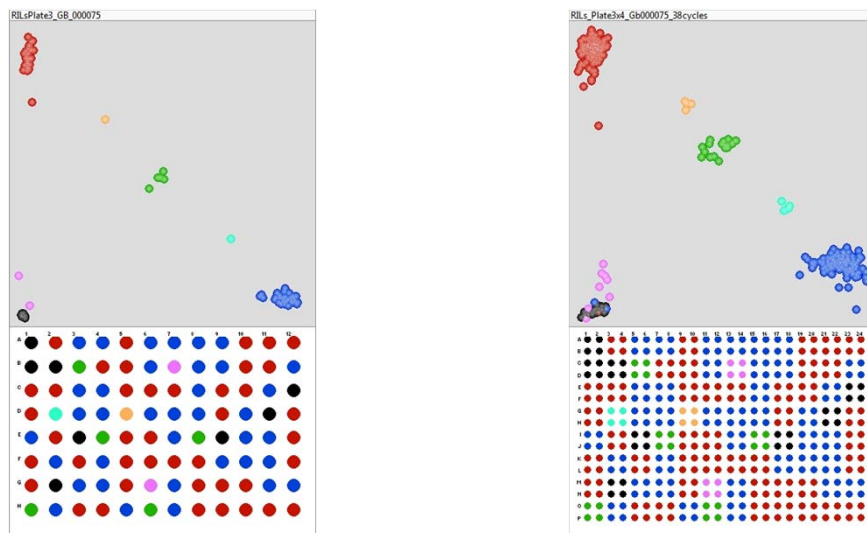


- BNL3279-CIR069 ~ 4Mbps
- MarkerA-MarkerB~500Kbps

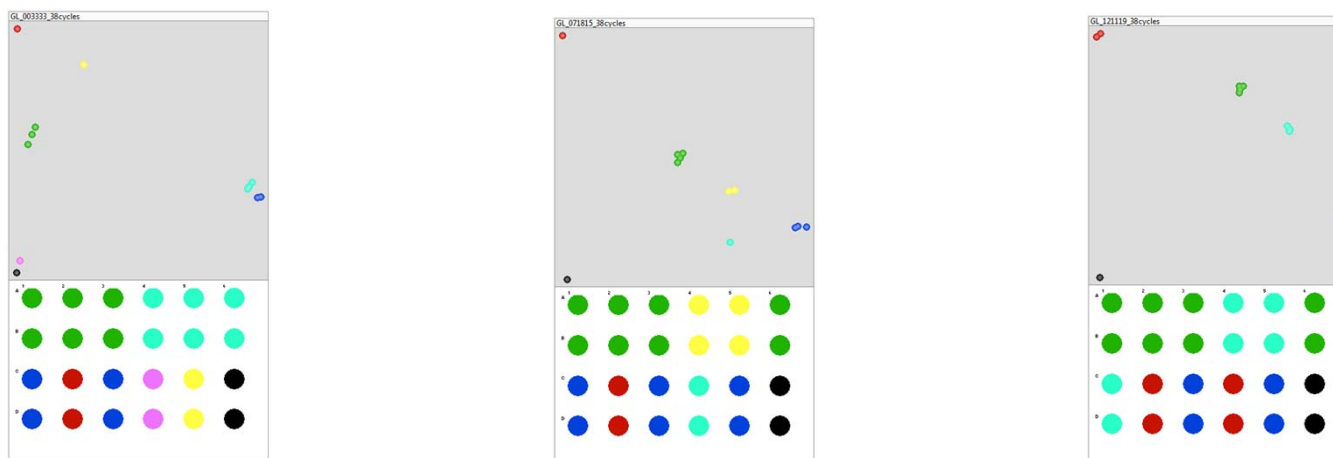
SNPs in Critical Region

- Localized 240 SNPs in BNL3279-CIR069 (4 Mb/Scaffold-7)
 - Class-I: 100
 - Class-II: 100
 - Class-III: 40
- There are 42 SNPs in MarkerA-MarkerB (500 kb/Scaffold-7)
 - Class-I: 21
 - Class-: 18
 - Class-III: 3
- ~70 SNPs of ~200 Class-I/II SNPs BNL3279-CIR069 region tested by assay

Assay Basis – Kbiosciences 96/384/(1536)



SSR/SNP-Screening 11-member Panel



Next Steps

- 2012 Summer:
 - Expand the number of SNPs in region of interest; test them
 - Large-scale screening of seed to identify recombinants of interest
- 2012 Fall: grow, retest markers and increase stocks of recombinants for phenotypic testing
- 2013: phenotypic testing

Related Activities to Mention

- *Ren*^{lon} in c21 (i.e., in homeolog)
- BARBREN resistance locus (loci) in c21 versus *Ren*^{lon}