DS1

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

X Zheng, J Quintana, DM Stelly, AA Bell and RL Nichols

Collaborations (so far):
- Van Deynze and Ashrafi: seq'g, assemblies, informatics
- Ulloa & Roberts: BACs and BAC seqs
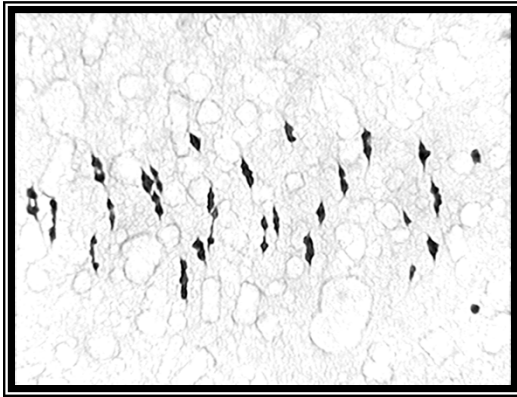- Yu: RIL map and access to RILs
- …

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DS1 Add authors & instns

David Stelly, 3/29/2012

Prior Work

Bell, Robinson, Dighe, Stelly, Nichols

- Created tri-species hybrid

- Mapped gene – designated Ren^{lon}

- Developed information and markers that enable MAS

- Used MAS to expedite introgression and breeding of Ren^{lon}

- Created multiple lineages

- LONREN-1 and LONREN-2, to enable widespread testing and breeding.

Marker **BNL3279_114** Scan on 34 BC$_6$S$_1$ Plants
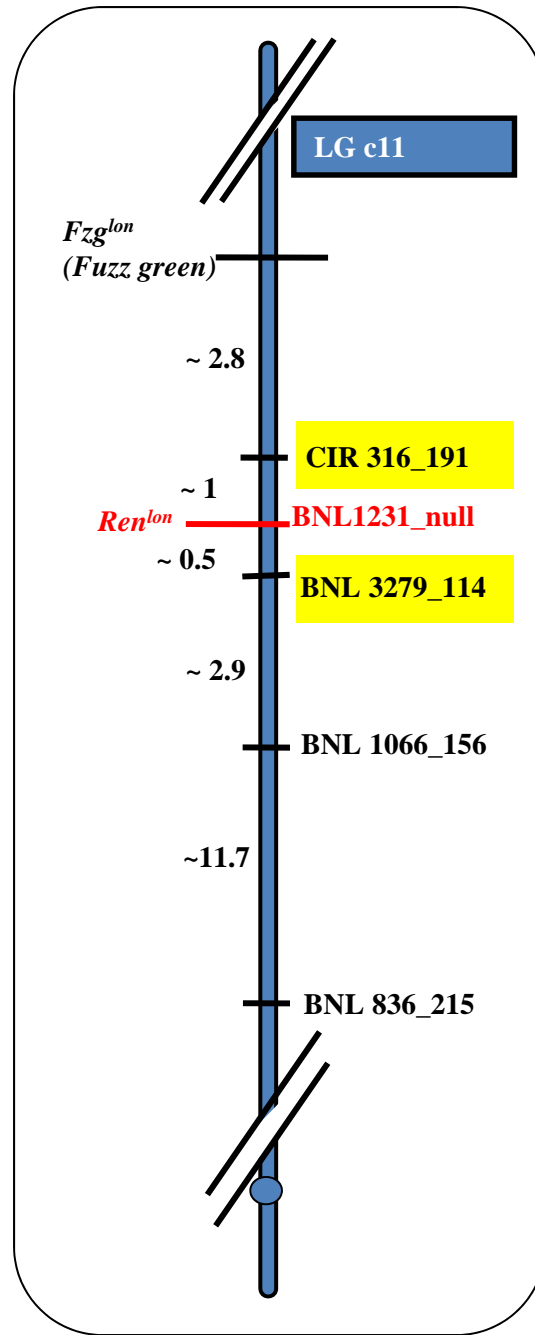
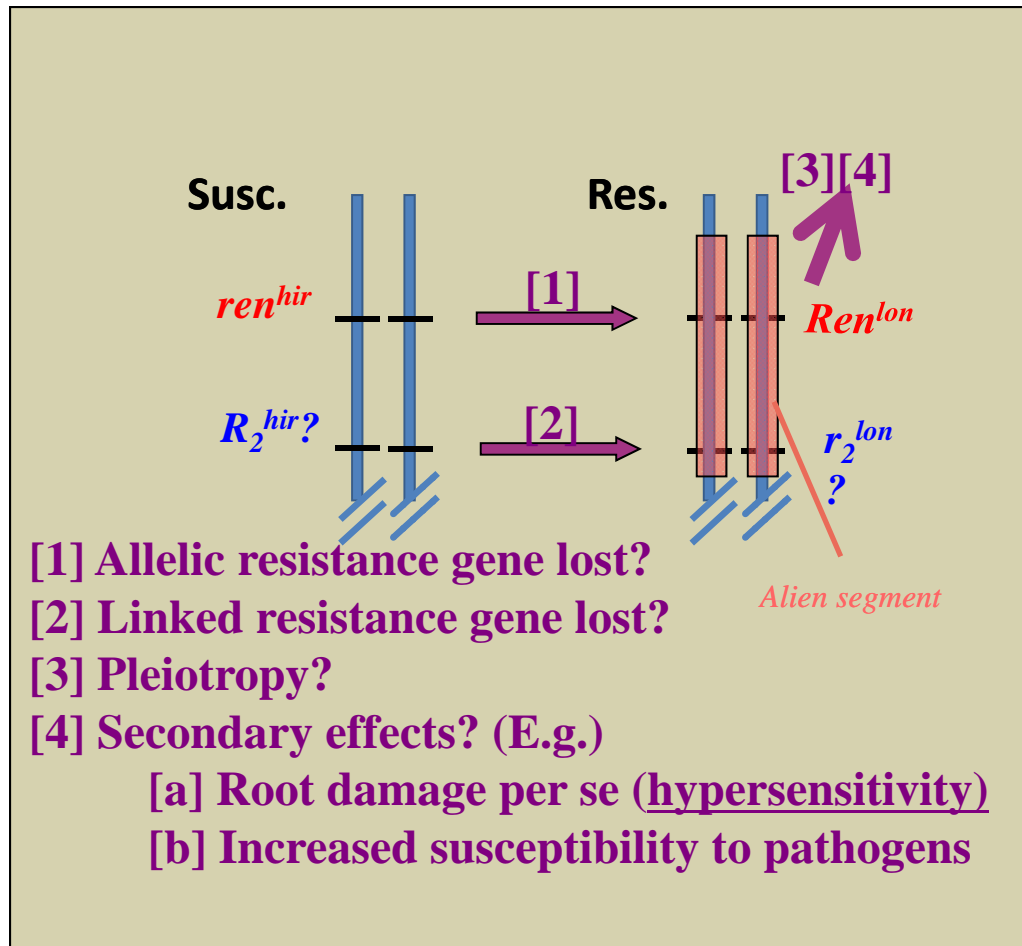# Testing of LONREN in diverse environments quickly revealed susceptibility to field-specific "Stunting"

- 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**

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DS2   David Stelly, 3/29/2012

Hypotheses for Stunting

Susc.   **Res.**   [3][4]

ren^{hir} [1] Ren^{lon}

R_2^{hir}?   [2] r_2^{lon}?

Alien segment

[1] Allelic resistance gene lost?
[2] Linked resistance gene lost?
[3] Pleiotropy?
[4] Secondary effects? (E.g.)
    [a] Root damage per se (<u>hypersensitivity</u>)
    [b] Increased susceptibility to pathogens

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.

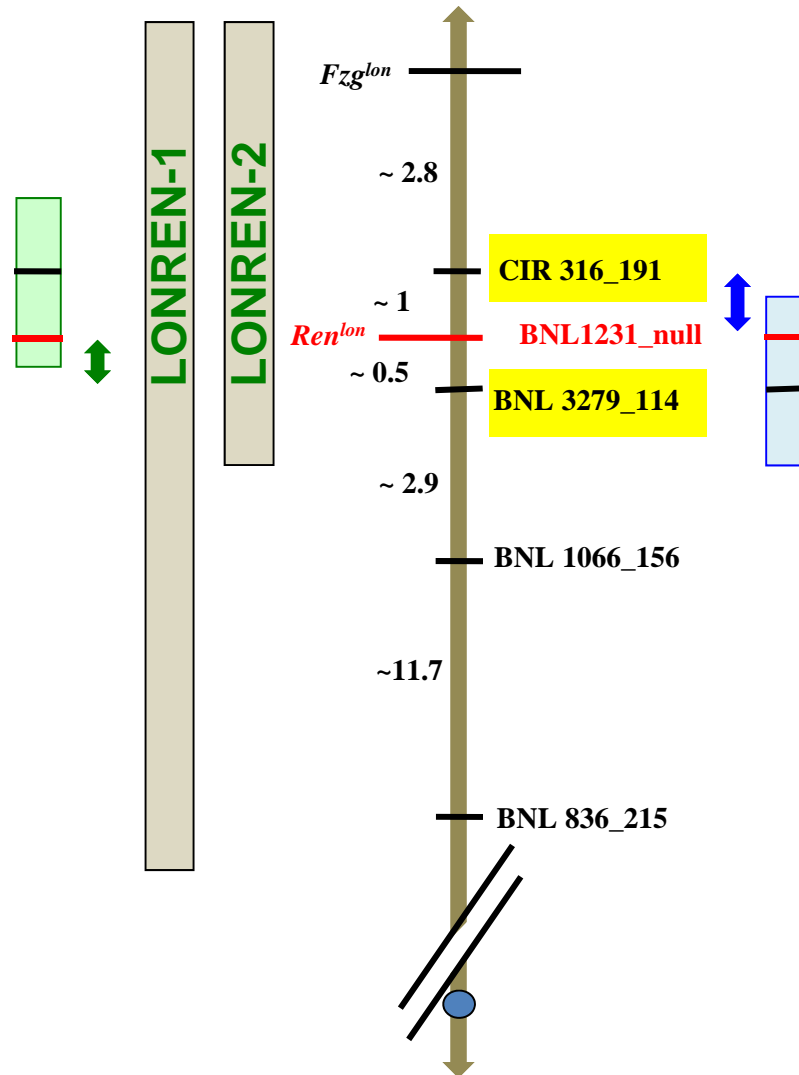A high resolution map is needed for this region.

Main Objectives

➢ **Highly resolve the *Ren^{lon}* and surrounding regions by <u>high-resolution recombination and linkage mapping</u>. (Extend to *Ren$_2$^{bar}*, *Ren$_3$^{bar}(?)*, *Ren$_4$^{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, <u>derive improved LONREN types with no or reduced stunting</u>**

➢ **Obtain recombinants involving the two sources of resistance genes for phenotypic analysis (LONREN, BARBREN)**

Region of intense interest.

Fzg^lon

~ 2.8

CIR 316_191

~ 1

Ren^lon   BNL1231_null

~ 0.5

BNL 3279_114

~ 2.9

BNL 1066_156

~11.7

BNL 836_215

Chr.11(A11)   Chr.21(D11)

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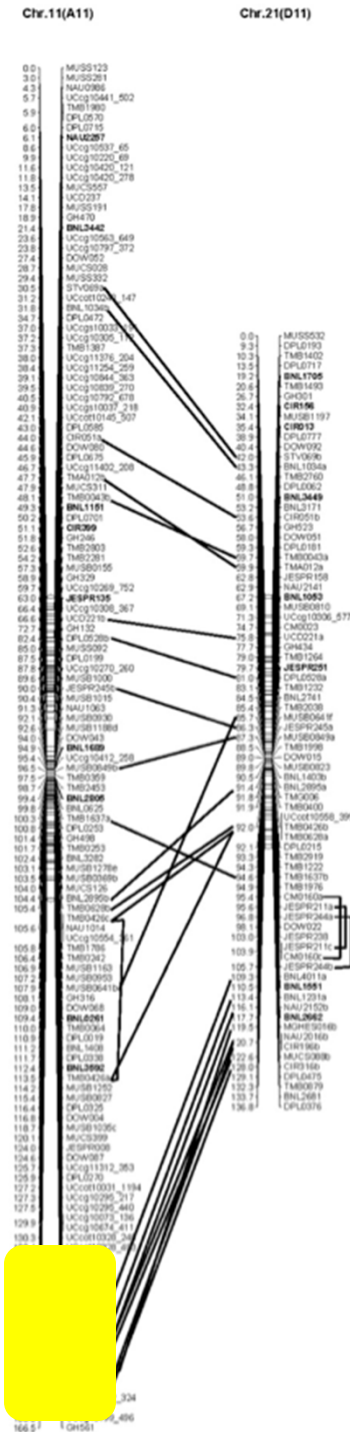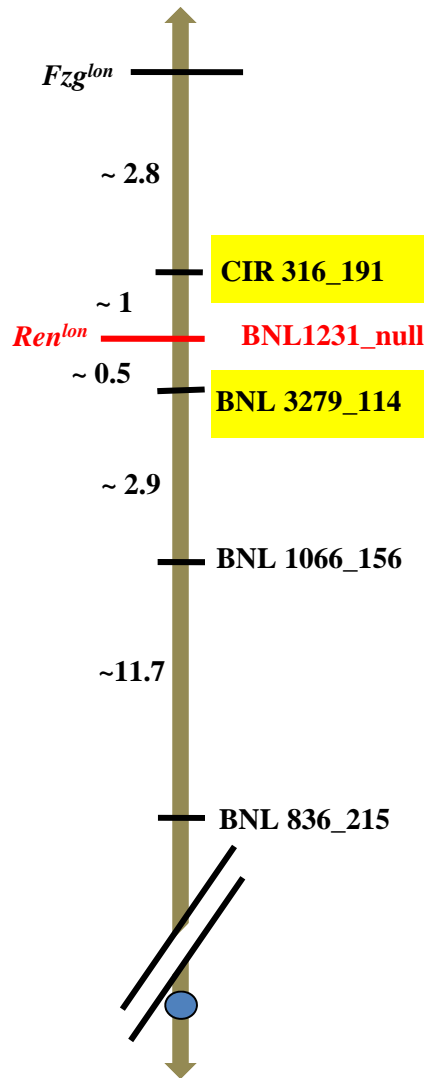
Strategies for Segment Reduction via Genetic Recombination

Single-recombination products

Use single pre-existing COs, to recover new single **homeologous** CO products.

Combine pre-existing proximal & distal recombinants to recover **homologous** CO products
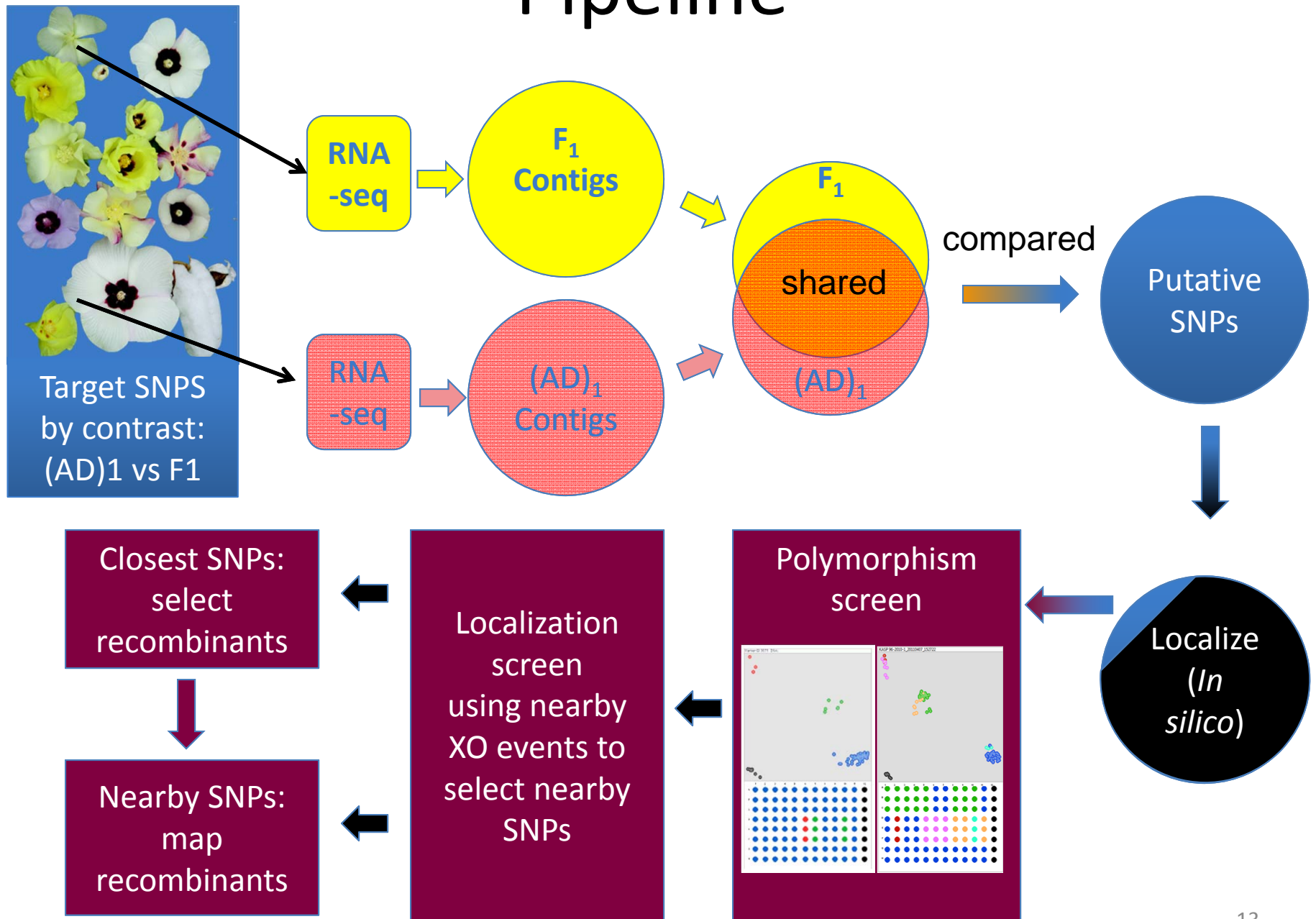
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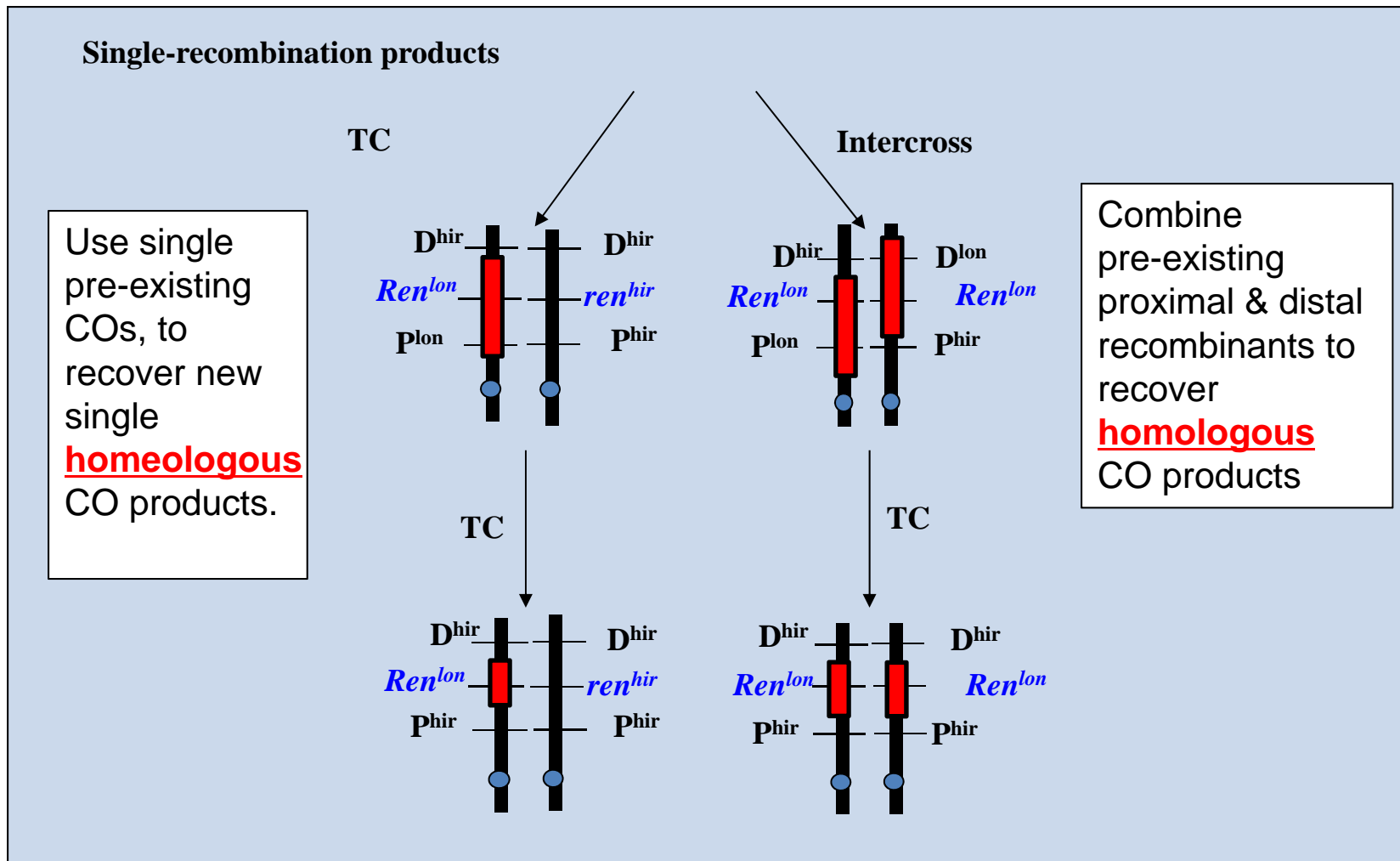
TC Seed to Reduce the Alien Segment

- 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"

Target SNPS by contrast: $(AD)1$ vs $F1$

RNA-seq → F_1 Contigs →

RNA-seq → $(AD)_1$ Contigs →

F_1 / shared / $(AD)_1$ → compared → Putative SNPs

Localize (*In silico*)

Polymorphism screen

Localization screen using nearby XO events to select nearby SNPs

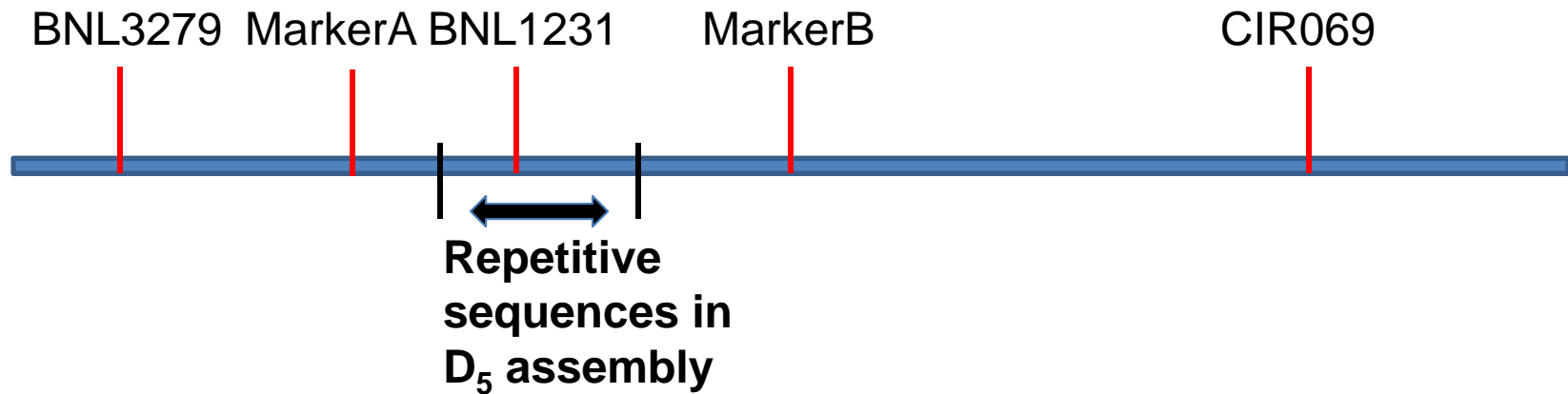Closest SNPs: select recombinants

Nearby SNPs: map recombinants

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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-Gl SNPs localization

Scaffold_7

BNL3279 MarkerA BNL1231 MarkerB CIR069

Repetitive sequences in D_5 assembly

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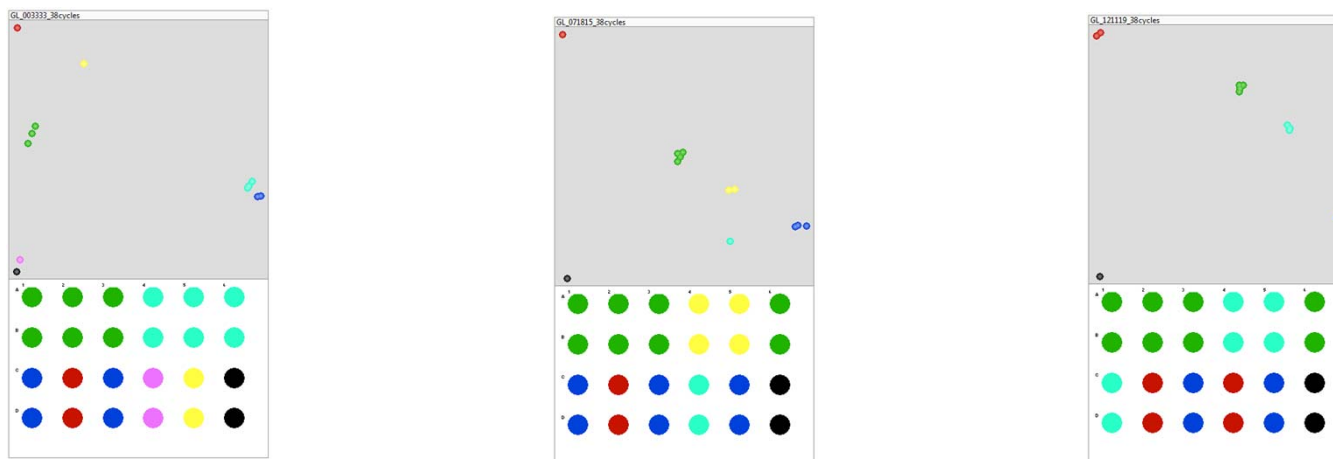- MarkerA-MarkerB~500Kbps

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)
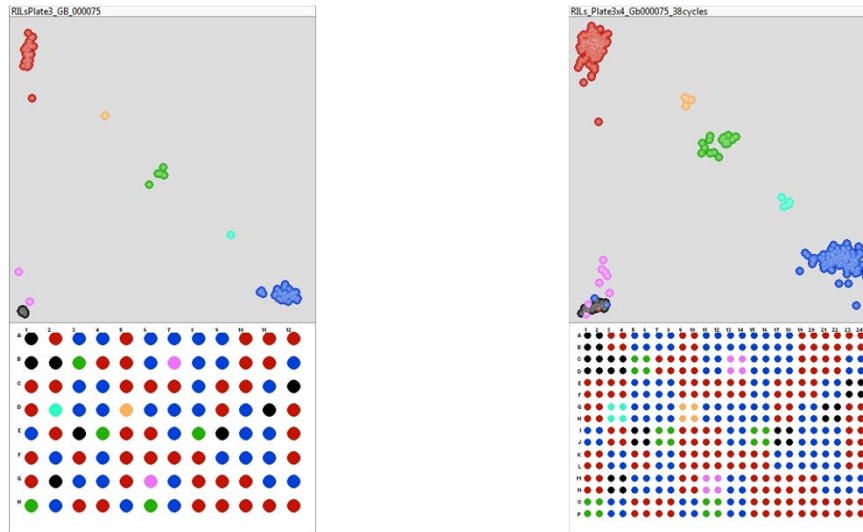
SSR/SNP-Screening 11-member Panel

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}*