Development of Genomic Tools for RKN Resistance Breeding in Cotton

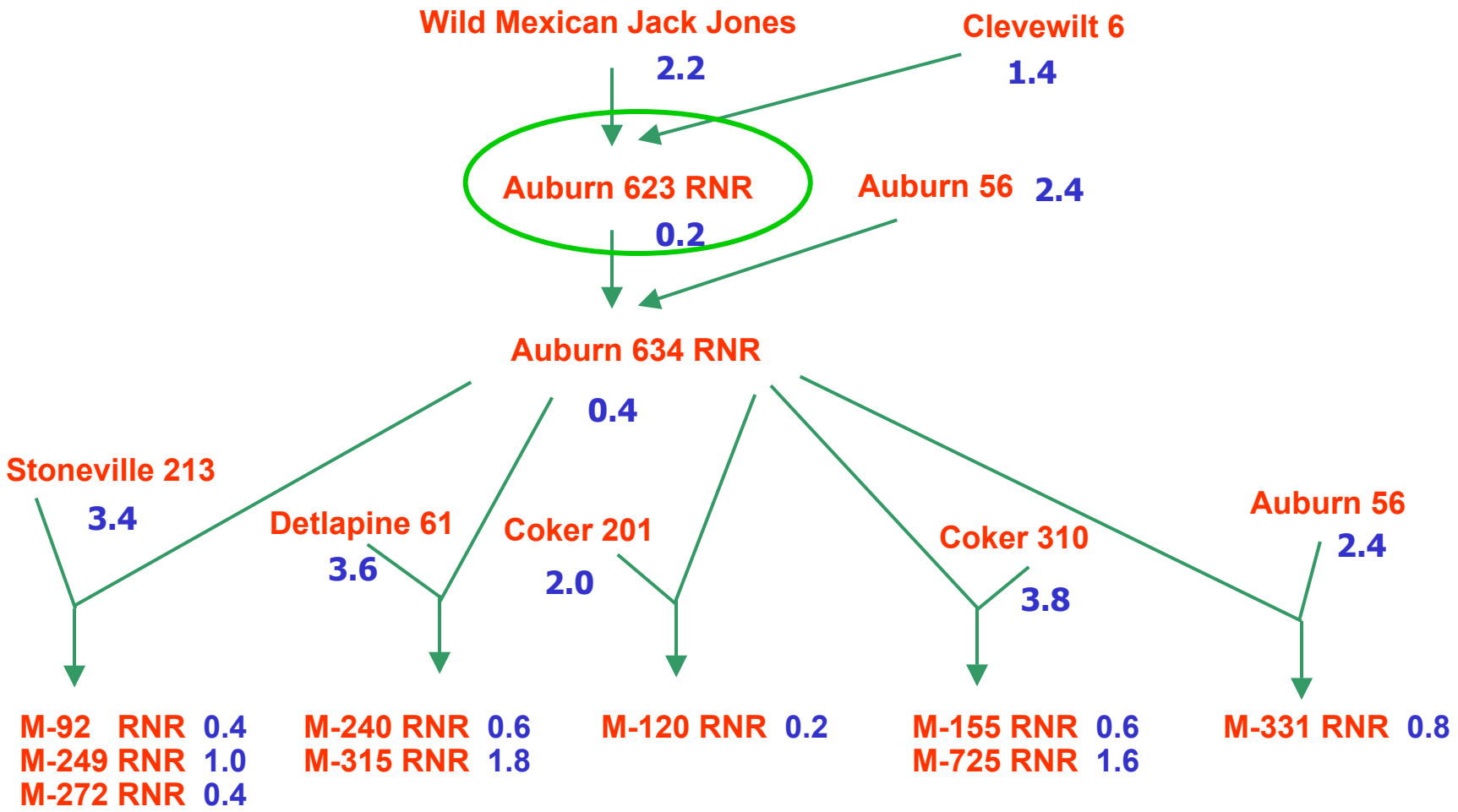
Dr. Hongbin Zhang

Department of Soil & Crop Sciences and
Institute for Plant Genomics & Biotechnology
Texas A&M University, College Station, Texas

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- **Root-knot Nematode Resistance**
- Mapping Populations
- PCR-based DNA Marker Development
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- Large-insert BAC libraries
- Integrated Physical and Genetic map construction
- Future Research Directions and Plans

Deltapine 16 Auburn 623



Pedigree of the cotton highly root-knot resistant (RNR) Auburn lines

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- Root-knot Nematode Resistance
- **Mapping Populations**
- PCR-based DNA Marker Development
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- Large-insert BAC libraries
- Integrated Physical and Genetic map construction
- Future Research Directions and Plans

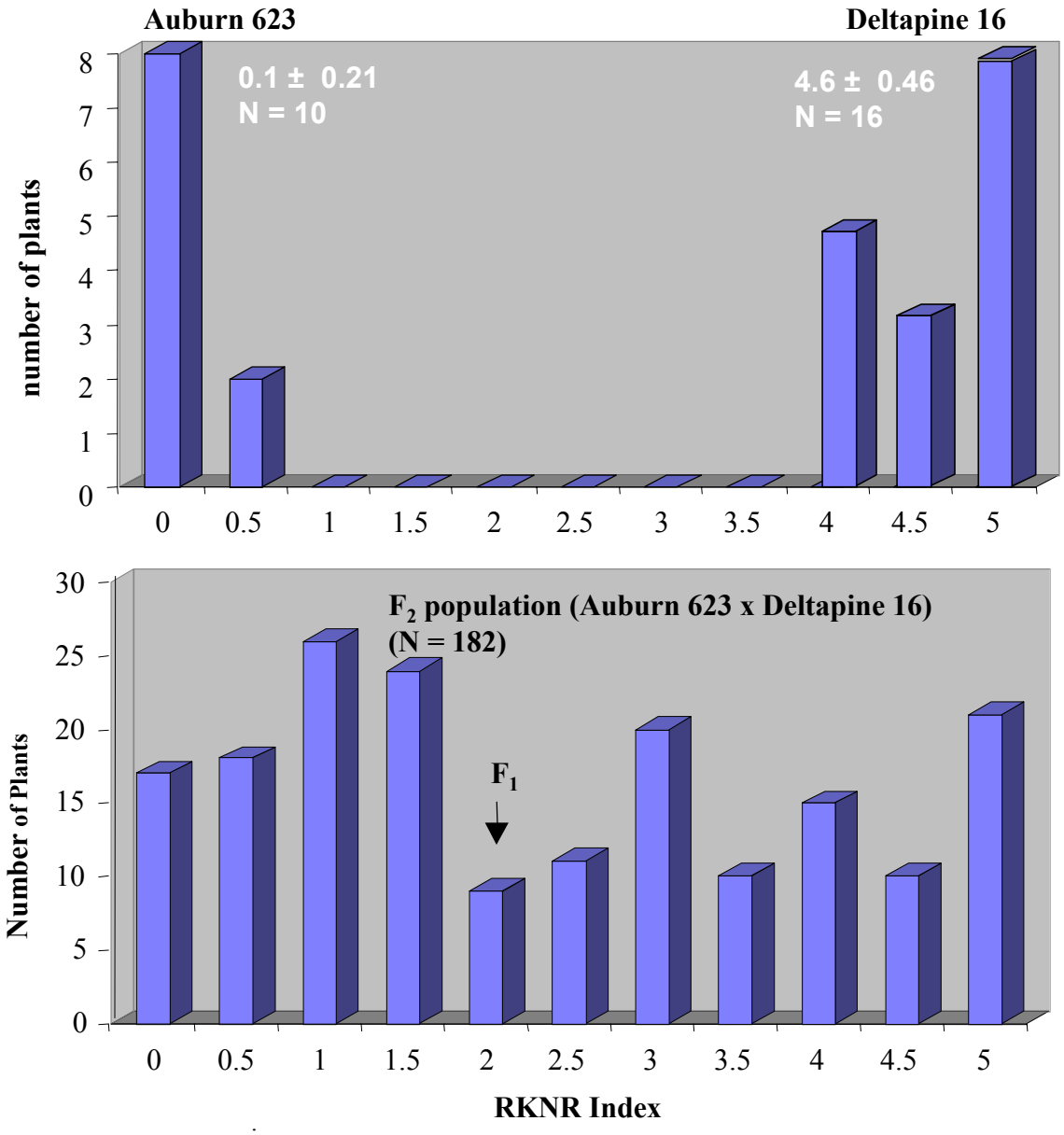
Mapping populations for RKN resistance

1. *G. hirsutum* x *G. hirsutum*

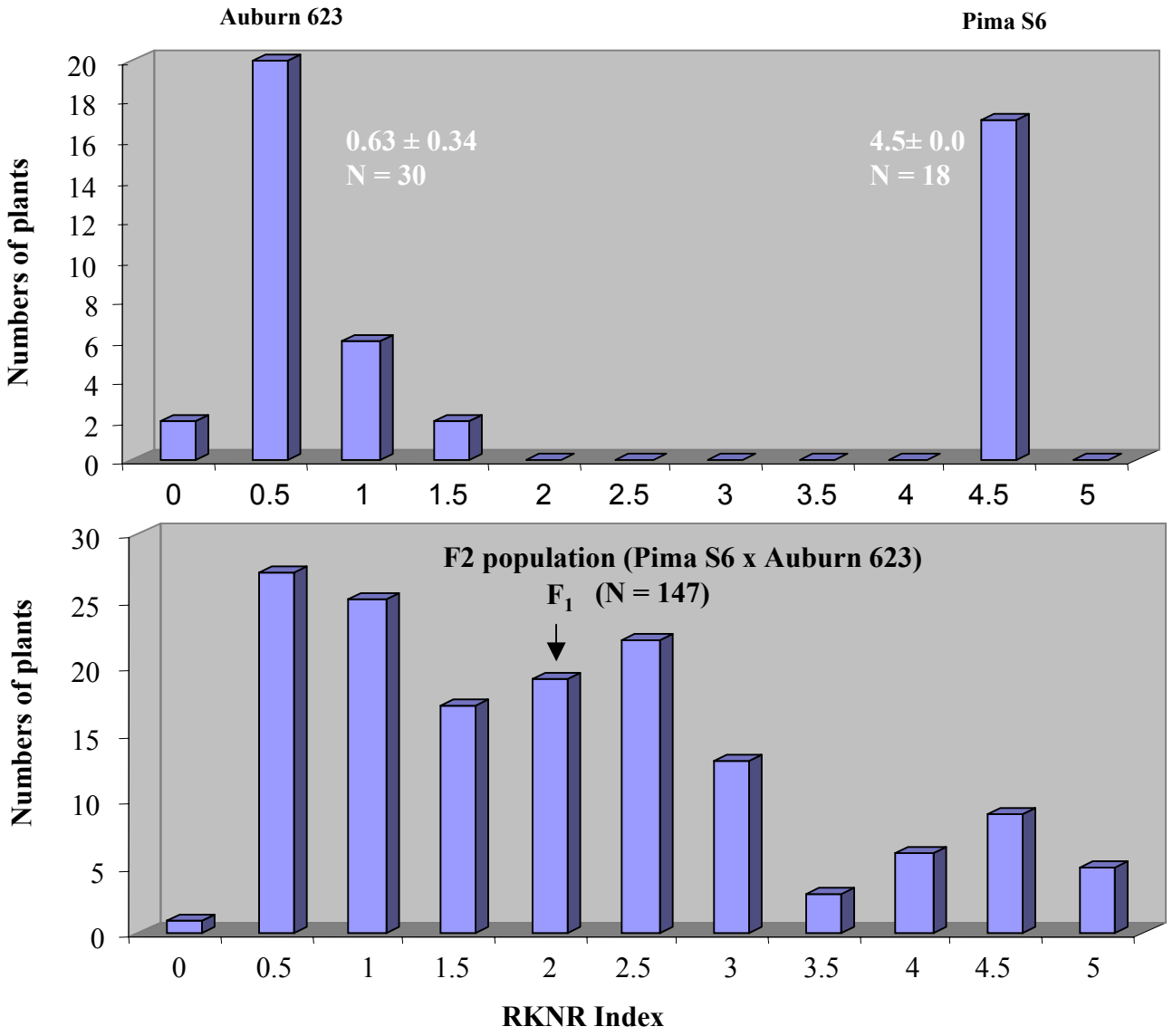
   Auburn 623 x Deltapine 16 and reciprocal cross: > 10,000 F_2
   Wild Mexican Jack Jones x Deltapine 16: > 5,000 F_2

2. *G. barbadense* x *G. hirsutum*

   Pima S6 x Auburn 623 and reciprocal cross: > 10,000 F_2
   Pima S6 x Auburn 623 RILs: 202 F_4 RILs



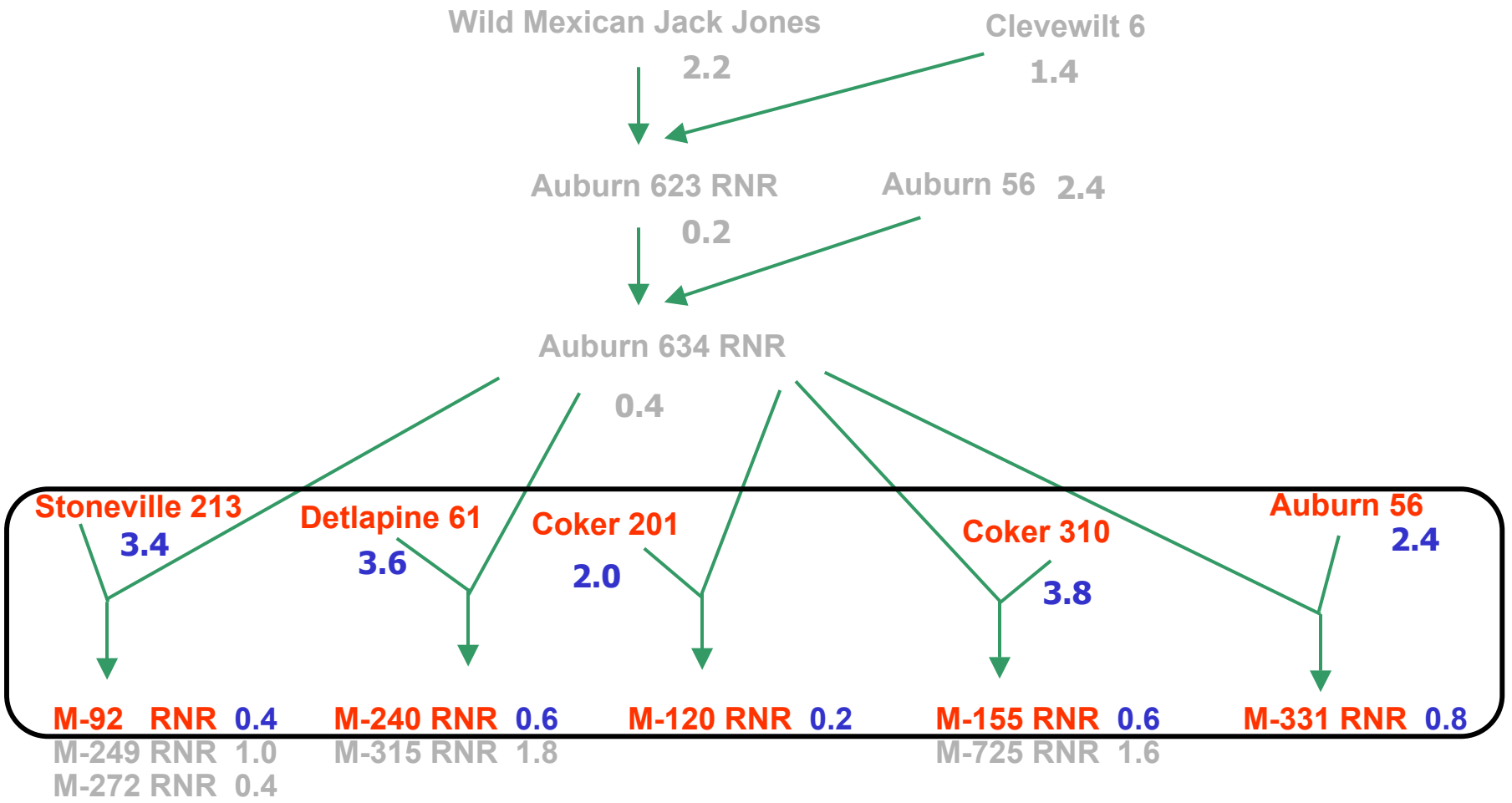
Segregation of the Auburn 623 x Deltapine 16 population



Segregation of the Pima S6 x Auburn 623 population

Development of Genomic Tools for RKN Resistance Breeding in Cotton

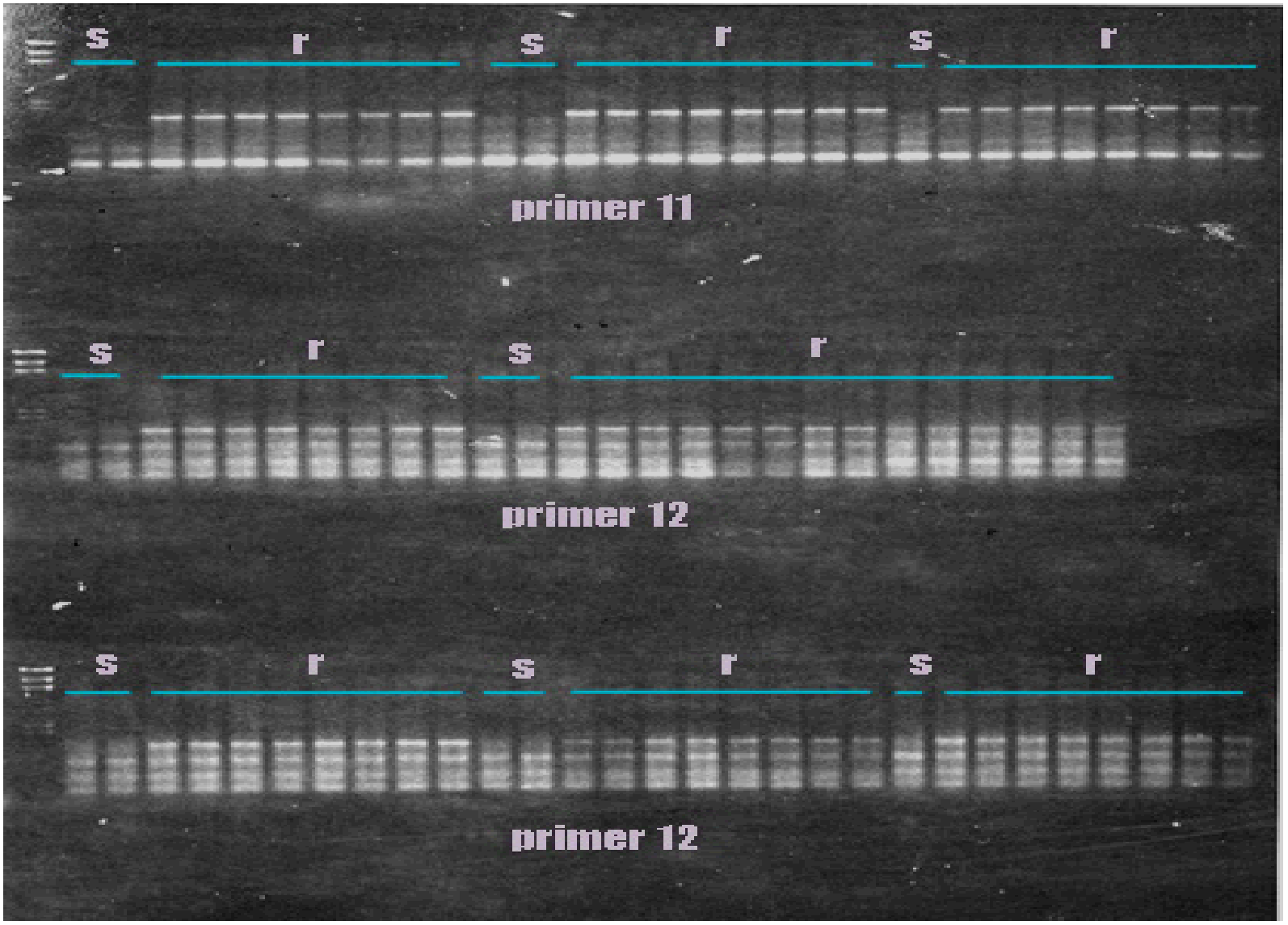
- Root-knot Nematode Resistance
- Mapping Populations
- **PCR-based DNA Marker Development**
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- Large-insert BAC libraries
- Integrated Physical and Genetic map construction
- Future Research Directions and Plans



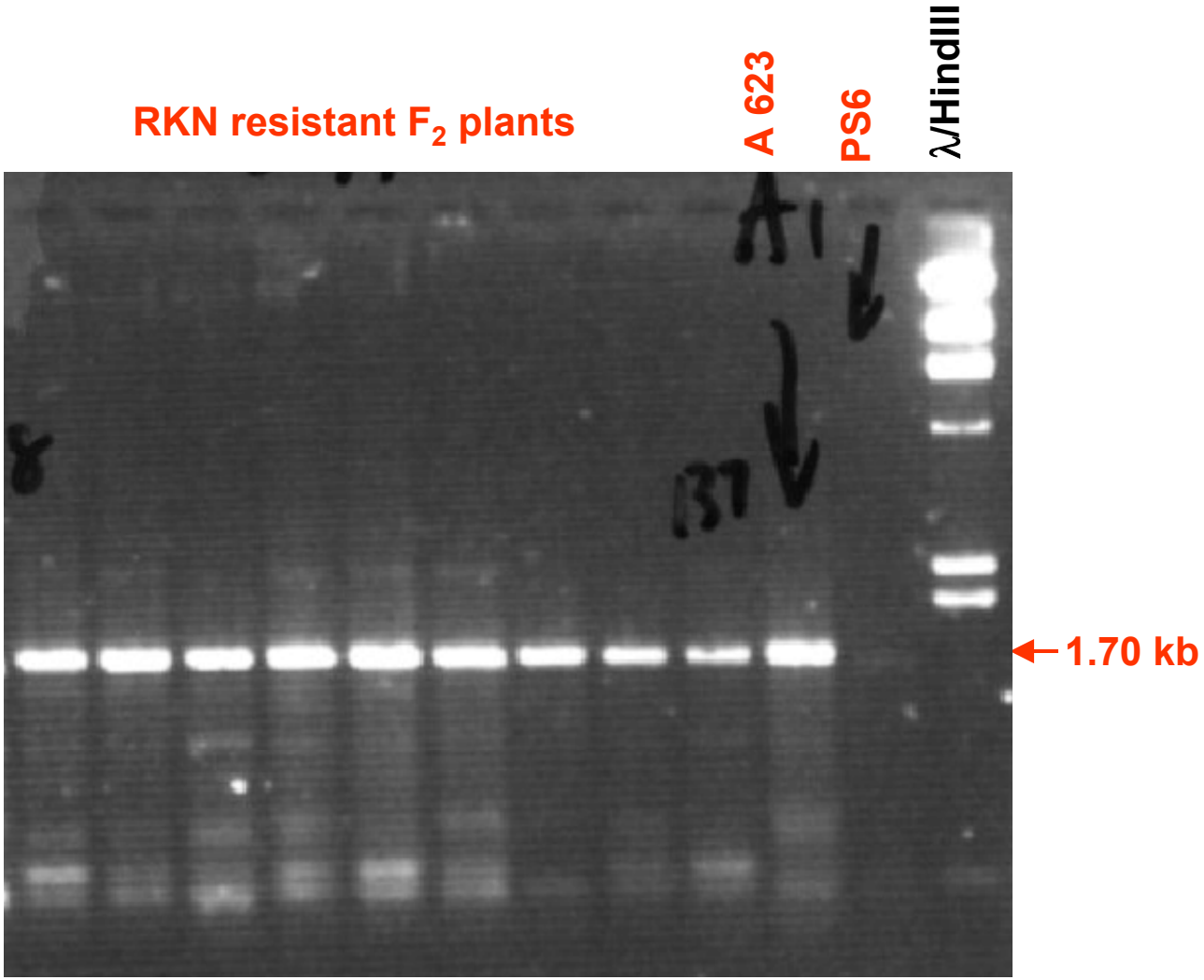
The Pedigree of the cotton highly root-knot resistant (RNR) Auburn lines

PCR Analysis of the Five Pairs of NILs of RKN Resistance with Oligo Primers

- A total of 700 oligo primers were screened
- Six oligo primers were identified to give polymorphic bands between the RKN resistance lines and the RKN susceptible lines



PCR analysis of the RKNR NILs with oligo primers



Screening the plants having RKNR index < 1.0 and randomly selected from the Pima S6 x Auburn 623 F_2 population with primer 11

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- Root-knot Nematode Resistance
- Mapping Populations
- PCR-based DNA Marker Development
- **Candidate Genes for Resistance to Different Pathogens, including Nematodes**
- Large-insert BAC libraries
- Integrated Physical and Genetic map construction
- Future Research Directions and Plans

TABLE 1 Classes of characterized R genes

Class/gene	Interaction (Host/pathogen)	Predicted protein structure	Complex locus[a]	Introgressed from wild species	Reference
1 *L*	Flax/*Melampsora lini*	TIR-NBS-LRR	No	No	(81)
M	Flax/*Melampsora lini*	TIR-NBS-LRR	Yes	No	(2)
N	Tobacco/TMV	TIR-NBS-LRR	Yes	Yes	(154)
P	Flax/*Melampsora lini*	TIR-NBS-LRR	Yes	No	(35)
RPP1	*Arabidopsis*/*Peronospora*	TIR-NBS-LRR	Yes	No	(14)
RPP5	*Arabidopsis*/*Peronospora*	TIR-NBS-LRR	Yes	No	(107)
RPS4	*Arabidopsis*/*Pseudomonas*	TIR-NBS-LRR	No	No	(46)
Bs2	Pepper/*Xanthomonas*	NBS-LRR	Yes	Yes	(136)
Dm3	*Lettuce*/*Bremia*	NBS-LRR	Yes	No	(96)
Gpa2/Rx1	Potato/*Globodera* Potato/PVX (*Rx1*)	NBS-LRR	Yes	Yes	(144) (5)
I2	Tomato/*Fusarium*	NBS-LRR	Yes	Yes	(104, 122)
Mi	Tomato/*Meloidogyne*/	NBS-LRR	Yes	Yes	(99)
	Macrosiphum	NBS-LRR	Yes	Yes	(117, 146)
Mla	Barley/*Blumeria*	NBS-LRR	Yes	No	(162)
Pib	Rice/*Magnaporthe*	NBS-LRR	Yes	No	(148)
Pi-ta	Rice/*Magnaporthe*	NBS-LRR	No	No	(18)
Prf[b]	Tomato/*Pseudomonas*	NBS-LRR	Yes	Yes	(118)
Rp1	Maize/*Puccinia*	NBS-LRR	Yes	No	(25)
RPM1	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No	(48)
RPP8/HRT	*Arabidopsis*/*Peronospora* *Arabidopsis*/TCV (*HRT*)	NBS-LRR	Yes	No	(89) (27)
RPP13	*Arabidopsis*/*Peronospora*	NBS-LRR	No	No	(11)
RPS2	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No	(9, 100)
RPS5	*Arabidopsis*/*Pseudomonas*	NBS-LRR	No	No	(149)
Rx2	Potato/PVX	NBS-LRR	Yes	Yes	(5)
Sw-5	Tomato/*Tospovirus*	NBS-LRR	Yes	Yes	(16)
Xa1	Rice/*Xanthomonas*	NBS-LRR	No	No	(158)
2 *Cf-2/5*	Tomato/*Cladosporium*	LRR-TM	Yes	Yes	(32)
Cf-4/9	Tomato/*Cladosporium*	LRR-TM	Yes	Yes	(69, 137, 141)
3 *Pto*	Tomato/*Pseudomonas*	Protein Kinase	Yes	Yes	(87)
4 *Xa21*	Rice/*Xanthomonas*	LRR-TM-Kinase	Yes	Yes	(129)
5 *HS1*$^{pro-1}$	Beet/*Heterodera*	Unique[c]	No	Yes	(20)
6 *Rpw8*	Arabidopsis/*Erisyphe*	Unique	Yes	No	(157)
7 *mlo*	Barley/*Blumeria*	Membrane Prot.[d]	No	No	(19)
8 *Hm1*	Maize/*Cochliobolus*	Toxin reductase	No	No	(68)

NBS = nucleotide binding site. LRR = leucine-rich repeat. TIR = domain with homology to the *Toll* gene of *Drosophila*, and the *Interleukin-1* receptor of mammals. TM = transmembrane domain. Domains are listed as they appear in the proteins from N to C terminal end.

[a]"Complex locus" indicates the gene belongs to a tightly linked family of highly homologous genes.

[b]*Prf* is required for *Pto* mediated resistance to *P. syringae* pv *tomato* strains carrying *avrPto* and for the *Fen* mediated, hypersensitive-like reaction to the organophosphate insecticide Fenthion.

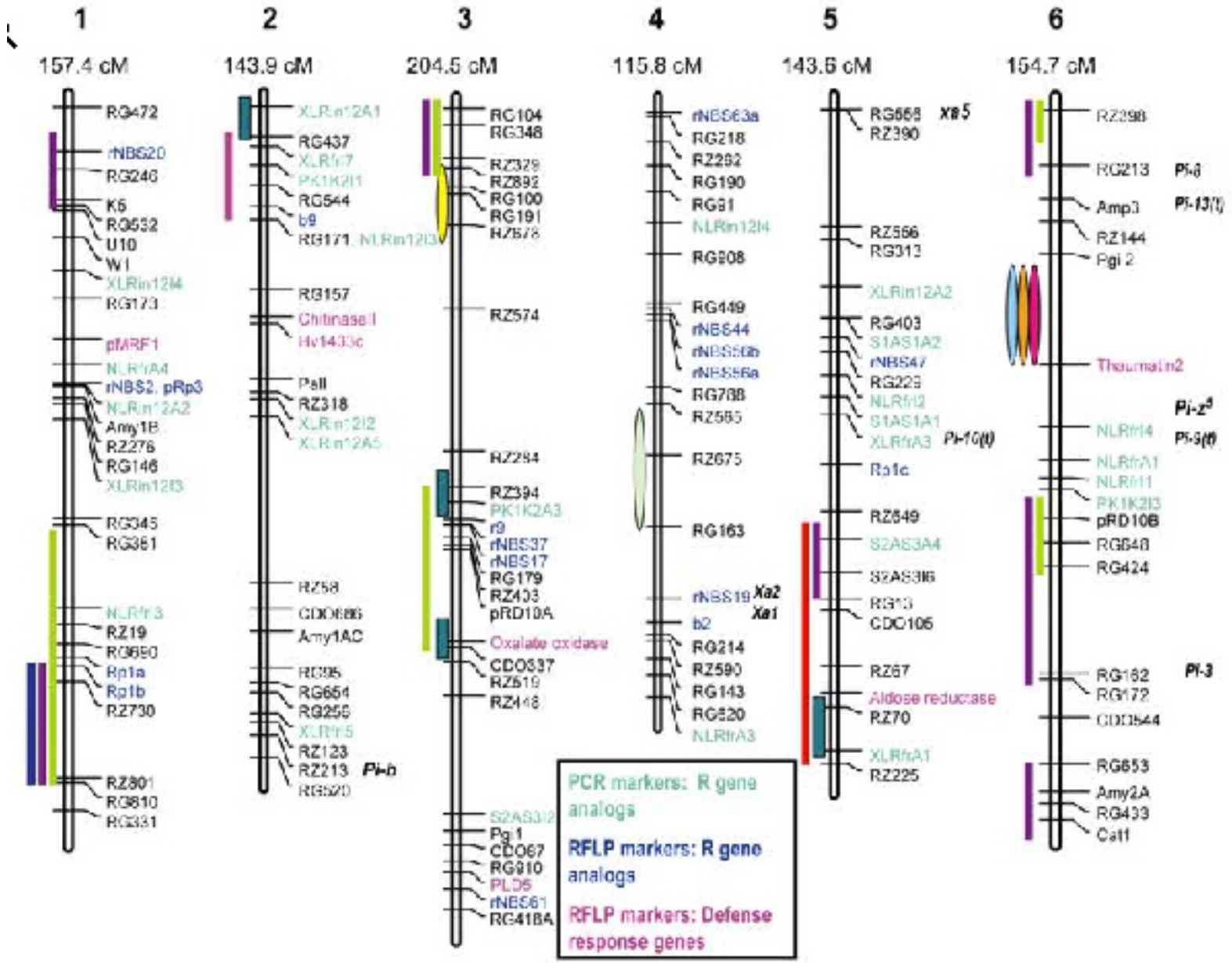
[c]The predicted *HS1*$^{pro-1}$ protein was originally reported to have a LRR-TM signature though it poorly fits the LRR consensus and has minimal similarity to other known resistance genes (40).

[d]Predicted 60-kDa protein is membrane anchored with at least 6 membrane spanning helices.

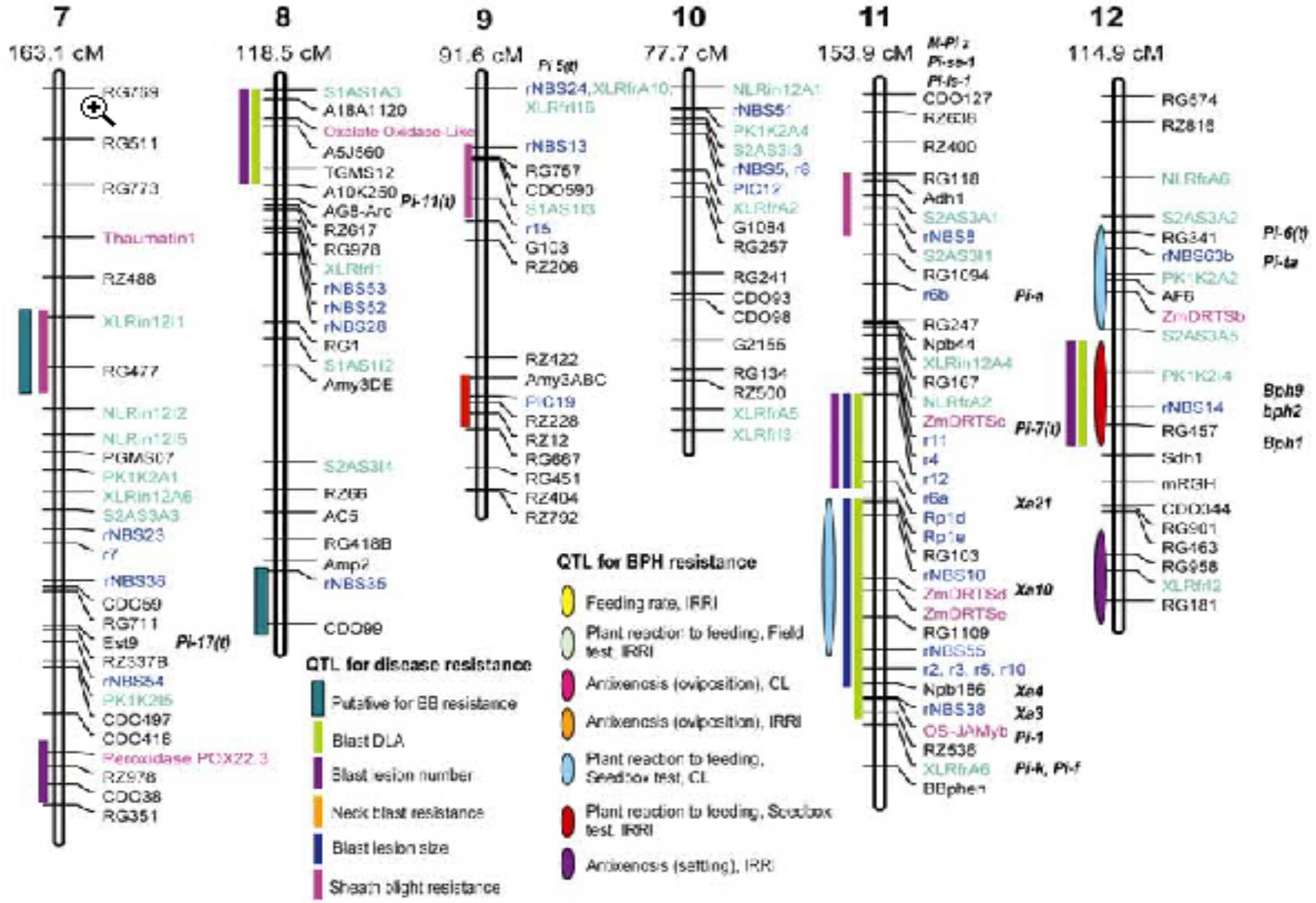
(Hulbert et al. 2001. Ann. Rev. Phytopathol. 39: 285-312)

Ramalingam et al. 2003. *Molecular Plant-Microbe Interactions* 16:14-24

Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice



(Ramalingam et al. 2003. MPMI 16:14-24)



(Ramalingam et al. 2003. MPMI 16:14-24)

NBS-LRR-encoding genes and their organization in the genome

Species	Genome size (Mb/1C)	No. of NBS-LRR genes	No. of loci	References
Arabidopsis	145	166	91	Richly et al. 2002
Japonica rice	430	500 - 750	>52	Goff et al. 2002 Meyers et al. 1999 Santos et al. 2002
Indica rice	430	500 – 750	>142	Meyers et al. 1999 Santos et al. 2002
Soybean	1,100	1,500 – 2,000	334	Wu et al. 2003

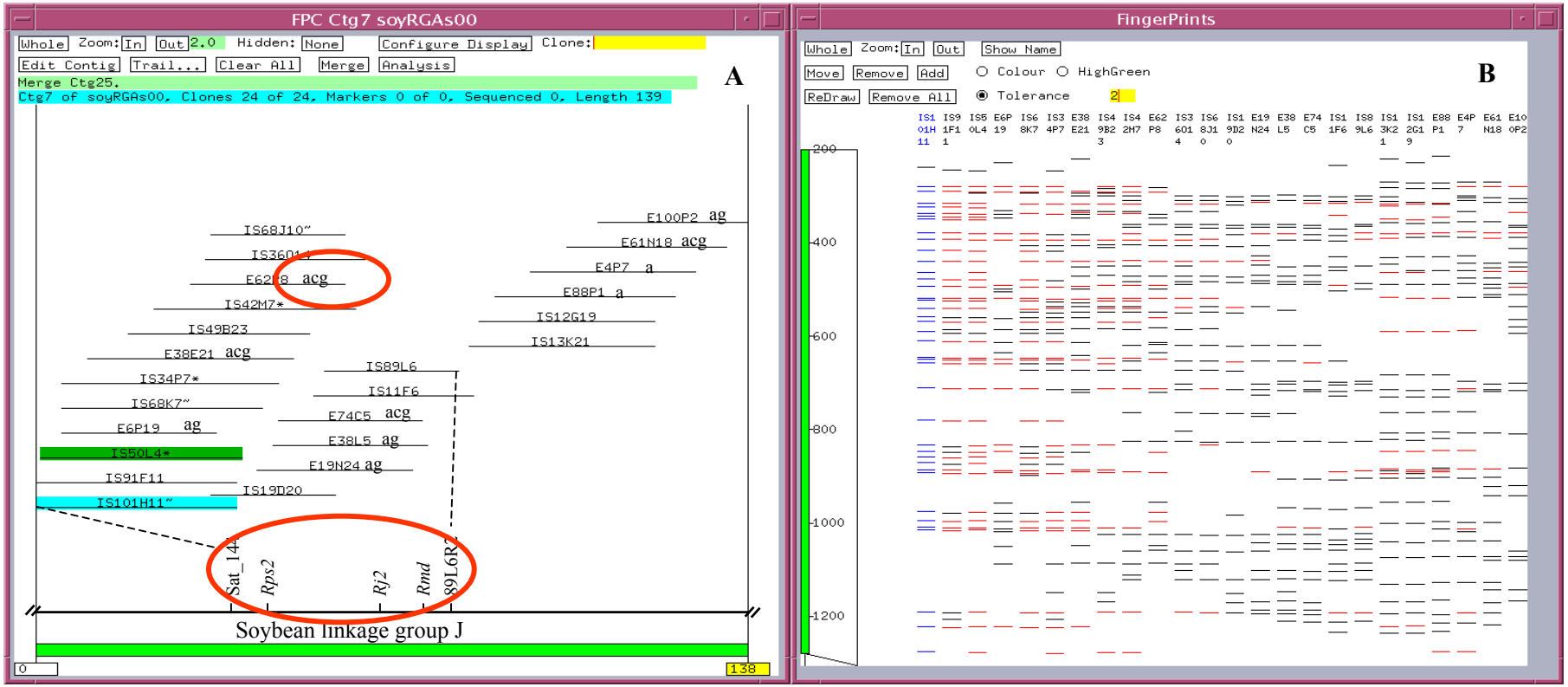
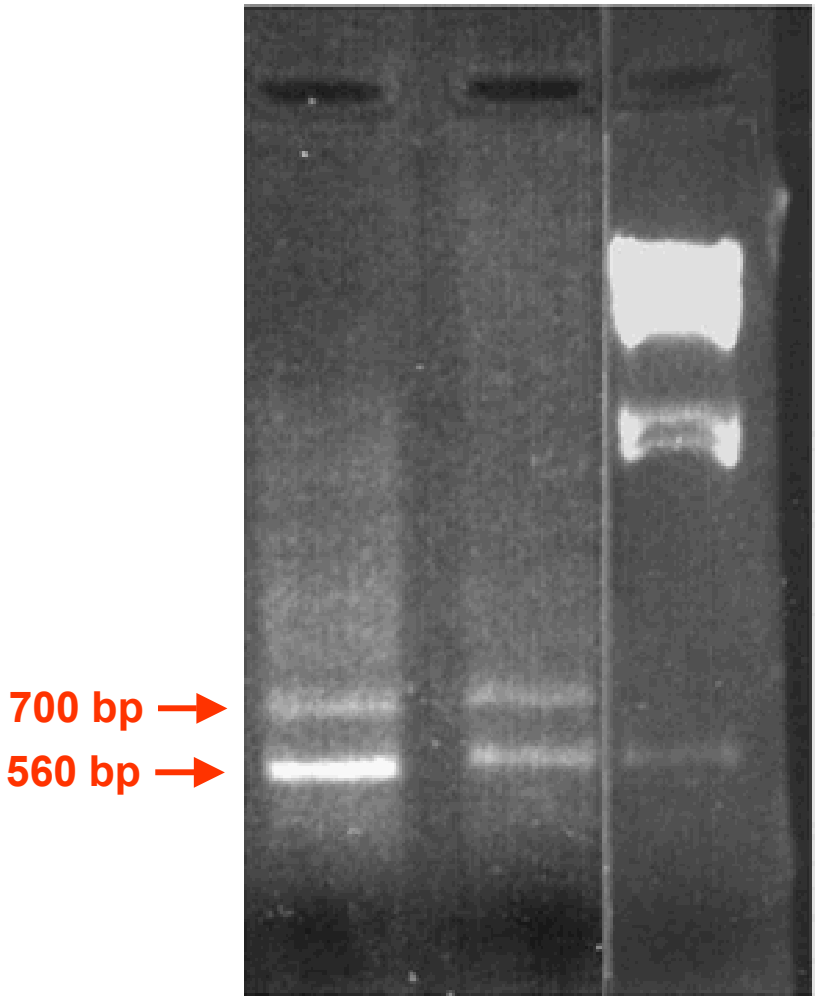
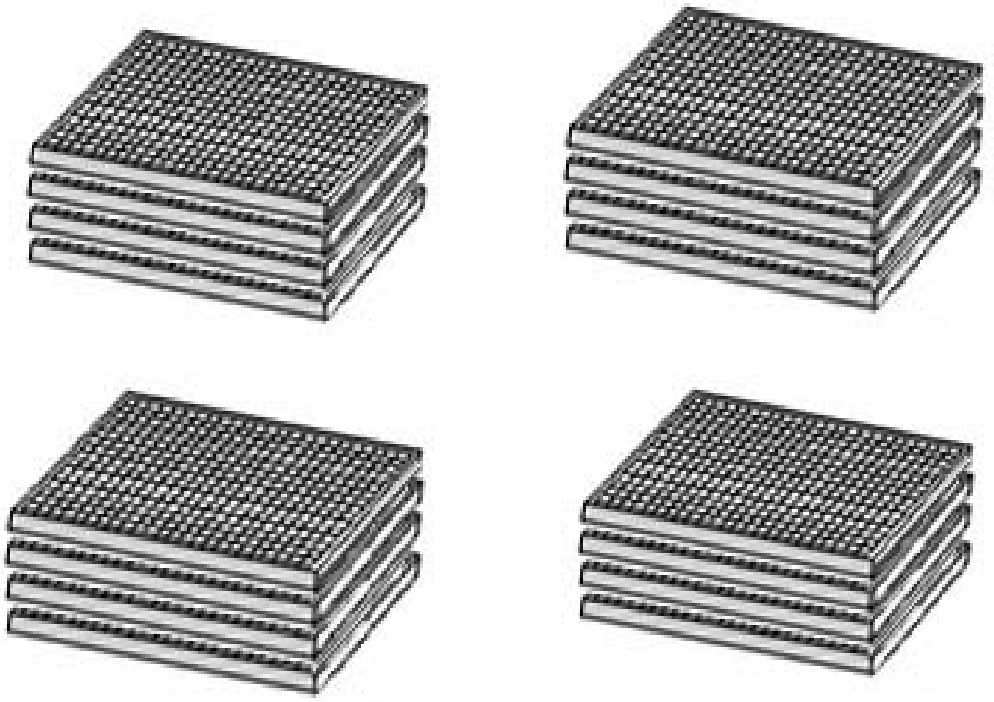


Fig. 1. Example of the soybean R gene cluster BAC contigs (A) and the digitized fingerprints of the contig BACs (B). This contig consists of 24 BACs, spanning 727 kb in physical length, and is mapped to the region of linkage group J of the soybean genetic map containing the genes conferring resistance to powdery mildew (*Rmd*) and *Phytophthora* stem and root rot (*Rps2*), and the gene for ineffective nodulation (*Rj2*). Ten of the 24 BACs prefixed with "E" were from the soybean cv. Forrest BAC library and 14 prefixed with "IS" from the soybean cv. Williams 82 BAC library. The "E" clones suffixed with a, ag or acg indicate the clones that were hybridized with probes RGA1, RGA1 and RGA7, or RGA1, RGA3 and RGA7, respectively. The locations of the RGAs in the "IS" clones were not studied.

(From Wu et al. 2003)



PCR products of cotton Auburn 623 genomic DNA amplified using the degenerate primer pair designed from the conserved motifs (NBS-LRR) of the cloned plant disease resistance gene-encoding proteins



Ordered library of candidate genes for resistance to fungal, nematode, bacterial, pest and viral pathogens in cotton

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- Root-knot Nematode Resistance
- Mapping Populations
- PCR-based DNA Marker Development
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- **Large-insert BAC libraries**
- Integrated Physical and Genetic map construction
- Future Research Directions and Plans

Table 1. Cotton BAC and BIBAC Libraries Constructed by the the USDA-ARS and the TAMU GENE*finder* Genomic Resources at College Station, Texas.

Genotype	Genome size (Mb/1C)	Mean insert size (kb)	No. of clones	Genome equivalents	Vector	Cloning
Tamcot HQ95	2,250	93	51,072	2.2x	pBeloBAC11	*Hind* III
Auburn 623	**2,250**	**140**	**44,100**	**2.7x**	**pBeloBAC11**	***Bam* HI**
TM-1NIL(ESP)	2,250	148	38,400	2.5x	pECBAC1	*Hind* III
		138	38,784	2.3x	pECBAC1	*Bam* HI
		142	38,400	2.4x	pECBAC1	*Eco* RI
TM-1	2,250	152	53,760	3.6x	pECBAC1	*Hind* III
		130	76,800	4.4x	pCLD04541	*Bam* HI
Total			**341,316**	**20.1x**		

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- Root-knot Nematode Resistance
- Mapping Populations
- PCR-based DNA Marker Development
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- Large-insert BAC libraries
- **Integrated Physical and Genetic map construction**
- Future Research Directions and Plans

DNA Markers

1. RFLP - Restriction Fragment Length Polymorphism
2. STS - Sequence Tagged Site
3. CAPS - Cleaved Amplified Polymorphic Sequences
4. RAPD - Randomly Amplified Polymorphic DNA
5. AFLP - Amplified Fragment Length Polymophism
6. SSR - Simple Sequence Repeat or Microsatellite
7. SNP - Single Nucleotide Polymorphism

Gene Mapping

The most popularly used method of mapping genes is genetic mapping that is based on:

[1] recombination frequency, and

[2] polymorphism

Arabidopsis	**125 Mb/1C**	**25,000 genes**
Rice	**430 Mb/1C**	**50,000 genes**
Cotton	**2,200 Mb/1C**	**50,000 – 80,000 genes**

Gene Isolation

- Library screening
- DNA subtraction and differential display
- EST and genome sequencing
- T-DNA or transposon tagging
- PCR-based gene candidate
- Positional cloning

Positional cloning

Gene genetic mapping

↓

Physical mapping (optional)

↓

Chromosome landing or walking

↓

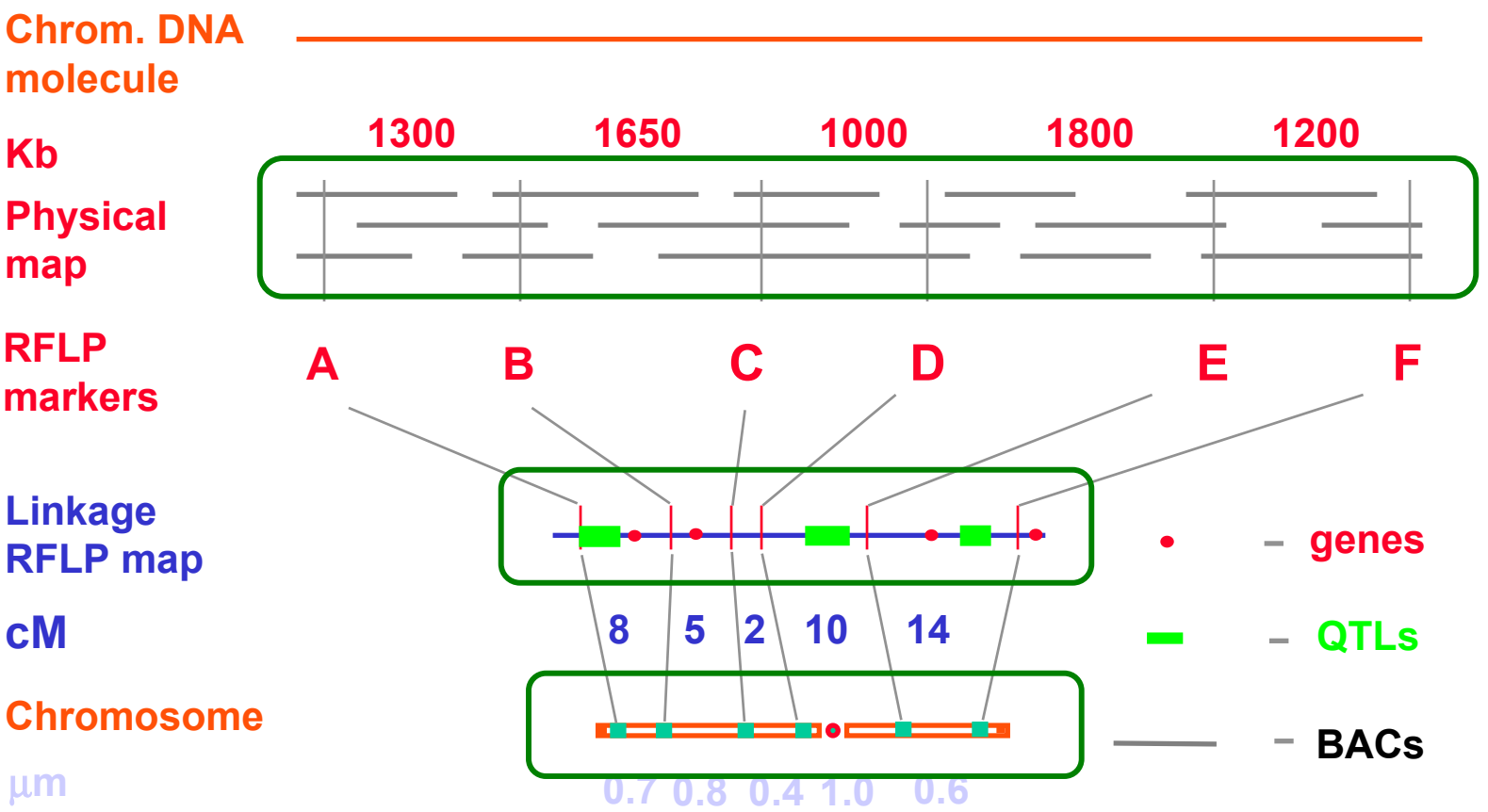
High-resolution mapping

↓

Gene Identification

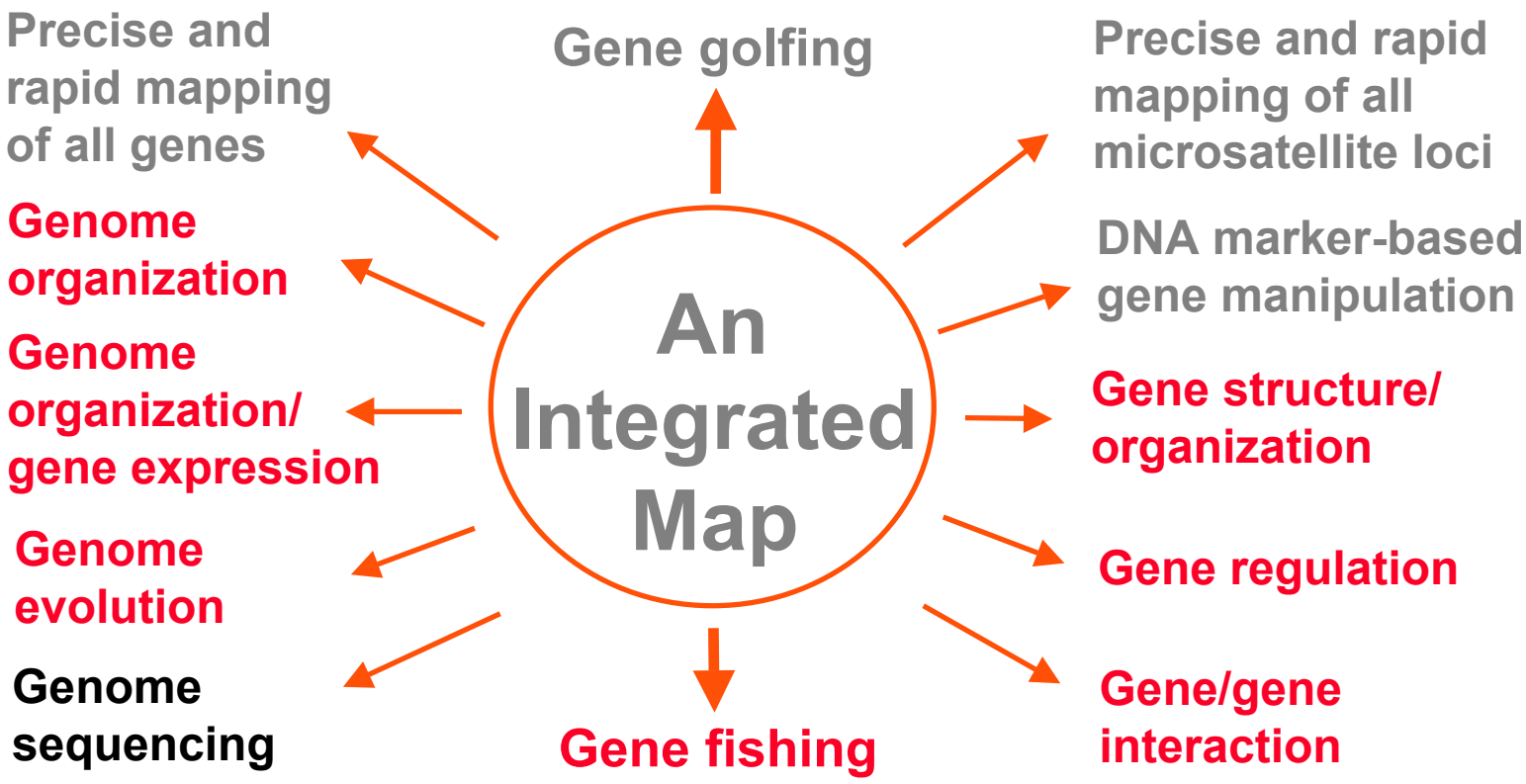
Integrative Physical Mapping

Physical mapping: Reconstruction of chromosomes from DNA fragments cloned in BACs, PACs, PBCs and/or YACs



Significance of An Integrated Map for Genome Research

It is a "freeway" for rapid isolation of numerous mapped genes and QTLs, and for many other genetic and biological studies



BAC-based Physical Maps Constructed (as of November 20, 2002)

Physical Maps Published

- Arabidopsis (Marra et al. 1999; Chang et al. 2001)
- Drosophila (Hoskins et al. 2000)
- Human (International Human Genome Mapping Consortium 2001)
- Indica rice (Tao et al. 2001)
- Japonica rice (Chen et al. 2002)
- Mouse (Gregory et al. 2002)

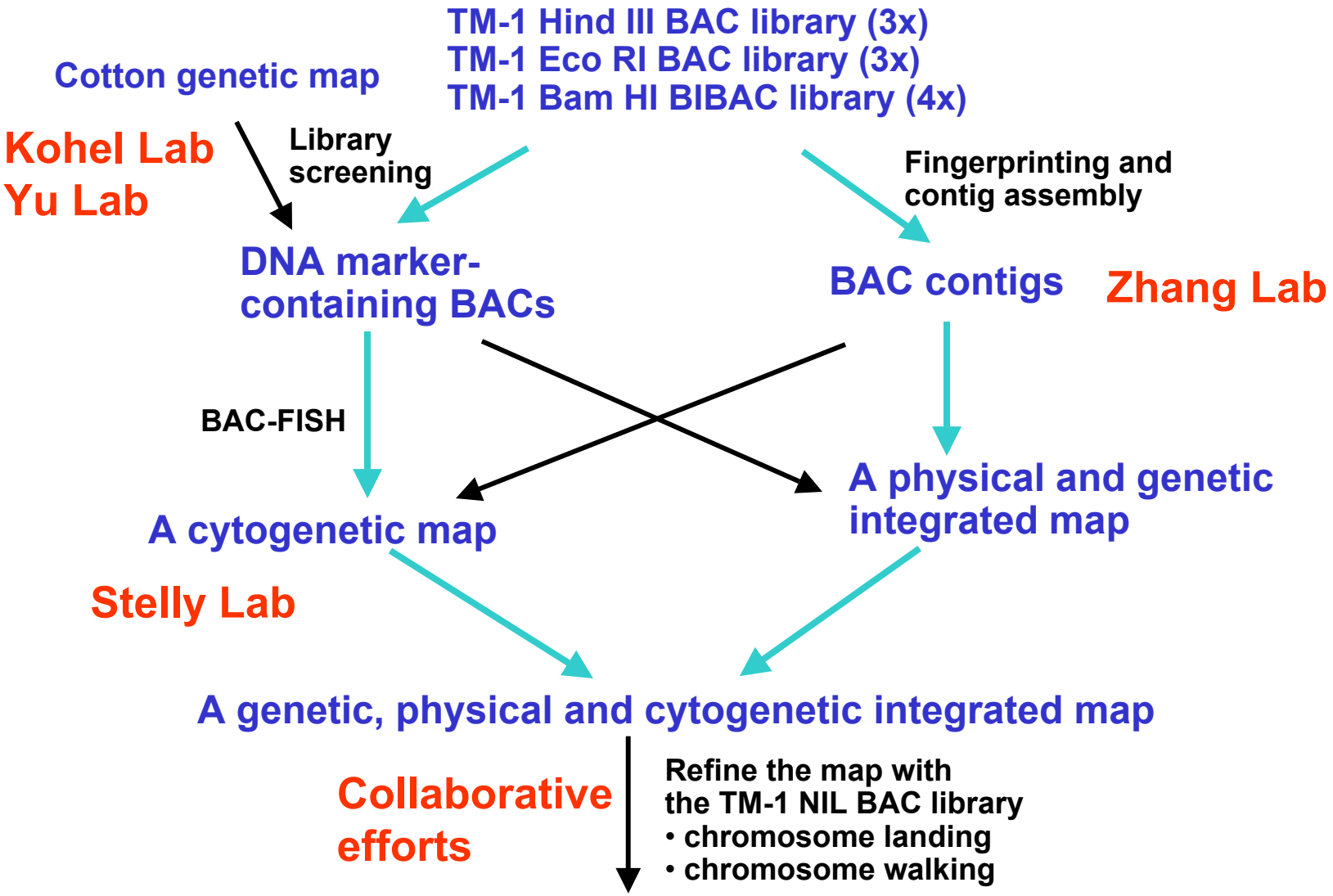
Physical Maps under Construction:

Soybean (NSF, IUSB - TAMU/SIU), maize (NSF - UMC/AU/UNJ), wheat D genome (NSF - UCD/TAMU), tomato (NSF - CU/AU), chicken (USDA, NIH - MSU/TAMU/WU)

Integrative Physical and Genetic Mapping of Agricultural Genomes (1997 – present)

PI/Co-PI: Hongbin Zhang

Species	BACs/ BIBACs	Progress	Funding Agencies
Indica rice	21,078	Tao et al. 2001	RF, THECB, TAES
Arabidopsis	10,368	Chang et al. 2001	NSF, THECB
Soybean	85,944	Wu et al. 2003	NSF, IUSB
Chicken	66,048	Ren et al. 2003	USDA, IFAFS
Japonica rice	23,040	Li et al. 2003	RF, TAES
Cotton	>200,000	Fingerprinting	



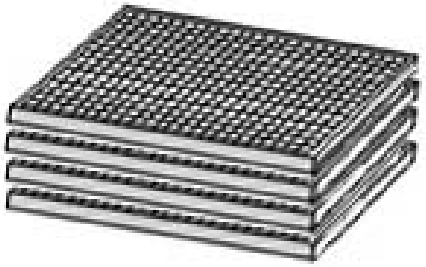
A strategy for integrative mapping of the cotton genome

Collaborative efforts:

Development of a Robust Integrated Physical and Genetic Map of the Cotton Genome

- If possible, please use the source BACs of the cotton integrated physical and genetic map under development so that your DNA markers or genes will be automatically incorporated into the cotton genetic and physical maps
- If a non-source BAC library of the map is used in your research and you could send the BAC clones to us, we could fingerprint and incorporate them into the cotton genetic and physical maps

In return, you will be able to use the integrated physical and genetic map in your research



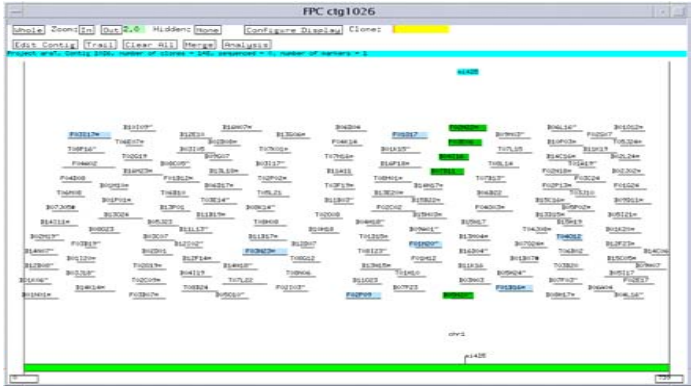
A BAC library

DNA isolation

Autogen 960

ABI 3100

Fingerprinting



A BAC-based map contig

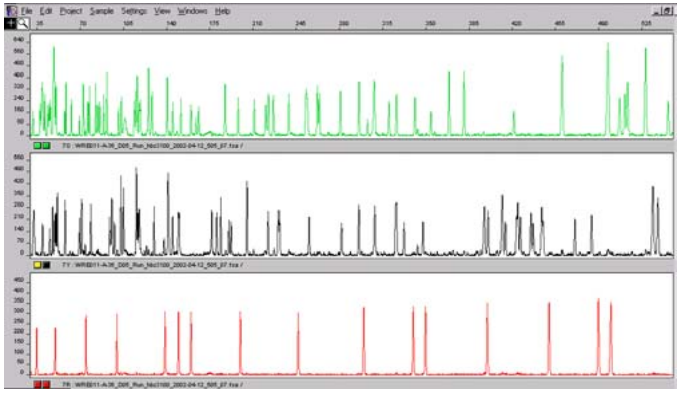
Contig assembly
(FPC)

BAC fingerprints from one channel

BAC 1

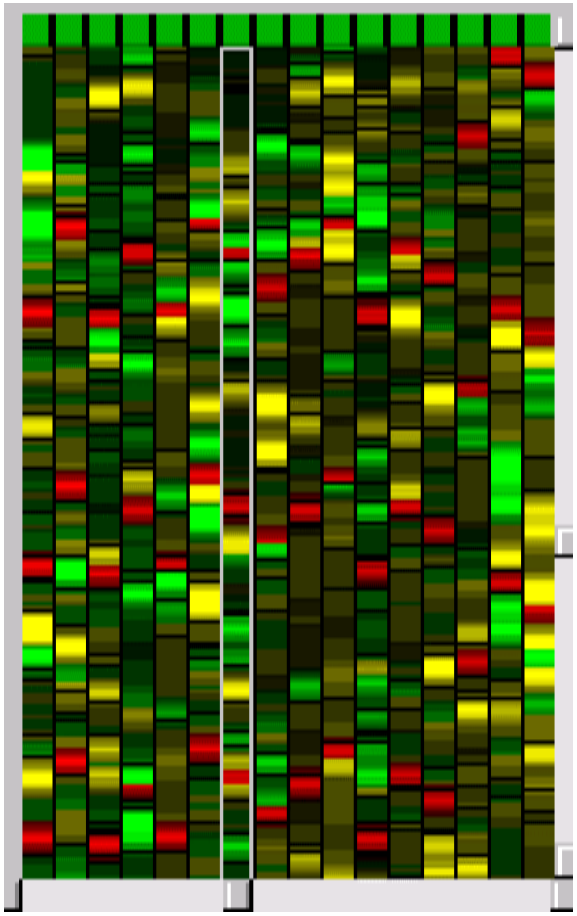
BAC 2

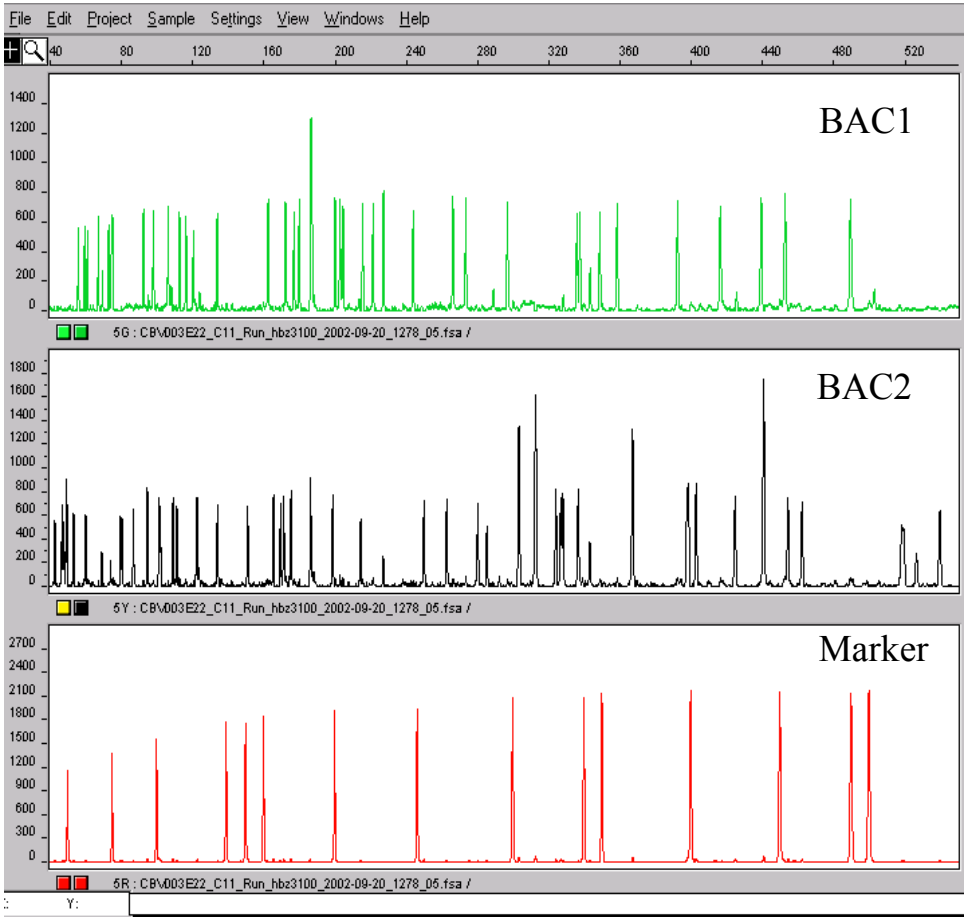
Internal
marker



Automated procedure for physical mapping with BACs

Three-enzyme kit





A: BAC fingerprint images B: BAC fingerprints from one channel of the ABI 3100 analyzer

BAC fingerprints generated by the thee-enzyme kit: Enzymes: *Hind* III, *Bam* HI and *Hae* III; one-tube one-step reaction; 2 BACs per channel of ABI 3100; and readable fragments range from 35 to 500 bases.

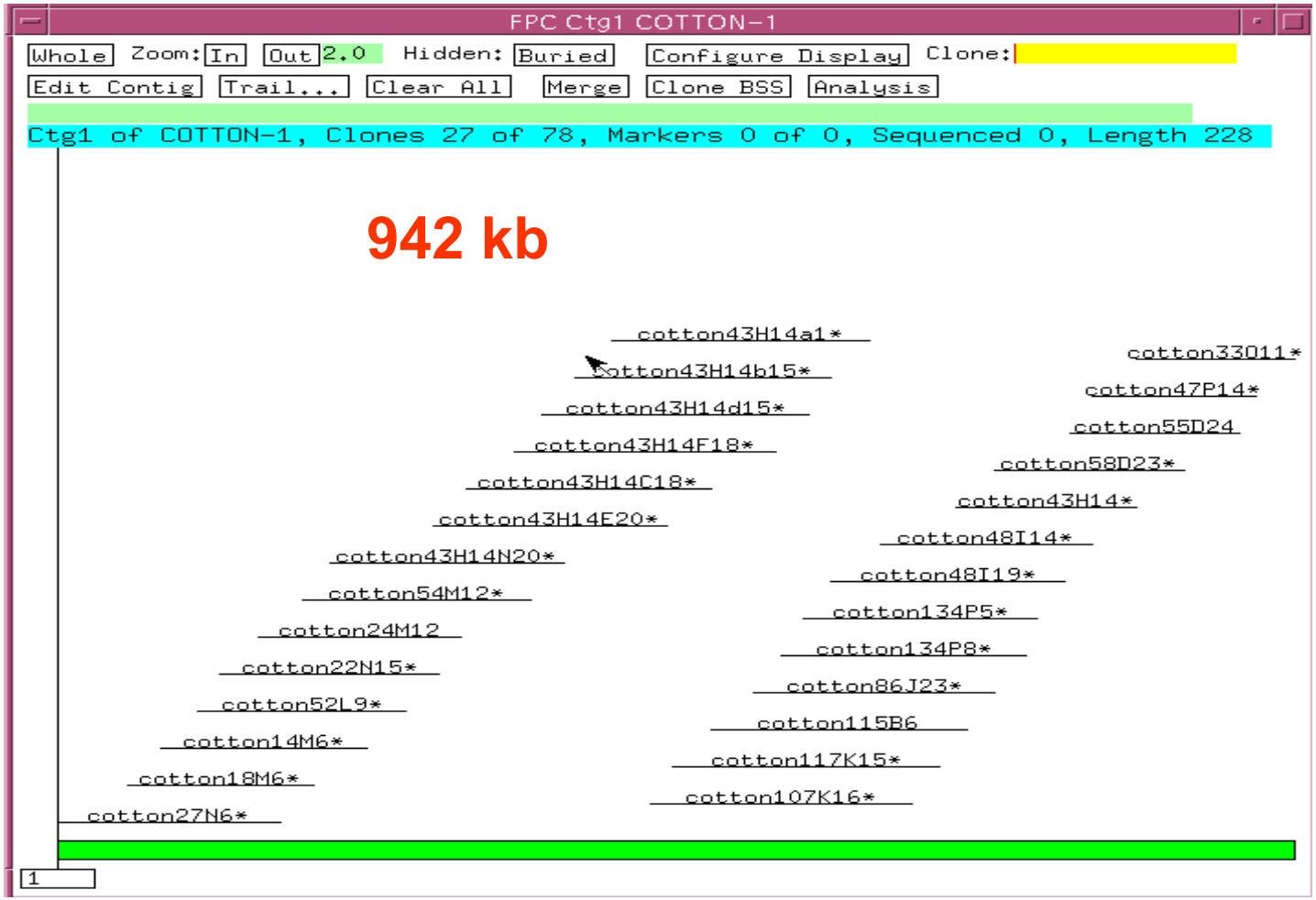
(Xu, Sun amd Zhang, unpublished)

Table 2. Progress of whole-genome physical mapping of the cultivated cotton (as of August 2003)

Genotype	Mean insert size (kb)	No. of clones	Genome equivalents	Vector	Cloning site	No. of clones fingerprinted
TM-1	152	53,760	3.6x	pECBAC1	*Hin*d III	23,040
	130	76,800	4.4x	pCLD04541	*Bam* HI	76,800
Total		130,560	8.0x			99,840 (6.1x)

Status of the Cotton physical map from automatic assembly

Date	August 18, 2003
Number of clones in FPC database	85,040
Coverage of the clones	5.6 X
Number of singletons	11,411
Number of contigs	5,466
Contigs containing	
> 200 clones	1
101 – 200 clones	0
51 – 100 clones	1
26 – 50 clones	11
10 – 25 clones	330
3 – 9 clones	3537
2 clones	1766



Example of the BAC/BIBAC contigs of the cotton TM-1 genome physical map

Development of Genomic Tools for RKN Resistance Breeding in Cotton

- Root-knot Nematode Resistance
- Mapping Populations
- PCR-based DNA Marker Development
- Candidate Genes for Resistance to Different Pathogens, including Nematodes
- Large-insert BAC libraries
- Integrated Physical and Genetic map construction
- **Future Research Directions and Plans**

Future Research Directions and Plans

1. Construct the high-density local genetic maps for the loci containing RKN resistance genes using the primers and the cotton disease resistance gene candidate clones identified, and other available DNA markers

2. Develop two or more PCR-based, user-friendly, highly polymorphic and closely linked DNA markers for each locus of the RKN resistance genes using the cotton physical map

   The genetic distance between the genes and the markers should be within 1.0 cM, reducing the probability of mis-selection by marker-assisted selection to <1%

Future Research Directions and Plans
(continued)

3. Establish an encyclopedia of the disease resistance genes and related sequences, including those for nematode resistance, in cotton
4. Identify and characterize all loci containing disease resistance genes and related sequences in the cotton genome using the cotton physical map
5. Isolate the genes conferring resistance to RKN and all other pathogens, including nematodes, fungi, bacteria, pests and viruses, that are important to cotton production using the cotton physical map by “gene fishing” and/or “gene golfing”

Acknowledgements

Ms. Limei He, TAMU

Dr. Shuku Sun, TAMU

Dr. Forest Robinson, USDA-ARS/TAMU

Dr. Charles C. Cook

Dr. Zhanyou Xu, TAMU and USDA-ARS

Ms. Lina Covaleda, TAMU/USDA-ARS

Dr. Russell Kohel, USDA-ARS/TAMU

Dr. John Yu, USDA-ARS/TAMU

Ms. Jianmin Dong, USDA-ARS/TAMU

Dr. Mi-Kyung Lee, TAMU

Dr. David M. Stelly, TAMU