

**ADVANCEMENT OF COTTON (*Gossypium*) RADIATION HYBRID
MAPPING TOOLS**

A Thesis

by

STEVEN MICHAEL TODD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Molecular and Environmental Plant Sciences

**ADVANCEMENT OF COTTON (*Gossypium*) RADIATION HYBRID
MAPPING TOOLS**

A Thesis

by

STEVEN MICHAEL TODD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	David Stelly
Committee Members,	John Ford
	John Yu
Chair of MEPS Faculty,	Marla Binzel

December 2006

Major Subject: Molecular and Environmental Plant Sciences

ABSTRACT

Advancement of Cotton (*Gossypium*) Radiation Hybrid Mapping Tools.

(December 2006)

Steven Michael Todd, B.A., Bradley University

Chair of Advisory Committee: Dr. David Stelly

The assembly of a robust structural genomics system requires the development and integration of multiple types of genome maps. This research focused on the development of a relatively new means of plant genome mapping, radiation hybrid mapping, for use in cotton genomics. Simple sequence repeat markers were genotyped onto an existing wide-cross whole-genome radiation hybrid panel for genome mapping of the *Gossypium barbadense* line '3-79'. A new mapping panel was created for genome mapping of the *G. hirsutum* line 'TM-1'. Carthagene software was compared to RHMAP and found to be superior in most regards.

A total of 92 simple sequence repeat markers were genotyped onto the mapping panel for *G. barbadense*. Data from 64 of the 92 markers were deemed robust and combined with pre-existing data to develop an expanded framework map, which provides partial coverage of 7 chromosomes and three unidentified linkage groups.

A new mapping population was created to allow mapping of the *G. hirsutum* genome. The population was developed by treatment of TM-1 pollen with 8 krad of radiation, which was used to make more than 1000 controlled cross-pollinations. From these, 979 bolls were harvested and seeds were planted until a population of 115 viable plants was obtained. Of these, 92 were selected at random for inclusion in the mapping panel.

Carthagene genome mapping software was evaluated and compared to the previously utilized RHMAP. Carthagene compared favorably in ease of use, calculation

speed, and reliability of results. As such, it is recommended for use for the RH mapping project.

DEDICATION

I dedicate this thesis to all those researchers who came before and all those who will come after. Without the hard work of those who came before, the ideas and inventions needed for this research would not exist. It is due to the efforts of past agricultural researchers that the fields of today bloom so awesomely.

I also dedicate this thesis to all researchers who will benefit from it in the future. I wish that someone will draw on the discoveries presented within to further the bounds of science and apply it to the benefit of humanity.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. David Stelly, for all his advice and support during my time here. I would also like to thank my advisory committee: Dr. John Ford and Dr. John Yu for their advice and assistance. I would also like to acknowledge Dr. Monica Menz and Dr. Alan Pepper for their technical advice during my research.

I would like to thank Dwaine “Wayne” Raska, for his help caring for my plants. I would also like to thank Jiamin Dong for her technical advice.

I would like to thank Cotton Incorporated for providing funding for the research and the TAMU Department of Soil and Crop Sciences for providing me with an assistantship. I would also like to thank the Plant Genomics Training Program for providing me with a fellowship during my first year of study at Texas A&M. I thank the Texas A&M Nuclear Science Center and the Electron Beam Food Research Facility for providing radiation treatments free of charge.

I want to thank my labmates for their advice, encouragement, and friendship during my studies: Alisher Abdullaev, Jason Anderson, Kelly Biddle, Nilesh Dighe, George Hodnett, Steven Hoffman, Brian Gardunia, Stella Kantartzi, Les Kuhlman, Fakhriddin Kushanov, Jewel Stroupe, and Dr. Suk-Hwan “Sam” Yang.

I would also like to acknowledge all the researchers who have offered words of advice and encouragement when needed, including, but not limited to: Dr. Harry Cralle, Dr. Osman Gutierrez, Dr. Lori Hinze, Dr. Russell Kohel, Dr. Barbara Triplett, Dr. C. Wayne Smith, and others too numerous to list.

I would also like to acknowledge the many student workers in the Stelly lab who helped care for my plants and those in the Yu lab who helped keep the lab running smoothly.

I would like to thank Dr. Wenxiang Gao for his seminal research efforts on the RH mapping project.

TABLE OF CONTENTS

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGMENTS.....	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES.....	viii
LIST OF TABLES	ix
INTRODUCTION.....	1
MATERIALS AND METHODS	11
Creation of TM-1 mapping panel.....	11
Genotyping of molecular markers on the 3-79 panel.....	11
Advances in software usage	14
RESULTS.....	15
Creation of TM-1 RH mapping panel	15
Genotyping of SSRs onto the 8-Krad 3-79 WWRH panel.....	18
Comparison of Carthagene and RHMAP software.....	18
DISCUSSION	23
SUMMARY	30
LITERATURE CITED	31
APPENDIX A	38
APPENDIX B	55
VITA	60

LIST OF FIGURES

FIGURE	Page
1 Gel scoring methods.....	14
2 Leaf variation in TM-1 mapping population.....	15
3 Whole plant phenotypic variation of the TM-1 8-krad mapping population.	16
4 Map created using Carthagene.	17
5 Comparison of radiation hybrid mapping retention models.....	22

LIST OF TABLES

TABLE		Page
1	A comparison of genome mapping technologies.	6
2	PCR conditions of primer pairs.	12
3	Comparison of syntenic groups obtained from two-point analysis using RHMAP and Carthagene.	20
4	Comparison between linkage maps and RH map.	27

INTRODUCTION

Cotton is the most important fiber crop in the United States and the world. In 2005, the most recent year for which statistics are available, nearly 5.6M hectares (13.8 M acres) of cotton were planted in the United States. From this, over 23M bales, with a raw product value over \$5.5B were harvested (USDA-NASS 2006). Worldwide in 2004, the most recent year for which data is available, over 32M hectares (79 M acres) were planted, producing nearly 95 M bales (USDA-NASS 2005, chap. 2). The economic importance of cotton clearly makes it a valuable target for study.

Commercially grown cotton consists primarily of two major types: *Gossypium hirsutum* (Upland cotton) and *G. barbadense* (Egyptian or Pima cotton). Upland cotton is grown throughout the US cotton belt, in the Southern United States stretching from Virginia and the Carolinas to California. While Pima cotton is grown primarily in Arizona, California, New Mexico and Texas. Upland cotton is known for high yields, adequate fiber quality, and a wide growing range. Pima cotton is known for very high fiber quality, but does not currently have the high yields and wide range associated with Upland cotton. Approximately 90% of cotton produced worldwide is *G. hirsutum* while 5-10% is *G. barbadense* (USDA-NASS 2005, chap. 2).

The importance of cotton, both as an economic staple and an evolutionary model has led to many scientific studies of the cotton genus. Modern genetic studies of cotton date to at least the early 20th century. Early cytogeneticists observed cotton chromosomes and distinguished the 26- and 52-chromosome groups (Denham 1924). Further research divided the cotton genus into several genome groups based on chromosome size, chromosome number, and chromosome behavior (Beasley 1941,

This thesis follows the style of Genetics.

Beasley 1942). Research over the ensuing decades has led to the current grouping of cotton species into the genome categories discussed below.

G. hirsutum and *G. barbadense* are two members of the fifty-member *Gossypium* (cotton) genus. This genus is found naturally on five continents: Africa, Asia, Australia, North America, and South America. The genus is divided into several genomes, each containing one or more species. These include the A, B, C, D, E, F, G and K genomes, as well as the AD tetraploid genome. Diversification of the *Gossypium* genus began between 5-15 million years ago (MYA) with the earliest known diversification event being the separation of the D genome group, likely following a trans-Atlantic voyage from Africa to Central America or the northern coast of South America. Following this event, several more diversification events occurred, resulting in the fifty cotton species known today. Of these fifty species, four are cultivated in various locations around the world. Today, *G. hirsutum* dominates world cotton production (Wendel and Cronn 2003).

Many studies have helped elucidate the evolutionary relationships among cotton species. Studies of cytoplasmic DNA have shown that modern 52-chromosome cottons arose from a single hybridization event and concomitant or subsequent polyploidization, 1-2 MYA somewhere in Latin America, near the Caribbean Sea. The male parent was a native D-genome species, to which the most closely related extant species is *G. raimondii*, while the female parent was most closely related to the modern A-genome species *G. herbaceum* and *G. arboreum* (Wendel 1989, Small and Wendel 1999). Structural genomics studies in cotton (Rong et al. 2004, Brubaker et al. 1999, Liu et al. 2001) seem to indicate that the most recent polyploidization event in *Gossypium* was not followed by massive genomic rearrangements. Although polyploidization is frequently followed by such rearrangements (Soltis and Soltis 1995, Song et al. 1995, Feldman et al. 1997, Leitch and Bennett 1997, Wendel 2000), there have been notable exceptions, e.g., *Spartina anglica* (Baumel et al. 2001, Baumel et al. 2002). The apparent genomic stability of polyploid cotton species calls for further verification and investigation. Previous studies of cotton structural phylogenetics have focused on the comparison of

the modern AD tetraploid structures with their modern A and D diploid cousins (Rong et al. 2004, Brubaker et al. 1999, Liu et al. 2001). Presumably, all five 52-chromosome cotton species arose from a single polyploidization event approximately 1-2 MYA (Wendel 1989, Small and Wendel 1999). Following this assumption, any differences between the 52-chromosome species must have arisen following the last episode of *Gossypium* polyploidization. Thus, the discovery of colinearity between *G. barbadense* and *G. hirsutum* will support the conclusions by previous authors (Rong et al. 2004, Brubaker et al. 1999, Liu et al. 2001), while the discovery of rearrangements will provide refuting evidence.

In recent years, the development of new genomic resources and technologies has led to improved understanding of the cotton genome. Structural genomics has advanced with the use of cytogenetic stocks (Stelly and Raska 2003, Stelly et al. 2004), linkage mapping (Reinisch et al. 1994, Brubaker et al. 1999, Lacape et al. 2003, Rong et al. 2004, Nguyen et al. 2004, Zhang et al. 2002, Mei et al. 2004, Lacape et al. 2005, Han et al. 2006) FISH (Hanson et al. 1995, Zhao et al. 1998, Ji et al. 1999, Zhao et al. 1998, Wang et al. 2006), and bacterial artificial chromosome (BAC) libraries (Tomkins et al. 2001). These technologies have allowed the identification of individual chromosomes, chromosome arms, and ordering of markers and BAC contigs along chromosomes.

Since their widespread use began in the late 1980s, molecular markers and linkage mapping have been widely used to improve knowledge of the cotton genome. Numerous linkage maps have been made of selected *Gossypium* species. Due to low levels of intraspecific polymorphism in both *G. hirsutum* and *G. barbadense*, most linkage maps have relied on interspecific crosses between the two species. These maps have used both restriction fragment length polymorphism markers (RFLPs) (Reinisch et al. 1994, Brubaker et al. 1999, Lacape et al. 2003, Rong et al. 2004) and simple sequence repeats (SSRs) (Lacape et al. 2003, Nguyen et al. 2004, Zhang et al. 2002) and, to a lesser extent, amplified fragment length polymorphisms (AFLPs) (Mei et al. 2004, Lacape et al. 2003). With few notable exceptions (Ulloa and Meredith 2000, Guo et al. 2006), these maps have relied on interspecific crosses to generate the polymorphism

levels needed for development of saturated maps. While this approach has provided important information regarding the genomic structures of both *G. hirsutum* and *G. barbadense*, it has also limited the ability to differentiate the genomes. Translocations and inversions which conceivably distinguish the two species may not be identified via interspecific linkage mapping. Furthermore, the coalescence of linkage groups such that their number equals the haploid chromosome number has proven difficult in cotton, perhaps due to large amounts of recombination in cotton. Only recently was a linkage map with the expected 26 linkage groups completed (Rong et al. 2004). The need for verification of the linkage maps, and a map with improved relationships to physical structures calls for the development and use of complementary technologies, especially those amenable to high-throughput analysis.

As genome mapping technologies have advanced, the number of molecular markers in cotton has rapidly expanded. Cotton researchers around the world have focused heavily on SSRs, because they are frequently codominant, cheap, and portable between populations. Several groups have contributed to SSR marker development, including: BNL (Liu et al. 2000), CIR (Nguyen et al. 2004), NAU (Zhang et al. 2002), JESPR (Reddy et al. 2001), Gh (Hoffman et al. 2006), and TMB (Yu et al. 2002) markers have all become available in recent years. Many of these efforts are represented in the on-line Cotton Microsatellite Database (CMD) found at <http://www.mainlab.clemson.edu/cmd/>.

The development of a consensus map will allow improved communication and collaboration between groups, allow cotton genomics to move to the next level, and help the research community prepare for genome sequencing. The presence of a unique platform for integration may encourage labs to combine their mapping and marker data on a single, non-competing platform. As a completely new, physically based system, radiation hybrid mapping can provide just such a platform. The research presented in this thesis continues on the work of Gao et al. 2004, 2006 by locating several Gh markers and NAU markers on the RH mapping population for *G. barbadense*.

In addition to aiding basic research in evolution and structural genomics, current maps are providing benefits to applied cotton research. Quantitative trait loci (QTLs) have been identified for traits, especially cotton fiber quality, using both interspecific crosses (Mei et al. 2004, Kohel et al. 2001) and intraspecific crosses (Ulloa and Meredith 2000). Such research is allowing improvements in cotton development via improved marker-assisted selection (MAS) (Abdurakhmonov et al. 2005, Zhang et al. 2003). Furthermore, mapping is providing information that may help cotton breeders overcome some of the difficulties associated with interspecific introgression. Introgression of traits from *G. barbadense* into *G. hirsutum* has proven notoriously difficult in cotton. However, few studies have been undertaken to identify the underlying mechanisms (Jiang et al. 2000). The identification of structural differences between the genomes will help identify the mechanisms responsible for limitations on interspecific introgression between cotton species.

While linkage mapping has provided many benefits to cotton genomics, it also has a variety of limitations. Linkage maps have been used to guide the development of physical maps. By providing a framework on which to align BAC contigs across libraries, linkage maps have enabled improved physical coverage of the cotton genome (Tomkins et al. 2001, Zhang personal communication). However, genetic linkage map distances are not closely related to physical distances, due, for example, to much higher crossover rates in euchromatic than heterochromatic regions. This phenomenon leads to a lack of resolution in some regions of the genome, e.g. pericentromeric regions, while the distances in others, e.g. gene-rich regions, are relatively inflated. In cotton, linkage mapping relied heavily on segregation from interspecific hybrids between *G. hirsutum* and *G. barbadense*, which has largely precluded their use for genome comparisons, i.e. (AD)₁ versus (AD)₂.

Several complementary mapping technologies have been developed to help overcome some of the limitations of linkage mapping. To expand understanding of the relationship between genetic and physical distances, recombination nodule maps have been created for maize (Anderson et al. 2003, Anderson et al. 2004). These utilize

pachytene bivalent spreads to count recombination nodules along chromosomes. By physically measuring the distance between recombination nodules, the average number of crossovers in a selected section of the genome was determined. This development is allowing improved integration of genomic architecture from linkage, cytogenetic, and molecular data.

Table 1: A comparison of genome mapping technologies. Various genome mapping technologies, their applications, resolutions, relative throughput rates, and selected references. These technologies can be combined to answer many important questions in structural genomics.

Technology	Scope of Application	Resolution	Throughput	References
RH mapping	widely used in animals, less used in plants	variable	high	Gao et al. 2004, Gao et al. 2006, Riera-Lizarazu et al. 2000
Linkage mapping	widely used	low	high	Rong et al. 2004, Hoffman et al. 2006, Nguyen et al. 2004
FISH	specialized uses in plants	variable	low	Wang et al. 2006, Kim et al. 2005
HAPPY mapping	rarely used	variable	high	Dear and Cook 1989, Thangavelu et al. 2003
RN mapping	rarely used	low	low	Anderson et al. 2003, Anderson et al. 2004
BAC contigs	widely used	very high	high	Tomkins et al. 2001
Cytogenetic stocks	specialized uses in plants	low	high	Liu et al. 2000

Another technology, HAPPY mapping, is especially promising for the high-resolution integration of linkage and physical maps. By irradiating high molecular weight DNA samples *in vitro* and then diluting them prior to genotyping, HAPPY mapping provides an additional technology for fine mapping. Furthermore, by removing the need for a host parent needed for both RH and linkage mapping, HAPPY mapping, eliminates the need for polymorphism. (Dear and Cook 1989, Thangavelu et al. 2003).

Thus, it has the potential to vastly increase the number of molecular markers which can be placed on a mapping panel.

RH mapping is a method of mapping that uses radiation to induce chromosome breakage. It relies on ionizing radiation to fragment chromosomes via double strand breaks. The frequency of these breaks along the length of a chromosome is related to the radiation dose. As a result of the chromosome breakage, some fragments of the chromosome and the markers located on those fragments are lost as the cell matures and divides. Following near-lethal radiation treatment, the target genome is rescued by uniting it with a host genome. High levels of polymorphism can be obtained by selecting a host genome of a different species than the irradiated genome. Genotyping is used to detect the presence or absence of markers from the target genome. By assuming that the probability of marker separation via chromosome breakage is inversely proportional to the distance between the markers, the relative distances between markers can be determined from the frequencies at which they are coincidentally lost or retained.

Radiation hybrid mapping has been used in human and animal genomics since the mid-1970s (Goss and Harris 1975). The technology developed over the next several years and played a major role in the mapping of the human genome. Modifications to the original procedure allowed the mapping of individual chromosomes via chromosome addition lines (Cox et al. 1990). Throughout the early and mid-1990s, RH mapping was used to create maps of the human genome in preparation for sequencing (Walter et al. 1994, Schuler et al. 1996, McPherson et al. 1997, reviewed in Kelavkar and Ketan 1998). Subsequently, RH mapping was applied to every major animal genome mapping project (Hawken et al. 1999, Rexroad et al. 1999, Williams et al. 2002, Watanabe et al. 1999, Chowdhary et al. 2003, Geisler et al. 1999, Van Etten et al. 1999). More recently, RH tools in animals have found valuable, widespread use in comparative genomics and evolutionary studies (Everts-van der Wind et al. 2005, Shimogiri et al. 2006, Yasue et al. 2006, Liu et al. 2005).

While RH mapping has played a large role in animal genomics for many years, only recently has the technology been applied to plant genomics. One of the earliest

applications of RH mapping to plant genomics involved the study of *Arabidopsis thaliana*. Irradiation of *Arabidopsis* ecotype Landsberg erecta followed by cross pollination with a multi-marker line allowed a proof of concept for the application of RH technology to plants (Vizir et al. 1994). Another proof-of-concept paper in the mid-1990s showed that RH mapping applied to plants could help overcome many of the deficiencies of traditional linkage mapping. RH mapping was used to create a map of tomato chromosome six with a special focus on the centromeric regions, which remain difficult to map via homologous recombination (Liharska et al. 1997). This paper showed that RH mapping could be applied to plant genomes to improve maps of certain areas.

Significant applications of RH technology to plant genomics have only recently occurred. A system to provide RH maps of individual maize chromosomes was described in 2000 by Riera-Lizarazu *et alibi*. The authors used oat-maize chromosome addition lines to isolate individual maize chromosomes. The irradiation of seeds to break up the maize chromosomes allowed the development of RH panels for maize chromosomes (Riera-Lizarazu et al. 2000). Another effort to apply RH technology to plants was published in 2002. Wardrop and her colleagues developed an *in vitro* system to map the barley genome. While the project did meet with some success, difficulties in developing *in vitro* plant cultures and removing chimeras precluded map development (Wardrop et al. 2002). More recently a system to apply radiation hybrid mapping to wheat was developed. This system allows RH mapping of a single wheat chromosome by irradiating the seed of chromosome substitution stocks of wheat followed by cross-pollination. These wheat studies have shown that RH mapping can play an important role in localizing ESTs within a small segment of the genome, an important step in the isolation and cloning of valuable genes (Hossain et al. 2004).

A modified RH-mapping approach was recently developed for whole-genome *in vivo* RH mapping of the cotton genome (Gao et al. 2004). This technology, dubbed Wide-cross Whole-genome Radiation Hybrid Mapping (WWRH), has been demonstrated on a small scale to be capable of yielding genome-wide maps for *G.*

barbadense and *G. hirsutum* (Gao et al. 2004, 2006). Gao et al. (2004) demonstrated feasibility of WWRH of the *G. hirsutum* genome based on segmentation by 5-Kr irradiated of pollen, and rescue by in vivo cross pollination of *G. barbadense*. The principles were confirmed through the reciprocal cross with segmentation by 8-Kr irradiation of *G. barbadense* pollen. Moreover, the higher dosage of irradiation led to clearly superior RH map resolution. These results suggested that the 26-chromosome AD genomes of *Gossypium* could be individually mapped and structurally compared by WWRH analysis, and that cotton RH mapping technologies might be further improved.

In his early efforts at RH mapping of cotton genome, Gao (2003) relied on RHMAP (Boehnke et al. 1996), which is fairly user friendly, but has the limitation of allowing the user to simultaneously analyze only 60 loci. This limitation significantly increases the time needed to compute a map, due to the need to input genotyping data from several different 60-locus subsets of the marker population. Furthermore, reliability is decreased as different subsets of loci may give different results. Since the beginning of the cotton RH mapping project, several new mapping software programs have been developed.

Newer RH mapping programs give superior results and faster analysis than many earlier versions of mapping software. Examples include TSP/CONCORDE, Flipper, and Carthagene. TSP/CONCORDE was developed around the principle of the “traveling salesperson problem” (TSP), and compares favorably to alternative programs for RH mapping (Argawala et al. 2000, Hitte et al. 2003). Another program, Flipper, has also been favorably reviewed (Crane and Crane 2004). Carthagene, like CONCORDE, resolves genotyping data using algorithms that expedite solutions to the traveling salesperson problem (de Givry 2005). It has given reliable results in previous tests, and has the ability to combine raw data from both linkage mapping and RH mapping projects, while most other programs rely on overlaying maps after their creation (Snelling et al. 2004).

RH mapping holds the promise of improving the cotton genome map, preparing for genome sequencing, advancing molecular breeding, and expanding evolutionary

studies. The work presented here will advance development of WWRH and cotton genomics on several fronts, through expansion of the *G. barbadense* map, creation of an improved *G. hirsutum* panel, and possible improvements of mapping software. Indeed, this research resulted in localization of 92 additional markers (87 SSRs) on an existing RH panel developed by Gao et al. (2006) for use in mapping *G. barbadense* line 3-79. A new RH panel was developed for whole-genome mapping of the genetic and genomic standard of *G. hirsutum*, line TM-1. Various RH mapping programs were set up, utilized, and compared for RH data analysis. Two programs: RHMAP and Carthagene, were set up and tested for speed, efficiency, and output using the RH mapping data. This will allow improved analysis as marker localization continues.

MATERIALS AND METHODS

Creation of TM-1 mapping panel

A *G. hirsutum* mapping population comparable to the existing *G. barbadense* mapping population was created using 8-kilorad dosage of gamma rays. A population of *G. hirsutum* line TM-1 and a population of *G. barbadense* line 3-79 were grown to maturity in a greenhouse. Upon flowering, flowers were stripped once to promote synchronized flowering among all population members.

During cross-pollination, TM-1 was used as a male parent and 3-79 was used as female parent. TM-1 flowers were harvested at approximately 7:30 am each morning and irradiated with gamma rays at the TAMU Nuclear Science Center starting at approximately 8 am each morning. Flowers were placed in a plastic bag and lowered into the radiation cell. Flowers were irradiated until a cumulative dose of 8 krad was measured (approximately 1 hour) using an LND ion chamber (LND, Inc.) located on the window. Following irradiation, the flowers were brought back to the greenhouse with cross-pollination occurring at approximately 11 am-1 pm. These steps were repeated M-F for 2 weeks. Over 1000 crosses were made, resulting in 979 mature bolls. Following boll maturation, which varied widely among bolls, seeds were harvested and ginned. The seed were then germinated in “rag dolls”, transferred to Jiffy peat pellets, and later transferred to pots.

Genotyping of molecular markers on the 3-79 panel

Genotyping was completed at the lab of Dr. John Yu at the USDA facility on F&B road, College Station, TX. SSR markers were selected based on demonstrated polymorphism between TM-1 and 3-79, availability of primer sets, and ease of use. This resulted in a selection of markers from multiple SSR sets: Gh (Hoffman et al. 2006), BNL (Liu et al. 2000), NAU (Zhang et al. 2002), and JESPR (Reddy et al. 2001) being genotyped onto the 92-plant mapping population. The mapping panel was genotyped for 87 markers. PCR was performed on an Eppendorf Mastercycler Gradient 5331 (Eppendorf International, Hamburg, Germany). Annealing temperatures were selected according to

performance using a survey on two selected RH panel members. Temperatures included 55 °C, 57°C, and 60 °C. Melting was performed at 94 °C for 30 seconds. Extension was performed at 72°C for either 50 seconds or 25 seconds, followed by a two-minute final extension time at 72°C. Annealing temperatures and extension times for individual markers are listed below.

Table 2: PCR conditions of primer pairs. The annealing temperature and the extension time are given for each primer pair. The marker name is listed in column 1, the annealing temperature is listed in column 2, and the extension time is listed in column 3. Other PCR conditions were constant between primer pairs and are listed in the text.

Marker	Annealing temp (°C)	Extension time (s)	Marker	Annealing temp (°C)	Extension time (s)
Gh			Gh 133	60	50
Gh 2	55	50	Gh 142	55	50
Gh 12	55	50	Gh 153	60	50
Gh 22	60	50	Gh 167	60	50
Gh 27	55	50	Gh 171	57	25
Gh 34	55	50	Gh 182	55	50
Gh 39	55	50	Gh 188	60	25
Gh 48	55	50	Gh 199	57	25
Gh 51	55	50	Gh 200	60	50
Gh 52	55	50	Gh 216	60	50
Gh 56	60	50	Gh 220	60	50
Gh 67	60	50	Gh 246	60	50
Gh 71	60	50	Gh 260	55	50
Gh 73	60	50	Gh 272	55	50
Gh 74	55	50	Gh 277	55	50
Gh 75	55	50	Gh 288	60	50
Gh 83	57	25	Gh 300	60	50

Table 2: Continued.

Marker	Annealing temp (°C)	Extension time (s)
Gh 96	60	50
Gh 98	60	50
Gh 109	60	50
Gh 110	60	50
Gh 111	60	50
Gh 112	60	50
Gh 118	60	50
Gh 119	60	50
Gh 129	60	50
Gh 132	60	50
Gh 459	60	50
Gh 462	60	50
Gh 465	57	25
Gh 466	55	25
Gh 471	57	25
Gh 484	57	25
Gh 495	57	25
Gh 498	60	50
Gh 506	60	50
Gh 513	60	25
Gh 523	57	25
Gh 537	57	25
Gh 539	57	25
Gh 548	57	25
BNL		
BNL 119	55	50
BNL 1034	55	50
BNL 3563	55	50

Marker	Annealing temp (°C)	Extension time (s)
Gh 302	60	50
Gh 329	60	50
Gh 330	60	50
Gh 336	60	50
Gh 345	60	50
Gh 354	60	50
Gh 428	60	50
Gh 441	60	50
Gh 443	60	50
Gh 449	60	25
JESPR		
JESPR 298	57	25
NAU		
NAU 859	55	50
NAU 864	57	25
NAU 882	55	50
NAU 884	55	25
NAU 920	57	25
NAU 986	55	25
NAU 1009	55	25
NAU 1014	55	50
NAU 1063	55	50
NAU 1190	57	25
NAU 1201	57	25
NAU 1231	52	25
NAU 1246	57	25
NAU 1278	57	25

PCR products were separated in an Owl separation systems gel box using 2% GenePure agarose and 2% GenePure high-resolution agarose (ISC BioExpress, Kaysville, UT). Gels were scored based on the presence or absence of the band size expected for 3-79 at the selected locus. A picture of a gel is below with an explanation of scoring procedures.

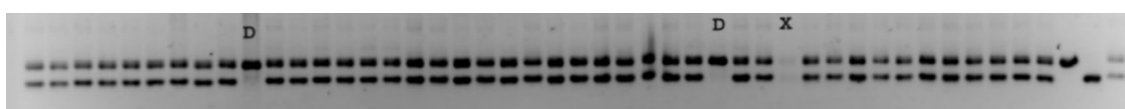


Figure 1: Gel scoring methods. A typical agarose gel. Each well represents one member of the RH population. The last well, which contains 2 bands, is a non-irradiated F₁. The second to last well is the 3-79 parent and the third to last well is the TM-1 parent. Note that two wells are marked with “D”, these are population members that have had the 3-79 marker deleted by irradiation. The well marked “X” is unscorable.

Advances in software usage

Carthagene was selected for data analysis due to its high performance ratings in past studies and its unique ability to combine linkage mapping data with RH mapping data, which will result in an improved consensus map (Schiex and Gaspin 1997, de Givry et al. 2004). Carthagene was compared to RHMAP, the program used in the original analysis of RH data for this project (Gao 2004). Additionally, the retention models in each program were compared to determine which gives the most reliable results for a selected syntenic group from chromosome 11. The same data set, for markers BNL 3442, BNL 1034, BNL 3431, BNL 4094, BNL 3411, BNL 1404, BNL 2632, BNL 1681, Gh 246, and BNL 2805, was analyzed using each available retention model. The retention models RHMAP were “equal retention”, “centromeric retention”, “left endpoint retention” and “general retention” on RHMAP and for Carthagene the “equal retention” model was used.

RESULTS

Creation of TM-1 RH mapping panel

Over 1000 interspecific crosses of *G. barbadense* line 3-79 flowers were made with pollen from irradiated TM-1 flowers, from which 979 bolls were harvested. There was a high occurrence of moths in the harvested seed, as reported by Gao et al. 2004. Seed from randomly selected crosses were planted until a mapping population of 115 plants was created. Phenotypes of the resulting plants were extremely varied in morphology and size (Figures 2,3).



Figure 2: Leaf variation in TM-1 mapping population. Four leaves taken from selected plants from the TM-1 mapping population. Variation in size, color, and shape are representative of the wide range of phenotypes.



Figure 3: Whole plant phenotypic variation of the TM-1 8-krad mapping population. Three plants from the TM-1 mapping population. All plants are the same age but vary widely in size, shape, and maturity.

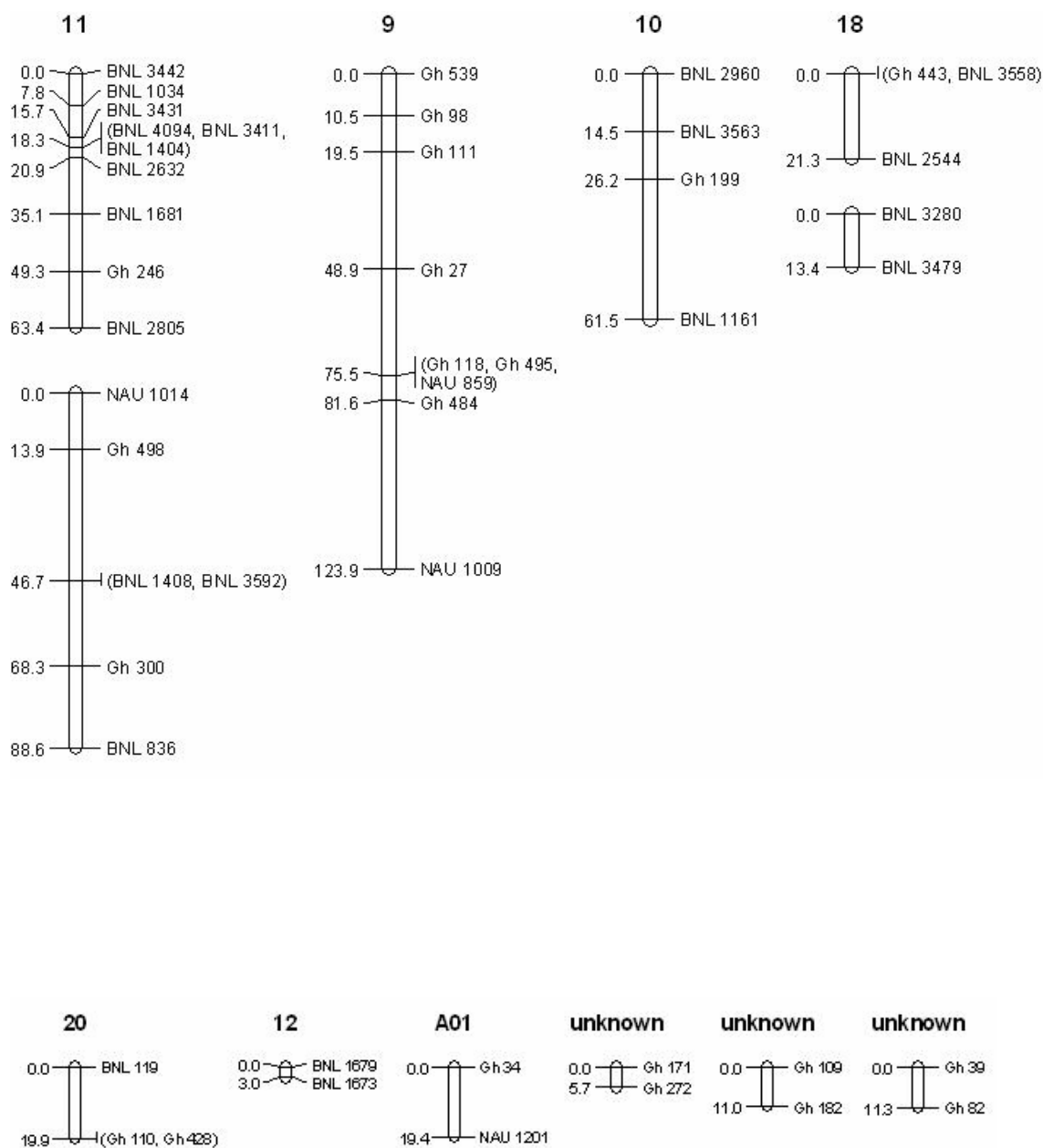


Figure 4: Map created using Carthagene. The syntenic groups shown above were identified using Carthagene. Distances are listed in centiRays. The syntenic groups listed were identified by comparison with known public markers contained in the respective syntenic groups and previously listed in the Cotton Microsatellite Database. Two separate syntenic groups were identified for chromosomes 11 and 18, respectively.

Genotyping of SSRs onto the 8-Krad 3-79 WWRH panel

The WWRH mapping panel of *G. barbadense* 3-79 was genotyped for 87 SSR molecular markers. Each marker was scored for the presence or absence of the 3-79 parental band. From the 87 markers, 64 were selected to form a framework map, based on the most reliably scored markers.

Comparison of Carthagene and RHMAP software

The program RHMAP (Boehnke 1996) was used in the original RH mapping project (Gao et al. 2004). Since that time, the new, highly rated program Carthagene, has been developed for creating RH maps. To prepare for future RH work, a comparison of the two programs was run to determine which one to use as the project continues.

RHMAP is a Fortran 77 program that runs on the LINUX (UNIX) operating system. The program contains three separate packages for various forms of data analysis. Rh2pt is a two-point analysis program that provides linkage group identities. Rhminbrk provides a minimum breaks analysis that can be used after the two point analysis. Rhmaxlik provides a maximum likelihood analysis. For this project only rh2pt and rhmaxlik analyses were performed.

Carthagene was first developed in 1995 and has been updated several times since. It can be run on either LINUX (UNIX) or Windows, for purposes of this study the Windows version was used.

I generally found Carthagene to be easier to use. The input requirements for Carthagene are much simpler and the output is in a more useable format. One limitation of the Carthagene is the inability to use any models other than the equal retention model. RHMAP can, on the other hand, make use of the equal retention model, centromeric retention model, left-endpoint retention model, or general retention model.

The various retention models give widely varying results. Of the four maps produced by the various retention models, the equal retention model from Carthagene was the shortest at only 63.4 cR. The left endpoint retention model in RHMAP provided a distance of 72.2 cR, the centromeric retention model provided a map of 73.1 cR and

the equal retention model gave a syntenic group length of 75.1 cR. Following analysis using Carthagene and RHMAP, maps were drawn using Mapchart (Voorrips 2002).

While differences in ease of use differentiated RHMAP and Carthagene, we can see that the programs also give different results. While adjusting the parameters for Carthagene does result in an increase in the number of linked markers, linkage distances greater than 40 cR are of questionable reliability, thus 40 cR was used as the maximum linkage threshold.

Table 3: Comparison of syntenic groups obtained from two-point analysis using RHMAP and Carthagene. Markers identified as syntenic using RHMAP (top table Carthagene at LOD score 4 and maximum distance of 40 cR. Gh 506 and Gh 548 are not identified as syntenic using Carthagene with the selected parameters. BNL 3895 is not identified as syntenic to any markers using Carthagene with the selected parameters. Markers differentially linked by RHMAP and Carthagene are denoted by the bold typeface

Syntenic Groups from RHMAP and Carthagene

RHMAP (LOD 4)

BNL 119, Gh 110, Gh 428
Gh 27, Gh 98, Gh 111, Gh 118, Gh 484, Gh 495, Gh 539, NAU 859, NAU 1009
Gh 34, NAU 1201
Gh 39, Gh 82
Gh 56, Gh 345
Gh 109, Gh 182
Gh 171, Gh 272
Gh 199 L, BNL 2960, BNL 1161, BNL 3563, BNL 3895
Gh 246, Gh 329, BNL 1681, BNL 3411, BNL 1404, BNL 3431, BNL 1034, BNL 3442, BNL 4094, BNL 2632, BNL 2805
Gh 300, Gh 498, NAU 1014, BNL 1408, BNL 3592, BNL 836
Gh 443, BNL 3558, BNL 2544
BNL 1673, BNL 1679
BNL 3280, BNL 3479
Gh 506, Gh 548

Table 3: Continued

Carthagene (LOD 4)

BNL 119, Gh 110, Gh 428

Gh 27, Gh 111, Gh 539, Gh 98, NAU 859, Gh 495, Gh 484, Gh 118, NAU 1009

Gh 34, NAU 1201

Gh 39, Gh 82

Gh 56, Gh 345

Gh 109, Gh 182

Gh 171, Gh 272

Gh 199 L, BNL 2960, BNL 1161, BNL 3563

Gh 246, Gh 329, BNL 1681, BNL 3411, BNL 1404, BNL 3431, BNL 1034, BNL 3442, BNL 4094, BNL 2632, BNL 2805

Gh 300, Gh 498, NAU 1014, BNL 1408, BNL 3592, BNL 836

Gh 443, BNL 3558, BNL 2544

BNL 1673, BNL 1679

BNL 3280, BNL 3479

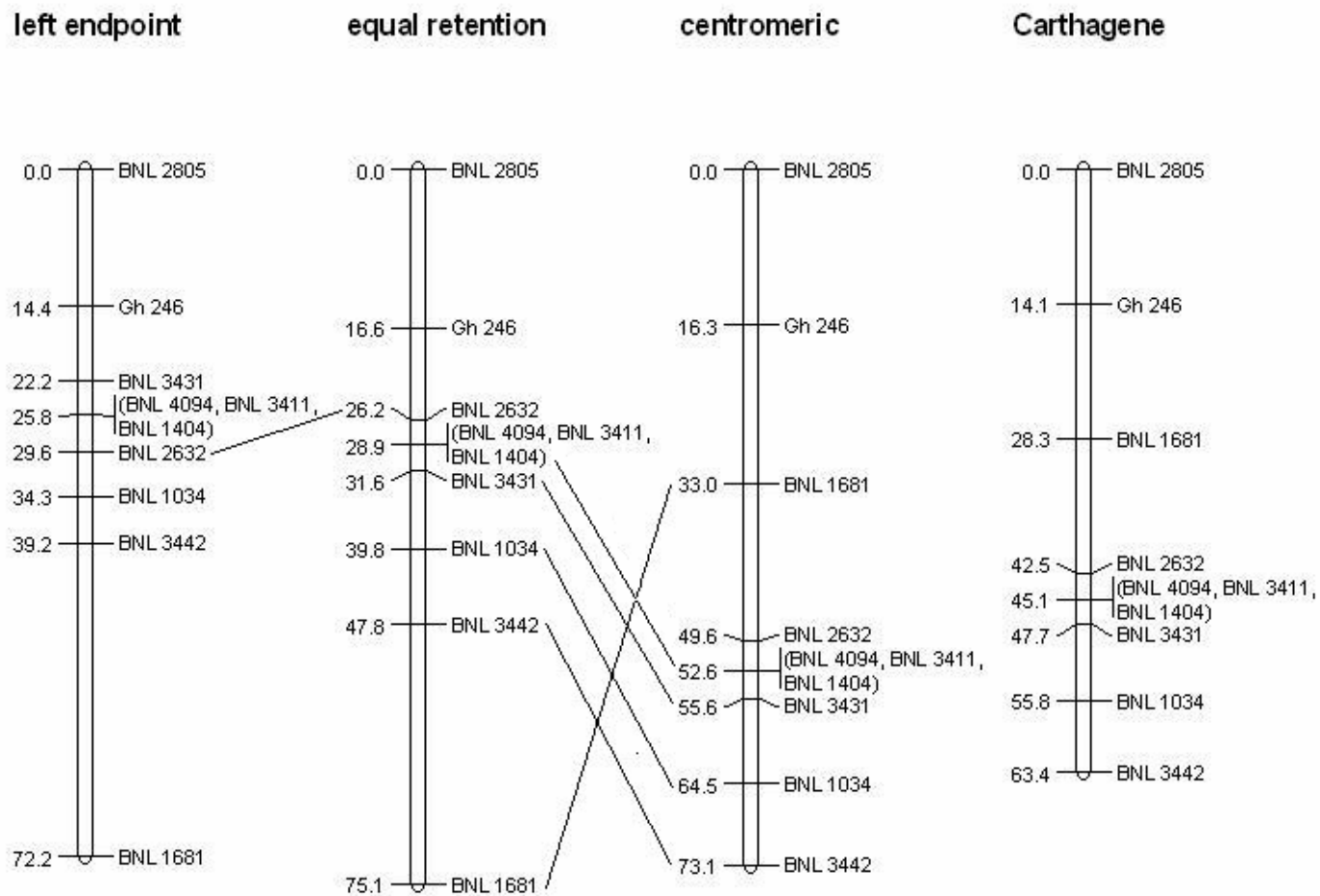


Figure 5 Comparison of radiation hybrid mapping retention models. The possible maps for a selected syntenic group from c11. Each map was created using the same input data, but was developed using three marker retention models from RHMMap left endpoint retention, equal retention, and centromeric retention, and a model from Carthagene (equal retention).

DISCUSSION

Results from this research contributed to development of radiation hybrid mapping technology for cotton by expanding the set of genotyped markers, creating a new mapping population for *G. hirsutum*, and exploring alternative analysis software. This thesis expands the previous RH map of *G. barbadense* (Gao et al. 2006) from 31 to 92 markers. Following genotyping, data analysis resulted in creation a map containing 47 markers across 7 known linkage groups, plus three additional syntenic groups for which chromosomes have not yet been identified. Future research is planned to identify the chromosomal location of these syntenic groups by locating markers on monosomic stocks and/or linkage mapping populations with identified linkage groups. The previous cotton RH map (Gao et al. 2006) has been adjusted and corrected with the new marker data. Two syntenic groups that were previously identified as chromosome 17 now have been shown to provide coverage of chromosome 11 (Gutierrez and Stelly, personal communication). A primary focus of further research will be to genotype more markers previously mapped to chromosome 11 (c11) in a attempt to connect these two syntenic groups and provide improved coverage of c11. A single syntenic group with 9 markers was identified as c09. A single syntenic group with 4 markers was identified as part of c10. Two syntenic groups with a total of 5 markers were identified as c18. Completion of chromosome 18 should be a significant focus of future research. Furthermore, syntenic groups of 2-3 markers have been identified for chromosomes 12, 13, and 20. Three syntenic groups of 2 markers each have been identified, but their chromosomal identities are currently unknown.

The data analysis has yielded a new map based on the combination of data from Gao et al. (2006) and newly genotyped SSR markers (Figure 3). The map confirms many aspects of the traditional linkage map. We have seen that adding additional markers to the mapping panel results in expansion and improved analysis of the syntenic groups.

The new 8-Kr WWRH panel of *G. hirsutum* cv. TM-1 will provide an excellent comparison to the 8-Kr WWRH of *G. barbadense*, and supercede the 5-Kr panel first reported by Gao et al. (2004). The new panel was obtained after making relatively large numbers of manual cross-pollinations, and germinating relatively large numbers of seed. The difficulty of obtaining viable seed after such high irradiation, and the extensive phenotypic variability observed in the population of WWRH plants suggest that the new WWRH panel will allow mapping of the genetic standard for *G. hirsutum* (TM-1). A population of 92 plants has been selected for genotyping.

We have also compared a range of retention models and software algorithms. The results of this analysis can be seen in Figure 5. Analysis using Carthagene has provided the shortest map, with a length of 63.4 cR. Carthagene utilizes the equal retention model for its analysis, making it interesting to note that the map produced by Carthagene most closely resembles the centromeric retention model produced using RHMAP. Meanwhile, the analysis using the equal retention model in RHMAP produced the longest map at 75.1 cR. This map also contains several differences in marker order from the Carthagene map, which are denoted with lines in Figure 2. Particularly as the number of loci analyzed increases, Carthagene has significant demonstrable advantages. It can be run on multiple operating systems (OS), reducing the need to utilize more than one OS. It can also analyze more than 60 markers at a time. In fact, it has been demonstrated to handle thousands of markers simultaneously. The Carthagene analysis results in a syntenic group with a \log_{10} likelihood of -65.24. The RHMAP \log_{10} -likelihoods of the same data set (from c11) are as follows: left endpoint: -61.37, equal retention: -66.14, centromeric retention: -64.40.

Several differences were found upon comparison to the map produced by Gao et al. (2006) which was produced by following the general retention model. Whereas they located the BNL 1161 marker between BNL 2960 and BNL 3563, my analysis places it 47 cR away from BNL 3563, and the closest marker to BNL 1161 among those currently genotyped on the panel is Gh 199, which is 35.3 cR away. It also placed BNL 2960 and BNL 3563 closer to each other, 14.5 cR apart. Our current map does not provide

evidence of association between BNL 1665 and any other markers which have been traced to c10. While this is not evidence that the marker is not on c10, this is another point of discordance with Gao et al. (2006). Our data also does not identify synteny between BNL 1066 and either BNL 836 or BNL 1408 and BNL 3592.

A comparison to current linkage maps reveals many similarities to our RH map (Table 4). A comparison to the recent linkage map by Han et al. (2006) shows many similarities between the linkage groups. Linkage group A03, which has been identified as c11 by both Wang et al. (2006), and Gutierrez and Stelly (personal communication), shares many similarities between the markers in the linkage groups. Common markers are: BNL 1408, NAU 1014, BNL 2632, BNL 4094, BNL 1681, BNL1404, BNL 3411, BNL 3431, and BNL 3442. However, we also locate BNL1034 on c11, while Han et al. (2006) place the marker on its homeologue, D02. Also, there are significant differences in the marker order between the maps for this linkage/syntenic group. These can likely be attributed to differences in parental genomes, panel creation and markers genotyped, as the groups used very different methods to create the mapping populations and genotyped several different markers. Additional comparisons to the Han et al. (2006) map can be made for chromosomes 10 and 18.

We have genotyped a number of markers which trace to c10, including BNL 256, BNL1665, BNL3895, BNL 1161, BNL 3563, and BNL 2960. Our map shows three of the above markers to be syntenic: BNL 2960, BNL 3563, and BNL 1161. These markers appear on the same order in both maps. While several more markers on both the Han et al. (2006) map and the RH map trace to c10, the Han map shows them distantly linked. It is likely the RH map is simply not saturated enough to indicate linkage between the rest of the markers on c10. A comparison of c18 reveals 4 common markers: BNL 3558, BNL 2544, BNL 3280, and BNL 3479. Our map shows these markers in two separate syntenic groups. The Han map shows the syntenic groups are separated from each other by 15.5 cM. This seeming discordance between the RH and linkage map implies that the crossover rates (cM/Mbp) for this section of c18 are extremely low and/or that susceptibility to radiation damage is very high. BNL 3479 and BNL 3280 could not be

separated by the linkage map, our map places these markers 13.4 cR apart. The Han map also shows BNL 3558 and BNL 2544 are 18.6 cM apart. This concordance between linkage and RH distances implies that these markers are in a higher recombination region of the chromosome. These comparisons between the RH and linkage maps suggest that continued analysis of c18 on the RH panel will improve the understanding the chromosome structure. The different maps of c18 provided by radiation hybrid technology and homologous recombination illustrate the potential future benefits of Carthagene. The program's ability to combine raw data from both linkage mapping and RH mapping populations may localize and order additional markers. While no such analysis was performed in this research, the clear differences between the technologies imply that future benefits would derive from the combination of data gained from each.

The map developed by Nguyen and colleagues (Nguyen et al. 2004) also bears several similarities to our map. On linkage group A03 (c11) common markers are: BNL 836, BNL 3592, BNL 1408, BNL 2805, BNL 4094, BNL 1681, BNL 3411, BNL 1404, BNL 3431, BNL 1034, and BNL 3442. A comparison of this linkage/syntenic groups between the two maps provides additional evidence of the complementarity of linkage mapping and RH mapping. Neither map was able to separate BNL 3592 and BNL 1408, providing evidence that both mapping populations have resolution limits. The linkage map however, was able to separate BNL 3411 and BNL 1404, although they are located only 3 cM apart whereas the RH map places them in the same bin. The fact that Nguyen et al. (2004) observed recombination (13 cM) between BNL 2805 and BNL 1458 whereas these loci were not detectably syntenic in the WWRH panel suggests that these two loci occur in a large segment with very low recombination. The linkage map (Nguyen et al. 2004) and the RH map (Gao et al. 2006) share 3 common c10 markers: BNL 2960, BNL 3563, and BNL 1161, all of which are linked and syntenic, and in the same order. This provides another good example of the concordance between RH and linkage mapping. The maps of c18 share 4 common markers, which exemplify the complementarity between linkage and RH mapping. In the RH map, these markers

appear in two unlinked syntenic groups: BNL 3558 and BNL 2544 are 21.3 cR apart and BNL 3280 and BNL 3479 are 13.4 cR apart. In the linkage map, BNL 3479 and BNL 3280 are only 2 cM apart while BNL 3558 and BNL 2544 are 30 cM apart. Meanwhile, BNL 3280 and BNL 3558 are 18 cM apart. The seeming discordance here implies that the segment between BNL 3479 and BNL 3280 is extremely low recombination. The segment between BNL 3280 and BNL 3558 is also fairly low recombination. The segment between BNL 3558 and BNL 2544 is a higher recombination area.

Table 4 : Comparison between linkage maps and RH map. The distances between selected marker pairs from two linkage maps (Nguyen et al. 2004, Han et al. 2006). Distances for the two linkage maps are provided in centiMorgans (cM), distances for the RH map are provided in centiRays (cR). UM indicates a marker that was not mapped on that population. NS indicates that the markers were genotyped but no synteny was found in the WWRH population.

Marker pair	Nguyen et al.	Han et al.	RH map
c11/Lg A03			
BNL 3442 - BNL 1034	13 cM	UM	7.8 cR
BNL 3442 - BNL 3431	40 cM	39.3 cM	15.7 cR
BNL 3431 - BNL 3411	10 cM	5 cM	2.6 cR
BNL 3411 - BNL 1404	3 cM	0.7 cM	0 cR
BNL 1404 - BNL 1681	27 cM	20.2 cM	16.8 cR
BNL 2632 - NAU 1014	UM	4.4 cM	NS
BNL 2805 - BNL 1408	13 cM	UM	NS
BNL 1408 - BNL 3592	0 cM	UM	0 cR
BNL 1408 - BNL 836	9 cM	UM	41.9 cR
c10			
BNL 2960 - BNL 3563	4 cM	3.3 cM	14.5 cR
BNL 3563 - BNL 1161	33 cM	14.6 cM	47 cR
c18			
BNL 3558 - BNL 2544	30 cM	18.6 cM	21.3
BNL 3558 - BNL 3280	15.5 cM	18 cM	NS
BNL 3280 - BNL 3479	0 cM	2 cM	13.4 cM

RH technology provides a complementary mapping tool to the more commonly used linkage mapping. RH mapping contains a physical component lacking in linkage mapping and isolates the mapping to a single genome. Current linkage mapping technologies do not allow comparable mapping of the cultivated cotton species *G. barbadense* and *G. hirsutum*. Most systems to date have relied on interspecific crosses to generate polymorphism levels required for saturated mapping. This has increased polymorphism rates but causes the resulting map to be influenced by interspecific genomic differences and their effect on crossover rates. Systems have also been developed to allow intraspecific linkage mapping between *G. hirsutum* parental lines. These eliminate the interspecific crossover effects, but face reduced polymorphism and do not preclude the influential effects of intraspecific genomic variation. To our knowledge, no intraspecific linkage mapping systems have been developed for *G. barbadense*. While there may be benefits to such systems, they would likely not allow comparative mapping of *G. hirsutum* and *G. barbadense*, as markers which are polymorphic among lines of one species may not show intraspecific polymorphism in the other.

While RH mapping has many advantages, it is not without its weaknesses. Empirically, we can assume that not all sections of the cotton genome are equally susceptible to radiation damage and/or retention. Euchromatic regions are more likely to face radiation breaks than the better shielded heterochromatic regions (Durante et al. 2002, Kawata et al. 2002, Kawata et al. 2004, Durante et al. 2004). The effects that these differences can have are clearly projected by Figure 2, which shows differences among the resulting maps, each based on different input assumptions about radiation damage and fragment retention patterns. The ability of plants to withstand radiation damage is likely correlated to deletion of vital genes. In the case of pollen, i.e. microgametophyte, damage to the generative nucleus would generally be independent of damage to the vegetative nucleus, so selection due to induced changes of genotype expression would potentially be low, for the gametophyte, but significant for the zygote

and endosperm. Endosperm effects would inferably affect zygote recovery but not the genotype, aside from potential epigenetic effects and physiological effects on development. Zygotic defects would inferably affect zygotic recovery, as well as zygotic genotype and expression.

Future work should heavily emphasize expanding marker analysis of both the TM-1 and 3-79 RH panels and their comparison. For future analysis I recommend use of Carthagene rather than RHMAP due to its faster analysis, ability to handle larger numbers of markers, and improved results.

SUMMARY

As the world's most important fiber crop, cotton plays a major role in the world economy. Furthermore, the evolutionary history of cotton provides unique insights into the importance of polyploidy in plants. As such, cotton is an important target for scientific study. Structural genomics studies have proven valuable in many crops, paving the way for genome sequencing, gene cloning, and marker assisted selection. In cotton, there have been many genome mapping studies, and these have made use of a variety of technologies, including linkage mapping, cytogenetic stocks, FISH, and BAC contig development. Each of these approaches has provided important information about the cotton genome, but the complexity of data demands development of a consensus map. Radiation hybrids have played an important role in the development of animal genomics in recent decades. They have allowed mapping of numerous animal genomes, but have been little used in plants, yet radiation hybrid mapping could facilitate consensus map development in cotton and improve overall map quality. This research builds on previous research into radiation hybrids in cotton. We have developed a new mapping population to allow mapping of the *Gossypium hirsutum* genetic standard inbred line 'TM-1'. We have localized additional markers onto the existing mapping panel for the *G. barbadense* line 3-79. We have utilized Carthagine data analysis software to provide improved analysis of existing data. A total of 92 new markers have been genotyped onto the RH panel for *G. barbadense* and data are available publicly for use in continued mapping efforts. Data analysis localized 47 of the markers to syntenic groups.

LITERATURE CITED

- Abdurakhmonov, I. Y., A. A. Abdullaev, S. Saha, Z. T. Buriev, D. Arslanov *et. al.* 2005 Simple sequence repeat marker associated with a natural leaf defoliation trait in tetraploid cotton. *J. Hered.* **96**: 644-653.
- Ananiev, E. V., O. Riera-Lizarazu, H. W. Rines and R. L. Phillips, 1997 Oat-maize chromosome addition lines: a new system for mapping the maize genome. *Proc. Natl. Acad. Sci. USA.* **94**: 3524-3529.
- Anderson, L. K., G. G. Doyle, B. Brigham, J. Carter, K. D. Hooker *et. al.* 2003 High-resolution crossover maps for each bivalent of *Zea mays* using recombination nodules. *Genetics* **165**: 849-865.
- Anderson, L. K., N. Salameh, H. W. Bass, L. C. Harper, W. Z. Cande *et. al.* 2004 Integrating genetic linkage maps with chromosome structure in maize. *Genetics.* **166**: 1923-1933.
- Argawala, R., D. L. Applegate, D. Maglott, G. D. Schuler and A. A. Schäffer, 2000 A fast and scalable radiation hybrid map construction and integration strategy. *Genome Res.* **10**: 350-364.
- Baumel, A., M. L. Ainouche, and J. E. Levasseur, 2001 Molecular investigations in populations of *Spartina anglica* C.E. Hubbard (Poaceae) invading coastal Brittany (France). *Mol. Ecol.* **10**: 1689-1701.
- Baumel, A., M. Ainouche, R. Kalendar, and A. H. Schulman, 2002 Retrotransposons and genomic stability in populations of the young allopolyploid species *Spartina anglica* C.E. Hubbard (Poaceae). *Mol. Biol. Evol.* **19**: 1218-1227.
- Beasley, J.O., 1942 Meiotic Chromosome Behavior in Species, Species Hybrids, Haploids, and Induced Polyploids in *Gossypium*. *Genetics* **27**: 25-54.
- Boehnke, M., K. Lunetta, E. Hauser, K. Lange, J. Uro and J. van der Stoep 1996. RHMAP: Statistical Package for Multipoint Radiation Hybrid Mapping. (Version 3.0). <http://csg.sph.umich.edu/boehnke/rhmap.php>.
- Boehnke, M., K. Lange and D. R. Cox, 1991 Statistical methods for multipoint radiation hybrid mapping. *Am. J. Hum. Genet.* **49**: 1174-1188.
- Brubaker, C. L., A. H. Paterson, J. F. Wendel, 1999 Comparative genetic mapping of allotetraploid cotton and its diploid progenitors. *Genome* **42**: 184-203.

- Chowdhary, B. P., T. Raudsepp, S. R. Kata, G. Goh, L. V. Millon *et. al.*, 2003 The first-generation whole-genome radiation hybrid map in the horse identifies conserved segments in human and mouse genomes. *Genome Res.* **13**: 742-751.
- Cox, D. R., M. Burmeister, E. R. Price, S. Kim and R. M. Myers, 1990 Radiation hybrid mapping: a somatic cell genetic mapping method for constructing high-resolution maps of mammalian chromosomes. *Science.* **250**: 245-250.
- Crane, C. F. and Crane, Y. M., 2004 Flipper: A general, high-capacity genetic mapping program. *Intl. Plant Animal Genome Conf.* San Diego, CA. computer: poster and demo C993.
- Dear, P. H. and P. R. Cook, 1989 Happy mapping: a proposal for linkage mapping the human genome. *Nucleic Acids Res.* **17**: 6795-6807.
- De Givry, S., M. Bouchez, P. Chabrier, D. Milan and T. Schiex, 2005 Carthagene: multipopulation integrated genetic and radiation hybrid mapping. *Bioinformatics.* **21**: 1703-1704.
- Denham, J. H. , 1924 The cytology of the cotton plant: II. Chromosome numbers of old and new world cottons. *Annals of Botany.* **38**: 433-438.
- Durante, M., K. George, H. Wu, F. A. Cucinotta, 2002 Karyotypes of human lymphocytes exposed to high-energy iron ions. *Radiat. Res.* **158**: 581-590.
- Endrizzi, J. E. 1966 Additional information on chromosomal structural changes and differentiation in *Gossypium*. *J. Arizona Acad. Sci.* **4**: 28-34.
- Everts-van der Wind A., D. M. Larkin, C. A. Green, J. S. Elliot, C. A. Olmstead *et. al.*, 2005 A high-resolution whole-genome cattle-human radiation hybrid map reveals details of mammalian chromosome evolution. *Proc. Natl. Acad. Sci. USA.* **102**: 18526-18531.
- Feldman, M., B. Liu, G. Segal, S. Abbo, A. A. Levy *et. al.*, 1997 Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. *Genetics* **147**: 1381-1387.
- Gao, W., Z. J. Chen, J. Z. Yu, D. Raska, R. J. Kohel *et. al.*, 2004 Wide-cross whole-genome radiation hybrid mapping of cotton (*Gossypium hirsutum* L.). *Genetics* **167**: 1317-1329.
- Geisler, R., G. J. Rauch, H. Baier, F. van Bebber, L. Bross *et. al.*, 1999 A radiation hybrid map of the zebrafish genome. *Nat. Genet.* **23**: 86-89.

- Goss, S. J. and H. Harris, 1975 New method for mapping genes in human chromosomes. *Nature*. **255**: 680-684.
- Gyapay, G., K. Schmitt, C. Fizames, H. Jones, N. Vega-Czarny *et. al.*, 1996 A radiation hybrid map of the human genome. *Hum. Mol. Genet.* **5**: 339-346.
- Han, Z., C. Wang, X. Song, W. Guo, J. Gou *et. al.*, 2006 Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. *Theor. Appl. Genet.* **112**: 430-439.
- Hanson, R. E., M. S. Zwick, S. Choi, M. N. Islam-Faridi, T. D. McKnight *et. al.*, 1995 Fluorescent *in situ* hybridization of a bacterial artificial chromosome. *Genome* **38**: 646-651.
- Hawken, R. J., J. Murtaugh, G. H. Flickinger, M. Yerle, A. Robic *et. al.*, 1999 A first generation porcine whole-genome radiation hybrid map. *Mamm. Genome* **10**: 824-830.
- Hitte, C., T. D. Lorentzen, R. Guyon, L. Kim, E. Cadieu *et. al.*, Comparison of MultiMap and TSP/CONCORDE for constructing radiation hybrid maps. *J. Hered.* **94**: 9-13.
- Hoffman, S. M., J. Z. Yu, R. J. Kohel, R. G. Cantrell, J. Xiao, A. E. Pepper. 2006 Characterization of 656 new SSR markers developed from *Gossypium hirsutum* sequences. *Intl. Plant Animal Genome Conf.* San Diego, CA. poster P157.
- Hossain, K. G., O. Riera-Lizarazu, V. Kalavacharla, M. I. VAlez, S. S. Maan, and S. F. Kianian, 2004 Radiation hybrid mapping of the species cytoplasm-specific (*scs^{ae}*) gene in wheat. *Genetics* **168**: 415-423.
- Jiang, C. -X., P. W. Chee, X. Draye, P. L. Morrell, C. W. Smith *et. al.*, 2000 Multilocus interactions restrict gene introgression in interspecific populations of polyploid *Gossypium* (cotton). *Evolution*. **54**: 798-814.
- Ji, Y., D. A. Raska, T. D. McKnight, M. N. Isalm-Faridi, C. F. Crane *et. al.*, 1997 Use of meiotic FISH for identification of a new monosome in *Gossypium hirsutum* L. *Genome*. **40**: 34-40.
- Ji, Y., W. A. Raska, M. DeDonato, M. N. Islam-Faridi, H. J. Price *et. al.*, 1999 Identification and distinction among segmental duplication-deficiencies by fluorescent *in situ* hybridization (FISH)-adorned multivalent análisis. *Genome* **42**: 763-771.
- Ji, Y., M. DeDonato, C. F. Crane, W. A. Raska, M. N. Islam-Faridi *et. al.*, 1999 New ribosomal RNA gene locations in *Gossypium hirsutum* mapped by meiotic FISH. *Chromosoma* **108**: 200-207.

Kawata, T., H. Ito, K. Motoori, T. Ueda, N. Shigematsu *et. al.*, 2002 Induction of chromatin damage and distribution of isochromatid breaks in human fibroblast cells exposed to heavy ions. *J. Radiat. Res.* **43: Suppl.** S169-S173

Lacape, J. –M., T. –B. Nguyen, S. Thibivilliers, B. Bojinov, B. Courtois *et. al.* 2003 A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome* **46:** 612-626.

Leitch, I. J. and M. D. Bennett, 1997 Polyploidy in angiosperms. *Trends Plant Sci.* **2:** 470-476

Liharska, T. B., J. Hontelez, A. van Kammen, P. Zabel, M. Kooreneef, 1997 Molecular mapping around the centromere of tomato chromosome 6 using irradiation-induced deletions. *Theor. Appl. Genet.* **95:** 969-974.

Liu, B., C. L. Brubaker, G. Mergeai, R. C. Cronn, and J. F. Wendel, 2001 Polyploid formation in cotton is not accompanied by rapid genomic changes. *Genome* **44:** 321-330.

Liu, S., S. Saha, D. Stelly, B. Burr, and R. G. Cantrell, 2000 Chromosomal assignments of microsatellite loci in cotton. *J. Hered.* **91:** 326-332.

Liu, W. S., K. Eyer, H. Yasue, B. Roelofs, H. Hiraiwa *et. al.*, 2005 A 12,000-rad porcine radiation hybrid (IMNpRH2) panel refines the conserved synteny between SSC12 and HSA17. *Genomics* **86:** 731-738.

McPherson, J. D., B. Apostol, C. B. Wagner-McPherson, S. Hakim, R. G. Del Mastro *et. al.*, 1997 A radiation hybrid map of human chromosome 5 with integration of cytogenetic, genetic, and transcript maps. *Genome Res.* **7:** 897-909.

Mei, M., N. H. Syed, W. Gao, P. M. Thaxton, C. W. Smith *et. al.*, 2004 Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). *Theor. Appl. Genet.* **108:** 280-291.

Nguyen, T. –B., M. Giband, P. Brottier, A. –M. Risterucci, J. –M. Lacape, 2004 Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. *Theor. Appl. Genet.* **109:** 167-175.

Reddy, O. U. K., A. E. Pepper, I. Abdurakhmonov, S. Saha, J. N. Jenkins *et. al.*, 2001 New dinucleotide and trinucleotide microsatellite marker resources for cotton genome research. *J. Cot. Sci.* **5:** 103-113.

- Reinisch, A.J., J. Dong, C.L. Brubaker, D.M. Stelly, J.F. Wendel, *et. al.*, 1994 A detailed RFLP map of cotton, *Gossypium hirsutum* x *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. *Genetics* **138**: 829-847
- Rexroad, C. E., J. S. Schlapfer, Y. Yang, B. Harlizius and J. E. Womack, 1999 A radiation hybrid map of bovine chromosome 1. *Anim. Genet.* **30**: 325-332.
- Reyes-Valdez, M. H., Y. Ji, C. F. Crane, J. F. Taylor, M. N. Islam-Faridi *et. al.*, 1996 ISH-facilitated analysis of meiotic bivalent pairing. *Genome.* **39**: 784-792.
- Riera-Lizarazu, O., M. I. Vales, E. V. Ananiev, H. W. Rines, and R. L. Phillips, 2000 Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. *Genetics* **156**: 327-339.
- Rong, J., C. Abbey, J. E. Bowers, C. L. Brubaker, C. Chang *et. al.*, 2004 A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission, and evolution of cotton (*Gossypium*). *Genetics* **166**: 389-417.
- Rungis, D., D. Llewellyn, E. S. Dennis and B. R. Lyon, 2005 Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. *Aust. J. Agr. Res.* **56**: 301-307.
- Saha, S., J. Wu, J.N. Jenkins, J.C. McCarty, Jr., O.A. Gutierrez *et. al.*, 2004 Effect of chromosome substitutions from *Gossypium barbadense* L. 3-79 in to *G. hirsutum* L. TM-1 on agronomic and fiber traits. *J. Cot. Sci.* **8**: 162-169.
- Saha, S., J. N. Jenkins, J. Wu, J. C. McCarty, O. A. Gutiérrez *et. al.*, 2006 Effects of chromosome-specific introgression in upland cotton on fiber and agronomic traits. *Genetics.* **172**: 1927-1938.
- Schuler, G. D, M. S. Boguski, E. A. Stewart, L. D. Stein, G. Gyapay *et. al.*, 1996 A gene map of the human genome. *Science* **274**: 540-546.
- Shimogiri, T., S. Kiuchi, H. Hiraiwa, T. Hayashi, Y. Takano *et. al.*, 2006 Assignment of 204 genes localized on HSA17 to a porcine RH (IMpRH) map to generate a dense comparative map between pig and human/mouse. *Cytogenet. Genome Res.* **112**: 114-120.
- Small, R. L. and J. F. Wendel, 1999 The mitochondrial genome of allotetraploid cotton (*Gossypium* L.). *J. Hered* **90**: 251-253.
- Soltis, D. E., and P. S. Soltis, 1995 The dynamic nature of polyploid genomes. *Proc. Natl. Acad. Sci. USA.* **92**: 8089-8091.

Song, K., P. Lu, K. Tang, and T. C. Osborn, 1995 Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc. Natl. Acad. Sci. USA* **92**: 7719-7723.

Stelly, D. and W. Raska, 2003 The cotton cytogenetics collection. *Proceedings of the Beltwide Cotton Conferences*. National Cotton Council of America, Memphis, TN, p. 834

Stelly, D. M., S. Saha, D. A. Raska, J. N. Jenkins, J. C. McCarty, Jr. *et. al.*, 2005 Registration of 17 upland (*Gossypium hirsutum*) cotton germplasm lines disomic for different *G. barbadense* chromosome or arm substitutions. *Crop Sci.* **45**: 2663-2665.

Thangavelu, M., A. B. James, A. Bankier, G. J. Bryan, P. H. Dear *et. al.*, HAPPY mapping in a plant genome: reconstruction and analysis of a high-resolution physical map of a 1.9 Mbp region of *Arabidopsis thaliana* chromosome 4. *Plant Biotechnol. J.* **1**: 23-31.

Tomkins, J. P., D. G. Peterson, T. J. Yang, D. Main, T. A. Wilkins *et. al.* 2001 Development of genomic resources for cotton (*Gossypium hirsutum* L.): BAC library construction, preliminary STC analysis, and identification of clones associated with fiber development. *Mol. Breeding.* **8**: 255-261.

Ulloa, M., and W. R. Meredith Jr., 2000 Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. *J. Cot. Sci.* **4**: 161-170.

USDA-NASS, 2005 Cotton, tobacco, sugar crops, and honey chap. 2 in *Agricultural Statistics 2005*, from United States Department of Agriculture website http://www.nass.usda.gov/Publications/Ag_Statistics/agr05/05_ch2.PDF

USDA-NASS, 2006 http://www.nass.usda.gov/Statistics_by_Subject/index.asp#C retrieved: July 17, 2006 from United States Department of Agriculture website.

Van Etten, W. J., R. G. Steen, H. Nguyen, A. B. Castle, D. K. Slonim *et. al.*, 1999 Radiation hybrid map of the mouse genome. *Nat. Genet.* **22**: 384-387.

Vizir, I. Y., M. L. Anderson, Z. A. Wilson, and B. J. Mulligan, 1994 Isolation of deficiencies in the *Arabidopsis* genome by γ -irradiation of pollen. *Genetics* **137**: 1111-1119.

Voorrips, R. E., 2002 MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity* **93**: 77-78.

- Walter, M. A., D. J. Spillett, P. Thomas, J. Weissenbach and P. N. Goodfellow, 1994 A method for constructing radiation hybrid maps of whole genomes. *Nat. Genet.* **7**: 22-28.
- Wang, K., X. Song, Z. Han, W. Guo, J. Z. Yu *et al.*, 2006 Complete assignment of the chromosomes of *Gossypium hirsutum* L. by translocation and fluorescence *in situ* hybrid mapping. *Theor. Appl. Genet.* **113**: 73-80.
- Wardrop, J., J. Snape, W. Powell and G. C. Machray, 2002 Constructing plant radiation hybrid panels. *Plant J.* **31**: 223-228.
- Watanabe, T. K., M. T. Bihoreau, L. C. McCarthy, S. L. Kiguwa, H. Hishigaki *et. al.*, 1999 A radiation hybrid map of the rat genome containing 5,255 markers. *Nat. Genet.* **22**: 27-36.
- Wendel, J. F., 1989 New world tetraploid cottons contain old world cytoplasm. *Proc. Natl. Acad. Sci. USA.* **86**: 4132-4136.
- Wendel, J. F., 2000 Genome evolution in polyploids. *Plant Mol. Bio.* **42**: 225-249.
- Wendel, J. F., and R. C. Cronn, 2003 Polyploidy and the evolutionary history of cotton. *Adv. Agron.* **78**: 139-186.
- Williams, J. L., A. Eggen, L. Ferretti, C. J. Farr, M. Gautier *et. al.*, 2002 A bovine whole-genome radiation hybrid panel and outline map. *Mamm. Genome* **13**: 469-474.
- Wu, H., Y. Furusawa, K. George, T. Kawata, and F. A. Cucinotta, 2002 Analysis of unrejoined chromosomal breakage in human fibroblast cells exposed to low- and high-LET radiation. *J. Radiat. Res.* **43**: **Suppl.** S181-S185.
- Yasue, H., S. Kiuchi, H. Hiraiwa, A. Ozawa and T. Hayashi 2006 Assignment of 101 genes localized in HSA10 to a swine RH (IMpRH) map to generate a dense human-swine comparative map. *Cytogenet. Genome Res.* **112**: 121-125.
- Zhang, J., W. Guo, and T. Zhang, 2002 Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) with a haploid population. *Theor. Appl. Genet.* **105**: 1166-1174.
- Zhang, T., Y. Yuan, J. Yu, W. Guo, and R. J. Kohel, 2003 Molecular tagging of a major QTL for fiber strength in upland cotton and its marker-assisted selection. *Theor. Appl. Genet.* **106**: 262-268.

APPENDIX A

Marker	RH 1	RH 2	RH 3	RH 4	RH 5	RH 6	RH 7	RH 8	RH 9	RH 10	RH 11	RH 12	RH 13	RH 14	RH 15	RH 16
BNL																
BNL 119	H	A	H	A	H	H	H	H	H	H	H	H	H	H	H	A
Gh																
Gh 2	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 12	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
Gh 22	-	H	H	H	H	H	-	H	H	H	H	H	H	H	H	H
Gh 27	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
Gh 34	H	H	A	H	H	H	H	H	A	H	H	A	H	H	H	H
Gh 39	H	A	H	H	H	H	H	H	H	A	A	H	H	H	H	A
Gh 48	H	A	H	A	H	H	H	-	H	-	-	H	H	H	H	A
Gh 51	H	H	H	H	H	H	H	H	H	H	A	H	A	H	H	H
Gh 52	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 56	-	-	H	H	H	H	H	H	H	H	H	H	H	A	H	H
Gh 58	H	H	H	H	H	H	H	H	H	H	H	H	-	H	-	H
Gh 67	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 71	H	H	H	H	A	H	H	H	H	H	H	H	A	H	-	H
Gh 73	H	H	H	-	H	H	H	H	H	H	H	H	H	H	H	H
Gh 74	A	A	A	H	H	H	H	H	-	A	H	H	H	A	-	A
Gh 75	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 82	H	A	H	H	H	H	H	H	H	A	A	H	H	H	H	A
Gh 83	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
Gh 96	H	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 98	A	H	H	-	H	H	A	H	H	H	H	H	H	H	H	H
Gh 109	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
Gh 110	-	A	H	A	H	H	H	H	H	H	H	H	H	H	-	A
Gh 111	-	-	-	H	H	H	A	H	H	H	H	H	H	H	-	H
Gh 112	-	H	H	H	H	H	A	H	H	H	H	H	H	A	-	H
Gh 118	-	-	-	H	-	H	-	H	-	H	-	H	-	H	-	H
Gh 119	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H
Gh 129	-	H	-	H	-	H	H	H	A	-	H	H	-	H	-	H
Gh 132	A	A	A	H	H	H	H	H	H	A	H	H	H	-	H	A
Gh 133	-	H	H	H	H	H	H	H	H	H	-	H	H	H	-	H
Gh 142	H	H	H	H	H	A	H	H	H	H	A	H	A	H	H	H
Gh 153	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 167	H	H	H	H	H	H	H	-	H	H	A	H	H	H	H	H
Gh 171	H	H	H	H	H	H	H	H	H	H	A	H	A	H	H	H
Gh 182	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
Gh 188	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 199	-	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H
Gh 199 L	-	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H

Marker	RH 17	RH 18	RH 19	RH 20	RH 21	RH 22	RH 23	RH 24	RH 25	RH 26	RH 27	RH 28	RH 29	RH 30	RH 31	RH 32
BNL																
BNL 119	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh																
Gh 2	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H
Gh 12	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
Gh 22	H	H	H	H	H	H	H	A	H	A	H	H	H	A	H	H
Gh 27	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
Gh 34	H	H	H	H	A	H	H	H	A	A	H	H	A	H	H	H
Gh 39	H	H	A	H	H	H	H	-	H	H	A	A	H	H	H	H
Gh 48	H	H	H	H	H	H	H	H	A	-	H	H	H	H	H	H
Gh 51	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A
Gh 52	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	-
Gh 56	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 58	H	-	H	A	H	H	H	H	H	H	H	H	H	H	H	H
Gh 67	H	H	H	H	H	H	H	H	H	H	H	H	H	-	-	-
Gh 71	H	H	H	H	H	H	H	-	-	A	H	H	H	-	H	H
Gh 73	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H
Gh 74	H	-	H	H	H	H	H	H	H	H	H	H	A	H	-	-
Gh 75	H	-	-	-	-	H	H	A	H	A	H	H	-	H	H	-
Gh 82	H	H	A	H	H	H	H	H	H	A	A	H	H	H	H	H
Gh 83	H	H	H	A	H	H	-	A	H	H	H	H	A	H	H	H
Gh 96	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H
Gh 98	H	H	H	H	H	H	H	H	H	H	H	-	H	H	A	H
Gh 109	H	H	H	H	A	H	H	H	H	-	H	H	H	H	H	H
Gh 110	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
Gh 111	H	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 112	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 118	-	-	H	A	-	A	H	H	H	-	H	H	H	H	H	H
Gh 119	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 129	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 132	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 133	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 142	H	H	H	H	H	A	H	H	H	-	H	A	H	H	H	A
Gh 153	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 167	H	H	H	H	H	H	H	H	H	A	A	H	H	H	A	H
Gh 171	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H
Gh 182	H	A	H	H	A	H	H	H	H	A	H	H	H	H	H	H
Gh 188	H	H	H	H	H	-	H	H	H	H	H	H	H	H	H	H
Gh 199	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 199 L	H	H	H	A	H	H	H	H	A	H	H	H	H	A	H	H

Marker	RH 33	RH 34	RH 35	RH 36	RH 37	RH 38	RH 39	RH 40	RH 41	RH 42	RH 43	RH 44	RH 45	RH 46	RH 47	RH 48
BNL																
BNL 119	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh																
Gh 2	A	H	H	H	H	H	-	H	H	H	H	H	H	H	H	H
Gh 12	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 22	H	H	H	H	A	-	H	H	H	H	H	H	H	H	H	H
Gh 27	H	A	H	H	H	A	H	H	H	H	A	A	A	H	H	H
Gh 34	H	A	H	H	H	H	A	H	H	H	H	A	H	H	H	H
Gh 39	A	H	H	H	A	A	H	H	H	A	H	A	H	H	H	A
Gh 48	A	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H
Gh 51	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
Gh 52	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
Gh 56	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	-
Gh 58	H	H	H	H	H	H	A	H	H	-	-	A	H	H	H	H
Gh 67	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H
Gh 71	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
Gh 73	A	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 74	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
Gh 75	H	H	A	H	A	A	H	H	H	H	H	H	H	H	A	-
Gh 82	A	H	H	H	A	A	H	H	H	A	H	A	A	H	H	A
Gh 83	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H
Gh 96	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 98	H	A	H	H	H	H	H	H	H	-	H	A	H	H	H	H
Gh 109	H	H	H	H	H	H	H	H	A	-	H	H	H	H	H	-
Gh 110	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 111	H	-	H	H	H	H	H	H	H	H	H	A	H	H	H	H
Gh 112	H	-	H	H	H	H	H	A	H	H	H	A	H	H	H	A
Gh 118	-	-	H	H	H	A	H	H	H	H	A	A	H	H	H	H
Gh 119	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 129	A	A	H	H	A	H	H	H	H	H	H	H	A	H	H	H
Gh 132	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
Gh 133	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 142	H	H	H	A	H	H	A	-	H	H	A	H	H	H	H	H
Gh 153	H	H	A	H	H	H	H	H	H	-	H	H	H	H	H	H
Gh 167	H	H	H	H	H	H	A	-	H	H	H	H	H	H	H	H
Gh 171	H	H	H	H	H	H	A	H	H	H	H	H	-	H	H	H
Gh 182	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H
Gh 188	H	H	-	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 199	H	H	H	H	A	H	H	H	A	H	H	H	H	H	H	-
Gh 199 L	H	H	H	H	H	H	A	H	H	H	H	A	H	H	H	H

Marker	RH 65	RH 66	RH 67	RH 68	RH 69	RH 70	RH 71	RH 72	RH 73	RH 74	RH 75	RH 76	RH 77	RH 78	RH 79	RH 80
BNL																
BNL 119	H	A	H	H	H	H	H	H	H	-	H	H	A	H	H	H
Gh																
Gh 2	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 12	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 22	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 27	H	H	A	A	A	H	H	H	H	H	H	A	H	H	H	H
Gh 34	H	-	H	A	H	H	A	A	H	H	H	H	H	H	H	H
Gh 39	A	H	A	H	A	H	H	H	H	H	H	H	H	A	A	H
Gh 48	H	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 51	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 52	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H
Gh 56	H	H	H	H	H	H	H	H	H	H	A	H	H	-	H	H
Gh 58	H	H	H	A	H	A	H	A	H	-	H	A	H	H	H	H
Gh 67	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H
Gh 71	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 73	H	H	H	H	H	H	H	H	H	H	H	H	-	H	H	H
Gh 74	H	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
Gh 75	H	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H
Gh 82	A	H	A	H	A	H	H	H	H	H	H	H	H	A	H	H
Gh 83	H	H	H	H	A	H	H	H	H	H	A	A	H	H	H	H
Gh 96	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 98	H	H	A	A	A	H	H	H	H	H	H	A	A	H	H	A
Gh 109	H	H	H	-	H	H	H	-	-	-	H	H	H	H	H	H
Gh 110	H	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 111	H	H	A	H	A	H	H	H	H	H	H	A	H	H	-	A
Gh 112	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 118	-	-	H	A	A	H	H	H	H	H	H	A	H	H	-	A
Gh 119	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 129	H	H	H	H	H	H	H	H	H	H	H	A	H	A	H	H
Gh 132	-	H	H	H	H	H	-	A	-	A	-	A	H	A	A	A
Gh 133	H	-	H	-	H	H	H	-	H	H	H	H	H	H	H	H
Gh 142	-	H	-	H	H	H	H	H	A	H	H	H	H	H	H	H
Gh 153	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 167	H	H	H	H	H	H	H	H	H	A	-	H	H	H	H	H
Gh 171	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 182	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H
Gh 188	-	H	-	H	A	H	H	H	H	H	A	H	H	H	-	H
Gh 199	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H
Gh 199 L	H	H	H	H	H	A	H	A	H	H	-	H	H	H	H	H

Marker	RH 1	RH 2	RH 3	RH 4	RH 5	RH 6	RH 7	RH 8	RH 9	RH 10	RH 11	RH 12	RH 13	RH 14	RH 15	RH 16
Gh 200	-	H	H	H	H	H	H	H	H	H	H	H	H	H	-	H
Gh 216	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 220	-	A	A	H	H	H	H	H	H	H	A	H	H	H	-	H
Gh 246	A	A	A	H	H	H	H	H	H	A	H	H	H	A	A	A
Gh 260	H	H	H	-	H	H	H	A	H	A	H	H	H	H	A	H
Gh 272	H	-	H	-	H	H	H	H	H	H	A	H	A	H	H	H
Gh 277	H	A	H	-	H	H	H	H	H	H	H	H	H	H	-	-
Gh 288	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 300	A	A	H	H	H	H	H	H	H	H	A	H	A	H	A	H
Gh 302	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 329	-	A	A	H	H	H	H	H	H	A	H	H	H	A	-	-
Gh 330	-	H	H	H	H	H	H	H	H	H	-	H	-	H	-	-
Gh 336	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 345	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
Gh 354	-	H	H	H	H	H	H	H	H	H	H	H	H	H	-	H
Gh 428	-	A	-	A	H	H	H	H	H	H	H	H	H	H	H	A
Gh 441	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 443	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 449	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 459	H	H	H	H	A	H	-	H	H	H	H	H	H	H	-	-
Gh 462	H	H	H	H	H	H	H	H	H	H	A	A	A	H	-	-
Gh 465	H	H	H	H	-	H	H	A	-	-	H	H	H	H	-	H
Gh 466	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 471	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
Gh 484	A	H	H	H	-	H	H	H	-	A	H	H	H	H	-	H
Gh 495	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
Gh 498	A	A	H	H	H	H	H	H	H	H	H	H	H	H	-	H
Gh 506	H	H	H	H	A	H	A	H	A	H	H	H	H	H	H	H
Gh 513	H	H	H	H	H	A	H	H	A	H	H	H	H	H	A	A
Gh 523	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 537	H	H	H	H	H	H	H	H	-	-	H	H	H	H	-	H
Gh 539	A	H	H	H	H	H	A	H	H	H	H	H	H	H	A	H
Gh 548	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
JESPR																
JESPR 298	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
NAU																
NAU 859	A	H	H	H	H	H	A	H	H	-	H	H	H	H	H	H
NAU 864	H	H	H	H	-	H	H	H	-	-	H	H	H	H	-	H
NAU 882	H	H	H	H	H	H	H	H	H	H	A	H	A	H	H	H
NAU 884	A	H	A	A	-	H	H	H	-	-	H	H	H	H	-	H
NAU 920	H	H	H	A	-	H	H	H	-	-	H	H	H	H	-	A
NAU 986	H	H	H	-	-	H	A	A	-	-	A	H	H	A	-	H

Marker	RH 17	RH 18	RH 19	RH 20	RH 21	RH 22	RH 23	RH 24	RH 25	RH 26	RH 27	RH 28	RH 29	RH 30	RH 31	RH 32
Gh 200	H	H	H	H	H	H	-	A	H	A	H	H	H	H	H	H
Gh 216	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H
Gh 220	-	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 246	H	H	H	H	H	H	H	H	H	H	-	H	-	H	H	H
Gh 260	H	H	H	A	H	H	H	H	H	H	H	H	A	-	H	H
Gh 272	H	H	H	H	H	H	H	H	H	A	H	H	H	H	A	H
Gh 277	H	H	H	A	H	H	H	H	H	H	H	H	H	-	H	H
Gh 288	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 300	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
Gh 302	H	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H
Gh 329	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 330	H	A	H	H	-	H	H	H	H	H	H	H	H	A	H	A
Gh 336	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
Gh 345	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 354	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 428	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
Gh 441	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 443	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H
Gh 449	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 459	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
Gh 462	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	-
Gh 465	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H
Gh 466	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 471	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 484	H	H	H	A	A	A	H	H	-	H	H	H	H	H	H	H
Gh 495	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H
Gh 498	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 506	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
Gh 513	H	H	A	H	H	H	H	H	H	-	H	A	H	H	H	A
Gh 523	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 537	H	H	H	H	A	H	A	H	-	H	H	H	H	H	H	H
Gh 539	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H
Gh 548	H	A	H	H	H	H	H	H	A	H	H	H	H	H	H	H
JESPR																
JESPR 298	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
NAU																
NAU 859	H	H	H	A	A	A	H	H	H	-	H	H	H	H	H	H
NAU 864	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H
NAU 882	H	H	A	H	H	H	H	H	H	H	A	A	H	H	H	A
NAU 884	H	H	H	H	H	H	A	H	-	H	H	H	A	H	H	H
NAU 920	A	H	H	H	H	H	H	A	-	H	H	A	H	A	H	H
NAU 986	H	H	H	H	A	H	A	H	-	A	A	H	H	A	H	H

Marker	RH 33	RH 34	RH 35	RH 36	RH 37	RH 38	RH 39	RH 40	RH 41	RH 42	RH 43	RH 44	RH 45	RH 46	RH 47	RH 48
Gh 200	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	-
Gh 216	H	H	A	H	A	A	H	H	H	H	H	H	H	H	A	A
Gh 220	A	A	H	-	H	H	H	H	H	H	H	H	H	H	H	A
Gh 246	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
Gh 260	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	A
Gh 272	H	H	H	H	H	H	A	H	H	H	H	H	H	-	H	H
Gh 277	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 288	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H	H
Gh 300	H	H	H	A	A	H	H	-	H	H	H	H	H	H	H	A
Gh 302	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H	H
Gh 329	H	H	H	H	A	H	A	A	H	H	H	A	H	H	-	-
Gh 330	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
Gh 336	H	H	H	H	A	A	H	H	H	H	H	H	H	H	A	A
Gh 345	A	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
Gh 354	H	H	H	H	H	H	H	H	H	H	H	H	H	-	H	H
Gh 428	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 441	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 443	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
Gh 449	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 459	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
Gh 462	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
Gh 465	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 466	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 471	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 484	H	A	H	H	H	A	H	H	H	H	A	A	H	H	H	H
Gh 495	H	A	H	-	H	A	H	H	-	-	-	A	H	-	H	-
Gh 498	H	H	H	H	A	H	H	A	H	-	H	H	H	H	H	H
Gh 506	H	H	H	-	H	H	H	A	H	-	-	H	H	H	H	-
Gh 513	H	H	H	H	H	H	H	H	H	A	H	H	H	H	-	A
Gh 523	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 537	H	H	H	H	H	H	H	H	A	H	H	H	H	H	A	H
Gh 539	H	A	H	H	H	H	H	A	H	H	H	A	H	H	A	H
Gh 548	A	H	H	H	H	H	H	A	H	H	H	A	H	H	H	H
JESPR																
JESPR 298	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
NAU																
NAU 859	H	A	H	H	H	A	H	H	H	H	A	A	H	-	H	H
NAU 864	-	H	A	H	H	H	A	H	H	H	H	H	H	H	H	H
NAU 882	H	H	H	A	-	A	H	H	H	H	H	H	A	H	H	H
NAU 884	A	A	H	H	A	H	H	H	H	H	H	A	A	-	H	H
NAU 920	A	H	H	A	H	-	H	H	A	H	A	H	H	A	H	H
NAU 986	H	H	H	H	H	H	H	H	A	H	H	H	A	-	A	H

Marker	RH 65	RH 66	RH 67	RH 68	RH 69	RH 70	RH 71	RH 72	RH 73	RH 74	RH 75	RH 76	RH 77	RH 78	RH 79	RH 80
Gh 200	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 216	H	H	H	H	H	H	H	H	A	H	H	H	H	A	H	H
Gh 220	A	A	H	H	H	H	H	H	H	H	-	H	H	H	A	A
Gh 246	A	H	H	H	H	H	A	H	A	H	H	H	H	A	A	A
Gh 260	H	H	H	H	-	H	H	H	H	H	H	H	H	H	H	H
Gh 272	H	A	H	H	H	H	H	H	H	H	H	-	H	H	H	H
Gh 277	H	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 288	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H
Gh 300	A	H	H	H	H	H	H	H	H	A	A	H	A	H	H	H
Gh 302	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H
Gh 329	A	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A
Gh 330	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	-
Gh 336	H	H	H	H	H	H	H	H	H	-	H	H	H	A	H	H
Gh 345	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
Gh 354	H	H	H	H	H	-	H	H	A	H	H	H	H	H	H	H
Gh 428	H	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H
Gh 441	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 443	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
Gh 449	H	H	H	H	H	H	H	H	H	H	A	H	H	H	-	H
Gh 459	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H
Gh 462	H	H	H	H	H	H	A	H	H	H	-	H	H	H	H	A
Gh 465	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
Gh 466	H	H	H	H	H	H	A	H	H	H	A	H	H	H	H	H
Gh 471	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
Gh 484	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	A
Gh 495	H	A	H	H	H	H	H	H	H	H	-	A	H	H	H	A
Gh 498	A	H	H	H	H	H	H	H	H	H	-	H	A	H	H	H
Gh 506	H	H	A	H	H	H	H	H	H	H	-	H	H	H	H	H
Gh 513	A	H	H	H	H	H	H	H	H	H	H	A	H	H	A	H
Gh 523	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Gh 537	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H
Gh 539	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	A
Gh 548	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
JESPR																
JESPR 298	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
NAU																
NAU 859	H	A	H	A	A	H	H	H	H	H	H	A	H	H	H	A
NAU 864	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
NAU 882	H	H	H	H	A	H	H	H	H	A	H	H	A	H	H	H
NAU 884	H	A	H	H	H	-	H	H	H	A	H	A	H	A	H	A
NAU 920	H	H	H	H	A	H	H	H	H	A	H	H	H	H	H	H
NAU 986	H	H	H	H	H	-	A	A	-	-	H	-	A	A	A	A

Marker	RH 81	RH 82	RH 83	RH 84	RH 85	RH 86	RH 87	RH 88	RH 89	RH 90	RH 91	RH 92
Gh 200	H	-	H	-	H	-	H	H	H	A	H	-
Gh 216	A	H	H	H	H	H	H	H	H	H	H	H
Gh 220	-	-	A	-	H	-	H	-	H	A	H	-
Gh 246	H	H	H	H	H	H	-	H	H	H	H	H
Gh 260	H	H	H	H	H	A	-	-	H	H	H	H
Gh 272	H	H	H	H	H	A	-	H	H	H	H	H
Gh 277	H	H	-	H	H	H	-	A	H	H	H	A
Gh 288	H	H	H	H	H	H	H	H	H	H	H	H
Gh 300	H	H	H	A	H	A	H	H	H	H	H	H
Gh 302	H	H	A	H	H	H	A	H	H	H	H	H
Gh 329	-	H	-	H	H	H	H	A	H	-	H	H
Gh 330	H	-	H	-	H	A	H	H	A	H	H	A
Gh 336	A	H	H	H	H	H	H	H	H	H	H	H
Gh 345	H	H	H	H	H	-	H	H	H	A	H	H
Gh 354	H	-	H	-	H	-	H	H	H	H	H	H
Gh 428	H	H	H	H	H	H	H	A	A	H	H	A
Gh 441	H	H	H	H	H	H	H	H	H	H	H	H
Gh 443	-	H	H	H	H	H	H	H	H	H	H	H
Gh 449	H	-	H	H	H	H	H	H	H	H	H	H
Gh 459	H	H	H	H	H	H	H	A	A	H	H	H
Gh 462	H	H	H	H	H	H	H	H	H	H	H	H
Gh 465	A	H	H	H	H	H	A	H	H	H	A	H
Gh 466	H	H	H	H	H	H	H	H	H	H	H	H
Gh 471	H	H	A	H	H	A	H	H	H	H	H	H
Gh 484	H	-	H	A	H	H	H	A	H	H	A	H
Gh 495	H	A	-	A	H	H	H	A	H	H	A	H
Gh 498	H	H	H	H	H	H	H	H	H	H	H	H
Gh 506	A	H	H	H	H	H	H	H	H	H	H	H
Gh 513	H	A	A	H	H	A	H	A	H	A	-	H
Gh 523	H	H	H	H	H	H	H	H	H	A	H	H
Gh 537	H	H	H	H	A	A	H	H	H	H	H	H
Gh 539	H	H	H	A	H	H	H	H	H	H	H	H
Gh 548	A	H	H	H	A	H	H	H	H	H	H	H
JESPR												
JESPR 298	H	H	H	H	H	H	H	H	H	H	H	H
NAU												
NAU 859	H	A	H	A	H	H	H	A	H	-	-	H
NAU 864	H	A	A	A	H	A	A	H	H	A	H	H
NAU 882	H	A	H	H	H	H	A	H	H	H	H	H
NAU 884	A	H	A	A	A	H	H	H	H	-	H	H
NAU 920	A	H	H	A	A	H	H	H	H	H	H	A
NAU 986	H	H	A	H	A	A	H	H	H	-	H	H

Marker	RH 17	RH 18	RH 19	RH 20	RH 21	RH 22	RH 23	RH 24	RH 25	RH 26	RH 27	RH 28	RH 29	RH 30	RH 31	RH 32
NAU 1009	H	H	H	A	A	A	H	H	-	H	H	H	H	A	H	H
NAU 1014	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
NAU 1063	H	H	H	H	H	H	H	H	A	H	H	H	A	H	H	H
NAU 1190	H	H	H	H	H	H	-	H	-	-	H	H	A	H	H	H
NAU 1201	H	H	H	H	A	H	H	H	A	H	H	H	A	A	H	H
NAU 1231	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H	H
NAU 1246	A	H	A	H	H	H	H	H	-	H	H	H	H	H	H	A
NAU 1278	H	H	H	A	H	H	H	A	-	H	H	H	H	H	H	H
BNL (Gao)																
BNL 2960	H	A	H	A	A	H	H	H	A	H	H	H	H	A	H	H
BNL 1161	H	H	H	A	A	H	H	H	A	H	H	H	H	A	H	H
BNL 3563	H	H	H	A	A	H	H	H	A	H	H	H	H	H	H	H
BNL 3895	H	H	H	A	A	H	H	H	A	H	H	H	H	A	H	A
BNL 1665	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
BNL 0256	H	H	H	A	H	H	A	H	A	H	H	H	H	A	H	H
BNL 1679	H	H	H	A	H	H	H	A	H	H	H	H	H	H	H	A
BNL 1673	H	H	H	A	H	H	A	A	H	H	H	H	H	H	H	A
BNL 2967	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A
BNL 3261	H	H	H	H	H	A	A	H	H	A	H	H	H	H	H	A
BNL 3955	H	H	H	A	H	H	H	H	H	H	H	H	H	H	A	H
BNL 2443	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
BNL 1681	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 3411	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 1404	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 3431	H	H	H	H	A	H	H	H	H	H	H	H	A	H	H	H
BNL 1034	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H
BNL 3442	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H
BNL 4094	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 2632	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 2805	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H
BNL 1408	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3592	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 1066	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	A
BNL 0836	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 2652	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
BNL 3280	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3479	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 1079	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3558	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
BNL 2544	H	H	H	H	H	A	H	H	H	A	H	H	H	H	H	H

Marker	RH 33	RH 34	RH 35	RH 36	RH 37	RH 38	RH 39	RH 40	RH 41	RH 42	RH 43	RH 44	RH 45	RH 46	RH 47	RH 48
NAU 1009	H	A	H	H	H	A	H	H	H	-	H	A	A	A	H	H
NAU 1014	H	H	H	H	A	H	H	A	H	H	H	H	H	H	H	H
NAU 1063	H	H	H	H	-	H	-	-	H	H	H	A	H	A	H	-
NAU 1190	A	A	H	H	A	H	H	H	H	H	H	H	A	H	H	H
NAU 1201	H	A	H	A	-	H	A	H	H	H	H	A	H	H	H	A
NAU 1231	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
NAU 1246	H	A	H	H	A	H	H	H	A	H	H	H	A	H	H	A
NAU 1278	H	H	H	H	H	H	H	H	-	H	A	H	H	H	H	A
BNL (Gao)																
BNL 2960	H	H	H	H	H	H	A	H	H	H	H	A	H	H	H	H
BNL 1161	H	H	H	H	H	H	A	H	H	H	H	A	H	H	H	H
BNL 3563	H	H	H	H	H	H	A	H	H	H	H	A	H	H	H	H
BNL 3895	H	H	A	H	H	H	A	H	A	H	H	H	H	H	H	H
BNL 1665	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
BNL 0256	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H
BNL 1679	H	H	H	H	H	H	H	H	A	H	A	H	H	H	H	A
BNL 1673	H	H	H	H	H	H	H	H	A	H	A	H	H	H	H	A
BNL 2967	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
BNL 3261	H	H	H	H	H	A	H	H	A	A	H	H	H	H	H	A
BNL 3955	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 2443	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 1681	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 3411	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 1404	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 3431	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 1034	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 3442	H	H	A	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 4094	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 2632	H	H	H	H	A	H	A	A	H	H	H	A	H	H	H	H
BNL 2805	H	H	H	H	A	H	A	A	H	H	H	H	H	H	H	H
BNL 1408	H	A	H	H	A	H	H	A	H	H	H	H	H	H	H	H
BNL 3592	H	A	H	H	A	H	H	A	H	H	H	H	H	H	H	H
BNL 1066	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	A
BNL 0836	H	A	H	H	A	H	H	H	H	H	H	H	H	H	H	A
BNL 2652	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3280	A	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H
BNL 3479	A	H	H	H	H	A	H	A	H	H	H	H	H	H	H	H
BNL 1079	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3558	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
BNL 2544	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H

Marker	RH 49	RH 50	RH 51	RH 52	RH 53	RH 54	RH 55	RH 56	RH 57	RH 58	RH 59	RH 60	RH 61	RH 62	RH 63	RH 64
NAU 1009	H	H	A	H	H	H	A	H	H	H	H	H	H	A	H	H
NAU 1014	H	H	H	H	H	H	A	H	H	H	H	H	H	A	H	H
NAU 1063	A	H	H	H	H	A	H	H	A	H	A	H	A	A	H	A
NAU 1190	H	H	H	A	A	A	A	-	H	A	H	H	H	A	H	H
NAU 1201	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
NAU 1231	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H
NAU 1246	A	H	-	H	H	H	H	A	A	H	H	H	H	H	H	H
NAU 1278	H	H	A	H	H	H	A	H	H	A	H	H	H	H	A	H
BNL (Gao)																
BNL 2960	H	H	H	A	H	H	A	H	H	H	H	H	H	A	H	H
BNL 1161	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3563	H	H	H	A	H	H	A	H	H	H	H	H	H	H	H	H
BNL 3895	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H
BNL 1665	H	H	H	A	H	H	H	H	H	H	A	H	H	H	H	H
BNL 0256	H	H	H	A	H	A	H	H	H	H	H	H	H	H	A	A
BNL 1679	H	H	A	H	H	H	A	H	H	A	H	H	H	A	A	H
BNL 1673	H	H	A	H	H	H	A	H	H	A	H	H	H	A	A	H
BNL 2967	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3261	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3955	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 2443	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H
BNL 1681	A	H	H	H	A	H	H	H	A	H	A	H	A	A	H	A
BNL 3411	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 1404	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 3431	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 1034	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 3442	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 4094	A	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 2632	H	H	H	H	A	A	H	H	A	H	A	H	A	A	H	A
BNL 2805	A	H	H	H	A	H	H	H	A	H	A	H	A	A	H	A
BNL 1408	H	H	H	H	H	H	A	H	A	H	A	H	H	A	H	H
BNL 3592	H	H	H	H	H	H	A	H	A	H	A	H	H	A	H	H
BNL 1066	H	H	A	H	H	H	A	H	A	H	A	H	H	A	H	H
BNL 0836	H	H	A	H	H	H	A	H	A	H	A	H	H	A	H	H
BNL 2652	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H
BNL 3280	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3479	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 1079	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3558	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
BNL 2544	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H

Marker	RH 65	RH 66	RH 67	RH 68	RH 69	RH 70	RH 71	RH 72	RH 73	RH 74	RH 75	RH 76	RH 77	RH 78	RH 79	RH 80
NAU 1009	H	A	H	A	A	-	A	A	A	A	H	A	H	H	H	A
NAU 1014	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
NAU 1063	A	H	H	H	H	H	A	H	A	H	H	H	H	A	A	A
NAU 1190	H	A	H	H	A	H	H	H	A	H	H	-	H	A	H	H
NAU 1201	H	H	H	A	H	H	A	A	H	H	H	H	H	H	H	H
NAU 1231	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
NAU 1246	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H
NAU 1278	H	H	H	H	H	H	A	H	H	H	H	H	H	H	A	A
BNL (Gao)																
BNL 2960	H	H	H	H	H	A	H	A	H	H	A	H	H	H	H	H
BNL 1161	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 3563	H	H	H	H	H	A	H	A	H	H	A	H	H	H	H	H
BNL 3895	H	H	H	H	H	A	H	A	H	H	A	H	H	H	H	H
BNL 1665	H	H	H	H	H	H	H	A	H	H	A	H	H	H	H	H
BNL 0256	H	H	H	H	H	A	H	H	H	A	A	H	H	H	H	A
BNL 1679	H	H	H	H	H	H	A	H	H	H	A	H	H	H	A	A
BNL 1673	H	H	H	H	H	H	A	H	H	H	A	H	H	H	A	A
BNL 2967	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H
BNL 3261	H	H	H	H	H	H	A	H	H	H	H	A	H	H	H	H
BNL 3955	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
BNL 2443	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
BNL 1681	A	H	H	H	H	H	A	H	A	A	H	H	H	H	H	A
BNL 3411	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
BNL 1404	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
BNL 3431	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
BNL 1034	A	H	H	H	H	H	A	H	A	A	H	H	A	A	A	A
BNL 3442	A	H	H	H	H	H	A	H	A	A	H	H	A	A	A	A
BNL 4094	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
BNL 2632	A	H	H	H	H	H	A	H	A	A	H	H	H	A	A	A
BNL 2805	A	H	H	H	H	H	A	H	H	H	H	A	H	A	A	A
BNL 1408	A	H	H	H	H	H	H	H	H	A	H	H	A	H	H	H
BNL 3592	A	H	H	H	H	H	H	H	H	A	H	H	A	H	H	H
BNL 1066	A	H	H	H	H	H	H	H	H	A	A	H	A	H	H	A
BNL 0836	A	H	H	H	H	H	H	H	H	A	H	H	A	H	H	H
BNL 2652	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H
BNL 3280	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H
BNL 3479	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H
BNL 1079	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H
BNL 3558	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
BNL 2544	H	H	H	H	H	A	H	H	H	H	H	H	H	A	H	H

APPENDIX B

Primer Name	Clone Name	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	GenBank Acc. No.
Gh				
Gh 2	LIB5327-016-A1-N1-G6_F_S	GAATTGAAGCAAACCTCATTAAATTACC	CTACCCTCATCTCATTCCAAAAAAC	DQ907949
Gh 12	LIB5327-032-A1-N1-G5_F_S	GGTTAAGTAAGTTCATGAGGTTTATTTG	CCAATATTTACTCCAAGCCAATC	DQ907959
Gh 22	LIB5327-032-A1-N1-F10_F_S	CAACTAAGGAAATGAAAATAGAAAAATC	GACTTAGTTTTATCTAGGTTTTCTCTTAGC	DQ907968
Gh 27	LIB5327-008-A1-N1-F6_F_S	CACTAGCATTGCTTTTTACTGG	GAAAATAATAGATAATGGAGGAGAACAAGG	DQ907972
Gh 34	LIB5327-008-A1-N1-B6_F_S	CCCTTTTGTTATCTAACTTCTGTTACTCCTAAC	CCTTTTGTTAGCTCTTCTATACTTGAATTCC	DQ907978
Gh 39	LIB5327-008-A1-N1-E7_F_S	CCAGTTTATAATAAGAATCATAGTTTGGTGG	CACATTCACCTCAAAGTCCATCAC	DQ907983
Gh 48	LIB5327-024-A1-N1-D2_F_S	CTGTTTCTTAACATGGGTTTTTTCC	CAAAACACTAATTGCAAAAATAAATTATATTTTG	DQ907990
Gh 51	LIB5327-027-A1-N1-D10_FS	GCACAATCACAGATTGGGA	GATTTTAGCTAACTGTATCGGTTCCG	DQ907993
Gh 52	LIB5327-027-A1-N1-G9_FS	CTTGATCCGGTAGAGGAGTGT	GCTTTCCACAGAAACCAATGG	DQ907994
Gh 56	LIB5327-027-A1-N1-H10_FS	TCCATTAGACAAAGTTTTCTAAAGTTC	TGAGACTTCCAACCAGATACAG	DQ907998
Gh 58	LIB5327-003-A1-N1-D10_FS	GACTTTGAGAGGGATTTTACAGTG	CACCCAATTTAAGCAGAAATTG	DQ908000
Gh 67	LIB5327-019-A1-N1-G4_FS	CAAGAGGGAAATCGACAAGG	CCCCTTCACCTATTAAGTCAACA	DQ908009
Gh 71	LIB5327-012-A1-N1-B10_FS	GTTTCATCACCATTTTCATCAGC	GAATCCATAGCTTGTTGCATTG	DQ908013
Gh 73	LIB5327-012-A1-N1-G4_FS	GCTGACTGCATTGGTTCCG	TGGGTCCTCTACCTAATAGCTGG	DQ908015
Gh 74	LIB5327-041-A1-N1-B7_FS	ACAGCCTATAATAAGATGCCACA	CAGTAGCCAGAACTTAAGCTATG	DQ908016
Gh 75	LIB5327-026-A1-N1-F6_FS	CGTCTGGATTGAACAGTGATC	CAACTTGATCTAACTATTGCATACG	DQ908017
Gh 82	LIB5327-035-A1-N1-B4_FS	GATACCTTTGTCACGAAGCTG	GCACCAATCAGTAAGTGCAAGTC	DQ908024
Gh 83	LIB5327-035-A1-N1-F7_FS	GGTTTGATCAGTTTGATGATTTAGC	CCGCGGAATCGTCAAC	DQ908025

Gh 96	LIB5327-036-A1-N1-E12_FS	TTCATAGACGTTTCGTTTATAACAAG	GCTGCCCATTCACTOCTC	DQ90803 8
Gh 98	LIB5327-028-A1-N1-D12_FS	CACCGCATCACCCAAATAGTAG	TCTTCCATATCTTCTCTTCTCC	DQ90804 0
Gh 109	LIB5327-038-A1-N1-F3_FS	CAAGAAGGAAATGGCTGAATTG	CAGACACCAGCTGTTGCC	DQ90805 1
Gh 110	LIB5327-029-A1-N1-E12_FS	ACCATCCCAAAGAATCATCCTC	ACTAAAACCAAGGCAATAAAGTG	DQ90805 2
Gh 111	LIB5327-038-A1-N1-A3_FS	GTTGCAACCTTGAAACCA	GGGTTGCCGTTAGACCAG	DQ90805 3
Gh 112	LIB5327-029-A1-N1-B9_FS	GGTTGGGTTTCCACAATAGC	TGTTGCAACCTTGAAACC	DQ90805 4
Gh 118	LIB5327-027-A1-N1-H1_PS	CGGAAGCTAGTGAAGGAGG	TCTTTCCTTGTTCTGGAGT	DQ90806 0
Gh 119	LIB5327-027-A1-N1-G2_PS	GTTGAAGCAAGTGAGGATCC	CGGTTATTGGTTCCATTAGTTCAGTCG	DQ90806 1
Gh 129	LIB5327-027-A1-N1-C3_PS	ACACAAGCGATCAACAAGG	GAAATGATGTGAGCTCTTGTTTC	DQ90807 1
Gh 132	LIB5327-003-A1-N1-D3_PS	TCATGGAACACCAAAGTTGGA	ACATGATAGATTATTCAGCAATGCA	DQ90807 4
Gh 133	LIB5327-003-A1-N1-E6_PS	TGTTTTCTCTCGGAAACTATAGACCA	CCAACCTTAAGAAGGAAGAGATACCA	DQ90807 5
Gh 142	LIB5327-019-A1-N1-G9_PS	GAGTCTCCTCCTCGCATG	TCAACCACACATCATAAGACCA	DQ90808 4
Gh 153	LIB5327-041-A1-N1-E10_PS	GGCTCAAATTTGCATTCCAG	CCATAGTTGGAAGCCATGAAG	DQ90809 5
Gh 167	LIB5327-026-A1-N1-H9_PS	CCATTACCTTTCACACCTCAAATTC	GAAAGATGGATATGCACATATGC	DQ90810 9
Gh 171	LIB5327-026-A1-N1-C12_PS	CCCTAAAGAGAAATCGGTATCCTC	CAAACCCAGAACTGGCTTC	DQ90811 3
Gh 182	LIB5327-035-A1-N1-B3_PS	AGCGCTTAGAAGTTGTCAATGTC	ACATGCCAACCTCTGACCTC	DQ90812 4
Gh 188	LIB5327-035-A1-N1-E1_PS	CGCAACTGTAAGCTATCTTATGG	TGCTTGTGGGAGTAATGGTG	DQ90813 0
Gh 199	LIB5327-004-A1-N1-E3_F_S AA	CAAAAAGAATATGAATGAGTCAATAGAC	CAATAAATGCCATAATCTTCAACTCAC	DQ90814 1
Gh 199 L	LIB5327-004-A1-N1-E3_F_S AA	CAAAAAGAATATGAATGAGTCAATAGAC	CAATAAATGCCATAATCTTCAACTCAC	DQ90814 1
Gh 200	LIB5327-004-A1-N1-C7	TCAAGTCTTTTTTTTAACTCAACATTC	CTTATTAGACTAGATCTTAGTTTGATC	DQ90814 2
Gh 216	LIB5327-004-A1-N1-F9	TCCACATTCCCATGCACTACTC	CTAAAACCTTATACATACAAAATGCAGC	DQ90815 8
Gh 220	LIB5327-020-A1-N1-H10	CAATTATTCACCTTCCAGGCTTCC	TGGATTTGAAAATCCATTGAACTCACC	DQ90814 2

Gh 246	LIB5327-013-A1-N1-H12	GCATCTTGTTGAGCCTTATAGGG	ACTTATCAAGTGATTTGCAGGTGAC	DQ90818 7
Gh 260	LIB5327-021-A1-N1-F1	GCATGGAATAAATTATGAAGTCACAGAC	GTATGGAAAGAGTTGAGGATGGAAG	DQ90820 0
Gh 272	LIB5327-021-A1-N1-A11	AACCGAAAAACCCCTAAATGTTGAG	TTTCAGAAAATATATCAAATGGGTTAGTTC	DQ90821 2
Gh 277	LIB5327-014-A1-N1-G2	TACTAAAACCAAGGCAATAAAGTGA	CACCACCTTCCATATATCTTGCTC	DQ90821 7
Gh 288	LIB5327-014-A1-N1-B10	CTATTCCACAAGCTTCATTCTGCAG	GGAGCACAATGAGGAAGTATACTG	DQ90822 8
Gh 300	LIB5327-030-A1-N1-A2	GGAAAACCCAAAATACATAAGAACCC	AGATTCTAACTTCCAGCAAGACATG	DQ90824 0
Gh 302	LIB5327-030-A1-N1-E8	ACTAGTATCATTAGGGTCAGTGAGC	CACTGGATGTGAAGGAAATGCTATC	DQ90824 2
Gh 329	LIB5327-006-A1-N1-G11	CAGCAGGCAGAAATCTTGTGATCG	CTTAAATTTCTCTCCCTCAAACCATC	DQ90826 8
Gh 330	LIB5327-022-A1-N1-B3	GATCATTTCAGCCCATAGTGTGC	GTTTCAGGGCTTCAAAGAGGCTC	DQ90826 9
Gh 336	LIB5327-022-A1-N1-B10	ACTAGGAGTTACATTGCATTTTGCC	CTAGCGCACAAAGGGCTATTTTGC	DQ90827 5
Gh 345	LIB5327-022-A1-N1-G11	ATTTGAGACTTCCAACCAGATACAG	TTCTAAAGTTCTCTTCTCTCAAACC	DQ90828 4
Gh 354	LIB5327-015-A1-N1-G11	CTTACCCATAAAACCCTAAATCTGAG	CTCTACATCCTTAAGAATTTCTTCTCC	DQ90829 3
Gh 428	LIB5327-040-A1-N1-C5_P_S_C	AAAATTCCAGTCGTGCTCAACTC	ACAAAGGTTGTCTGTTTGATTCTGAAG	DQ90836 7
Gh 441	LIB5327-032-A1-N1-D7_P_S	GATCGGTAATGTTTCGTAACCCTAC	AGAATTAGGTATAGAGGTTGGTGCG	DQ90838 0
Gh 443	LIB5327-032-A1-N1-B2_P_S	TATCAGAATCAATATGCACAGGTTGAG	CTAAAGAATTATTGTTGGAACCAGACG	DQ90838 2
Gh 449	LIB5327-032-A1-N1-B7_P_S	CATTGCTGTAGACCATTTGCTTTAAG	GTTATGAATCGAAAGCTTGTTTAGGC	DQ90838 8
Gh 459	LIB5327-008-A1-N1-D6_P_S	AGGTGAGGAATCCATAGCTTGTTG	CCTAGTTCATCACCATTTTCATCAGC	DQ90839 8
Gh 462	LIB5327-024-A1-N1-A7_P_S	GAAAGGTTTTAGCATATACCACTTAAGG	GTTAAATTCGGTTAAGGAAGATGGATC	DQ90840 1
Gh 465	LIB5327-024-A1-N1-G7_P_S	AAGTCAAAGGAAGAGACGCTTCG	AAATTCACCTTCTGGCAGTGACAC	DQ90840 4
Gh 466	LIB5327-024-A1-N1-B3_F_S	AATCTGCATGTGCCAATACACTGG	CATCACTCCATGTTACAGTTGAGG	DQ90840 5
Gh 471	LIB5327-024-A1-N1-C1_F_S_C	CAGGCATCAACTAGCATTGAAAACG	ATCTTCTGATCTCTATTAGCTACAACG	DQ90841 0
Gh 484	LIB5327-009-A1-N1-C5_P_S	CCTTTTGCCTTTATTGCTTGCTTGG	CCAAGATGACAAACACACGTGAATC	DQ90842 3

Gh 495	LIB5327-009-A1-N1-G10_F_S	AAACTCTTAGCCTTGTCCATGAAAG	TGATCAAAGATGGGAGAAAAGAGTC	DQ90843 4
Gh 498	LIB5327-009-A1-N1-H12_P_S_C	ATTTAGACTAGTTGATAGTGATAAGGAC	ACAACATCAACCATATCTATATGCATTC	DQ90843 7
Gh 506	LIB5327-025-A1-N1-E7_F_S	TGGAGAATCCAAGTAAAGTAGCGAC	ATCTGCTGTAATAGGAACCACAAGG	DQ90844 5
Gh 513	LIB5327-025-A1-N1-A12_P_S	TTAACTCTACAAGCGATGGGATCG	TCTCAAAGCCGACAAACTGTTAG	DQ90845 2
Gh 523	LIB5327-001-A1-N1-B8_P_S_C	GGAACAATTGAAGAAGACGATATAAGG	CTTGGATGGACTATGGAACTGTG	DQ90846 2
Gh 537	LIB5327-017-A1-N1-B4_F_S	GTTGGGTGGCAATTCCTTTTAGATC	AAAGCTAATCCCTATACCTTTTCTCG	DQ90847 6
Gh 539	LIB5327-025-A1-N1-E8_F_S	AGTTCGTGCCTTTGATACTGAAGG	CAAACGAAGTGAATGTTAGTCTATTCCG	DQ90847 8
Gh 548	LIB5327-017-A1-N1-E8_P_S	CCATCATTATTTTACCTTTGCCTCTC	GGTGGTTTTGCACCATCGTTTAAG	DQ90848 7
BNL				
BNL 119		CGATCCTTCTTATTCTCATCTCTC	GAAACACTTCTTCACAAATCCTAAT	
JESPR				
JESPR 298		GATGCCCTCGTGTTAAAG	GGACCTTCGGAATAATTACC	AF351536
NAU				
NAU 859	GA_Ed0084F12r	CAGGCTTCATCTTTTTGGAC	CATTGGATCCTAGTGGGAAG	BQ414273
NAU 864	GA_Ed0079F04r	GGATTAATTAGCCCCACAT	TCTTTTTCAGCTTGGGTCT	BQ413859
NAU 882	GA_Ed0064G12r	ATCATCCATTAGGCACCAAC	GAGGGAAGAAGCAGCTAACA	BQ412880
NAU 884	GA_Ed0059H08r	AGAGCTGGAGGACATAACAAA	CGCAGATAAAGGATGGATTT	BQ412605
NAU 920	GA_Ed0031D07r	CATCCTAACCCAAAACAAGA	TTGGAGCATTGAAATTACCC	BQ410493
NAU 986	GA_Ed0098G11f	AAACAAAACAGCTCCTCGTT	ATCCGATCGGTAGCATCTT	BQ406802
NAU 1009	GA_Ed0087G06f	CATCGATCCAAGAGGAATTT	CAGACTCCATATATCAAGTTCAAGC	BQ405858
NAU 1014	GA_Ed0084D08f	GCCTCCACTTGTTTTCTACC	GGCACCCATATCAGAAGAAG	BQ405578
NAU 1063	GA_Ed0051D06f	CACACTACCCCTTTTCTT	AGCAGGTTTACGGTTGTTGT	BQ402750
NAU 1190	GA_Ea0022I09f	CCATGTCCGTATCCATGTTA	TAAGGCAAGATAGGGTCAGG	BG443893
NAU 1201	GA_Ea0017K23f	CCGATATCTTACTTTCCAACCT	AAGGGGTTTGAAGGTTATC	BG442619
NAU 1231	GA_Ea0006A19f	TTGAGACCAAAAACATGTGG	GCTCATTTTGTATCTGAACTCTG	BG440134
NAU 1246	GA_Eb0020P18f	TGCGCACTAAAGAAAAAGAA	GAACAAAATGAATACCCACA	BF274421
NAU 1278	GA_Ea0026D24f	ATCATGGAACCTGGTTGTTT	ATGAATTGCGGAGTCTAAGG	BE052577

BNL				
BNL 2960		TAAGCTCTGGAGGCCAAAAA	CCATTTCAATTTCAAGCATACG	
BNL 1161		CATCTCCTCTGGAAAGAGCG	ATGAAGCAGCACATTCCATG	
BNL 3563		AAGCATAAACTTGACACAAGCC	AATGGGCAAGAAAAGGGAAC	
BNL 3895		CGCTCTTGGTCATGGATTTT	GCCAAGCTCACTGGAAGAAC	
BNL 1665		CAGAACCAACATACTTTCTACGG	ATGTGCAAAAACCTTGATGTGG	
BNL 0256		TTTTGCTCCATTTTTTTGCC	TTTATTAATTTTCGTTTAGCTCCG	
BNL 1679		AATTGAGTGATACTAGCATTTACGC	AAAGGGATTTGCTGGCAGTA	
BNL 1673		CTCTTAATGCTTGGCCTTGG	AGTACCGGACTCGGCACTAT	
BNL 2967		GAAACTTCGGATGACTTTTGC	TCGACTAGGATCTATTTCAAGATG	
BNL 3261		AAACGGAAACGAAGAAGGGT	CCCAAACCTGTCTCACCAAC	
BNL 3955		AGAGATGCAATGGGATCGAC	ATGTGATAATGCGGGGAATG	
BNL 2443		TTTATTGGTCGGTCTTTGCC	TTAGGGTGTTCTTTGGGCAC	
BNL 1681		GTGTGTGGGTGTGCATGTTT	TGGGGAGACTTTATCACGCT	
BNL 3411		TTTTACTCTCTCTCCTGTCTCC	GTTTCCATTTGCGATGAGCT	
BNL 1404		CGAGAGCCCCTAACAGAAA	CCATTTGTTTTTCCCCTT	
BNL 3431		TCAACCAAGCAACCAATTCA	ATGTATAGAGATAGATTGAAAAGGGG	
BNL 1034		TTGCTTCAATGGAAAACCC	CGTCGCAAAGTTGAGAATCA	
BNL 3442		CATTAGCGGATTTGTCGTGA	AACGAACAAAGCAAAGCGAT	
BNL 4094		ATGCTGCGGAGTGCATATCT	AAATTGATTTTCATGCCGGAG	
BNL 2632		CGTGTCTCCAGACCAACAAA	GGGAGTTGAAGCCGACATAA	
BNL 2805		AGTTTGGAAATTACAATAAATGTA	CCAAGGTCGGTTCGGTACTA	
BNL 1408		AAGGGAGAGAAACGGAGAGC	CATTTACCTCTCCCACCAC	
BNL 3592		GTTCTAGTCTCTTTCTTTATGGGC	TTGATTGAGATGCCAATGGA	
BNL 1066		ACATTTCCACCCAAGTCCAA	ACTCTATGCCGCTCTCGTA	
BNL 0836		ATCTTGTTGATTTTCTGACTACAGG	CAGACATTTCCCTTCCTTGA	
BNL 2652		TTCATATTCTAGCCTGAGTCC	GCGATAATCCTTCCAGGGAT	
BNL 3280		GCAGAAGTCCACTTGTGTTG	AGAAAATGGGTTGTGCTTGG	
BNL 3479		AGTGGGTTGGACTTTTCATGC	CACGGGCTTTTTTTTTTCA	
BNL 1079		TCATACTCTTTCATCTAGCGCG	AAAGGAATCCAGGTGAGCCT	
BNL 3558		AAGCAAATCATGATGAACATACG	TGCGAAGAGTAGCTCTGCTG	
BNL 2544		GCCGAAACTAAAACGTCCAA	TCCTTACTACTAAGCAGCCG	

VITA

Steven Michael Todd received his Bachelor of Arts degree in biochemistry from Bradley University in Peoria, IL in May 2003. He received his master's degree in molecular and environmental plant sciences from Texas A&M University in College Station, TX in December 2006. His professional interest is the application of biotechnology to crop improvement.

Mr. Todd may be contacted at:

Steven Todd

Monsanto Leland Agronomy Center

4006 Old Leland Rd

Leland, MS 38756

His email address is steve.m.todd@monsanto.com