A rat genetic map constructed by representational difference analysis markers with suitability for large-scale typing

(polymorphic markers/dot blot hybridization/quantitative trait loci)

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ABSTRACT **Representational difference analysis (RDA)** was applied to isolate chromosomal markers in the rat. Four series of RDA [restriction enzymes, BamHI and HindIII; subtraction of ACI/N (ACI) amplicon from BUF/Nac (BUF) amplicon and vice versa] yielded 131 polymorphic markers; 125 of these markers were mapped to all chromosomes except for chromosome X. This was done by using a mapping panel of 105 ACI \times BUF F₂ rats. To complement the relative paucity of chromosomal markers in the rat, genetically directed RDA, which allows isolation of polymorphic markers in the specific chromosomal region, was performed. By changing the F₂ driver-DNA allele frequency around the region, four markers were isolated from the D1Ncc1 locus. Twenty-five of 27 RDA markers were informative regarding the dot blot analysis of amplicons, hybridizing only with tester amplicons. Dot blot analysis at a high density per unit of area made it possible to process a large number of samples. Quantitative trait loci can now be mapped in the rat genome by processing a large number of samples with RDA markers and then by isolating markers close to the loci of interest by genetically directed RDA.

The laboratory rat, *Rattus norvegicus*, is widely used in many fields of biomedical research, such as carcinogenesis, neurological disease, diabetes (1), hypertension (2), and autoimmune diseases (3). The rat's relatively large body size when compared with that of the mouse is one of its advantages; it allows the collection of larger tumors, which facilitates the detailed analyses of histology and genetic alterations. The rat's higher capacity for cognition and memory is another important feature and allows the clarification of brain functions (4, 5). When we focus on these features, rat, as well as mouse, genome studies become very important.

However, genetic markers in the rat have been very sparse, and the genetic map presently available needs much improvement. Recently, two research groups reported (6, 7) on the presence of collections of simple sequence length polymorphism (SSLP) markers. However, the two series have not been integrated with each other and the total number of rat SSLP markers described so far is much smaller than for the mouse and man. This poses problems for fine chromosomal mapping.

Many interesting phenotypes, such as cancer susceptibilities, abnormal blood pressure levels, and enhanced capacity for learning, are measured as continuous variables, namely, quantitative traits, usually controlled by multiple genetic loci, called quantitative trait loci (QTL) (8). To identify QTL, a large number of F_1 backcross or F_2 animals must be analyzed (9). When genotyping is carried out using SSLP markers, DNA samples from these animals must be subjected to electrophoresis after PCR amplification. Thus, an increase in sample number is directly linked with escalating labor and financial costs. A new type of genetic marker suitable for handling a large number of samples may be valuable for the progress of genome studies on QTL.

Representational difference analysis (RDA) was developed to identify differences between two complex genomes (10). It utilizes a subtractive hybridization method by using representations (amplicons) of the genomes that have a reduction in complexity. The representations are generated by a PCR-based size selection process applied to the restriction fragments of both genomes (10). In this way, probes can be isolated for the detection of genetic lesions in cancer including rearrangements, amplifications, and losses (11). Further, exogenous DNA sequences such as viruses (12, 13) and polymorphic markers among different strains (10) can be easily isolated. Moreover, RDA is suitable for isolating polymorphic markers in a specific chromosomal region [genetically directed RDA (GDRDA)] (14). Two pools of DNA from F_2 animals can be prepared so that they have different genotypes (AA vs. BB) only in a specific chromosomal region. RDA performed on these two pools will yield polymorphic markers specifically in the region of interest.

In the present study, we isolated many polymorphic markers with high efficiency by RDA and constructed a rat chromosomal map by integrating these with other chromosomal markers. We also showed the usefulness of GDRDA in isolating specific markers in a defined chromosomal region. We further showed that the genotypes of a large number of samples, which are essential for QTL analysis, can be efficiently determined by using these markers with the dot blot method.

MATERIALS AND METHODS

RDA. RDA was performed as described by Lisitsyn *et al.* (10). The restriction enzymes *Bam*HI and *Hin*dIII and their corresponding anchor primers (10) were used for digestion of the DNA samples and subsequent PCR amplification to prepare amplicons. After three rounds of competitive hybridization, the final PCR products were again digested with a restriction enzyme to remove adapters, and then the digest obtained was cloned into pBluescript II (Stratagene). The pBluescript II had been previously digested with an appropriate restriction enzyme and treated with calf intestinal alkaline phosphatase. The clones obtained were used as probes for Southern blot analysis.

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Abbreviations: RDA, representational difference analysis; GDRDA, genetically directed RDA; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; SSLP, simple sequence length polymorphism; cM, centimorgan(s). To whom reprint requests should be addressed at: Carcinogenesis

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Southern Blot Analysis. Genomic DNA (10 μ g) was digested with an appropriate restriction enzyme and the purified product was run through 0.9% agarose gel electrophoresis and blotted onto a nylon filter (Hybond-N; Amersham). Probe DNA was prepared by purifying the insert of each RDA clone through a SeaPlaque GTG gel (FMC), and then by labeling it with [α -³²P]dCTP by random DNA labeling (MultiPrime; Amersham). Prehybridization and hybridization were performed as described (15).

Linkage Mapping Using ACI/N 4 BUF/Nac F_2 Rats. A total of 105 F_2 rats were produced by crossing ACI/N (ACI) and BUF/Nac (BUF) (CLEA Japan, Osaka). DNA was extracted from their livers by the phenol and chloroform method as described (16). Their genotypes were determined using SSLP (7, 17, 18), arbitrarily primed-PCR (AP-PCR) (F.C., T.U., M.T., Y.H., T. Su, and M.N., unpublished data), and RDA markers. A multipoint linkage analysis of these polymorphic markers was performed with MAPMAKER version 3.0 (19).

FIG. 1. RDA of DNAs from ACI and BUF rats. (A) Agarose gel electrophoresis of BamHI RDA products. Aliquots $(2 \mu g)$ of the tester RDA amplicon (lane 1), driver DNA amplicon (lane 2), and RDA products of the first (lane 3), second (lane 4) and third (lane 5) competitive hybridization-amplification steps were electrophoresed in a 2% NuSieve gel. Hae III/ ϕ X174 DNA size markers (lane M) are indicated in base pairs. (B) Southern blot of the RDA product. The blots from A were probed with RDA probe M1-8. (C) Hybridization of clone M1-8 with BamHI digested genomic DNA from ACI (lane 1), BUF (lane 2), and ACI \times BUF F₂ rats (lanes 3-13). M1-8 was mapped to chromosome 17 by using a 105 ACI \times BUF F₂ mapping panel and designated D17Ncc3.

Dot Blot Analysis. Amplicons were prepared from ACI and BUF and their F_2 rats in the same manner as tester/driver amplicon. Each amplicon $(1 \ \mu g)$ in 20 μl of Tris/EDTA was mixed with 20 μl of 20× standard saline citrate $(1 \times SSC = 0.15$ M sodium chloride/0.015 M sodium citrate, pH 7) and 1 μl of black indian ink; a 1- μl aliquot was blotted onto a nylon filter using a 96-well blotting device (Kriplanker; Washington University, St. Louis) at a density of eight dots in an area of 9 mm \times 9 mm.

Nomenclature. Loci defined by RDA were named in accordance with the rat nomenclature committee (20). For example, D1Ncc5 refers to a locus corresponding to the 5th marker on chromosome 1, isolated at The National Cancer Center Research Institute.

RESULTS

Isolation of Polymorphic Markers by RDA. RDA was performed using the amplicons prepared from *Bam*HI and

Table 1. Number of isolated clones, independent clones, and polymorphic clones in the four series of RDA and GDRDA

	DNA amplicon		No. of clones				
Restriction enzyme	Tester	Driver	Total	Independent	Polymorphic (% independent clones)		
RDA							
BamHI	ACI	BUF	70	40	36 (90)		
	BUF	ACI	64	32	24 (75)		
HindIII	ACI	BUF	177	67	42 (62)		
	BUF	ACI	161	35	29 (83)		
Subtotal GDRDA			472	174	131 (75)		
BamHI	ACI	Pool-1*	6	6	2		
	ACI	Pool-2*	3	3	2		
Subtotal			9	9	4		
Total			481	183	135 (74)		

*Ten rats selected from 105 ACI \times BUF F₂ rats as shown in Fig. 3A.

HindIII digests of ACI and BUF rat genomic DNA. Using these paired sets of amplicons, four series of RDA were performed by subtracting the ACI amplicon from the BUF amplicon and the BUF amplicon from the ACI amplicon. Fig. 1 A and B shows a representative series of concentrations of a polymorphic fragment subjected to RDA by subtracting the *Bam*HI amplicon of BUF from that of ACI. After three rounds of competitive hybridization, the final PCR products were cloned into pBluescript II and the restriction fragment length polymorphism (RFLP) was determined by Southern blot anal-

Chr.1		Chr.3		Chr.7		Chr.12		Chr.18	
Maker Dis	stance (cM)	Marker Dista	ance (cM)	Marker Dis	tance (cM)	Marker Dis	tance (cM)	Maker Dist	arice (cM)
D1Mah16	22.8	D3Mah9	6.8	*D7Ncc9	5.8	*D12Ncc3	7.8	*D18Ncc1	9.7
*D1Ncc16		D3Kvo2	1.4	*D7Ncc11	10.5	D12Mit5	12.2	Ttr	12.8
	22.8	*D3Ncc5	13.7	D7Mit7	4.8	Planh	10.4	ADC	20.5
		D3Mit9	2.5	*D7Ncc7	12.9	D12Mah5	1.6	Adrb2	5.6
Maker Dis	stance (cM)	*D3Ncc3	1.6	*D7Ncc4	7.8	*D12Ncc1	15.4	Gja1	0.0
*D1Ncc15	11.9	D3Ncc1	15.7	*D7Ncc3	14.7	*D12Ncc2		Ólf	7.3
*D1Ncc24	1.3	*D3Ncc4	0.9	*D7Ncc5	20.0		47.4	D18Mgh3	3.3
Ton	0.0	*D3Ncc8	1.6	*D7Ncc8	8.5			*D18Ncc2	3.4
Kik1	11.1	*D3Ncc7	6.0	*D7Ncc6	5.4	Chr 12		*D18Ncc3	
*D1Ncc13	2.8	Scn2a1	15.9	D7Mit5	6.2	011.13			62.7
D1Ncc1	0.0	D3Ncc2	5.4	Myc	0.0	Marker D	istance (cM)		
*D1Ncc8	0.0	*D3Ncc6	10.2	*D7Ncc1	12.6	D13Kvo3	6 9	Chr.19	
*D1Ncc9	1.2	Ivd	7.1	D7Mit14	7.9	D13Ncc1	64		
*D1Ncc18	3.7	D3Kyo5	2.1	*D7Ncc12	4.2	*D13Ncc5	61	Maker Dist	ance (cM)
*D1Ncc17	15.3	*D3Ncc9	21.7	*D7Ncc10	6.6	Ren	16.3	*D19Ncc2	7.7
*D1Ncc22	4.1	A	17.8	D7Mgh1	9.4	D13Ncc2	4.5	Hp	2.7
C	0.0	Svp2	3.9	Ela1	1.4	D13Ncc3	21.8	Tat	17.6
DINCC10	2.0	PCK1		Prpn	14.0	*D13Ncc6	2.1	D19Mit3	8.5
DINCC14	15.0		134.3	*D7Ncc2	450.7	Atp1a2	1.1	*D19Ncc1	
PIHrei	2.2				152.7	D13Ncc4	1.2		36.5
DINCCZ	0.0	Chr.4				D13Mit4	5.9		
DINCCII	1.3			Chr.8		*D13Ncc8	14.1	01-00	
DINCC25	3.3	Marker Dist	tance (cM)			*D13Ncc7	*****	Chr.20	
DINCC3	1.3	116	14.7	Marker Dist	tance (cM)		86.4	Makes Diet	
Lincc19	0.1	*D4Ncc6	13.2	Acaa	9.0			Tofe	auce (GM)
L3/1 Mul2	0.0	D4Ncc1	8.8	D1N64	4.2	Chr.14		11110	4./ o 4
Myla Dibleed	1.3	D4Mgh14	15.4	Mylc1v	6.9			DZUNCCZ	0.4
DINCC4	1.3	*D4Ncc11	13.2	Rbp2	5.8	Maker Dis	tance (cM)	*D20NCCJ	3.5
DINCOL	1.0	D4Ncc2	2.9	D8Ncc1	8.8	*D14Ncc4	5.6	*D20NCC4	10.3
DINCES	1.9	Cpa2	6.2	D8N136	22.3	*D14Ncc3	12.5	D20MUH	21.3
*D1Ncc20	10.5	Try1	0.0	Apoc3	5.6	D14Mit3	9.6	DAGMIN	51.3
*D1Ncc26	5.8	D4Ncc3	5.8	Thy1	1.4	н	2.9		51.5
*D1Ncc27	9.8	*D4Ncc10	6.0	*D8Ncc2	0.7	D14Ncc1	6.0		
*D1Ncc12	6.4	*D4Ncc9	2.9	D8Mit3	1.9	*D14Ncc2	0.0	Chr.X	
D1Ncc7	22.4	*D4Ncc12	0.0	*D8Ncc3	4.4	CSNA	0.8		
*D1Ncc23	7.9	Fabp1	4.5	*D8Ncc8	10.4	Afp	0.0	Maker Dist	ance (cM)
*D1Ncc28	9.6	-D4NCC8	9.2	*D8Ncc4	21.7	AID	5.7	DXNcc1	28.7
D1Mah12	12.0	Spr	10.7	*D8Ncc7	0.0	<u>HSD</u>		Cbpi	18.0
D1Mit14	13.4	Ampp	5.0	DBNcc6	18.6		43.2	_Pfkfb1	
		D4NCC4	0.1	"DUNCCS					46.7
*D1Ncc21		*DANoo7	11.6						
*D1Ncc21	181.2	*D4Ncc7	11.6		121.9	Chr.15			
*D1Ncc21	181.2	*D4Ncc7 Ret *D4Ncc5	11.6 24.1 6.5		121.9	Chr.15			
*D1Ncc21	181.2	*D4Ncc7 Ret *D4Ncc5 Pthlb	11.6 24.1 6.5	Chr.9	121.9	Chr.15 <u>Maker Dist</u>	ance (cM)		
<u>*D1Ncc21</u> Chr. 2	181.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u>	11.6 24.1 6.5 	Chr.9	121.9	Chr.15 <u>Maker Dist</u> *D15Ncc2	ance (cM) 16.1		
<u>*D1Ncc21</u> Chr. 2	181.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u>	11.6 24.1 6.5 160.9	Chr.9 <u>Marker Dis</u> i	121.9	Chr.15 <u>Maker Dist</u> *D15Ncc2 *D15Ncc1	<u>ance (cM)</u> 16.1 17.8		
<u>*D1Ncc21</u> Chr. 2 <u>MakerDist</u>	181.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u>	11.6 24.1 6.5 160.9	Chr.9 <u>Marker Dis</u> D9Mit1	121.9 tance (cM) 15.9	Chr.15 <u>Maker Dist</u> *D15Ncc2 *D15Ncc1 *D15Ncc4	ance (cM) 16.1 17.8 6.3		
<u>*D1Ncc21</u> Chr. 2 <u>Maker Dist</u> D2Mit2	181.2 tance (cM) 13.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5	11.6 24.1 6.5 160.9	Chr.9 <u>Marker Dis</u> D9Mit1 *D9Ncc1	121.9 tance (cM) 15.9 3.8	Chr.15 <u>Maker Dist</u> *D15Ncc2 *D15Ncc1 *D15Ncc4 D15Mgh1 *D15Mgh1	ance (cM) 16.1 17.8 6.3 1.6		
<u>*D1Ncc21</u> Chr. 2 <u>Maker Dist</u> D2Mit2 *D2Ncc5	181.2 181.2 13.2 13.2	*D4Ncc7 Ret *D4Ncc5 <u>PthIh</u> Chr.5	11.6 24.1 6.5 160.9	Chr.9 <u>Marker Dis</u> D9Mit1 "D9Ncc1 D9Mgh2	121.9 tance (cM) 15.9 3.8 16.3	Chr.15 <u>Maker Dist.</u> *D15Ncc2 *D15Ncc4 D15Ncc4 D15Ncc3 *D15Ncc3	ance (cM) 16.1 17.8 6.3 1.6 16.0		
<u>*D1Ncc21</u> Chr. 2 <u>Maker Dist</u> D2MIt2 *D2Ncc5	181.2 181.2 13.2 13.2 13.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 Marker Dist	11.6 24.1 6.5 160.9	Chr.9 <u>Marker Dis</u> D9Mit1 [*] D9Ncc1 D9Mgh2 D9Mit3	121.9 tance (cM) 15.9 3.8 16.3 5.2	Chr.15 *D15Ncc2 *D15Ncc4 *D15Ncc4 D15Ncc4 D15Ncc3 *D15Ncc3 *D15Ncc5	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5		
*D1Ncc21 Chr. 2 Maker Dist D2Mit2 *D2Ncc5	181.2 181.2 13.2 13.2 13.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5 Marker_Distr D5Mit9 D5Mit9	11.6 24.1 6.5 160.9 ance (cM) 17.6 22.0	Chr.9 <u>Marker Disi</u> D9Mit1 "D9Ncc1 D9Mgh2 D9Mit3 "D9Ncc2	121.9 tance (cM) 15.9 3.8 16.3 5.2 12.0	Chr. 15 <u>Maker Dist</u> *D15Ncc2 *D15Ncc4 D15Ncc4 D15Ncc3 *D15Ncc5 D15Ncc5 D15Ncc5	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8		
*D1Ncc21 Chr. 2 Maker Dist *D2Ncc5 Maker Dist	181.2 181.2 13.2 13.2 13.2 tance (cM)	D4Ncc7 Ret D4Ncc5 Pthlh Chr.5 Marker Distu D5Mit9 D5Mit11 Glut1	11.6 24.1 6.5 160.9 ance (cM) 17.6 23.0 22.3	Chr.9 <u>Marker</u> Dis DOM/11 "D9Ncc1 D9Mgh2 D9Mit3 "D9Ncc2 "D9Ncc3	121.9 tance (cM) 15.9 3.8 16.3 5.2 12.0	Chr.15 Maker Distr "D15Ncc2 "D15Ncc4 D15Mc64 D15Mc63 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8		
<u>*D1Ncc21</u> Chr. 2 <u>Maker Dist</u> <u>D2Mit2</u> <u>*D2Ncc6</u> <u>*D2Ncc8</u>	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Distr D5Mit9 D5Mit11 Glut1 Fie2	11.6 24.1 6.5 ——	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mgh2 D9Mit3 "D9Ncc2 <u>"D9Ncc3</u>	121.9 15.9 3.8 16.3 5.2 12.0 53.2	Chr. 15 *D15Ncc2 *D15Ncc4 *D15Ncc4 D15Mcc4 D15Ncc3 *D15Ncc3 *D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh6	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8		
<u>D1Ncc21</u> Chr. 2 <u>Maker Dist</u> <u>D2Mit2</u> <u>D2Ncc5</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u>	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2 0.0 2.5 9.6	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5 <u>Marker Disk</u> D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1	11.6 24.1 65 160.9 17.6 23.0 22.3 2.9 8.1	Chr.9 <u>Marker Dist</u> D9Mit1 D9Mgh2 D9Mit3 "D9Mic2 <u>"D9Nic2</u> <u>"D9Nic2</u>	121.9 15.9 3.8 16.3 5.2 12.0 53.2	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 D15Mgh1 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh6	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.5 17.8 15.8 		
<u>D1Ncc21</u> Chr. 2 <u>Maker Dist</u> <u>D2Mit2</u> <u>D2Ncc5</u> <u>D2Ncc8</u> <u>D2Ncc8</u> <u>D2Ncc7</u> <u>D2Ncc9</u> <u>M1C5</u>	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 15.5 9.6 1.6	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Distr</u> D5Mit9 D5Mit1 Ela2 D5Ncc1 D5Ncc1 D5Ncc1	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5	Chr.9 <u>Marker Dis</u> DOMIt1 D9Mcc1 D9Mgh2 D9Mit3 "D9Ncc2 <u>"D9Ncc3</u> Chr.10	121.9 15.9 3.8 16.3 5.2 12.0 53.2	Chr.15 <u>Maker</u> Dist "D15Ncc2 "D15Ncc4 D15Mgh1 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh6	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.5 17.8 15.8 106.9		
D1Ncc21 Chr. 2 D2Mt2 D2Nt2 D2Ncc5 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc9 Mt1pb D2Ncc9	181.2 181.2 13.2 13.2 13.2 tance (cM) 0.0 2.5 9.6 1.6 8.2	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 Marker_Distr D5Mit9 D5Mit9 D5Mit9 D5Mit11 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2	11.6 24.1 6.5 	Chr.9 <u>Marker Dis</u> DOM/it1 "D9Nec1 D9Mgh2 D9Mit3 "D9Nec2 "D9Nec3 Chr.10	tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2	Chr.15 Maker Distr "D15Ncc2 "D15Ncc4 D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9		
<u>D1Ncc21</u> Chr. 2 <u>Maker Dist</u> <u>D2Mit2</u> <u>D2Ncc5</u> <u>D2Ncc5</u> <u>D2Ncc7</u> <u>D2Ncc9</u> <u>M11pb</u> <u>D2Ncc10</u> <u>Prir</u>	181.2 181.2 13.2 14.6 14.2 14.4	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5 <u>Marker Disk</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3	Chr.9 <u>Marker Disi</u> D9Mit1 D9Mgh2 D9Mit3 D9Mit3 D9Mit23 <u>D9Mit23</u> Chr.10 <u>Marker Dis</u>	121.9 tance (CM) 15.9 3.8 16.3 5.2 12.0 53.2 stance (CM)	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 D15Mgh1 "D15Ncc3 D15Mgh4 D15Mgh4 D15Mgh4 Chr.16	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 17.8 17.8 17.8 106.9		
<u>D1Ncc21</u> Chr. 2 <u>D2Mit2</u> <u>D2Ncc5</u> <u>D2Ncc8</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u>	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Distr D5Mit9 D5Mit1 Glut1 Ela2 D5Ncc1 D5Ncc2	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mgh2 D9Mit3 D9Mcc2 <u>D9Ncc3</u> Chr.10 <u>Marker Dis</u> D10Mit5	121.9 tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc4 D15Mgh1 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 Chr.16 Maker Dist	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9 ance (cM)		
D1Ncc21 Chr. 2 D2Mt2 D2Mt2 D2Ncc5 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc1 D2Ncc1 Cpb	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0	*D4Ncc7 Ret *D4Ncc5 Pthlh Chr.5 Marker Distu D5Mit9 D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2	11.6 24.1 6.5 	Chr.9 <u>Marker Dis</u> DOM/11 "D9Nec1 D9Mgh2 D9Mit3 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2 "D9Nec2"	tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4	Chr.15 <u>Maker District</u> <u>'D15Ncc2</u> <u>'D15Ncc4</u> <u>D15Mc64</u> <u>D15Mc63</u> <u>'D15Ncc5</u> <u>D15Mgh3</u> <u>D15Mgh4</u> <u>D15Mgh6</u> Chr.16 <u>Maker District</u>	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0		
<u>D1Ncc21</u> Chr. 2 <u>D2Mit2</u> <u>D2Nic2</u> <u>D2Ncc5</u> <u>D2Ncc7</u> <u>D2Ncc9</u> <u>Mt1pb</u> <u>D2Ncc10</u> <i>Cpb</i> <u>D2Ncc1</u> <i>Cpb</i> <u>D2Mc10</u>	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 1.6 8.2 14.4 8.3 0.0 3.5	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5 <u>Marker Distr</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6	11.6 24.1 6.5 	Chr.9 <u>Marker Dist</u> D9Mit1 "D9Ncc1 D9Mgh2 D9Mit3 "D9Ncc2 "D9Ncc2 "D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 "D10Mcc1 D10Mgh10	tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2 53.2 stance (cM) 16.2 14.4 11.6	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc3 D15Mgh4 D15Mgh4 D15Mgh6 Chr.16 <u>Maker Dist</u> "D16Ncc6 D16Mgh4	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6		
<u>D1Ncc21</u> Chr. 2 <u>D2Mit2</u> <u>D2Ncc5</u> <u>D2Ncc8</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc7</u> <u>D2Ncc70</u> <u>P1r</u> <u>D2Ncc10</u> <i>Ptr</i> <u>D2Ncc10</u> <i>Ptr</i> <u>D2Ncc15</u>	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 3.5 3.4	D4Ncc7 Ret D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Ncc2 Chr.6 Marker Dist	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mec1 D9Mit3 D9Mit3 D9Mec2 <u>D9Nec2</u> <u>D9Nec3</u> Chr.10 <u>Marker Dis</u> D10Mit5 D10Nec1 D10Nec2	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc4 D15Mgh1 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 Chr.16 <u>Maker Dist</u> "D16Ncc6 D16Mgh4 D16Kyo1	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0		
D1Ncc21 Chr. 2 D2Mit2 D2Mit2 D2Ncc8 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Cpb D2Mgh5 D2Mgh5 D2Ncc2	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Distr</u> D5Mit9 D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker Dis</u> Inter District	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8	Chr.9 <u>Marker Dis</u> DOM/11 *D9Ncc1 D9Mgh2 D9Mit3 *D9Ncc2 *D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 *D10Ncc1 D10Mgh10 *D10Ncc2 Myh3	tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4	Chr.15 Maker Distr "D15Ncc2 "D15Ncc4 D15Mc61 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh6 Chr.16 Maker Distr "D16Ncc6 D16Ncc6 D16Ncc4	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5		
D1Ncc21 Chr. 2 D2Mit2 D2Mit2 D2Ncc6 D2Ncc6 D2Ncc7 D2Ncc9 Mt1pb D2Ncc10 Prir D2Ncc1 Cpb D2Ncc15 D2Ncc2 D2Ncc2 D2Ncc2	181.2 181.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 16.6 14.4 8.3 0.0 3.5 3.4 10.1 15.4	*D4Ncc7 Ret *D4Ncc5 <u>Pthih</u> Chr.5 <u>Marker_Dist</u> D5Mit9 D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker_Dist</u> Ighe	11.6 24.1 6.5 	Chr.9 <u>Marker Disi</u> D9Mgh2 D9Mgh2 D9Mcc1 D9Mcc2 <u>D9Ncc2</u> <u>D9Ncc2</u> Chr.10 <u>Marker Dis</u> D10Mit5 D10Mcc1 D10Mcc1 D10Ncc2 Myh3 D10Ncc4	121.9 tance (CM) 15.9 3.8 16.3 5.2 12.0 53.2 stance (CM) 16.2 14.4 11.6 3.4 2.4 0.0	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Ncc6 D16Ncc4 "D16Ncc4 "D16Ncc5	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.5 16.2		
D1Ncc21 Chr. 2 D2Mit2 D2Ncc5 D2Ncc8 D2Ncc8 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc11 Cpb D2Ncc13 D2Ncc21	tance (cM) 13.2 13.1	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Ncc1 D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0	Chr.9 <u>Marker Dis</u> D9Mic1 D9Mic2 D9Mic3 D9Nic2 <u>D9Nic2</u> <u>D9Nic2</u> <u>D9Nic2</u> <u>D10Nic2</u> D10Mit5 D10Nic1 D10Nicc1 D10Nicc2 Myh3 D10Nicc4 Syb2	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc4 D15Mgh1 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh6 Chr.16 <u>Maker Dist</u> "D16Ncc6 D16Mgh4 D16Kyo1 "D16Ncc5 "D16Ncc5 "D16Ncc5	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3		
D1Ncc21 Chr. 2 D2Mit2 D2Mit2 D2Ncc8 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc115 D2Ncc13 D2Ncc12	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Distriture</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker District</u> 106Ncc3 *D6Ncc3	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1	Chr.9 <u>Marker Dis</u> DOM/11 "D9Mcc1 D9Mgh2 D9Mit3 "D9Ncc2 "D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 "D10Ncc1 D10Mgh10 "D10Ncc2 Myh3 "D10Ncc4 Syb2 Tp53	tance (cM) 15.9 3.8 16.3 5.2 12.0 53.2 tance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 0.0 1.1	Chr. 15 <u>Maker District</u> 'D15Ncc2 'D15Ncc4 D15Mc63 'D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr. 16 <u>Maker District</u> 'D16Ncc6 D16Mgh4 D16Ncc4 'D16Ncc4 'D16Ncc9 D16Ncc2	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7		
D1Ncc21 Chr. 2 D2Mit2 D2Nit2 D2Ncc5 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Cpb D2Mgh5 D2Mgh5 D2Ncc13 D2Ncc11 D2Ncc12 Fga	tance (cM) 13.2 0.0 2.5 9.6 1.6 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Dist</u> D5Mit9 D5Mit11 Glut1 Ela2 D5Mcc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker Dist</u> Ighe *D6Ncc3 *D6Ncc8 *D6Ncc8	11.6 24.1 6.5 160.9 ance (cM) 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6	Chr.9 <u>Marker Disi</u> D9Mgh2 D9Mgh2 D9Mgh2 D9Mrt3 'D9Ncc2 'D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 'D10Ncc1 D10Mgh10 'D10Ncc2 Myh3 'D10Ncc4 Syb2 Tp53 'D10Ncc3	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 0.0 1.1 	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Ncc5 "D16Ncc5 "D16Ncc5 "D16Ncc2 "D16Ncc7	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9		
D1Ncc21 Chr. 2 D2Mit2 D2Nit2 D2Ncc5 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc10 D2Ncc15 D2Ncc13 D2Ncc11 D2Ncc12 Fga Fgg	tance (cM) 13.2 13.5 3.4 10.1 15.4 3.1 2.9 0.0 0.0	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Ela2 D5Ncc1 D5Ncc1 D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 *D6Ncc3 *D6Ncc9 Ckb	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5	Chr.9 <u>Marker Dis</u> D9Mic1 D9Mec1 D9Mic2 D9Mic3 "D9Nec2 <u>"D9Nec2</u> <u>"D9Nec2</u> <u>"D9Nec2</u> <u>"D9Nec2</u> <u>"D9Nec2</u> <u>"D9Nec5</u> D10Mic5 "D10Nec4 Syb2 Tp53 <u>"D10Nec3</u>	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 1.1 49.1	Chr. 15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr. 16 <u>Maker Dist</u> "D16Ncc6 D16Mgh4 D16Kyo1 "D16Ncc5 "D16Ncc5 "D16Ncc7 "D16Ncc7	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5		
D1Ncc21 Chr. 2 D2Mit2 D2Ncc5 D2Ncc5 D2Ncc6 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc11 Cpb D2Ncc13 D2Ncc13 D2Ncc12 Fga Fgg Pkir	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 2.27	*D4Ncc7 Ret *D4Ncc5 Pthlh Chr.5 Marker Distr D5Mit9 D5Mit1 Glut1 Ela2 D5Ncc1 D5Ncc2 Chr.6 Marker Dist Ighe *D6Ncc3	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (CM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4	Chr.9 <u>Marker Dis</u> DOMIt1 D9Mcc1 D9Mgh2 D9Mit3 D9Mcc2 D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 D10Mit5 D10Ncc1 D10Ncc4 Syb2 Tp53 D10Ncc3	121.9 15.9 3.8 16.3 5.2 12.0	Chr. 15 <u>Maker</u> Distr "D15Ncc2 "D15Ncc4 D15Ncc5 D15Ngh1 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh6 Chr. 16 <u>Maker</u> Distr "D16Ncc6 D16Mgh4 D16Ncc6 D16Mgh4 D16Ncc6 D16Ncc4 "D16Ncc2 "D16Ncc2 "D16Ncc2 "D16Ncc2 "D16Ncc3	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 		
DINCC21 Chr. 2 D2MI2 D2MI2 D2NIC2 D2NCC5 D2NCC7 D2NCC9 Mt1pb D2NCC10 Prir D2Ncc10 Prir D2Ncc15 D2Ncc15 D2Ncc13 D2Ncc13 D2Ncc11 D2Ncc12 Fgg Fgg Pkir D2Ncc12 Fgg Pkir D2Ncc12	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 22.7 8.8 8.8	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Disk</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker Disk</u> ighe *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc9 Ckb D6Mgh3 D6Mgh3 D6Mgh3 D6Mgh4	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9	Chr.9 <u>Marker Dis</u> D9Mgh2 D9Mgh2 D9Mrt3 'D9Ncc2 'D9Ncc2 'D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 'D10Ncc4 Syb2 Tp53 'D10Ncc3 Chr.11	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 0.0 1.1 49.1	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Ncc5 "D16Ncc4 "D16Ncc5 "D16Ncc2 "D16Ncc2 "D16Ncc3 "D16Ncc3	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 16.2 26.3 8.7 2.9 9.5 94.7		
D1Ncc21 Chr. 2 Chr. 2 D2Mit2 D2Ncc5 D2Ncc8 D2Ncc8 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc10 Cpb D2Ncc1 Cpb D2Ncc12 Cpb D2Ncc12 Fga Fgg Pkir D2Ncc12 Fga Fgg Pkir D2Ncc3	tance (cM) 13.2 13.4 10.1 15.4 3.1 2.9 0.0 0.0 2.7 8.8 10.4	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Ncc1 D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 *D6Ncc9 Ckb D6Mgh3 D6Mgh4 *D6Ncc10 *D6Ncc9	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 1.1 11.6 30.5 3.4 1.9 3.9	Chr.9 <u>Marker Dis</u> D9Mic1 D9Mic2 D9Mic3 D9Mic2 <u>D9Mic2</u> <u>D9Mic2</u> <u>D9Mic2</u> <u>D9Mic2</u> <u>D9Mic2</u> <u>D9Mic2</u> <u>D10Mic2</u> <u>D10Mic1</u> D10Mgh10 <u>D10Ncc2</u> <u>Myn3</u> <u>D10Ncc4</u> <u>Syb2</u> <u>Tp53</u> <u>D10Ncc3</u> Chr.11	121.9 15.9 3.8 16.3 5.2 12.0	Chr.15 <u>Maker Dist</u> "D15Noc2 "D15Noc2 "D15Noc4 D15Mgh1 "D15Noc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr.16 <u>Maker Dist</u> "D16Noc6 D16Mgh4 D16Kyo1 "D16Noc5 "D16Noc5 "D16Noc2 "D16Noc8 "D16Noc8 "D16Noc8 "D16Noc8 "D16Noc8	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7		
DINCC21 Chr. 2 Maker Dist D2Mit2 D2Ncc5 D2Ncc5 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc11 Cpb D2Ncc13 D2Ncc13 D2Ncc12 Fga Fgg Fkir D2Ncc1 D2Ncc12 Fga Fgg Pkir D2Ncc3 D2Ncc12 D2Ncc12 D2Ncc12 D2Ncc12 D2Ncc12 D2Ncc13 D2Ncc12 D2Ncc12 D2Ncc13 D2Ncc12 D2Ncc12 D2Ncc12 D2Ncc13 D2Ncc13 D2Ncc13 D2Ncc12 D2Ncc13 D2Ncc3	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 2.27 8.8 10.4 4.5	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit1 Glut1 Ela2 D5Ncc1 D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 *D6Ncc3 *D6Ncc3 *D6Ncc9 Ckb D6Mgh3 D6Mgh4 *D6Ncc10 *D6Ncc12	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mcc1 D9Mgh2 D9Mit3 D9Ncc2 D9Ncc3 D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 D10Ncc1 D10Ncc1 D10Ncc4 Syb2 Tp53 D10Ncc3 Chr.11 Marker Dis	121.9 15.9 3.8 16.3 5.2 12.0	Chr.15 <u>Maker</u> Dist "D15Ncc2 "D15Ncc4 D15Ncc5 D15Ngh1 "D15Ncc5 D15Ngh4 D15Ngh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Ncc6 D16Ngc4 "D16Ncc4 "D16Ncc4 "D16Ncc2 "D16Ncc2 "D16Ncc3 "D16Ncc3	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 D2Mit2 'D2Ncc8 'D2Ncc8 'D2Ncc8 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc10 Prir D2Ncc11 D2Ncc12 'D2Ncc13 'D2Ncc13 'D2Ncc12 Fgg Pkir D2Ncc3 D2Ncc3 D2Ncc3 D2Ncc3 D2Ncc3 D2Nicc3 D2Ncc4 'D2Ncc4'	Isi.2 181.2 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 2.2.7 8.8 10.4 4.5 3.3 0.0	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Dist</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 <u>Marker Dist</u> Ighe *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc9 Ckb D6Mgh4 D6Mgh4 D6Ncc10 *D6Ncc12 D6Ncc12 D6Ncc12 D6Mit2	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0	Chr.9 <u>Marker</u> Disi D9Mgh2 D9Mgh2 D9Mrt3 'D9Ncc2 'D9Ncc2 'D9Ncc3 Chr.10 <u>Marker</u> Dis D10Mrt5 'D10Ncc1 D10Ncc1 D10Ncc1 D10Ncc2 Myh3 'D10Ncc4 Syb2 Tp53 'D10Ncc3 Chr.11 <u>Marker</u> Dis 'D11Ncc6	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 1.1 49.1 stance (cM) 2.8	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Ncc5 "D16Ncc4 "D16Ncc5 "D16Ncc2 "D16Ncc3 "D16Ncc3 "D16Ncc3 "D16Ncc3	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 D2Ncc5 D2Ncc8 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc11 Cpb D2Ncc12 Fga Fgg Pkir D2Ncc13 D2Ncc12 Fga Pgy Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga Pkir D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc5 D2Ncc12 Fga D2Ncc5 D2Ncc5 D2Ncc5 D2Ncc12 Fga D2Ncc5	tance (cM) 13.2 13.3 0.0 3.3 0.0 2.7 8.8 10.4 4.5 3.3 0.0	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Ngh9 *D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 *D6Ncc9 Ckb D6Mgh3 D6Mgh4 *D6Ncc10 *D6Ncc10	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0 2.8	Chr.9 <u>Marker Dis</u> D9Mic1 D9Mic21 D9Mic2 D9Mic3 D9Mic2 <u>D9Nic2</u> <u>D9Nic2</u> <u>D9Nic2</u> <u>D10Mic5</u> D10Mic5 D10Mic5 D10Nicc1 D10Mic1 D10Nicc2 Myn3 <u>D10Nicc4</u> Syb2 Tp53 <u>D10Nicc3</u> Chr.11 <u>Marker Dis</u> <u>D10Nicc3</u> Chr.11	121.9 15.9 3.8 16.3 5.2 12.0	Chr.15 <u>Maker Dist</u> "D15Noc2 "D15Noc2 "D15Noc4 D15Mgh1 "D15Noc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr.16 <u>Maker Dist</u> "D16Noc6 D16Mgn4 D16Kyo1 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc6 D16Noc6 D16Noc6 D16Noc6 "D16Noc6 D16Noc7 D16Noc6 D16Noc6 D16Noc7 D16Noc6 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16Noc6 D16Noc7 D16	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 16.5 16.5 16.2 26.3 8.7 26.3 8.7 9.5 94.7		
DINCC21 Chr. 2 Maker Dist D2Mit2 D2Nec5 D2Nec5 D2Nec7 D2Nec7 D2Nec7 D2Nec10 Prir D2Nec10 Prir D2Nec10 Prir D2Nec13 D2Nec13 D2Nec13 D2Nec13 D2Nec12 Fga Fgg Pkir D2Nec3 D2Nec5 D2Nec6 D2Nec6 D2Nec6 D2Nec14 D2Nec64 D2Nec64	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 22.7 8.8 10.4 4.5 3.3 0.0	*D4Ncc7 Ret *D4Ncc5 Pthlh Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ele2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 Marker Dist Ighe *D6Ncc3 *D6Ncc8 *D6Ncc9 Ckb D6Mgh3 D6Mcc10 *D6Ncc12 D6Mcc11 *D6Ncc11	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0 2.8 2.0	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mcc1 D9Mcc1 D9Mc2 D9Mc22 D9Ncc3 Chr.10 <u>Marker Dis</u> D10Mit5 D10Mcc1 D10Mcc1 D10Mcc1 D10Mcc2 Myh3 D10Ncc4 Syb2 Tp53 D10Ncc3 Chr.11 <u>Marker Dis</u> Chr.11 <u>Marker Dis</u> D11Mcc6 D11Mgh2 Sst	121.9 15.9 3.8 16.3 5.2 12.0	Chr. 15 <u>Maker</u> Dist "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr. 16 <u>Maker</u> Dist "D16Ncc6 D16Mgh4 D16Ncc6 D16Mgh4 D16Ncc6 D16Mcc7 "D16Ncc4 "D16Ncc2 "D16Ncc3 "D16Ncc3 Chr. 17 <u>Maker</u> Dist	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7 ance (cM)		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 'D2Ncc8 'D2Ncc8 'D2Ncc6 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc1 D2Ncc1 Cpb D2Ncc13 'D2Ncc12 'D2Ncc13 'D2Ncc13 'D2Ncc12 'D2Ncc14 D2Ncc12 'D2Ncc15 D2Ncc22 'D2Ncc12 'D2Ncc12 'Fgg Pkir D2Ncc3 D2Ncc3 D2Ncc4 'D2Ncc4 'D2Ncc5 D2Ncc4 'D2Ncc4 'D2Ncc4	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 22.7 8.8 10.4 4.5 3.3 0.0 3.3.0	*D4Ncc7 Ret *D4Ncc5 <u>Pthlh</u> Chr.5 <u>Marker Disk</u> D5Mit9 D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc2 Chr.6 <u>Marker Disk</u> 'D6Ncc3 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc8 *D6Ncc9 Ckb D6Mgh4 D6Msc10 *D6Ncc12 D6Ncc12 *D6Ncc11 *D6Ncc15	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0 2.8 2.0 1.2	Chr.9 Marker Disi D9Mgh2 D9Mgh2 D9Mkt3 'D9Ncc2 'D9Ncc2 'D9Ncc2 'D9Ncc2 'D9Ncc3 Chr.10 Marker Dis D10Mcc1 D10Mcc1 D10Ncc4 Syb2 Tp53 'D10Ncc3 Chr.11 Marker Dis 'D11Ncc6 D11Mgh2 Sst Kng	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 1.1	Chr.15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc3 "D15Ncc3 "D15Ncc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Mcc5 "D16Ncc4 "D16Ncc5 "D16Ncc2 "D16Ncc2 "D16Ncc3 "D16Ncc3 "D16Ncc3 "D16Ncc4 "D17Ncc4 "D17Ncc4	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 16.5 106.5 16.5 16.2 26.3 8.7 2.9 9.5 94.7 ance (cM) 12.9		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 D2Ncc5 D2Ncc8 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc11 Cpb D2Ncc13 D2Ncc12 Fga Fgg Pkir D2Ncc3 D2Ncc12 Fga Psir D2Ncc3 D2Ncc12 Fga Pkir D2Ncc3 D2Ncc14 D2Ncc4 D2Ncc4	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 0.0 2.7 8.8 10.4 4.5 3.3 0.0 133.0	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit9 D5Mit11 Giut1 Eia2 D5Ncc1 D5Ncc1 D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 D6Ncc9 Ckb D6Msh3 D6Msh3 D6Msh4 *D6Ncc10 *D6Ncc11 *D6Ncc11 *D6Ncc15 *D6Ncc14	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 1.1 11.6 30.5 3.4 1.9 3.4 1.9 3.9 0.6 1.0 2.8 2.0 1.2 7.2	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mic1 D9Mic2 D9Mit3 D9Mic2 <u>D9Nic2</u> <u>D9Nic2</u> <u>D9Nic2</u> <u>D10Mit5</u> D10Mit5 D10Mit5 D10Mit5 D10Mic1 D10Mic1 D10Mic2 Myn3 <u>D10Nic2</u> Myn3 <u>D10Nic2</u> Syb2 Tp53 <u>D10Nic3</u> Chr.11 <u>Marker Dis</u> <u>D10Nic2</u> Sit <u>D11Mic6</u> D11Mic5 D11Mic5	121.9 15.9 3.8 16.3 5.2 12.0	Chr.15 <u>Maker Dist</u> "D15Noc2 "D15Noc2 "D15Noc4 D15Mgh1 "D15Noc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Moc6 D16Mgh4 D16Kyo1 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc6 D16Noc6 D16Noc6 D16Noc6 "D16Noc6 D16Noc6 "D16Noc6 D16Noc6	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 16.5 16.2 26.3 8.7 9.5 94.7 94.7		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 D2Ncc5 'D2Ncc6 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc7 'D2Ncc10 Prir D2Ncc110 D2Ncc12 'D2Ncc111 'D2Ncc12 Fga Pkir D2Ncc3 D2Mic63 D2Mic63 D2Ncc14 D2Ncc24	tance (cM) 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 22.7 8.8 10.4 4.5 3.3 0.0 133.0	*D4Ncc7 Ret *D4Ncc5 Pthlh Chr.5 Marker Dist D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 Marker Dist Ighe *D6Ncc3 *D6Ncc3 *D6Ncc3 Ckb D6Mgh4 *D6Ncc10 *D6Ncc12 D6Ncc12 D6Ncc12 D6Ncc13	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0 2.8 2.0 1.2 7.2 11.5	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mcc1 D9Mcc1 D9Mit3 D9Mcc2 <u>D9Ncc3</u> <u>D9Ncc3</u> <u>D9Ncc3</u> <u>D10Ncc3</u> <u>D10Ncc4</u> <u>Syb2</u> <u>T053</u> <u>D10Ncc3</u> <u>Chr.11</u> <u>Marker Dis</u> <u>D11Ncc6</u> <u>D11Mgh2</u> <u>Sst</u> Kng <u>D11Mcc5</u> <u>D11Mgh2</u>	121.9 15.9 3.8 16.3 5.2 121.9 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 1.1 49.1 stance (cM) 2.8 7.5 0.0 8.2 11.3 1.1	Chr. 15 <u>Maker Dist</u> "D15Ncc2 "D15Ncc2 "D15Ncc4 D15Mcd D15Ncc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh6 Chr. 16 <u>Maker Dist</u> "D16Ncc6 D16Mgh4 D16Ncc6 D16Mcc9 D16Ncc5 "D16Ncc5 "D16Ncc3 "D16Ncc3 Chr. 17 <u>Maker Dist</u> "D17Ncc4 D17Ncc4 D17Ncc4 D17Ncc4 D17Ncc4 D17Ncc4	ance (cM) 16.1 17.8 6.3 1.6 16.0 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7 2.9 94.7		
D1Ncc21 Chr. 2 Maker Distipute D2Mit2 "D2Ncc6 "D2Ncc7" D2Ncc7 "D2Ncc7" D2Ncc7 "D2Ncc7" "D2Ncc7" "D2Ncc7" "D2Ncc7" "D2Ncc7" "D2Ncc7" "D2Ncc7" "D2Ncc13" "D2Ncc12" Fgg Fkir D2Nsc3 D2Ncc4 D2Ncc4	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.4 8.3 0.0 3.5 3.4 10.1 15.4 3.1 2.9 0.0 22.7 8.8 10.4 4.5 3.3 0.0 23.0	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker_Disk D5Mit9 D5Mit11 Glut1 Ela2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 Marker_Dis Ighe *D6Ncc3 *D6Ncc8 *D6Ncc8 *D6Ncc9 Ckb D6Mgh3 D6Mgh4 D6Mcc10 *D6Ncc11 *D6Ncc11 *D6Ncc15 *D6Ncc15 *D6Ncc15 *D6Ncc15 *D6Ncc15 *D6Ncc15	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 0.0 1.1 11.6 30.5 3.4 1.9 3.9 0.6 1.0 2.8 2.0 1.2 7.2 11.5 13.4	Chr.9 Marker Disi D9Mgh2 D9Mgh2 D9Mkt3 'D9Ncc2 'D9Ncc2 'D9Ncc2 'D9Ncc3 Chr.10 Marker Dis D10Mcc1 D10Ncc1 D10Ncc4 Syb2 Tp53 'D10Ncc3 Chr.11 Marker Dis 'D10Ncc5 D11Mcc5 D11Ncc5 D11Ncc5 D11Ncc5 D11Ncc5 D11Ncc5 D11Ncc5 D11Ncc5 D11Ncc4 Sat	121.9 15.9 3.8 16.3 5.2 12.0 53.2 stance (cM) 16.2 14.4 11.6 3.4 2.4 0.0 0.0 1.1	Chr.15 <u>Maker Dist</u> "D15Noc2 "D15Noc2 "D15Noc3 "D15Noc5 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D15Mgh4 D16Moc5 "D16Noc4 "D16Noc2 "D16Noc4 "D17Noc4	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 1.6 2.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7 ance (cM) 12.9 5.6 8.8 3.5 0.1		
D1Ncc21 Chr. 2 Maker Dist D2Mit2 D2Ncc5 D2Ncc8 D2Ncc7 D2Ncc7 D2Ncc7 D2Ncc10 Prir D2Ncc10 Prir D2Ncc10 Prir D2Ncc12 Fga Fgg Pkir D2Ncc3 D2Ncc12 Fga Pkir D2Ncc3 D2Ncc12 Fga Pkir D2Ncc3 D2Ncc14 D2Ncc4	tance (cM) 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 13.2 14.2 15.4 3.1 2.9 0.0 2.7 8.8 10.4 4.5 3.3 0.0 12.7 13.0	*D4Ncc7 Ret *D4Ncc5 Pthih Chr.5 Marker Dist D5Mit9 D5Mit11 Giut1 Eia2 D5Ncc1 D5Mgh9 *D5Ncc2 Chr.6 Marker Dis Ighe *D6Ncc3 D6Ncc9 Ckb D6Msc3 D6Ncc9 Ckb D6Msc4 D6Ncc10 *D6Ncc11 *D6Ncc11 *D6Ncc11 *D6Ncc13 *D6Ncc14 *D6Ncc14 *D6Ncc14 *D6Ncc2 D6Mgh7	11.6 24.1 6.5 160.9 17.6 23.0 22.3 2.9 8.1 19.5 93.3 stance (cM) 6.8 0.0 1.1 11.6 30.5 3.4 1.9 3.4 1.9 3.9 0.6 1.0 2.8 2.0 1.2 7.2 11.5 13.4 7.1	Chr.9 <u>Marker Dis</u> D9Mit1 D9Mic1 D9Mic2 D9Mit3 D9Mic2 <u>D9Nic2</u> <u>D9Nic2</u> <u>D9Nic2</u> <u>D9Nic2</u> <u>D10Nic2</u> <u>D10Nic5</u> <u>D10Nicc1</u> <u>D10Nicc1</u> <u>D10Nicc1</u> <u>D10Nicc2</u> <u>Myn3</u> <u>D10Nicc3</u> <u>D10Nicc3</u> <u>Chr.11</u> <u>Marker Dis</u> <u>D11Nicc5</u> <u>D11Micc4</u> <u>S11Migh4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nicc4</u> <u>D11Nic</u>	121.9 15.9 3.8 16.3 5.2 12.0	Chr.15 <u>Maker Dist</u> "D15Noc2 "D15Noc2 "D15Noc2 "D15Noc3 D15Mgh1 "D15Noc5 D15Mgh3 D15Mgh4 D15Mgh4 D15Mgh4 D16Moc6 D16Mgh4 D16Kyo1 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc5 "D16Noc6 D16Noc6 D16Noc6 "D16Noc6" "D16Noc6 "D16Noc6" "D17Noc6" "D17Noc6	ance (cM) 16.1 17.8 6.3 1.6 15.5 17.8 15.8 106.9 ance (cM) 11.0 16.5 16.2 26.3 8.7 2.9 9.5 94.7 94.7 12.9 5.6 8.8 3.5 9.1 4.1		
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FIG. 2. Chromosomal assignment of the RDA markers. Three coat colors, 109 SSLP markers reported previously (7, 17, 18), and 28 AP-PCR markers were used as anchor loci. Numbers to the right of the loci are the distances estimated between two loci in cM. An asterisk indicates the polymorphic markers isolated in this study. Markers whose order is supported by a logarithm of odds of 2.0 or higher are indicated in boldface type.

ysis of ACI and BUF genomic DNA. Fig. 1C shows a representative result from Southern blot analysis probed with an isolated clone, M1–8, which was designated *D17Ncc3* after linkage mapping.

The characteristics of the clones recovered by pBluescript II by four series of RDA are summarized in Table 1. In total, 472 clones were recovered, 174 of them were independent and were not cross hybridized with each other. Of the 174 clones, 131 showed RFLP between ACI and BUF rats by Southern blot analysis. There was no major difference in the yields given by the two restriction enzymes used.

Chromosomal Assignment of the RDA Markers. A total of 105 ACI × BUF F_2 rats were used as a mapping panel. Because this panel corresponds to 210 meiosis, chromosomal markers could be potentially mapped at a resolution of 0.5 centimorgan (cM). The 105 F_2 rats were genotyped for 140 anchored loci, including 3 coat colors and 61 previously reported SSLP markers (17), 45 SSLP markers described by Jacob *et al.* (7), 3 SSLP markers reported by Remmers *et al.* (18), and 28 AP-PCR markers and RDA markers isolated in this study.

The previously reported markers and 131 RDA markers fell into 21 large linkage groups and 2 additional small linkage groups, covering all 21 rat chromosomes with an average spacing of 8 cM. Chromosomal assignment of these linkage groups was made based on published data on the 128 anchored loci. Of the 131 RDA markers, 125 fell into one of the 20 rat chromosomes with logarithm of odds scores of more than 5.0 (Fig. 2), and 6 were not linked by the same criteria to any of the 23 linkage groups.

GDRDA. To overcome the paucity of chromosomal markers in the rat, we applied GDRDA. The region around the *D1Ncc1* locus on chromosome 1 was targeted because there were only a few polymorphic markers between ACI and BUF (*Ton, Kal, D1Ncc1, C, Lsn*; see Fig. 3A Upper).

Two series of GDRDA were carried out; a tester amplicon was prepared from a *Bam*HI digest of ACI DNA and two *Bam*HI driver amplicons were prepared from two DNA pools of F_2 rats selected according to their genotype. Fig. 3A shows the frequency of ACI- and BUF-type alleles around *D1Ncc1*. In the first series, the BUF genomic DNA allele frequencies were 100% at the *Ton*, *D1Ncc1*, and *C* loci and 85% at the *Lsn* locus. In the second series, they were 100% at the *D1Ncc1* locus and 70% at the *C* and *Ton* loci. A longer region of chromosome 1 around *D1Ncc1* was targeted in the first series whereas only a region close to *D1Ncc1* was targeted in the second series.

After three rounds of RDA, clear bands were visible after ethidium bromide staining in both the first and second series (Fig. 3B). The bands were cloned into pBluescirpt II, and each clone was examined for RFLPs. Two of the six clones in the



FIG. 3. Isolation of polymorphic markers linked to the loci of interest by GDRDA. (A) Schema of the genotypes of the animals used to prepare driver amplicons. Ten F₂ rats having the BUF genotype in the region around D1Ncc1 were selected from 105 ACI \times BUF F₂ rats. Allele frequency of the two DNA pools used in the two series of GDRDA was indicated; open bars indicate the allele from ACI and solid bars indicate the allele from BUF. Polymorphic markers identified by GDRDA are boxed. (B) Agarose gel electrophoresis of GDRDA products (series 1). Aliquots $(2 \mu g)$ of tester BamHI DNA amplicon (lane 1), driver DNA amplicon (lane 2), and RDA products of the first (lane 3), second (lane 4), and third (lane 5) hybridization amplification were electrophoresed in a 2% NuSieve gel. Arrowheads indicate the bands examined for RFLP by Southern blot analysis of the genomic DNA. Asterisk indicates the exogenous plasmid DNA added as a positive control. Hae III/ ϕ X174 DNA size markers (lane M) are indicated in base pairs.

first series and two of the three clones in the second series were proved to be polymorphic by Southern blot analysis of ACI and BUF genomic DNA. These four polymorphic clones were mapped using the 105 F_2 mapping panel (Fig. 3A Upper). The two clones isolated in the first series were mapped at 24.2 cM (D1Ncc10) and 42.5 cM (D1Ncc11) away from D1Ncc1, and the two clones isolated in the second series were mapped at 0 cM (D1Ncc8 and D1Ncc9) away from D1Ncc1, with no recombination in the 105 F_2 mapping panel.

Dot Blot Analysis. Most of the RDA markers showed a big difference in size between the tester and driver strains of genomic DNA in Southern blot analysis, as observed with M1-8 (D17Ncc3) (Fig. 1C). This suggested that most of the RDA markers could be used as probes for the dot blot analysis of tester-strain amplicons, because the corresponding driver strain restriction DNA fragment might be too large to be amplified by PCR. Prepared from 105 ACI \times BUF F₂, inbred ACI and BUF DNA amplicons were blotted in duplicate onto a nylon membrane of 72 mm \times 36 mm using Kriplanker. Hybridization with a ³²P-labeled probe gave a clear positive or negative signal (Fig. 4). Test hybridization of the $105 F_2$ rats in this manner showed complete concordance with the result obtained by Southern blot analysis of genomic DNA (data not shown). Twenty-seven RDA markers were examined to show whether or not they could provide information about the dot blot amplicons. Of these, 25 proved to be informative (data not shown); thus about 90% of the RDA markers were considered to be appropriate for large-scale analysis.

DISCUSSION

In the present study, we isolated 131 polymorphic markers by RDA, and 126 of them could be mapped to 1 of 20 chromosomes by linkage analysis using an F_2 mapping panel with logarithm of odds scores of more than 5.0. None were mapped to chromosome X. To our knowledge, this is the first time a large-scale isolation of polymorphic markers by RDA has been reported. Using a series of RDAs (one enzyme with one combination of tester and driver amplicon), 32 to 67 independent clones were isolated, and 62% to 90% of them were polymorphic between two inbred rats (Table 1). This indicates that RDA is a very efficient way to isolate polymorphic markers. Considering that the number of polymorphic clones



FIG. 4. Dot blot analysis with a probe isolated by RDA. (A) Hybridization of the filter blotted with amplicons from $105 \text{ ACI} \times \text{BUF } F_2$, ACI, and BUF rats with an RDA probe (D1Ncc15). (B) Arrangement of the filter with the amplicons. Four samples were blotted in duplicate in a 9 mm × 9 mm square (positions a-d), and 32 squares were arranged in eight (A-H) rows and four columns (1-4). Amplicons of ACI and BUF rats were blotted in position d of D1 and position d of D2.

isolated was almost the same in the four series of RDA, it can be inferred that RDA, when used with other restriction enzymes, will yield almost the same number of markers. The distribution of RDA markers across the genome appears to be relatively uniform. They can thus be considered flexible when choosing portions of the genome. The use of different restriction enzymes will change the region of the genome targeted.

Our map integrates the RDA markers with the existing SSLP maps of Yamada *et al.* (6) and Jacob *et al.* (7), which as a result, provides much more information on the rat genome. The total length of our map is about 2000 cM, which is comparable to the estimate of Jacob *et al.* (7). Since our 105 ACI \times BUF F₂ rat mapping panel includes 210 meiosis, it could provide a resolution of 0.5 cM. We also determined the chromosomal loci of the *Ret* (21) Apc (22), and Tp53 (F.C., T.U., M.T., T. Su, and M.N., unpublished data) genes using this mapping panel.

Twenty-five of the 27 RDA polymorphic markers examined gave information about the dot blots of F_2 DNA amplicons. Since the technology required for high-density blotting (e.g., 864 dots on a 72 mm × 108 mm filter) is available, several times this number can be processed at the same time in a small hybridization bag. This gives a great advantage to RDA markers over SSLP markers, considering that it is becoming more important to map QTL using a large number of samples. Markers isolated by subtraction of interspersed repetitive sequence PCR products (IRS–PCR markers) can also be used as probes for dot blot analysis (23). Thus, integration of IRS–PCR markers with RDA markers may provide a powerful tool.

We have demonstrated that we can obtain markers in the intended regions if appropriate F_2 rats are selected in the preparation of driver DNA amplicons. We propose the following methods as a simple and rapid approach to mapping a target trait: (*i*) roughly screen the loci linked to a phenotype with markers suitable for large-scale analysis, such as RDA and IRS-PCR markers and (*ii*) generate more detailed markers linked to the loci of interest using GDRDA, or SSLPs near the loci. Mapping of QTL and positional cloning in the rat will be accelerated.

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