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# A High-Density Integrated Genetic Linkage and Radiation Hybrid Map of the Laboratory Rat

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The laboratory rat (*Rattus norvegicus*) is a key animal model for biomedical research. However, the genetic infrastructure required for connecting phenotype and genotype in the rat is currently incomplete. Here, we report the construction and integration of two genomic maps: a dense genetic linkage map of the rat and the first radiation hybrid (RH) map of the rat. The genetic map was constructed in two  $F_2$  intercrosses (SHRSP  $\times$  BN and FHH  $\times$  ACI), containing a total of 4736 simple sequence length polymorphism (SSLP) markers. Allele sizes for 4328 of the genetic markers were characterized in 48 of the most commonly used inbred strains. The RH map is a  $\text{lod} \geq 3$  framework map, including 983 SSLPs, thereby allowing integration with markers on various genetic maps and with markers mapped on the RH panel. Together, the maps provide an integrated reference to >3000 genes and ESTs and >8500 genetic markers (5211 of our SSLPs and >3500 SSLPs developed by other groups). [Bihoreau et al. (1997); James and Tanigami, RHdb (<http://www.ebi.ac.uk/RHdb/index.html>); Wilder (<http://www.nih.gov/niams/scientific/ratgbase>); Serikawa et al. (1992); RATMAP server (<http://ratmap.gen.gu.se>)] RH maps (v. 2.0) have been posted on our web sites at <http://goliath.ifrc.mcw.edu/LGR/index.html> or <http://curatools.curagen.com/ratmap>. Both web sites provide an RH mapping server where investigators can localize their own RH vectors relative to this map. The raw data have been deposited in the RHdb database. Taken together, these maps provide the basic tools for rat genomics. The RH map provides the means to rapidly localize genetic markers, genes, and ESTs within the rat genome. These maps provide the basic tools for rat genomics. They will facilitate studies of multifactorial disease and functional genomics, allow construction of physical maps, and provide a scaffold for both directed and large-scale sequencing efforts and comparative genomics in this important experimental organism.

Defining the function of the ~100,000 mammalian genes is a major goal for biologists in the next century. The mouse is widely used for understanding gene function, owing to the availability of genetic and genomic infrastructure together with the ability to perform gene knockout and knockin studies. However, the mouse lags behind other species with respect to physiological characterization and does not correlate with human

clinical characteristics for a large number of diseases. The rat is arguably the most commonly used model for physiological studies. Accordingly, the rat is likely to have a key role in the interpretation of mammalian genomes.

Over the past century, the rat has been a key animal for biomedical research; since 1966 nearly 500,000 manuscripts using rats have been published [PubMed, National Center for Biotechnology Information (NCBI) search using "rat" as the key word (<http://www.ncbi.nlm.nih.gov/PubMed/medline.html>)]. The rat has served as a primary model in biochemistry, neurobiol-

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ogy, nutrition, pharmacology, physiology, and other fields. There are numerous genetic diseases and disorders identified in >200 inbred strains of rat (Greenhouse et al. 1990). These genetic models have been used extensively for the study of alcohol preference, autoimmunity, behavior, cancer, dental diseases, diabetes, eye disorders, hematological disorders, hypertension, metabolic disorders, neurobiology, renal failure, reproduction, skeletal disorders, and toxicology (Robinson 1965; Lindsey 1979; Gill et al. 1989). Given the wealth of biological and biomedical information, the rat has the potential to have a major role in functional genomics and pharmacogenetics, both through studies within the organism and through comparative genomics.

Over the past 3 years, there has been an explosion in the rat genetic and genomic infrastructure. Several large-insert genomic libraries (Cai et al. 1997; Haldi et al. 1997; Woon et al. 1998), which are key resources for physical mapping, positional cloning, and transgenesis, have been constructed. More than a dozen normalized cDNA libraries have been made (B. Soares, pers. comm.), from which >90,000 rat expressed sequence tags (ESTs; from dbEST, NCBI) have been sequenced. Nearly 10,000 genetic markers, primarily simple sequence length polymorphisms (SSLPs), have been developed by several groups [Serikawa et al. 1992; Jacob et al. 1995; Bihoreau et al. 1997; Brown et al. 1998; RATMAP server (<http://ratmap.gen.gu.se>); M. James and A. Tanigami, RHdb (<http://www.ebi.ac.uk/RHdb/index.html>); R. Wilder, pers. comm. and <http://www.nih.gov/niams/scientific/ratgbase>], although a minority have been mapped previously. A rat radiation hybrid (RH) panel has been generated, from which it is possible to develop an RH map that would allow a PCR-based method for localizing any sequence within the genome. A key limitation, however, has been the absence of a tool to enable investigators to integrate these reagents in a coordinated and efficient fashion.

To provide such a tool for genome-wide mapping and integration, we have created two integrated genomic maps: a dense genetic map and a RH framework map. This provides a level of genomic integration that was available only in the human and just now is becoming available for the mouse. The genetic and RH maps will serve as a backbone for (1) placing quantitative trait loci (QTLs) for disease models from different crosses onto a common map; (2) integration of ESTs onto a common map; and (3) comparative mapping between rat, human, and mouse.

## RESULTS

### Genetic Linkage Maps

In generating the first genetic linkage map of the rat, we determined that the average polymorphism rate

between 12 inbred strains was 50% (Jacob et al. 1995). Whereas mouse genetic maps can be constructed in interspecific crosses having a polymorphism rate of 90%, no interspecific mapping crosses are available for the rat. Accordingly, we chose to generate genetic linkage maps from two different  $F_2$  intercrosses (SHRSP  $\times$  BN and FHH  $\times$  ACI) which enabled us to collectively map >65% of all markers.

We mapped a total of 4736 SSLP markers, yielding a genetic map spanning 1477 cM in the SHRSP  $\times$  BN and 1527 cM in the FHH  $\times$  ACI (Kosambi map units). The average genetic length is 1503 cM between the most distal markers, or ~1515 cM when allowing for the expected genetic distance beyond the most distal markers. The genetic length is remarkably close to the length calculated in our first genetic map in 1995 (Jacob et al. 1995), which consisted of 431 genetic markers (Table 1). However, the length is >24% shorter than an integrated map, merging data from multiple published crosses, reported by Bihoreau et al. (1997). The increased length is likely the result of map expansion produced during the integration process. Some expansion was also seen in an integrated genetic map produced by our group (Brown et al. 1998).

As for the mouse (Dietrich et al. 1996) and human (Dib et al. 1996) genetic maps, the rat SSLP markers appear to be distributed in a relatively uniform fashion across the genome (Table 1), with two notable exceptions. There was a clear deficit of markers on chromosome X, an observation that was reported previously for the rat (Jacob et al. 1995; Bihoreau et al. 1997; Brown et al. 1998) and for other mammalian species (Dib et al. 1996; Dietrich et al. 1996). There was also a significant excess of markers on chromosome 10, a trend which was observed (but fell below statistical significance) in our previous integrated map (Brown et al. 1998).

### Allele Characterization

Use of the genetic markers in a given cross requires knowledge of polymorphism of markers between the strains. It would be impractical for an investigator to characterize all or most of the 5211 loci developed by our group. To facilitate the use of the markers and maps by a larger community, we determined the allele sizes for 4328 of our 5211 SSLPs in 48 of the most commonly studied inbred strains of the rat (Table 2). These 48 strains provide a total of 1128 pairwise combinations of crosses. These rat strains represent a large collection of disease models. A useful polymorphism was defined to be a difference  $\geq 3$  bp. The average rate of useful polymorphism between strains was 46%, and the average number of alleles was determined to be 6 (range 2–16 alleles).

The allele data can also be used to generate haplotypes for use in comparing ancestral similarity between

**Table 1.** A Comparison of the Genetic Lengths of Various Maps

Chrom.	Genetic length (avg.)	Percent of genome		Observed no. of markers (union) <sup>c</sup>	Expected no. of markers	s.d.	Z-score	SHR × BN <sup>c</sup>	Integrated map <sup>d</sup>	Integrated map <sup>e</sup>
		genetic map <sup>a</sup>	physical map <sup>b</sup>							
1	141.4	9.4	10.0	483	432	20.8	2.4	151.0	173.6	181.9
2	110.6	7.4	8.9	379	385	19.6	-0.3	157.0	133.8	144.8
3	94.0	6.3	6.8	280	294	17.1	-0.8	104.0	103.9	147.0
4	104.2	6.9	6.6	282	285	16.9	-0.2	105.0	102.3	154.4
5	97.6	6.5	6.5	249	281	16.8	-1.9	116.0	100.4	118.2
6	72.8	4.8	5.6	221	242	15.6	-1.4	76.0	77.7	83.7
7	79.1	5.3	5.0	228	216	14.7	0.8	75.0	103.2	124.6
8	83.8	5.6	4.7	227	203	14.3	1.7	97.0	86.5	112.9
9	78.9	5.3	4.5	193	195	13.9	-0.1	41.0	79.3	85.0
10	92.9	6.2	4.3	262	186	13.6	5.6	94.0	98.9	62.1
11	49.2	3.3	3.7	110	160	12.6	-3.9	50.0	59.0	62.0
12	43.1	2.9	2.5	112	108	10.4	0.4	54.0	47.8	55.7
13	45.9	3.1	3.9	193	169	13.0	1.9	56.0	51.9	68.7
14	68.8	4.6	3.9	143	169	13.0	-2.0	43.0	55.2	79.7
15	68.8	4.6	3.7	166	160	12.6	0.5	49.0	47.4	92.6
16	45.9	3.1	3.4	127	147	12.1	-1.6	17.0	23.2	56.1
17	50.3	3.3	3.3	180	143	11.9	3.1	50.0	61.3	99.8
18	52.3	3.5	3.2	147	138	11.8	0.7	44.0	53.9	85.0
19	44.3	2.9	2.6	115	112	10.6	0.2	38.0	28.3	44.3
20	36.7	2.4	2.2	77	95	9.8	-1.9	47.0	54.5	56.5
X	42.0	2.8	5.4	149	233	15.3	-5.5	45.0	35.9	83.2
Total	1502.6	100.0	100.0	4323	4353	66.0	-0.5	1509.0	1578.2	1998.2

<sup>a</sup>Jacob et al. (1995).<sup>b</sup>Robinson (1965).<sup>c</sup>Includes only Rat markers, as they were generated randomly, whereas all other markers were chosen based on previous map data.<sup>d</sup>Brown et al. (1998).<sup>e</sup>Bihoreau et al. (1997).

strains. For this purpose, we declared a shared haplotype to be three or more consecutive markers within the same genetic bin (~1.3 cM) having the same allele size in two strains. Evaluation of a selection of haplotypes revealed that long-shared haplotypes (>10 consecutive markers) were more prevalent in closely related strains known to be derived from the same progenitor. Also, as would be expected, strains with a higher polymorphism rate shared fewer haplotypes. These preliminary analyses suggest that shared haplotypes could be used to assess the ancestral relationships among the strains.

### RH Map

RH mapping provides a powerful alternative means of mapping genes (Cox et al. 1990; Gyapay et al. 1996) without the need for first identifying a polymorphism. Based on our data, the rat RH cells retain an average of 25% of the rat genome, with a fragment size of 3.8 Mb. We omitted 12 of the 106 rat RH hybrids to conform to a 96-well format, including 6 hybrids with very low retention (<0.5%) between hybrids 1–100, as well as hybrids 101–106.

Of an RH data set containing 5359 SSLP vectors, 983 were incorporated into a RH framework map for the rat (Fig. 1, poster). The framework map for each

chromosome was constructed using the RHMPPER computer program with loci ordered with an odds ratio of 1000:1 (lod score for order  $\geq 3.0$ ). The framework map spans 19,368 cR, with 1 cR<sub>3000</sub> equaling ~155 kb. Figure 1 illustrates how the RH framework map correlates with the genetic maps. An initial comparison of the maps revealed that the RH maps for chromosomes 6, 10, and 13 did not have full telomeric representation. Review of the vectors for all markers on chromosomes 10 and 13 revealed very high retention of these regions in the RHs, higher than was acceptable according to our screening criteria (see Methods). The high retention of rat DNA for markers on chromosome 10 is likely due to the presence of the thymidine kinase gene, which is selected for in the generation of RHs; the reason underlying the high retention on chromosome 13 is unknown. The top three markers of chromosome 6 span the gap at the end of the chromosome. As an independent validation, the RH maps were evaluated using the MultiMap program. Although the two programs yielded slight differences in map length, the order of the loci was supported.

### Placement Maps

We were able to place an additional 3876 markers, including SSLPs, genes, and ESTs relative to the RH

**Table 2.** Commonly Studied Rat Disease Models for which we Determined SSLP Allele Sizes

Strain	Complete strain name	Disease model for
ACI	ACI	spontaneous tumors, urogenital abnormalities, aplasia of one kidney
AVN	AVN/Orl	immunology, autoimmunity
BB(DR)	BBDR/WorAp Diabetic Resistant	diabetes resistant
BB(DP)	BBDP/WorAp Diabetic Prone	diabetes (type 1)
BC/CPBU	BC/CpbU	low frequency of audiogenic seizures
BDIX	BDIX/Han	carcinogenesis, teratogenesis
BDVII	BDVII/Cub	immunology
BN/CUB-LX	BN/Cub-lx	blood pressure, cancer
BN/SSN	BN/SsNHsd	blood pressure, cancer
BP	BP/Cub	cancer, immunology
BUF	BUF/Pit	autoimmunity, cancer, drug abuse
COP	COP/OlaHsd	cancer, immunology
DA	DA/Pit	autoimmunity
FHH	FHH/Eur	blood pressure, renal disease, bleeding diathesis
F344	F344/Pit	cancer, immunology, retinal degeneration in aged rats, drug abuse
GH	GH/Omr	blood pressure, cardiac hypertrophy
GK	GK	diabetes (type II)
DRY	Donryu/Melb	cancer, neoplastic lesions in 120 week old rats
M520	M520/N	cancer, nephritis, immunology
IS/Kyo	IS/Kyo	congenital malformations
WN	WN/N	cancer, severe chronic nephritis
LH	LH	blood pressure
LE	LE/Mol	metabolic disorders
LEW	LEW/Pit	autoimmunity, blood pressure, drug abuse
LOU/C	LOU/CHan	cancer
LN	LN	blood pressure
MHS	MHS/Gib	blood pressure
MNR	MNR/N	alcohol, stress
MNRA	MNRA/Har	stress
MNS	MNS/Gib	blood pressure, renal disease
MR	MR/Pit	alcohol, stress, drug abuse
NEDH	NEDH/K	pheochromocytoma
NP	NP9	alcohol
ODU	ODU/N	dental disease
OKA	OKA/Wsl	blood pressure
OM	OM/Han	cancer, obesity, retinal degeneration
P	P5C	alcohol
PVG	PVG/Pit	autoimmunity
SD	SD/Rij	blood pressure, drug abuse
SHR	SHR/OlaHsd	blood pressure
SR/JR	SR/Jrlpcv	blood pressure
SHR-SP	SHRSP/Riv	blood pressure, stroke
SS/JR	SS/JrMcv	blood pressure
WAG	WAG/RijKyo	cancer
WF	WF/Pit	cancer
WIST	WIST/Nhg	cancer
WKY	WKY/OlaHsd	blood pressure, drug abuse
WTC	WTC/Kyo	congenital malformations

framework maps (data not shown). These vectors had a threshold lod  $\geq 15$  for initial chromosome assignments and were placed with a lod  $\geq 3$  relative to the framework maps. The data set used for placement contained all RH vectors (SSLPs, genes, and ESTs) regardless of the number of 2s and 1s, and 0s. The remaining markers could not be uniquely placed according to these criteria.

#### Comparison of Maps

In total, 681 of the 983 framework markers on the RH map were also mapped in one or both genetic maps. These loci, shown in red in Figure 1, enable error

checking of the order of the loci. There were 38 conflicts in order, and these were small differences in local order that likely reflect the uncertainties in the various maps.

#### Integration of Other Maps

The placement of >4800 markers onto a common RH map provides the ability to integrate the various genetic maps constructed to date, as well as providing a reagent to integrate future maps.

#### DISCUSSION

Over the past 4 years, rat genomics has progressed from

an initial genetic map of 431 genetic markers to the creation of an International Rat Genome Project. Although this effort has been quite successful, there has been limited effort to date to integrate the various reagents. Here, we report two integrated genomic maps: a dense genetic map, and the first RH map of the rat. Together, these maps provide basic infrastructure for rat genomics.

The total length of the genetic map is closer to 1500 cM than the 2000 cM estimated by several groups (Serikawa et al. 1992; Bihoreau et al. 1997). It is now clear that our initial genetic linkage map provided reasonably accurate estimates of the total genetic length and good coverage of the vast majority of the genome. However, efforts to integrate diverse maps from multiple groups, with limited numbers of markers in common, appear to have inflated the total genetic length (Table 1). The relationship between genetic distance and physical distance appears to be  $\sim 1.9$  Mb/cM, assuming that the rat genome contains 3 billion base pairs.

The large number of markers (4328) for which allele sizes have been determined, together with software tools available on our web site, should assist investigators in selecting appropriate polymorphic markers to be used in particular crosses. For this purpose, polymorphic markers were defined as having allele size differences of at least 3 bp. (We used the standard of  $\geq 3$ -bp differences, rather than the minimum 2-bp differences between CA repeats, because the scoring of 2-bp differences among the 48 strains was less reliable.) It is important to note that many substrains of rat carry the same name. Consequently, allele sizes may not be identical in some strains with the same name. When possible, we have given the exact substrain used to generate these data (Table 2).

The availability of an RH map, integrated with a dense genetic map containing the most commonly used markers, is valuable for several reasons. First, these maps enable the integration of diverse genetic markers, including 5211 SSLPs developed by us, 2508 SSLPs developed at Otsuka Pharmaceutical Company (A. Tanigami, RHdb), 248 developed at Oxford (Bihoreau et al. 1997; M. James, RHdb), and 384 developed at the National Institutes of Health (NIH) (R. Wilder, pers. comm., <http://www.nih.gov/niams/scientific/ratgbase>); this enables investigators to identify genetic markers in regions of interest without the need to characterize large numbers of markers in their particular cross. Second, the high-resolution map provides a starting point for developing a complete rat physical map, containing ordered large-insert clones spanning the genome. Finally, the RH map provides a means to rapidly localize any new locus, such as a gene, without the need to identify a polymorphism. Loci can be mapped simply by submitting the RH vector to a server

on one of our web sites. This allows large-scale EST mapping, which facilitates positional cloning and comparative mapping (among different rat crosses and different species). Together with a group at the University of Iowa led by V. Sheffield and B. Soares, we are currently performing RH mapping on nearly 10,000 rat ESTs and genes.

The maps presented here provide a level of genomic integration that was available previously only in the human and just now is becoming available for the mouse. The genetic and RH maps serve as a backbone for the integration of ESTs and greatly facilitate comparative mapping among rat, human, and mouse. All of the basic genetic and genomic tools now exist that are needed to design approaches for determining and sequencing important regions of the rat genome containing QTLs for common human diseases. These tools allow biomedical researchers to maximally utilize the rat as a model system, as well as to link together rat, mouse, and human studies.

## METHODS

### Generation of Simple Sequence Repeats

Female rat genomic DNA (WKY) was digested with the restriction enzymes *AclI*, *HaeIII*, or *RsaI* (New England BioLabs). DNA digests were size-separated twice using 3% NuSieve GTG agarose gels (FMC Bioproducts); two size fractions (250–450 and 450–700 bp) were collected. DNA was extracted and purified using the QIAquick gel extraction kit (Qiagen), cloned into M13mp18 (Pharmacia), transformed using XL-I Blue (Stratagene) or JM101 cells, and plated at a density of  $\sim 250$  plaques per 110 cm<sup>2</sup>. Clones containing a simple sequence repeat (SSR) were identified by performing plaque hybridization and processed as described previously (Jacob et al. 1995). Libraries determined to contain phage with the majority of inserts in the 250- to 500-bp range were utilized to identify and characterize SSLPs.

DNA was isolated from the phage clones by automated solid-phase reversible immobilization assay (SPRI) (DeAngelis et al. 1995) and sequenced using ABI377 DNA sequencers (Applied Biosystems) with an automated version of the manufacturer's *Taq* cycle sequencing protocol. From the sequence, PCR primers flanking the SSRs were designed with Primer 3.0 (Rozen and Skaletsky 1996, 1997) to generate a product of 70–400 bp, with a  $T_m$  between 55°C–63°C, devoid of apparent secondary structures, potential for primer-dimer formation, or repetitive elements (LINE, B1, B2, ID). In addition, sequence for each clone was screened against an internal database to avoid duplicate occurrences of the same locus. PCR primers were ordered from Research Genetics, Inc., and aliquots are available for purchase by the research community.

### Selection of Strains

The SHRSP  $\times$  BN F<sub>2</sub> intercross (D. Ganten) was selected because of the rate of polymorphism and because it allowed integration with the German Rat Genome Project. The FHH  $\times$  ACI F<sub>2</sub> intercross (A. Provoost) was selected to increase the number of polymorphic markers on the two maps, because the parental strains were derived from progenitor strains different than SHRSP  $\times$  BN. The 48 strains listed in

Table 2 were selected because of their utility in studying a large number of common human diseases. Investigators interested in more details about these strains are encouraged to visit The Jackson Laboratories web site: <http://www.informatics.jax.org/rat/>. It should be noted that these are the official strain designations; names such as Brown Norway are no longer used (Levan et al. 1995).

### Construction of Genetic Linkage Maps

Progeny from two independent  $F_2$  intercrosses (SHRSP  $\times$  BN; 45 animals, or FHH  $\times$  ACI, 46 animals) were genotyped by radiolabeled PCR reactions. The products were visualized by autoradiography following electrophoresis on polyacrylamide gels, as described previously (Dietrich et al. 1996). The following thermocycling protocol was used: initial denaturation at 92°C for 1 min, followed by 35 cycles of 92°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a final extension period at 72°C for 5 min. The presence of polymorphism was initially assayed by amplifying genomic DNA from the four inbred strains used for the two  $F_2$  intercrosses. Markers found to be polymorphic in one or both crosses were then amplified in the corresponding  $F_2$  intercross progeny.

Linkage analysis was performed by using the MAPMAKER computer program (Lander et al. 1987). Linkage assignment was confirmed by using a stringent lod threshold for linkage on each chromosome (lod > 5.0). Framework markers were ordered with a LOD threshold of 3.0. All other markers were placed in their most likely positions relative to this framework. Potential errors were detected by looking for double crossovers and using the MAPMAKER automatic error detection features (Lander et al. 1987). Marker order was compared between the two mapping crosses and the RH map. Discrepancies in marker order between the genetic and RH maps were checked by verifying the data on the original autoradiograph and corrected, if necessary.

The number of meioses—90 for  $F_2$  (SHRSP  $\times$  BN) and 92 for  $F_2$  (FHH  $\times$  ACI)—allows markers to be resolved to an average resolution of ~1.1 cM in either cross. Total genetic length was calculated by measuring the intermarker distance for each chromosome (Table 1) and adding one-half the average intermarker distance (0.3 cM) to each telomere. Orientation of the chromosomes is based on FISH analysis performed by Szpirer et al. (1998).

### Allele Characterization

Allele sizes for 4328 of the markers were determined for the 48 strains using an internal size standard placed in every other lane. The gel marker (BioVentures, Murphreesboro, TN) was end-labeled using T4 ligase and [ $\gamma$ - $^{32}$ P]ATP (6000/Ci mmole). Genetic markers were characterized using essentially the same protocol as described above with the following modifications: Both primers were labeled, and the thermocycler program consisted of an initial denaturation at 93°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, with a final extension period at 72°C for 3 min.

The complete polymorphism information, primer names, and sequences are available at the web sites of the Whitehead Institute/MIT Center for Genome Research (<http://www.genome.wi.mit.edu/rat/public/>), the Medical College of Wisconsin (<http://goliath.ifrc.mcw.edu/rat/>), and the Jackson Laboratories (<http://www.informatics.jax.org/rat/>).

### Construction of RH Map

The rat RH panel was developed in the laboratory of Dr. Peter Goodfellow (L. McCarthy, Oxford University, UK; unpubl.). The panel was generated by using a cell line derived from a female Sprague Dawley rat that was exposed to 3000 rads, employing a protocol similar to that used in the generation of the GeneBridge 4 human radiation panel (Gyapay et al. 1996). DNAs to be used for mapping additional loci are available from Research Genetics Inc. (Huntsville, AL). To conform to both the 192- and 384-well format, we chose to use 94 of 106 hybrids for mapping, based on their retention frequencies, together with rat and hamster DNA controls. DNA from RH cell lines (20 ng) was PCR amplified in 10- $\mu$ l reactions containing 1.4 pmole of each primer, 250  $\mu$ M each dNTP, and 0.25 units of *Taq* polymerase. The PCR was performed using a Tetrad thermocycler in an oil-free system (MJ Research) with the following protocol: 3 min denaturing at 93°C, and 35 cycles of 30 sec at 94°C, 30 sec at 48°C, and 30 sec at 72°C, with a final 3-min extension at 72°C. Samples were mixed with loading dye and electrophoresed on 2% agarose gels (1  $\times$  TBE) containing 0.001% (vol/vol) GelStar nucleic acid gel stain (FMC) and imaged using the Eagle Eye still video system (Stratagene). The collective set of scores (0 = no amplification; 1 = amplification; 2 = uncertain) for a single marker is called an RH vector. All markers were assayed in duplicate to reduce errors (Lunetta et al. 1995), and a consensus was generated from the duplicate vectors; a score of 2, in this case, indicates uncertain or discordant results.

Framework markers were selected by restrictive criteria. RH vectors with not more than four discrepancies between the duplicate vectors and neither too few (<10) nor too many positives (>39) were used as framework markers. The same criteria were used when integrating into our data set 4494 genetic markers (1605 developed by our group) placed in RHdb, a public RH database at the European Bioinformatics Institute (<http://www.ebi.ac.uk/RHdb/index.html>). Where markers were assayed by more than one group, we used our RH vector when available, to enrich for markers assayed using a consistent protocol. The RH map data set contains 5359 vectors—2537 were SSLPs developed by us for this work, 2508 were SSLPs developed by Otsuka Pharmaceutical Company (A. Tanigani, unpubl.), 248 were developed by Oxford University (Bihoreau et al. 1997; M. James, unpubl.), 58 were developed by NIH (R. Wilder, pers. comm., <http://www.nih.gov/niams/scientific/ratgbase>), and 8 were developed by other groups (Table 3). Two exceptions were made to our criteria for framework markers. The q telomere of chromosome 10 and the p telomere of chromosome 13 contain markers with >40 1s in order to extend the full chromosome length. All RH vectors, including SSLPs, genes, and ESTs, were used in the placement maps, with the duplicate markers serving as internal controls.

Whole-genome RH maps were constructed by using RHMAPPER and MultiMap computer programs (Matise et al. 1994; Stein et al. 1995). The framework map was constructed using RHMAPPER, initially without reference to the Whitehead genetic maps. Initially, the markers were separated by D number, and framework maps were built with an lod threshold for order of  $\geq 3$ . Marker order on the RH map was evaluated independently using the MultiMap program. For final error checking, the RH marker order was compared to the marker order in the genetic maps. The RH maps (v. 2.0) have been posted on our web site at <http://goliath.ifrc.mcw.edu/LGR/index.html> or <http://www.curatools.curagen.com/>

**Table 3.** Source of SSLPs Listed by the Genetic or RH Maps

SSLP type	WIBR/MIT CGR (SHRSP × BN)	WIBR/MIT CGR (FHH × ACI)	WIBR/MIT CGR (Union of two crosses)	Integrated <sup>a</sup>	SHR × BN <sup>b</sup>	Union of all crosses	RH map <sup>c</sup>
Rat	3462	1836	4323	0	0	4323	2103
Mit/Mgh	186	117	220	639	401	649	434
Arb <sup>d</sup>	138	122	189	0	0	189	58
Got <sup>e</sup>							2508
Wox <sup>e</sup>							248
Other <sup>f</sup>	4	1	4	38	30	50	8
Total	3790	2076	4736	677	431	5211	5359

<sup>a</sup>Brown et al. (1998).<sup>b</sup>Jacob et al. (1995).<sup>c</sup>Includes both framework markers (Fig. 1) and placement markers (not shown in Fig. 1).<sup>d</sup><http://www.nih.gov/niams/scientific/ratgbase><sup>e</sup><http://www.ebi.ac.uk/RHdb/index.html><sup>f</sup>Markers from GenBank and various other researchers.

ratmap. Both web sites provide an RH mapping server where investigators can localize their own RH vectors relative to this map. The raw data have also been deposited in the RHdb database.

### Marker Nomenclature

Locus names for SSLPs are in accordance with the Rat Nomenclature Committee (Levan et al. 1995). For example, D1Rat1 refers to a locus on chromosome 1 isolated and mapped by the large-scale mapping project at the Whitehead Institute/MIT Center for Genome Research and Medical College of Wisconsin. Other loci, such as RNO-D1Mit3, have the additional prefix RNO to denote *R. norvegicus* and, therefore, to avoid confusion with other loci developed by the same laboratory in other organisms (such as the mouse, human, or zebrafish). Fortunately, the Rat designation appears to be unique; therefore, RNO is currently not necessary for these loci. Markers with the symbol Mit are from the laboratory of E.S. Lander and coworkers at the Whitehead Institute/MIT Center for Genome Research; those with the symbol Mgh are from the laboratory of H. Jacob et al. at the Massachusetts General Hospital; markers with the Mcw symbol are from the laboratory of H. Jacob et al. at the Medical College of Wisconsin; markers with the symbol Arb are from the laboratory of R. Wilder, E. Remmers, and colleagues at the National Institute of Arthritis and Musculoskeletal and Skin Diseases; markers with the symbol Wox are from M. James and M. Lathrop at Oxford University; markers with the symbol Got are from A. Tanigami at Otsuka Pharmaceutical Company; markers representing gene sequence from GenBank utilize their GenBank gene name. For other previously published loci, we have used the designation given by the original authors, even when they do not necessarily conform to the standards established by the nomenclature committee (Levan et al. 1995).

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Generation of SHRSP × BN F<sub>2</sub> Intercross: Detlev Ganten (Director); Margit Knoblauch (Project leader).

FHH × ACI F<sub>2</sub> Intercross: Abraham Provoost (Director).

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Robert G. Steen, Anne E. Kwitek-Black, Christopher Glenn, Jo Gullings-Handley, William Van Etten, O. Scott Atkinson, Diane Appel, Simon Twigger, Melanie Muir, Tim Mull, Mary Granados, Mushira Kissebah, Kerri Russo, Robbin Crane, Michael Popp, Marc Peden, Tara Matise, Donna M. Brown, Jian Lu, Stephen Kingsmore, Peter J. Tonellato, Steve Rozen, Donna Slonim, Peter Young, Margit Knoblauch, Abraham Provoost, Detlev Ganten, Steven D. Colman, Jonathan Rothberg, Eric S. Lander, and Howard J. Jacob.

L. McCarthy was mistakenly affiliated with Oxford University, UK in the above-mentioned paper. The correct affiliation should be Cambridge University, UK.



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