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Genetics

# 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, *Bam*HI and *Hin*dIII; 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 × BUF $F_2$ 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 $F_2$ 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).

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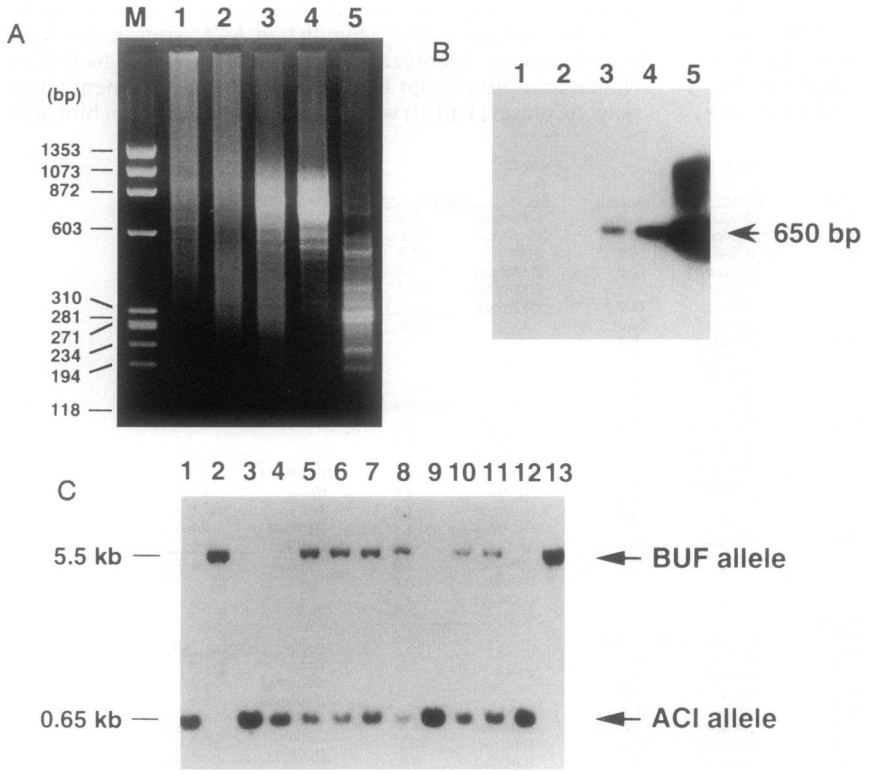


FIG. 1. RDA of DNAs from ACI and BUF rats. (*A*) Agarose gel electrophoresis of *Bam*HI RDA products. Aliquots (2 μg) of the tester RDA amplicon (lane 1), driver DNA amplicon (lane 2), and RDA products of the first (lane 3), second (lane 4) and third (lane 5) competitive hybridization-amplification steps were electrophoresed in a 2% NuSieve gel. *Hae* III/φX174 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 *Bam*HI digested genomic DNA from ACI (lane 1), BUF (lane 2), and ACI × BUF $F_2$ rats (lanes 3–13). M1–8 was mapped to chromosome 17 by using a 105 ACI × BUF $F_2$ mapping panel and designated *D17Ncc3*.

**Southern Blot Analysis.** Genomic DNA (10 μg) 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-$^{32}$P]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).

**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 μg) in 20 μl of Tris/EDTA was mixed with 20 μl of 20× standard saline citrate (1× 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 × 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 | | | | | |
| *Bam*HI | ACI | BUF | 70 | 40 | 36 (90) |
| | BUF | ACI | 64 | 32 | 24 (75) |
| *Hin*dIII | ACI | BUF | 177 | 67 | 42 (62) |
| | BUF | ACI | 161 | 35 | 29 (83) |
| Subtotal | | | 472 | 174 | 131 (75) |
| GDRDA | | | | | |
| *Bam*HI | 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 × BUF $F_2$ rats as shown in Fig. 3*A*.

*Hin*dIII 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

| Maker | Distance (cM) |
|---|---|
| **D1Mgh16** | 22.8 |
| ***D1Ncc16** | ------- |
| | 22.8 |

| Maker | Distance (cM) |
|---|---|
| *D1Ncc15 | 11.9 |
| *D1Ncc24 | 1.3 |
| **Ton** | 0.0 |
| **Klk1** | 11.1 |
| *D1Ncc13 | 2.8 |
| **D1Ncc1** | 0.0 |
| ***D1Ncc8** | 0.0 |
| ***D1Ncc9** | 1.2 |
| ***D1Ncc18** | 3.7 |
| *D1Ncc17 | 15.3 |
| ***D1Ncc22** | 4.1 |
| **C** | 0.0 |
| ***D1Ncc10** | 2.0 |
| *D1Ncc14 | 15.0 |
| **PTHrel** | 2.2 |
| **D1Ncc2** | 0.0 |
| ***D1Ncc11** | 1.3 |
| *D1Ncc25 | 3.3 |
| D1Ncc3 | 1.3 |
| *D1Ncc19 | 0.1 |
| **Lsn** | 0.0 |
| **Myl2** | 1.3 |
| D1Ncc4 | 1.3 |
| **Tnt3** | 0.0 |
| **D1Ncc5** | 1.9 |
| D1Ncc6 | 2.0 |
| *D1Ncc20 | 10.5 |
| ***D1Ncc26** | 5.8 |
| *D1Ncc27 | 9.8 |
| ***D1Ncc12** | 6.4 |
| **D1Ncc7** | 22.4 |
| *D1Ncc23 | 7.9 |
| *D1Ncc28 | 9.6 |
| D1Mgh12 | 12.0 |
| D1Mit14 | 13.4 |
| ***D1Ncc21** | ------- |
| | 181.2 |

Chr. 2

| Maker | Distance (cM) |
|---|---|
| **D2Mit2** | 13.2 |
| ***D2Ncc5** | ------- |
| | 13.2 |

| Maker | Distance (cM) |
|---|---|
| *D2Ncc8 | 0.0 |
| *D2Ncc7 | 2.5 |
| ***D2Ncc9** | 9.6 |
| **Mt1pb** | 1.6 |
| ***D2Ncc10** | 8.2 |
| **Prlr** | 14.4 |
| **D2Ncc1** | 8.3 |
| Cpb | 0.0 |
| **D2Mgh5** | 3.5 |
| ***D2Ncc15** | 3.4 |
| D2Ncc2 | 10.1 |
| ***D2Ncc13** | 15.4 |
| *D2Ncc11 | 3.1 |
| *D2Ncc12 | 2.9 |
| **Fga** | 0.0 |
| **Fgg** | 0.0 |
| **Pklr** | 22.7 |
| **D2N91** | 8.8 |
| **D2Ncc3** | 10.4 |
| **D2Mit15** | 4.5 |
| **D2Ncc6** | 3.3 |
| ***D2Ncc14** | 0.0 |
| D2Ncc4 | ------ |
| | 133.0 |

Chr.3

| Marker | Distance (cM) |
|---|---|
| **D3Mgh9** | 6.8 |
| **D3Kyo2** | 1.4 |
| *D3Ncc5 | 13.7 |
| **D3Mit9** | 2.5 |
| *D3Ncc3 | 1.6 |
| D3Ncc1 | 15.7 |
| ***D3Ncc4** | 0.9 |
| *D3Ncc8 | 1.6 |
| *D3Ncc7 | 6.0 |
| **Scn2a1** | 15.9 |
| D3Ncc2 | 5.4 |
| ***D3Ncc6** | 10.2 |
| **Ivd** | 7.1 |
| **D3Kyo5** | 2.1 |
| *D3Ncc9 | 21.7 |
| **A** | 17.8 |
| **Svp2** | 3.9 |
| **Pck1** | ------ |
| | 134.3 |

Chr.4

| Marker | Distance (cM) |
|---|---|
| **Il6** | 14.7 |
| *D4Ncc6 | 13.2 |
| D4Ncc1 | 8.8 |
| **D4Mgh14** | 15.4 |
| *D4Ncc11 | 13.2 |
| D4Ncc2 | 2.9 |
| **Cpa2** | 6.2 |
| **Try1** | 0.0 |
| D4Ncc3 | 5.8 |
| *D4Ncc10 | 6.0 |
| *D4Ncc9 | 2.9 |
| *D4Ncc12 | 0.0 |
| **Fabp1** | 4.5 |
| *D4Ncc8 | 9.2 |
| **Spr** | 10.7 |
| **Ampp** | 5.0 |
| D4Ncc4 | 0.1 |
| ***D4Ncc7** | 11.6 |
| **Ret** | 24.1 |
| *D4Ncc5 | 6.5 |
| **Pthlh** | ------ |
| | 160.9 |

Chr.5

| Marker | Distance (cM) |
|---|---|
| **D5Mit9** | 17.6 |
| **D5Mit11** | 23.0 |
| **Glut1** | 22.3 |
| **Ela2** | 2.9 |
| D5Ncc1 | 8.1 |
| **D5Mgh9** | 19.5 |
| ***D5Ncc2** | ------- |
| | 93.3 |

Chr.6

| Marker | Distance (cM) |
|---|---|
| **Ighe** | 6.8 |
| *D6Ncc3 | 0.0 |
| *D6Ncc8 | 0.0 |
| *D6Ncc9 | 1.1 |
| **Ckb** | 11.6 |
| **D6Mgh3** | 30.5 |
| **D6Mgh4** | 3.4 |
| ***D6Ncc10** | 1.9 |
| ***D6Ncc9** | 3.9 |
| *D6Ncc12 | 0.6 |
| **D6Mit2** | 1.0 |
| *D6Ncc11 | 2.8 |
| ***D6Ncc4** | 2.0 |
| *D6Ncc15 | 1.2 |
| ***D6Ncc14** | 7.2 |
| ***D6Ncc13** | 11.5 |
| ***D6Ncc2** | 13.4 |
| **D6Mgh7** | 7.1 |
| ***D6Ncc5** | 2.6 |
| ***D6Ncc7** | 5.3 |
| ***D6Ncc6** | 23.1 |
| **D6Ncc1** | 8.4 |
| **D6Mit5** | ------ |
| | 129.6 |

Chr.7

| Marker | Distance (cM) |
|---|---|
| *D7Ncc9 | 5.8 |
| ***D7Ncc11** | 10.5 |
| **D7Mit7** | 4.8 |
| ***D7Ncc7** | 12.9 |
| *D7Ncc4 | 7.8 |
| *D7Ncc3 | 14.7 |
| ***D7Ncc5** | 20.0 |
| ***D7Ncc8** | 8.5 |
| ***D7Ncc6** | 5.4 |
| **D7Mit5** | 6.2 |
| **Myc** | 0.0 |
| *D7Ncc1 | 12.6 |
| **D7Mit14** | 7.9 |
| ***D7Ncc12** | 4.2 |
| *D7Ncc10 | 6.6 |
| **D7Mgh1** | 9.4 |
| **Ela1** | 1.4 |
| Prph | 14.0 |
| ***D7Ncc2** | ------- |
| | 152.7 |

Chr.8

| Marker | Distance (cM) |
|---|---|
| **Acsa** | 9.0 |
| **D1N64** | 4.2 |
| **Mylc1v** | 6.9 |
| **Rbp2** | 5.8 |
| **D8Ncc1** | 8.8 |
| **D8N136** | 22.3 |
| **Apoc3** | 5.6 |
| **Thy1** | 1.4 |
| *D8Ncc2 | 0.7 |
| **D8Mit3** | 1.9 |
| *D8Ncc3 | 4.4 |
| ***D8Ncc8** | 10.4 |
| ***D8Ncc4** | 21.7 |
| *D8Ncc7 | 0.0 |
| ***D8Ncc6** | 18.6 |
| ***D8Ncc5** | ------- |
| | 121.9 |

Chr.9

| Marker | Distance (cM) |
|---|---|
| **D9Mit1** | 15.9 |
| ***D9Ncc1** | 3.8 |
| **D9Mgh2** | 16.3 |
| **D9Mit3** | 5.2 |
| ***D9Ncc2** | 12.0 |
| ***D9Ncc3** | ------- |
| | 53.2 |

Chr.10

| Marker | Distance (cM) |
|---|---|
| **D10Mit5** | 16.2 |
| ***D10Ncc1** | 14.4 |
| **D10Mgh10** | 11.6 |
| ***D10Ncc2** | 3.4 |
| **Myh3** | 2.4 |
| *D10Ncc4 | 0.0 |
| Syb2 | 0.0 |
| Tp53 | 1.1 |
| ***D10Ncc3** | ------- |
| | 49.1 |

Chr.11

| Marker | Distance (cM) |
|---|---|
| *D11Ncc6 | 2.8 |
| **D11Mgh2** | 7.5 |
| **Sst** | 0.0 |
| **Kng** | 8.2 |
| ***D11Ncc5** | 11.3 |
| **D11Mgh4** | 1.1 |
| ***D11Ncc4** | 5.4 |
| *D11Ncc2 | 6.9 |
| D11Ncc1 | 27.2 |
| **Mox2** | 33.6 |
| *D11Ncc3 | 11.2 |
| *D11Ncc7 | 6.2 |
| **D11Mit2** | ------ |
| | 121.6 |

Chr.12

| Marker | Distance (cM) |
|---|---|
| ***D12Ncc3** | 7.8 |
| **D12Mit5** | 12.2 |
| **Planh** | 10.4 |
| **D12Mgh5** | 1.6 |
| ***D12Ncc1** | 15.4 |
| ***D12Ncc2** | ------- |
| | 47.4 |

Chr.13

| Marker | Distance (cM) |
|---|---|
| **D13Kyo3** | 6.9 |
| D13Ncc1 | 6.4 |
| *D13Ncc5 | 6.1 |
| **Ren** | 16.3 |
| D13Ncc2 | 4.5 |
| D13Ncc3 | 21.8 |
| *D13Ncc6 | 2.1 |
| Atp1a2 | 1.1 |
| D13Ncc4 | 1.2 |
| **D13Mit4** | 5.9 |
| *D13Ncc8 | 14.1 |
| ***D13Ncc7** | ------- |
| | 86.4 |

Chr.14

| Marker | Distance (cM) |
|---|---|
| *D14Ncc4 | 5.6 |
| ***D14Ncc3** | 12.5 |
| **D14Mit3** | 9.6 |
| **H** | 2.9 |
| D14Ncc1 | 6.0 |
| *D14Ncc2 | 0.0 |
| CSNA | 0.8 |
| **Afp** | 0.0 |
| **Alb** | 5.7 |
| **Bsp** | ------- |
| | 43.2 |

Chr.15

| Marker | Distance (cM) |
|---|---|
| *D15Ncc2 | 16.1 |
| ***D15Ncc1** | 17.8 |
| ***D15Ncc4** | 6.3 |
| **D15Mgh1** | 1.6 |
| *D15Ncc3 | 16.0 |
| ***D15Ncc5** | 15.5 |
| **D15Mgh3** | 17.8 |
| **D15Mgh4** | 15.8 |
| **D15Mgh6** | ------- |
| | 106.9 |

Chr.16

| Marker | Distance (cM) |
|---|---|
| ***D16Ncc6** | 11.0 |
| **D16Mgh4** | 1.6 |
| **D16Kyo1** | 2.0 |
| *D16Ncc4 | 16.5 |
| ***D16Ncc5** | 16.2 |
| ***D16Ncc9** | 26.3 |
| **D16Ncc2** | 8.7 |
| *D16Ncc7 | 2.9 |
| *D16Ncc8 | 9.5 |
| ***D16Ncc3** | ------- |
| | 94.7 |

Chr.17

| Marker | Distance (cM) |
|---|---|
| ***D17Ncc4** | 12.9 |
| ***D17Ncc2** | 5.6 |
| **D17Mit2** | 8.8 |
| ***D17Mit3** | 3.5 |
| **Chrm3** | 9.1 |
| **D17Mit5** | 6.1 |
| ***D17Ncc1** | ------- |
| | 45.9 |

Chr.18

| Maker | Distance (cM) |
|---|---|
| ***D18Ncc1** | 9.7 |
| **Ttr** | 12.8 |
| **Apc** | 20.5 |
| **Adrb2** | 5.6 |
| **Gja1** | 0.0 |
| **Olf** | 7.3 |
| **D18Mgh3** | 3.3 |
| ***D18Ncc2** | 3.4 |
| ***D18Ncc3** | ------- |
| | 62.7 |

Chr.19

| Maker | Distance (cM) |
|---|---|
| ***D19Ncc2** | 7.7 |
| **Hp** | 2.7 |
| **Tat** | 17.6 |
| **D19Mit3** | 8.5 |
| ***D19Ncc1** | ------- |
| | 36.5 |

Chr.20

| Maker | Distance (cM) |
|---|---|
| **Tnfa** | 4.7 |
| ***D20Ncc2** | 8.4 |
| ***D20Ncc3** | 3.5 |
| ***D20Ncc4** | 18.3 |
| *D20Ncc1 | 27.3 |
| **D20Mit1** | ------- |
| | 51.3 |

Chr.X

| Maker | Distance (cM) |
|---|---|
| **DXNcc1** | 28.7 |
| **Cbpl** | 18.0 |
| **Pfkfb1** | ------- |
| | 46.7 |

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. 1*C* 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. 3*A 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. 3*A* 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. 3*B*). The bands were cloned into pBluescirpt II, and each clone was examined for RFLPs. Two of the six clones in the

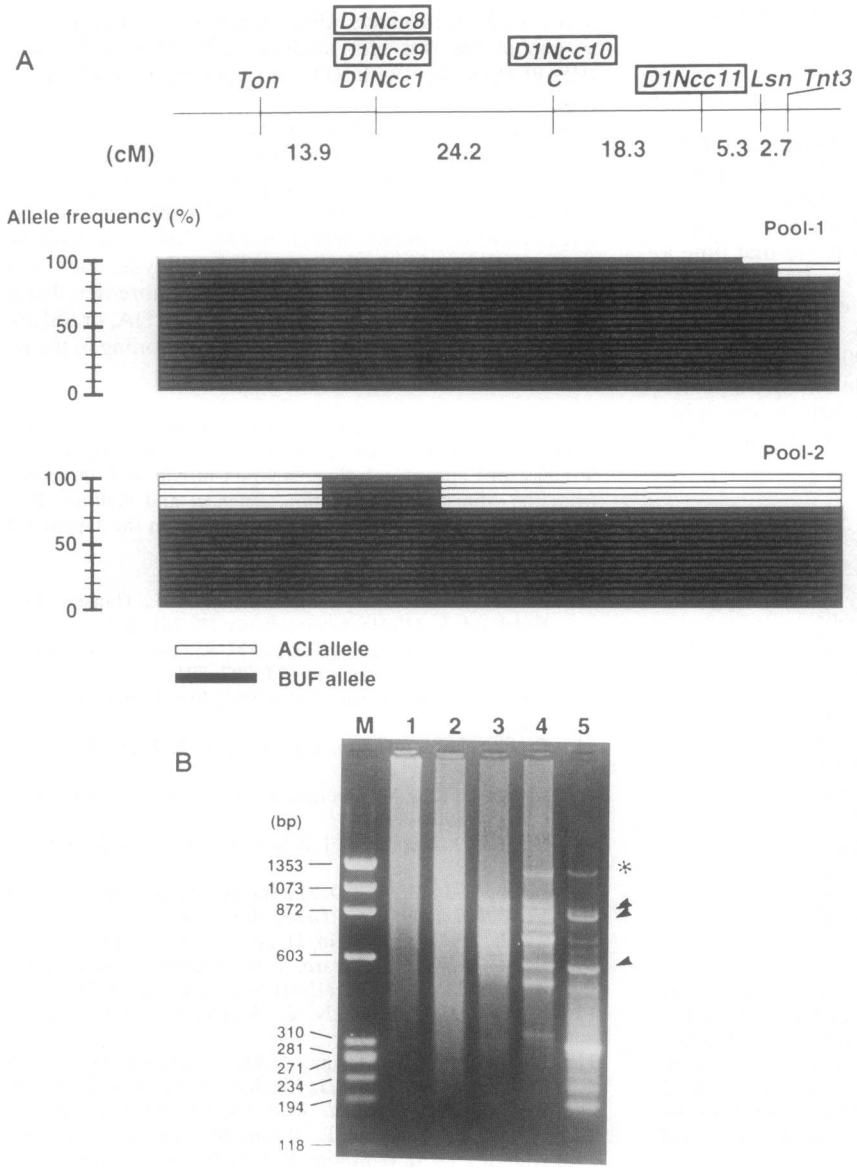


FIG. 3. Isolation of polymorphic markers linked to the loci of interest by GDRDA. (*A*) Schema of the genotypes of the animals used to prepare driver amplicons. Ten $F_2$ rats having the BUF genotype in the region around *D1Ncc1* were selected from 105 ACI × BUF $F_2$ rats. Allele frequency of the two 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 μg) of tester *Bam*HI 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/φX174 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. 3*A 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 × BUF $F_2$, inbred ACI and BUF DNA amplicons were blotted in duplicate onto a nylon membrane of 72 mm × 36 mm using Kriplanker. Hybridization with a $^{32}$P-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 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.

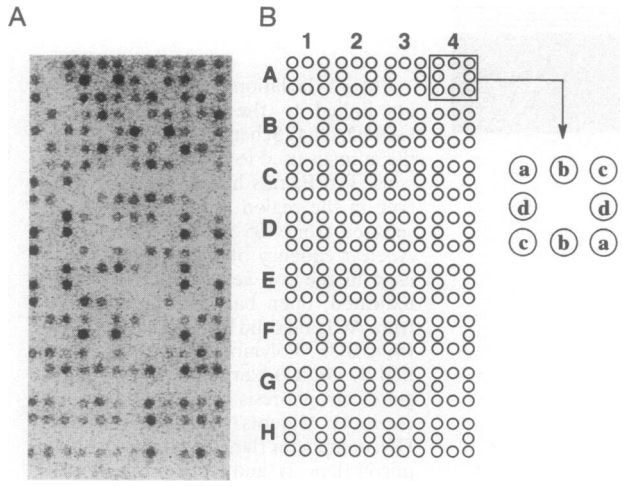


FIG. 4. Dot blot analysis with a probe isolated by RDA. (*A*) Hybridization of the filter blotted with amplicons from 105 ACI × BUF $F_2$, ACI, and BUF rats with an RDA probe (*D1Ncc15*). (*B*) Arrangement of the filter with the amplicons. Four samples were blotted in duplicate in a 9 mm × 9 mm square (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.

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 × BUF $F_2$ 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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