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# *Mus spretus*-Specific LINE-1 DNA Probes Applied to the Cloning of the Murine *Pearl* Locus

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**LINE-1 is the major family of long, interspersed, repetitive DNA sequences found in mammalian genomes. The mouse species *Mus spretus* contains large LINE-1 subfamilies that are distinguishable from the LINE-1 elements of laboratory *Mus domesticus* strains by their content of particular nucleotide differences. Oligonucleotides containing these differences act as *M. spretus*-specific LINE-1 hybridization probes. We have used these probes as a novel genetic tool in conjunction with an interspecific hybrid congenic mouse, in which the *M. spretus* allele of the *pearl* gene has been transferred onto a *M. domesticus* background. From a lambda library prepared from this congenic mouse, four clones were isolated by hybridization to the *M. spretus*-specific probes. After derivation of genetic markers from these clones, two of them were found to be linked to the *pearl* gene. These markers are the first two of up to 75 that could be isolated to support cloning the *pearl* gene. Considering the interspersed nature of LINE-1, we propose that species-specific LINE-1 probes could also be used to isolate markers for many other target genes.** 

## INTRODUCTION

Hundreds of murine genetic variants are studied in biomedical research as a means of understanding human physiological dysfunctions (Lyon and Searle, 1989). For example, the *pearl* mouse used in this study has vision abnormalities similar to some forms of human congenital stationary night blindness (Pinto *et al.*, 1985). *Pearl* was originally described as an autosomal recessive mutation causing hypopigmentation of the coat and the eyes (Sarvella, 1954). It exhibits a defect in the optokinetic nystagmus (Balkema *et al.*, 1981; Mangini *et al.*, 1985), a 100-fold reduction of retinal sensitivity in the dark-adapted state (Balkema *et al.*, 1983), and altered binding of somatostatin by the retina (Kossut *et al.*, 1990).

We would like to clone the *pearl* gene. Because of the lack of a candidate protein and the lack of tissues known to differentially express the gene, we have chosen a positional cloning approach. By making use of breeding manipulations available in the murine genetic system, we want to map genetic markers sufficiently close to the *pearl* gene that they will be able to support the subsequent cloning of the gene. Positional cloning requires some form of chromosomal walking, which is laborious. Consequently, we want to begin from a marker that is very close to the *pearl* gene. To increase the probability of finding such a marker, we have implemented a method for isolating a batch of markers that are linked to the *pearl* gene.

In this report, *Mus spretus*-specific repetitive sequence probes are used in conjunction with an interspecific *pearl* congenic mouse to selectively isolate LINE-1-containing clones near the *pearl* gene. The interspecific congenic mouse is a hybrid between a C57BL/6J mouse bearing the *pearl* mutation and *M. spretus*. We refer to C57BL/6J as *M. domesticus*, although it is more exactly an inbred artificial hybrid between the subspecies *M. musculus domesticus* and *M. musculus musculus* (Bonhomme, 1986). *M. spretus* is a separate species that can still interbreed with *M. domesticus* in the laboratory and produce fertile female offspring (Bonhomme *et al.*, 1978, 1979), although it apparently has not done so in nature for 3 to 5 Myr (Bonhomme *et al.*, 1984). The congenic hybrid was bred such that the wildtype allele of *pearl* from *M. spretus* and its surrounding DNA were genetically transferred to the *M. domesticus* background by repeated backcrossing and phenotypic selection for the coat color. The interspecific congenic mouse was then made homozygous for the *M. spretus* wildtype allele of the *pearl* gene.

The repetitive sequence that we have used to follow the transferred *M. spretus* DNA was LINE-1, an abundant repetitive DNA sequence family whose members

Sequence data from this article have been deposited with GenBank under Accession Nos. L07331 and L07332.

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are interspersed among many different genes (for reviews see Skowronski and Singer, 1986; Edgell *et al.*, 1987; Hutchison *et al.*, 1989; Di Nocera and Sakaki, 1990). LINE-1 is found in all mammals; however, studies of LINE-1 evolution revealed that subfamilies containing defining base differences exist in individual species of animals (Martin *et al.*, 1985). This led to the design of *M. spretus*-specific oligonucleotide hybridization probes, oMS496 and oMS416C, detecting about 9000 LINE-1 elements each in the *M. spretus* genome (Rikke *et al.*, 1991; Rikke and Hardies, 1991).

Although each probe also detects a sizable background in laboratory strains of *M. domesticus*, we have demonstrated that 7500 LINE-1 elements are detected by both probes in *M. spretus*, whereas only about 30 LINE-1 elements are detected by both probes in the laboratory strain C57BL/6J. Consequently, it was expected that the application of the probes to a library from the *pearl* congenic mouse would yield a number of doubly positive clones, many of which would have originated from the *M. spretus* segment surrounding the *pearl* gene. In this report, we have characterized four such double positives and found that two of them were linked to the *pearl* gene.

## MATERIALS AND METHODS

*Mouse strains.* The interspecific congenic strain was bred starting with the following parents: The *M. spretus* parent was an inbred wildtype strain SPRET/Ei obtained from Dr. Eva Eicher, The Jackson Laboratory. The *M. domesticus* was C57BL/6JPin *pe/pe*, in which the *pe* mutant, which had originally arisen on a C3H background (Sarvella, 1954), had been transferred to a C57BL/6J background. The congenic animal was bred by (1) crossing a *M. spretus* female with a *M. domesticus* C57BL/6JPin *pe/pe* male, (2) backcrossing a female F1 to a C57BL/6JPin *pe/pe* male to produce the N2 generation, (3) choosing an N2 heterozygote by its wildtype coat color to further backcross to C57BL/6J *pe/pe* to create the N3 generation, (4) continuing to backcross until reaching the N12 generation, and (5) inbreeding heterozygotes from the N12 generation to create an interspecific congenic strain homozygous for the *M. spretus* wildtype allele of the *pearl* locus in an otherwise *M. domesticus* background. This latter line, formally B6–*spretus* $pe^{+Pin}/pe^{+Pin}$, has been successfully inbred for 12 additional generations.

*The congenic library.* The interspecific congenic mouse library, having a complexity of 150,000 plaque-forming units, was constructed from a partial *Mbo*I digest of B6–*spretus* $pe^{+Pin}/^{+Pin}$ (N12) DNA using the bacteriophage λ vector EMBL3. Construction and screening were performed as previously described (Rikke and Hardies, 1991), with the following modification. The wash temperature used after hybridization to oMS496 was 43–45°C, which represents a minor upward adjustment in stringency that gave a signal more suitable for this screening. Plaque density for screening with oMS496 ranged from 2300 to 4700 per 150-mm plate. Plaque density for rescreening with oMS416C was 20–100 per 100-mm plate. The stringency wash for oMS416C was 50°C, as previously described (Rikke and Hardies, 1991). All screening was done in duplicate and employed the signal amplification procedure of Woo (1979).

*Probes for Southern blot hybridization.* A 1.5-kb *Eco*RI/*Hin*dIII fragment from EMBL3 clone PL1 was subcloned into M13mp18 to make mPL1A. The 1.5-kb fragment originated from a 7-kb *Eco*RI fragment from PL1 which does not contain the LINE-1 element. The Marker 1 RFLP probe was PCR amplified from mPL1A using vector primers flanking the insert. A nonrepetitive 2.5-kb *Eco*RI fragment of EMBL3 clone PL2 was cloned into pUC119 to make pUCPL2X. pUCPL2X was directly labeled and used as the Marker 2 RFLP probe. A nonrepetitive 1.8-kb *Eco*RI fragment of EMBL3 clone PL3 was cloned into pUC119 to make pUCPL3A, which was directly labeled and used as the Marker 3 RFLP probe. A 6.2-kb *Eco*RI/*Sal*I fragment from EMBL3 clone PL4 was cloned into pUC119 to make pUCPL4B, which was directly labeled and used as the Marker 4 RFLP probe. The probes were labeled with $^{32}P$ by nick translation.

*Sequencing of sequence tagged sites.* DNA sequencing was on double-stranded circular templates using primers to vector regions flanking the insert or using custom-synthesized internal primers. The dideoxy chain termination method was performed as described (Johnston-Dow *et al.*, 1987; Toneguzzo *et al.*, 1988). The sequence reported for Marker 2 comes from just inside the insert in pUCPL2X next to the universal priming site. All of the sequence reported was confirmed by sequencing back on the opposite strand. The sequence reported for Marker 3 starts about 100 bp from the end of the insert in pUCPL3A next to the reverse priming site of the vector. It starts just beyond a CA dinucleotide repeat near the end of the insert. All but the last 33 bases of the sequence reported were confirmed by sequencing back on the complementary strand. No matches to these sequences were found in a FASTA search of the GenBank rodent library (Pearson and Lipman, 1988; Benton, 1990).

*Screening of hamster/mouse somatic cell hybrids.* Hamster/mouse somatic cell hybrids were kindly provided by Dr. C. A. Kozak. They were derived from a Chinese hamster–BALB/c mouse hybrid that was previously characterized to contain mouse Chromosomes 7, 13, 15, and X (Bowes *et al.*, 1989; Travis *et al.*, 1989; Danciger *et al.*, 1990). The X chromosome was excluded using selection with azaguanine (Littlefield, 1966). A series of isolates was scored for the remaining mouse chromosomes using probes for *Dhfr* to mark Chromosome 13, *Gpi-1* to mark Chromosome 7, and *Int-1* to mark Chromosome 15. Isolates containing Chromosome 15 and Chromosomes 13 + 15 were used in this study.

PCR amplifications were done using 2 ng of the cloned DNA from which the STS was sequenced as a positive control, 1 μg of BALB/c *M. domesticus* genomic DNA, 0.4 μg of DNA from a mouse/hamster somatic cell hybrid containing BALB/c Chromosomes 13 and 15, or 0.5 μg of DNA from a mouse/hamster somatic cell hybrid containing only BALB/c Chromosome 15. PCR reaction components were the following: 1 × PCR buffer (50 m*M* KCl, 10 m*M* Tris–Cl, pH 8.7, 0.01% gelatin), 2.0 m*M* $MgCl_2$, 200 μ*M* of each deoxynucleoside triphosphate, 100 pmol of each primer, and 1.7 units *Taq* polymerase (Perkin Elmer Cetus). Perfect Match (0.7 μl; Stratagene) was added to the reactions involving the Marker 2 STS primers. Reactions were conducted in 50-μl volumes. Amplifications were carried out for 35 cycles using the following PCR profile: denaturation for 1 min at 96°C, annealing for 30 s at 58°C, and extension for 1 min at 72°C.

*Calculation of genetic distance from recombination frequency.* To calculate the lod score for linkage given 0 recombinants of 25, it was assumed that unlinked markers would coseggregate 50% of the time, that the probability of unlinked markers coseggregating 25 times in a row would be $0.5^{25}$, and that the lod score would therefore be $-\log_{10}(0.5^{25})$. Confidence intervals for the distance between the markers was estimated as follows: $\theta$ is the recombination fraction, defined as 1/100 the distance in cM. $(1 - \theta)^{25}$ for any distance, $\theta$, gives the likelihood of obtaining no recombinants of 25 offspring at that distance. Integrating $(1 - \theta)^{25}$ over $\theta = 0$ to $x$ and normalizing by the integral over $\theta = 0$ to 0.5 simplify to $(1 - x)^{26} = 1 - C$, where $C$ is the desired confidence interval, and $x$ is the distance within which the markers are expected to fall. Since $(1 - 0.11)^{26} = 0.05$, 11 cM is the distance within which the markers must lie with 95% confidence. Similarly, $(1 - \theta_m)^{26} = 0.5$ defines the median distance, such that the true distance has a 50% chance of being greater than $\theta_m$ and a 50% chance of being less than $\theta_m$.

## RESULTS

### *Screening a Library from the Interspecific Congenic*

The interspecific congenic mouse was used to construct a recombinant DNA library. A portion of this li-

brary was screened with two *M. spretus*-specific LINE-1 probes, oMS496 and oMS416C, sequentially. Of these two oligonucleotide probes, oMS416C is more difficult to work with because it has a narrower window of stringencies that discriminate between the *spretus* and the *domesticus* LINE-1 sequences and hence a lower signal-to-noise ratio when plaque lifts are screened. We found that first purifying oMS496 positive plaques and then rescreening them with oMS416C was more convenient than trying to apply oMS416C to filters with the original library imprint.

A total of 44,000 plaques were screened with oMS496. Assuming an average insert size of 15 kb and a genome size of $3 \times 10^6$ kb, the portion of the genome screened with oMS496 alone was approximately 22%. Seventy-five replicate positives were identified. Of these, 53 were individually isolated and probed with oMS416C, which led to the identification of the four clones described below. Because only 71% (53/75) of the oMS496 positives were reprobed with oMS416C, the fraction of the genome screened with both oMS496 and oMS416C was therefore estimated to be about 16% ($0.71 \times 0.22$).

This screening yielded four candidate *pearl* locus clones, named PL1, PL2, PL3, and PL4, that were positive for both probes. The probes, when used together, have previously been shown to detect 7500 copies in the *M. spretus* genome and a background of about 30 copies in the *M. domesticus* genome (Rikke and Hardies, 1991). Based on the number of backcrosses used to develop the interspecific congenic mouse, approximately 1% of the *M. spretus* genome should have been transferred to the *M. domesticus* background (Flaherty, 1981). If the *M. spretus* segment also carried 1% of the 7500 *M. spretus*-specific LINE-1 elements, then 75/(30 + 75) or about $\frac{3}{4}$ of doubly positive clones should have come from the *M. spretus* parent.

To be sure that each of the four clones was really doubly positive, we conducted partial sequencing of their LINE-1 elements (data not shown). PL1, PL2, and PL3 each contained the 496 and 416 sequence variants as expected. However, PL4 did not contain the 416 sequence variant. This appeared to contradict the observation that PL4 was consistently positive by plaque hybridization to oMS416C during plaque purification, including a plate made from the phage preparation from which the DNA was prepared. To explore this discrepancy further, PL4 DNA was compared to PL1 DNA by blot hybridization with oMS416C (not shown). By increasing the stringency wash from 50 to 54°C, we found that the hybridization signal from PL4 could be eliminated while PL1 still hybridized. We believe that a complete match to the oMS416C probe is not present anywhere in PL4, but that there is a partial match to some extraneous sequence that makes it a particularly bright false positive. This result follows a variety of problems we have experienced in working with the narrow stringency window associated with oMS416C and indicates that special care should be taken with this probe. Of our four clones, then, only three are truly positive for both oMS416C and oMS496, and hence only three are good candidates to come from the *pearl* differential chromosomal segment.

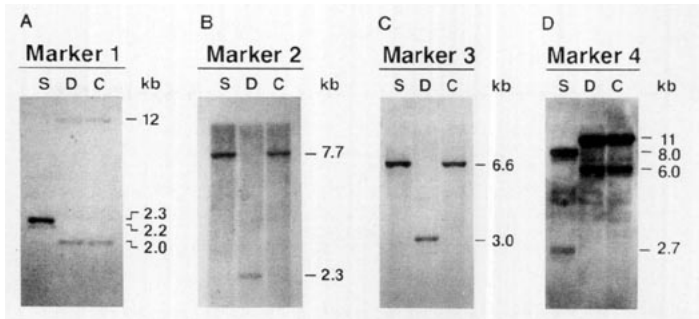


**FIG. 1.** Southern blot hybridization showing that Markers 2 and 3 from the interspecific congenic mouse are *M. spretus*-specific. Each lane contains 7 μg of genomic DNA from *M. spretus* (lane **S**), the *M. domesticus* pearl mutant (C57BL/6J $pe^{+Pin}/pe^{+Pin}$) (lane **D**), or the interspecific congenic mouse, which has a wildtype *M. spretus* pearl locus in an *M. domesticus* background (lane **C**). Each DNA sample was digested for 3 h with 140 units of restriction enzyme. The figure was made from an unedited electronic image of photographs taken of each autoradiogram. **(A)** A 1.5% agarose gel with *Taq*I digests probed for Marker 1. **(B)** A 1.2% agarose gel with *Msp*I digests probed for Marker 2. **(C)** A 1.2% agarose gel with *Taq*I digests probed for Marker 3. **(D)** A 1.2% agarose gel with *Msp*I digests probed for Marker 4.

### *Mapping of the Recovered Clones*

To test for a *M. spretus* origin of each candidate clone, we first subcloned a nonrepetitive DNA fragment from each. This fragment or the subclone itself was then used as a hybridization probe in a Southern analysis of *Msp*I- or *Taq*I-digested genomic DNA (Fig. 1). Each probe detected a restriction fragment length polymorphism (RFLP) between *M. spretus* and *M. domesticus*. Hybridization of each of the probes to DNA from the interspecific congenic mouse showed that Markers 2 and 3 from PL2 and PL3, respectively, hybridized to *M. spretus* alleles. Therefore, PL2 and PL3 came from the *M. spretus* parent along with the *pearl* gene. Because PL2 and PL3 had cosegregated with the *M. spretus pearl* phenotype during 11 generations of backcrossing, the probability that they should be linked to the *pearl* gene was high (Flaherty, 1981).

Physical linkage of Markers 2 and 3 to the *pearl* gene was tested by examining mouse/hamster somatic cell hybrids. To implement a polymerase chain reaction (PCR)-based assay for the presence of Markers 2 and 3, we first sequenced a small segment from each. The resulting sequences are shown in Fig. 2. A pair of PCR primers matching the left end and complementary to the right end of these sequences was synthesized as indicated in Fig. 2. These primers were found to specifically PCR-amplify this segment from the genome and therefore to constitute a sequence-tagged site (STS) (Olson *et al.*, 1989). DNA from the hamster/mouse cell lines was then subjected to PCR amplification with these primers to see if Markers 2 and 3 were located on Chromosome 13 as is the *pearl* gene (Lyon and Searle, 1989). Both markers were found to be present in a somatic cell hy-

**A**

**STS for Marker 2**

ATGGTAAACAGCAAAGGCTGATGTCTAAGTTTGTTCTCTGACCTCCACAC
ACTGTGGCATACACCTGCAGGAAGGAGTAGCAGTCTGTATTCAGAAGGAT
TCTTCTGAATGTATCTAAAAAAAAAAATTTCCACCTTGCATACATATCACA
TTCATTTACATTTACCTTTCTTGTGGTTCTTAGGTTTTAGGTTTTAGAAT
AAGTCAGGCTCCAGCTCTGTAGTTGTTTAGCCATTAAAGCATTCTTAACT
GATTTCCATATGAATAGATGCTATGCCTTGCTATAATGTAAATAGCTTCT
AAGGATACAATAAACTGGTGTTTATACACTCTTAGGAAACAGCTCTATCA
GTGACTTTTTCAGAGGCACAGGAAAAGCTTAT

**B**

**STS for Marker 3**

TATGGTGGTTTCTCCATGTAGTCCTAGTTCTCAGTCATCTGAGGCAGAAG
GATGCCCTTGCTACAAAGCCAACTGACTAGAAGAAGACCCTGCTTCAAAA
CAACATTCCAGTATATAAAATAAAATAGGCAGACTTGCCATCTTTTCACC
CCTCTTCCTTATCTATTTTGGATTATCCATATGTATACGATTGACTCCTG
TTTTTTGTTAGACCCTATTAATTTGGTTTCTTCCTACCTGTAACTAGCAA
CACGAATCATCTGAGGCTGTTTAATAAAGTATTAAGACCAATCAGGGAGT
ATTGGAGATAATCACTAATACTTGT

**FIG. 2.** STSs for markers linked to *pearl*. Underlined regions represent PCR primers used to amplify each STS. **(A)** 382-bp STS for Marker 2 from subclone pUCPL2X. **(B)** 325-bp STS for Marker 3 from subclone pUCPL3A.

brid containing mouse Chromosomes 13 and 15, but not in a somatic cell hybrid containing Chromosome 15 alone (Fig. 3). Therefore, Markers 2 and 3 are present on Chromosome 13 and are physically linked to the *pearl* gene.

Genetic linkage of Markers 2 and 3 to the *pearl* gene was confirmed by screening an interspecific congenic mouse backcross panel for the same RFLP alleles shown in Fig. 1. The backcross panel was produced by mating the interspecific congenic mouse with a homozygous *M. domesticus pearl* mutant to yield a heterozygous interspecific congenic mouse having the dominant *M. spretus* wildtype phenotype. The heterozygous *pearl* congenic was then backcrossed with the *M. domesticus pearl* mutant. Of the 13 wildtype offspring and 12 mutant offspring, there was no recombination between either marker and the *pearl* gene; all of the wildtype offspring were heterozygous for the RFLP alleles and all of the mutant offspring were homozygous for the *M. domesticus* RFLP alleles (data not shown). This result of no recombinants out of 25 mice indicates tight genetic linkage, with a lod score of 7.5 and 95% confidence that the two markers and *pearl* fall within an 11-cM region. The median distance, within which the two markers and *pearl* have a 50% chance of lying, is 3 cM.

## DISCUSSION

We have established that *M. spretus*-specific LINE-1 probes can be used to isolate clones that are linked to a target gene that has been transferred into the differential locus of an interspecific congenic mouse. By requiring that candidate clones hybridize to two separate *M. spretus*-specific probes, we have succeeded in reaching a situation where two of three doubly positive candidate clones actually were derived from *M. spretus* DNA in the differential chromosomal segment. By comparison, only 1% of clones picked from the genome at random would be expected to fall within the differential chromosomal segment, assuming that it was an average size of 16 cM. Enrichment to a success rate of $\frac{2}{3}$ is consistent with the maximum performance that can be expected from this technique according to an analysis of the *spretus*-like background in the C57BL/6 genome (Rikke and Hardies, 1991).

These two clones linked to *pearl* are the first of many that we hope to isolate by this method. If the differential chromosomal segment is 1% of the genome, it should contain on the order of 75 LINE-1 elements that could be isolated in this way. Since two markers were found in a screen covering only 16% of the genome (see Results), there should be a minimum of at least 13 markers to find. However, we have no reason to believe that we have exhausted even this partial library. The tight linkage of the first two markers to *pearl* suggests that this particular differential chromosomal segment may be smaller than the average of 16 cM (Flaherty, 1981). However, since we do not yet know if recombination rates are suppressed in this region for *spretus* × *domesticus* crosses, it would be prematurely optimistic to conclude that our differential chromosomal segment is substantially less than 1% of the genome at this time. In any case, the density of available markers should be about two per megabase, suitable for supporting chromosome walking with yeast artificial chromosomes (Burke *et al.*, 1987).

### *Applicability to Other Loci*

This method of isolating chromosomal markers linked to *pearl* was designed to support cloning the gene with-

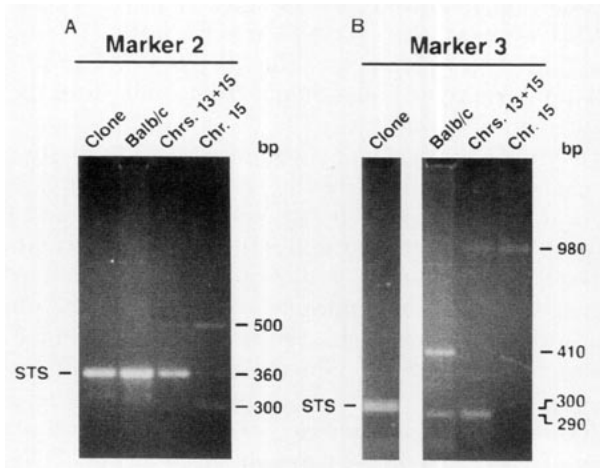


**FIG. 3.** PCR assay showing that the STSs corresponding to Markers 2 and 3 are present on Chromosome 13. The figure was made from unedited electronic images. Sizes indicated were determined according to size markers and differ slightly from the sequenced lengths of the STSs. **(A)** Marker 2 STS amplification using primers indicated in Fig. 2A. The STS corresponds to the 360-bp fragment. The Marker 2-positive control clone is pUCPL2X. **(B)** Marker 3 STS amplification using primers indicated in Fig. 2B. The STS from the clone is the 300-bp fragment. The Marker 3-positive control clone is pUCPL3A. An STS length polymorphism exists between BALB/c (the strain from which the somatic cell hybrids were derived) and the *M. spretus* clones. Southern blot hybridization of the BALB/c lane with Marker 3 verified that the 290-bp fragment corresponds to the 300-bp STS from *M. spretus*.

out knowing or assuming the biochemical cause of the phenotype. This is especially helpful in the case of the *pearl* phenotype. Because the *pearl* mouse displays multiple abnormalities in different tissues, it has been difficult to pinpoint a biochemical cause. In addition to the *pearl* mouse, there are many other murine genetic variants that have phenotypes for which the biochemical cause is not understood. Considering the interspersed nature of LINE-1, it is reasonable that there are also species-specific LINE-1 elements near the genes responsible for those phenotypes.

The major limitation to the application of *spretus*-specific LINE-1 probes in this way is the availability of the interspecific congenic mouse. Breeding the interspecific congenic takes 12 generations spread over several years and is not always successful. Given the success of current methods of saturating the entire genome with markers (e.g., Dietrich *et al.*, 1992), and the impending contribution of YAC contig mapping, one would probably not choose to start breeding an interspecific congenic at this time solely to access LINE-1 markers. However, it is our hope that LINE-1 markers will assist the mapping of those few *spretus* segments that have been transferred into *domesticus* backgrounds.

How near LINE-1 elements will be to different target genes depends on the details of the LINE-1 distribution. It is now clear that LINE-1 elements are not distributed randomly throughout the genome. *In situ* hybridization results of Boyle *et al.* (1990) indicate that LINE-1 elements are present in Giemsa (G) bands, but absent in reverse (R) bands of mouse chromosomes. Human chromosomes have also been shown to have this pattern of LINE-1 distribution (Korenberg and Rykowski, 1988). Therefore, one might expect markers based on LINE-1 elements to be hard to find near genes in R bands.

Further examination of the literature suggests that distribution of LINE-1 elements may be tied more closely to the underlying isochores than to the cytogenetically staining bands themselves. GC-poor isochores are large regions (>300 kb) of DNA that have a homogeneous guanine + cytosine composition of 39–43% in mice (Bernardi, 1989; Mouchiroud *et al.*, 1991). DNA from the GC-poor isochores has been fractionated and shown to be heavily enriched in LINE-1 elements (Soriano *et al.*, 1983). On the other hand, GC-rich isochores constitute 40% of the genome, contain 66% of the genes, and contain few LINE-1 elements.

Fortunately, R-bands do not appear to be monolithic regions devoid of GC-poor isochores and hence devoid of LINE-1 elements. Human prophase banding has resolved small G-banding regions within the traditional R-bands, indicating that there is heterogeneity in R-band composition (Yunis, 1976, 1981; Sawyer and Hozier, 1986; Bernardi, 1989). Based on a total of 2000 bands visualized, it has been estimated that individual GC-rich isochores may average only about 1300 kb in length (Yunis, 1981; Holmquist, 1989). There are examples, such as the $\beta$-globin locus, of genes that are cytogenetically R-banding (Magenis *et al.*, 1985), but which correspond to a GC-poor isochore at the molecular level (Bernardi *et al.*, 1985) and contain many LINE-1 elements (Collins and Weissman, 1984; Shehee *et al.*, 1989). Other examples of R-bands containing LINE-1 elements include 11p13 (Rose *et al.*, 1990) and 19q13.3 (Martin-Gallardo *et al.*, 1992). An analysis of compositional heterogeneity of Chromosome 21 supports the conclusion that GC-poor isochores are interspersed within R-bands (Gardiner *et al.*, 1990). Even genes in R-bands may be expected to fall within a megabase or so of a region with LINE-1 elements. Therefore, we propose that species-specific LINE-1 probes could be used to support the isolation of markers linked to a variety of other target genes, even those from substantial R-banding regions.

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