Single Nucleotide Polymorphism Markers for Genetic Mapping in *Drosophila melanogaster*

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For nearly a century, genetic analysis in *Drosophila melanogaster* has been a powerful tool for analyzing gene function, yet *Drosophila* lacks the molecular genetic mapping tools that recently have revolutionized human, mouse, and plant genetics. Here, we describe the systematic characterization of a dense set of molecular markers in *Drosophila* by using a sequence tagged site-based physical map of the genome. We identify 474 biallelic markers in standard laboratory strains of *Drosophila* that span the genome. Most of these markers are single nucleotide polymorphisms and sequences for these variants are provided in an accessible format. The average density of the new markers is one per 225 kb on the autosomes and one per megabase on the X chromosome. We include in this survey a set of P-element strains that provide additional use for high-resolution mapping. We show one application of the new markers in a simple set of crosses to map a mutation in the *hedgehog* gene to an interval of <1 Mb. This new map resource significantly increases the efficiency and resolution of recombination mapping and will be of immediate value to the *Drosophila* research community.

The development of genome-based tools for genetic mapping has made possible increasingly sophisticated genetic studies in many eukaryotes and has contributed to rapid increases in the rate of discovery of new genes and gene functions. In particular, dense maps of polymorphic markers are in use in humans (Wang et al. 1998; Cargill et al. 1999) and mice (Lindblad-Toh et al. 2000), and currently are being developed in many other vertebrates. Similar resources have been deployed in well-studied and genetically powerful model organisms, including *Saccharomyces cerevisiae* (Winzeler et al. 1998), *Arabidopsis thaliana* (Cho et al. 1999), and *Caenorhabditis elegans* (Koch et al. 2000). It is clear that a dense map of molecular markers is now an important tool for genetic analyses in any organism.

Traditional strategies for meiotic recombination mapping in *Drosophila melanogaster* rely on a chromosome carrying multiple dominant or recessive marker mutations with visible phenotypes. These visible phenotypes are often laborious to score and may interfere with the phenotype of the mutant of interest. Most importantly, because mutations with easily scored, viable phenotypes are relatively infrequent, the mapping resolution available using this approach is limited. A much higher degree of interstrain variation is available at the molecular level, and modern methods for scoring molecular variants offer the advantages of high throughput and automated scoring (Landegren et al. 1998). In addition, the alleles of such markers are codominant and usually phenotypically neutral. Although microsatellites exist in *Drosophila*, they occur infrequently and show relatively low rates of polymorphism (Schug et al. 1997). By far the most common types of molecular variation are single nucleotide polymorphisms and insertion/deletion polymorphisms, hereafter collectively referred to as single nucleotide polymorphisms (SNPs). The interstrain level of sequence polymorphism in *Drosophila* is relatively high (Begun and Aquadro 1993; Moriyama and Powell 1996). Thus, sufficient variation exists in *Drosophila* to develop a high-density map. Recently, a set of 69 SNP markers in a collection of strains used for quantitative trait loci (QTL) mapping purposes was described (Teeter et al. 2000).

We describe here the systematic discovery of a set of genome-wide SNP markers in a collection of commonly used laboratory strains of *Drosophila*, and we provide the sequences of these markers in an accessible format that allows immediate use by the research community. We also show the use of this resource by mapping a mutation in the *hedgehog* gene to a small interval.

RESULTS

Survey of Interstrain Polymorphism

We first evaluated several *Drosophila* strains for levels of sequence polymorphism. Many strains are in use in the *Drosophila* research community, and there are no widely used standard mapping strains. We therefore

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Article published on-line before print: *Genome Res.*, 10.1101/gr.178001. Article and publication are at www.genome.org/cgi/doi/10.1101/gr.178001.

11:1100–1113 ©2001 by Cold Spring Harbor Laboratory Press ISSN 1088-9051/01 $5.00; www.genome.org

selected a few strains with the aim of identifying strains highly polymorphic relative to each other. The polymorphism rate in *Drosophila* varies among specific strain pairs, with the greatest variation observed between East African and other populations (Begun and Aquadro 1993). Therefore, strain selection might significantly affect the rate of SNP discovery. We also wanted to use a series of strains containing single mapped P-element insertions for fine-scale mapping (Cutforth and Rubin 1994; Spradling et al. 1995). Thus, another important consideration in our strain selection was the level of sequence polymorphism relative to these P-element strains.

Using genomic DNA from six wild-type strains (Barcelona, Capetown, Hikone, Pyrenees, w;iso2;iso3, and a P-element containing strain) as templates, we compared sequences from 24 third chromosome sequence tagged sites (STSs) from the P1-based physical map of the genome (Kimmerly et al. 1996). The results shown in Table 1 indicate that the rate of sequence variation between any two strains ranges from 2.1 per kilobase (1 polymorphism/476 bp) to 5.2 per kilobase (1 polymorphism/192 bp, Table 1A), in agreement with previous studies. Although the rate of sequence polymorphism varies depending on the strains compared, much of the observed polymorphism occurs in local clusters, a single STS containing as many as five SNPs. For purposes of genetic mapping, one needs only a single polymorphism per point in the genome. Thus, for the purposes of this study, a better measure of the interstrain polymorphism rate is the percentage of STSs that contain at least one SNP (Table 1B). Although the absolute polymorphism rate observed varies nearly 2.5-fold, from 2.1 differences/kb to 5.2 differences/kb, the percentage of polymorphic STSs varies less than two-fold, from 33% to 63%. In addition, the polymorphism rates relative to the P-element containing strain vary less, ranging from 33% to 50%. Because we observed a largely random distribution of sequence variation and similar levels of relative polymorphism among pairwise sets of strains, we chose to focus our genome-wide discovery efforts on three commonly used strains-Canton S, Oregon R, and w; iso2; iso3. We also selected a series of 17 strains that contain single P-element insertions at evenly spaced intervals across the genome.

Genome-Wide SNP Discovery

We set out to identify a set of SNP markers spanning the genome, with a goal of identifying at least one SNP every 500 kb. We used mapped STSs as a source of genomic sequences (Kimmerly et al. 1996). At the time this project was initiated, these STSs were the only mapped sequence elements with a genome-wide distribution of the required density. The cytological map positions of STSs were inferred from the P1 clones from which they were derived. We chose STSs evenly distrib-

Table 1. Analysis of Sequence Variation among Six Wild-Type *Drosophila* Strains

A. Total number of differences per kilobase

	BEP	ISO	BAR	CAP	HIK	PYR
BEP	-					
ISO	2.5	-				
BAR	4.5	3.7	-			
CAP	4.3	3.5	3.1	-		
HIK	2.9	3.1	5.2	4.8	-	
PYR	3.5	2.1	3.5	4.5	4.1	-

B. Relative percentage of STSs with polymorphisms

	BEP	ISO	BAR	CAP	HIK	PYR
BEP	-					
ISO	38%	-				
BAR	50%	46%	-			
CAP	42%	38%	50%	-		
HIK	33%	38%	54%	50%	-	
PYR	50%	42%	42%	63%	50%	-

Twenty-four STSs from the third chromosome were sequenced in the following strains: w;iso2;iso3 (ISO), Barcelona (BAR), Capetown (CAP), Hikone (HIK), Pyrenees (PYR), and a P-element strain (BEP). The total amount of sequence examined was 5.16 kb. Data are represented as (A) total number of polymorphisms per kilobase, and (B) percentage of STSs containing polymorphisms.

uted across each cytological division for screening. We took two approaches to polymorphism identification. First, a set of 1016 STSs was amplified from isogenized versions of Canton S and Oregon R and then sequenced to identify candidate polymorphisms. Second, a partially overlapping set of ~1050 STSs was screened for polymorphisms between w;iso2;iso3 and the 17 P-element strains by using denaturing high performance liquid chromatography (DHPLC; Underhill et al. 1997). DHPLC is a simple and robust screening tool and is also useful for genotyping SNPs in recombination mapping experiments (see Fig. 2 below; Spiegelman et al. 2000). Polymorphisms identified via DHPLC were verified and characterized by DNA sequencing.

Sequence data from a total of ~1500 STSs from these two discovery approaches were assembled into a single data set. We identified polymorphisms both by visual inspection and automatically as high-quality discrepancies by using `Phred` quality scores in the `Phrap` assembly viewer `Consed` (Gordon et al. 1998). Table 2 displays the sequences of 109 STSs for which sequence was derived from all strains used in the genome-wide survey. In addition, we aligned each consensus STS sequence to the Release 1 version of the *Drosophila* genome sequence to identify the allele in the *y;cn bw sp* strain (Adams et al. 2000). These data define 279 polymorphisms, of which 225 are single nucleotide substitutions, 17 are small insertion/deletions, 8 are dinucleotide substitutions, and 29 are more complex substitutions. Among the single nucleotide substitutions, transitions outnumber transversions by 55% to 45%, and ~22% of these substitutions occur at CpG dinucleotides. These data are similar to those observed in human and mouse genomes and may be evidence that similar mutagenic mechanisms are at work in *Drosophila* (Moriyama and Powell 1996), despite the fact that there is no deficit of CpG dinucleotides and little evidence for cytosine methylation that explains the bias seen in vertebrate genomes (Hacia et al. 1999). Note that the STSs analyzed here represent randomly sampled genomic sequence, so this analysis does not take into account effects of coding versus noncoding sequence. Approximately 40% of these SNPs create restriction site polymorphisms and thus are accessible to scoring by a simple restriction digestion (Table 2). In addition to the sequences provided in Table 2, the sequences for 365 STSs that define an additional ~750 polymorphisms in pairwise subsets of the strains are available at http://www.fruitfly.org.

The chromosome-wide distribution of the polymorphisms identified in this study is represented in Figure 1. The distribution is relatively even across the genome, with an average density of one SNP marker per 225 kb on the autosomes and one per megabase on the X chromosome. The lower density of SNPs discovered on the X chromosome is due primarily to a lower density of available STSs. The region flanking the centromere of chromosome 3, including cytological divisions 77 through 83, contains far fewer polymorphisms per kilobase than the rest of the genome. All available STSs in this region were screened in the w;iso2;iso3/P-element strain comparison, and additional comparison of nearly 100 kb of sequence revealed a lack of polymorphism between these strains (data not shown). This observation is consistent with previous studies indicating that levels of sequence polymorphism correlate with recombination rates in *Drosophila* (Begun and Aquadro 1992; Charlesworth 1996). However, we do not observe a similar reduction of variation near the centromere of chromosome 2.

Mapping with SNPs

Having identified a sufficiently dense set of SNPs, we designed a simple strategy to use these markers for mapping single gene mutations (Fig. 2). This strategy requires two to four generations and informative assays for 10–20 SNPs. In the first step, the mutation of interest is mapped relative to six markers that span the chromosome at 8–10 Mb intervals (Fig. 2A), by using 96 unselected, chromosome-wide recombinants. These markers can be SNPs, transposon insertions, or visible markers. Once the interval in which the mutation lies is defined, SNPs are tested on the appropriate recombinants to further refine the position of the gene (Fig. 2B,C). Using this strategy, we mapped two independent recessive lethal mutations isolated in a screen for suppressors of a human p21 overexpression phenotype. These mutations were placed between the STSs Dm1601 and Dm1655 on chromosome 3, an interval of <1 Mb (Fig. 2C) and subsequently were shown to be alleles of the *hedgehog* gene (Tabata and Kornberg 1994).

To achieve the high-resolution mapping suitable for positional cloning, we have used the P-element strains listed in Table 2 in a strategy to select many white (−) recombinants in a small region (Cutforth and Rubin 1994; Spradling et al. 1995; see Discussion).

Discussion

Determining the locations of mutations in the genome is a critical component of genetic analysis. We have identified a set of 474 SNP markers spanning the *Drosophila* genome and showed their use as a new and powerful resource for genetic mapping. The density of available SNPs is high, so the mapping resolution achievable with SNPs is much greater than with traditional, phenotypic markers. With the example of the mutations in *hedgehog*, we show the ease with which mutations can be localized to intervals of ~1 Mb, and we routinely use this approach to map genes to intervals of <500 kb. If the mutation is mapped relative to a P-element insertion in the initial mapping, a chromosome that contains the P element linked to the muta-

Table 2. List of SNP Sequences Identified

				X CHROMOSOME						
STS	Cytological location	Flanking sequence	Nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1 Q1028	P strain 2	Release 1 v:cnbwsp	RFLP?
Dm3513	1F1–1F3	TCACT–GGAAC	78	G(42)	C(42)	G(46)	G(51)		G	
		GGACA–CTCAA	90	G(42)	A(44)	G(35)	G(35)		G	AluI
		ACCCA–ACACT	144	C(42)	A(44)	C(56)	C(56)		C	
		ACAAA–ATGGG	174	A(47)	T(44)	A(40)	A(35)		A	
Dm1729	2B8–2B9	GAGCT–CTCGA	477	T(56)	C(56)	T(40)	T(42)		T	SacI
Dm0127	2F1–2F6	GTCGT–AGGGG	1447	C(37)AGT(40)	C(40)AGG(56)	C(30)AGT(42)	(A/C,22)AGG(33)		AAGG	
		CGCTC–CGCTC	1450	C(56)	T(43)	C(32)	C(35)		C	AciI BsrBI
Dm0106	3A6	TCTGT–GAACG	122, 125	G(37)TGA(37)	A(56)TGG(42)	G(45)TGA(34)	A(35)TGG(34)		ATGG	
Q1028	**3D–E**						**Q1028**	**Q1034**		
Dm3247	4C7–4C8	AGTTT–CTGCG	1607	T(42)	C(56)	C(24)	C/T(22)	C/T(11)	T	
		CAGTC–GTGAA	1628	C(37)	G(37)	G(25)	G(28)	C/G(23)	C	
		GTGAA–ACGCC	1634	C(51)	T(44)	T(25)	C/T(19)	T/C(37)	C	
		GTTTT–CCGAA	1766	C(56)	T(56)	T(44)	T(42)	T(20)	C	
Dm1954	4E1	ACAAT–ATCGA	149	T(45)	A(56)	A(42)	T(56)	T(40)	T	Tsp5091
Dm3001	5C1–5C2	AATTC–GGATC	695	G(27)	G(35)	A(41)	G/A(22)	G(30)	G	
		TCCAA–ACTTT	704	T(37)	T(41)	G(50)	T(30)	T(33)	T	
		ATTTT–ACAAT	888	T(30)	C(56)	T(42)	C(34)	C(42)	C	
Q1034	**6E**						**Q1034**	**Q1030**		
Dm2006	7A2–7A5	CCCAG–ACATG	582	T(56)	C(45)	T(30)	T/C(26)	T/C(17)	T	RsaI
		TACCT–CCCCC	618	DEL(56)	C(56)	DEL(98)	DEL(98)	DEL(98)	DEL	
		TCTTG–C	624	C(37)	C(46)	C(31)	A/C(26)	A/C(33)	C	
		C–CAATC	626	G(40)	A(45)	G(20)	G(42)	G(42)	G	
		ACTCG–CCAGC	647	A(40)	A(35)	A(42)	A/T(30)	A/T(29)	A	TaqI BspGI
Dm1849	7D10–7D16	GGTT–TCCCT	180	A(30)	A(51)	T(28)	T(28)	T(28)	A	
Dm0426	8C1–8C2	CATAT–TA	1223	A(51)	A(51)	A(42)	G/A(24)	G/A(28)	A	
		TA–GTACA	1226–7	T(51)G(42)	T(51)G(42)	T(44)G(42)	T/C(17), G/A(17)	T/C(18), G/A(18)	TA	
		ACATA–GTACT	1234	T(56)	T(56)	T(48)	C/T(17)	C/T(11)	T	NdeI RsaI SnaBI
		TACTT–ATGTTG	1241	G(42)	A(51)	G(42)	A(40)	A(42)	A	MseI
		ACAAC–TACAA	1352	T(40)	T(40)	T(42)	A/T(23)	A/T(14)	T	
Dm3169	8D1–8D5	GCCGC–CACGA	1280	T(45)	T(40)	T(44)	C/T(13)	C/T(19)	T	BsrBI
		CCCGT–TGCTC	1373	G(37)	A(29)	G/A(37)	G/A(30)	G/A(39)	G	
Dm1980	9B1–9B2	GGTGA–TTCAC	35	A(48)	C(40)	C(40)	C(40)	C(40)	C	EcoRI Tsp5091
		ATCTA–CAAAA	62	A(51)	A(43)	T(40)	A(40)	A(45)	T	
		GCAAA–CATAA	95, 98, 101	A(51)TTA(51)AAG(56)	C(45)TTC(51)AAA(56)	C(35)TTC(40)AAA(40)	C(29)TTC(34)AAA(25)	C(29)TTC(27)AAA(27)	CTTCAAA	MseI Tsp5091
Q1030	**10E**						**Q1030**	**Q1035**		
Dm3746	10F7–10F8	TACAC–TCTCT	79–80	G(51)C(51)	A(51)A(51)	G(40)C(40)	A(45)A(46)	A(35)A(35)	AA	
		TCACA–CCGTA	137	G(42)	T(43)	G(35)	T(44)	T(37)	T	
Dm2024	11B1–11B2	GAAC$(A)_9$–$(A)_5$GAT	172	A(47)	C(44)	A	C(56)	C(42)	C	
Q1035	**13E9**						**Q1035**			
Dm0478	13A8–13A9	TGGCA–TTGGC	70	A(40)	A(51)	G(35)	A(39)		A	MunI Tsp5091
		AGACT–TAGTT	145	A(45)	A(45)	C(35)	A(37)		A	BfaI

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1 Q1028	P strain 2	Release 1 v:cnbwsp	RFLP?
Dm3491	13D1–E4	CATTG–CCGCC	60	T(46)	T(46)	C(46)	T(39)		T	EclI
		CACTG–ACGAG	170	A(56)	G(40)	A(35)	A(35)		A	BspGI
Dm1911	14B3–14B4	TGAAT–GAATG	1134	A(46)C(42)	A(40)C(37)	G(43)T(40)	G(21)T/C(21)		GT	
Dm3790	15B1–15B2	TGTGC–CTTAA	106	C(44)	A(45)	A(37)	C(50)		C	ApaLI CviRI
		ACTAT–CGCCT	221	C(40)	T(40)	T(40)	T(45)		T	AciI EciI
Dm0509	16F5–16F8	GAGGG–CCGCT	58	G(37)	T(45)	G(37)	T(40)		T	HaeIII SimI
Q1035	**17C**									
Dm1846	17A1–17A2	ATTTC–ACTTG	2150	A(37)	C(45)	A(42)	A(46)		C	
Dm2754	17B5–17C4	AGTAG–GTATG	402	C(35)	C(38)	C(56)	T(34)		C	
		CATTT–CAGCG	423	C(33)	T(42)	C(40)	T(22)		C	
		TGCGG–TTCTC	444	G(42)	T(38)	G(40)	T/G(34)		GT	
		CAAAA–ACCGG	465	A(34)	A(34)	T(56)	T(26)		T	
		CCGTT–GCTAA	512	T(27)	T(35)	T(40)	C(51)		C	
Dm0505	19E1–19E3	GCTGC–TGGCT	90	A(42)	A(42)	A(30)	A(30)		A	CviRI NIaIII
		CGGAG–ATATG	170	C(44)	T(56)	C(56)	C(37)		C	NdeI
		ATATA–TATTG	190	A(56)	A(42)	A(42)	A(42)		A	
Dm0529	20B1–20B2	GGCTC–GTGTC	837	G(45)	T(45)	T(42)	G(56)		G	

2nd Chromosome

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1 Q1037	P strain 2	Release 1 y:cnbwsp	RFLP?
Dm0447	21D1–21D2	TCTCT–ACGGC	449	C(37)	C(40)	C(42)	T(17)		C	
		TCTCT–GACCT	485, 487	C(32)TT(32)	*(98)T*(98)	*(98)T*(98)	*(98)T*(98)		CTT	
Dm0375	21E3–21F3	TACGG–AGTGT	32	A(29)	A(27)	G(30)	A(27)		A	BscGI
		GTCGC–CTGGT	103	C(32)	C(33)	A(21)	C(37)		C	
Q1037	**22D1–D2**						**Q1037**	**Q1040**		
Dm2641	23A1–23A3	ACTTG–CCCAG	32	A(40)	A(51)	G(56)	A(47)	A(42)	G	SimI HaeIII
		TCCAT–AAGAA	107	G(56)	G(56)	A(48)	A(48)	A(46)	G	NiaIII
		GGCCA–CGGCT	174	G(35)	A(40)	G(37)	G(37)	G(35)	A	AciI
Dm0611	23E1–23E4	ACAAA–CTTTA	133, 135	A(56)AC(42)	T(45)AG(40)	T(51)AG(45)	T(45)AG(45)	T(51)AG(51)	TAG	AluI
		ATCTT–TATAG	153	C(48)	C(46)	T(56)	C(46)	C(46)	C	
		ACTCG–TGTGT	169	C(51)	C(51)	C(27)	G(56)	G(56)	C	
		GCCAC–CAGTT	186	T(45)G(45)	C(51)C(51)	T(34)G(34)	T(40)G(45)	T(51)G(51)	CC	CviRI PstI
Dm2235	25A3	TATTA–AAATC	141	ND	ND	G(48)	A(42)	A(37)	A	MseI
		CATAT–GATAT	156–163	DEL(98)	DEL(98)	DEL(98)	TATAATAT(42)	TATAATAT(42)	TATAATAT	NdeI
		GTAAT–ATTGT	174	A(40)	T(35)	T(56)	A(40)	A(42)	A	
		ATTGG–TTG	233	T(56)	T(56)	T(56)	C(44)	C(56)	T	
		TTG–AGTAA	237–238	G(56)A(56)	G(56)A(56)	G(56)A(56)	T(42)T(40)	T(42)T(40)	GA	
Dm2207	26D1–26D2	TCCGT–TCCAG	28	G(51)	G(51)	G(40)	A(45)	A(45)	G	BspGI
		TCTTC–GT	118	G(56)	T(56)	T(42)	G(44)	G(43)	G	
		GT–TAGTT	121	G(51)	A(56)	A(37)	G(35)	G(40)	G	
		CGTCC–AGACG	148	T(51)	C(51)	C(40)	C(46)	C(46)	T	BfaI
		AGGTA–TCGTT	163	G(40)	G(45)	G(40)	C(40)	C(45)	G	RsaI
Dm3015	29A2	AACTG–CTTTT	23	T(40)	C(56)	T(34)	T(35)	C(42)	C	
		TGTTT–AAAGT	114	A(56)	G(56)	A(44)	A(56)	G(56)	G	DraI MseI
		AACAC–CGGTT	147	T(39)	A(56)	T(35)	T(39)	A(38)	A	
		CAACC–GGTGC	171	T(31)	A(32)	A/T(14)	A(56)	A(42)	A	
Dm0274	29A5–29B4	GGTAC–CGGTA	741	G(29)	G(35)	G(38)	T(42)	G(37)		AciI TthI
		TTAAT–CAATT	805	G(40)	G(42)	G(51)	A(44)	G(42)		CviRI
		GCCGA–AGGTG	832	C(40)	C(40)	C(42)	T(46)	C(56)	T	

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1	P strain 2	Release 1 y:cnbwsp	RFLP?
Q1040	**29C1–C2**						**Q1040**	**Q1042**		
Dm0434	30A3–30A6	TTTTT–AACAT	73–75	A(35)A(35)A(35)	T(35)A(35)A(34)	A(21)A(27)A(27)	T(21)T(27)T(29)	T(4)T(4)T(4)	TAA	
		TAGGC–AAAAA	113–114	A(56)A(56)	A(47)C(47)	A(13)A(13)	DEL(98)	DEL(98)	AC	
Dm0652	31F3–32A1	AGCAA–CGAAA	742–743	C(9)A(9)	C(37)A(37)	C(24)A(24)	T(46)T(33)	C(24)A(25)	CA	BsbI
		AGTTT–AACGA	821	A(51)	A(51)	A(42)	G(42)	A(26)	A	DraI MseI PmeI
		TTATG–AATGC	900	G(51)	G(56)	G(44)	C(37)	G(42)	G	CviRI
		TACAA–TTGTC	929	C(51)	T(56)	T(48)	T(13)	T(44)	T	Tsp5091
Dm4048	32A1–32A2	CATAC–TTAGA	113	T(56)	A(56)	A(42)	A(35)	T(42)	A	
		GGTTG–CGATA	168	C(46)	G(42)	G(37)	G(35)	C(35)	G	
Dm3374	33A1–33A2	GATAT–GCAGT	733	T(51)	G(40)	G(44)	G(42)	T(44)	G	CviRI
		TCCCA–CGGAT	758–759	A(29)A(42)	G(34)T(42)	G(42)T(42)	G(26)T(35)	A(24)A(46)	GT	
		TAAAA–GAAAT	769	T(47)	C(51)	C(56)	C(42)	T(44)	C	
		GCATT–ACACA	802	G(40)	A(51)	A(56)	A(33)	G(33)	A	MseI
		CACAG–GAA	809	C(37)	T(56)	T(44)	T(42)	C(33)	T	
		GAA–GTACG	813	T(42)	A(56)	A(42)	A(51)	T(42)	A	
Dm2112	33A1–33A2	TAAAA–TTATG	67	T(35)	G(42)	T(56)	T(56)	G(45)	T	Tsp5091
		AACAT–AATCG	104	A(42)	T(45)	A(56)	A(56)	A(42)	A	
		GAGAA–AGCGG	125	A(56)	G(51)	A(42)	A(46)	G(42)	A	BsrBI
Dm0240	33A3–33A8	ATGTG–GTGCA	514	C(24)	C(24)	T(56)	C(37)	C(47)	T	
		CATAT–TTTGG	546	DEL(27)	DEL(52)	DEL(48)	GTAT(44)	DEL(33)	DEL	NdeI
Dm2479	33D4	ATAGT–GGCAA	75	A(17)	A(40)	A(40)	A(45)	T(40)		
		TTTTC–CATTG	110	G(37)	G(56)	G(44)	G(56)	T(44)	G	
Dm3635	34C1–34C2	CCGCA–AATGC	1494	C(27)	C(27)	T(40)	C(44)	C(42)	T	
		GCTTG–ATAAA	1552	A(27)	G(14)	A(42)	A(56)	G(42)	A	
		AAAAT–TAAGA	1560	G(33)	T(13)	G(56)	G(44)	T(29)	G	MseI Tsp5091
Dm0393	34D1–34D6	TAACA–TAATT	50	A(42)	A(42)	T(37)	A(33)	A(37)	A	MseI VspI
		ACTGC–GTGAG	191–192	T(46)C(33)	A(42)A(42)	A(48)A(56)	A(29)A(29)	T(38)C(38)	TC	BssSI CviRI
Dm0012	35A1–35A2	AATTT–TAAAG	74	T(42)	G(23)	T(42)	G(51)	T(44)	T	DraI MseI
Q1042	**35E1–E2**						**Q1042**	**Q1805**		
Dm0043	35F1–35F2	GATGG–CAGTC	60	A(30)	A	A(35)	A(40)	T(37)	A	
Dm2451	36B3–36C1	TCCTT–AGACA	108	G(40)	A(45)	G(44)	A(56)	A(32)	G	AflII MseI
		AGAAT–AA	125	T(51)	C(45)	T(40)	C(56)	C(56)	T	
		AA–AGT	128, 130	A(40)AT(40)	T(45)AA(51)	A(45)AT(56)	T(56)AA(51)	T(56)AA(40)	AAT	
		AGT–AAT	134	T(40)	C(56)	T(45)	C(51)	C(38)	T	
		AAT–CCCAA	138–139, 141, 143, 145	*(98)G(56)T*(98)T* (98)TC(40)	A(45)T(45)TA(45) TA(51)TT(56)	*(98)G(56)T*(98)T* (98)TC(56)	A(45)T(45)TA(51) TA(40)TT(40)	A(56)T(56)TA(46) TA(38)TT(40)	*GT*T*TC	
		TCAAA–GTGAA	157	C(42)	T(56)	C(51)	T(56)	T(56)	C	TaII
		GTGAA–GTACT	163–164	A(42)T(42)	*(38)*(38)	A(56)T(56)	*(98)*(98)	*(98)*(98)	AT	ScaI
Dm0353	39A3–39A7	CTGCT–CAGCT	26	G(40)	A(51)	G(40)	A(42)	A(42)	G	CviRI PatI
		TTCCG–GTATC	41	A(51)	C(40)	C(42)	C(42)	C(37)	C	AciI ThaI
		CTCGA–ACAGC	60	C(47)	T(45)	C(40)	C(42)	C/T(21)	C	
		CTGTG–TTTTC	85	G(51)	A(42)	A(51)	A(51)	A(56)	A	
		TGGGA–AAATC	212	A(56)	A(56)	A(56)	T(42)	A(47)	A	
Dm0627	40A6–40B2	GCAAT–TCTGA	304	A(33)	T(56)	T(25)	T(51)	T(40)	T	Tsp5091
Dm0975	41C1–41C6	GCGTG–CGTGC	140–143	****(40)	****(40)	CGTG(51)	****(51)	****(40)	****	
Dm3495	42A1–42A2	CGGGG–CTTGT	83	G(56)	T(37)	G(35)	G(51)	G/T(17)	T	SimI
		CGAGA–GTCAG	132	T(45)	C(46)	T(20)	C(16)	C(19)	C	AatII TaII
Dm2609	42A8–42A16	TTTTT–AAATT	77–78	*(43)A(40)	T(47)A(40)	T(56)A(56)	T(56)T(56)	T(56)A(23)	*A	
Dm2106	42B4–42C2	ACCGA–GCTAC	114	A(56)	G(45)	G(39)	G(25)	A(22)	A	AluI
		AGTTT–TAAGC	140	C(51)	C(51)	T(56)	C(56)	C(56)	C	MseI
		AGCAG–AGTCC	148	G(56)	G(56)	A(34)	G(34)	G(32)	G	
		TGCGA–GGAGA	160	C(46)	C(46)	T(35)	C(34)	C(35)	C	BccI
		GCTTT–ACAGG	176	C(42)	C(51)	T(39)	C(40)	C(40)	C	

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1	P strain 2	Release 1 v:cnbwsp	RFLP?
Q1805	**43E9**						**Q1805**	**Q1049**		
Dm0735	43E12–43F2	CCAAG–TGCGC	74	C(51)	T(40)	C(40)	T(34)	T(34)	T	AluI
		ATCTA–GACAT	97	C(42)	T(46)	C(40)	C(45)	C(36)	T	Pfl1108I
Dm0746	44B1	GTATC–GTTCT	140–145	GCACGG(51)	*****T(56)	GCACGG(33)	GCACGG(40)	GCACGG(51)	GCACGG	BscGI
Dm2209	44D1–44D2	CAATA–AAGTA	128	A(45)	G(51)	A(26)	G/A(10)	G(29)	G	
		TATCA–AACAT	155	A(46)	G(40)	A(35)	G/A(16)	G(32)	G	
Dm0722	44D5–44E2	CACCG–TTACT	101, 104	G(56)TTT(51)	*(41)TTC(45)	G(32)TTC(14)	G/*(11)TTC/T(11)	*(39)TTC(40)	*TTC	MspI PinAI
Dm3845	46B1–46B2	TTTTT–AATAC	159	A(39)	C(51)	A(51)		C(35)	C	DraI MseI
Dm0340	47D6–47E2	GCGAA–TTTTA	205	T(28)	A(32)	T(37)	T(40)	T(40)	T	
Dm0084	48C5–48D2	CTCAT–CAGCT	762	T(56)	T(51)	T(56)	C(35)	T(49)	T	
		TTATT–GAGAG	772	C(51)	C(56)	A(44)	C(42)	C(47)	C	TaqI
		TAAGT–ATTTC	808	A(51)	G(51)	G(40)	G(40)	G(40)	A	Tsp509I
		TGAGA–GCCGA	866	C(51)	C(51)	C(40)	C(42)	T(40)	C	
Q1049	**49D1–D3**						**Q1049**	**Q1047**		
Dm2480	50A3–50A4	CCTAA–GACGT	1032	A(51)	DEL(51)	DEL(53)	A(35)	A(33)	A	
		TATCC–ACAAC	1060	C(37)	T(37)	T(51)	C(44)	C(42)	C	
Dm0064	50C23–50D1	TTTAC–TTTCT	119	A(37)	T(31)	A(40)	A(31)	A(37)	A	
		GCTAA–TTACT	140	G(33)	A(29)	G(40)	A(40)	A(40)	G	Tsp509I
		TATTA–TACAT	154	T(40)	A(30)	T(43)	A(35)	A(42)	T	MseI VspI
		TTTTT–AAAAA	164	G(9)T(13)	A(36)A(36)	G(35)T(50)	G(27)A(44)	G(42)A(44)	GT	DraI MseI
Dm3709	51E10–51E11	AGAAT–GAATC	83, 85, 87–90	C(35)AG(35)AATAA(42)	*(98)A*(98)A****(98)	*A*A****	C(31)AG(29)AATAA(35)	C(35)AG(35)AATAA(37)	CAGAATAA	
Dm0788	51E5–51E8	AAAAA–CAACA	1012	C(40)	T(51)	T(33)	C(26)	C(42)	C	
Dm2663	51E5–51E8	TCATT–TTTTT	156	A(42)	G(22)	G(36)	A(42)	A(42)	A	
Dm0754	52B1–52B2	AATCA–ATATA	916	C(33)	T(51)	T(37)	T(44)	T(46)	C	
Dm2192	54A2–54B1	AAATG–TGGTT	608	T(56)	G(56)	T(42)	G(37)	G(51)	T	
		ATTTC–GGAAT	639	G(51)	C(42)	G(32)	G(48)	G(51)	G	BspEI MspI
Dm2427	54C3–54C11	TCGCT–CCTAC	185	T(42)	A(53)	T(36)	T(45)	T(40)	T	
Dm3490	54E8–54F2	AGTGT–TTTTT	169	T(44)	*(56)	T(56)	T(56)	T(46)	A	
Dm0851	55A2–55B1	GAGTT–CGAGT	737	G(56)	A(42)	A(35)	A(40)	A(17)	A	
Dm1720	55A2–55B1	ACTGG–GAAAA	251	G(56)	A(48)	G(35)	G(27)	G(14)	G	
		TAAGT–TGCCA	271	C(40)	C(45)	C(21)	G(35)	G(38)	C	
Dm2631	55A2–55B1	TTTTA–TTGCG	161	T(46)	T(51)	T(20)	G(56)	G/T(11)	T	
		TGCCA–CATCG	179	T(40)	C(37)	T(34)	T(34)	T/C(28)	T	
Q1047	**57A3**							**Q1047**		
Dm0602	56B1–56B2	ATCCC–CCGGT	80	T(44)	G(40)	T(23)		T(44)	T	AciI
		GGATT–CTC	95	A(37)	G(56)	A(35)		A(33)	A	
		CTC–TTCT	99	T(33)	C(56)	T(42)		T(33)	T	
		TTCT–GGCAG	104	T(56)	G(56)	T(42)		T(35)	T	
		GGTGT–TTCCG	152	A(56)	G(56)	A(50)		A(40)	A	
		CTGGG–CACCT	178	C(40)	A(56)	C(42)		C(40)	C	HaeIII
		AGTTC–GTTTG	221	T(56)	C(56)	T(37)		T(17)	T	
Dm0800	56B1–56B2	AAAA–TAAAA	1298	T(42)	T(40)	A(51)		T(45)	A	
		TTTAT–AAGTG	1319	T(31)	A(42)	T(45)		T(33)	T	MseI
		CTAGG–AATAT	1336, 1339	A(29)TAC(25)	A(43)TAC(17)	T(40)TAA(51)		A(12)TAC(24)	TTAA	MseI
		AATAT–ATATA	1345	G(32)	C(35)	G(51)		G(29)	G	
		ATAGT–GCAAC	1364	A(10)	A(29)	G(45)		A(15)	G	
Dm0824	57A4–57A6	GAGCT–GGCGC	64	G(42)	T(42)	G(44)		T(42)	T	
		TATAT–CAGCT	198, 200	A(51)TT(42)	*(51)T*(51)	A(51)TT(51)		*(98)T*(98)	*T*	
Dm0063	57E2–57E8	TTAGC–TAAAC		G(35)	G(45)	G(34)			G	

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1	P strain 2	Release 1 y:cnbwsp	RFLP?
Q1047	**57A3**							**Q1047**		
Dm2274	58A4	CCCCC–TTTTC	129–138	TATTTTCCCG(42)	GA********(56)	AATTTTCTCG(56)		GA********(30)	T	BscGI
Dm1544	58B10–58C4	GGTGA–GGTAT	99	C(19)	C(51)	C(42)		C/T(13)	C	BccI
		GTGGT–AACGA	144	A(33)	A(56)	G(56)		G/A(17)	A	
Dm1639	58F2–58F7	TTCCA–CGGAT	87, 90	A(40)ATC(39)	G(35)ATT(56)	A(35)ATC(37)		A(40)ATC(40)	AATC	BspEI MspI
		ATGAT–GGTTT	175	C(40)	G(56)	C(56)		C(30)	C	DpnI Sau3AI BccI
Dm2101	59A1–59A3	AGTAA–TAGAA	690	T(11)	T(45)	G(42)		A(37)	T	Tsp509I
		ACTTC–TTACT	811	A(40)	G(51)	T(42)		G(42)		
		CAGCC–CTGGA	858	G(22)	T(37)	G(40)		T(32)	G	AclI
Dm2487	59F2	TATCT–ATACT	49	A(40)	G(45)	A(40)		A(33)	A	
		ATTCC–AACAA	71	A(46)	A(45)	G(51)		A(30)	G	
		AGGCA–GCGAT	107	T(51)	C(39)	T(42)		T(42)	T	NiaiII SphI ThaI
		CAGAC–CCGCA	173	A(40)	G(35)	G(40)		G(35)	G	
		CAGCA–ACGGA	182	G(33)	C(43)	C(56)		C(42)	C	
Dm0327	60B2–60B10	TATGTGTG–TGTGTGCT	100–101	T(39)A(39)	DEL(98)	DEL(98)		DEL(98)	TA	
Dm2247	60D10–60D11	TTCGG–GGGCG	158	C(42)	T(56)	T(44)		T(42)	C	AclI

3rd Chromosome

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1Q1050	P strain 2	Release 1 y:cnbwsp	RFLP?
Dm2467	61C1–61C2	ATCTT–ACTGG	219	T(45)	T(46)	T(29)	A(22)		A	MseI
		CTGTC–TTTTT	235	*(56)	T(48)	T(27)	T(30)			
Dm0688	61D1–61D2	AATTT–GGAGA	203	A(51)	C(56)	A	C		A	
		AGAAT–TGGAA	252	G(51)	C(51)	G	C		G	
Q1050	**62B4–62B5**						**Q1050**	**Q1052**		
Dm3835	62F1–62F2	CAACA–GCTAA	202	G(40)	G(42)	G(42)	A(44)	A(42)	G	
Dm2200	6303	GCCGC–TTCCC	151	C(45)	T(45)	C(29)	T(30)	T(42)	C	
		TTCCC–CCCTC	157	A(45)	G(51)	A(44)	G(42)	G(37)	G	AclI
Dm2086	65A6–65A10	TACGA–AAGCT	35	T(33)	C(37)	C(47)	T(38)	T	T	
		TATTT–AAA	85–86	C(35)A(12)	*(98)A(51)	*(98)C(56)	C(15)A/C(11)	CA	CA	
		AAA–TTTAA	90–94	TTT(51)AA(45)	***(98)A*(98)	***(98)A*(98)	TTT(24)AA(28)	TTTAA	TTTAA	
		TTAAT–TTATA	101–110	ATATATTTAA(48)	*T*TA***A*(98)	*T*TA***A*(98)	ATATATTTAA(33)	ATATATTTAA	ATATATTTAA	
		TTATA–GTATT	116–128	TATATAATATATA(56)	*(98)	*(98)	TATATAATATATA(28)	TATATAATATATA	TATATAATATATA	
		ATTAA–GTATA	136	T(42)	C(40)	C(42)	T(42)	T	T	VspI TaII
Dm0929	67A1–67A2	TAATG–GTTT	508	T(56)	C(56)	C(42)	C(18)	C(42)	C	
		GTTT–GTGGT	513	A(40)	T(42)	A(40)	T(24)	T(44)	T	
Dm2202	67C10–67D1	CATTT–CCGAA	880	G(42)	A(40)	G(40)	A(37)	A(35)	G	
Q1052	**68A1–68A2**						**Q1052**	**Q1060**		
Dm2215	68B1–68B2	ATTCA–CTAAT	593, 596	G(40)TTC(38)	G(42)TTC(38)	T(56)TTT(40)	G(35)TTC(28)	G(42)TTC(40)	GTTC	
		TTCCC–TATCC	607	T(42)	T(37)	A(42)	T(42)	T(46)	T	
		TTCAC–AATA	619	A(45)	A(37)	G(30)	A(42)	A(42)	A	
		ATAA–AAC	624	G(47)	G(42)	A(44)	G(42)	G(44)	G	
		AAC–TTCAC	628	A(47)	A(44)	T(47)	A(42)	A(42)	A	
		TCAAC–ATTTT	641	T(45)	T(40)	C(42)	T(38)	T(40)	T	
		GTTTT–GTGGG	753	T(45)	T(40)	C(42)	T(42)	T(42)	T	
Dm2544	69D1	TACTT–CGCTG	642	G(27)	G(40)	G(40)	A(51)	A(40)	G	HhaI

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1	P strain 2	Release 1 v:cnbwsp	RFLP?
Dm3239	71F1–71F2	AGAGC – GAACT	73	C(40)	C(37)	T(35)	T(35)	C(40)	T	AluI
		GTGTG – GAGAT	88	G(46)	G(51)	A(30)	A(42)	G(40)	G	
		AATTC – TCCTA	114–132	**T****A**G ******G*(56)	**T****A**G ******G*(44)	AATTTTTAATGT TTCTAGG(33)	AATTTTTAATGT TTCTAGG(37)	**T****A**G ******G*(35)	AATTTTTAATGT TTCTAGG	BfaI MseI Tsp509I
Dm1809	72C1–72D6	AGGCG – AGGAT	714	C(40)	C(39)	A(19)	A	C	C	HhaI
Q1060	**73A1–A4**						**Q1060**	**Q1056**		
Dm3626	74F4–75A2	AGTGG–AAAGC	1267	A(30)	A(56)	G(56)	A(33)	A(56)	A	
		ATGGG – GGTGG	1301	A(22)	T(51)	T(56)	A(42)	T(47)	T	
Dm2220	76B6–76B10	GCAAT – CCGAT	124	T(16)	T(29)	G(33)	T(22)	T(42)	G	Tsp509I
Dm3089	76C3	CGATG – TTTAT	51	*(98)	*(98)	T(51)	*(98)	*(98)	T	
		AATAT – TA	156, 158	A(51)TG(51)	A(45)TG(45)	G(44)TA(44)	A(40)TG(45)	A(56)TG(56)	GTA	
		TA – TATAT	161–167	TATGTAG(45)	TATGTAG(45)	CTACGTA(56)	TATGTAG(42)	TATGTAG(37)	CTACGTA	
Q1056	**83A7–A8**						**Q1056**	**Q1058**		
Dm1568	86A5	TTGCA – CAAGA	155	A(51)	A(38)	C(27)	A(33)	A(30)	A	
		AACTC – TCCAC	165	C(56)	A(46)	C(31)	A(35)	C(42)	A	
Dm3326	87F13 – 88A1	GCTGG – AAATA	71–91	ACATGAACTTAT TCAGCA ACG(35)	********** **********(98)	ACATGAACTTAT TCAGCAACG(31)	****** *********(98)	****** ********(98)	ACATGAACTTAT TCAGCAACG(31)	BspGI
Dm3478	88A1	TCCGT – CAGCA	90	G(37)	G(35)	A(37)	G(37)	G(40)	A	CviRI RsaI
Dm0337	89C2–89C5	TCCTG – TATAT	856	C(51)	G(56)	G(31)	C(40)	C(42)	G	
Dm2709	89D2–89D4	CAGGG – GATGA	668	A(15)	G(56)	A(42)	A(48)	A(44)	A	
		GAGCT – TCCAT	677, 680, 683	C(27)CTG (46)TAT(39)	G(30)CTC(33) TAC(42)	A(42)CTG(38) TAT(38)	C(56)CTG(42) TAT(56)	C(42)CTG(46) TAT(42)	ACTGTAT	SacI
		ATGAT – CCGCG	692	A(37)	T(56)	A(34)	A(42)	A(35)	A	
		CCGCG – CCCAT	698	A(37)	G(35)	A(30)	A(37)	A(31)	A	HaeIII SacII SimI
		GCCGA – GAATG	710–711	G(42)C(42)	A(41)A(41)	G(38)C(38)	G(46)G(46)	G(42)C(42)	GC	
		GAATG–AGCGA	717, 720–722	A(41)GAA(56) A(56)G(56)	C(27)GAC(38) T(38)C(38)	A(42)GAA(46) A(42)G(44)	A(42)GAA(56) A(56)G(51)	A(42)GAA(56) A(56)G(46)	A	
		AGCGA – CAGAG	731, 734, 737, 740	G(32)GAG(44) CAC(45) GTG(45) TGT(45)	A(48)GAA(37) CAG(45) GTT(51)TGC(45)	G(46)GAG(37) CAC(42) GTG(44)TGT(42)	G(51)GAG(46) CAC(44) GTG(40)TGT(51)	G(56)GAG(48) CAC(37) GTG(45)TGT(56)	GGAGCACGTGTGT	PmlI TaiI
Dm2494	89F4–90A2	GGATG – CATCC	503	C(38)	C(20)	C(56)	T(44)	C(42)	C	BccI
		TCGGG – TACTA	623	C(37)	A(51)	C(39)	A(40)	C(35)	C	
		GATTT – CAACT	659	G(45)	G(45)	G(42)	C(29)	G(35)	G	CviRI
		ATGCT – AGCTA	678	T(45)	A(51)	T(38)	A(30)	T(42)	T	
Dm3952	90D1–90E2	AATTC – TTTTT	717	T(27)	T(30)	T(44)	*(98)	*(98)	*	
Q1059	**91A1–A2**						**Q1058**	**Q1059**		
Dm3293	92D1–91D2	TTGTG – GACCC	94	T(51)	C(40)	C(35)	C(42)	T(42)	C	
		CTGAG – ACTGA	112	G(56)	C(42)	C(38)	C(42)	G(35)	C	
		GGAAT – AGAAT	183	T(40)	C(45)	C(35)	C(35)	T(33)	C	Tsp509I
Dm1705	92D1–92D2	GATAT – CCAAT	473	C(51)	C(42)	T(42)	T(42)	C(28)	C	EcoRV
		TCATA – AAG	518	C(33)	C(28)	A(42)	A(33)	C(28)	C	
		AAG – CGGAT	522	C(42)	C(56)	A(37)	A(44)	C(42)	C	
		TGCGG – AGATC	617	T(45)	G(51)	G(26)	G(17)	G(32)	G	
Dm2772	94C1–94C5	CATTT – GGCGG	409	C(45)	C(40)	T(40)	T(42)	C(42)	C	

Table 2. (*Continued*)

STS	Cytological location	Flanking sequence	Relative nucleotide position	Oregon R	Canton S	w:iso2:iso3	P strain 1	P strain 2	Release 1 v:cnbwsp	RFLP?
Dm1690	94E6–94E12	AGTTT–GGATT	802	G(56)	G(56)	T(56)	G(33)	G(9)	G	
		TAAAT–TGGAA	872	T(44)	T(45)	T(42)	G(36)	G(9)	T	Tsp509I
		AACAT–TCTGA	902–903	A(44)T(48)	A(51)T(51)	A(40)T(45)	T(42)A(42)	T(9)A(9)	TA	
		TCTTT–GCCTG	916	T(56)	C(40)	C(40)	C(44)	C/T(9)	T	
		GCCTG–TTATT	922	T(56)	A(56)	A(45)	A(40)	T(9)	T	
		TTATT–AAGTG	940–950	*******A***(98)	*******A***(98)	*******A***(98)	TGTTCTTATTG(37)	TGTTCTTATTG(11)	******A***	MseI
		AGTGA–ATTGT	957	C(51)	T(56)	T(44)	T(27)	T(11)	C	
		GTATT–TTCAA	966	C(56)	C(56)	C(56)	T(42)	T(20)	C	
		AATAA–TTAGT	996	T(45)	T(43)	T(40)	A(17)	A(16)	T	
		TTAGT–TATAT	1002	A(51)	A(51)	A(40)	T(42)	T(33)	A	
		TATAT–TAAAC	1008	A(51)	A(51)	C(40)	C(17)	C(20)	C	
Dm1592	95D1–95D6	AGACA–TTCAC	911	C(56)	T(44)	T(31)	C(56)	C(56)	C	
Dm0667	98E11–98F3	TTTAA–ATTTC	1032	C(29)	G(35)	G(56)	G(42)	C(30)	G	
		CTAGC–TTGAA	1042	C(51)	T(56)	T(46)	T(37)	C(35)	G	AluI
		GTCCG–CGA	1081	A(29)	A/G(21)	A(26)	G(33)	A(14)	G	
		CGA–TTTAG	1085–1100	**T*T***A***G(36) A**(45)	GGTATGGCAT TCTATG(37)	GGTATGGCAT TCTATG(48)	GGTATGGCAT TCTATG(42)	**T*T***A*** G(42)A**(98)	GGTATGGCAT TCTATG(48)	
		AAACT–TTTTT	1138, 1142	T(56)ATTG(37)	G(56)ATTA(51)	G(44)ATTA(45)	G(37)ATTA(37)	T(22)ATTG(15)	GATTA	
		CAGAA–ATTGA	1156	A(56)	T(51)	T(30)	T(29)	A(40)	T	Tsp509I SspI
		TTGGC–AAAAT	1170	C(42)	A(51)	A(44)	A(44)	C(26)	A	HaeIII MscI
		AAAAT–TATTT	1176, 1178	A(38)CG(40)	G(56)CA(56)	G(50)CA(37)	G(30)CA(35)	A(32)CA(21)	GCA	SnaBI TaII CviRI NsiI
Q1058	**96F10–F11**						**Q1058**	**Q1059**		
Dm1657	97C1–97C3	CATGG–CGCAT	968	C(37)	C(40)	A(30)	C(38)	C(24)	C	AciI HaeIII
		AGTTT–TGTTT	1024	G(56)	T(56)	G(56)	G(37)	G(37)	T	
		GCCCC–CCATC	1049	G(40)	A(40)	A(40)	A(37)	G(40)	A	AclI
Dm2473	99D1–99D2	GTCGC–GAATC	1029	*(19)	T(40)	T(30)	T(40)	C(40)	C	NruI ThaI
		ATTGG–CCGGA	1049–1049, 1052	C*(11)T(11)CAG(22)	T(40)T(40)CAA(40)	T(23)T(25)CAA(23)	T(38)T(37)CAA(33)	T(35)C(35)CAG(33)	CTTCAA	DrdII
Dm2288	100C1–100C4	CTGCT–CATTT	1040	C(42)	T(44)	T(47)	C(21)	C(29)	C	
		TTGGC–GTTCC	1110–1113, 1116	****GG*(98)	GTTT(40)GGT(40)	GTTT(42)GGT(38)	****GG*(98)	****GG*(98)	ATTTGGT	AciI
		CAAAG–TGCGT	1139	T(51)	C(37)	C(42)	T(42)	T(56)	C	AluI

List of SNP sequences identified among CS, OreR, w;iso2;iso3, y;cnbwsp, and pairwise combinations of 17 single P-element insertion strains. Identified SNPs are listed according to the STS from which they were derived. For each SNP entry, the approximate cytological location and ~10 bp of sequence flanking the polymorphic site are indicated. Cytological locations are derived from the P1-based genome physical map (Kimmerly et al. 1996) and confirmed by comparison of the STS sequence to a database of Release 1 genome sequences with associated cytological locations (Adams et al. 2000). "Nucleotide Position" refers to nucleotide positions within sequence assemblies and is included to provide relative, rather than absolute, positions for each polymorphism. The identity of the dimorphic base for each SNP is listed for each strain, and the corresponding Phred quality score of that base in the sequence trace is listed parenthetically. Sequences from the *y;cn bw sp* strain were derived from the Release 1 sequence, where available. Restriction endonucleases listed under "RFLP?" are those for which the recognition site is present in one allele and absent in the other. The location of P-element insertions (designated as Q1040, etc.) is indicated; SNP sequences are listed between each pairwise set of P-element strains that can be used for eye-color-based selection of recombinants in that interval. All polymorphisms between the w;iso2;iso3 and P-element strains were initially detected by Denaturing HPLC. For additional data, see http://www.fruitfly.org/SNP/index.html.

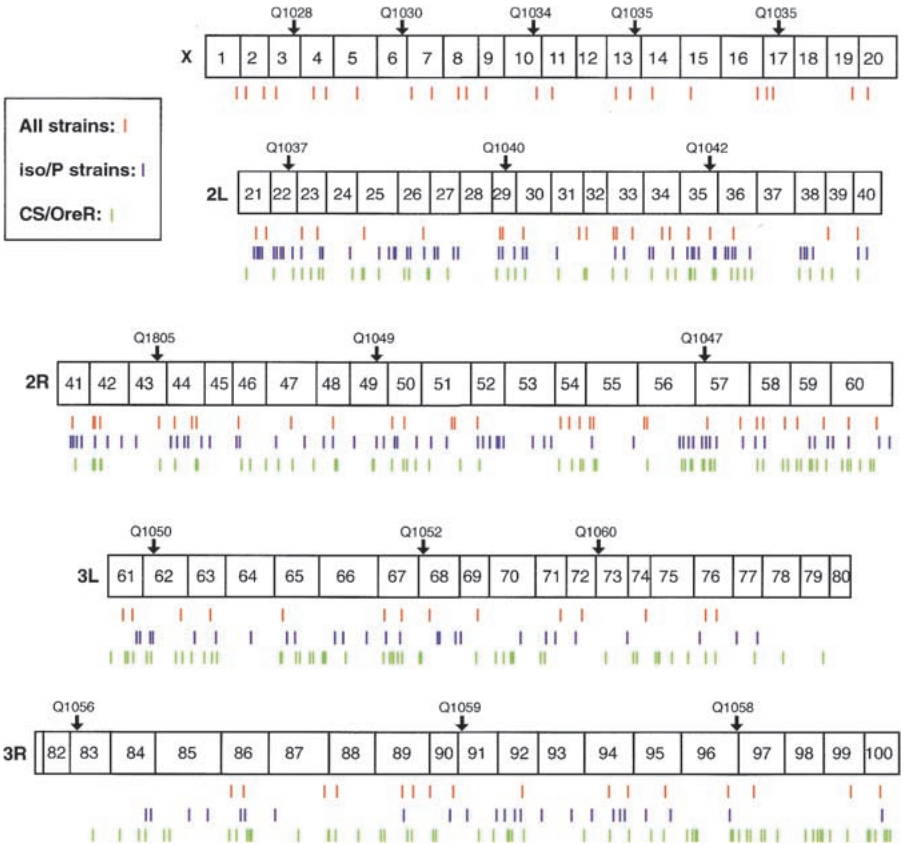

Figure 1 Distribution in the *Drosophila* genome of SNPs identified in this study. The euchromatic portion of the genome is represented by horizontal bars, with the extent of each cytological division representing the genomic extent as estimated by Sorsa (1988). The positions of SNP markers identified in this study are represented by vertical hatch marks: SNPs for which sequence has been determined in all strains are represented by red hatch marks; those that were identified in pairwise subsets of strains are indicated by blue or green. The distribution is relatively even throughout the genome, with the exception of cytological divisions 78–83 near the centromere of chromosome 3. Strain designations (e.g., Q1040) and a small downward arrow indicate the positions of P elements useful for fine-scale mapping.

tion can be used for finer scale localization. For instance, a useful approach is to generate many recombinants between the P[w +] elements shown in Table 2, which can be selected easily using the white eye color phenotype (Cutforth and Rubin 1994; Spradling et al. 1995). In a P[w +] interval of ~10 Mb, 500 selected recombinants result in one recombination event every 20 kb on average. Using this approach and a combination of the SNPs reported here and newly discovered SNPs in a selected interval, we have localized a single mutation to <25 kb, an interval in which DNA sequencing is a realistic method for identifying mutations (D.A. Ruddy and M.C. Ellis, unpubl.).

Although the paucity of molecular variation and recombination events near the centromeres is currently a limitation for recombination mapping, new techniques based on male-specific recombination for mapping relative to P-element insertion sites may provide a solution (Chen et al. 1998). A commonly used but relatively low-resolution approach to mapping mutations is complementation testing with chromosomal deficiencies, which sometimes can be confounded by synthetic interactions. Existing large collections of P-element insertion mutants also can be used in complementation tests with new, unmapped mutations, an approach that sometimes will obviate the need for high-resolution recombination mapping (Spradling et al. 1999). However, the spectrum of genes that can be recovered by insertional mutagenesis is limited by insertion site biases. Point mutations are useful for many genetic analyses and are recoverable only by chemical mutagenesis. A combined approach that makes use of both recombination mapping with SNPs and analysis of transposon insertions in candidate genes is an efficient approach to positional cloning.

Another advantage of SNPs as markers is the ability to use standardized and scalable genotyping methods. We have found DHPLC to be a useful technology for SNP scoring, as it is a technically simple and robust technology that is inexpensive to operate and well suited to detection of heteroduplex DNA molecules derived from heterozygous individuals. Assay technologies that do not require specialized instrumentation, such as PCR followed by restriction site polymorphism analysis, can be applied to many of the polymorphisms identified in this study. This approach has been shown to be effective for SNP mapping in *C. elegans* (S. Wicks, pers. comm.). A vast array of technologies for genotyping SNPs has been developed, a partial sampling of which includes systems based on primer extension, oligonucleotide ligation, or nuclease assays (Landegren et al. 1998), and various microarray formats (Hirschhorn et al. 2000; Pastinen et al. 2000).

The set of SNPs described here can be used in mapping crosses involving any strain. For example, a mutation in an unknown genetic background could be mapped in two sets of crosses to any two of the isogenic strains described here, using SNP markers that distinguish the two mapping strains. Because SNP

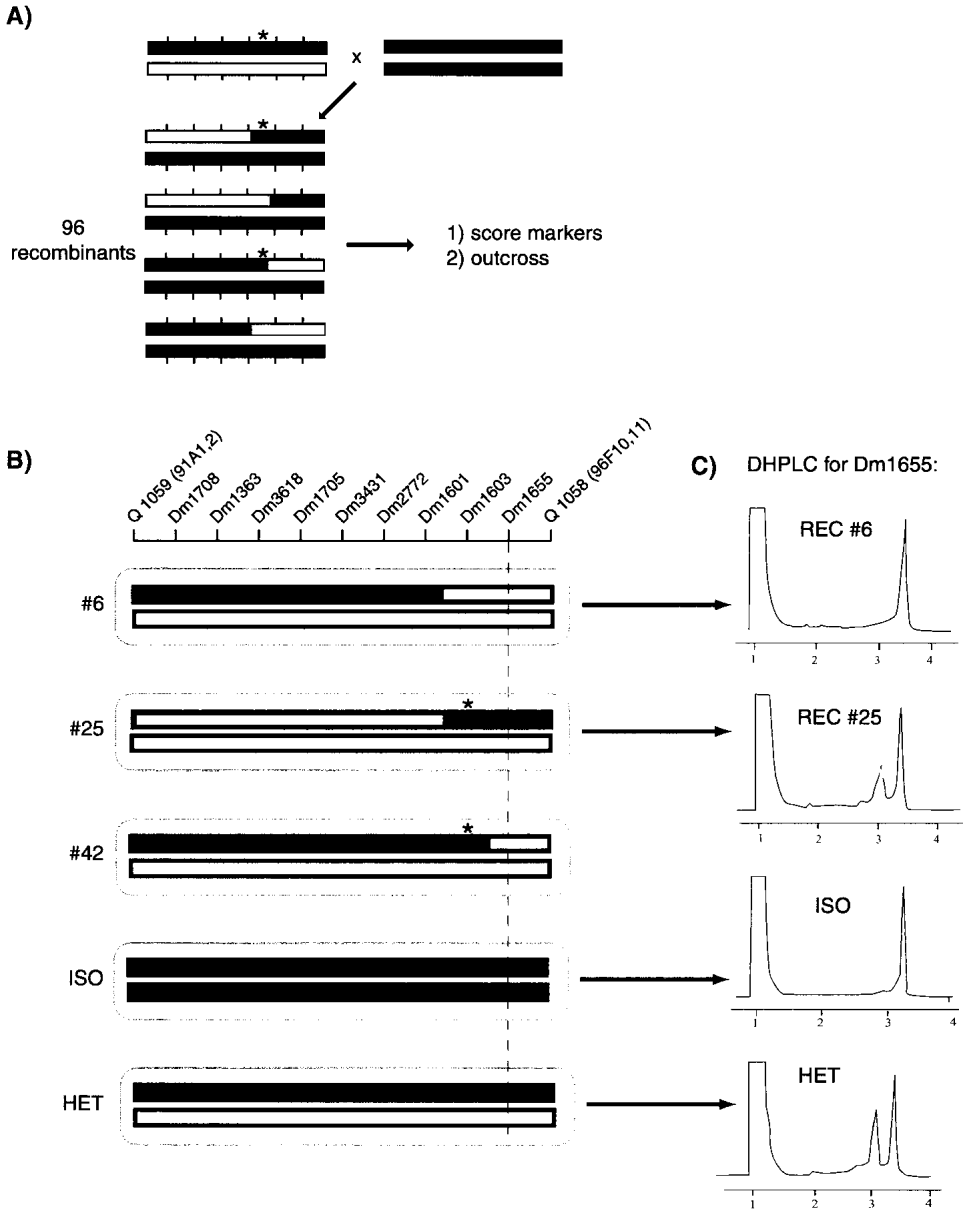

Figure 2 Recombination mapping of a recessive lethal mutation by using SNP markers. Chromosomes (bars) and molecular markers (vertical hatch marks) are shown. The mapping process occurs in two stages. (*A*) The mutation (asterisk) induced in the w;iso2;iso3 background (black bar) that has been mapped previously to a chromosome and balanced is mapped relative to a polymorphic mapping strain (open bar). Single flies heterozygous for the mutation-carrying chromosome and the mapping chromosome are crossed to flies homozygous for the parental w;iso2;iso3 strain to generate 96 recombinant flies. The four recombinant classes are represented. Each recombinant fly strain is assayed for a low-density set of markers that span the chromosome. These markers can be of any type, including SNPs or P-element insertions. We have typically tested six markers spaced at ~10-Mb intervals on these 96 recombinants, for a total of 576 assays. Each recombinant also is assayed for presence or absence of the mutation by outcrossing. From the outcross data and initial marker data on this set of 96 recombinants, the mutation can be assigned to an interval of 10–20 Mb. (*B*) A higher density set of SNPs then is assayed on recombinants from *A* that break in the appropriate interval. An example of mapping a mutation in the *hedgehog* gene is shown. SNP markers are indicated by the STSs from which they are derived. Two P elements (Q 1059 and Q 1058) that were used to localize these mutations also are indicated. The chromosomal compositions of three recombinant (#6, #25, and #42) and two control (ISO and HET) flies are represented. Asterisks indicate chromosomes that carry the mutation as defined by outcrossing. The SNP markers shown were scored using DHPLC. The recombinants shown delimit the position of these mutations to between Dm1601 and Dm1655, a region of ~984 kb. Subsequent complementation testing showed that these mutations are alleles of the *hedgehog* gene, which lies between Dm1601 and Dm1655. (*C*) DHPLC scoring of SNPs. PCR products were amplified from recombinants and analyzed under partially denaturing conditions. Data are shown for Dm1655, a C/T dimorphism. Run time in minutes is shown on the *X* axis and ultraviolet absorbance on the *Y* axis. Dm1655 was analyzed from the following strains: w;iso2;iso3 (ISO), a w;iso2;iso3/Q1059 heterozygote (HET), and *hedgehog* recombinants 6 and 25 (#6 and #25). In this example, the sample from recombinant 25 shows a heteroduplex pattern and therefore is scored as a heterozygote. (DHPLC) denaturing high performance liquid chromatography.

markers are biallelic, essentially all SNPs will distinguish the unknown strain from one or the other mapping strain, so essentially all markers will be informative in one cross or the other. Furthermore, genotyping assays can be used to identify a subset of the SNPs presented here that differentiate two unknown strains. However, the development of standardized *Drosophila* mapping resources may benefit from the selection of standardized strains and a standard set of SNPs that can be scored using a widely available and easily accessible scoring technology.

With the complete genomic sequence now available, we can look toward rapid developments in genomics-based approaches to biological problems in *Drosophila*. For example, a higher density set of SNPs could be developed to enable even higher resolution mapping strategies. Additional large-scale SNP discovery is now very straightforward using the reference *Drosophila* genome sequence as a guide. SNPs will be useful in other mapping applications, including characterization of complex traits, QTL mapping, and loss-of-heterozygosity approaches to defining deletion end points. SNPs also may be useful as *Drosophila* strain identifiers. Finally, SNPs may have use in genomic sequence-based screening approaches, whereby randomly mutagenized chromosomes are screened for molecular lesions (Bentley et al. 2000).

METHODS

Drosophila Strains

Wild-type *Drosophila* strains were obtained from the Bloomington Stock Center. Canton S and Oregon R were obtained from the laboratory of G.M. Rubin; single P-element insertion strains were obtained from C. Goodman and G.M. Rubin and were derived from an enhancer trap screen performed in their laboratories. Canton S, Oregon R, and w;iso2;iso3 were made isogenic for the indicated chromosomes (X, 2, or 3) according to standard techniques, with the exception of Canton S and Oregon R isogenic chromosome 2 stocks, which were obtained from T. Laverty and G.M. Rubin. All P-element strains and newly isogenized wild-type strains are available from the Bloomington Stock Center.

Molecular Biology

Drosophila genomic DNA was isolated either from adult populations or single recombinant flies according to standard techniques. STSs were amplified from genomic DNA preparations using either standard or touchdown PCR (Don et al. 1991) to facilitate amplification of most STSs under a single set of conditions.

Identification of Sequence Variants

The STSs used in this study were developed by the Berkeley *Drosophila* Genome Project (BDGP; Kimmerly et al. 1996), and were selected based on map position inferred from the P1 clones and contigs with which they are associated. STS sequences, primer sequences, and PCR conditions are available on the BDGP Web site (http://www.fruitfly.org). STS sequences were compared among different strains by using the two approaches discussed below.

DNA sequencing

One thousand sixteen STSs were selected for sequencing in the Canton S and Oregon R strains based on the following criteria: primer annealing temperature of 58°C, PCR product length of >180 bp, and reliable amplification in STS content mapping experiments. STSs were PCR amplified with AmpliTaq Gold polymerase (Applied Biosystems) by using PCR conditions described in individual STS reports available at http://www.fruitfly.org. PCR products were treated with exonuclease I and shrimp alkaline phosphatase to degrade primers and free nucleotides (Werle et al. 1994). Treated products were sequenced using the PCR primers and BigDye terminator sequencing chemistry (Applied Biosystems) and analyzed on ABI 377 sequencing machines. Sequences were assembled using the `Phred`/`Phrap`/`Consed` package (Ewing and Green 1998; Gordon et al. 1998; http://genome.washington.edu). Candidate polymorphisms were detected both by inspection of traces and by automated detection of high-quality sequence discrepancies in `Consed`. High-quality sequence was obtained from 796 of the 1016 STSs, and 309 of these contained at least one polymorphism. The sequences in Canton S and Oregon R of 49 of these polymorphic STSs have been reported previously (Teeter et al. 2000).

DHPLC

Approximately 1050 STSs between 180 and 200 bp in size were selected and amplified by PCR from the P-element and w; iso2; iso3 strains. Successful amplification was confirmed by gel electrophoresis. For each STS, the products amplified from the two strains were mixed in a 1 : 1 (v/v) ratio. The mixed products were denatured for 5 min at 95°C and reannealed slowly to create heteroduplex molecules. Presence or absence of heterozygosity was analyzed using DHPLC (Oefner and Underhill 1998) under the following conditions: Samples were run on a metal-free HPLC system (Varian Chromatography Systems) and fitted with a column capable of high-resolution DNA separations; the Eclipse and Helix columns (Varian Chromatography Systems) or the DNASep column (Transgenomic, Inc.) were used. Chromatographic separations were performed using a uniform gradient (1.8% acetonitrile/min for DNAsep columns, 4%/min for other columns), and all STSs were screened at 52°C, 54°C, 56°C, 58°C, and 60°C to determine the optimum temperature for heteroduplex detection. STSs positive for heterozygosity were DHPLC-analyzed in the homozygous strains and sequenced to identify the variant base.

Sequences from both approaches were assembled using `Phred` and `Phrap` and analyzed in `Consed` to generate the sequences represented in Table 2 and at http://www.fruitfly.org. All STSs containing SNPs were sequenced on both strands; in some cases, this resulted in single-stranded coverage of SNPs near the ends of STSs. The SNPs listed in Table 2 were sequenced in the strains that contain the two flanking P elements to identify those useful for fine-scale mapping.

Recombination Mapping

Alleles of the *hedgehog* gene were isolated in a screen for ethyl methanesulfonate-induced mutants that suppress a p21 overexpression phenotype. These mutations were induced in a w;iso2;iso3 strain, and balanced mutant stocks were established over TM3 or TM6 by standard methods. The mutations were localized initially by crossing to a mapping chromosome

that contained six molecular polymorphisms at evenly spaced intervals. By analyzing 96 random recombinants for both (1) the allele at each marker, and (2) the presence or absence of the mutant gene, the position of the mutation along the chromosome can be established to within a 10–20-Mb interval of the chromosome between the molecular markers. SNP markers in the appropriate interval were amplified from each recombinant and analyzed using DHPLC to generate the haplotypes shown in Figure 2.

ACKNOWLEDGMENTS

We thank Brett Milash for establishing an STS location database at Exelixis, Ross Buchholz and Wes Miyazaki for generating *hedgehog* recombinants, the Exelixis sequencing group for sequencing support, Bruce Kimmel and Mike Palazzolo for advice and discussions, and Gerald M. Rubin for support on NIH grant P50-HG00750. This work was funded in part by grant BG94-206 to E.M.R., and from Rhone-Poulenc to R.A.H.

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Received January 5, 2001; accepted in revised form March 22, 2001.