A Genetic Mapping System in *Caenorhabditis elegans* Based on Polymorphic Sequence-Tagged Sites

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ABSTRACT

We devised an efficient genetic mapping system in the nematode *Caenorhabditis elegans* which is based upon the differences in number and location of the transposable element Tc1 between the Bristol and Bergerac strains. Using the nearly completed physical map of the *C. elegans* genome, we selected 40 widely distributed sites which contain a Tc1 element in the Bergerac strain, but not in the Bristol strain. For each site a polymerase chain reaction assay was designed that can distinguish between the Bergerac Tc1-containing site and the Bristol "empty" site. By combining appropriate assays in a single reaction, one can score multiple sites within single worms. This permits a mutation to be rapidly mapped, first to a linkage group and then to a chromosomal subregion, through analysis of only a small number of progeny from a single interstrain cross.

SEQUENCE-tagged sites (STSs), which have recently been proposed as common landmarks for genomic physical mapping (OLSON *et al.* 1989), are short, unique genomic regions that are easily detected by polymerase chain reaction (PCR) amplification (SAIKI *et al.* 1988). A primary virtue of STSs is that they can be distributed among laboratories as a DNA sequence database, obviating the transfer and storage of biological materials.

Polymorphic genomic regions have also been converted into STSs by appropriately designed PCR assays. These are valuable as easily scored genetic markers and as points of correspondence between physical and genetic maps. Variable numbers of repetitive mono-, di- and trinucleotide motifs (LITT and LUTY 1989; TAUTZ 1989; WEBER and MAY 1989), referred to as microsatellites, and variable lengths of the polydeoxyadenylate tracts within individual *Alu* elements (ECONOMOU *et al.* 1990) are examples of polymorphisms that have been rapidly scored through appropriately designed PCR assays. A large number of microsatellites in the mouse genome have been systematically cloned and converted into polymorphic STSs with the goal of producing a polymorphic STS map (CORNALL *et al.* 1991).

Here we report the systematic development of a polymorphic STS map in the nematode *Caenorhabditis elegans*. The polymorphisms exploited in this scheme are copies of the transposable element Tc1 which are present at dispersed loci in the high Tc1-copy number Bergerac strain (approximately 500 copies), but absent from these same locations in the low Tc1-copy number Bristol strain (30 copies) (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983).

Previously, Tc1s have been used in efforts to molecularly clone specific genes, both by direct transposon tagging (MOERMAN, BENIAN and WATERSTON 1986) and by genetic mapping of the Bristol/Bergerac Tc1 polymorphisms nearest a gene to identify molecular clones from the region (RUVKUN *et al.* 1989). Conversely, cloned genes have been placed on the linkage map by using Tc1 polymorphisms as genetic tags (FILES, CARR and HIRSH 1983).

To develop a genome-wide genetic mapping strategy, individual randomly cloned Bergerac Tc1s were positioned within the nearly completed *C. elegans* genomic physical map (COULSON *et al.* 1986, 1988, 1991). PCR assays were then developed for a subset of the Tc1s, selected to give markers well distributed throughout the genome. The assays yield a product when the Tc1 is present (Bergerac DNA) but give no product when the Tc1 is absent (Bristol DNA). The resulting STS map has sufficient density to allow a new mutation to be mapped to a chromosomal subregion through analysis of a limited number of progeny from a single interstrain cross. Since the STS markers do not have an associated phenotype and can be scored directly by PCR with DNA released from single animals or embryos, they offer advantages beyond a simple reduction in the number of crosses needed to map new mutations. These are demonstrated here in mapping experiments with lethal and *X*-linked mutations.

MATERIALS AND METHODS

Strains, genes and mutations: All mutations used in mapping experiments were generated in the Bristol N2 background. Most have been described by BRENNER (1974)

and by HODGKIN et al. (1988); the genes and corresponding mutations are listed below for each chromosome.

Chromosome *I*: *dpy-5(e61)*, *unc-29(e1072)*, *unc-75(e950)*, *unc-101(m1)*, *unc-95(su33)*, *lev-11(x12)*.

Chromosome *II*: *sqt-2(sc3)*, *dpy-10(e128)*, *unc-4(e120)*, *unc-52(st572)* (B. WILLIAMS and R. WATERSTON, unpublished results), *unc-52(e444)*.

Chromosome *III*: *unc-45(e286ts)*, *dpy-1(e1)*, *dpy-18(e364)*.

Chromosome *IV*: *dpy-9(e12)*, *deb-1(st385)* (BARSTEAD and WATERSTON 1991), *unc-22(s12)*.

Chromosome *V*: *unc-60(e677)*, *dpy-11(e224)*, *unc-23(e25)*, *dpy-21(e428)*, *rol-9(sc148)*.

Chromosome *X*: *dpy-3(e27)*, *unc-27(e155)*, *unc-90(e1463)*, *pat-(st558)* (B. WILLIAMS and R. WATERSTON, unpublished results).

Two Bergerac strains, RW7000 and DP13, were used to generate the Bristol/Bergerac hybrid animals required for all polymorphic STS mapping experiments. DP13 is a derivative of RW7000 obtained after maintenance for several years as an independent strain in another laboratory. There are only two differences between the strains with respect to the STS polymorphisms described here: (1) Tc1 *eP64 III* is present in DP13 but absent from RW7000, and (2) Tc1 *stP128 V* is present in RW7000, but absent from DP13 (see RESULTS).

Techniques for culture and genetic analysis were as described by BRENNER (1974). All crosses were at 20° except for those with *unc-45(e286ts)* homozygotes, which were set up at 15° and then shifted to 20° after 2 days.

Cloning, physical mapping and sequencing of Bergerac Tc1 insertion sites: Most of the Bergerac Tc1s were isolated from a library of RW7000 genomic DNA in vector λ1059 by hybridization with Tc1 sequences (I. MORI, D. MOERMAN and R. H. WATERSTON, unpublished results). DNA minipreps (HELMS *et al.* 1987) from positive clones were subjected to fingerprint analysis (COULSON *et al.* 1986) to place them in the *C. elegans* genomic physical map. Clones which could not be positioned by fingerprinting were radiolabeled by random priming (Prime-It kit, Strategene, La Jolla, California) and hybridized using standard procedures to a gridded set of YAC clones that correspond to most of the *C. elegans* physical map (COULSON *et al.* 1991). For clones mapping to advantageous positions, the sequence of DNA flanking the Tc1 was determined directly from miniprep DNA using primer 618 (see Table 1), which is complementary to Tc1 sequence (ROSENZWEIG, LIAO and HIRSH 1983a), by using a linear amplification sequencing method (MURRAY 1989), as modified (CRAXTON 1991). The same method was used directly with plasmids SS#2 (M. BEANAN and S. STROME, unpublished results) and peP64BO (D. PILGRIM, unpublished results) to determine sequence flanking Tc1 polymorphism *Tcbn2 I* and *eP64 III*, respectively. DNA sequence flanking polymorphism *maP1 II* was obtained directly from plasmid pAPP-HB4 (PAPP, ROUGVIE and AMBROS 1991) using Tc1 primer 618 and a standard double stranded sequencing method (Sequenase Kit, USB, Cleveland, Ohio). Sequence flanking the Tc1 polymorphism *bP1 V* was taken directly from ROSENZWEIG, LIAO and HIRSH (1983b). For Tc1 polymorphisms *hP4 I*, *mgP21 III* and *sP4 IV*, sequence of flanking DNA was obtained from Bergerac subclones pCeh50 (STARR *et al.* 1989), mgP21 (FINNEY, RUVKUN and HORVITZ 1988) and pCes233 (BAILLIE, BECKENBACH and ROSE 1985) respectively, by further subcloning to place the *Eco*RV site from the Tc1 inverted repeats adjacent to sequencing primer sites of vector pBS⁻ (Strategene, La Jolla, California), and double strand sequencing with the Sequenase kit (USB, Cleveland, Ohio).

PCR methods: The primers used for PCR are listed in Table 1. They include primer 618, which is complementary to a sequence within the Tc1 element, and primers specific for each Tc1 insertion site. Each of the latter is complementary to DNA sequences that flank the Tc1 insertion site and will work in combination with primer 618 to amplify a DNA fragment of defined size from the Bergerac Tc1-containing site, but not from the "empty" Bristol site.

DNA for PCR assay was prepared from individual animals using methods adapted from BARSTEAD, KLEIMAN and WATERSTON (1991). Single animals were picked up with a platinum wire and each placed in a 2.5-μl drop of lysis buffer [60 μg/ml proteinase K in 10 mM Tris (pH 8.2), 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20 and 0.05% gelatin) in the cap of a separate 0.5-ml tube suitable for PCR. The drops were then moved to the bottom of the tubes by a brief microfuge spin, frozen (−70°, 15 min), and after the addition of a mineral oil overlay, heated (60°, 1 hr followed by 95°, 15 min). After cooling to 4°, 22.5 μl of a "master mix" were pipetted on top of the mineral oil overlay. The mix is formulated to bring the reaction volume to 25 μl with these final conditions: 25 pmol of internal Tc1 primer 618 and 25 pmol each for the appropriate flanking sequence primers, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dCTP, 0.2 mM dGTP and 0.6 unit Taq polymerase (Perkin Elmer Cetus, Norwalk, Connecticut). A brief microfuge spin was used to move the "master mix" through the mineral oil overlay, and the reactions were rapidly heated (within 1 min) to 94° and cycled 30 times: 94° for 30 s, 58° for 1 min, and 72° for 1 min. Failure to promptly bring the tubes to 94° after the brief microfuge spin results in a significant increase in background bands that can complicate interpretation of the data. Gel lanes were loaded with 10 μl of each reaction.

Individual unhatched late-stage embryos were also used for single PCR reactions. Eggs were picked up directly from a culture plate using a 5-μl capillary tube which had been pulled to a convenient inner diameter (50–100 μm) and filled with chitinase solution [20 mg/ml chitinase (Sigma C-6137) in 50 mM NaCl, 70 mM KCl, 2.5 mM $MgCl_2$ and 2.5 mM $CaCl_2$]. They were then immediately transferred to worm lysis buffer, and processed as described above. The same capillary tube was used for multiple transfers without significant cross-contamination of DNA.

Amplification products were separated using standard nondenaturing 6% acrylamide minigels (Mighty Small II, Hoeffer Scientific Instruments, San Francisco, California).

Mapping of new mutations: Since Bergerac males of the RW7000 strain do not mate efficiently, all crosses used to generate Bristol/Bergerac hybrids had to be carried out beginning with Bristol males. Consequently, different procedures were required to generate the hybrids when mapping autosomal and *X*-linked mutations. For the former, F_1 hybrids were generated in two steps: (1) homozygous (nonlethal mutations) or heterozygous (lethal mutations) Bristol hermaphrodites were crossed with Bristol males, and (2) 10–15 male progeny were then mated with 20–30 Bergerac hermaphrodites. F_1 hermaphrodites were then picked to separate plates, and the mutant heterozygotes were recognized when they produced homozygous mutant F_2 self-progeny. The mutant F_2 animals (dead embryos for the lethal mutations) were assayed individually for Bergerac Tc1s by PCR.

For *X*-linked mutations, where passage through an *XO* male would be difficult, the F_1 hybrid was generated as follows: (1) Bristol/Bergerac hybrid males, which have a single *X* chromosome of Bergerac origin, were generated by mating Bristol males with Bergerac hermaphrodites. (2) These males were mated to the Bristol strain carrying the mutation to be mapped. F_1 hermaphrodite progeny were

TABLE 1

Primer sequences for Tc1 polymorphisms

Primer	Polymorphism	Band size (bp)	Sequence (5′ → 3)
618			GAA CAC TGT GGT GAA GTT TC
LG *I*			
093	*stP124*	115	GAC GCA GAC AGA CGA AGT G
734	*hP4*	130	CGG AAA TAT TAT CAG CAC AGC
473	*TCbn2*	150	GCA TAA AAA GCC TCC AAG TCA
LG *II*			
543	*stP196*	133	CCA AAA GTT TAA AGG AAA TGA AGC
363	*stP100*	198	GGA AAC CAA GAA CAT TGG ACG
133	*stP101*	118	CGC CTG ATT TTT CCA GGT GC
816	*stP50*	180	TCC AGA TAT CAT ATA GCT TGT TC
886	*stP36*	153	CAC TGT CTT GTC GAT ACC
437	*stP98*	269	AAG TAG AAA AAT TGC CTT GCG
723	*maP1*	234	CCA ATT TTC CGG AAG TTT TCG
LG *III*			
570	*eP64*	137	TAA TAA TTT GTC AGG AAA CGA G
845	*stP19*	216	ACA AGC GGG TCT ACT GAA CC
587	*mgP21*	165	GGA ACA AAA GTG CCT TGG G
401	*stP127*	233	GCA TCG ATA CAA GTG GAA GC
548	*stP120*	149	AAT AAT CAG TGA AGC CTC ATG
800	*stP17*	183	CTC GAT GTG TCT CAA TAG TTC C
LG *IV*			
815	*stP13*	236	CCC ACA ACC TTT TGC TAC AAC
056	*stP51*	281	GTT CGT TTT TAC TGG GAA GG
931	*stP44*	209	CCA TCG TTT GTG TCT AGA GTC
653	*sP4*	179	TTT CTG TTT TGT GCT TAG ACG
510	*stP5*	261	GGA TTA TTA CCG TCT TAC GCA
536	*stP35*	149	GCA GTC TCT AAT AGA GCT GC
LG *V*			
813	*stP3*	153	GCT GCA TTT CCA TTC ATG CAG
440	*stP192*	290	GCA CGC TGA GAG TAA GTG C
997	*stP23*	135	TTG TCA ACT ATT TTA CAG CGA G
736	*bP1*	119	AAC ACA TTT AGG TAA TGT AGC AC
998	*stP6*	170	TCA CAA TCG ATG ACT AAG TAC TGG
862	*stP18*	203	TTG AAC TTC TCC CAC TCC TC
438	*stP108*	135	AAA GAT AAA CGC GCT TTT TGG
439	*stP105*	152	GGG TAG TTG TTC ATG TCT CG
652	*stP128*	200	GCA ACG CTT TGT GGA TCT G
LG *X*			
930	*stP41*	193	TGT CTA CTT ACC TTA ACT TAC C
888	*stP40*	229	GTA TGA GCT AAT TGT ACC CTC
449	*stP156*	143	TGG AGG ATT CGG GCG ATT G
112	*stP33*	260	CGT CTA GTC GTG TGT TTC C
453	*stP103*	209	GAC GAA AAG AGG TAC ACG AG
454	*stP129*	160	CCA CTT ATT GCC ACT TTT TGG
450	*stP61*	176	GAA TTG GTG TCC GGA ACA C
452	*stP72*	112	CTT GAA AAT ACC ATG GCA TAC
887	*stP2*	127	CAA AAC GGT ATA CTC TGG TG

picked to separate plates, and mutant heterozygotes were recognized by their homozygous mutant self-progeny (F_2). When mapping lethal mutations, each F_1 parent was tested for the *X*-linked Bergerac Tc1s before the F_2 progeny were assayed. In this way any nonhybrid F_1 animals (self-progeny of the original Bristol strain) were recognized and their progeny discarded.

Genetic mapping of Tc1s: A procedure similar to standard 3-factor mapping was used to confirm the genetic map positions of the STSs. In each cross two visible marker mutations (*a* and *b* in the following examples) were used to mark a single Bristol chromosome of an F_1 Bristol/Bergerac hybrid. The hybrid was then allowed to self, and individual F_2 animals were scored for Tc1s by multiplex PCR. (Since the STSs behave as dominant genetic markers, F_2 animals were scored directly. This is in contrast to standard 3-factor mapping, which requires an F_3 generation to permit homozygosity of the recessive mutation that is being mapped.) The F_1 hybrids were constructed as described above for single autosomal or *X*-linked mutations starting with double mutant homozygotes. Hybrid F_1 double heterozygotes were recognized by the appearance of double mutant F_2 self-progeny.

Map information was obtained in several ways from analysis of F_2 animals. First, we selected for recombination events between the visible markers; F_2 recombinants of genotypes *a+/ab* and *+b/ab* were picked and assayed by PCR. By comparing the subsets of Tc1s inherited on the selected recombinant chromosomes (*a+* or *b+*) of these animals, the Tc1s within the marker interval were ordered. Since the

STSs behave as dominant genetic markers, one or more Tcls that were inherited on the unselected chromosome (*ab*), due to a recombination event outside of the visible marker interval, occasionally complicated this analysis. These events were identified easily and usually directly confirmed by PCR analysis of individual F_3 progeny (F_2 animals were picked to separate plates and allowed to self before they were lysed for PCR analysis). Multiple F_3 animals of the *ab/ab* genotype were tested in these instances, and all were positive for the Tcl(s) inherited on the unselected chromosome.

Map information was also obtained by analysis of F_2 double mutant progeny (*ab/ab*). The Tcls detected in these animals were placed outside of the visible marker interval and sometimes ordered relative to each other with these data; Tcls inside could only be inherited on a chromosome that was double recombinant within the visible marker interval. Similar mapping data was also obtained from Tcls inherited on the unselected chromosomes of recombinant F_2 animals, as described above.

The four crosses used to map STSs on chromosome *I* are presented in Table 2. Tcl *stP124* maps within the *dpy-5* to *unc-29* interval, as indicated by analysis of animals recombinant for these marker mutations (*dpy-5* [8/36] *stP124* [28/36] [*unc-29 hP4*]). Similarly, Tcls *hP4* and *TCbn2* map within the *unc-75* to *lev-11* interval ([*stP124 unc-75*] [33/50] [*hP4 TCbn2*] [17/50] *lev-11*), but we failed to recover recombinants that ordered these Tcls relative to each other, suggesting that they are close together. Tcl *hP4* maps to the left of *unc-95*, as shown by animals recombinant for *dpy-5* and *unc-95* ([*dpy-5 stP124*] [37/45] *hP4* [8/45] *unc-95*), and may also be left of *unc-101* (*dpy-5* [3/84] *stP124* [79/84] *hP4* [2/84] *unc-101*).

Three crosses were used to map STSs on chromosome *II* (Table 3). Animals recombinant for the markers *sqt-2* and *unc-4* order *stP100*, *stP196*, *stP101* and *stP50* on the left half of the linkage group and place all but *stP100* within the marker interval ([*stP100 sqt-2*] [25/63] *stP196* [21/63] *stP101* [10/63] *stP50* [7/63] *unc-4*). These data suggest that *stP100* may be left of *sqt-2*, but do not rule out a position close to and right of this marker. The map positions of *stP196* and *stP101* are left of *dpy-10*, as shown by chromosomes that were nonrecombinant for *dpy-10* and *unc-52* but positive for these Tcls (recovered in Dpy Uncs). On the right half of linkage group *II*, chromosomes recombinant for markers *dpy-10* and *unc-52* order *stP36*, *stP98* and *maP1*, positioning all within the *dpy-10* to *unc-52* marker interval ([*stP50 dpy-10*] [4/130] *stP36* [4/130] *stP98* [16/130] *maP1* [106/130] *unc-52*). The map position of *stP36* is further refined by chromosomes recombinant between *dpy-10* and *unc-4*, which place it left of *unc-4* (*dpy-10* [4/25] *stP36* [21/25] *unc-4*).

Table 4 shows the mapping data for STSs on chromosome *III*. Animals recombinant for *unc-45* and *dpy-1* place *eP64* between these markers (*unc-45* [10/64] *eP64* [54/64] *dpy-1*, while nonrecombinant animals positive for *stP19*, *stP120*, *mgP21*, *stP127* and *stP17* place these Tcls right of *dpy-1*. Animals recombinant for *unc-45* and *dpy-18* order *stP19*, *stP120*, *mgP21*, *stP127* and *stP17*, and place all but *stP17* within the *unc-45* to *dpy-18* interval (*unc-45* [49/69] *stP19* [7/69] *stP120* [2/69] *mgP21* [2/69] *stP127* [9/69] [*dpy-18 stP17*]. Tcl *stP17* is right of *dpy-18*, as shown by chromosomes nonrecombinant for *unc-45* and *dpy-18*, but positive for this Tcl, which were recovered in Dpy Uncs and as the unselected chromosome in Dpy non-Uncs.

A cross with markers *dpy-9* and *unc-22* was used to map STSs on chromosome *IV* (Table 5). Animals recombinant for these markers order *stP13*, *stP51* and *stP44* within the *dpy-9* to *unc-22* interval and place *sP4*, *stP5* and *stP35* to the right of *stP44* (*dpy-9* [39/52] *stP13* [6/52] *stP51* [1/52] *stP44* [6/52] [*unc-22 sP4 stP5 stP35*]). Tcls *stP5* and *stP35* are right of *unc-22* and *sP4*, as indicated by a chromosome that was nonrecombinant for *dpy-9* and *unc-22*, negative for *sP4*, but positive for *stP5* and *stP35* (recovered as the unselected chromosome in one non-Dpy Unc animal). Tcl *sP4* was not resolved from *unc-22* in this cross.

The chromosome *V* mapping data are presented in Table 6. Tcl *stP3* is within the *unc-60* to *dpy-11* interval, as

TABLE 2

Linkage group *I* mapping data

Genotype of hybrid parent; phenotype of self-progeny	Detection of STS			
	stP124	*hP4*	*TCbn2*	*n*
dpy-5 unc-29/++[a]				
Dpy non-Unc	+	+		6
	−	+		16
	−	−		2[b,c]
				24
Non-Dpy Unc	−	−		1
	−	+		1[d]
	+	−		7
	+	+		3[d]
				12
Dpy Unc	−	−		35
	−	+		17
				52
unc-75 lev-11/++[a]				
Unc non-Lev	−	+	+	15
	−	−	−	7[e]
	+	−	−	4[f]
				26
Non-Unc Lev	+	−	−	18
	+	+	+	6
				24
dpy-5 unc-95/++[a]				
Dpy non-Unc	−	+		21
	−	−		2[b]
				23
Non-Dpy Unc	+	−		16
	+	+		6
				22
dpy-5 unc-101/++[a]				
Dpy non-Unc	+	+		1
	−	+		36
	−	−		1[b]
				38
Non-Dpy Unc	−	−		2[b]
	+	−		43
	+	+		1
				46

[a] Mutant chromosome from the Bristol background; wild-type chromosome from the Bergerac background.

[b] Unlinked Tcls were detected and served as internal positive controls for these reactions (not shown).

[c] The absence of *hP4* may be due to a double crossover event or to Tcl excision.

[d] Tcl *hP4* was inherited on the unselected chromosome or through a double crossover on the selected chromosome.

[e] For six of these animals either detection of an unlinked Tcl in the same reaction, or failure to detect *stP124*, *hP4* and *TCbn2* in multiple F_3 animals, suggest these negative results are not simply due to failed PCR assays.

[f] Analysis of F_3 animals indicates that *stP124* was inherited on the unselected chromosome.

TABLE 3

Linkage group *II* mapping data

Genotype of hybrid parent; phenotype of self-progeny	Detection of STS							
	stP100	*stP196*	*stP101*	*stP50*	*stP36*	*stP98*	*maP1*	*n*
sqt-2 unc-4/++[a]								
Sqt non-Unc	−	+	+	+		+	+	3
	−	−	+	+		+	+	5
	−	−	−	+		+	+	1
	−	−	−	−		+	+	1
								10
Non-Sqt Unc[b]	+	−	−	−				22
	+	+	−	−				16
	+	+	+	−				9
	+	+	+	+				6
								53
dpy-10 unc-52/++[a]								
Dpy non-Unc				−	+	+	+	4
				−	−	+	+	2
				−	−	−	+	7
				−	−	−	−	50
								63
Non-Dpy Unc				+	+	−	−	2
				+	+	+	−	9
				+	+	+	+	56
								67
Dpy Unc		−	−	−	−	−	−	23
		+	−	−	−	−	−	2
		+	+	−	−	−	−	1
								26
dpy-10 unc-4/++[a]								
Dpy non-Unc	−		−		+		+	1
	−		−		−		+	4
	+		−		−		+	3[c]
								8
Non-Dpy Unc	+		−		−		−	1[d]
	+		+		−		−	2
	+		+		+		−	14
								17

[a] Mutant chromosome from the Bristol background, wild-type chromosome from the Bergerac background.

[b] Assays for *stP98* and *maP1* were performed on only 14 of these animals and *maP1* was detected in one of them; this Tc1 was probably inherited on the unselected chromosome.

[c] Analysis of F_3 animals indicates that *stP100* was inherited on the unselected chromosome.

[d] Tc1 *stP101* may have been lost by excision; loss by recombination would require either a triple recombinant selected chromosome, or a double recombinant selected chromosome in combination with a single recombinant unselected chromosome.

indicated by chromosomes recombinant for these markers (*unc-60* [104/170] *stP3* [66/170] *dpy-11*). Tc1 *stP192* maps close to *dpy-11*; we failed to detect a recombination event between *dpy-11* and *stP192* in animals recombinant for *dpy-11* and *unc-60* to the left (*unc-60* [170/170] [*dpy-11 stP192*]), or *dpy-11* and *unc-23* to the right ([*stP192 dpy-11*] [22/22] *unc-23*). Data from the second cross place *stP23* within the *dpy-11* to *unc-23* interval (*dpy-11* [12/22] *stP23* [10/22] *unc-23*). Tc1s *bP1*, *stP6*, *stP18*, *stP108* and *stP105* are ordered and positioned within the *dpy-11* to *rol-9* interval by recombinants for these markers (*dpy-11* [10/54] *bP1* [2/54] *stP6* [7/54] *stP18* [8/54] *stP108* [13/54] *stP105* [14/54] *rol-9*). Animals recombinant for *dpy-21* and *rol-9* further refine the map positions of *stP108* and *stP105*, placing them right of *dpy-21*; these recombinants also position *stP128* to the right of *stP105*, but left of *rol-9* (*dpy-21* [2/84] *stP108* [21/84] *stP105* [43/84] *stP128* [18/84] *rol-9*).

For the *X*-linkage group (see Table 7), chromosomes recombinant for *dpy-3* and *unc-27* order *stP41*, *stP40*, *stP156*, *stP33* and *stP103*, placing all but *stP41* within the marker interval ([*stP41 dpy-3*] [18/58] *stP40* [15/58] *stP156* [9/58] *stP33* [5/58] *stP103* [11/58] *unc-27*); *stP41* was inherited on the unselected chromosome in one Dpy non-Unc animal (see footnote *b*), placing it left of *dpy-3*. Chromosomes that were nonrecombinant for *dpy-3* and *unc-27*, but positive for one or more of the Tc1s *stP129*, *stP61*, *stP72* and *stP2* (recovered in non-Dpy Uncs as the unselected chromosome), place these STSs to the right of *unc-27* and order them with one exception; a recombination event between *stP61* and *stP72* was not recovered, so these Tc1s cannot be ordered relative to each other.

RESULTS

Identification of Bergerac Tc1s: Because the Bergerac strain of *C. elegans* has many more Tc1s than

TABLE 4

Linkage group *III* mapping data

Genotype of hybrid parent; phenotype of self-progeny	Detection of STS						
	eP64	*stP19*	*stP120*	*mgP21*	*stP127*	*stP17*	*n*
unc-45 dpy-1/++[a]							
Unc non-Dpy	+	+	+	+	+	+	7
	−	+	+	+	+	+	21
	−	+	−	+	+	+	1[b]
							29
Non-Unc Dpy	−	−	−	−	−	−	2[c]
	−	−	−	−	+	+	1[d]
	+	−	−	−	−	−	18
	+	−	−	−	−	+	10[d]
	+	+	+	+	+	+	4[e]
							35
Unc Dpy	−	+	+	+	+	+	4
	−	−	−	−	−	+	7
	−	−	−	−	−	−	9
							20
unc-45 dpy-18/++[a]							
Unc non-Dpy		+	+	+	+	+	30
		−	+	+	+	+	3
		−	−	−	+	+	1
		−	−	−	−	+	6
							40
Non-Unc Dpy		−	−	−	−	−	19
		+	−	−	−	−	1
		+	−	−	−	+	3[f]
		+	+	−	−	−	2
		+	+	+	−	−	1
		+	+	+	+	−	2
		+	+	+	+	+	1[f]
							29
Unc Dpy		−	−	−	−	−	7
		−	−	−	−	+	6
							13

[a] Mutant chromosome from the Bristol background; wild-type chromosome from the Bergerac background.

[b] Absence of *stP120* is most likely due to Tc1 excision; loss by recombination would require a triple recombinant selected chromosome, or the combination of a double recombinant selected chromosome and a single recombinant unselected chromosome.

[c] One of these animals produced several F_3 progeny, and each tested negative for all of the chromosome *III* STSs.

[d] Tc1s *stP127* and/or *stP17* were probably inherited on the unselected chromosome.

[e] Test of F_3 progeny from two of these animals confirms that *stP19*, *stP120*, *mgP21*, *stP127* and *stP17* were inherited on the unselected chromosome.

[f] Since *stP17* maps to the right of *dpy-18*, this Tc1 was probably inherited on the unselected chromosome.

TABLE 5

Linkage group *IV* mapping data: *dpy-9 unc-22/++*

Phenotype of self-progeny[a]	Detection of STS						
	stP13	*stP51*	*stP44*	*sP4*	*stP5*	*stP35*	*n*
Dpy non-Unc	+	+	+	+	+	+	16
	−	+	+	+	+	+	3
	−	−	+	+	+	+	1
	−	−	−	+	+	+	2
	−	−	−	−	−	−	3[b]
							25
Non-Dpy Unc	−	−	−	−	−	−	22
	−	−	−	−	+	+	1[c]
	+	−	−	−	−	−	3
	+	+	+	−	−	−	4
							30

[a] Mutant chromosome of the parental strain from the Bristol background; wild-type chromosome from the Bergerac background.

[b] Probably failed reactions.

[c] Tc1s *stP5* and *stP35* were most likely inherited on the unselected chromosome.

the standard Bristol strain, almost every Bergerac Tc1 should be a polymorphic site between the two strains. We chose seven previously cloned Bergerac Tc1s for development as polymorphic STSs because of their well dispersed positions on the *C. elegans* physical map. To increase this number, we undertook to position 200 Tc1-hybridizing clones which had been previously isolated from a Bergerac genomic library.

Initially, these clones were subjected to restriction fragment length fingerprint analysis (COULSON *et al.* 1986) and this information was used to place them on the physical map. However, many of the clones had too few fingerprint bands to allow positioning; these, along with other problem clones, were also hybridized against a gridded set of YAC clones representing most of the physically mapped genome (COULSON *et al.* 1991). The 200 clones identified 73 independent sites (Figure 1), indicated as vertical lines below the physical map of each chromosome. Precise positions are available within the ACEDB/CEMAP database (R. DURBIN and J. THIERRY-MIEG, personal communication).

Because much of the physical map has been correlated with the genetic map through markers mapped both physically and genetically, the physical map position allowed us to infer the genetic map position of most clones. Those with relatively dispersed genetic map positions were selected for further study, as were all the Tc1-containing clones that mapped to contigs of unknown genetic map location. The latter were chosen since they were likely to be located in the less well characterized regions of the genome. STS markers *stP3 V*, *stP128 V*, and *stP41 X* (see below) were developed from this second set of clones.

Conversion to STSs: For each clone of interest, sequence of the DNA flanking the Tc1 site was determined, usually by priming from a site within the Tc1 (see MATERIALS AND METHODS for details). From the sequence, an oligonucleotide primer was designed to work in combination with a constant primer site within the Tc1. The primer pair was tested in the polymerase chain reaction to ensure that the pair gave only a single major band from the phage clone and Bergerac genomic DNA, and no product from Bristol genomic DNA. These primers then served to assay for the Bergerac, Tc1-containing site. These sites are highlighted by boxes in the Figure 1, which shows a

TABLE 6

Linkage group *V* mapping data

Genotype of hybrid parent; phenotype of self-progeny	Detection of STS									
	stP3	*stP192*	*stP23*	*bP1*	*stP6*	*stP18*	*stP108*	*stP105*	*stP128*	*n*
unc-60 dpy-11/++[a]										
Unc non-Dpy	+	+	+	+	+	+				55
	+	+	+	+	+	−				1[b]
	+	+	−	+	+	+				1[c]
	−	+	+	+	+	+				26
	−	+	+	−	+	+				1[d]
										84
Non-Unc Dpy	−	−	−	−	−	−				40
	−	−	−	−	−	+				1[e]
	−	−	−	−	+	+				3[e]
	−	−	−	+	+	+				2[e]
	−	−	+	+	+	+				1[e]
	+	−	−	−	−	−				37
	+	−	−	+	+	+				2[e]
										86
dpy-11 unc-23/++[a]										
Dpy non-Unc	+	−	+	+	+					2[f]
	−	−	+	+	+					4
	−	−	−	+	+					4
	−	−	−	−	+					1[g]
										11
Non-Dpy Unc	+	+	−	−	−					6
	+	+	+	−	−					4
	+	+	+	−	+					1[h]
										11
dpy-11 rol-9/++[a]										
Dpy non-Rol		−		+	+	+	+	+		5
		−		−	+	+	+	+		2
		−		−	−	+	+	+		5
		−		−	−	−	+	+		4
		−		−	−	−	−	+		6
		−		−	−	−	−	−		4
										26
Non-Dpy Rol		+		−	−	−	−	−		5
		+		+	+	−	−	−		2
		+		+	+	+	−	−		4
		+		+	+	+	+	−		7
		+		+	+	+	+	+		10
										28
dpy-21 rol-9/++[a]										
Dpy non-Rol		+		−	−		−	+	+	1[i]
		−		−	−		−	+	+	5
		+		−	−		−	−	+	1[i]
		−		−	−		−	−	+	11
		−		−	−		−	−	−	3
		+		+	−		−	−	−	1[i]
		+		+	+		−	−	−	2[i]
										24
Non-Dpy Rol		+		+	+		−	−	−	2
		+		+	+		+	−	−	15
		+		+	+		+	+	−	31
		+		+	+		+	+	+	12
										60
Dpy Rol		−		−	−		−	−	−	15
		+		+	−		−	−	−	1
		+		+	+		−	−	−	1
										17

[a] Mutant chromosome from the Bristol background; wild-type chromosome from the Bergerac background.
[b] The absence of *stP18* in this recombinant may be due to a double crossover event or Tc1 excision.
[c] The absence of *stP23* is probably due to Tc1 excision.
[d] The absence of *bP1* is probably due to Tc1 excision.
[e] Tc1s *stP23* and/or *bP1* and/or *stP6* and/or *stP18* were inherited on the unselected chromosome or a double recombinant selected chromosome.
[f] Tc1 *stP3* was inherited on the unselected chromosome or on a double recombinant selected chromosome.
[g] The absence of *bP1* is probably due to Tc1 excision.
[h] Tc1 *stP6* was inherited on the unselected chromosome or on a double recombinant selected chromosome.
[i] Tc1s *stP23* and/or *stP192* and *stP3* were inherited on the unselected chromosome or on a double recombinant selected chromosome.

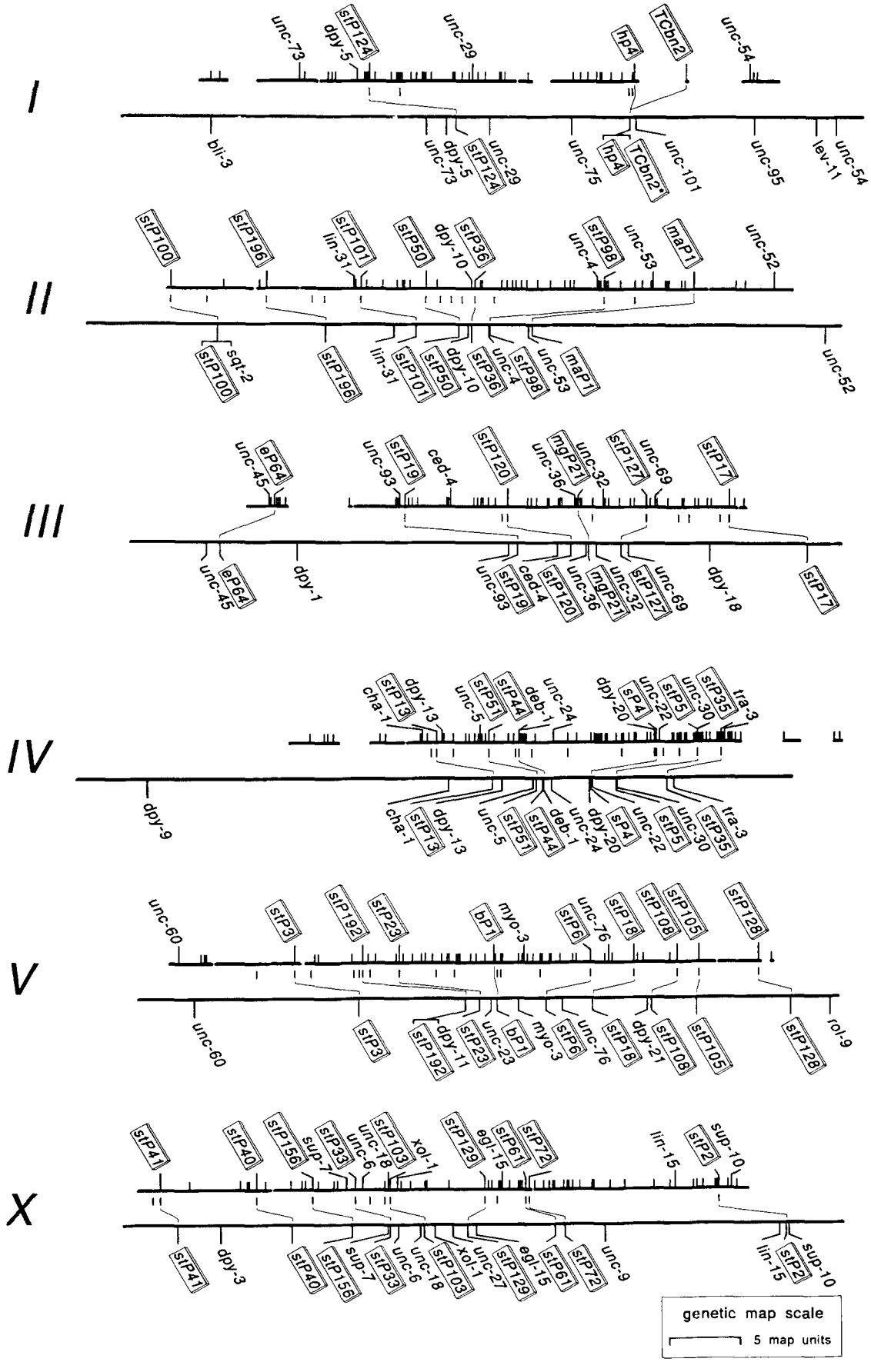

TABLE 7

Linkage group *X* mapping data: *dpy-3 unc-27*/++

Phenotype of self-progeny[a]	Detection of STS									
	stP41	*stP40*	*stP156*	*stP33*	*stP103*	*stP129*	*stP61*	*stP72*	*stP2*	*n*
Dpy non-Unc	+	+	+	+	+	+	+	+	+	1[b]
	−	+	+	+	+	+	+	+	+	4
	−	−	+	+	+	+	+	+	+	14
	−	−	−	+	+	+	+	+	+	5
	−	−	−	−	+	+	+	+	+	3
	−	−	−	−	−	+	+	+	+	4
										31
Non-Dpy Unc	+	−	−	−	−	−	−	−	−	10
	+	−	−	−	−	−	−	−	+	2[c]
	+	−	−	−	−	−	+	+	+	1[c]
	+	+	−	−	−	−	−	−	−	1
	+	+	+	−	−	−	−	−	−	2
	+	+	+	−	−	−	−	−	+	1[c]
	+	−	+	−	−	−	−	−	−	1[d]
	+	+	+	+	−	−	−	−	−	2
	+	+	+	+	+	−	−	−	−	2
	+	+	+	+	+	−	−	−	+	2[c]
	+	−	+	+	+	−	−	−	−	1[d]
	+	−	+	+	+	−	−	−	+	1[c,d]
	+	−	+	+	+	+	+	+	+	1[d,e]
										27

[a] Mutant chromosome of the parental strain from the Bristol background; wild-type chromosome from the Bergerac background.
[b] Test of F_3 progeny shows that *stP41* was inherited on the unselected chromosome; this places *stP41* to the left of *dpy-3*.
[c] Analysis of F_3 progeny were performed for three out of seven of these animals testing positive for *stP61*, *stP72* and/or *stP2*; the results show that these Tc1s were inherited on the unselected chromosome.
[d] The absence of *stP40* is probably due to Tc1 excision; loss by recombination would require a triple crossover event.
[e] Analysis of F_3 progeny suggests that *stP129*, *stP61*, *stP72* and *stP2* were inherited on the unselected chromosome.

physical (top) and genetic (bottom) map of each chromosome.

Multiplex PCR: To implement an efficient genetic mapping strategy, STS band sizes were chosen which permit appropriate multiplex PCR assays. Various assay sets have been developed which either detect a single Tc1 on each autosome (Figure 2, Chromosome Assignment Set) or multiple Tc1s on individual chromosomes (Figure 2, linkage group (LG) *I*, LG *II*, LG *III*, LG *IV*, LG *V*–right, LG *V*–left, and LG *X*).

Each multiplex assay can be used with either of the related Bergerac strains RW7000 or DP13, with the following caveat. Tc1 *eP64 III* is present only in DP13 (see Figure 2, LG *III*), and *stP128 V* is present only in RW7000 (STS *stP128* failed to amplify from DP13 DNA, data not shown); all of the other Tc1s shown in Figure 1 have been detected in both strains. Control reactions with Bristol DNA show that all of these Tc1s are absent from the Bristol N2 strain (Figure 2, rightmost lane of each panel).

PCR artefacts: PCR artefacts that might complicate mapping experiments include false negatives due to reaction failure and false positives due either to contamination of samples or to amplification of background bands that happen to comigrate with one or more STS bands.

False negatives could be detected by including an assay for a nonpolymorphic site. This kind of internal positive control would allow failed reactions to be easily distinguished from animals that simply did not inherit any of the Tc1s normally detected by the multiplex assay. In practice, this information was not necessary for efficient mapping (see below), so we decided that it was better to minimize the number of primers in each reaction.

Although we did not see false positive bands attributable to cross contamination of DNA samples, amplification of background bands comigrating with one or more of the STS bands was observed on occasion in early experiments (not shown). These bands were

FIGURE 1.—Physical (top line) and genetic (bottom line) maps of chromosomes *I*, *II*, *III*, *IV*, *V* and *X*. Bergerac Tc1s that have been converted to polymorphic STSs are highlighted with boxes; their genetic map positions are based on physical map data and genetic data from crosses presented in MATERIALS AND METHODS. Lines extending above the physical maps mark the positions of physically mapped genes; those which were used in this study are labeled. Lines just below the physical maps mark the positions of Tc1-hybridizing Bergerac clones. The physical map for each chromosome is scaled to chromosome size; these are estimated to be 18 megabases (mb) (*I*), 14 mb (*II*), 16 mb (*III*), 19 mb (*IV*), 22 mb (*V*) and 18 mb (*X*). Our genetic data place *TCbn2 I* near *hP4*, but the (*) indicates that it has not been ordered relative to *hP4* or to *unc-101* and *unc-95*.

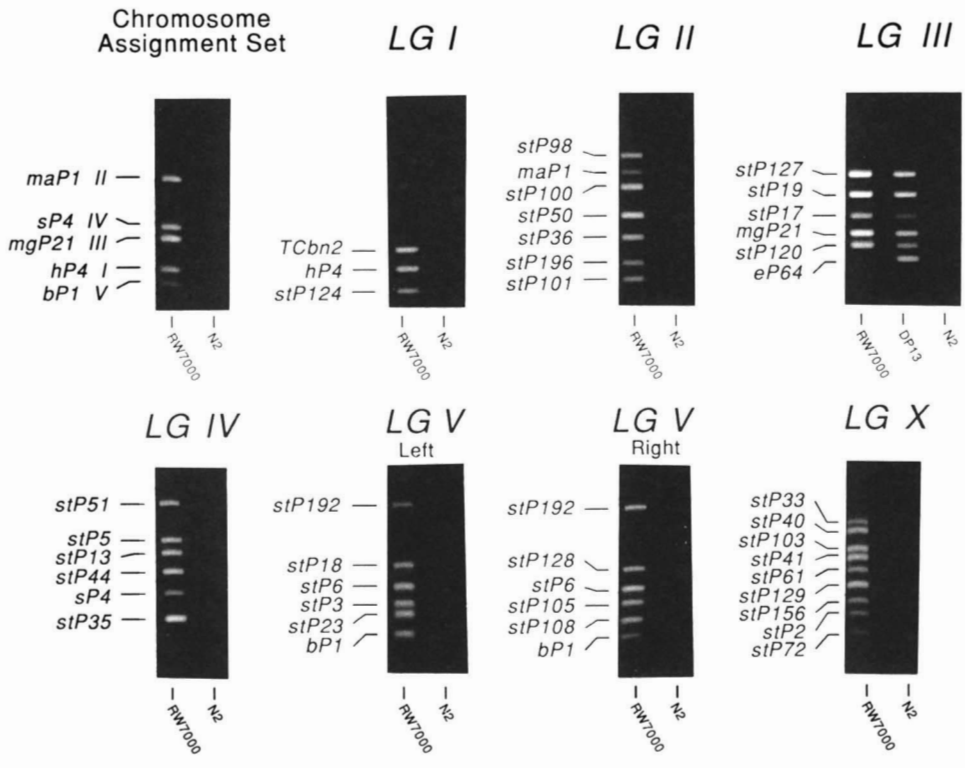

FIGURE 2.—Multiplex PCR assays for Bergerac Tc1s. Each PCR assay was performed on the DNA of a single animal and the products resolved on a standard 6% mini-acrylamide gel. STS band sizes and genetic map positions can be found in Table 1 and Figure 1, respectively. In reactions with DNA from Bergerac strain RW7000 the chromosome assignment assay set detects a single Tc1 on each autosome, while sets LG *I*, LG *II*, LG *III*, LG *IV*, LG *V* (left), LG *V* (right) and LG *X* detect multiple Tc1s on the corresponding individual linkage groups. Results were identical for the closely related Bergerac strain DP13 (not shown), with two exceptions. Tc1 *eP64* was detected in DP13, but not in RW7000 (see LG *III*), and Tc1 *stP128* was detected in RW7000, but not in DP13. It should be noted that when mapping with strain DP13 it is possible to substitute the *stP18* assay for the *stP128* assay within the LG *V* (right) assay set, due to similar band sizes. In control reactions with Bristol N2 DNA no bands were amplified by any of the multiplex assays.

usually very faint and did not confuse interpretation of the data. To confirm that these animals were indeed negative for the STS bands in question, we tested several self progeny from each, in some instances with a simpler mixture of primers. The appearance of background bands is not surprising given the complex mixture of primers in each multiplex reaction, including a primer to the high copy number Tc1 sequence. In subsequent experiments we found that by heating the PCR reactions soon after the components were mixed (see MATERIALS AND METHODS for details), the intensity of background bands was reduced to the point that they rarely caused difficulty.

STS genetic map position: The STSs in regions of the physical map densely covered with markers could often be assigned to a relatively narrow genetic interval. These STS map positions were tested in crosses by marking the appropriate Bristol chromosome of an F_1 Bristol/Bergerac hybrid with two visible marker mutations and then scoring recombinant and nonrecombinant F_2 animals for linked Tc1s by multiplex PCR. The data from these crosses (see MATERIALS AND METHODS) were consistent with the inferred STS map positions, with one exception. *stP36* had been given a physical map position on chromosome *X* with marginal fingerprint data; once placed genetically on chromosome *II*, an alternative match was recognized as correct.

For the STSs in less densely marked regions of the physical map, and for those on contigs not assigned a chromosomal location, more extensive crosses were used to establish the genetic positions (see MATERIALS AND METHODS), and these data improved the contiguity of the physical map. For example, on the right end of chromosome *V* our mapping data for *stP108*, *stP105* and *stP128* positioned the associated contigs relative to each other. In several cases the genetic data led to the recognition and correction of errors in the physical map. In particular, our genetic mapping of *hP4 I*, *stP100 II* and *stP196 II* repositioned the associated contigs.

Spontaneous Tc1 excision in Bristol/Bergerac hybrids: Rates of Tc1 excision in Bristol/Bergerac stains can be similar to or even enhanced relative to those in Bergerac mutator strains (MORI, MOERMAN and WATERSTON 1988, 1990).

In the course of verifying Tc1 map positions, we observed possible spontaneous excision of Tc1s *stP101 II* (Table 3, footnote *d*), *stP120 III* (Table 4, footnote *b*) *stP23 V* (Table 6, footnote *c*), *bP1 V* (Table 6,

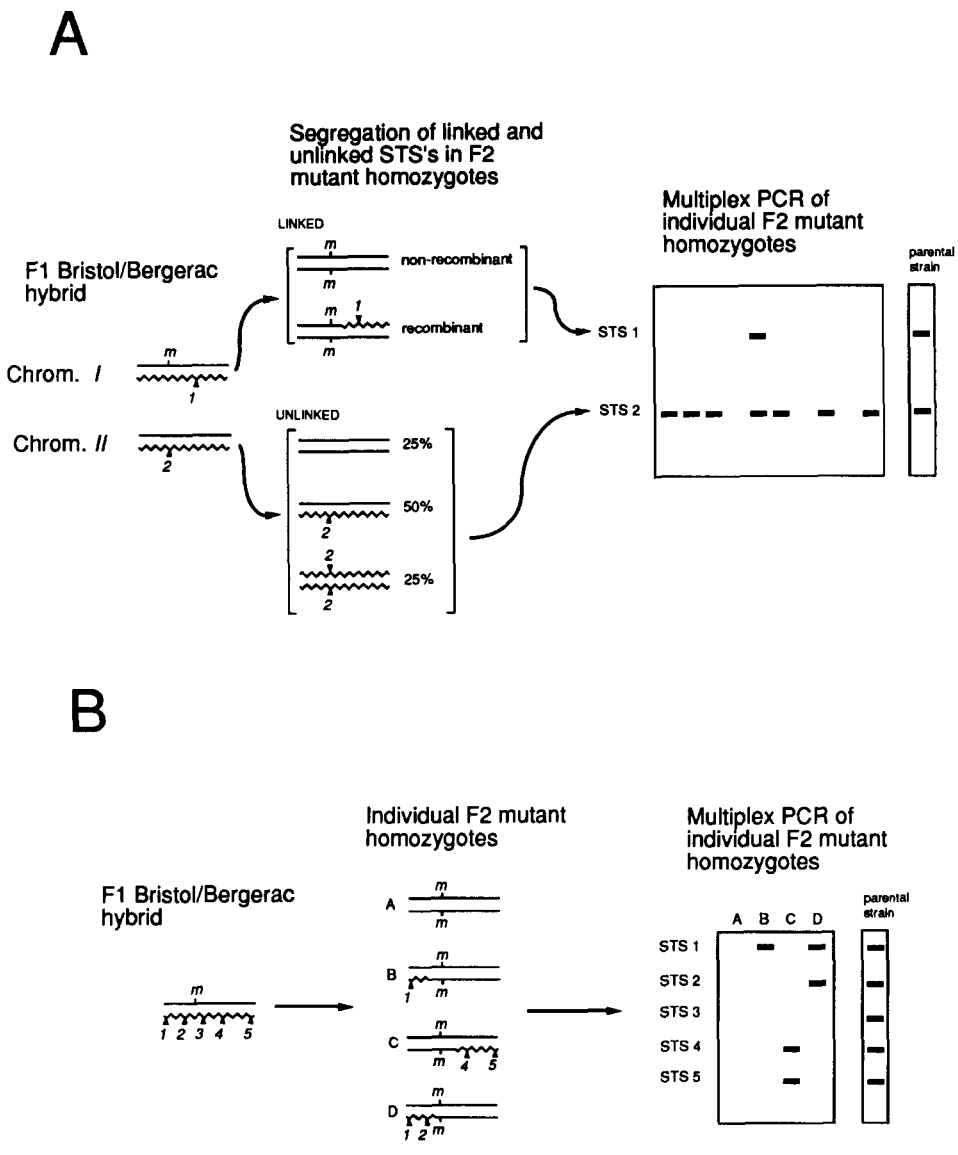

FIGURE 3.—Two-step polymorphic STS mapping strategy. (**A**) A new mutation, m, is first mapped to a chromosome by using a single STS to mark each autosome, STSs 1 and 2 in this example. A Bristol/Bergerac F_1 hybrid (Bristol DNA = straight line, Bergerac DNA = jagged line) that is heterozygous for the new mutation is constructed and allowed to produce self progeny. As diagramed, segregation of linked and unlinked Tc1s among the homozygous mutant progeny will be different. Unlinked Tc1 2 is inherited by 75% of the animals, while linked STS 1 is partially or completely "excluded" from the mutant homozygotes because it is inherited only when there is a crossover between the mutation and the STS. Each F_2 mutant animal is scored for the Tc1s using a single multiplex PCR reaction. The diagramed acrylamide gels shows that only 1 of 10 animals inherited Tc1 1, indicating linkage, while 7 of 10 inherited STS 2, indicating nonlinkage. (**B**) Second STS mapping step. Additional F_2 mutant homozygotes are assayed for several linked Bergerac Tc1s, STSs 1, 2, 3, 4 and 5, by multiplex PCR. Genotypes of some expected F_2 mutant homozygotes (A–D) are diagramed along with the corresponding results of multiplex PCR assays on DNA from these animals. By using the PCR data to infer positions of crossovers between the Bristol and Bergerac chromosomes, mutation m can be placed between STSs 2 and 4; alternative positions are ruled out because they would require more complex patterns of recombination.

footnotes *d* and *g*) and *stP40* (Table 7, footnote *d*). In some cases we failed to detect the Tc1 in a multiplex PCR assay of DNA from a single F_2 animal, while Tc1s to either side were detected. A trivial explanation for these results is a partial failure of the multiplex assay, but we have not seen this type of failure in any of numerous control reactions using single Bergerac worms. Consequently, it is more likely that these Tc1s have either excised or been lost through recombination. The latter is unlikely for the *stP101*, *stP120*, *stP23* and *bP1* events, since each would require either a triple recombinant chromosome or fortuitous combinations of a double recombinant chromosome and a single recombinant homolog in which the necessary intervals for recombination are relatively small; loss by recombination cannot be ruled out, however. In contrast, for *stP40* enough events have been observed to provide compelling evidence of excision. Loss of this marker occurred in three of the 14 animals in which it could have been detected, and to invoke recombination would require a triple recombinant chromosome in each instance.

With the possible exception of the *stP40* excision events, Tc1 excision frequency is low enough that it does not pose a significant complication when using the Tc1s as genetic markers. The method used to initially assign a new mutation to a linkage group (see below) would be unaffected by low levels of spontaneous Tc1 excision. Subsequent mapping to a chromosomal subregion might be more seriously affected, but in practice the level of redundant information obtained from these crosses make it likely that rare excision events would be readily recognized and not lead to a incorrect map position assignment.

Mapping strategy: To map a recessive mutation generated in the Bristol background, a Bristol/Bergerac hybrid strain that is heterozygous for the unmapped mutation is constructed and allowed to self-fertilize (see MATERIALS AND METHODS for details). Approximately 25% of the progeny will be homozy-

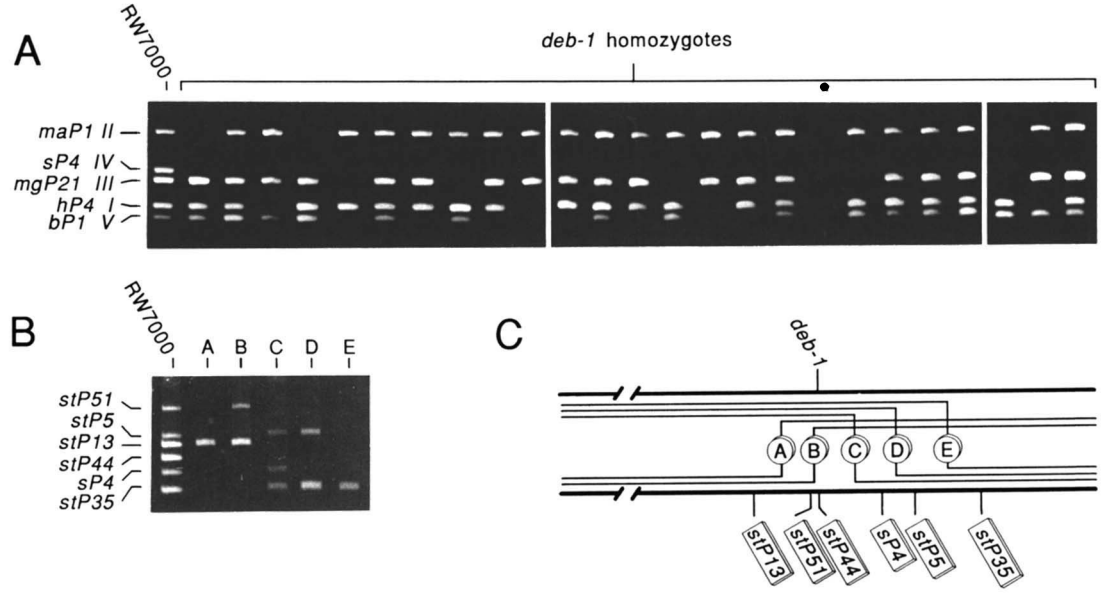

FIGURE 4.—Test mapping of lethal mutation *deb-1(st385)*. (**A**) Multiplex PCR assays of individual *deb-1* homozygotes from a Bristol/Bergerac heterozygous mutant parent. The chromosome assignment assay detects Tc1s marking chromosomes *I*, *II*, *III*, *IV* and *V*. Each Tc1 is detected in approximately 75% of the animals except for *sP4 IV*, which was detected in none of them, indicating linkage. A control reaction with RW7000 DNA shows that the multiplex assay set detects all five of the Tc1s. The (*) indicates a failed PCR reaction. (**B**) Additional *deb-1* homozygotes assayed for several Tc1s on linkage group *IV*. Lanes A–E each show a representative reaction for each type of animal with at least one STS band. (**C**) Proposed recombination events between the Bristol (top line) and Bergerac (bottom line) chromosomes of the F_1 hybrid which produce the F_2 classes A–E [class A (n = 4), class B (n = 1), class C (n = 1), class D (n = 3) and class E (n = 1)]. This analysis places *deb-1* between *stP51* and *stP5*, which corresponds with its previously established genetic and physical map positions.

gous for the mutation, and these animals are picked into separate tubes, their DNA is released by lysis, and then each sample is used in a separate multiplex PCR assay that can detect several Bergerac Tc1s. Mapping is accomplished in two successive steps, each using a different multiplex assay. In the first step the animals are assayed for five Tc1s, one marking each autosome. As illustrated in Figure 3A the STSs behave as dominant genetic markers, so the unlinked Tc1s will be detected in 75% of the animals. In contrast, the linked Tc1 will be detected in significantly fewer of the mutant homozygotes since this requires recombination between the mutation and the Tc1. An STS marker for the *X* chromosome is unnecessary because *X*-linkage is detected when constructing the Bristol/Bergerac hybrid, due to hemizygosity of *C. elegans* males for the *X* chromosome.

In the second mapping step additional animals are assayed for Bergerac Tc1s with dispersed locations on the appropriate chromosome. As illustrated in Figure 3B, multiplex PCR on individual animals allows recombination events to be located and the mutation mapped to a chromosomal subregion.

Mapping autosomal mutations: To illustrate the strategy and demonstrate its effectiveness for mapping lethal mutations, we mapped two autosomal genes whose physical and genetic map positions are well established. In the first experiment we mapped the lethal mutation *deb-1(st385)*; mutant animals either arrest in late embryogenesis or hatch to yield misshapen L1 larvae. Individual mutant self progeny (n = 25) from the appropriate Bristol/Bergerac hybrid were tested with the chromosome assignment assay set (Figure 4A). Each Tc1 except for *sP4 IV* was detected in approximately 75% of the animals; *sP4* was detected in none of them, placing *deb-1* on chromosome *IV* near this STS marker. It should be noted that one lane was negative for all five autosomal markers [Figure 4A (*)], probably due to a failed PCR reaction.

In the second mapping step we assayed 33 additional *deb-1* homozygotes with the LG *IV* assay set. Tc1s were detected in only 10 animals, confirming linkage to chromosome *IV*. These recombinants fell into the five classes illustrated in Figure 4B. The simplest interpretation of these data is illustrated in Figure 4C, which shows the likely crossover events between the Bristol and Bergerac chromosomes of the F_1 hybrid which correspond to the F_2 classes A–E. This interpretation places *deb-1* between *stP51* and *sP4*, agreeing with its known map position. It should be noted that a recombination event between *deb-1* and *stP44* was not detected, so *deb-1* cannot be ordered with respect to this marker. This failure is not surprising given the limited number of animals tested and physical map data which suggest that *deb-1* is very tightly linked to *stP44* (see Figure 1).

We chose the lethal mutation *unc-52(st572)* (arrest phenotype similar to *deb-1(st385)*) as a more stringent test of the first STS mapping step since approximately

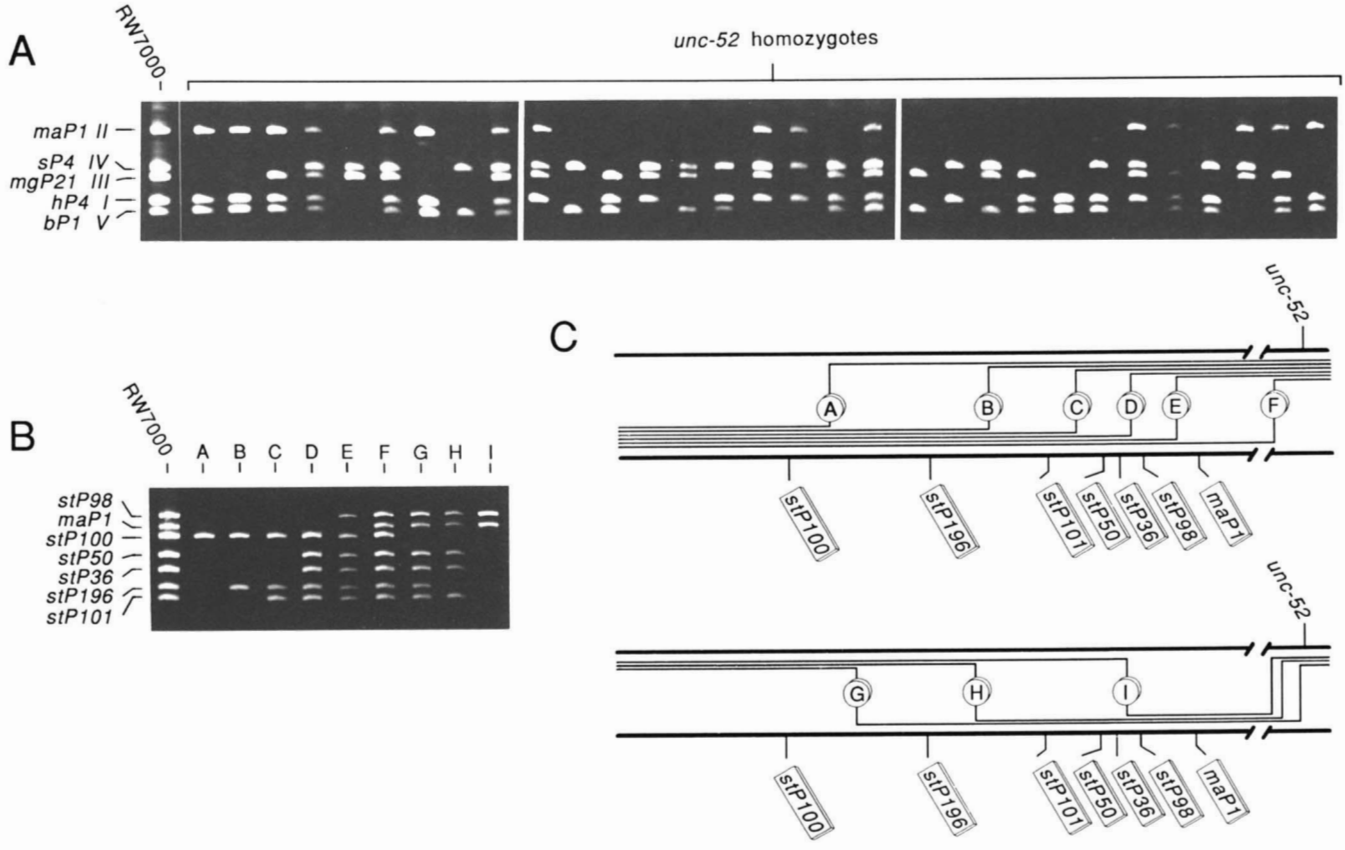

FIGURE 5.—Test mapping of lethal mutation *unc-52(st572)*. (**A**) Individual *unc-52(st572)* homozygotes from a Bristol/Bergerac parent assayed for Bergerac Tc1s on linkage groups *I*, *II*, *III*, *IV* and *V*. Tc1 *maP1 II* is detected in significantly fewer than 75% of the animals, indicating linkage to this marker. In a control reaction with RW7000 DNA each of the autosomal Tc1s is detected. (**B**) Additional *unc-52* homozygotes assayed for multiple Tc1s on chromosome *II*. Lanes A–I show representative reactions for each type of F_2 animal with one or more STS bands [class A (n = 6), class B (n = 2), class C (n = 2), class D (n = 2), class E (n = 1), class F (n = 9), class G (n = 2), class H (n = 1), class I (n = 1)]. (**C**) Proposed recombination events between the F_1 Bristol (top line) and Bergerac (bottom line) chromosomes which produce F_2 classes A–F and G–I.

20 map units separate it from the chromosome *II* marker *maP1*. Reactions with DNA from 36 of the 39 animals tested yielded 1 or more bands (Figure 5A, three lanes with no bands are not shown and were assumed to be failed PCR reactions), and analysis of this data clearly indicates linkage to *maP1* (detection frequency $R = 0.47$, $\chi^2 = 12.75$). Marginally significant linkage to *sP4 IV* ($R = 0.59$, $\chi^2 = 3.92$) and *mgP21 III* ($R = 0.56$, $\chi^2 = 5.64$) was also detected in this data set, however.

Since the strongest linkage was to chromosome *II*, 41 additional *unc-52* homozygotes were tested with the LG *II* multiplex assay in the second mapping step. The 26 animals that were positive for one or more Tc1s fell into the nine classes illustrated by representative reactions in Figure 5B. The simplest interpretation of these data places *unc-52* right of *maP1*, consistent with its established map position; inferred crossover events corresponding to each class are illustrated in Figure 5C.

Mapping *X*-linked mutations: Mapping experiments with lethal mutation *pat-(st558)*, which has not been mapped before, and *unc-90(e1463)*, which has an established map position, illustrate the mapping on the *X* chromosome. An analysis of 39 *pat-(st558)* homozygotes (phenotype similar to the *deb-1* and *unc-52* lethals) yielded 25 that were positive for *X*-linked Tc1s, and these animals fell into the six classes illustrated by representative reactions in Figure 6A. The simplest interpretation of these data place *pat-(st558)* to the right of *stP72* (Figure 6B). Since *stP2* was not detected in any of the animals, *pat-(st558)* cannot be ordered relative to this marker, but must be close to it.

A similar mapping experiment was performed with *unc-90(e1463) X*, which is a semidominant mutation causing uncoordinated movement. Assays of 101 homozygous mutant progeny from the Bristol/Bergerac hybrid strain yielded four classes of animals testing positive for Bergerac Tc1s (Figure 6A). As indicated by the diagram in Figure 6C, these data position *unc-90* between *stP103* and *stP61*, which agrees with its established map position. Recombinant animals which could position *unc-90* with respect to *stP129* were not recovered in this experiment.

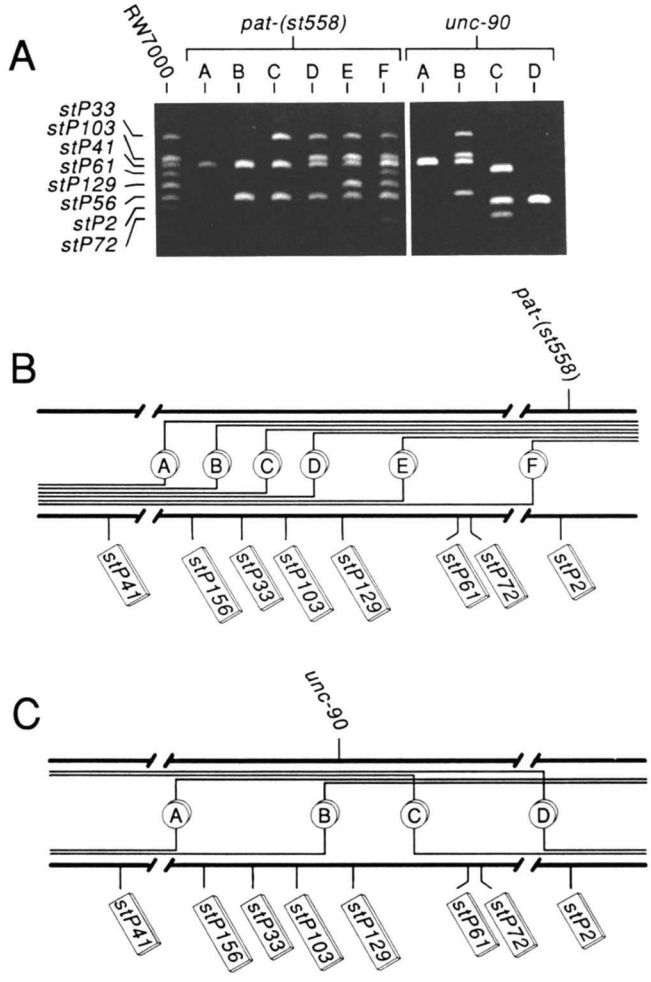

FIGURE 6.—(**A**) Mapping of lethal mutation *pat-(st558)* and semidominant mutation *unc-90(e1463)* on the *X* chromosome. In separate experiments *pat-(st558)* and *unc-90* F_2 homozygotes from the appropriate F_1 Bristol/Bergerac hybrid strains were assayed for *X*-linked Bergerac Tc1s. Those which tested positive at least one Tc1 fell into six classes (A–F) in the *pat-(st558)* experiment [class A (n = 4), class B (n = 2), class C (n = 1), class D (n = 4), class E (n = 1), class F (n = 13); an assay for *stP40* was not included in this experiment], and four classes (A–D) in the *unc-90* experiment [class A (n = 21), class B (n = 2), class C (n = 2), class D (n = 26)]; a representative reaction is shown for each class. (**B**) Proposed recombination events responsible for F_2 classes in the *pat-(st558)* experiment; this simplest interpretation of the data places *pat-(st558)* right of *stP72*, but fails to order it with respect to *stP2*. (**C**) Proposed recombination events responsible for F_2 classes A–D in the *unc-90* experiment. This simplest interpretation of the data places *unc-90* between *stP103* and *stP61*, corresponding to its previously established genetic map position.

DISCUSSION

The polymorphic STS mapping strategy offers significant advantages over standard genetic mapping procedures in *C. elegans*. In the latter only a few visible markers can be used simultaneously due to the small number of phenotypes that are both distinct and easily scored. Consequently, several crosses are often used to place a mutation on a linkage group, and these must be followed by a succession of 3-factor crosses that refine map position. In contrast, the STS mapping strategy permits the simultaneous use of 40 genetic markers and potentially many more as additional polymorphic STSs are developed. Through efficient scoring of single animals by multiplex PCR, a new mutation can be mapped to a chromosomal subregion after analysis of a small number of progeny from a single cross with only one mutant phenotypic class. At this point visible markers can be chosen for detailed 3-factor analysis.

The savings in number of crosses is helpful, particularly when a mutation presents special difficulties, such as lethal, sterile, maternal effect, suppressor or *X*-linked mutations.

Absence of an associated visible phenotype for STS markers offers additional advantages. This feature avoids difficulties that can arise when scoring a mutant phenotype in a background of several visible marker phenotypes (when mapping a suppressor mutation, for example) and also simplifies mapping on the *X* chromosome. The latter is complicated in standard mapping procedures due to the hemizygosity of *C. elegans* males. Mutant *XO* males are often ineffective in mating, so visible markers on the *X* chromosome must usually be used in conjunction with sexual transformation mutations in genes such as *tra-1* (HODGKIN and BRENNER 1977), which can produce *XX* males. In contrast, as shown in test crosses presented here, all nine STS markers for the *X* chromosome can be introduced easily into a mapping strain by using Bristol/Bergerac hybrid males.

Aside from facilitating multifactor mapping, STS markers allow particularly efficient and sensitive mapping strategies because of their behavior as genetic dominants and their suitability for homozygosity mapping. Although some dominant visible markers are available in *C. elegans*, most mapping is accomplished with recessive markers and strategies that require significantly more cross progeny to be scored in order to detect linkage.

The advantages of the STS mapping method, including the ability to directly score dead embryos by PCR, were demonstrated here for lethal alleles of *deb-1* and *unc-52*. If one were to map these same mutations by standard methods, the first step would be to construct F_1 animals that are *trans*-heterozygotes for the lethal mutation and for several visible mutations that mark individual chromosomes. Since it is not possible to score the F_2 lethal homozygotes for the marker phenotypes, viable F_2 siblings (heterozygous for the lethal mutation or homozygous wild-type at this locus) would have to be analyzed instead by cloning them and scoring the phenotypes of the self progeny that they segregate in the F_3 generation. It takes 3 days to produce this extra generation, including several hours at the dissecting microscope cloning the F_2s and then scoring F_3 phenotypes. This compares with the elapsed time of 6 hr, including approximately 2 hr of

bench time, it takes to pick the F_2 homozygotes, perform PCR, and run the gels in an STS mapping experiment. At this point the comparative elapsed times from the first cross are approximately 9 days for the standard mapping method and 6 days for the STS method. Significantly more time is saved in the subsequent mapping phase, since 3-factor crosses must be set up for the standard mapping experiment, requiring at least an additional 9–12 days by a very conservative estimate and substantially more time for the *X*-linked mutation *pat-(st558)*. This contrasts with the STS mapping experiment in which the mutations were mapped to a chromosomal subregion in a single additional day; more F_2 mutant homozygotes from the original cross were simply scored with the appropriate chromosome specific multiplex PCR assay.

Because they are common landmarks to the genetic and physical maps, polymorphic STSs are also very useful when using linkage data to identify DNA clones within the physical map that are likely to contain a gene of interest. Systematic development of additional STS markers should enhance their usefulness for this purpose, and such polymorphic STSs might be used in an extension of the mapping procedure presented here. To continue mapping with STS markers on a finer scale, it would probably be most efficient to use a visible marker in *cis* to the mutation to be mapped. This would allow crossover events in the interval between mutation and visible marker to be readily recognized and the appropriate progeny scored by multiplex PCR for Tc1s in the region. This approach is directly analogous to the one we used to confirm STS genetic map position in crosses with multiple visible markers.

Until a high density polymorphic STS map is available, fine scale mapping with Tc1 polymorphisms for the purpose of cloning a gene from the physical map can be accomplished using the procedure described by RUVKUN *et al.* (1988). Multiple backcrosses produce a strain in which the region containing the gene of interest is derived from the Bergerac background while the remainder of the genome is Bristol. The Bergerac Tc1s are then genetically mapped by forcing recombination in the appropriate interval; segregation of multiple Tc1s is scored simultaneously by Southern blots of DNA isolated from the recombinant strains. Sequences flanking Tc1 polymorphisms mapping nearest to the gene can subsequently be used to identify DNA clones in the physical map.

The polymorphic STSs developed here have been useful tools for physical map building. They served as genetic tags which helped confirm the local order of the physical map, and in some cases they placed previously unassigned DNA contigs on a chromosome.

Finally, the polymorphic STSs may permit new strategies for genetic analysis in *C. elegans*. One of these is the mapping of multiple genetic factors involved in the inheritance of quantitative traits (LANDER and BOTSTEIN 1989). This has been intractable by standard methods, but may be feasible using the STS markers, since the segregation of many of these markers can be scored in a single cross.

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