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Short-interval traffic lines: versatile tools for genetic analysis in *Arabidopsis thaliana*

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Abstract

Traffic lines are transgenic stocks of Arabidopsis thaliana that contain a pair of linked seed-specific eGFP and DsRed markers. These stocks were originally developed for the purpose of studying recombination, but can also be used to follow the inheritance of unmarked chromosomes placed in *trans* to the marked chromosome. They are particularly useful for this latter purpose if the distance between markers is short, making double recombination within this interval relatively rare. We generated 163 traffic lines that cover the *Arabidopsis* genome in overlapping intervals of approximately 1.2 Mb (6.9 cM). These stocks make it possible to predict the genotype of a plant based on its seed fluorescence (or lack thereof) and facilitate many experiments in genetic analysis that are difficult, tedious, or expensive to perform using current techniques. Here, we show how these lines enable a phenotypic analysis of alleles with weak or variable phenotypes, genetic mapping of novel mutations, introducing transgenes into a lethal or sterile genetic background, and separating closely linked mutations.

Keywords: balancer chromosome; fluorescent-tagged lines; recombination; myb75; fie; val1; Plant Genetics and Genomics

Introduction

Identifying a mutation or polymorphism of interest in the progeny of a genetic cross is central to genetic analysis. If the molecular identity or chromosomal location of the mutation/polymorphism is known, this can be done using molecular methods. However, these methods are destructive and time-consuming and require resources that may not be available in some circumstances. We developed a set of transgenic lines of Arabidopsis thaliana that make it possible to visually determine the genotype of seeds in a segregating population prior to planting (Wu et al. 2015). Each transgenic line—termed a "Traffic Line" (TL) because of its green, red, and yellow phenotypes—possesses a pair of linked pNAP::eGFP and pNAP::DsRed transgenes. The reporters in these transgenes are under the regulation of the seed-specific Napin promoter and thus produce, respectively, green or red fluorescent seeds (Stuitje et al. 2003). Such lines were originally developed for the purpose of studying recombination (Melamed-Bessudo et al. 2005), but also make it possible to follow the transmission of the chromosome segment that is in trans to marked segment (Wu et al. 2015). Indeed, a TL can be used much like a balancer chromosome (Casso et al. 2000; Hentges and Justice 2004) to follow the segregation of genes located in a defined region of the genome.

Recombination between the transgenes in a TL is useful if the goal is to study factors that influence recombination (Melamed-Bessudo and Levy 2012; Ziolkowski et al. 2015, 2017; Saini et al. 2020; Nageswaran et al. 2021; Kbiri et al. 2022). However,

recombination is disadvantageous if the TL is being used to follow the segregation of an unmarked chromosome segment in *trans* to the TL because double recombination within the interval generates an unmarked chromosome that may be genetically different from the original unmarked chromosome. Recombination is minimized if the interval between the markers in a TL is relatively short. Such "short-interval" TLs are not only useful as balancers but also facilitate experiments that rely on the ability to detect recombination in small, defined chromosomal regions. These include studies of the factors that influence recombination frequency, removing second-site mutations/polymorphisms linked to a gene of interest, and fine mapping a QTL or a newly induced mutation known only by its phenotype.

Our initial set of TLs contained transgenes located an average of 4.8 Mb (21 cM) from each other (Wu *et al.* 2015). Here, we describe a collection of 163 TLs that covers the *Arabidopsis* genome in much shorter overlapping segments. In addition, we present examples of how TLs facilitate genetic analysis by providing a rapid, visual method for determining the genotype of plants in a segregating population.

Materials and methods Plant material

The recombinant lines described here are in the Columbia (Col) accession and were produced from transgenic lines containing

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sequenced insertions of *pNAP::eGFP* and *pNAP::DsRed*, as described previously (Wu et al. 2015).

Microscopy

Dry seeds were observed with a Leica MZ FLIII fluorescent stereomicroscope, using long-bandpass GFP (excitation 480/40 nm, barrier 510 nm), short-bandpass GFP (excitation 470/40 nm, barrier 525/50 nm), and DsRed (excitation 546/12 nm, barrier ET560 nm) filter sets. The long-band pass filter was particularly useful because it transmits enough red light to enable simultaneous scoring of GFP and DsRed fluorescence.

Producing a TL

Plants containing a *pNAP::eGFP* insertion were intercrossed with a plant containing a linked *pNAP::DsRed* insertion, and the progeny of this cross were then crossed as pollen parents to Col. Progeny from this cross were scored for red fluorescent, green fluorescent, nonfluorescent, and green and red fluorescent seed to measure the recombination distance between the 2 transgenes; seeds that expressed both green and red fluorescence were planted and allowed to self-pollinate. Self-pollinated seeds displayed varying intensities of green, red, and green and red fluorescence. Seeds with the strongest green and red fluorescence were planted to select for plants homozygous for the recombinant *pNAP::eGFP* and *pNAP::DsRed* chromosome, and seed stocks were established from these homozygous plants.

Construction of FIE-HA and its transformation into fie-11

A 4.1-kb FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) genomic region without the stop codon was fused to 6xHA and cloned into the P1 position of the pAGM4723 vector in the Golden Gate system; Basta resistance was cloned into the P2 position in this vector (Engler et al. 2014). The primers used in cloning are listed in Supplementary Table 2. TL 3.31 was crossed to *fie*-11 heterozygotes (genotyped using the *fie*-11 F and *fie*-11 R1 primers) and the *fie*-11 heterozygotes from this cross were selected for further analysis. Moderately fluorescent seeds from the *fie*-11 heterozygote's progeny were planted and the FIE-HA construct was transformed into these plants. Fully developed nonflorescent seeds from the T0 population were planted and single T-DNA insertion lines were selected from the T2 generation by Basta selection. Plants that had 2 copies of *fie* -11 and *FIE-HA* were identified by quantitative PCR using a Bio-Rad CFX 96 real-time System.

Results

TLs were generated from a collection of sequenced pNAP::eGFP and *pNAP::DsRed* T-DNA insertions, using the approach described in Wu et al. (2015) and summarized the Materials and Methods. The chromosomal position of these TLs is illustrated schematically in Fig. 1, along with the locations of the TLs described previously. The insertion sites of the transgenes used in the construction of the new lines, and the physical and recombination distance between them are presented in Table 1 and Supplementary Table 1. Although these recombination distances are sometimes based on relatively few individuals, they are of interest because they represent the frequency of recombination across the entire genome within an inbred background. On average, the physical distance between transgenes was 1.2 Mb, and the recombination distance was 6.9 cM. This yields an average recombination rate of 5.6 cM/ Mb (Table 1), which is higher than has been observed in crosses between different accessions (Singer et al. 2006; Salomé et al.

2012). A few lines (e.g. TL1.55, TL2.20) displayed an unusually high rate of recombination whereas others displayed an unusually low rate of recombination compared to other lines in the same region of the genome. Although TLs with unusually low rates of recombination could reflect the actual rate of recombination in this region, this phenomenon could also be due to small chromosomal rearrangements associated with one or both of the transgenes in the TL. Transgenes associated with reciprocal translocations or large inversions were eliminated by screening T1 plants for semisterility, but this approach cannot detect small rearrangements.

Using a TL to genotype seeds

It is often important to be able to distinguish different genotypes in a segregating population. This is particularly important if one wants to compare the phenotype of mutants with weak or poorly penetrant phenotypes with their wild-type siblings. In Arabidopsis, this is typically done by performing allele-specific PCR on plants after germination. To demonstrate that TLs can be used to perform this task, we took advantage of a semidominant allele of CHLORINA 42 (ch42-4sd) that has a yellow-green phenotype when heterozygous and is albino when homozygous. Plants heterozygous for ch42-4sd were crossed to TL4.22, a line that spans the CH42 locus and contains transgenes located 1.8 Mb from each other. Yellow-green progeny from this cross were allowed to selffertilize, and nonfluorescent, moderately fluorescent red and green, and strongly fluorescent red and green seed were selected (Fig. 2). One hundred percent of the nonfluorescent seeds produced albino seedlings, 97% of the moderately fluorescent seeds produced yellow-green seeds, and 97% of the strongly fluorescent seeds produced dark-green seedlings. Thus, TLs are a reliable tool for determining the genotype of a seed prior to planting.

Using TLs for phenotypic analysis

Determining the phenotype of a mutation in a known gene can be problematic if the mutation has a weak phenotype or is present in a genetic background different from the one that is commonly used in a laboratory. The latter problem can occur if the mutation was generated in a different accession (e.g. Ler instead of Col), or is in a mutagenized background, which may contain other linked and unlinked mutations. One way to get around this problem is to compare the phenotype of mutant and wild-type siblings in the same family. On average, these individuals will share the same background, even if this background is not completely uniform.

To test the usefulness of TLs for comparing the phenotype of genetically different siblings, we examined the phenotype of myb75-1 in a moderately heterogeneous genetic background. This mutation was of particular interest because it has been reported to delay the transition from juvenile to adult growth (vegetative phase change) under short days, but not long days (Meng et al. 2021). myb75-1 is a Ds-induced allele originally isolated in the Nössen accession (Teng et al. 2005). We crossed myb75-1 to Col 5 times and then crossed these myb75-1 plants to TL1.55, a TL that spans a 1-Mb interval containing the MYB75 locus. Nonfluorescent and moderately fluorescent seeds produced by TL1.55/myb75-1 plants were grown separately in the same flat under long-day conditions. As shown in Fig. 3, nonfluorescent seeds had slightly, but significantly, later abaxial trichomes than their fluorescent siblings. This result demonstrates that myb75-1 delays vegetative phase change under both long-day and shortday conditions.



Fig. 1. Location of short-interval (right) and long-interval (left) TLs on the chromosomes of the Columbia accession of Arabidopsis thaliana.

Using TLs to map a novel mutation

In addition to being useful for tracking the segregation of known genes, TLs can also be used to map novel mutations or natural variants. As an example, we used a set of long-interval TLs (Wu et al. 2015) to map a novel juvenilized mutant present in a transgenic stock of Col obtained from the Arabidopsis Biological Resource Center. This mutation was unlinked to the known T-DNA insertion in this line, and a subsequent "mapping by bulked genome sequencing" approach failed to identify a polymorphism that segregated with this mutation. As an alternative approach, we crossed the mutant to a set of long-interval TLs spanning the Arabidopsis genome, and determined if the mutant phenotype was linked to markers in these lines by planting 12-15 nonfluorescent and a similar number of strongly fluorescent seeds from the F2 progeny of these crosses. In most cases, wild-type and mutant seedlings were present in both the fluorescent and nonfluorescent seed classes in approximately equal ratios, with wild-type seedlings typically being more abundant than mutant seedlings (the expected ratio is 3/ 4 wild type:1/4 mutant for an unlinked TL; Table 2). In contrast, all of the fluorescent seeds from the cross to TL4.6 had a wild-type phenotype, and almost all of the nonfluorescent seeds had a mutant phenotype. This result suggests that the mutation is located within, or close to, the segment marked by TL4.6.

Using TLs to rescue a lethal or sterile mutation with a transgene

It is often desirable to generate a transgenic line carrying an epitope-tagged protein (e.g. a reporter protein, or an affinity-tagged protein). Ideally, the epitope-tagged construct should be transformed into a line carrying a null mutation of the corresponding gene to demonstrate that the construct is able to rescue the mutant phenotype and ensure that the only functional copy

of the protein is the version produced by the transgene. This is straightforward when the homozygous mutant is viable and fully fertile, and can therefore be directly transformed with the construct. It is more difficult when plants homozygous for the mutation are lethal or sterile. In this case, the simplest approach is to transform the construct into wild-type plants and then introduce the construct into a homozygous mutant background by several generations of crossing. This is a time- and space-consuming process and requires a considerable amount of molecular genotyping.

TLs facilitate this process by providing a convenient method for identifying plants homozygous and heterozygous for lethal/ sterile mutations. As an example, we used a TL to generate a line carrying an HA-tagged version of FIE, an essential component of the PRC2 complex (Ohad et al. 1999). Plants homozygous for the null allele, fie-11, are embryonic lethal, making it necessary to maintain this mutation in heterozygous condition (Guitton et al. 2004). We generated a balanced stock by crossing fie-11/+ plants to TL3.31 and identified the fie-11/TL3.31 plants from this cross using PCR primers specific for fie-11. We then selected moderately fluorescent green and red seed from these plants (which were expected to be fie-11/TL3.31), and used floral dipping to transform these plants with a T-DNA construct containing pFIE::FIE-HA and Basta-resistance as a selectable marker (Fig. 4a). Approximately 75% of the T1 seeds resulting from this experiment were fluorescent and were expected to either be heterozygous for fie-11 or homozygous wild type (Fig. 4). Most of the remaining seeds were shriveled and dead, as expected because fie-11 is lethal in homozygous condition. We also identified 14 nonfluorescent, viable seeds (Fig. 4a). These T1 seeds were planted, and their progeny were screened for Basta-resistance. Three of these families segregated 1/4 dead seed and were sensitive to Basta indicating that the parental T1 seeds were

Table 1. Genomic location and recombination distance between the *pNAP::eGFP* (CG) and *pNAP::DsRed* (CR) insertions in short-interval TLs.

ABRC ID	Line #	Upper marker	Upper marker	Lower marker	Lower marker	Length of interval (nt)	Progeny of test cross (Col × dsRed/GFP)						cM/mB
			position		position		dsRed	GFP	dsRed + GFP	Nonfluor.	Total		
Chr. 1													
CS72863	1.29	CR1297	77,685	CG308	1,882,501	1,804,816	147	151	19	44	361	19.1	10.6
CS72864	1.30	CG427	92,431	CR924	995,883	903,452	196	194	21	12	423	7.8	8.6
CS72865	1.31	CR924	995,883	CG308	1,882,501	886,618	88	89	3	9	189	6.4	7.2
CS72866	1.32	CR924	995,883	CG246	2,326,356	1,330,473	34	29	3	5	71	11.3	8.5
CS72867	1.33	CR1161	1,778,732	CG945	3,030,311	1,251,579	117	125	7	15	264	8.3	6.6
CS72868	1.34	CG945	3,030,311	CR1312	4,270,245	1,239,934	108	108	8	8	232	6.8	5.5
CS72869	1.35	CR62	3,852,828	CG384	4,767,960	915.132	123	126	14	7	270	7.8	8.5
CS72870	1.37	CR1312	4.270.245	CG538	5.608.046	1.337.801	82	76	1	4	163	3.1	2.3
CS72871	1.38	CG384	4,767,960	CR710	5.897.006	1.129.046	87	88	9	3	187	6.4	5.7
CS72872	1.39	CR1078	4,777,894	CG538	5.608.046	830,152	103	107	4	2	216	2.8	3.4
CS72873	1.40	CG538	5.608.046	CR30	6.899.263	1.291.217	151	166	11	9	337	5.9	4.6
CS72875	1.42	CG926	6.122.779	CR1416	7.179.633	1.056.893	214	196	1	3	414	1.0	0.7
CS72874	1.41	CR30	6.899.263	CG698	7.931.648	1.032.385	156	137	11	8	312	6.1	5.9
CS73210	1 71	CR434	6 995 077	CG298	8 534 178	1 539 101	118	118	15	11	262	99	6.4
CS72876	1.43	CG698	7.931.648	CR94	9.321.582	1.389.934	197	176	12	19	404	7.7	5.5
CS72877	1 44	CG914	9 171 797	CR75	10 678 341	1 506 544	76	77	6	15	174	12.1	8.0
CS72878	1 45	CR94	9 321 582	CG712	11 325 954	2 004 372	115	108	7	12	242	7.8	39
CS72879	1.15	CR75	10 678 341	CG510	11 820 335	1 141 994	120	116	8	4	248	4.8	4.2
CS72880	1.10	CG510	11 820 335	CR1111	13 207 868	1 387 533	108	86	6	7	207	63	4 5
CS72881	1 48	CR1111	13 207 868	CG30	15 662 695	2 454 827	155	153	5	2	315	2.2	0.9
CS72882	1.40	CC30	15,207,000	CR78	17 324 946	1 662 251	86	79	7	2	174	5.2	3.1
CS72883	1.40	CC514	16 346 787	CR1348	17,524,540	1 271 931	96	123	ģ	2	222	5.6	4.4
CS72884	1.51	CR78	17 324 946	CC529	17 952 797	627 851	141	169	6	-	200	3.0	5 Q
CS72885	1.52	CD801	17,324,940	CC669	20 100 577	2 214 890	175	140	20	3	338	5.7	2.1
CS72885	1.55	CP1002	10 802 010	CC289	20,100,377	1 286 272	90	96	20	10	202	7 0	5.1 6.1
CS72880	1.54	CC 280	21 020 122	CB265	21,009,102	1,200,272	122	1/2	27	38	202	21.4	21.2
CS72880	1.55	CC505	21,009,102	CR54	22,097,009	250 725	101	22	5	20	100	21.4	4.2
C372033	1.00	CDOCA	21,309,119	CC26	22,333,034	1 015 240	115	122	0	2	190	5.0	4.Z
C372000	1.50	CC620	22,037,003	CBOSO	23,112,417	1,013,340	117	112	0	/	232	5.9	5.0 6.0
CS72000	1.57	CC2EE	22,923,792	CR939	23,074,330	1 042 201	161	113	0	0	245	0.0 E 0	10.9
CS72900	1 50	CB030	23,434,133	CC17	24,337,020	1,042,091	101	144	11	11	102	3.U 11 E	4.0
CS72090	1.30	CDG12	23,074,330	CCE70	25,044,505	1,170,429	00 70	02 71	11	11	201	11.5	9.0 E 0
CS72901	1.70	CC47	24,771,403	CB776	25,070,292	1,100,029	150	1/2	4	12	221 221	6.5	5.9
C572691	1.59	CD776	25,044,905	CC1020	23,333,013	1 254 902	100	145	0	12	221	0.2	0.0
CS72692	1.01	CC/20	25,955,019	CD1050	27,300,421	1,334,602	106	109	11	0 F	254	0.1	6.U
CS72093	1.02	CG459 CC1020	20,100,900	CRISSO	27,404,040	1,505,140	95	104	10	2 10	214	7.0	5.4 0.4
CS72694	1.05	CD11EE	27,306,421	CR905	20,213,013	905,194	97 E7	90	4	1Z F	209	7.0	0.4
CS72895	1.04	CRIIDD	27,990,551	CG549	20,043,139	1 202,020)/ 1/0	1/1	10	20	201	2.5	0.1
CS72690	1.00	CCOFT	20,213,013	CD1112	29,390,303	1,362,930	140	141	11	20	5Z1 211	10.0	7.2
CS72697	1.00	CG03/	20,957,550	CC204	29,747,475	769,917	102	101	11 E	5	262	2.2	7.0
Cbr 2	1.07	GRIIIZ	29,747,475	66394	50,275,249	525,770	121	151	C	C	202	5.0	1.2
CG72002	2 1 2	CCE01	2 1 1 4	CP140	000 600	OEC ECC	100	OE	10	11	242	11 0	12.0
CS72902	2.15	CD1000	Z,114	CK140	050,000	600,000 F00.070	120 11E	00 110	10	11	242	11.9 C 4	10.9
CS72903	2.14	CR1026	444,033	CCE21	1 020 262	1 120 502	160	100	9	10	249	7.1	1Z.Z
CS72904	2.15	CCE21	1 020 767	CB1014	1,303,202	1,130,362	105	170	9	10	206	7.1 E /	0.5
CS72905	2.10	CCE21	1,969,202	CR1014	4,022,367	2,033,123	100	1/9	9	12	200	5.4 E 4	2./
CS72900	2.17	CG331	1,969,202	CRI04/	4,525,167	2,333,923	11/	105	2	10	252	5.4 7 1	2.1
CS72907	2.19	CRIU4/	4,525,187	CG559	0,051,980	1,526,799	115	110	0 17	10	253	12.0	4./
CS72908	2.20	CG559	6,051,986	CR896	7,229,424	1,1/7,438	106	113	1/	13	249	12.0	10.2
CS72909	2.21	CG1024	6,262,040	CR13	7,242,222	980,182	113	104	5	8	230	5.6	5./
CS/2910	2.22	CR13	7,242,222	CG635	9,379,120	2,136,898	138	1/0	11	11	330	6./	3.1
CS/2911	2.23	CR128	8,6/7,163	CG46	9,738,512	1,061,349	162	139	6	3	310	2.9	2./
CS/2912	2.24	CR114/	9,656,849	CG882	10,781,018	1,124,169	180	1//	10	14	381	6.2	5.5
CS/2913	2.25	CR441	9,976,629	CG515	11,/28,603	1,/51,9/4	110	136	13	4	263	6.5	3./
CS/2914	2.26	CR441	9,976,629	CG6	11,048,948	1,0/2,319	2/6	281	14	10	581	4.1	3.8
CS/2915	2.27	CR640	10,01/,/80	CG515	11,/28,603	1,/10,823	269	243	1/	6	535	4.2	2.5
CS72916	2.28	CR640	10,017,780	CG6	11,048,948	1,031,168	258	295	13	17	583	5.1	4.9
CS72917	2.29	CG601	10,291,848	CR1026	10,807,952	516,104	128	136	4	7	275	4.0	7.8
CS72918	2.30	CG771	11,608,725	CR929	12,506,436	897,711	152	145	9	10	316	6.0	6.7
CS72919	2.31	CG866	12,387,640	CR1124	13,622,737	1,235,097	83	95	3	1	182	2.2	1.8
CS72920	2.33	CR1124	13,622,737	CG405	14,782,362	1,159,625	157	125	5	9	296	4.7	4.1
CS72921	2.34	CR1381	14,566,497	CG637	15,436,302	869,805	103	93	6	10	212	7.6	8.7
CS72922	2.35	CG405	14,782,362	CR841	16,251,113	1,468,751	191	225	24	18	458	9.2	6.3
CS72923	2.36	CG637	15,436,302	CR1117	17,170,719	1,734,417	141	143	8	9	302	5.6	3.2
CS72925	2.38	CG951	16,663,782	CR1145	18,556,060	1,892,278	167	141	24	28	360	14.4	7.6
CS72924	2.37	CR1117	17,170,719	CG404	19.103.853	1.933.134	75	83	3	4	165	4.2	2.2

(continued)

ABBCID Line II marker marker position Lower marker position Lower position Lower marker position Lower position Lower position <thlower position Lower position</thlower 	Table 1. Co	'able 1. Continued												
br. br. <th>ABRC ID</th> <th>Line #</th> <th>Upper marker</th> <th>Upper marker position</th> <th>Lower marker</th> <th>Lower marker position</th> <th>Length of interval (nt)</th> <th></th> <th></th> <th>Progeny of test (Col × dsRed/</th> <th>cross GFP)</th> <th></th> <th>сM</th> <th>cM/mB</th>	ABRC ID	Line #	Upper marker	Upper marker position	Lower marker	Lower marker position	Length of interval (nt)			Progeny of test (Col × dsRed/	cross GFP)		сM	cM/mB
Date: Section Control Control <thcontrol< th=""> <thcontrol< th=""> <thcon< th=""><th></th><th></th><th></th><th>•</th><th></th><th>-</th><th></th><th>dsRed</th><th>GFP</th><th>dsRed + GFP</th><th>Nonfluor.</th><th>Total</th><th></th><th></th></thcon<></thcontrol<></thcontrol<>				•		-		dsRed	GFP	dsRed + GFP	Nonfluor.	Total		
CS72926 3.1 CC445 45,383 CR1110 727,37 652,714 71 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 74 76 76 74 76 74 76 74 76 74 76 74 76 74 76 76 75 75 76 76 77 76 76 76 77 76 <	Chr. 3													
Lar/247 3.18 CH426 5.4.382 CH2/16 C/16 C/16 <thc 16<="" th=""> C/16 C/16</thc>	CS72926	3.17	CG465	45,383	CR1110	707,793	662,410	152	145	14	11	322	7.8	11.8
Lab / Lab Lab <thlab< th=""> Lab <thlab< th=""> <thlab<< td=""><td>CS/292/</td><td>3.18</td><td>CG465</td><td>45,383</td><td>CR921</td><td>6/2,39/</td><td>627,014</td><td>/1</td><td>66</td><td>3</td><td>/</td><td>14/</td><td>6.8</td><td>10.8</td></thlab<<></thlab<></thlab<>	CS/292/	3.18	CG465	45,383	CR921	6/2,39/	627,014	/1	66	3	/	14/	6.8	10.8
CST2980 2.2.2 CR24 1.2.2 CR24 1.2.2 2.2.4 3.8 1.0.2 CST2981 3.2.3 CR82 1.1.7.1464 CGS0 2.5.3.7.44 1.0.3.7778 3.9 100 5 18 2.2.8 8.1 6.0 CST2983 3.3.3 CR527 1.1.7.1464 CGS0 2.5.3.7.44 1.0.3.7778 3.9 15 18 2.2.8 1.4.0 CST2984 3.3.4 CG421 3.2.9.4.66 CR71 4.3.3.3.47 1.9.4.82 0.9 16 5 18 4.2.2 3.1 1.4.0 3.6 3.4 4.3.3.3.47 1.9.4.82 18 3 11 4.40 4.0 4.4 1.5. 5 1.4.4 4.0.7 4.4.3 5 5 3 3.3.3 2.9 3.3.2 2.9 8.4 6.7 7.5 2 6 1.5. 3.3.2 3.3.2 2.9 3.4 6.7 7.5 2 6.7 3.3.2 2.9 3.4 6	CS72928	3.19	CC211	90,264	CG834 CD021	/38,368	648,104 E41.0E9	98	93 11E	2	3 12	196	2.6	4.0
CSC2981 2.42 CBSC CSC2982 2.22 CBSC CSC2982 2.22 CBSC CSC2982 2.22 CBSC CSC2983 2.44 CSC2984 2.35 CSC2984 2.35 CSC2983 2.34 CGC4944 1.984,068 CSC2983 3.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2983 3.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2984 2.35 CGSC2985 2.36 CGSC2985 2.36 CGSC2985 2.36 CGSC2984 2.30 CCSC2946 2.33 CGSC2984 2.30 CCSC2984 2.32 CGSC2985 2.32 CGSC2985 2.32 CGSC2985 2.32 CGSC2985 2.32 CGSC2984 2.30 CCSC2944 2.33 CCSC2947	CS72929	3.20 2.21	CB021	130,439	CR921	1 502 406	541,958	129	115	12	13	269	9.3	17.2
CST2791 3.3 CSG7 1.46578 CSG 2.57243 3.40 1.031914 85 60 10 4 188 7.4 7.7 CS7293 3.3 CG44 1.593466 CR51234 3.025.68 1.402.2283 89 95 9 9 9 2.8 2.2 3.1 4.0 CS72944 3.25 CG51 1.59346 CG71 1.85 1.88 3 1.4 4.00 4.2 4.4 CS72945 3.27 CG719 3.385,285 CR14 5.403,042 1.074116 125 6 1.00 4.2 4.4 3.5 CS72934 3.33 CG521 S.0407,686 1.273461 125 1.06 7 2.6 1.03 3.23 3.23 3.23 3.23 3.24 2.6 2.13 3.52 1.08 1.072496 1.13444 1.08 1.8 1.53 1.11 2.13 3.23 3.23 3.23 3.24 2.6 2.27	CS72930	3.21 3.21	CR921	072,597	CG446 CC50	1,595,400	921,009	111	109	5	13	244	9.0 Q 1	10.6
cszpzes s.a. s.a. s.a. cszpzes s.a.	CS72931	3.22	CR627	1 496 828	CG50	2,528,742	1,031,278	85	207	10	13	188	7.4	7.2
CSX2924 2.5. CCAD 2.5.28./A2 CR2235 3.30 CR2355 5. 8 2.22 3.1 4.0 CS72956 3.27 CG319 3.36.286 CR3 4.33.342 965.057 185 198 3 14 400 4.2 4.4 CS72957 3.28 CR73 4.33.342 CC44 5.60.37.68 1.273.426 116 122 5 6 140 4.4 3.5 CS72987 3.30 CR124 5.60.37.68 CR1416 6.741.102 151 165 7 3 2.29 6.4 4.4 3.5 CS72987 3.30 CR24 5.60.37.68 CR1474 7.45.89 118 133 11 119 7.5 2 6 10.3 8.29 10.7 8.22 10.8 7.7 14.4 10.7 11.2 11.3 10.7 11.3 11.3 11 11.3 12.3 10.7 11.3 11.3 11 11.2	CS72933	3.25	CG446	1 593 406	CR631	3 025 689	1 432 283	89	95	9	9	202	89	6.2
CSX2929 3.26 CC212 3.280.40 CV2 4.393.342 1.04982 2.09 196 5 10 4.20 3.6 3.4 CSX2936 3.27 CG319 3.344 CG310 5.440.370 1.944.180 11 6 187 9.1 4.7 CSX2936 3.28 CR3 4.30.342 CC24 5.60.764 116 122 5 6 140 0.0 4.2 4.4 CSY2948 3.29 CC41170 5.31.548 CC42 8.07.244 135 139 165 6 3 2.99 3.3 2.29 3.3 CC42 3.5 139 161 1 149 2.99 3.4 4.6 7.7 16.4 16.7 133 2.25 10.8 139 161 11 2.29 3.3 2.29 3.3 2.29 3.3 2.20 2.21 1.3 14 11 2.23 2.14 3.4 10 2.21 2.23	CS72934	3.25	CG50	2.528.742	CR352	3.300.254	771.512	208	201	5	8	422	3.1	4.0
CSX2936 3.27 CC3919 3.365,285 CHX2 4.330,342 965,057 185 188 3 14 400 4.2 4.4 CS72321 3.28 CRX3 4.330,342 CC24 5.560,768 1.273,426 116 122 5 6 249 4.4 3.5 CS72938 3.30 CC24 5.503,768 CR1405 5.61,383 1.15,6126 106 5 3.29 3.3 229 8.4 6.7 CS72940 3.31 CR1045 8.76,128 N.12,794 8.11,819 91 105 7 4 213 3.7 4.5 CS72941 3.35 CR1276 8.74,224 7.55,431 1.020 1.38 13 11 229 3.4 6.7 7.1 1.04 10 1.04 1.02 1.04 10 1.04 1.0 1.04 1.0 1.04 1.0 1.04 1.0 1.04 1.0 1.04 1.0 1.04 1.0 <td>CS72935</td> <td>3.26</td> <td>CG21</td> <td>3,280,460</td> <td>CR73</td> <td>4,330,342</td> <td>1,049,882</td> <td>209</td> <td>196</td> <td>5</td> <td>10</td> <td>420</td> <td>3.6</td> <td>3.4</td>	CS72935	3.26	CG21	3,280,460	CR73	4,330,342	1,049,882	209	196	5	10	420	3.6	3.4
CS72913 3.51 CR1470 3,493,140 CC310 5,403,760 1,273,426 16 122 5 6 129 4.4 3.55 CS72938 3.29 CR1120 5,531,946 CG291 6,264,102 912,156 67 75 2 6 150 5.3 2.39 3.3 2.9 CS72940 3.31 CR1045 6,761,383 CC448 8,017,809 1.256,476 139 135 7 18 229 8.4 6.7 CS72941 3.32 CC244 2,072,494 2.02,647 8,949,279 714,643 123 11 11 293 75 1.04 CS72943 3.34 CC654 8,296,846 CR1076 9,11,400 1.118,041 161 131 12 11 120 11 120 136 12 144 648 741 CS72944 3.45 CC200 7,473,40 CC331 9,41,400 1,41,400 1,41,400 1,41,400	CS72936	3.27	CG919	3,365,285	CR73	4,330,342	965,057	185	198	3	14	400	4.2	4.4
CS72937 3.28 CR73 4.330.342 CC24 5.503.768 1.212 5 6 249 4.4 3.5 CS72938 3.30 CC24 5.503.768 CR104 5.671.83 1.157.615 125 106 5 3 239 3.3 299 8.4 6.7 CS72940 3.31 CR104 5.671.33 CC44 8.017.809 125.6426 139 135 7 18 239 8.4 6.7 CS72940 3.33 CR104 8.474.84 125.6426 138 13 11 237 7.5 10.4 CS72944 3.33 CR1076 8.474.84 10.049 138 133 11 237 5.4 10.4 129 10.4 129 13.6 10.4 129 13.6 10.4 120 13.6 10.4 120 120 124 14 4.4 3.5 10.4 120 120 124 4.4 3.5 11.5	CS73211	3.51	CR1420	3,493,180	CG310	5,440,370	1,947,190	86	84	11	6	187	9.1	4.7
CS27938 3.29 CR1120 5,581,946 CC991 6,264,102 912,156 67 75 2 6 150 5.3 5.8 329 33.2 2 CS27944 3.31 CR1045 6,661,383 CC472 807,2294 815,819 9 106 2 6 213 3.7 4.5 CS72944 3.32 CR272 807,2294 C1056 8368,977 914,664 129 131 11 123 7.5 1.0 CS72944 3.33 CR1076 874,227 CR2737 914,668 154 129 134 12 138 9 10 286 6.6 7.2 23 352 1.0.8 97 CS72945 3.35 CR1076 941,450 CR1076 941,450 1.1.132,039 1.00,714 1.24 1.3 1.4 12 213 1.4 6.8 7.1 233 27 5.4 5.6 252 4.3 2.0 1.0 2.4 4.6 5.8 7.4 9 5.5 6.222 4.3 2.0	CS72937	3.28	CR73	4,330,342	CG24	5,603,768	1,273,426	116	122	5	6	249	4.4	3.5
CST2939 3.30 CC244 5.603,768 CR1045 6.761,383 1.157,615 125 106 5 3 239 3.3 2.9 CST2944 3.32 CC531 7.256,475 CR729 8.072,294 815,819 99 106 2 6 213 3.7 4.5 CST2944 3.33 CR293 8.072,294 CG1076 8.042,424 725,434 113 14 129 3.6 6.6 7.2 CST2943 3.35 CR1267 8.743,244 725,433 3.0 1.31 14 12 311 8.4 6.9 CST2943 3.55 CR1267 8.744,242 CG331 9.946,151 1.202,909 1.54 1.31 1.4 12 3.1 8.4 6.7 CST2944 3.44 CC331 1.946,017 1.146,203 9.00 1.16 1.5 1.4 4.8 2.5 4.5 CST2944 3.40 CC331 1.946,030 CC447	CS72938	3.29	CR1120	5,351,946	CG991	6,264,102	912,156	67	75	2	6	150	5.3	5.8
CST2940 3.31 CR1045 6,761,383 CC43 8,017,809 1,256,475 CR29 8,072,294 215,817 11 129 8,4 6,7 CST2941 3.52 CC431 8,071,809 CR1267 8,072,294 C1056 8,086,977 914,668 129 138 9 10 286 6,6 7.2 CST2944 3.35 CR1076 9,417,490 1,118,644 146 168 15 2.3 352 10.4 4.6 7.2 CST2944 3.35 CR1076 9,417,490 1,118,644 140 114 12 311 8.4 6.7 CST2944 3.35 CR1076 9,417,490 C10318,203 900,713 127 130 4 10 211 4 4.6 5.8 7.1 4.33 4.5 1.7 1.2 14 4.6 5.8 7.1 10 1.2 1.4 4.5 1.0 1.0 1.0 1.1 1.2 1.4	CS72939	3.30	CG24	5,603,768	CR1045	6,761,383	1,157,615	125	106	5	3	239	3.3	2.9
CS:7941 3.32 CCS371 2/254/3* CR/29 8/0/2244 815,819 99 106 2 6 213 3.7 4.5 CS73212 3.33 CR279 8/07,294 CG1006 8/86,977 914,683 129 138 9 10 286 6.6 7.2 10.4 CS72943 3.35 CR1207 8/14,207 1.118,614 120 311 14 12 311 8.4 6.8 CS72943 3.55 CR1207 8/14,207 5/14,614 106 118 37 2.5 8 6 6.36 7.2 4.4 4.5 7 10 4 10 211 4.4 4.6 7 7 6 2.2 4.4 4.3 7.0 9 5 3.4 1.0 231 4.4 10 324 4.7 7 6 2.2 4.4 4.3 2.0 2.2 5 7 4 10 3.4 7	CS72940	3.31	CR1045	6,761,383	CG43	8,017,809	1,256,426	139	135	7	18	299	8.4	6.7
LS:3.212 3.5.2 C(A3 8.017,203 CR12b 8./43,442 725,433 118 153 19 102 265 6.66 7.2 CST2944 3.34 C(GS4 8.298,446 CR1076 9.417,490 1.118,644 146 168 15 23 352 10.3 9.7 CST2944 3.35 CR1076 9.414,490 CC396 10.318,203 900,713 127 130 4 10 221 5.2 5.8 CST2944 3.35 CR1076 9.414,400 CS906 10.318,203 RR121 1.1120,015 813,812 210 210 14 446 5.8 7.1 5.6 5.5 1.580,464 2.107,914 116 1.46 5.6 6.75 4.9 5.5 1.580,468 2.710,144 176 5 6.5 6.25 4.9 5.5 1.580,468 2.710,144 178 15 5.3 6.4 4.0 4.2 6.2 5.2 6.2 6.2	CS/2941	3.32	CG521	/,256,4/5	CR/29	8,0/2,294	815,819	99	106	2	6	213	3./	4.5
LS:2942 3.33 CR/29 8.296.397 914,663 129 138 9 10 286 6.6 7.2 CS72943 3.53 CR1267 8.296,846 CR076 9.11,740 1.118,644 146 168 15 23 552 10.8 9.7 CS72314 3.54 CR1267 8.296,840 C10215 811,812 210 12 14 446 6.58 7.1 CS72344 3.54 CG931 9.946,151 CR10215 811,812 210 12 14 446 5.8 7.1 CS72944 3.38 CR830 CR3211 3.805,656 CR512 1.809,565 1.66,202 1.99,393 157 174 9 5 345 4.0 4.2 CS72949 3.41 CR320 1.74,873 9.943,930 1.033,447 155 177 15 5 352 5.7 5.4 CS72951 3.43 CR847 18,415,973 9.92,930	CS/3212	3.52	CG43	8,017,809	CR126/	8,743,242	/25,433	118	153	11	11	293	/.5	10.4
LS:J.293 3.34 C.C.B34 8.J.39.40 C.C.B71 3.9.41 J.18.044 146 103 2.3 3.32 10.8 9.7 CS72131 S.S. CR1207 9.441.440 CG391 3.946.151 LC20.99 154 131 14 10 2.71 5.2 5.8 CS72244 S.S. CR2321 S.S. CR1201 13.09.056 1.07.057 1.01.44 16 14 10 2.11 4.4 5.8 CS72244 S.S. CR800 1.2,643.03 CG312 13.809.566 1.0,62.02 4.94 4.5 1.0 4.5 1.7 CS7244 S.S. CG323 1.2,04.94 CR1404 1.8,04.84 2.17.01.91 1.45 6 10 3.34 4.0 2.35 4.5 4.0 4.20 2.20.31.41 1.74 5 5 2.56 4.5 4.50 2.57.25 5.1 1.50 10 3.34 4.7 7 5.6 5.5 2.50	CS72942	3.33	CR/29	8,072,294	CG1026	8,986,977	914,683	129	138	9	10	286	6.6	7.2
CS72241 3.33 CR1267 6.9417.430 C0296 10.318.231 1.42 1.44 1.03 1.04 1.04 1.05 1.04 1.04 1.05 1.04 1.04 1.04 1.05 1.04 1.04 1.05 1.04 1.04 1.05 1.04 <	CS72943	3.34	CB1267	8,298,840	CC021	9,417,490	1,118,644	140	108	15	23	35Z	10.8	9.7
CST2214 3.54 CGS00 9.14,153 CCN004 10.20,075 10.04,644 106 114 3 7 2.06 4.4 4.3 CS72245 3.36 CG906 10.318,033 CH1121 11.132,015 813,812 210 210 12 14 446 5.8 7.1 CS72244 3.38 CGR800 12,403,63 CO912 13,809,565 1,75,750 140 156 6 2.2 4.3 2.0 CS72447 3.39 CG312 13,809,565 1,70,1918 115 126 6 2.2 4.3 2.0 CS72448 3.41 CG329 17,20,400 CR437 18,145,873 945,334 157 174 9 5 3.45 CG803 18,71094 1.29,8144 178 15 5 3.22 5.7 5.4 CS72955 3.44 CR895 18,71094 1.050,755 1.328 177 15 5 3.22 5.7 5.4	CS73213	2.25	CR120/	0,745,242	CC906	9,940,151	1,202,909	104	130	14	12	271	0.4 5 0	0.9 5 8
CS72946 3.36 CC2906 10318.203 CR1121 11,132,015 C912 13,809,565 2,677,550 140 156 4 10 310 316 5,7 4,5 CS72346 3.38 CR880 CG312 13,809,565 1,66,202 419 451 1 4,85 5,7 4,9 CS72344 3.39 CG312 13,809,565 15,980,483 2,170,918 115 126 5 6 252 4,3 2,0 CS72344 3.40 CG320 17,402,480 CG325 19,9320 1,118,14 44 6 6 7 13 6.1 5.5 CS72954 3,44 CR837 14,51,15 5 322 5.7 5.4 CS72954 3,44 CR837 14,15,87 2,003,150 1,053,447 155 10 7 226 6.3 6.0 CS72954 3,46 CG332 2,104,086 CR845 2,219,925 1,170,839 163 181 14 44 372 7.5 6.4 CS72955 3,48 <	CS73214	3.55	CC931	9 946 151	CR1004	10,318,203	1 014 644	106	114	3	7	230	4.4	J.8 4 3
CS72215 3.55 CR1121 11.132.015 CC12 13.809.565 1.66.20 140 156 4 10 310 4.5 17 CS72947 3.39 CCG912 13.809.565 1.66.20 19 451 1 4 875 5.7 4.9 CS72944 3.40 CG252 17.452.950 CR895 18.751.094 1.280.440 178 14.5 6 10 310 4.7 3.6 CS72949 3.41 CG520 17.452.950 CR895 18.751.094 1.280.414 178 14.5 6 10 310 4.7 3.6 CS72950 3.42 CR1041 18.408.060 CG252 19.99.20 10.51.256 13.3 9 9 266 6.3 6.0 CS72953 3.45 CR031 18.498.969 CG610 12.471.664 1.572.965 110 125 10 7 25 6.4 4.7 CS72955 3.46 CG312 <	CS72945	3 36	CG906	10 318 203	CR1121	11 132 015	813 812	210	210	12	14	446	5.8	7 1
CS72946 338 C(R880 12/64/3 (s5 CC912 13/809/565 1.166/202 419 451 1 -4 875 5.7 49 CS72947 3.30 CG312 13/809/565 CR55 15/80/483 21/0/918 115 126 5 6 252 4.3 2.0 CS72948 3.40 CG329 17/200/480 CR1437 18,145/87 3.166/202 11/18/214 44 106 6 7 213 6.1 55 CS72951 3.43 CR1437 18,145/873 CG520 19,199/202 1,15/1,126 133 9 9 286 6.3 6.0 CS72954 3.44 CR895 12,471,664 12,72/2955 110 125 10 7 25 6.7 4.3 CS72954 3.46 CG913 22,485,149 CR339 22,490,705 50,3,449 92 99 11 3 205 6.8 13.5 CS72957 4.8	CS73215	3.55	CR1121	11.132.015	CG912	13.809.565	2.677.550	140	156	4	10	310	4.5	1.7
CS72947 3.39 CC312 13:goo_fes CR437 8:145.87.3 9:5.93 157 174 9 5 345 4.0 4.2 CS72948 3.41 CGS20 17,452,950 CR895 18,751,094 1.298,144 178 145 6 10 339 4.7 3.6 CS72949 3.41 CR1041 18,006,06 CG225 19,199,320 1.015,144 14 10.6 6 7 21.3 6.1 5.5 CS72951 3.44 CR487 18,145,873 91.93,20 1.051,256 13.3 9 9 266 6.3 6.0 CS72953 3.45 CR030 19,802,350 CR1015 2.11,01,575 1.328,225 84 83 4 7 178 6.2 4.7 CS72954 3.46 CR134 2.406,157 934,493 112 127 9 10 258 7.5 6.4 CS72957 3.49 CG691 2.406,157 934,493 112 127 9 10 258 7.5 6.4	CS72946	3.38	CR880	12.643.363	CG912	13.809.565	1.166.202	419	451	1	4	875	5.7	4.9
CS72948 3.40 CG329 17.200.480 CR1437 18.145.873 945.393 157 174 9 5 345 4.0 4.2 CS72949 3.41 CGS20 17.45.295 CR895 R3751.094 L289.144 178 145 6 10 339 4.7 3.6 CS72950 3.42 CR1041 18.080.606 CG292 19.199.320 1.053.447 155 173 15 5 352 5.7 5.4 CS72953 3.45 CR800 19.898.690 CG691 2.1471.664 1.572.965 10 125 10 7 725 6.7 4.3 CS72954 3.46 CG932 21.471.664 CR1154 2.2406.157 C937.49 9 11 3 205 6.8 1.35 CS72957 3.49 CG913 2.2853.491 CR33 376.780 291.219 171 145 10 15 341 7.3 25.1 CS72957 3.48 CR133 376.780 291.219 171 115 10 13	CS72947	3.39	CG912	13,809,565	CR55	15,980,483	2,170,918	115	126	5	6	252	4.3	2.0
CS72949 3.44 CGS20 17.452.950 CR895 18.751.094 1.298.144 178 145 6 10 339 4.7 3.6 CS72950 3.44 CR1437 18.145.873 CG925 19.199.320 1.053.447 155 173 9 9 266 6.3 6.0 CS72958 3.44 CR893 19.802.350 CR1015 2.1130.575 1.328.225 84 83 4 7 17.8 6.2 4.7 CS72958 3.45 CR903 19.888.699 CR6601 2.219.925 1.10 125 10 7 252 6.7 4.3 CS72955 3.44 CG893 2.149.106 CR815 2.290.97.05 50.35.49 2.99 91 13 205 6.8 13.5 CS72959 4.8 CR943 85.561 CG873 376.780 291.219 171 146 18 23 351 2.2 10.1 CS72964 4.10 CG373 376.780 291.219 171 145 10 15 341 7.3	CS72948	3.40	CG329	17,200,480	CR1437	18,145,873	945,393	157	174	9	5	345	4.0	4.2
CS72950 3.42 CR1041 18.080,606 CG925 19.199.320 1,118,714 94 106 6 7 213 6.1 5.5 CS72951 3.43 CR1471 18.145,873 CG925 19.99.320 1,053,447 155 177 15 5 552 5.7 5.4 CS72952 3.44 CR803 19.898,699 CG650 19.802,350 1,522,55 110 125 10 7 252 6.7 4.3 CS72954 3.45 CR903 19.898,699 CG611 21,471,664 1572,955 110 125 10 7 252 6.7 4.7 7.9 CS72957 3.48 CR1154 22,406,157 CG927 22,907,079 503,549 92 9 11 3 205 6.8 13.5 CS72957 3.48 CR943 85,561 CG873 376,780 CP11,21 143 146 2 42 25.1 10.1 503,814 31.22 10.1 15.7 4.1 7.3 25.1 CS72959 4.8 <	CS72949	3.41	CG520	17,452,950	CR895	18,751,094	1,298,144	178	145	6	10	339	4.7	3.6
CS72951 3.44 CR1437 18,145,873 CC925 19,199,320 1,051,265 135 133 9 9 286 6.3 6.0 CS72958 3.50 CC650 19,802,350 CR1015 21,130,575 1,328,225 84 83 4 7 178 6.2 4.7 CS72953 3.45 CR903 19,898,699 CG691 21,471,664 1,572,965 110 125 10 7 252 6.7 4.3 CS72955 3.47 CG691 21,471,664 CR154 22,405,157 934,493 112 127 9 10 258 7.4 7.9 CS72955 3.48 CR143 22,853,491 CR389 22,974,799 121,308 143 146 2 4 295 2.0 165 Chr.4 CG913 328,551 CG873 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72959 4.8 CR943 85,561 CG873 376,780 2,814,455 1,324,210 14 <td>CS72950</td> <td>3.42</td> <td>CR1041</td> <td>18,080,606</td> <td>CG925</td> <td>19,199,320</td> <td>1,118,714</td> <td>94</td> <td>106</td> <td>6</td> <td>7</td> <td>213</td> <td>6.1</td> <td>5.5</td>	CS72950	3.42	CR1041	18,080,606	CG925	19,199,320	1,118,714	94	106	6	7	213	6.1	5.5
CS72952 3.44 CR895 18/51,094 CC6650 19,802,350 1,051,256 133 9 9 286 6.3 6.0 C572958 CS72953 3.45 CR903 19,898,699 CG691 21,471,664 1,572,965 110 125 10 7 252 6.7 4.3 CS72954 3.46 CG921 21,471,664 CR1154 22,406,157 934,493 112 127 9 10 258 7.4 7.5 CS72955 3.47 CG691 21,471,664 CR1154 22,974,799 121,30 143 146 2 4.295 2.0 16.5 CS72957 3.48 CR143 85,561 CG873 376,780 C1130 1.586,387 1,209,607 148 18 23 335 12.2 10.1 1.5 41 7.3 25.1 CS72959 4.8 CR43 85,561 CG873 376,780 CR1130 1.586,487 1.209,41 12 11 40 4.3 25.1 12.2 10.1 150 43.6 65.3<	CS72951	3.43	CR1437	18,145,873	CG925	19,199,320	1,053,447	155	177	15	5	352	5.7	5.4
CS72958 3.50 CG650 19,802,350 CR1015 21,471,664 1.572,965 110 125 10 7 252 6.7 4.3 CS72953 3.45 CR9032 21,049,086 CR865 22,219,925 1.170,839 163 181 14 14 372 7.5 6.4 CS72956 3.48 CR1154 22,406,157 CG292 22,909,706 503,549 92 99 11 3 205 6.8 15.5 CS72956 3.48 CR154 22,407,799 121,308 143 146 2 4 295 2.0 16.5 CS72956 4.8 CR943 85,561 CG873 376,780 291,219 144 145 10 15 341 7.3 25.1 1.0 CS72962 4.11 CC509 1,138,245 CR1628 2,381,455 1,242,210 174 151 13 10 348 6.6 5.3 CS72976 4.12 CA1628 2,381,455 CG870 5,120,474 55 1120,474 15	CS72952	3.44	CR895	18,751,094	CG650	19,802,350	1,051,256	135	133	9	9	286	6.3	6.0
CS72953 3.45 CR903 19,898,699 CC691 21,471,664 1,572,955 110 / 252 1,67 6.4 CS72955 3.47 CG691 21,471,664 CR1154 22,406,157 934,493 112 127 9 10 258 7.4 7.9 CS72955 3.48 CR1154 22,406,157 CG272 22,909,706 503,549 92 99 11 3 205 6.8 13.5 CS72957 3.49 CC813 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72950 4.8 CR943 85,561 CG873 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72950 4.9 CG507 1,138,245 CR1130 1,586,387 1,209,607 146 148 183 333 12.2 10.1 CS72954 4.10 CG490 998,987 CR1628 2,381,455 1,232,359 85 63 11 11 170 12.9	CS72958	3.50	CG650	19,802,350	CR1015	21,130,575	1,328,225	84	83	4	7	178	6.2	4.7
CS72954 3.46 CG932 21,049,066 CK805 22,219,925 1,170,839 103 181 14 15 14 15 13 10 15 13 10 14 15 13 11 11 16 16 16 14 14 14 14 14 14 14 14 <	CS/2953	3.45	CR903	19,898,699	CG691	21,4/1,664	1,5/2,965	110	125	10	/	252	6./	4.3
CS72955 3.44 CG691 7.1471,664 CK1154 22,406,157 934,493 112 127 9 10 228 7.4 7.9 CS72955 3.49 CG913 22,853,491 CR389 22,974,799 121,308 143 146 2 4 295 2.0 16.5 Chr. 4 CS72956 4.8 CR943 85,561 CG873 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72956 4.8 CR943 85,561 CG873 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72956 4.10 CG490 98,987 CR1628 2,381,455 1,243,210 174 151 13 10 348 6.6 5.3 CS72963 4.12 CR1628 2,381,455 CG300 5,452,727 3071,272 126 129 15 10 280 8.9 2.9 CS72976 4.13 CG305 5,503,814 3,861 6,573,205 1,204,78	CS/2954	3.46	CG932	21,049,086	CR865	22,219,925	1,170,839	163	181	14	14	3/2	7.5	6.4
CS72950 3.49 CCR1134 22,483,491 CCR389 22,974,709 121,308 143 146 2 4 295 2.0 16.5 Chr. 4 CS72957 3.49 CCR373 376,780 CCR173 1,586,387 1,209,607 146 148 18 23 335 12.2 10.1 CS729561 4.10 CG490 998,987 CR1628 2,381,455 1,382,468 177 201 12 11 401 5.7 4.1 CS729563 4.12 CR1628 2,381,455 L230,50 1,22,359 85 63 11 11 170 12.9 4.1 CS729763 4.32 CR1628 2,381,455 CC350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72978 4.32 CR1628 2,381,455 CC350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS729764 4.13 CG350 5,503,814 GR46 6,573,205 1,120,478	CS72955	3.4/ 2.40	CD11EA	21,4/1,664	CC027	22,406,157	934,493	112	127	9	2	258	/.4 6 0	1.9 12 E
$ \begin{array}{c} \text{Ch}, 4 \\ \text{CS72959} & 4.8 \\ \text{CR943} & 85,561 \\ \text{CG873} & 376,780 \\ \text{CR1130} \\ \text{CR56387} & 376,780 \\ \text{CR1130} \\ \text{CR1130} \\ \text{CR56387} \\ \text{CR52961} \\ \text{4.10} \\ \text{CG490} \\ \text{CG400} \\ \text{CG490} \\ \text{CG490} \\ \text{CG400} \\ \text{CG490} \\ \text{CG400} \\ $	CS72950	2.40 2.40	CC012	22,400,137	CB380	22,909,700	505,549 121 208	92 143	99 146	11	3	205	2.0	15.5
CS72959 4.8 CR943 85,561 CG873 376,780 291,219 171 145 10 15 341 7.3 25.1 CS72960 4.9 CC873 376,780 CR1130 1,586,387 1,209,607 146 148 18 23 335 12.2 10.1 CS72961 4.10 CG490 998,987 CR1628 2,381,455 1,382,468 177 201 12 11 401 5.7 4.1 CS72963 4.12 CR1628 2,381,455 CG350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72974 4.32 CR1628 2,381,455 CG350 5,505 1,20,478 95 124 10 4 233 6.0 5.4 CS72964 4.13 CG350 5,505 126,317 156 167 15 20 367 9.5 5.1 CS729264 4.14 CR142	Chr 4	J. T J	00713	22,055,451	GIGOD	22,374,733	121,500	145	140	2	-1	200	2.0	10.5
CS72960 4.9 CG873 376,780 CR1130 1,586,387 1,209,607 146 148 18 23 335 12.2 10.1 CS72961 4.10 CG490 998,987 CR1628 2,381,455 1,382,445 1,223,210 174 151 13 10 348 6.6 5.3 CS72963 4.12 CR1628 2,381,455 CG350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72978 4.32 CR1628 2,381,455 CG310 5,452,727 3,071,272 126 129 15 10 280 8.9 2.9 CS72964 4.13 CG350 5,503,814 CR861 6,573,205 1,120,478 95 124 10 4 233 6.0 5.4 CS72965 4.14 CR1422 6,395,655 CR1305 7,544,169 585,137 165 167 15 20 367 9.5 5.1 CS73218 4.36 CG8 6,955,655 CR1305 7,544,169 <t< td=""><td>CS72959</td><td>48</td><td>CR943</td><td>85 561</td><td>CG873</td><td>376 780</td><td>291 219</td><td>171</td><td>145</td><td>10</td><td>15</td><td>341</td><td>73</td><td>25.1</td></t<>	CS72959	48	CR943	85 561	CG873	376 780	291 219	171	145	10	15	341	73	25.1
CS72961 4.10 CG490 998,987 CR1628 2,381,455 1,382,468 177 201 12 11 401 5.7 4.1 CS72962 4.11 CG509 1,138,245 CR1628 2,381,455 CG350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72978 4.32 CR1628 2,381,455 CG310 5,452,727 3,071,272 126 129 15 10 280 8.9 2.9 CS72979 4.33 CG910 5,452,727 3,071,272 126 129 15 10 280 8.9 2.9 CS72964 4.13 CG350 5,503,814 CR861 6,573,205 1,102,478 95 124 10 4 233 6.0 5.5 CS72965 4.14 CR142 6,030,331 CG8 6,955,655 CR1600 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS72966 4.16 CR1660 7,522,130 CG916 8,750	CS72960	4.9	CG873	376,780	CR1130	1,586,387	1,209,607	146	148	18	23	335	12.2	10.1
CS72962 4.11 CG509 1,138,245 CR1628 2,381,455 1,243,210 174 151 13 10 348 6.6 5.3 CS72963 4.12 CR1628 2,381,455 CG350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72978 4.32 CR1628 2,381,455 CG910 5,452,727 CR861 6,573,205 1,120,478 95 124 10 4 233 6.0 5.4 CS72964 4.13 CG30,331 CG8 6,955,655 925,324 110 123 7 8 248 6.0 6.5 CS7216 4.34 CR1422 6,895,655 CR1606 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS72964 4.16 CR1606 7,522,130 C66,475 118 130 3 6 257 3.5 6.2 CS72966 4.16 CR1606 7,522,130 C66,475 118 130 3 4 170<	CS72961	4.10	CG490	998,987	CR1628	2,381,455	1,382,468	177	201	12	11	401	5.7	4.1
CS72963 4.12 CR1628 2,381,455 CG350 5,503,814 3,122,359 85 63 11 11 170 12.9 4.1 CS72978 4.32 CR1628 2,381,455 CG910 5,452,727 3,071,727 126 129 15 10 280 8.9 2.9 CS72979 4.33 CG910 5,452,727 CR861 6,573,205 1,100,478 95 124 10 4 233 6.0 5.4 CS72965 4.14 CR142 6,030,331 CG8 6,955,655 925,324 110 123 7 8 248 6.0 6.5 CS73216 4.34 CR1422 6,895,645 CR1600 7,524,130 566,475 118 130 3 6 257 3.5 6.2 CS73217 4.35 CG8 6,955,655 CR1600 7,524,130 566,475 118 130 3 6 257 3.5 6.2 2572,956 4.17 4.1 3.3 CS72966 4.16 CR1606 7,524,169 <	CS72962	4.11	CG509	1,138,245	CR1628	2,381,455	1,243,210	174	151	13	10	348	6.6	5.3
CS72978 4.32 CR1628 2,381,455 CG910 5,452,727 3,071,272 126 129 15 10 280 8.9 2.9 CS72979 4.33 CG910 5,452,727 CR861 6,573,205 1,104,789 95 124 10 4 233 6.0 5.4 CS72964 4.13 CG350 5,503,814 CR861 6,573,205 1,069,391 138 128 12 11 289 8.0 7.5 CS72965 4.14 CR1422 6,030,331 CG8 6,955,655 925,324 110 123 7 8 248 6.0 6.5 CS73216 4.34 CR1422 6,855,655 CR1600 7,524,169 588,514 95 104 9 5 213 6.6 11.2 CS72966 4.16 CR1600 7,524,169 CG513 12,137,086 4,592,917 229 256 51 61 597 18.8 4.1 CS72968 4.19 CG916 8,750,678 CR31 1,2137,086 4,592,077	CS72963	4.12	CR1628	2,381,455	CG350	5,503,814	3,122,359	85	63	11	11	170	12.9	4.1
CS72979 4.33 CG910 5,452,727 CR861 6,573,205 1,120,478 95 124 10 4 233 6.0 5.4 CS72964 4.13 CG350 5,503,814 CR861 6,573,205 1,069,391 138 128 12 11 289 8.0 7.5 CS72965 4.14 CR1422 6,895,545 CR160 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS73216 4.36 CG8 6,955,655 CR1600 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS73266 4.16 CR1600 7,524,169 588,514 95 104 9 5 13 6.6 15.2 CS72966 4.17 CR1305 7,544,169 CG513 12,28,548 78 85 3 4 170 4.1 3.3 CS72966 4.10 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 9 256 5.5	CS72978	4.32	CR1628	2,381,455	CG910	5,452,727	3,071,272	126	129	15	10	280	8.9	2.9
CS72964 4.13 CG350 5,503,814 CR861 6,573,205 1,069,391 138 128 12 11 289 8.0 7.5 CS72965 4.14 CR142 6,030,331 CG8 6,955,655 165 167 15 20 367 9.5 5.1 CS73217 4.35 CG8 6,955,655 CR1660 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS73218 4.36 CG8 6,955,655 CR1600 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS72966 4.16 CR1600 7,522,130 CG916 8,750,678 1,228,548 78 85 3 4 170 4.1 3.3 CS72968 4.19 CG916 8,750,678 CR3 1,440,981 2,690,303 84 69 10 6 169 9.5 5.5 4.9 CS72969 4.20 CG916 8,750,678 CR3 1,440,981 2,690,303 84 69 <td>CS72979</td> <td>4.33</td> <td>CG910</td> <td>5,452,727</td> <td>CR861</td> <td>6,573,205</td> <td>1,120,478</td> <td>95</td> <td>124</td> <td>10</td> <td>4</td> <td>233</td> <td>6.0</td> <td>5.4</td>	CS72979	4.33	CG910	5,452,727	CR861	6,573,205	1,120,478	95	124	10	4	233	6.0	5.4
CS72965 4.14 CR142 6,030,331 CG8 6,955,655 925,324 110 123 / 8 248 6.0 6.5 CS73216 4.34 CR1422 6,895,541 CG916 8,750,678 1,855,137 165 167 15 20 367 9.5 5.1 CS73218 4.36 CG8 6,955,655 CR1600 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS72966 4.16 CR1660 7,522,130 CG916 8,750,678 1,228,548 78 85 3 4 170 4.1 3.3 CS72968 4.19 CG916 8,750,678 CR3 11,440,981 2,690,303 84 69 10 6 169 9.5 5.5 4.9 CS72969 4.20 CG916 8,750,678 CR3 11,440,981 2,690,303 84 69 10 6 169 9.5 5.5 4.9 CS72970 4.21 CR1108 9,875,815 CI_23 1,122,137 11	CS72964	4.13	CG350	5,503,814	CR861	6,573,205	1,069,391	138	128	12	11	289	8.0	7.5
CS 3216 4.34 CR1422 6,895,541 CG916 8,750,678 1,855,137 165 167 15 20 367 9.5 5.1 CS 73217 4.35 CG8 6,955,655 CR1305 7,524,169 586,575 118 130 3 6 257 3.5 6.2 CS 72216 4.16 CR1660 7,522,130 CG916 8,750,678 1,228,548 78 85 3 4 170 4.1 3.3 CS 72966 4.17 CR1305 7,544,169 CG513 12,137,086 4,592,917 229 256 51 61 597 18.8 4.1 CS 72969 4.20 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 5 9 256 5.5 4.9 CS 72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS 72971 4.25 CG262 12,337,463 CR800 13,192,747 85,284 <	CS/2965	4.14	CR142	6,030,331	CG8	6,955,655	925,324	110	123	/	8	248	6.0	6.5
CS73217 4.35 CG8 6,955,655 CR1860 7,522,130 566,475 118 130 3 6 257 3.5 6.2 CS73218 4.36 CG8 6,955,655 CR1305 7,544,169 588,514 95 104 9 5 213 6.6 11.2 CS72966 4.16 CR1660 7,522,130 CG916 8,750,678 1,28,548 78 85 3 4 170 4.1 3.3 CS72967 4.17 CR1305 7,544,169 CG513 12,137,086 4,592,917 229 256 51 61 597 18.8 4.1 CS72968 4.19 CG916 8,750,678 CR3 11,440,981 2,690,303 84 69 10 6 169 9.5 3.5 CS72970 4.20 CG916 8,750,678 CR1108 9,875,815 1,127,177 117 125 5 9 256 5.5 4.9 CS72971 4.23 CR1279 10,825,656 CG2 11,872,893 1,997,078 79	CS/3216	4.34	CR1422	6,895,541	CG916	8,750,678	1,855,137	165	16/	15	20	367	9.5	5.1
CS75216 4.35 CC63 6,953,053 CK1505 7,944,169 380,514 95 104 9 5 215 6.6 11.2 CS72966 4.16 CR1600 7,522,130 CG916 8,750,678 1,228,548 78 85 3 4 170 4.1 3.3 CS72967 4.17 CR1305 7,544,169 CG513 12,137,086 4,592,917 229 256 51 61 597 18.8 4.1 CS72969 4.20 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 5 9 256 5.5 4.9 CS72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS72971 4.23 CR1279 10,825,656 CG2 11,872,893 1,047,237 132 149 6 5 292 3.8 3.6 CS72972 4.25 CG262 12,337,463 CR800 13,192,747 855,284 133	CS/321/	4.35	CGS	6,955,655	CR1660	7,522,130	500,475	118	104	3	b F	257	3.5	0.Z
CS72967 4.10 CR1000 7,524,150 CG510 8,750,078 1,220,346 78 83 3 4 170 4.1 3.5 CS72967 4.17 CR1305 7,544,169 CG513 12,137,086 4,592,917 229 256 51 61 597 18.8 4.1 CS72968 4.19 CG916 8,750,678 CR3 11,440,981 2,690,303 84 69 10 6 169 9.5 3.5 CS72969 4.20 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 5 9 256 5.5 4.9 CS72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS72971 4.23 CR1279 10,825,656 CG2 11,872,893 1,047,237 132 149 6 5 292 3.8 4.4 CS72974 4.25 CG262 12,727,651 CR1394 14,380,651 1,653,000	CS73218	4.30		0,955,055	CR1305	7,544,169	588,514 1 220 E40	95 70	104	9	5	213	0.0 1 1	11.2
CS72968 4.19 CG916 8,750,678 CR3 11,440,981 2,690,303 84 69 10 6 169 9.5 3.5 CS72969 4.20 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 5 9 256 5.5 4.9 CS72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS72971 4.23 CR1279 10,825,656 CG2 11,872,893 1,047,237 132 149 6 5 292 3.8 3.6 CS72972 4.25 CG262 12,337,463 CR800 13,192,747 855,284 133 117 7 3 260 3.8 4.4 CS72973 4.26 CG29 12,727,651 CR1394 14,380,651 1,653,000 118 131 6 10 265 6.0 3.6 CS72974 4.27 CR1394 14,380,651 CG507 15,542,832 1,162,181 1	CS72967	4.10	CR1305	7,522,150	CG513	12 137 086	1,220,340	70 229	256	51	61	597	4.1 18.8	5.5 4.1
CS72969 4.20 CG916 8,750,678 CR1108 9,875,815 1,125,137 117 125 5 9 256 5.5 4.9 CS72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS72970 4.23 CR1279 10,825,656 CG2 11,872,893 1,047,237 132 149 6 5 292 3.8 3.6 CS72972 4.25 CG262 12,337,463 CR800 13,192,747 855,284 133 117 7 3 260 3.8 4.4 CS72973 4.26 CG29 12,727,651 CR1394 14,380,651 1,653,000 118 131 6 10 265 6.0 3.6 CS72974 4.27 CR1394 14,380,651 CG507 15,542,832 1,162,181 193 200 13 10 416 5.5 4.7 CS72975 4.29 CR1452 15,399,547 CG1020 16,460,866 1,061,319	CS72968	4 19	CC916	8 750 678	CR3	11 440 981	2 690 303	84	69	10	6	169	95	35
CS72970 4.21 CR1108 9,875,815 CG2 11,872,893 1,997,078 79 93 8 12 192 10.4 5.2 CS72971 4.23 CR1279 10,825,656 CG2 11,872,893 1,047,237 132 149 6 5 292 3.8 3.6 CS72972 4.25 CG262 12,337,463 CR800 13,192,747 855,284 133 117 7 3 260 3.8 4.4 CS72973 4.26 CG29 12,727,651 CR1394 14,380,651 1,653,000 118 131 6 10 265 6.0 3.6 CS72974 4.27 CR1107 13,813,834 CG864 14,954,316 1,140,482 163 149 6 7 325 4.0 3.5 CS72974 4.27 CR1394 14,380,651 CG507 15,542,832 1,162,181 193 200 13 10 416 5.5 4.7 CS72975 4.29 CR1452 15,399,547 CG1020 16,460,866 1,061,319	CS72969	4 20	CG916	8 750 678	CR1108	9 875 815	1 125 137	117	125	5	9	256	55	49
CS729714.23CR127910,825,656CG211,872,8931,047,237132149652923.83.6CS729724.25CG26212,337,463CR80013,192,747855,284133117732603.84.4CS729734.26CG2912,727,651CR139414,380,6511,653,0001181316102656.03.6CS732194.37CR110713,813,834CG86414,954,3161,140,482163149673254.03.5CS729744.27CR139414,380,651CG50715,542,8321,162,18119320013104165.54.7CS729754.29CR145215,399,547CG102016,460,8661,061,319141138792955.45.1CS729764.30CG102016,460,866CR109217,743,5991,282,73313914611183149.27.2CS729774.31CG75317,649,518CR145118,296,565647,047115104782346.49.9Chr. 5CS729805.20CG4586,161CR775657,523571,3621491438113116.110.7CS729815.21CR775657,523CG491,307,945650,42211490693097.411.4 <td>CS72970</td> <td>4.21</td> <td>CR1108</td> <td>9.875.815</td> <td>CG2</td> <td>11.872.893</td> <td>1.997.078</td> <td>79</td> <td>93</td> <td>8</td> <td>12</td> <td>192</td> <td>10.4</td> <td>5.2</td>	CS72970	4.21	CR1108	9.875.815	CG2	11.872.893	1.997.078	79	93	8	12	192	10.4	5.2
CS72972 4.25 CG262 12,337,463 CR800 13,192,747 855,284 133 117 7 3 260 3.8 4.4 CS72973 4.26 CG29 12,727,651 CR1394 14,380,651 1,653,000 118 131 6 10 265 6.0 3.6 CS72973 4.26 CG29 12,727,651 CR1394 14,380,651 1,653,000 118 131 6 10 265 6.0 3.6 CS72974 4.27 CR1394 14,380,651 CG507 15,542,832 1,162,181 193 200 13 10 416 5.5 4.7 CS72975 4.29 CR1452 15,399,547 CG1020 16,460,866 1,061,319 141 138 7 9 295 5.4 5.1 CS72976 4.30 CG1020 16,460,866 1,061,319 141 138 7 9 295 5.4 5.1 CS72976 4.30 CG1020 16,460,866 CR1092 17,743,599 1,282,733 139 146	CS72971	4.23	CR1279	10,825,656	CG2	11,872,893	1,047,237	132	149	6	5	292	3.8	3.6
CS729734.26CG2912,727,651CR139414,380,6511,653,0001181316102656.03.6CS732194.37CR110713,813,834CG86414,954,3161,140,482163149673254.03.5CS729744.27CR139414,380,651CG50715,542,8321,162,18119320013104165.54.7CS729754.29CR145215,399,547CG102016,460,8661,061,319141138792955.45.1CS729764.30CG102016,460,866CR109217,743,5991,282,73313914611183149.27.2CS729774.31CG75317,649,518CR145118,296,565647,047115104782346.49.9Chr. 5CS730125.54CG4586,161CR775657,523571,3621491438113116.110.7CS729805.20CG4586,161CR906992,521906,360138125192230413.514.9CS729815.21CR775657,523CG491,307,945650,42211490693097.411.4	CS72972	4.25	CG262	12,337,463	CR800	13,192,747	855,284	133	117	7	3	260	3.8	4.4
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CS72974 4.27 CR1394 14,380,651 CG507 15,542,832 1,162,181 193 200 13 10 416 5.5 4.7 CS72975 4.29 CR1452 15,399,547 CG1020 16,460,866 1,061,319 141 138 7 9 295 5.4 5.1 CS72976 4.30 CG1020 16,460,866 CR1092 17,743,599 1,282,733 139 146 11 18 314 9.2 7.2 CS72977 4.31 CG753 17,649,518 CR1451 18,296,565 647,047 115 104 7 8 234 6.4 9.9 Chr. 5 CS73012 5.54 CG45 86,161 CR775 657,523 571,362 149 143 8 11 311 6.1 10.7 CS72980 5.20 CG45 86,161 CR906 992,521 906,360 138 125 19 22 304 13.5 14.9 CS72981 5.21 CR775 657,523 CG49 1,307,945 650,422	CS73219	4.37	CR1107	13,813,834	CG864	14,954,316	1,140,482	163	149	6	7	325	4.0	3.5
CS72975 4.29 CR1452 15,399,547 CG1020 16,460,866 1,061,319 141 138 7 9 295 5.4 5.1 CS72976 4.30 CG1020 16,460,866 CR1092 17,743,599 1,282,733 139 146 11 18 314 9.2 7.2 CS72977 4.31 CG753 17,649,518 CR1451 18,296,565 647,047 115 104 7 8 234 6.4 9.9 Chr. 5 CS73012 5.54 CG45 86,161 CR775 657,523 571,362 149 143 8 11 311 6.1 10.7 CS72980 5.20 CG45 86,161 CR906 992,521 906,360 138 125 19 22 304 13.5 14.9 CS72981 5.21 CR775 657,523 CG49 1,307,945 650,422 114 90 6 9 309 7.4 11.4	CS72974	4.27	CR1394	14,380,651	CG507	15,542,832	1,162,181	193	200	13	10	416	5.5	4.7
CS/29/6 4.30 CG1020 16,460,866 CR1092 17,743,599 1,282,733 139 146 11 18 314 9.2 7.2 CS72977 4.31 CG753 17,649,518 CR1451 18,296,565 647,047 115 104 7 8 234 6.4 9.9 Chr. 5 CS73012 5.54 CG45 86,161 CR775 657,523 571,362 149 143 8 11 311 6.1 10.7 CS72980 5.20 CG45 86,161 CR906 992,521 906,360 138 125 19 22 304 13.5 14.9 CS72981 5.21 CR775 657,523 CG49 1,307,945 650,422 114 90 6 9 309 7.4 11.4	CS72975	4.29	CR1452	15,399,547	CG1020	16,460,866	1,061,319	141	138	.7	9	295	5.4	5.1
CS/29// 4.31 CG/53 1/,649,518 CR1451 18,296,565 64/,04/ 115 104 7 8 234 6.4 9.9 Chr. 5 CS73012 5.54 CG45 86,161 CR775 657,523 571,362 149 143 8 11 311 6.1 10.7 CS72980 5.20 CG45 86,161 CR906 992,521 906,360 138 125 19 22 304 13.5 14.9 CS72981 5.21 CR775 657,523 CG49 1,307,945 650,422 114 90 6 9 309 7.4 11.4	CS72976	4.30	CG1020	16,460,866	CR1092	17,743,599	1,282,733	139	146	11	18	314	9.2	7.2
CS73012 5.54 CG45 86,161 CR775 657,523 571,362 149 143 8 11 311 6.1 10.7 CS72980 5.20 CG45 86,161 CR906 992,521 906,360 138 125 19 22 304 13.5 14.9 CS72981 5.21 CR775 657,523 CG49 1,307,945 650,422 114 90 6 9 309 7.4 11.4	CS/2977	4.31	CG/53	17,649,518	CR1451	18,296,565	647,047	115	104	/	8	234	6.4	9.9
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	CS72981	5.21	CR775	657.523	CG49	1,307.945	650.422	114	90	6	9	309	7,4	11.4

ABRC ID	Line #	Upper	Upper	Lower	Lower	Length of	Progeny of test cross						cM/mB
		marker	position	marker	position	intervar (ing				GII)			
							dsRed	GFP	dsRed + GFP	Nonfluor.	Total		
CS72982	5.22	CR906	992,521	CG345	2,076,237	1,083,716	160	176	14	14	364	7.7	7.1
CS72983	5.23	CG49	1,307,945	CR1156	2,874,982	1,567,037	106	96	15	15	232	12.9	8.2
CS72984	5.24	CR947	1,483,635	CG892	3,078,592	1,594,957	137	149	9	14	309	7.4	4.6
CS72985	5.25	CR1597	1,684,117	CG892	3,078,592	1,394,475	117	141	13	11	282	8.5	6.1
CS72986	5.26	CR1156	2,874,982	CG738	3,592,092	717,110	132	137	6	7	282	4.6	6.4
CS72987	5.27	CG892	3,078,592	CR41	4,735,563	1,656,971	135	137	8	13	293	7.2	4.3
CS72988	5.28	CR41	4,735,563	CG719	5,915,491	1,179,928	165	144	8	6	323	4.3	3.6
CS72989	5.29	CG767	5,240,098	CR1645	6,274,480	1,034,382	96	84	1	5	186	3.2	3.1
CS73220	5.55	CG495	6,043,335	CR1336	7,987,368	1,944,033	140	135	11	19	305	9.8	5.1
CS72990	5.31	CG855	6,585,030	CR1336	7,987,368	1,402,338	166	183	11	11	371	5.9	4.2
CS73221	5.56	CG855	6,585,030	CR1635	8,515,260	1,930,230	149	146	14	16	325	9.2	4.8
CS72991	5.32	CR1336	7,987,368	CG4	8,647,153	659,785	165	177	3	9	354	3.5	5.3
CS72992	5.33	CR1635	8,515,260	CG597	9,890,330	1,375,070	202	188	16	17	423	7.8	5.7
CS72993	5.34	CG358	13,229,304	CR29	16,780,239	3,550,935	163	155	34	39	391	18.7	5.3
CS72994	5.36	CG16	15,254,136	CR29	16,780,239	1,526,103	199	207	15	21	442	8.1	5.3
CS72995	5.37	CR29	16,780,239	CG621	17,850,058	1,069,819	304	318	18	19	659	5.6	5.2
CS72996	5.38	CR29	16,780,239	CG518	18,678,795	1,898,556	163	155	34	39	391	18.7	9.8
CS72997	5.39	CG518	18,678,795	CR39	20,491,443	1,812,648	203	203	15	18	439	7.5	4.1
CS72998	5.40	CG605	19,327,782	CR39	20,491,443	1,163,661	94	98	5	1	198	3.0	2.6
CS72999	5.41	CR39	20,491,443	CG326	21,542,180	1,050,737	99	91	3	5	198	4.0	3.8
CS73000	5.42	CR983	21,542,180	CG720	22,522,561	980,381	167	194	15	12	388	6.9	7.0
CS73001	5.43	CG326	21,542,180	CR1603	22,170,459	628,279	178	183	6	13	380	5.0	8.0
CS73002	5.44	CR58	21,803,754	CG19	22,594,347	790,593	48	56	2	2	108	3.7	4.7
CS73010	5.52	CR1603	22,170,459	CG354	23,038,968	868,509	232	216	6	6	460	2.6	3.0
CS73003	5.45	CG19	22,594,347	CR1081	23,501,247	906,900	213	213	13	10	449	5.1	5.6
CS73004	5.46	CR1114	23,415,403	CG608	24,596,395	1,180,992	106	120	2	2	230	1.7	1.4
CS73005	5.47	CR1081	23,501,247	CG608	24,596,395	1,095,148	209	206	8	4	427	2.8	2.6
CS73006	5.48	CG668	24,115,449	CR59	25,204,540	1,089,091	153	135	11	9	308	6.5	6.0
CS73007	5.49	CR59	25,204,540	CG769	25,742,640	538,100	121	113	6	6	246	4.9	9.1
CS73011	5.53	CG454	25,204,540	CR911	26,171,385	966,845	129	127	17	9	282	9.2	9.5
CS73008	5.50	CG769	25,742,640	CR106	26,333,257	590,617	165	123	12	15	315	8.6	14.6
CS73009	5.51	CG769	25,742,640	CR715	26,684,741	942,101	171	205	28	23	427	11.9	12.6



Fig. 2. Self-progeny of a *ch42-4sd*/TL4.22 plant, planted according to the seed fluorescence phenotype. Nonfluorescent seeds are expected to be predominantly *ch42-4sd/ch42-4sd* and albino, moderately fluorescent seeds are expected to be predominantly *ch42-4sd/TL4.22* and yellow-green, and strongly fluorescent seeds are expected to be predominantly TL4.22/TL4.22 and dark green. Seedlings that do not conform to the expected phenotype are circled.

heterozygous for fie-11 and not transgenic. These seeds were likely the result of double recombination within the marked segment which transferred the wild-type allele of FIE to an unmarked chromosome. The remaining 11 families segregated 1/4 or less dead seeds, and all the viable seeds were resistant to Basta. Six of the families had very few dead seed, suggesting that they contained multiple, independent, *p*FIE::FIE-HA insertions. Five families had 1/4 dead seed, implying that the parental T1 seed was homozygous for *fie*-11 and heterozygous for a single *p*FIE::FIE-HA insertion. Seedlings from at least 6 plants in each of these single-insertion families were treated with Basta to identify families homozygous for the transgene. Subsequent quantitative PCR analysis of the dosage of *fie*-11 and FIE-HA (primer sequences in Supplementary Table 2) demonstrated that these lines were indeed *fie*-11/*fie*-11 *p*FIE::FIE-HA/.

An alternative approach is useful for lethal or sterile mutations whose seeds are phenotypically normal (Fig. 4b). In this case, it is impossible to identify which nonfluorescent seeds contain the transgene of interest because transgenic seeds are visually indistinguishable from nontrangenic seeds. Transgenic plants can be identified after germination using a selectable marker (e.g. Basta resistance) present on the transgene, but if the transformation efficiency is relatively low this will require selecting and planting a large number of nonfluorescent seeds, which is tedious. Alternatively, one can screen a large number of T1 plants for the presence of the selectable marker and then screen the T2 seeds produced by these plants for seed fluorescence. Approximately 1/4 of the plants containing the selectable marker should have nonfluorescent seeds and be homozygous for the lethal/sterile mutation.

Using TLs to separate tightly linked mutations

Next-generation sequencing has revealed that many induced mutations are associated with linked second-site mutations (Schneeberger et al. 2009; Enders et al. 2015; Thole and Strader 2015; Lup et al. 2021). Determining whether the phenotype of these lines is attributable to one or more of these linked mutations can be problematic if the mutations are so tightly linked that they do not readily segregate from each other. We identified an EMSinduced late vegetative phase change mutant that contained missense mutations in 2 members of the PRC1 complex, VAL1 and AtBMI1A (Molitor and Shen 2013; Merini and Calonje 2015). Null alleles of each gene have a much weaker effect on vegetative phase change than this double mutant, so we assumed that the relatively strong phenotype of the double mutant was attributable to the combined effects of these missense mutations. One way to test this hypothesis is to separate these mutations by recombination and examine their individual phenotypes. However, VAL1 and AtBMI1A are located only 45.5 kb from each other on chromosome 2, making this an extremely time- and resource-intensive experiment to perform by molecular genotyping.

We selected 12 strong red, moderate green, and 12 strong green, moderate red seeds from the F2 progeny of plants heterozygous for TL2.31 and *val1 atbmi1a* (Fig. 5). Because these genes are located ca. 600 Mb from both the *pNAP::eGFP* and *pNAP::DsRed* transgenes, we expected approximately equal numbers of *val1*



Fig. 3. The first leaf with abaxial trichomes in the self-progeny of a *myb75-1*/TL1.55 plant, planted according to seed fluorescence phenotype. Nonfluorescent seeds are expected to be predominantly *myb75-1/myb75-1* and moderately fluorescent seeds are expected to be predominantly *myb75-1*/TL1.55. Error bars = SEM; ** = P < 0.01, 2-tailed Student's t-test, n > 45.

atbmi1a/++ and ++/++ recombinants and a much smaller number of val1 +/++ and + atbmi1a/++ recombinants. Consistent with this expectation, we obtained only 2 val1 +/++ recombinants and no + atbmi1a/++ recombinants. Unexpectedly, we found that the number of recombinants between pNAP::eGFP and val1 was 3 times greater than the number of recombinants between pNAP::DsRed and val1 (18 vs 6). This was surprising because val1 atbmi1a are equidistant from these transgenes. This suggests that the pNAP::DsRed insertion in TL2.31 (CR1124) is associated with a small rearrangement that suppresses recombination. This hypothesis is supported by the observation that the cM/Mb ratio in TL2.31 is significantly less than the ratio in a different line (TL2.32) that spans the same region (Table 1).

Subsequent analysis of the phenotype of the val1 single mutant (val1-5sd) revealed that it is semidominant (Fouracre et al. 2021), and that its phenotype is identical to the phenotype of the original val1-5sd atbmi1a double mutant. This result suggests that—in contrast to our initial hypothesis—the missense mutation in AtBMI1A makes no contribution to the phenotype of the double mutant and may be phenotypically silent. It also suggests that val1-5sd interferes with the function of VAL2 or other members of the PRC1 complex, as null alleles of VAL1 have a weaker phenotype than val1-5sd (Fouracre et al. 2021).

Discussion

The value of linked fluorescent transgenes for studies of recombination has been recognized for some time (Melamed-Bessudo *et al.* 2005; Berchowitz and Copenhaver 2008). The utility of such transgenes for following the segregation of chromosome segments in trans to the marked chromosome is less well appreciated, but is an equally important feature of these lines. This property relies on the fact that a nonfluorescent chromosome segregating from a doubly marked chromosome will either represent a nonrecombinant chromosome, or a chromosome that has undergone double recombination within the marked interval (Wu *et al.* 2015). The frequency of double recombination decreases with the distance between markers, so it is beneficial to use TLs that define relatively small intervals if the goal is to use the TL as a balancer.

For an interval of 6.9 cM—the average for the TLs described here—the maximum frequency of double recombination is 0.035 \times 0.035, or approximately 1/1000 chromosomes, although this number is likely to be significantly lower due to crossover interference (Berchowitz and Copenhaver 2010; Salomé *et al.* 2012). This means that the nonfluorescent chromosomes produced by a plant heterozygous for a short-interval TL are very likely to have the genotype of the non-TL chromosome. Furthermore, for any single nucleotide within this interval (e.g. a mutation or polymorphism), the probability that a nonfluorescent chromosome will carry the parental form of this nucleotide increases the farther the nucleotide is from the midpoint of the marked interval. This is because the probability that double recombination will replace the allele on the nonfluorescent chromosome with the allele

Table 2. Mapping a recessive mutation using a genome-wide set of long-interval TLs.

Seed phenotype		Number of WT: mutant progeny produced by TL/mutant plants													
	TL1.9	TL1.13	TL2.5	TL3.8	TL3.5	TL3.11	TL4.1	TL4.4	TL4.6	TL5.8	TL5.12	TL5.14			
Fluorescent Nonfluorescent	5:1 5:2	9:3 10:2	11:3 9:3	9:2 9:2	9:1 4:3	9:3 8:3	9:2 8:4	8:1 4:7	12:0 1:12 [°]	11:2 11:1	5:6 9:4	11:4 10:4			

The high frequency of mutant seedlings among nonfluorescent seeds indicates that the mutation is within, or near, this TL.



Fig. 4. Rescuing lethal or sterile mutations with a transgene. a) Moderately fluorescent seeds from a *fie*-11/TL3.31 plant were selected and the resulting plants were transformed with a *pFIE*-HA *BaR* T-DNA construct by floral dipping. The expected genotypes of the nonfluorescent seeds from these plants are shown. Fully formed (i.e. viable) seeds will either be homozygous for *fie*-11 and hemizygous for *pFIE*-HA *BaR*, or be heterozygous for *fie*-11 as a result of double recombination within the interval marked by TL3.31. Given the relatively low frequency of transformation, this latter class of seeds will probably lack *pFIE*-HA *BaR*. b) Using a TL to rescue a seedling lethal or sterile mutant with a transgene. This approach is useful if the mutation does not affect seed morphology or viability, making it impossible to distinguish transgenic from nonransgenic seeds prior to planting.



Fig. 5. Using a TL to separate closely linked mutations. F2 seeds from a *val1 bmi1a*/TL2.31 plant were screened for seeds that displayed strong red and moderate green fluorescence, or strong green and moderate red fluorescence. The genotype of these seeds was determined by examining the molecular genotype of their F2 progeny, using allele-specific PCR primers.

from the TL decreases as one moves away from the center of the interval; i.e. the closer a gene is to one of the transgenes in a TL, the less susceptible it is to double recombination. Thus, even relatively large TLs can be useful for gene mapping, as we found in the case of a novel phase change mutation that we mapped to the bottom of chromosome 4. Single fluorescent transgenes are also useful for following polymorphisms/mutations in trans to the transgene if the polymorphisms/mutations is sufficiently close to the transgene (Wu *et al.* 2015).

Although recombination within a TL is disadvantageous if the TL is being used to follow the segregation of a chromosome in trans to the TL, this feature makes it possible to dissect relatively small chromosomal regions. As described here, we were able to recombinationally separate mutations located only 45 kb (ca. 0.03 cM) from each other by visual screening seeds for fluorescence intensity. This process only took a few hours. In contrast, screening the same F2 population for recombinants by PCR would have required hundreds or possibly thousands of quantitative PCR reactions, at considerable time and expense. The ability to rapidly identify and then rescue recombinants in a small chromosomal region is not only useful for dissociating second-site mutations but also facilitates fine mapping of mutations or natural variants that have been localized to specific regions of the genome. It is particularly useful for mapping variants with weak phenotypes because the dosage sensitivity of the fluorescent markers makes it possible to identify plants homozygous for recombinant chromosomes in F2 populations. This facilitates determining which allele of the variant the chromosome possesses because the phenotype of this allele is likely to be strongest when it is homozygous.

An ideal TL is one in which the transgenes are not subject to silencing, are expressed strongly and uniformly in dry seeds, display clear differences in fluorescence intensity in 1 and 2 doses, and are inserted in functionally silent positions in the genome. Unfortunately, this is difficult to achieve. It is easy to produce T-DNA-transformed lines in *Arabidopsis*, but a large number of these lines contain more than one insertion, and many contain a translocation or other type of large rearrangement at the site of the T-DNA insertion (Clark and Krysan 2010; Wu et al. 2015). Consequently, only some of the primary transgenic lines are useful for the production of TLs. The existence of small inversions that do not lead to semisterility but which eliminate or reduce recombination is another problem. These are useful if the TL is being used as a balancer, but are problematic if the TL is being used to identify recombination in a defined interval, as was the case in our experiment with the val1 atbmi1a double mutant. Finally, there is the problem of transgene silencing. Some of the transgenes we identified silenced after several rounds of propagation, and the frequency of this phenomenon increased for transgenes located close to the centromere. Indeed, it was sometimes necessary to abandon a TL late in the process of generating or amplifying the line because one or both of the markers underwent silencing. Unfortunately, this behavior is typical of transgenes in plants, and only repeated propagation will demonstrate if the transgenes in a TL are sufficiently stable for prolonged use. An expanded collection of pNAP::eGFP and pNAP::DsRed insertions will enable us to minimize these problems in future collections of TLs. Despite these limitations, the collection of TLs we have generated enable many experiments that are difficult or sometimes impossible to perform, and therefore represent a useful addition to the resources available for genetic analysis in A. thaliana.

Data availability

Short-interval TLs are available as homozygous stocks from the Arabidopsis Biological Resource Center (ABRC), under the stock numbers provided in Table 1. The ABRC stock numbers for the long-interval lines described previously are provided in Wu *et al.* (2015). The transgenes used to construct these lines are available from the ABRC, under the stock numbers provided in Supplementary Table 1. Other genetic stocks described in this article are also available from the ABRC or from the corresponding author.

Supplemental material is available at G3 online.

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Conflicts of interest

None declared.

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