

MAPPING OF RIPENING-RELATED OR -SPECIFIC cDNA CLONES OF TOMATO

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**Mapping of ripening-related or -specific cDNA clones of tomato**  
(*Lycopersicon esculentum*)

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## Summary

Nineteen ripening-related or -specific clones from *Lycopersicon esculentum* were mapped via RFLP analysis using an F<sub>2</sub> population from the cross *L. esculentum* X *L. pennellii* and cDNA or genomic clones of known map location. The map produced using cDNA and genomic clones of known map location corresponded well with previously published maps of tomato. The number of loci detected for each ripening-related or -specific clone varied from one to seven. These loci were located on all twelve chromosomes of the tomato genome. There was no significant clustering of ripening related or specific genes. Regions of very low recombination were observed.

The clone for polygalacturonase (TOM6) mapped to a single region on chromosome 10, the same chromosome as the *nor* and *alc* ripening mutants. To fine map this chromosome, two backcross populations were produced from the cross of *L. esculentum* x *L. pimpinillifolium*, in which the *esculentum* parents used were homozygous for either *alc* or the *nor*. The coding region for polygalacturonase is functionally unlinked to either of these two ripening mutants

**Key words:** RFLP's, clustering, nor, alc

## Introduction

The transformation of unpalatable green fruit to edible, marketable tomatoes involves the coordinated physical and chemical changes known as ripening. In order to study the genetic regulation of tomato fruit ripening, Slater et al. (1985) isolated 19 classes of ripening-related or -specific clones (referred to as TOM clones) from a cDNA library prepared from mRNA from ripening tomato pericarp. Using similar techniques, Mansson et al. (1985) produced 4 clones (referred to as CGN clones). Most of the cloned genes are of unknown function. One of the clones (TOM6) is a gene coding for polygalacturonase (Grierson et al. 1986), another (TOM13) is wound inducible and is suspected to be a component of the ethylene biosynthesis pathway (Holdsworth et al. 1987).

Five of the TOM clones showed 2 patterns of mRNA accumulation during ripening. Levels of messenger RNAs corresponding to TOMs 5 and 6 rose to a maximum when lycopene content was 38 mg/g fresh weight (corresponding to a fully orange fruit), then declined slowly. The expression of mRNAs homologous to TOMs 13, 36, and 99 were similar, rising rapidly at first, declining, then rising again to peak expression at a lycopene content also of 38 mg/g (Maunder et al. 1987).

The fifteen clones TOMs 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 96, 99, 111, and 114 were used to study the effects of the mutant alc on ripening processes (Mutschler et al. 1988). The level of messenger RNA homologous to TOM99

was not affected by *alc*. The other clones showed varying degrees of reduction of the levels of homologous mRNA. TOM5 exhibited the least reduction (to 66% of normal), and pTOM6 the greatest (to 10% of normal).

Since an excellent RFLP map of the tomato genome is already available (Bernatzky and Tanksley 1986b), the ripening-related and ripening-specific clones could be readily mapped. Several articles provide thorough explanations of the underlying theory of mapping using restriction fragment length polymorphisms (RFLPs) (Beckman and Soller 1983, 1986). A map of the ripening related or specific clones would provide important information as to: 1. the number of loci homologous to each ripening clone; 2. whether there is any clustering of ripening-related genes; 3. whether there is any clustering of loci exhibiting similar developmental expression patterns; 4. whether any of the clones map to a location near that of a known gene affecting fruit traits.

Few polymorphisms are found among domestic *Lycopersicon esculentum* lines, necessitating the use of interspecific crosses for mapping this species (Helentjaris et al. 1985; Miller 1989). Polymorphisms are easily detected between *L. esculentum* and *L. pennellii*, and a cross of these two parents has previously been used for isozyme and RFLP mapping (Tanksley and Rick 1980; Bernatzky and Tanksley 1986a, 1986b).

## Materials and Methods

### *Plant materials*

F<sub>2</sub> seeds were obtained by selfing F<sub>1</sub> plants from the cross *Lycopersicon esculentum* cv. 'New Yorker' x *L. pennellii* (LA 716). Leaf tissue of parents, F<sub>1</sub>, and F<sub>2</sub> plants was harvested, washed in 0.05% Tween 20, frozen in liquid nitrogen, and stored at -80°C until DNA extractions were made.

For the backcross populations, chromosome marker stocks *L. esculentum* *hy-u-h-alc* and *u-nor* were used to make F<sub>1</sub> hybrids with *L. pimpinellifolium* (LA2189). The F<sub>1</sub> plants were backcrossed to the *esculentum* parent. Fresh leaf tissue harvested from parents, F<sub>1</sub>, and BC<sub>1</sub> plants was used for DNA isolations.

### *DNA preparation*

For the F<sub>2</sub> population, DNA was extracted using the procedure of Vallejos et al. (1986), substituting 20 mM sodium bisulfite for the EDTA and  $\beta$ -mercaptoethanol in the extraction buffer, and eliminating the cesium purification steps. For the backcross populations the method of Doyle and Dickson (1987) was used.

### *Genomic blots*

10 mg of DNA from each plant was digested with restriction enzymes (Dra I, Eco RI, Eco RV, Hind III, Sst I, Xba I, or Xmn I), following the manufacturer's suggested conditions, with the addition of 2-4 mM spermidine to increase cutting efficiency. DNA fragments were loaded into 0.8% agarose gels and run overnight at 25 volts (Maniatis et al. 1982). Gels were blotted onto Zetabind membrane (AMF CUNO, Inc.), using an alkaline transfer procedure.

### *Clones used*

The mapping clones, total genomic (TG) and cDNA (CD) clones of known chromosomal location, were generously supplied by S. Tanksley (Bernatzky and Tanksley 1986a, 1986b). Ripening-specific DNA clones were obtained from two sources, the TOM clones from D. Grierson (Slater et al. 1985), and the CGN clones from Calgene (Mansonn et al. 1985).

### *Probe preparation*

Insert DNA was isolated following digestion to release insert. Insert and vector DNA were separated in low-melting-point agarose and the insert fragments were random primed, using the Multiprime kit (Amersham). Probe was purified by chromatography over G-75 Sephadex (Maniatis et al.

1982).

### *Hybridizations*

Genomic blots were prehybridized in 6x SSC, 0.5% SDS, 5x Denhardt's solution, and 100 mg/ml denatured salmon sperm DNA for 4 to 24 hours at 65C. Probe was then added, and hybridizations left for 24 to 48 hours at 65C. Filters were washed through serial washings down to 0.5x SSC, 0.1% SDS at 65C (Maniatis et al. 1982). Filters were exposed to X-ray film, with an intensifying screen (DuPont Cronex Lightning Plus HC), and developed after 1 to 7 days.

### *Linkage analysis*

The data were analyzed using the Mapmaker program (Lander et al. 1987). The "three point" command was used to determine the correct sequence for each linkage group. Where additional markers were mapped, the "ripple" function was used to confirm orders. The Kosambi function of genetic distance was used.

### *Homology testing*

In cases where no recombinant plants were detected for 2 clones, tests were undertaken to determine whether these clones were homologous. Inserts of each clone were isolated from the plasmid vector (as described under *probe preparation*), and digested with a combination of restriction enzymes

to obtain a restriction map of the insert, with each of the inserts being used as the probe on twin blots. To support the results of these homology tests, survey blot patterns of DNA of both parents and the F<sub>1</sub> digested with 5 enzymes were compared, using the clones in question as probes.

## Results and Discussion

### *The genetic linkage map*

The complete map, created using both the mapping clones and the ripening-related clones, spans 1158 centimorgans (cm) (Fig. 1). The nineteen ripening-specific or -related clones, TOMs 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 94, 99, 111, 114, and 129, and CGNs 9-24, 24-3, and 28-8, mapped to 38 loci. None of the three CGN clones used was a duplicate of a TOM clone. The ripening-related and -specific loci are located on all twelve chromosomes. Because the mRNA homologous to most of these clones has been shown to be affected by the ripening mutant *alc*, the effects of *alc* on mRNA level must be transactive.

Nine of the ripening clones (47%) map to one locus each, six (32%) map to two loci each, two map to three loci, TOM25 maps to four loci, and TOM38 maps to seven loci (Fig. 1). There is generally good agreement between the number of loci detected by RFLP analysis and the reported number of gene products detected by hybrid select translation as reported by Slater et al. (1985). The clones TOMs 5, 6, and 13 hybridized to one translation product

and TOMs 36 and 99 hybridized to two translation products each. RFLP analysis detected one locus for TOMs 6 and 13, two loci for TOMs 5 and 99, and three loci for TOM36.

The numbers of regions homologous to a clone may over- or underestimate the number of loci actually coding for a message. Not all of the loci detected by a clone are necessarily active coding genes; some of the loci may be pseudogenes, incomplete or mutated copies of the active gene.

Furthermore, each fragment with homology to a clone may contain more than one copy of the gene (Bernatzky and Tanksley 1986a).

The clones homologous to large numbers of loci, such as TOM25 and TOM38, may code for proteins needed in great abundance or at different stages during ripening or may indicate duplications. Unfortunately, the expression patterns of mRNA's homologous to TOM25 and 38 during ripening have not yet been characterized. The proportions of ripening clones mapping to 1, 2, or more loci agree well with the results of Bernatzky and Tanksley (1986a), who found that of 34 random cDNA clones from a leaf library, 53% corresponded to a single locus, and 32% corresponded to 2 loci in the tomato genome. Since the spread of numbers of regions homologous to ripening clones is the same as that for random leaf cDNA clones, the spread of copy number does not provide evidence that the ripening clones with more regions have multiple copies for a reason related to ripening. However, loci homologous to TOM 25 and TOM 38 are tightly linked on both chromosomes six and nine (3 and 4 cm, respectively), indicating the

possibility of a duplication on these two chromosomes.

*Suspected clustering of ripening cDNA clones*

The ripening-related and -specific loci are found on all twelve chromosomes of the tomato genome. Some regions homologous to ripening clones are found singly, others are in pairs or triplets (Table 1). No plants recombinant for the two loci were observed in a population of 72 plants for TOMs 31 and 41 on chromosome one, TOM25C and CGN24-3A on chromosome two, and TOMs 99B and 111 on chromosome three. Homology tests and comparison of polymorphism patterns using 5 restriction enzymes showed that all three of these pairs of clones were non-homologous.

There are other groups of closely linked loci -- TOMs 36A and 88B are within approximately 4 cm on chromosome five; TOMs 25D and 38B are within approximately 3 cm on chromosome six; TOMs 4 and 13 are within approximately 4 cm on chromosome seven, and TOM25A and TOM38G are within approximately 4 cm on chromosome nine. The triplet TOM25A, 99B, and 111 are within a 4 cm range, and TOMs 92, 94A, and CGN28-8B are all within approximately 3 cm. However, the first triplet of clones is in a region of reduced recombination (Bernatzky and Tanksley 1986b) and the second triplet of clones may be in or near the region of reduced recombination near Aps2 (Rick 1969).

The question whether the degree of clustering observed is statistically significant was examined. The analysis made several assumptions: 1. the ripening loci are unrelated by descent; 2. there is a fixed tomato genome size, which may be estimated by current information; 3. every region in the genome has an equal chance of containing a ripening locus; and 4. recombination is equally likely at any location throughout the genome. The first assumption is supported by the fact that the clones are non-homologous. The tomato genome has previously been estimated to be 1200 cm (Bernatzky and Tanksley 1986b; Tanksley and Hewitt 1988). Since more recent data indicate a genome size of 1500 cm (S. Tanksley, personal communication), both values are considered in this study.

The question of the randomness of locus position can be formulated as a problem of distributing balls into cells as follows: a set of  $n$  balls is distributed independently at random into a sequence of  $r$  cells, numbered 1 to  $r$ . A non-negative integer is chosen for the threshold value  $m$ . The choice of  $m$  could be based upon the smallest resolvable distance of recombination, which is a function of the population size used (Tanksley et al. 1988). A *near-match* occurs when two balls are assigned to cells whose distance is  $m$  or less; the special case of  $m=0$ , when two balls are assigned to the same cell, is a *match*. Let  $k$  be the number of near-matches observed when  $n$  balls are distributed into  $r$  cells. The probability distribution of the number of near-matches is derived under the null hypothesis of independence and randomness. Under the alternative hypothesis, that there is clustering among the locations of the balls, the number of near-matches tends to be greater than under the null hypothesis. The null

hypothesis is therefore rejected if the observed  $k$  lies in the upper 5% tail of the null distribution of  $k$ .

In this experiment,  $n = 38$  (the number of loci homologous to the ripening clones), and  $r = 1200$  or  $1500$  (the size of the tomato genome). Two values for  $m$  are of interest,  $m=0$ , which corresponds to the occurrence of cosegregating pairs, and  $m=5$ , which corresponds to groups of loci linked within 5 cm. The latter value of  $m$  is of interest because the minimum resolvable distance for an F<sub>2</sub> population of 72 plants is approximately 5 cm (Tanksley et al. 1988). Both analyses are illustrated in Figure 2 and Table 1. If each ball has 1200 possible locations, then  $1200^{38}$  arrangements of 38 balls are possible. A combinatorial argument establishes a rule for counting the exact number of these possible arrangements having  $k$  near-matches, with a specified threshold  $m$  (Schwager 1989).

For  $m = 0$ , the question is essentially: if 38 balls are distributed independently and at random into 1200 cells, what is the probability of at least  $k$  matches? This question reduces to a classical occupancy problem, determining the distribution of the number of empty cells. Since three cosegregating pairs were observed,  $k = 3$ . The probability of exactly 3 matches is 0.0163, and the probability of 3 or more matches is 0.0186 (Table 2). Thus, at a significance level of 5%, one would conclude that the loci are not distributed randomly. However, since one of the pairs (TOM99B and TOM111) is located within a region of reduced recombination, the assumption of equal likelihood of recombination throughout the tomato

genome is violated. Omitting this questionable pair, the probability of two or more exact matches is 0.1122, leading one to conclude that the ripening loci are distributed randomly. When a genome size of 1500 cm is considered, the same conclusions are drawn with  $k=3$  and  $k=2$  (Table 2).

For  $m = 5$ , the question becomes: if 38 balls are distributed independently and at random into 1200 cells, what is the probability of at least  $k$  near-matches? There are ten pairs of tightly linked loci, therefore  $k = 10$ .

Computational complexity makes calculations unmanageable at such high levels of  $m$  and  $k$ . Table 2 compares the calculated values and results from a computer simulation up to  $k = 4$ . The values are essentially identical, therefore the simulation values are used for  $k$  greater than 4.

As can be seen from Table 2, the probability of 10 or more near-matches within a genome of size 1200 is estimated to be 0.047, a borderline result when using a 5% significance level. However, 4 of these pairs are within suspected regions of reduced recombination, and the probability of 6 or more near-matches is 0.568. With a genome size of 1500, the probability of 10 or more near-matches is estimated to be 0.010, and the probability of 6 or more near-matches is 0.346. Therefore, there is no substantial evidence for functional clustering of ripening-related loci.

A similar analysis could be performed for any number of clones ( $n$ ), any size genome ( $r$ ), and any near-match threshold ( $m$ ). The number of near-match pairs ( $k$ ) needed to constitute significant evidence of clustering

increases with both increasing number of clones and increasing genome size.

*Comparison to existing map*

It is of interest to compare the framework map, which was generated using the mapping clones, to the previous maps produced by the originators of these clones (Bernatzky and Tanksley 1986b; Tanksley et al. 1988). Neither of the maps most recently published by Tanksley et al. was produced using the Mapmaker program. For uniformity, more recent estimates of genetic distances, obtained from the Mapmaker program, are used for these comparisons (S. Tanksley, personal communication).

The extreme similarity between our maps and the Tanksley maps is striking. The gene orders are identical for all clones located on both maps. Most of the linkage distances calculated between markers (the Kosambi function of the recombination fraction) also agree quite well. For example, on chromosome one, our estimates of linkage between loci CD15, TG21, CD12, and TG53 are 28, 17, and 41, compared with distances of 21, 15, and 45, respectively, on the Tanksley map. On the long arm of chromosome ten, we calculate as the distances between four of the markers CD34B, CD72, CD5, and CD32B, 14, 26, and 4, compared with 14, 31, and 5, respectively, on Tanksley's map.

In a few cases the distances on the two maps do not seem to correspond

well. For example, on chromosome five, estimates of linkage between the four mapping clones CD31, CD38B, TG60, and TG69 are 23, 40, and 11 cm, while estimates from Tanksley's lab are 12, 25, and 24 cm respectively. On chromosome seven, linkage estimates between the four clones CD48, CD54, TG61, and TG113 are 36, 14, and 13 cm, while those from Tanksley's lab are 61, 25, and 4 cm respectively.

Considering the number of distances being estimated, the differences in map distances are most likely attributable to sampling error. Differences could also be a result of differing population sizes or of errors in scoring. Large variations among populations for recombination fractions have been observed in both tomato (A. Patterson, personal communication), and in rice (S. McCouch, personal communication). Differences in recombination could be due to a number of environmental or other factors affecting meiosis.

#### *Locations of ripening-related loci versus classical ripening genes*

The ripening-specific or -related loci homologous to the CGN and TOM clones may be related to previously identified and mapped visual ripening mutants. Table 3 provides a list of possible linkages between ripening cDNA loci and visible ripening mutants. It is extremely difficult to compare RFLP maps to the classical tomato linkage map, due to the scarcity of common markers. Therefore, any markers within the same general region are listed, but further testing is needed before drawing any significant conclusions. Some of the more interesting possibilities include

the possible linkage of TOM36A and TOM38C to *rin* (ripening inhibitor) on chromosome five, of TOM25A, TOM38G, TOM88C, TOM99A, or CGN24-3B on chromosome nine to *Nr* (Never-ripe), or of TOM6 (the PG clone) to *alc* (alcobaca), *u* (uniform shoulder), or *nor* (non-ripening) on chromosome ten.

Two backcross populations were analyzed to determine whether PG is closely linked to *alc*, *u*, or *nor*. The localization of TOM6 versus the ripening mutants could not be studied in an intraspecific cross of *L. esculentum* because of a lack of polymorphisms within the species. This agrees with previous studies (Helentjaris et al. 1985; Miller 1989). A cross of *L. esculentum* x *L. pennellii* also could not be used, because the latter parent produces green fruit, and many of the BC1 fruit lack *esculentum*-type color development, making analysis of ripening mutants impossible. Therefore, BC1 populations were obtained from the interspecific cross between *L. esculentum* and the red-fruited species, *L. pimpinellifolium*.

One backcross population segregated for the visible markers *u* and *nor*. The other segregated for *hy* (homozygous yellow), *u*, *h* (hairless), and *alc*. Restriction polymorphisms were not easy to locate; when surveyed with 16 enzymes, the *alc* population exhibited polymorphisms for 7 out of 11 chromosome 10 clones, and the *nor* population exhibited polymorphisms for only 5 out of 11 chromosome 10 clones. The inability to detect polymorphisms for two of the clones in the *nor* population, for which polymorphisms were detected in the *alc* population, is attributed to

heterogeneity in the *L. pimpinellifolium* parent, rather than within the *nor* line itself.

The RFLP maps of chromosome 10 from these two populations agreed well with the map generated from the F<sub>2</sub> population (Figure 3), as well as with the published maps (Mutschler et al. 1987; Tanksley et al. 1988). When both the RFLP and visual traits are analyzed together, it is apparent that TOM 6 is many map units away from *u*, *alc* and *nor*, and is in fact functionally unlinked.

#### *Single gene ratios*

Upon first examination of the F<sub>2</sub> RFLP data, it is obvious that some of the loci do not fit the 1:2:1 ratio expected of co-dominant genes, or the 3:1 ratio expected with a presence/absence marker. Significant deviations from an expected single-gene ratio will affect the recombination fraction, and thus the map distance calculated. Seven chromosomes have regions in which one or more clones do not fit the expected ratio (Figure 4).

Three regions of ratio distortion are evident on chromosomes 1, 10, and 12. These segregation distortions agree with work by Bernatzky and Tanksley (1986b), Zamir and Tadmor (1986), Gadish and Zamir (1987), and Rick (1969). The cluster on chromosome 10 is of particular interest, because of the directional nature of the aberrancy; the distortion increases markedly for loci more distal on the short arm of the chromosome. Similar patterns

of ratio distortion were observed during the mapping of the *Ge* (Gamete eliminator) and *X* (gametophytic factor) loci in *L. esculentum* (Rick 1966, 1970; Alexander 1972). The backcross population used to analyze the ripening clone and mutants on chromosome 10 more thoroughly did not exhibit aberrant segregation ratios. Data from previous papers (Rick 1969) do not indicate a lack of aberrant segregation in backcross populations of *L. esculentum* x *L. pennellii*, therefore the lack of aberrancies on chromosome 10 detected in the BC population is probably due to the use of *L. pimpinellifolium* in the cross, rather than *L. pennellii*. The presence of aberrancies in the F<sub>2</sub> is likely to be an effect of the interaction of *pennellii* and *esculentum* genomes.

Segregation patterns not fitting Mendelian ratios are common in interspecific crosses (Grant 1975). This could be due to many factors affecting the plant life cycle between sporogenesis and seed germination. In the case of *L. esculentum* x *L. pennellii*, Gadish and Zamir (1986) suggested that the deviations are due to differential zygotic lethality.

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## References

- Alexander MP (1972) Inheritance of *X* locus in tomato. *Indian J Genet* 32:90-98
- Beckmann JS, Soller M (1983) Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and cost. *Theor Appl Genet* 67:35-43
- Beckmann JS, Soller M (1986) Restriction fragment length polymorphisms and genetic improvement of agricultural species. *Euphytica* 35:111-124
- Bernatzky R, Tanksley SD (1986a) Majority of random cDNA clones correspond to single loci in the tomato genome. *Mol Gen Genet* 203:8-14
- Bernatzky R, Tanksley S (1986b) Towards a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* 112:887-898
- Doyle JJ, Dickson EE (1987) Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715 - 722
- Gadish I, Zamir D (1987) Differential zygotic abortion in an interspecific *Lycopersicon* cross. *Genome* 29:156-159

Grant V (1975) Genetics of flowering plants. Columbia University Press,  
New York

Grierson D, Tucker GA, Keen J, Ray J, Bird CR, Schuch W (1986)  
Sequencing and identification of a cDNA clone for tomato  
polygalacturonase. Nuc Acids Res 14:8595-8603

Helentjaris T, King G, Slocum M, Siedenstrang C, Wegman S (1985)  
Restriction fragment polymorphisms as probes for plant diversity and  
their development as tools for applied plant breeding. Plant Molecular  
Biology 5:109-118

Holdsworth M, Bird C, Ray J, Schuch W, Grierson D (1987) Structure  
and expression of an ethylene related mRNA from tomato. Nuc Acids  
Res 15:731-739

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE,  
Newburg L (1987) MAPMAKER: An interactive computer package for  
constructing primary genetic linkage maps of experimental and natural  
populations. Genomics 1:174-181

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A  
laboratory manual. Cold Spring Harbor Laboratory. Cold Spring  
Harbor, NY

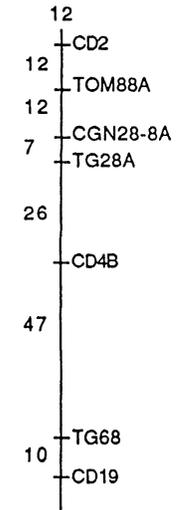
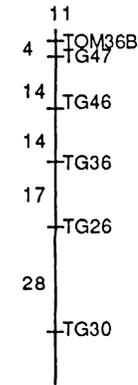
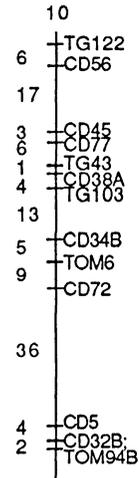
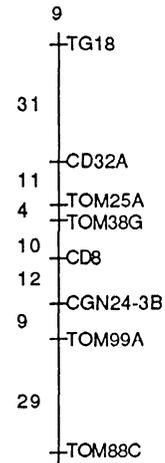
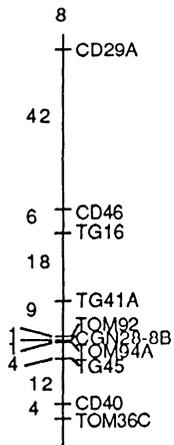
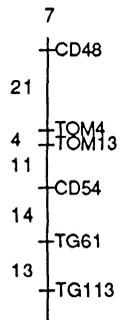
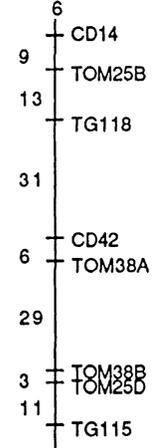
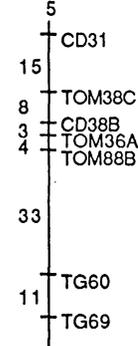
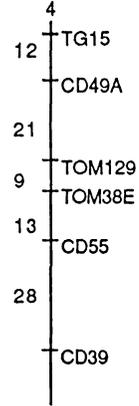
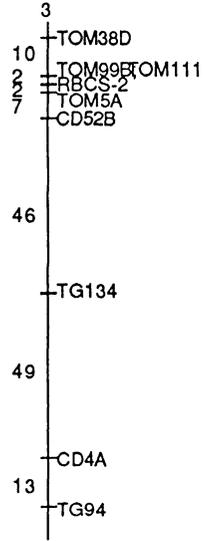
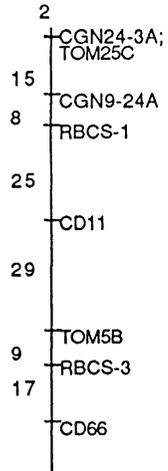
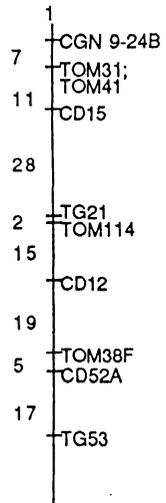
- Mansson PE, Hsu D, Stalker D (1985) Characterization of fruit specific cDNAs from tomato. *Molec Gen Genet* 200:356-361
- Maunders MJ, Holdsworth MJ, Slater A, Knapp JE, Bird CR, Schuch W, Grierson D (1987) Ethylene stimulates the accumulation of ripening-related mRNAs in tomatoes. *Plant Cell and Envir* 10:177-184
- Miller JC (1989) RFLP analysis of phylogenetic relationships, genetic variation, and factors affecting the detection of polymorphism in the genus *Lycopersicon*. Ph D diss, Cornell University
- Mutschler MA, Guttieri M, Kinzer S, Grierson D, Tucker G (1988) Changes in ripening-related processes in tomato conditioned by the *alc* mutant. *Theor Appl Genet* 76:285-292
- Mutschler MA, Tanksley SD, Rick CM (1987) 1987 Linkage maps of the tomato. *Reports of the Tomato Genet. Coop* 37: 5-34
- Rick CM (1966) Abortion of male and female gametes in the tomato determined by allelic interaction. *Genetics* 53:85-96
- Rick CM (1969) Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum*: segregation and recombination. *Genetics* 62:753-768

- Rick CM (1970) The tomato *Ge* locus: linkage relations and geographic distribution of alleles. *Genetics* 67:75-85
- Schwager SJ (1989) The probability distribution of near-matches of balls in an ordered sequence of cells. (in preparation)
- Slater A, Maunder MJ, Edwards K, Schuch W, Grierson D (1985) Isolation and characterization of cDNA clones for tomato polygalacturonase and other ripening-related proteins. *Plant Molec Biol* 5:137-147
- Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble solids content in tomato - a re-examination. *Theor Appl Genet* 75:811-823
- Tanksley SD, Miller JC, Paterson A, Bernatzky R (1988) Molecular mapping of plant chromosomes. In: Gustafson JP, Appels R (eds) *Chromosome structure and function*. Plenum Press, New York pp 157-173
- Tanksley SD, Rick CM (1980) Isozymic gene linkage map of the tomato: applications in genetics and breeding. *Theor Appl Genet* 57:161-170

Vallejos CE, Tanksley SD, Bernatzky R (1986) Localization in the tomato genome of DNA restriction fragments containing sequences homologous to the rRNA (45S), the major chlorophyll A/B binding polypeptide and the ribulose biphosphate carboxylase genes. *Genetics* 112:93-105

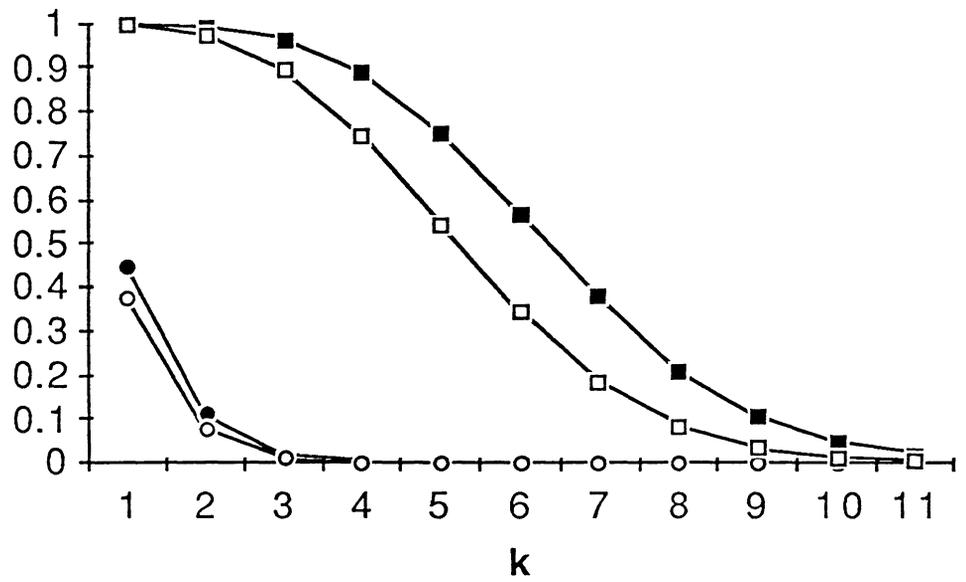
Zamir DZ, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. *Bot Gaz* 147:355-358

**Fig. 1.** RFLP map of tomato genome using clones of known map location (CD, RBCS, and TG) and ripening-specific or -related clones (CGN, or TOM). The numbers above the chromosomes indicate chromosome number.

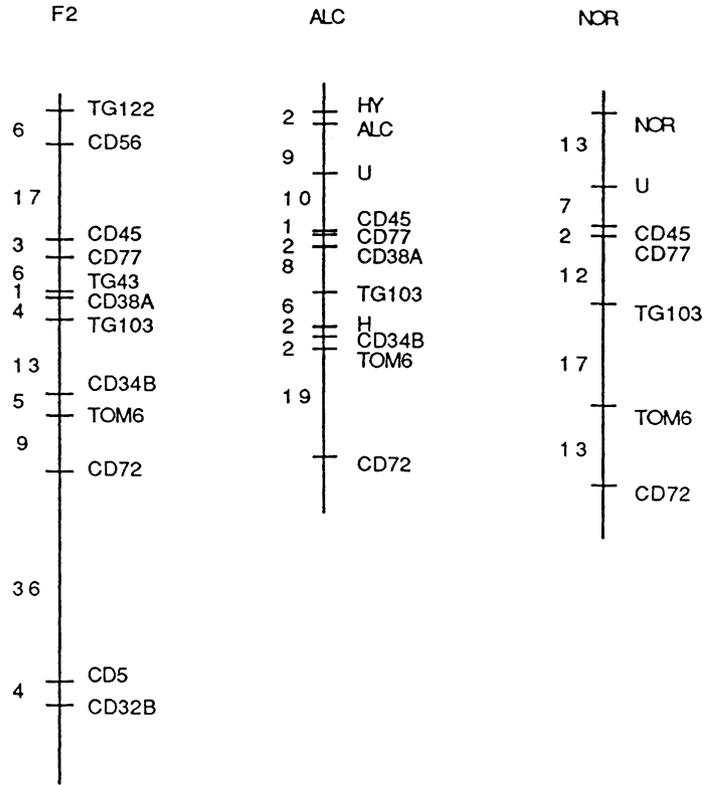


**Fig. 2** The probability of at least  $k$  near-matches, at varying levels of  $m$  and  $r$ , with  $n = 38$ . At  $r = 1200$ ,  $\bullet$  and  $\blacksquare$  indicate  $m = 0$  and  $5$ , respectively. At  $r = 1500$ ,  $\circ$  and  $\square$  indicate  $m = 0$  and  $5$ , respectively.

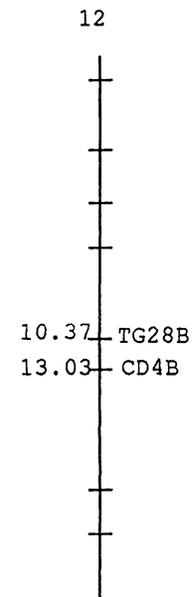
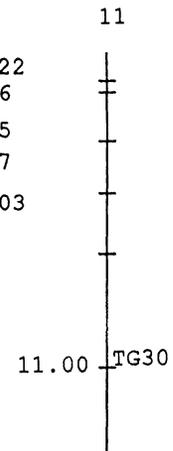
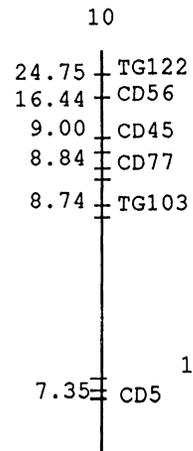
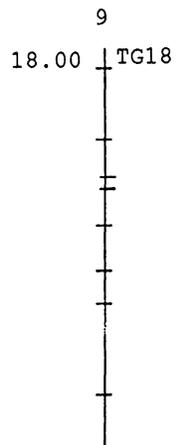
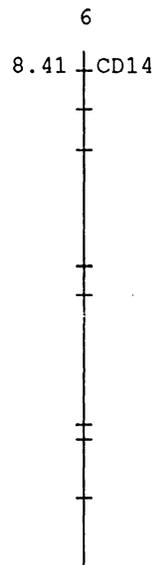
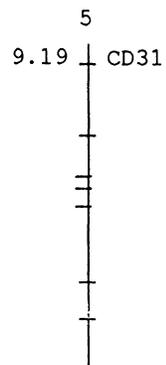
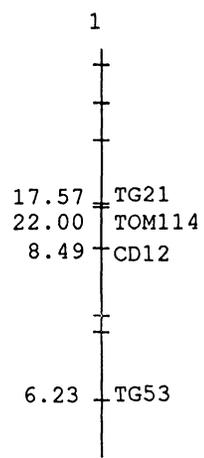
Probability  
of at least  $k$   
near  
matches



**Fig. 3** Maps of chromosome 10 using an *L. esculentum* X *L. pennellii* F<sub>2</sub> population, and (*L. esculentum* X *L. pimpinellifolium*) X *L. esculentum* BC populations segregating for *alc* and *nor*.



**Fig. 4** Loci at which segregation does not fit Mendelian ratios for a single gene at 95% confidence level. Chi-square values are listed to left of each locus. Unlabeled bars indicate loci at which segregation fits Mendelian ratios for a single gene.



**TABLE 1:** Probabilities of observing exactly  $k$  near matches, obtained from calculation and computer simulation\*

$k$	$m=0; r=1200$		$m=0; r=1500$	
	simulation	calculation	simulation	calculation
0	0.5527	0.5532	0.6229	0.6233
1	0.3358	0.3343	0.3007	0.2995
2	0.0936	0.0939	0.0669	0.0669
3	0.0158	0.0163	0.0088	0.0092
4	0.0020	0.0020	0.0007	0.0009
5	0.0001	0.0003	0	0.0002

$k$	$m=5; r=1200$		$m=5; r=1500$	
	simulation	calculation	simulation	calculation
0	0.0008	0.0008	0.0038	0.0039
1	0.0072	0.0074	0.0252	0.0253
2	0.0292	0.0299	0.0782	0.0772
3	0.0763	0.0760	0.1485	0.1476
4	0.1357	0.1360	0.2000	0.1989
5	0.1829		0.2000	
6	0.1890		0.1591	
7	0.1617		0.1006	
8	0.1085		0.0524	
9	0.0616		0.0221	
10	0.0299		0.0074	
11	0.0114		0.0022	
12	0.0039		0.0006	
13	0.0013		0.0002	
14	0.0004		0.0000	

\* Computer simulation (by random number generation) was run 100,000 times to obtain the simulation probabilities

**TABLE 2:** Possible linkages between regions homologous to ripening clones and visible ripening mutants

Chromosome:	One	Two	Three	Five	Six
Ripening clones:	CGN9-24B TOM 31 TOM41 TOM114	TOM5B	TOM5A TOM99B TOM111	TOM36A TOM38C TOM88A	TOM25B TOM25D TOM38A TOM38B TOM88B
Ripening mutants (position):	Never-ripe-2 (-) unpigmented fruit epidermis (30)	peach (67)	yellow flesh (29)	apricot (-) ripening inhibitor (0)	Beta-carotene (106) old gold crimson (106)
Chromosome:	Seven	Eight	Nine	Ten	Twelve
Ripening clones:	TOM4 TOM13	CGN28-8B TOM36C TOM92 TOM94A	CGN24-3B TOM38G TOM88C TOM99A	TOM6 TOM94B	CGN28-8A
Ripening mutants (position):	green stripe (5)	green flesh (44)	Never-ripe (-)	alcobaca (5) Fruit-stripe (16) non-ripening (15) tangerine (95) uniform (19)	high pigment (-)