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RFLP mapping of partially sequenced leaf cDNA clones in maize

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Abstract We report here the results of mapping a set of 92 leaf cDNA clones in maize. The ends of each of these cDNA clones have previously been partially sequenced, and the sequence comparison has revealed the putative function for 28 clones. It is expected that the RFLP map developed using these expressed sequence tags will be of great importance for future maize genome analysis, such as for PCR-based gene mapping or gene function identification.

Key words Maize · Linkage map · Sequence tagged sites · Expressed sequence tags

Introduction

A RFLP based genetic map for maize consisting of 215 markers has been developed previously by us using an immortalized F_2 mapping population between CO159 and Tx303 (Gardiner et al. 1993). Of 215 mapped markers, 35 are cDNA clones with identified gene functions. These cDNA clones have proved useful in aligning the molecular map with the classical genetic map since the map locations for several of these functionally defined probes are found to correspond with loci that had previously been genetically defined. For example, Wright et al. (1992) showed that a cDNA clone encoding sequence for tryptophan synthase β mapped to the chromosome regions where orange pericarp loci had previously been located.

Recently, 130 maize cDNA clones randomly selected from a mature leaf library have been partially sequenced (Keith et al. 1993). Sequence comparison revealed that over 20% of these clones showed similarity to previously sequenced maize genes or genes from other organisms. In the study presented here, RFLP analysis was done on a selection of 92 of these clones. The results described here will not only help to improve the resolution of our RFLP map, but the mapped cDNA clones will also allow us to find correspondence with genetically mapped traits.

Source and characterization of the cDNA clones

The origin of these clones, their partial sequencing, a list of database matches, and the GenBank accession numbers of identified clones have previously been published (Keith et al. 1993). Nine additional clones giving database matches were identified as a result of recent (ending Sept. 17, 1993) database searches using the BLAST server (Alt-

Table 1 Maize cDNA clones with sequence similarity to functional genes

Clone number[a]	RFLP number[b]	Gene description
csuh17	umc314	RNA binding protein
csuh19	umc315	Cold regulated gene
csuh63	umc343	DNA J protein
csuh74	umc349	Ferredoxin
csuh111	umc368	Glutathione reductase
csuh137	umc374	MADS box gene
csuh146	umc379	Putative membrane ATPase, ftsH
csuh148	umc381	Calnexin
csuh149	umc382	A short chain alcohol dehydrogenase

[a] Clone number is the California State University, Hayward, series used for partial sequencing
[b] Probe number is the umc series used for RFLP analysis

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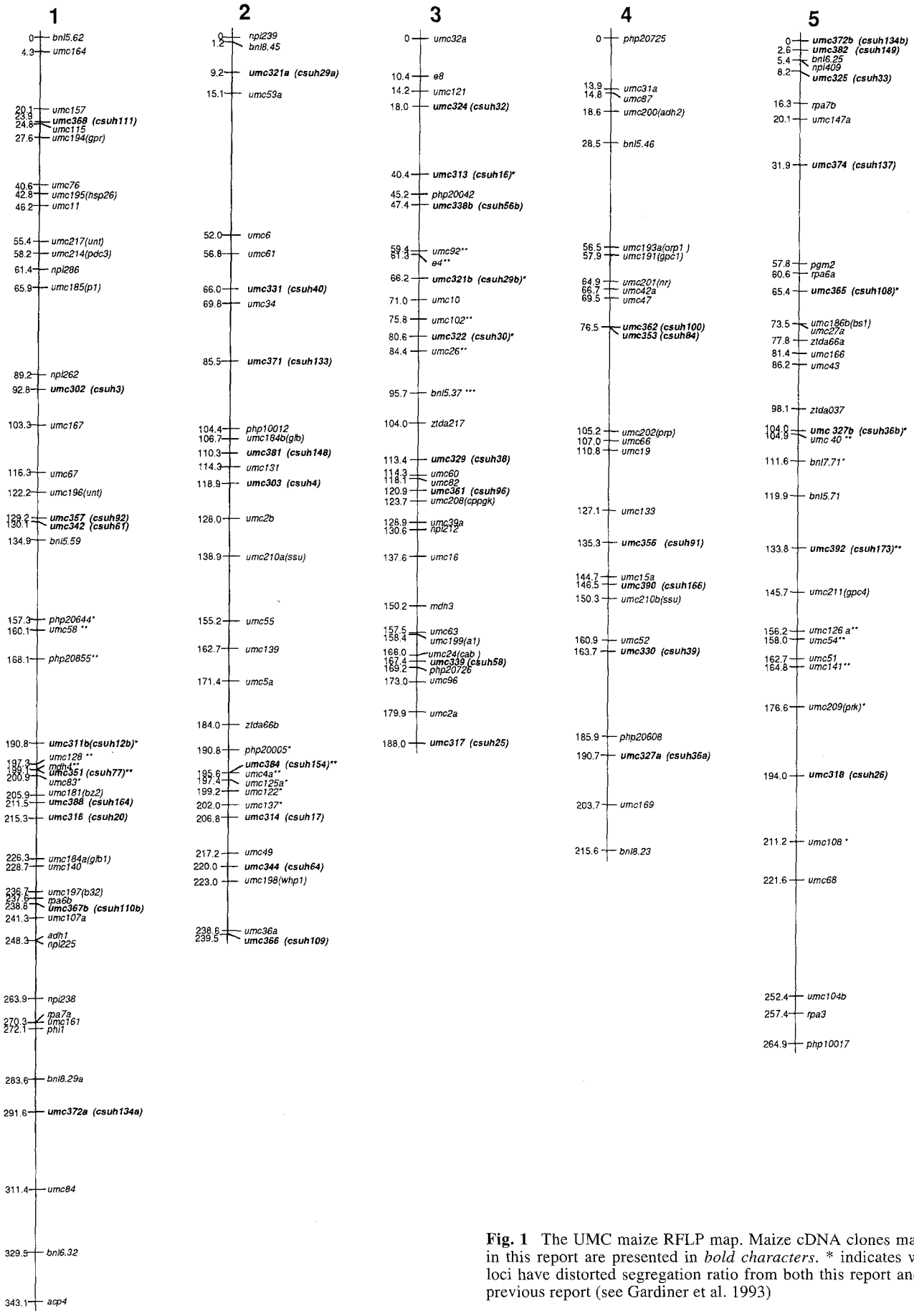
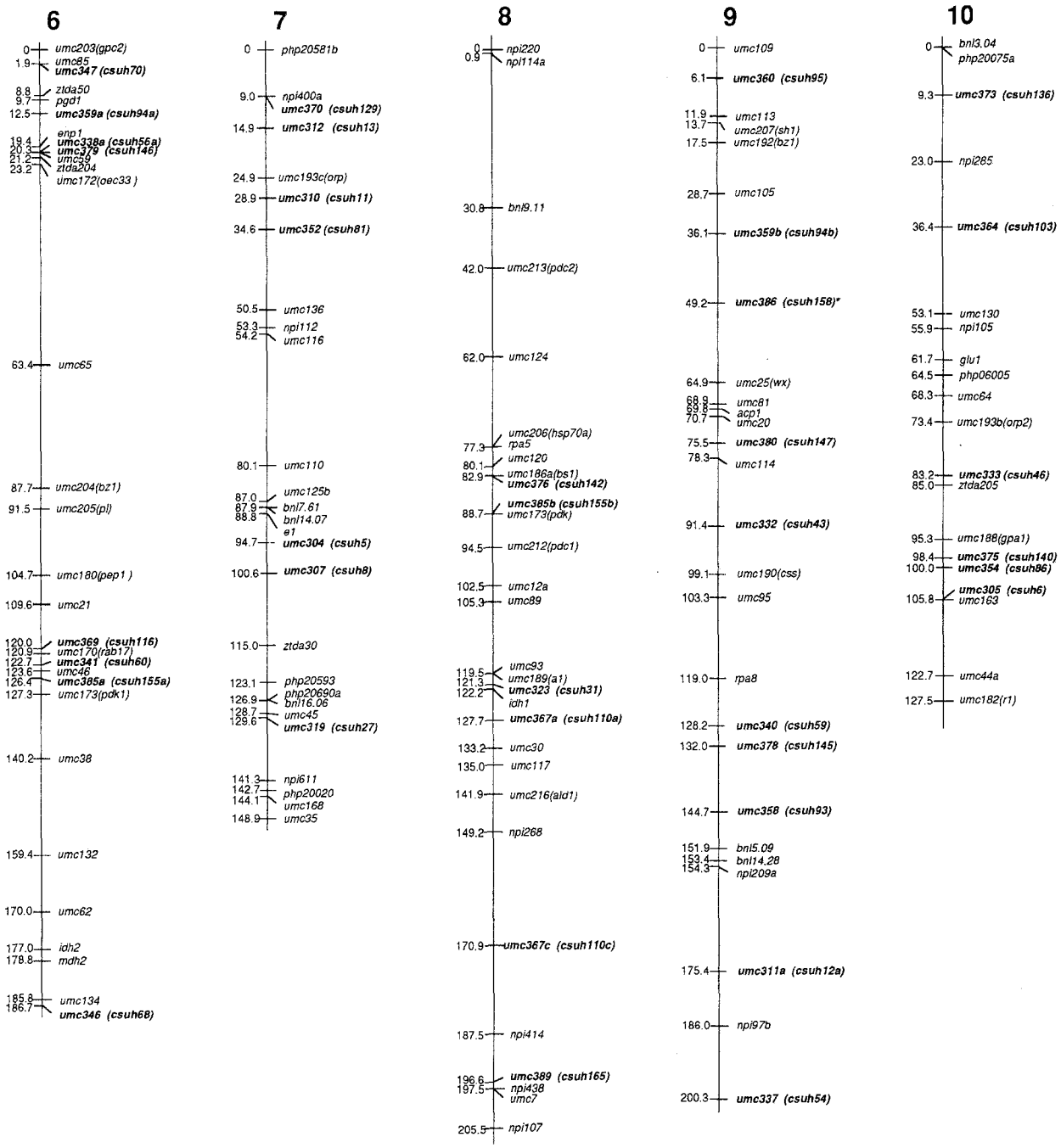


Fig. 1 The UMC maize RFLP map. Maize cDNA clones mapped in this report are presented in *bold characters*. * indicates where loci have distorted segregation ratio from both this report and our previous report (see Gardiner et al. 1993)



For legend of Fig. 1, see left

schul et al. 1990) at the National Center for Biotechnology Information (Table 1).

Clone information and sequence data availability

GenBank accession numbers and dbEST numbers have been issued to all of the cDNA clones used in this study. Clone information, including GenBank accession number, RFLP mapping data and other related information for each cDNA clone can be retrieved directly from the Maize Genome Database through Gopher (see Maize Genet Coop Newsl 63:170, 1993). The sequence data and sequence analysis results can be accessed from GenBank through Biology Gopher at Indiana University.

RFLP analysis

Eight restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI, *Dra*I, *Bgl*II, *Xba*I and *Sst*I, were used to screen for polymorphism between CO159 and Tx303. Seventy-eight clones showed polymorphisms with at least one enzyme, while among the 14 clones detecting no polymorphism, at least 7 of them showed a smear or unrecognizable banding pattern. Altogether, 70 out of 92 clones hybridized to

single- or low-copy sequences; 63 of these were polymorphic and given preference for RFLP mapping. RFLP mapping was attempted for probes giving complex banding patterns as well, but only the interpretable bands were scored.

Probe availability

All the cDNA clones described in this report can be released for general use and are available from the author (S Chao, FAX: 314-874-4063, e-mail: agronsc@mizzou1.missouri.edu).

Probe nomenclature

The cDNA clones were given a umc number from 301 to 392 followed by their original clone number in parenthesis. For instance, umc301(csuh2) denotes that the probe used for RFLP analysis is umc301 and that the original clone used for partial sequencing is number 2 of the California State University, Hayward, series.

Linkage analysis and RFLP map evaluation

Linkage analyses were performed using MAPMAKER (Lander et al. 1987; the Macintosh version supplied by Du Pont). The new segregation data were added to a preexisting dataset (Gardiner et al. 1993). The 'group' command with a LOD score of 3.0 and a recombination fraction of 0.4 was first applied. Then the placement of new markers was done by using the 'multipoint/try' command. The best fit order was used to add 71 cDNA probes (or 78 loci) to our present RFLP map (Fig. 1).

The mapping results indicate that the distribution of these cDNA clones seems to be at random on the map. One probe (umc334) showed no linkage to any of the ten chromosomes using a LOD score of 3, indicating substantial regions of the maize genome may still be unmarked. A few cDNAs were mapped to the ends of the chromosomes, and these may facilitate the mapping of telomere markers. Locus umc351 has sequence similarity to malate dehydrogenase and was mapped to the same site as the isozyme locus *mdh4* on chromosome 1 long arm, confirming the function associated with this clone. Chi-square goodness-of-fit- tests were done for all of the mapped cDNA clones and those with distorted segregation ratios are listed in Table 2. It is interesting to note that these skewed cDNA markers are located in the chromosomal regions where their neighboring markers were previously shown to also have skewed segregation ratios (Gardiner et al. 1993). Locus umc386 on chromosome 9 was an exception, this clone contains a sequence for enolase. When compared with other maize RFLP maps (for example, the 1993 version of the map of B. Burr, as distributed) it is obvious that on our map the region flanked by umc105 and umc25(wx) on the short arm of chromosome 9 is expanded. The distorted segregation at umc386 locus, which mapped in between these 2 markers, may contribute to the expansion. The placement of additional RFLP markers in this region or examination of the isozyme segregation pattern should clear up this ambiguity.

Table 2 Maize cDNA clones with distorted segregation ratio

Clone no.	Chromosome	Genotypes			χ^2
		Tx	Tx/CO	CO	
umc311b(csuh12b)[a]	1	35		21	4.67*
umc351(csuh77)	1	6	28	22	9.14**
umc384(csuh154)	2	15	37	4	10.11**
umc313(csuh16)	3	8	37	11	6.11*
umc321b(csuh29b)	3	10	38	8	7.29*
umc322(csuh30)	3	10	39	7	8.96*
umc365(csuh108)	5	6	37	13	7.54*
umc327b(csuh36b)	5	10	39	7	8.96*
umc392(csuh173)	5	9	40	7	10.43**
umc386(csuh158)	9	6	38	12	8.43*

[a] The locus was tested for goodness of fit to a 3:1 ratio
* $P<0.05$, ** $P<0.01$

Conclusion

The collected results of many cDNA sequencing projects (Grausz and Auffray 1993; Sikela and Auffray 1993) show that partial sequencing is an efficient way of identifying genes. It is clear that additional maize genes will also be identified using this approach. The end sequences of each cDNA clone also provide STS (sequence tagged site) information necessary for polymerase chain reaction (PCR)-based maize genome analysis. Such an STS-based strategy has been demonstrated in plants such as rice (Williams et al. 1991), barley (Tragoonrung et al. 1992) and *Arabidopsis* (Konieczny and Ausubel 1993). In addition, the mapped genes can not only be used as markers for genetic or physical mapping of the maize genome, they can also serve as candidate gene loci for mutant phenotypic traits.

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Note added in proof

A recent agreement among laboratories has made some changes in the nomenclature for maize RFLP markers. The mapped cDNA clones reported here have been renamed as 'csu' clones; the clone number remains the same but both umc and csuh names will become obsolete.