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Abstract

A method for finding the genetic map position of cloned DNA fragments

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A method for finding the genetic map position of cloned DNA fragments.

Some genes cannot be readily mapped by classical methods. Examples are genes of unknown function, multiple genes of identical function, or any other genes for which mutants have not been found. We describe a method to genetically map any cloned DNA (gene or otherwise). The method uses restriction fragment length polymprphisms (RFLPs) as genetic markers, and does not rely on gene function. The logic of the approach we describe below has been worked out in detail for the more complex case of a diploid organism by Botstein et al. (1980, Am. J. Human Genet. 32: 314-331) following work by others on isoenzyme polymorphisms.

Principle: A cross of a laboratory strain of Neurospora crassa with a nominally "Oak Ridge" genetic background and carrying several conventional markers is made to a wild-collected strain which has not been inbred with laboratory strains. Such a cross is, in a sense, "marked" not only by the conventional markers, but by thousands of nucleotide differences scattered throughout the genome. The differences will be useful for mapping if they result in restriction site differences between the two strains. Any cloned DNA fragment can be genetically mapped if a restriction fragment length difference is found in the homologous genomic DNA. To map the DNA, it is only necessary to be able to classify each random progeny from such a cross as having the allele from one parent or from the other. The allele assignments are then compared with the allele segregation of markers that have already been mapped (These can be the conventional markers that were used originally in the cross, or the can be other molecular markers that have been mapped subsequently relative to the conventional markers.) Linkage is detected in the usual way: by recombination between two markers being significantly less than 50%.

Practice: The Crosses: We have made and examined a number of such crosses, but the bulk of our data are from two of them. In each of these cases, the "exotic", wild-collected strain was Mauriceville-lc-A, P538 (FGSC #2225). Two different laboratory strains with largely or partly Oak Ridge ancestry were used as parents: (I) al-2;nuc-2;arg-12;cot-1;inl - a (FGSC #4411). This strain was crossed to Mauriceville strain and random progeny spores, presumably all from different asci , were isolated and cultures prepared from them. These cultures were sorted by color and morphology so that about half the progeny included in the experiment would be of each allele. Progeny were then scored for the nutritional markers. arg-12 and nuc-2, which are quite closely linked, segregated together in all cases, so only arg-12 is recorded below. (The battery of strains; to make a convenient kit, the Mauriceville parent is included here among the sequentially-numbered strains as #4416, even though it is also present in the FGSC as #2225.) Segregation of markers is listed in Table I. (2) "multicent-2-a", a strain marked near the centromere of all linkage groups except VI, and carrying several other conventional markers as well. This strain is un-2;arg-5;thi-4;pyr-1;lys-1;inl;nic-3, ars-1 - a (FGSC #4488). Progeny from ordered tetrads were scored for these markers. From each ascus, cultures from two nonsister spores from the same half of the ascus were studied further and ultimately deposited in the Fungal Genetics Stock Center. If these two are concordant for a RFLP, segregation is first-division; if discordant, the ascus is classified as second-division. By comparison of the RFLP type with the segregation of any one of the conventional markers, each ascus can also be classified as parental ditype, non-Parental ditype, or tetratype. Segregation of markers is listed in Table II. Note that in the case of ascus E, an isolate from each of the four spore pairs has been deposited. This allows one to see whether the gene being studied is undergoing Mendelian segregation.

Detailed Procedure

A. Steps that need only be done once and have already been done:
   1. Perform the crosses, isolate progeny.
   2. Score for conventional and molecular markers.

B. Steps that need only be done once in each lab:
   1. Grow the set of parental and progeny strains from one or both of the two crosses. The strains are grown at 25° C in' Vogel's medium with 2% sucrose, supplemented in the first cross with 1 mM L-arginine and 50 µg/ml inositol, and for the second cross with 1 mM L-citrulline, 1 mM uridine, 1 mM L-lysine, 50 µg/ml inositol, and 2 µg/ml each of thiamine HCl and nicotinamide. (Citrulline rather than arginine is used to avoid competition with lysine for uptake.)

C. Steps that need to be done for each cloned DNA segment that is to be mapped:
   1. Prepare plasmid or phage DNA carrying the segment and label it radioactively by nick translation or other means.
   2. Prepare digests of DNA from the two parental strains of a cross, with several arbitrarily chosen restriction enzymes to find one that will show a usable RFLP.
   3. Prepare Southern blots from agarose gel.electrophoresis of these digests and probe.
   4. Choose the best enzyme, digest an aliquot of DNA from progeny as well as from parentals, blot, probe and score. (In practice, we often skip steps 2 and 3 and arbitrarily choose one or more enzymes to try simultaneously with parentals and progeny. The amount of polymorphism, at least in the region of the 5S rDNA genes, is large enough so that this is a reasonably efficient strategy.)

Illustrative Example: Mapping the Hypothetical Gene, "X".

The enzyme EcoRI shows a good RFLP with the two parental strains of the cross of al-2;nuc-2,arg-12; cot-1;inl - a to Mauriceville-lc - A. A genome blot having the parentals in lanes 01 and 06 and progeny in the other lanes shows the following (Figure 1).

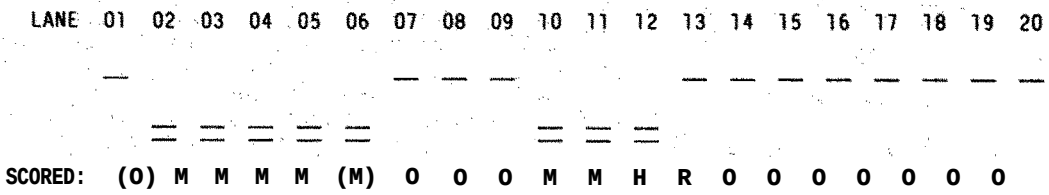

Figure 1. -- Segregation of a hypothetical molecular marker.

The scoring strip can be compared with the information listed for previously scored genes. In the present case, it is obvious that Gene X segregates in the same way as mating type (A/a) and therefore is at or near that gene on LG I (see Table II). It is also easy to map genes into this cross even if they are on chromosomes that were not previously marked by conventional markers. This has been done by crossing "Mauriceville" to an "Oak Ridge" strain that is suitably marked (e.g., in LG VI with chol-2, ylo-1, trp-2), scoring for the linked molecular marker (5S gene 50), and then mapping the 5S gene 50 into the original cross. A marker from the right arm of LG III, 5S gene 45, has similarly been mapped into the cross.

Other Crosses:

We have made a number of other crosses that are somewhat more satisfactorily marked on particular chromosomes, and in which 5S genes have been sometimes more closely mapped than with the two general-purpose crosses listed here. We have not deposited these in the FGSC because of the large number of them involved, and because of our belief that it would be better to expand the data base on progeny of one or two crosses only. One set of crosses that has been useful to us allows detection of a cloned gene at or near the tip of any arm except IIIL This is done with insertional translocations, which move a distal portion of one chromosome to another chromosome arm. Crossing of such a strain to Mauriceville-lc - A allows isolation of a partial diploid which is heterozygous for any genes that are covered by duplication. While there is not yet a translocation on hand in which each arm of each chromosome has functioned as a donor, every arm except LG IIIL has acted either as a donor and/or recipient. Thus a gene at the tip of a linkage group can be identified either by its heterozygosity in the case of a donor, or by its tight linkage to the junction, in the case of a recipient. Progeny of some or all of these crosses will be deposited if there seems to be interest in them.

Making a Rough Map:

Data from these two crosses and a number of others have allowed us to make a composite map of the 5S genes and the rDNA (nucleolus organizer). This is shown in Figure 2. Fine detail of the gene order is somewhat uncertain, being based on differences of one or two crossovers, and the genetic distances are not shown. Even with these limitations, the method allows rough mapping to be done quickly and easily. If the strains are used in other laboratories, the data base will expand and even mapping that has already been done will become increasingly accurate.

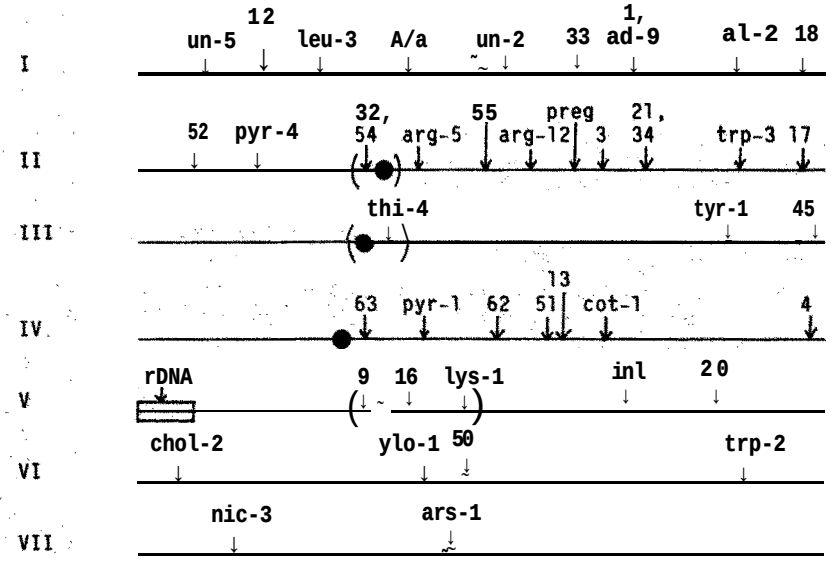

Figure 2. -- Map of some conventional and molecular markers. Distances are not to scale (see text), and minor errors in order are possible.

TABLE I

Segregation of conventional markers and of 5S and of rDNA in the cross of *al-2;arg-12,nuc-2;cot-1;inl*-a x Mauriceville-1c-A

M = like Mauriceville parent, O = "Oak Ridge" parent. Those shown with a dash could not be scored because of poor restriction digestion or background interference. Genes represented only by numbers are 5S DNAs.

		LG I						LG II						LG III	LG IV					LG V					LG VI
isol.	FGSC#	12	A/a	33	1	al-2	18	52	32, 54	55	arg-12	21, 34	17	45	63	62	13, 51	cot-1	4	rDNA	16	9	inl	20	50
1	4411	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
2	4412	0	M	M	M	M	M	0	0	0	0	0	0	0	M	M	M	M	0	M	M	M	0	0	M
3	4413	M	M	M	M	M	M	0	0	0	0	0	0	0	M	M	M	M	M	0	0	0	0	0	M
4	4414	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	0	0	0	M	M	M
5	4415	M	M	M	M	M	M	0	M	M	M	M	M	0	0	0	0	M	M	0	0	0	0	0	0
6	4416	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)	(M)
7	4417	M	0	0	0	0	0	0	0	M	M	M	M	0	M	M	M	M	M	0	0	0	M	M	M
8	4418	0	0	0	0	0	0	M	M	M	M	M	0	M	M	M	M	M	M	M	M	M	M	0	M
9	4419	0	0	0	0	0	0	0	0	0	0	0	0	M	M	M	M	M	0	M	M	M	M	M	0
10	4420	M	M	M	0	0	0	0	0	0	0	0	0	0	M	M	M	M	0	0	M	M	M	M	0
11	4421	M	M	M	M	M	M	0	0	0	M	M	M	0	M	M	0	0	-	0	M	M	M	M	M
12	4422	0	M	M	M	M	M	0	0	0	0	M	M	0	0	0	0	0	0	0	M	M	M	M	M
13	4423	0	0	0	M	M	0	0	M	M	M	M	M	M	0	0	0	0	M	M	M	M	M	M	M
14	4424	0	0	0	0	0	0	0	0	0	0	0	M	0	0	0	0	0	M	0	0	0	M	M	M
15	4425	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	M	M	M	M	0	0	M
16	4426	0	0	0	0	0	0	M	M	M	M	M	0	M	0	0	0	0	0	0	0	0	M	M	0
17	4427	M	0	0	0	0	0	0	0	0	0	M	M	M	0	0	0	0	M	M	0	0	0	M	0
18	4428	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	M	0	M	M	M	M	M
19	4429	-	0	0	0	0	0	0	M	M	M	M	-	0	0	0	0	0	M	M	M	M	M	M	M
20	4430	-	0	M	0	0	0	0	0	M	M	M	-	M	0	0	0	0	M	M	M	M	M	M	M

TABLE II

Segregation of markers in cross 2
(See text and legend of Table I.)

		LG I						LG II						
isol.	FGSC#	12	A/a	m-2	33	1	18	52	32	arg-5	55	3	34	17
A1	4450	M	M	M	M	M	M	O	M	M	M	O	O	O
A4	4451	O	O	M	M	O	O	M	M	M	M	M	M	M
B6	4452	M	M	M	M	M	M	O	O	O	O	O	O	O
B7	4453	M	M	M	M	M	O	O	O	O	O	O	O	O
C1	4454	M	O	O	O	M	O	-	M	M	M	M	M	M
C4	4455	O	O	O	M	O	O	M	M	M	M	M	M	M
D5	4456	M	M	M	M	O	O	O	O	O	O	O	O	O
D7	4457	O	M	M	M	M	M	O	O	O	O	O	M	M
E1	4458	M	M	M	M	M	M	M	M	M	M	M	M	M
E3	4459	M	M	M	M	M	M	M	M	M	M	O	O	O
E5	4460	O	O	O	O	O	O	O	O	O	O	O	O	O
E7	4461	O	O	O	O	O	O	O	O	O	O	M	M	M
F1	4462	M	M	M	M	-	M	O	O	O	O	O	M	O
F3	4463	M	M	M	M	O	O	O	O	O	O	O	O	M
G1	4464	M	M	M	M	M	O	M	M	M	M	M	M	O
G4	4465	O	M	M	M	O	M	M	M	M	M	M	M	M
H5	4466	O	O	M	M	M	M	M	M	M	M	M	M	M
H7	4467	M	M	M	M	M	M	M	M	M	M	M	O	O
I6	4468	M	O	M	M	M	O	M	M	M	M	O	O	O
I8	4469	M	M	M	M	M	M	M	M	M	M	M	M	O
J1	4470	M	M	M	M	M	M	O	M	M	M	M	M	M
34	4471	M	M	M	M	M	O	M	M	M	M	M	M	M
K1	4472	O	O	O	O	O	O	O	M	M	M	O	O	O
K4	4413	O	O	O	O	M	O	M	M	M	M	M	M	M
L1	4474	O	O	O	M	M	M	O	O	M	M	M	M	O
L4	4475	O	O	O	O	O	O	M	M	M	M	M	M	M
M5	4476	O	O	O	O	O	M	M	M	M	M	M	M	M
M8	4477	O	O	O	O	O	O	M	M	M	M	O	O	O
N2	4478	O	O	O	O	O	O	O	O	O	O	O	O	-
N3	4479	-	O	O	O	-	M	O	O	O	M	M	M	M
O2	4480	O	O	M	M	M	O	-	O	O	O	O	O	M
O4	4481	M	M	M	M	O	M	M	O	O	O	O	O	O
P1	4482	O	O	M	O	O	O	O	O	O	O	O	O	O
P4	4483	M	M	M	O	M	M	O	O	O	O	O	O	M
Q2	4484	M	O	O	O	O	O	M	M	M	M	M	M	M
Q4	4485	O	O	O	O	O	O	M	M	M	M	M	M	M
R1	4486	M	M	M	M	O	O	O	O	O	O	O	O	O
R4	4487	M	M	M	M	M	M	O	O	O	O	M	M	M

TABLE II (Continued):

	LG III		LG IV							LG V						LG VI	LG VII	
isol.	thi-4	45	63	pyr-1	62	51	13	4	Isol.	rDNA	l6	9	lys-1	inl	20	50	nit-3	ars-1
Al	O	M	M	M	M	M	M	M	Al	O	M	M	M	M	O	M	M	O
A4	O	O	M	M	O	O	O	O	A4	M	M	M	M	M	M	M	O	O
B6	O	M	M	M	M	M	M	M	B6	M	O	O	O	O	O	M	O	O
B7	O	O	O	O	O	O	O	O	B7	O	O	O	O	O	O	M	O	O
Cl	M	O	M	M	M	M	M	M	Cl	M	O	O	O	M	M	M	M	M
C4	M	O	M	M	O	O	O	O	C4	M	M	M	O	O	M	M	M	M
D5	M	O	O	O	O	O	O	O	D5	M	O	O	O	O	O	M	M	M
D7	O	M	O	O	O	O	O	M	D7	O	O	O	O	M	M	M	M	M
El	O	O	M	M	M	M	M	M	El	M	O	O	O	O	O	O	O	O
E3	O	M	M	M	M	M	M	O	E3	O	O	O	O	O	O	O	O	O
E5	M	O	O	O	O	O	O	O	E5	M	M	M	M	M	M	M	M	M
E7	M	M	O	O	O	O	O	M	E7	O	M	M	M	M	M	M	M	M
Fl	M	M	O	O	O	O	O	O	Fl	M	-	M	M	M	M	-	O	O
F3	M	O	O	O	O	O	O	M	F3	M	M	M	M	O	M	M	O	O
Gl	M	M	M	M	M	M	O	O	G1	M	O	O	M	M	M	O	O	O
G4	M	M	M	M	M	M	M	M	G4	O	O	O	M	M	M	O	M	O
H5	M	O	O	O	O	O	O	O	H5	M	M	M	M	M	M	O	O	O
H7	M	O	O	O	M	M	M	O	H7	O	M	M	M	M	M	O	O	O
I6	O	M	M	M	O	O	O	O	I6	O	–	O	O	O	O	O	M	O
I8	O	O	M	M	O	O	O	M	I8	M	-	O	O	M	M	O	O	O
Jl	M	M	M	M	M	M	M	O	Jl	M	–	M	M	O	O	O	O	O
J4	M	O	M	M	O	O	O	O	J4	O	–	M	M	M	M	O	O	O
Kl	O	O	M	M	M	M	M	O	Kl	M	M	M	M	M	M	O	O	O
K4	O	M	M	M	M	M	M	M	K4	M	M	M	M	O	M	O	O	O
Ll	O	O	M	M	M	M	M	O	Ll	M	O	O	O	O	O	M	O	M
L4	O	O	M	M	M	M	M	M	L4	O	O	O	O	M	M	M	M	M
M5	O	O	M	M	M	M	M	O	M5	M	M	M	M	M	M	O	M	M
M8	O	M	M	M	M	M	M	M	M8	O	M	M	M	M	M	O	O	M
N2	M	O	O	O	M	O	O	O	N2	O	O	O	O	O	M	O	O	O
N3	M	O	-	O	O	O	O	-	N3	O	-	O	O	M	-	-	O	O
O2	M	O	O	O	O	O	O	M	O2	M	M	M	M	O	O	M	M	M
O4	M	M	O	O	M	M	M	O	O4	M	M	M	M	M	M	M	M	M
P1	M	M	M	M	M	M	M	O	Pl	O	M	M	M	M	M	M	O	O
P4	O	O	O	O	O	O	O	M	P4	M	-	M	M	M	O	M	O	O
Q2	M	O	M	M	M	M	M	M	Q2	M	-	M	M	O	O	O	M	M
Q4	M	M	M	O	O	O	O	M	Q4	M	-	M	M	M	M	O	M	M
Rl	O	M	M	M	M	M	M	M	RI	O	O	O	O	M	M	M	O	O
R4	O	O	M	M	M	M	M	O	R4	M	O	O	O	O	O	M	O	O

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