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Restriction map of a 35-kb *HLA* fragment constructed by nested deletion 'drop-out' mapping

(Transposon; transposition; γδ; Tn*1000*; clone; deletion factory)

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SUMMARY

An efficient method for generating detailed restriction maps of large cloned DNA segments is demonstrated. The mapping strategy entails comparing restriction fragments from a parent clone and from nested deletion derivatives of that clone. In a set of deletion plasmids of decreasing size, an individual fragment will be lost, or 'drop-out', according to its position in the cloned fragment. In this demonstration, nested deletions were generated in both directions in a 35-kb DNA segment from the human leukocyte antigen (*HLA*) region by intramolecular transposition of an engineered γδ (Tn*1000*) element present in a special 'deletion factory' cloning vector [Wang et al., Proc. Natl. Acad. Sci. USA 90 (1993) 7874–7878]. Fifteen plasmids with deletions extending in one direction and eleven plasmids with deletions extending in the opposite direction were digested singly by each of four restriction enzymes. A total of 36 cleavage sites were mapped in the 35-kb *HLA* fragment. This drop-out approach using nested deletions provides a simple and efficient means of mapping restriction sites, genes and other features of interest in cosmid-sized cloned DNA segments or DNAs.

INTRODUCTION

Detailed restriction maps are valuable for aligning clones and for locating genes and other features of special interest. Their utility in large-scale mapping has been demonstrated (e.g., Olson et al., 1986; Kohara et al., 1987; Riles et al., 1993; Smith et al., 1994). However, restriction maps are so laborious to construct by conventional methods that they are usually bypassed in large scale gene mapping and sequencing projects.

We recently described pDUAL and pDELTA cosmid vectors that were designed for generating nested deletions in vivo (Wang et al., 1993a,b). In these 'deletion factory' vectors, intramolecular movement of an engineered bacterial transposon, γδ (Tn*1000*), yields clockwise and counterclockwise deletion plasmids that can be selected on simple bacteriological media. The aim of the present study was to demonstrate the use of nested deletions for constructing detailed restriction maps of cosmid-sized cloned DNAs.

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Abbreviations: *amp*, Ap^R-encoding gene; Ap, ampicillin; bp, base pair(s); Cm, chloramphenicol; *HLA*, human leukocyte antigen-encoding gene(s); *kan*, Km^R-encoding gene; kb, kilobase(s) or 1000 bp; Km, kanamycin; MCS, multiple cloning site(s); *ori*, origin(s) of DNA replication; ^{R}, resistance/resistant; ^{S}, sensitive/sensitivity; *sacB*, Suc^S-encoding gene; Sm, streptomycin; *strA (rpsL)*, Sm^S-encoding gene; Suc, sucrose; Tc, tetracycline; *tet*, Tc^R-encoding gene; Tn, transposon; γδ, Tn gamma delta (Tn*1000*).

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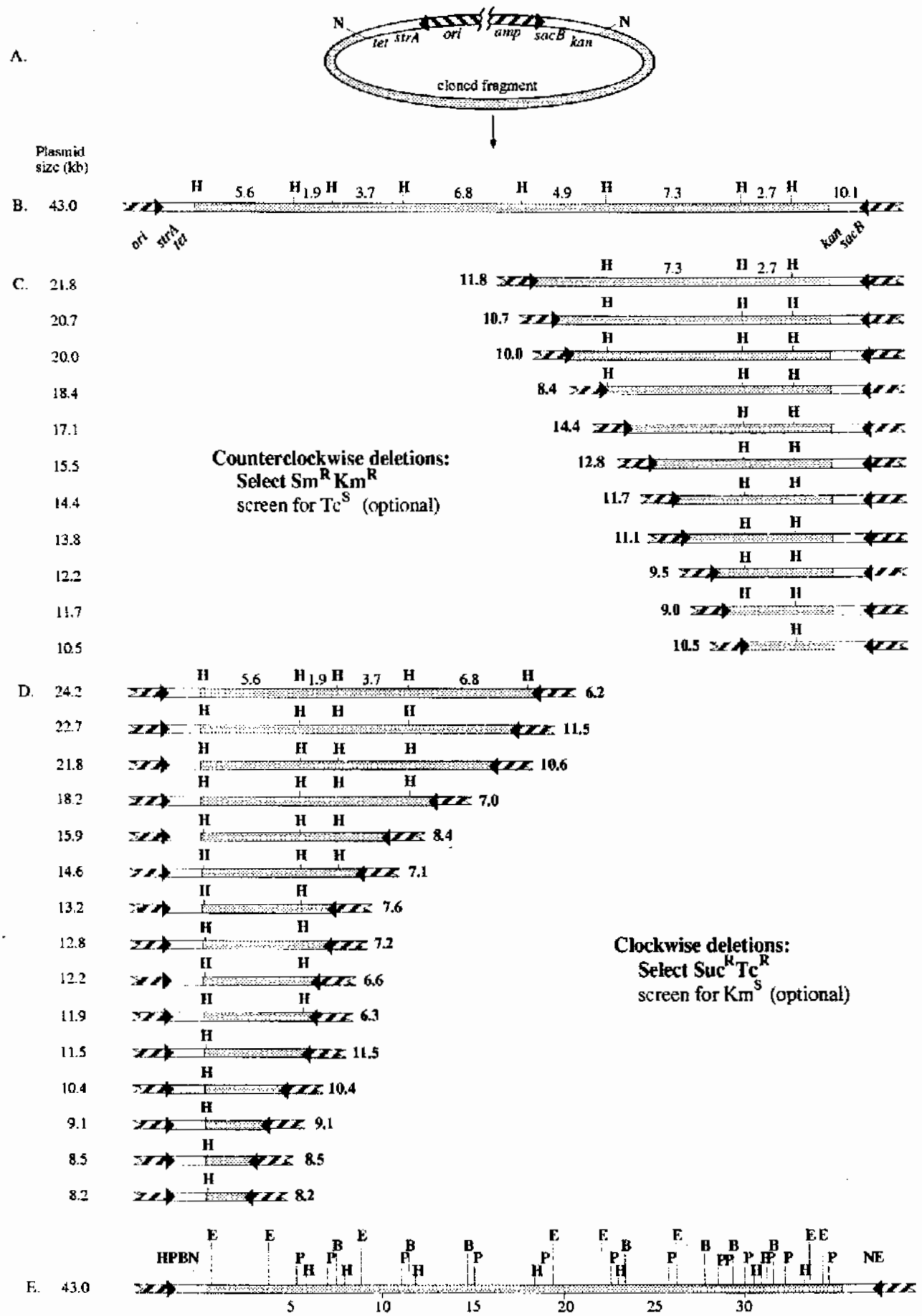
A.
N
tet
strA
ori
amp
sacB
kan
N
cloned fragment
Plasmid size (kb)
B. 43.0
H 5.6 H 1.9 H 3.7 H 6.8 H 4.9 H 7.3 H 2.7 H 10.1
ori
strA
tet
kan
sacB
C. 21.8
20.7
20.0
18.4
17.1
15.5
14.4
13.8
12.2
11.7
10.5
11.8
10.7
10.0
8.4
14.4
12.8
11.7
11.1
9.5
9.0
10.5
Counterclockwise deletions:
Select Sm^R Km^R
screen for Tc^S (optional)
D. 24.2
22.7
21.8
18.2
15.9
14.6
13.2
12.8
12.2
11.9
11.5
10.4
9.1
8.5
8.2
6.2
11.5
10.6
7.0
8.4
7.1
7.6
7.2
6.6
6.3
11.5
10.4
9.1
8.5
8.2
Clockwise deletions:
Select Suc^R Tc^R
screen for Km^S (optional)
E. 43.0
HPBN
NE
5
10
15
20
25
30

Fig. 1. Generation of a *Hin*dIII restriction map of pL14DEL1 by drop-out mapping. Symbols: ◄ and ►, 40-bp inverted transposon γδ ends in vector; ZZZ and SSS, vector segments interior to transposon ends; ———, vector segments between transposon ends and cloned fragment; ▒▒▒▒, cloned *HLA* fragment. (A) Circular depiction of the parental 43-kb clone. The *Not*I (N) sites were generated by cloning a *Not*I fragment into the unique *Not*I site of the vector. (B) Linear depiction of the parental clone. The ordered *Hin*dIII (H) sites are shown. Numbers represent the *Hin*dIII-fragment sizes (in kb). (C) Counterclockwise deletion derivatives from colonies selected on Sm + Km plates and screened for Tc^S. Fusion fragment sizes (bold, in kb) are listed to the left of the map of the deletion derivative. (D) Clockwise deletion derivatives from colonies selected on Suc + Tc plates and screened for Km^S. The data tabulated below were used to order the fragments. Fusion fragment sizes (bold, in kb) are listed to the right of the map of the deletion derivative.

Sizes of *Hin*dIII fragments in pL14DEL1 and in deletion derivatives (see panels B and D)

	Plasmid (kb)	Restriction fragments (kb)								Fusion
		Parental								
Parent plasmid	43.0	10.1	7.3	6.8	5.6	4.9	3.7	2.7	1.9	
Suc^RTc^R (clockwise deletions)	24.2			6.8	5.6		3.7		1.9	6.2
	22.7				5.6		3.7		1.9	11.5
	21.8				5.6		3.7		1.9	10.6
	18.2				5.6		3.7		1.9	7.0
	15.9				5.6				1.9	8.4
	14.6				5.6				1.9	7.1
	13.2				5.6					7.6
	12.8				5.6					7.2
	12.2				5.6					6.6
	11.9				5.6					6.3
	11.5									11.5
	10.4									10.4
	9.1									9.1
	8.5									8.5
	8.2									8.2

(E) *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H) and *Pst*I (P) restriction map generated from single digestion data. The data were compiled as shown above. The adjusted single-enzyme restriction maps were overlaid to create a four-enzyme restriction map. The restriction sites on the diagonals are from the MCS. The kb coordinates for the cloned fragment are shown below the fragment. **Methods**: Bacteria were grown on Luria agar containing appropriate antibiotics and counterselectable agents at the following concentrations (per ml): 20 μg Cm, 50 μg Km, 100 μg Sm, 5% Suc (w/v) and 20 μg Tc. One-half of these concentrations was used in broth. Deletions were isolated and plasmid size was estimated essentially as described elsewhere (Wang et al., 1993a; b; Krishnan et al., 1995). After transposition was induced, cells were plated on Suc + Tc medium to isolate clockwise deletions, or on Sm + Km medium to isolate counterclockwise deletions (*sacB* encodes Suc^S, and *strA (rpsL)* encodes Sm^S in a Sm^R strain). Most colonies that survive on the selection plates contain plasmids with a deletion that extends into the cloned fragment. To ensure that only deletions were analyzed, an optional screening step was taken: Sm^R Km^R colonies were screened for Tc^S; Suc^R Tc^R colonies were screened for Km^S. The exceptions (a few percent in cosmid-sized clones) are due to spontaneous mutations or to deletions with endpoints in the vector *sacB* or *strA* genes. Plasmid pL14DEL1 (Krishnan et al., 1995) contains a 35-kb segment of *HLA* DNA that had been recloned from a cosmid as a *Not*I fragment (using vector *Not*I sites) into the pDUAL 'deletion-factory' vector, pDELTA1 (Wang et al., 1993a,b). For restriction mapping, DNA of pL14DEL1 and of representative deletion derivatives was digested singly with *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I (N. E. Biolabs, Beverly, MA) according to the manufacturer's instructions, and electrophoresed in 0.7% agarose gels. The restriction fragments generated by *Hin*dIII digestion of pL14DEL1, of the clockwise deletion plasmids, and of the counterclockwise deletion plasmids were first aligned by size, as tabulated above, then by their 'drop-out' pattern, as depicted in parts C and D.

EXPERIMENTAL AND DISCUSSION

(a) Drop-out mapping of *Hin*dIII sites in a 35-kb HLA segment

Deletions extending either clockwise or counterclockwise from a transposon end into sites in the cloned DNA in pL14DEL1 (Krishnan et al., 1995) were selected simply by resistance to Suc or Sm, as described in the legend to Fig. 1. A set of 26 plasmids was chosen for study. These plasmids ranged in size from 8.2 to 24.2 kb, and had lost 17 to 33 kb of *HLA* DNA plus 2 kb of vector DNA adjacent to one or the other transposon end (Fig. 1).

*Hin*dIII digestion of pL14DEL1 generated eight fragments (sizes: 1.9 to 10.1 kb), while digestion of the deletion plasmids generated from one to five fragments (sizes: 1.9 to 14.4 kb). Each deletion plasmid had lost the 10.1-kb fragment, but most retained one or more of the other parental fragments, and all contained a single unique fusion fragment ranging in size from 6.2 to 14.4 kb. Since the vector (pDELTA1) was 8 kb (Wang et al., 1993a,b), and contained a *Hin*dIII site in the MCS (at the left in Fig. 1B), the only fragment large enough to include the vector was the 10.1-kb fragment. Therefore, this fragment contains vector DNA plus 2.1 kb of *HLA* DNA. The single unique fragment obtained from each deletion derivative, but not from the parent plasmid, was a vector-target fusion fragment formed by intramolecular γδ transposition. The restriction fragments are first aligned by plasmid size vs. fragment size, comparing the parent clone and derivatives that contained counterclockwise and clockwise deletions, as illustrated in the legend to Fig. 1.

The data summarized in Fig. 1D, indicate that all 15 clockwise deletion plasmids had lost four (10.1, 7.3, 4.6, and 2.7 kb) of the eight *Hin*dIII fragments. Only the largest of the derivative plasmids retained the 6.8-kb fragment, with the 3.7-, 1.9- and 5.6-kb fragments dropping out successively with decreasing plasmid size. These data, by themselves, demonstrate a partial fragment order (left to right) of 5.6, 1.9, 3.7, 6.8, [10.1, 7.3, 4.9, 2.7] kb, but do not reveal the order of the fragments in brackets.

The data summarized in Fig. 1C indicate that all eleven counterclockwise deletion plasmids had lost six (10.1, 6.8, 5.6, 4.9, 3.7 and 1.9 kb) of the eight *Hin*dIII fragments in

pL14DEL1. The other two fragments 'dropped-out' in the order 7.3, 2.7 kb, with decreasing plasmid size. These data, by themselves, demonstrate a partial fragment order (left to right) of [10.1, 6.8, 5.6, 3.7, 1.9], 7.3, 2.7 kb, but do not reveal the order of the fragments in brackets.

Within each group of plasmids that yielded the same number of fragments, the size of the unique fusion fragment decreased in parallel with plasmid size. For example, the top four deletion plasmids in Fig. 1C comprise one set, in which the fusion fragment decreased from 11.8 to 8.4 kb, depending on the deletion endpoint in the 4.9-kb fragment.

The data cumulatively indicate a partial fragment order (left to right) of 5.6, 1.9, 3.7, 6.8, 7.3, 2.7 [10.1, 4.9] kb. As described above, the parental 10.1-kb fragment contains the vector plus 2.1 kb of the cloned fragment, and is always changed in size in deletion derivatives. Although the 4.9-kb fragment was not present in this set of deletion plasmids, it was inferred to be in the central part of the cloned segment, between the 6.8- and 7.3-kb fragments because all other restriction fragments align, leaving a central gap and an unassigned 4.9-kb fragment. This fragment is truncated in the three largest plasmids in the counterclockwise deletion set (Fig. 1C), and in the largest plasmid from the clockwise deletion set (Fig. 1D). Its assignment to the central region was confirmed by drop-out analysis of additional deletion plasmids that retained the 4.9-kb fragment (data not shown). Together, these data yield the unambiguous fragment order, counterclockwise from the MCS, of 5.6, 1.9, 3.7, 6.8, 4.9, 7.3, 2.7, 10.1 kb (Fig. 1B).

(b) Construction of a four-enzyme restriction map

Digestion of pL14DEL1 with each of three additional restriction enzymes (*Bam*HI, *Eco*RI and *Pst*I) yielded a single fusion fragment plus one or more fragments matching those in the parent clone, similar to the drop-out pattern described for *Hin*dIII, above. The sizes of the largest fragments could not be determined accurately due to inherent limitations of agarose gel electrophoresis. Therefore, when there was a discrepancy between the sizes estimated for a single plasmid using different enzymes, the estimate based on the enzyme generating the smallest fragments was used, and the sizes estimated for large fragments from other digestions were adjusted to give the same plasmid size.

The use of four different restriction enzymes avoided the need for double digestions or pulsed field gel electrophoresis to determine the sizes of the largest fragments. The adjusted single-enzyme restriction maps were overlaid to create the four-enzyme restriction map depicted in Fig. 1E.

(c) Conclusions

(*1*) Restriction maps facilitate contig assembly, gene localization, and directed sequencing, but the construction of such maps is generally bypassed because ordering restriction fragments using standard methods is so laborious. The data presented here demonstrate that arrays of nested deletion plasmids allow rapid mapping of restriction sites in large cloned DNA segments. We compared the pattern of restriction fragments obtained with the parent *HLA* clone, and with 26 derivatives that had either clockwise or counterclockwise deletions. Single digestions with each of four restriction enzymes (Fig. 1C, D and data not shown) were sufficient to generate the restriction map shown in Fig. 1E. The ease of isolating nested deletion derivatives and of analyzing single digestion data makes the approach used here more attractive than partial digestions or a combination of single and double digestions of fewer deletion plasmids.

(*2*) Potential sources of ambiguity or error in drop-out restriction analysis include: (*i*) incomplete digestion; (*ii*) scoring pairs of restriction sites as single sites because of their proximity to each other; (*iii*) analyzing too few deletion derivatives to tag all restriction fragments; and, (*iv*) a nonrandom distribution of deletion endpoints due to γδ transposition specificity or to inadvertent selection for smaller clones during bacterial growth (as found here: see Fig. 1).

(*3*) Most drop-out mapping steps are automatable for large-scale efforts: (*i*) ordered sets of deletions can be obtained by size fractionating large pools of independent deletion plasmids in agarose gels (Berg et al., 1994; Sugino and Morita, 1994; P. Karlovsky, F.L. and C.M.B., unpublished); (*ii*) plasmid DNA extraction, restriction enzyme digestion and agarose gel loading can be robotized; and, (*iii*) computer-assisted methods for estimating fragment sizes from gel mobility and localizing restriction sites in individual clones and contigs are available or under development. Therefore, drop-out mapping is amenable to use in large-scale, as well as small-scale, projects.

(*4*) Restriction maps of large DNA segments that are obtained by the drop-out approach described here should be much easier to generate and probably more accurate than maps generated by fragment alignment from highly redundant libraries (Olson et al., 1986; Riles et al., 1993; Smith et al., 1994), or by optical mapping (Meng et al., 1995).

(*5*) The drop-out mapping approach is not limited to DNA cloned in special pDUAL vectors, but can also be used, albeit less efficiently, with transposon-containing vectors that yield deletions in only one direction (Ahmed, 1984, 1987; Peng and Wu, 1986; Prentki et al., 1991; Sugino and Morita, 1994). Nor is drop-out mapping limited to deletions generated by intramolecular transposi-

tion; it can be applied equally to sets of nested deletions generated by random hopping of a special transposon and then recombination between sites engineered into the transposon and vector (Sternberg, 1994) or by in vitro methods (Smith and Birnstiel, 1976; Henikoff, 1984; Eberle, 1993; Ahmed, 1994).

(6) Drop-out mapping will also be useful for locating specific genes or DNA sequences by complementation or by hybridization.

(7) The unique vector sequences adjacent to mapped target sequences at the deletion endpoints are valuable as primer-binding sites for sample sequencing (feature mapping) (Ahmed, 1984, 1994; Peng and Wu, 1986; Wang et al., 1993a,b; Sugino and Morita, 1994; Krishnan et al., 1995) and should be particularly valuable for closing gaps and analyzing repetitive DNAs (Berg et al., 1994).

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