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Application Note

Drop-out restriction mapping: Using nested deletions and single restriction enzyme digestions to obtain high-resolution cosmid maps

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DETAILED RESTRICTION maps are valuable for aligning overlapping clones in a contig, for mapping genes, and for directed sequencing. The most common methods for constructing restriction maps of cloned fragments involve a) digesting the clone with different restriction enzymes and aligning the fragments generated by sets of single and double digestions and b) linearizing the clone, partially digesting it, and visualizing the series of fragments that contain one or the other end of the clone plus a variable amount of target DNA. The first method depends on trial and error, the fitting together of double-digestion and two sets of single-digestion fragments. The second method depends on using just the right amount of enzyme to obtain a well-defined restriction digestion ladder. Both methods are difficult to scale up from plasmid to cosmid-sized or larger clones. Therefore, restriction maps are laborious to construct by conventional methods and are rarely used in large-scale gene analysis and sequencing projects.

The authors have developed a simpler (and probably more economical) method for generating unambiguous restriction maps that takes advantage of nested deletions generated in DNA cloned in one of the deletion factory pDUAL/pDELTA vectors (*Figure 1a*).[1,2] Detailed restriction maps can be constructed simply by comparing the restriction frag-

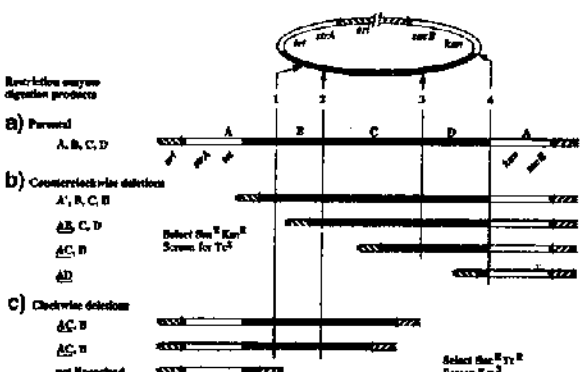
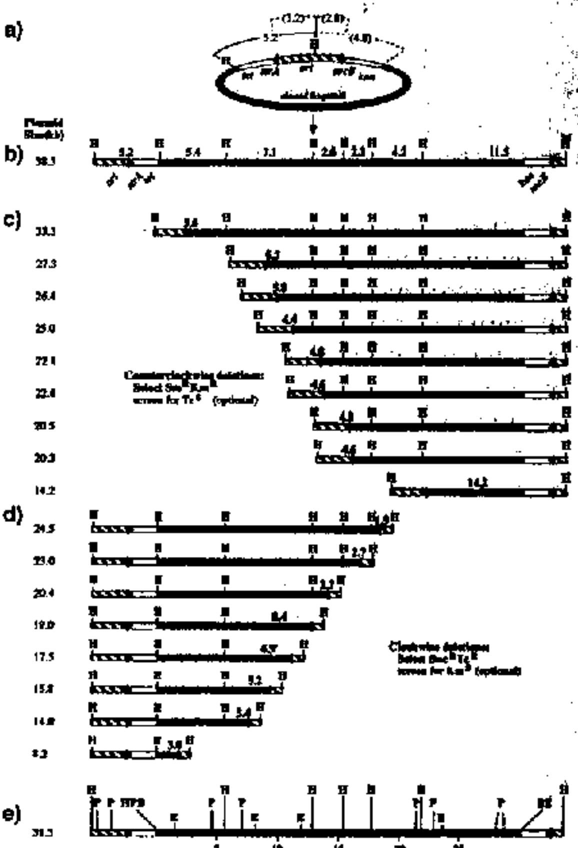


Figure 1 Expected phenotypes and restriction fragment profiles of hypothetical pDUAL/pDELTA clone deletion derivatives. Most colonies that survive on the selection plates (described in Table 1 footnote) contain plasmids with a deletion that extends into the cloned fragment. The exceptions (usually less than 10%) generally fall into two classes: 1) spontaneous mutation in the sacB (Suc^S) or strA (rpsL) (Sm^S) genes and 2) deletions with endpoints in the vector DNA. The vertical lines depict one restriction site at the cloning site and three in the cloned fragment. Letters A–D depict the four restriction fragments in the parental clone (note that fragment A includes the vector and the left end of the clone fragment). A' indicates a truncated fragment; heavy underlines indicate fusion fragments. a) Parent plasmid circular (top) and linearized at a site in the vector (below). b,c) Deletion derivatives. Every γδ-generated deletion reduces the size of the vector component (A), and most deletions generate fusion fragments (letters underlined on the left) between the remainder of the A fragment and one of the restriction fragments in the cloned portion. Note that the transposition target size



structed simply by comparing the restriction fragments present in the parent clone with those present in sets of nested deletion derivatives: The fragments disappear (drop out) in the order in which they are aligned in the parent clone.[3] These nested deletions are also valuable for sample sequencing (feature mapping)[4] at known positions in cosmid-sized cloned DNAs. Both clockwise and counterclockwise deletion plasmids can be selected on simple bacteriological media.

The nested deletions used in the authors' studies are generated by in vivo intramolecular movement of an engineered bacterial transposon, γδ (Tn*1000*). Transposons are small discrete DNA segments that move from site to site in DNA without any requirement for homology. Their movement between DNA molecules can yield simple insertions or fusions of the donor and target molecules bounded by copies of the transposon, while their movement within a single molecule yields deletions and inversions that start at a transposon end (see Refs. 5 and 6). The pDUAL/pDELTA vectors have been constructed to select for deletions that extend either clockwise or counterclockwise from a transposon end (Figure 1).

the cloned portion. Note that the transposition target size (deletion endpoint) can be determined approximately by the size of the deletion plasmid and more accurately by the restriction fragment pattern.

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Construction of a HindIII *restriction map of pTherm6 by drop-out mapping*

pTherm6 is a pDUAL cosmid clone containing a 28.3-kb *Thermotoga neapolitana* segment. *Hin*dIII digestion generated seven fragments (sizes from 2.3 to 11.5 kb). Since the vector contains two *Hin*dIII sites, one in the multiple cloning site (MCS) and the other within the vector, two of these fragments were from the vector: a 5.2-kb fragment and a >4.8-kb fragment, the size of which depends on the site of the nearest *Hin*dIII site in the cloned fragment (*Figure 2a*). Counterclockwise deletions will truncate the 5.2-kb fragment and create a new fusion fragment with the target DNA, while clockwise deletions will have the same effect on the >4.8-kb fragment (Figures 1 and 2).

Two sets of nested deletions, one that extended clockwise and the other that extended counterclockwise, were isolated. Digestion of the deletion plasmids generated from one to six fragments (sizes from 1.9 to 14.2 kb) (*Table 1*). Most deletion plasmids retained one or more of the parental fragments, and all contained a single unique fusion fragment.

Figure 2 *Generation of* HindIII *restriction map of pTherm6 by drop-out mapping. Symbols: ◄►, 40-bp inverted transposon γδ ends in vector; diagonally marked bars, vector segments interior to transposon ends; open bars, vector segments between transposon ends and cloned fragment; stippled bar,* Thermotoga neapolitana *fragment;* PstI *(P); B,* BamHI*; E,* EcoRI*; H,* HindIII*; P,* PstI*; vertical lines, restriction sites; dashed lines, ambiguous* PstI *site. a) Circular depiction of the parental 38.3-kb clone, with the* HindIII *(H) sites in the vector shown. The numbers represent the* HindIII *fragment (5.2) or partial fragment (4.8) sizes (in kb) within the vector. b) Linear depiction of the parental clone linearized at the vector* HindIII *site, with the ordered* HindIII *(H) sites shown. Numbers represent the* HindIII *fragment sizes (in kb). c) Counterclockwise deletion derivatives from colonies selected on Sm+Km medium and screened for Tc^S. Fusion fragment sizes (in kb) are listed in bold on the map of the deletion derivative. The data in Table 1 were used to order the fragments. d) Clockwise deletion derivatives from clones selected on Suc+Tc medium and screened for Km^S. e) A four-enzyme restriction map generated by overlaying single digestion data. The restriction sites on diagonals are from the MCS. The kb coordinates for the cloned fragment are shown below the fragment.*

The restriction fragments were first arranged by plasmid size versus fragment size, for counterclockwise and clockwise deletions (Table 1). The data summarized in Table 1*b* indicate that all nine counterclockwise deletion plasmids had lost the 5.2-kb vector fragment and a 5.4-kb fragment. Therefore, the 5.4-kb fragment must be the first clone fragment deleted in counterclockwise deletions. The 5.2-kb vector fragment suffers a deletion of the portion that is outside the transposon end, and the remainder forms a fusion with the DNA adjacent to the deletion endpoint. While the largest counterclockwise deletion plasmid had lost only the 5.2- and 5.4-kb fragments and gained a 5.6-kb fusion fragment (Table 1*b*, line 1, and Figure 2*c*, line 1), the 7.1-, 2.6-, 2.3-, 4.2-, and 11.5-kb fragments dropped out in that order in successively smaller deletion plasmids. Although the

Table 1

Sizes of *Hin*dIII fragments in pTherm6 and in deletion derivatives[a]

	Plasmid size (kb)	Restriction fragments (kb) Parental							Fusion
a) Parent plasmid	38.3	11.5	7.1	5.4	5.2	4.2	2.6	2.3	
b) Sm^R and Km^R (counter-clockwise deletions)	33.3	11.5	7.1			4.2	2.6	2.3	5.6
	27.3	11.5				4.2	2.6	2.3	6.7
	26.4	11.5				4.2	2.6	2.3	5.8
	25.0	11.5				4.2	2.6	2.3	4.4
	22.8	11.5				4.2		2.3	4.8
	22.6	11.5				4.2		2.3	4.6
	20.5	11.5				4.2			4.8
	20.3	11.5				4.2			4.6
	14.2								14.2
c) Suc^R and Tc^R (clockwise deletions)	24.5		7.1	5.4	5.2		2.6	2.3	1.9
	23.0		7.1	5.4	5.2		2.6		2.7
	20.4		7.1	5.4	5.2				2.7
	19.0			5.4	5.2				8.4
	17.5			5.4	5.2				6.9
	15.8			5.4	5.2				5.2
	14.0			5.4	5.2				3.4
	8.2				5.2				3.0

[a]DNA from genomic *Thermotoga neapolitana* NS-E, a thermophilic bacterium, was partially digested with *Bam*HI and relegated into the unique *Bam*HI site in a pDUAL vector using standard techniques.[7] Bacteria were grown on L agar containing appropriate antibiotics and contraselectable agents at the following concentrations: (per milliliter) 20 µg Cm, 50 µg Km, 100 µg Sm, 5% wt/vol Suc, 20 µg Tc. In liquid medium, one-half of these concentrations was used. Deletions were isolated and plasmid size was estimated

didate. These data, by themselves, demonstrate a fragment order (left to right, starting from the internal *Hin*dIII site) of 5.2, 5.4, 7.1, 2.6, 2.3, 4.2, and 11.5 kb (Figure 2*c*).

The clockwise deletions (Table 1*c* and Figure 2*d*) confirm the restriction fragment order deduced above.

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 second to fourth deletion plasmids in Table 1*b* comprise one set, in which the parental 7.1-kb fragment became part of a fusion fragment that decreased from 6.7 to 4.4 kb, depending on the deletion endpoint in the 7.1-kb fragment.

Construction of a four-enzyme restriction map

Digestion of these plasmids with each of three additional restriction enzymes (*Bam*HI, *Eco*RI, and *Pst*I) yielded data (not shown) similar to those presented for *Hin*dIII, above. The only uncertainty in these data involved the two right-most *Pst*I fragments (5.8 and 5.0 kb). Neither is truncated by a deletion, and both are large enough to be the vector-clone hybrid fragment because internal *Pst*I sites in the vector make the right-most *Pst*I fragment 4 kb or larger.

scale as well as small-scale projects. Many drop-out mapping steps are potentially automatable for large-scale efforts. Drop-out restriction maps of large DNA segments should be much easier to generate and probably more accurate than maps generated by fragment alignment from highly redundant libraries[8–10] or by optical mapping.[11] Locating specific genes or DNA sequences by complementation or by hybridization can also be accomplished with drop-out mapping. The unique vector sequences adjacent to mapped target sequences at the deletion endpoints are valuable as primer-binding sites for sample sequencing (feature mapping).[1,2,4,12–15] Deletion endpoints at mapped sites should be particularly valuable for closing gaps and for analyzing repetitive DNAs.[5]

References

1. Wang G, Blakesley RW, Berg DE, Berg CM. pDUAL: a transposon-based cosmid cloning vector for generating nested deletions and DNA sequencing templates in vivo. Proc Natl Acad Sci USA 1993; 90:7874–8.
2. Wang G, Berg CM, Chen J, et al. Creating nested deletions for sequencing cosmid DNAs. Focus 1993; 15:47–9.
3. LaBanca F, Krishnan BJ, Chaplin DD, Berg DE, Berg CM. Restriction map of a 35-kb *HLA* fragment by nested deletion "drop-out" mapping. Gene 1995; 164:335–9.
4. Krishnan BR, Jamry I, Berg DE, Berg CM, Chaplin DD. Construction of a genomic DNA "feature map" by sequencing from

drop-out data do not indicate the order of the 4.2- and 11.5-kb fragments, the 11.5-kb fragment was the vector-clone hybrid fragment because the vector portion of the hybrid fragment was larger than the 4.2-kb can-

essentially as described elsewhere[1-4]. After transposition was induced, cells were plated on Suc+Tc medium to isolate clockwise deletions or on Sm+Km medium to isolate counterclockwise deletions (*sac*B 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 performed: Sm^R and Km^R colonies were screened for Tc^S; Suc^R and 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 endpoint in the *sacB* or *strA* genes of the vector. Plasmid DNA was isolated by standard alkali lysis procedures.[7] For restriction mapping, DNA of pTherm6 and of representative deletion derivatives was digested singly with *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I (Life Technologies, Bethesda, MD) according to the manufacturer's instructions and electrophoresed in 0.7% agarose gels. Potential sources of ambiguity or error in drop-out restriction analysis include 1) obtaining an incomplete digestion; 2) scoring pairs of restriction sites as single sites because of their proximity to each other; 3) analyzing too few deletion derivatives to tag all restriction fragments; and 4) obtaining a nonrandom distribution of deletion endpoints due to γδ transposition specificity or to inadvertent selection for smaller clones during bacterial growth.

There was some variation in plasmid size determined by the different enzyme due to the 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 adjusted single-enzyme restriction maps were overlaid to create the four enzyme restriction map depicted in Figure 2*e*.

Conclusions

Arrays of nested deletion plasmids allow efficient mapping of restriction sites in large cloned DNA segments. By comparing the patterns of single digestion restriction fragments obtained from a *Thermotoga neapolitana* clone and 17 deletion derivatives, the authors were able to generate the four-enzyme restriction map shown in Figure 2*e*, with only a single ambiguity. The precise location of this site could be easily determined by digestion of a smaller counterclockwise deletion plasmid or by double digestion (most easily by *Eco*RI/*Pst*I digestion of the 14.2-kb counterclockwise deletion plasmid).

Drop-out mapping is amenable to use in large-

nested deletions: application to the HLA class I region. Nucl Acids Res 1995; 23:117-22.
5. Berg CM, Wang G, Isono K, Kasai H, Berg DE. Transposon-facilitated large scale DNA sequencing. In: Adams MD, Fields C, Venter JC, eds. Automated DNA sequencing and analysis. London: Academic Press, 1994: 51-9.
6. Berg CM, Berg DE. Transposable elements as tools for molecular analyses in bacteria. In: Sherratt D, ed. Mobile genetic elements: frontiers in molecular biology. Oxford: Oxford University Press, 1995:38-68.
7. Sambrook J Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
8. Olson MV, Dutchik JE, Graham MY, et al. Random-clone strategy for genomic restriction mapping in yeast. Proc Natl Acad Sci USA 1986; 83:7826-30.
9. Riles L, Dutchik JE, Baktha A, et al. Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics 1993; 134:81-150.
10. Smith MW, Holmsen AL, Wei YH, Peterson M, Evans GA. Genomic sequence sampling: a strategy for high resolution sequence-based physical mapping of complex genomes. Nature Genetics 1994; 7:40-7.
11. Meng X, Benson K, Chadra K, Huff EJ, Schwartz DC. Optical mapping of lambda bacteriophage clones using restriction endonucleases. Nature Genetics 1995; 9:432-8.
12. Ahmed A. Use of transposon-promoted deletions in DNA sequence analysis. J Mol Biol 1984; 178:941-8.
13. Ahmed A. Double-origin vectors for isolating bidirectional deletions useful in DNA sequence analysis. Gene 1994; 141:71-3.
14. Peng Z G, Wu R. A simple and rapid nucleotide sequencing strategy and its application in analyzing a rice histone 3 gene. Gene 1986; 45:247-52.
15. Sugino Y, Morita M. A new DNA cloning/sequencing vector with a built-in mechanism for generation of nested deletions using transposon Tn3. Gene 1994; 148:169-70.