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Use of oligonucleotides to generate large deletions

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We have found that methods for generating specific mutations and small insertions and deletions are not suitable for making large deletions. The following simple procedure has yielded several different precise deletions in the range of 1.1-4.1 kb using single-stranded templates. An overall efficiency of 10% is obtained, allowing for screening by colony or plaque hybridization using a standard labeled probe derived from each region to be deleted. Restriction mapping and/or sequencing through the breakpoint confirms that the correct deletion has been made.

Oligonucleotide: Generally, a 30-mer "clamp" is synthesized such that the first 15 bp "arm" is immediately upstream of the region to be deleted, and the last 15 bp arm is immediately downstream. The length of either arm is adjusted such that the calculated hybridization temperature (T_H) is approximately 42° using the formula $T_H=2^o x(\#A+T)+4^o x(\#G+C)-3^o$ (1).

Template: Either a pure single-stranded template or a mixture of single-stranded plasmid and helper, such as pEMBL (2), can be used, purified as for dideoxy sequencing. The helper can be excluded during the transformation step by using an F- host.

Annealing: Mix 5 pmol phosphorylated oligonucleotide, 0.5 pmol template, 20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl in a 10 µl volume. Heat 5 min 65° and transfer to T_H for 20 min.

Extension: Add 10 µl solution containing 2 units Klenow polymerase. and 3 units T4 DNA ligase in 20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.8 mM each dNTP, 1 mM ATP. Continue at T_H for at least 1 hr. Stop by addition of EDTA to 25 mM, heating to 70° for 10 min. Add NaCl to 0.15 M, add 2.5 vol ethanol, chill on dry ice, pellet 5 min in a microcentrifuge, rinse in 70% ethanol and dry.

S1 nuclease treatment: Dissolve the pellet in 10 µl 10 mM Tris-HCl pH 8, 1 mM EDTA. Add 15 µl 30 mM potassium acetate pH 4.6, 0.25 M NaCl, 1 mM $ZnSO_4$, 5% glycerol, 7 units (Vogt, i.e., 1 min) S1 nuclease. After 20 min at room temperature, add 2 µl 0.3 M Tris-OH, 0.05 M EDTA and heat 10 min at 70°.

Transformation: Add the entire sample to 60 ul Ca^{++}-treated cells. Using a pEMBL plasmid, 100-500 colonies are obtained.

References: 1. Hanahan, D. and Meselson, M. (1983) Meth. Enzymology 100:333.
2. Dente, L., Cesareni, G. and Cortese, K. (1983) Nucl. Acids Res. 11:1645.

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