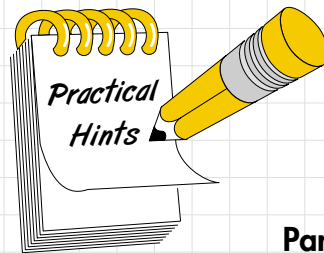


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The QIAGEN Guide to Analytical Gels

Part X: Restriction endonuclease digestion of DNA

This article continues our series aimed at providing useful hints for gel analysis. The series will continue in the next issue of QIAGEN News with a look at Southern blotting.

Principle of restriction digestion

Before Southern blotting and hybridization, DNA is usually digested with restriction endonucleases. This yields DNA fragments of a convenient size for downstream manipulations.

Restriction endonucleases are bacterial enzymes that bind and cleave DNA at specific target sequences. Type II restriction enzymes are the most widely used in molecular biology applications. They bind DNA at a specific recognition site, consisting of a short palindromic sequence, and cleave within this site, e.g., AGCT (for *AluI*), GAATTC (for *EcoRI*), and so on. Isoschizomers are different enzymes that share the same specificity and in some cases, the same cleavage pattern.

Tip Isoschizomers may have slightly different properties that can be very useful. For example, the enzymes *MboI* and *Sau3A* have the same sequence specificities, but *MboI* does not cleave methylated DNA, while *Sau3A* does. *Sau3A* can therefore be used instead of *MboI* where necessary.

Selecting suitable enzymes

The following factors need to be considered when choosing suitable restriction enzymes:

- ◆ Fragment size
- ◆ Blunt-ended/sticky-ended fragments
- ◆ Methylation sensitivity
- ◆ Compatibility of reaction conditions (when carrying out digests with more than one enzyme)

Fragment size

Restriction enzymes with shorter recognition sequences cut more frequently than those with longer recognition sequences. For example, a 4 base pair (bp) cutter will cleave, on average, every 4^4 (256) bases, while a 6 bp cutter cleaves every 4^6 (4096) bases.

Tip Use 6 bp cutters for mapping genomic DNA, YACs, BACs, or P1s, as these give fragments in a suitable size range for cloning.

Blunt-ended/sticky-ended fragments

Some restriction enzymes cut both DNA strands at the same position, creating blunt-ended DNA fragments. However, the majority of enzymes make cuts staggered on each strand, resulting in a few base pairs of single-stranded DNA at each end of the fragment, known as “sticky” ends. Some enzymes create 5' overhangs and others create 3' overhangs. The type of digestion affects the ease of downstream cloning:

- ◆ Sticky-ended fragments can be easily ligated to other sticky-ended fragments with compatible single-stranded overhangs, resulting in efficient cloning.
- ◆ Blunt-ended fragments usually ligate much less efficiently, making cloning more difficult. However, any blunt-ended fragment can be ligated to any other, so blunt-cutting enzymes are used when compatible sticky-ended fragments cannot be generated — for example, if the polylinker site of a vector does not contain an enzyme site compatible with the fragment being cloned.

Methylation

In mammalian DNA, some cytosine nucleotides are methylated, mainly those occurring as part of the dinucleotide sequence CpG. The CpG dinucleotide occurs about five times less frequently in mammalian DNA than would be expected by chance, and most restriction enzymes with a CpG dinucleotide in their recognition site do not cleave if the cytosine is methylated. Therefore many enzymes with CpG in their recognition site, such as *EagI*, *NotI*, and *SalI*, cleave mammalian DNA only rarely.

However, methylation patterns differ in different species, affecting the choice of restriction enzyme:

- ◆ Methylation patterns differ between bacteria and eukaryotes, so restriction patterns of cloned and uncloned DNA may differ.
- ◆ *Drosophila*, *Caenorhabditis*, and some other species do not possess methylated DNA, and have a higher proportion of CpG dinucleotides than mammalian species. Rare-cutter enzymes therefore cleave more frequently in these species.
- ◆ Plant DNA is highly methylated, so for successful mapping in plants, choose enzymes that either do not contain a CpG dinucleotide in their recognition site (e.g., *DraI* or *SspI*), or enzymes that can cleave methylated CpG dinucleotides, such as *BamHI*, *KpnI*, or *TaqI*.

Compatibility of reaction conditions

If a DNA fragment is to be cut with more than one enzyme, both enzymes can be added to the reaction simultaneously provided that they are both active in the same reaction buffer and at the same reaction temperature. If the enzymes do not have compatible reaction conditions, carry out one digestion, purify the reaction products, for example using the QIAquick® PCR Purification Kit, and then perform the second digestion.

Components of a restriction digest

- ◆ Water
- ◆ DNA
- ◆ Buffer
- ◆ Enzyme

DNA

The amount of DNA digested depends on the downstream application: for mapping of cloned DNA, 0.2–1 µg DNA per reaction is adequate, while for Southern blotting of mammalian genomic DNA, a minimum of 10 µg DNA per reaction is recommended.

Tip DNA should be free of contaminants such as phenol, chloroform, ethanol, detergents, or high salt concentrations (e.g., using DNeasy® Kits), as these may interfere with restriction endonuclease activity.

Enzyme

One unit of restriction endonuclease completely digests 1 µg of substrate DNA in 1 hour. However, supercoiled plasmid DNA generally requires more than 1 unit/µg to be digested completely. Most researchers add a ten-fold excess of enzyme to their reactions in order to ensure complete cleavage.

Tip Ensure that the restriction enzyme does not exceed more than 10% of the total reaction volume, as otherwise the glycerol in which the enzyme is supplied may inhibit digestion.

Reaction volume

Most digests are carried out in a volume between 10 and 50 µl. (Reaction volumes smaller than 10 µl are susceptible to pipetting errors, and are not recommended.)

Setting up a restriction digest

1. Pipet reaction components into a tube and mix well by pipetting.

Tip Thorough mixing is extremely important.

Tip The enzyme should be added last and kept on ice when not in the freezer.

Tip When setting up large numbers of digests, make a reaction master mix consisting of water, buffer, and enzyme, and pipet aliquots into tubes containing the DNA to be digested.

2. Centrifuge the tube briefly to collect the liquid at the bottom.
3. Incubate the digest in a water bath or oven, usually for 1–4 hours at 37°C. Restriction enzymes isolated from thermophilic bacteria require higher incubation temperatures, e.g., 50 – 65°C.
4. For some downstream applications it is necessary to heat-inactivate the enzyme after digestion. The majority of enzymes that have an optimal incubation temperature of 37°C are inactivated by heating the reaction to 65°C for 20 minutes after digestion.

Tip Some restriction enzymes are not fully inactivated by heat treatment. The new MinElute™ Reaction Cleanup Kit from QIAGEN provides complete removal of restriction enzymes and salts following digestion.

The QIAGEN Guide to Analytical Gels will continue in the next issue of *QIAGEN News* with a look at Southern blotting. If there is any other information you would like to see on these pages of *QIAGEN News*, please let us know by calling QIAGEN Technical Services or your local distributor.

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