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7.13 Experimental Microbial Genetics

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Restriction digests

There are two basic types of restriction digests that you will perform during this course. An analytical restriction digest is done when you need to use the information from the digest to create a map of your DNA or to be sure that you have the correct plasmid. A preparative restriction digest is done when you need to prepare a large amount of particular DNA fragment. The key difference is scale. Generalized protocols for both are described below.

REAGENTS

Restriction enzymes:

Stored in -20°C . The enzymes are very expensive and we all must share the same stock. **DO NOT CROSS-CONTAMINATE ENZYMES. ALWAYS KEEP THEM ON ICE.**

Enzyme buffers and 100x (10 mg/ml) BSA

These are supplied by New England BioLabs and are coded into 4 different types of buffer. Refer to the NEB catalogue for the correct buffer for each enzyme.

10 mg/ml RNase A

A 1000X stock for digesting away the residual RNA present in DNA minipreps – be sure that the RNaseA is DNase-free. If using RNase A that is not specifically indicated to be DNase -free, boil it for 10 minutes when preparing the stock.

Guidelines

For analytical digests:

1. Use a reaction volume of 20 μl .
2. Digest about 0.5 μg of DNA - about 1-5 μl of miniprep DNA.
3. Keep the volume of enzymes $<10\%$ the volume of the reaction to avoid interference from glycerol in the enzyme. Use about 5 units of enzymes per digest.
4. To see small fragments (smaller than 500bp), include ribonuclease A at 1 $\mu\text{g}/\text{ml}$.
5. The best resolution on agarose gels will be for fragments between 250 and about 4,000 bases (0.25-4 kb). Try to design a digest that generates fragments of this size since it is hard to see really small fragments.
6. To decide on the digestion conditions look up the enzyme(s) in the New England Biolabs Catalogue. This is not just a normal catalogue but is instead an invaluable reference book. Different enzymes have different temperature and buffer optima. Be sure to check the temperature of digestion and the recommended buffer for each enzyme you plan to use; include BSA if recommended.
7. **ALWAYS RUN SIZE MARKERS ON YOUR GEL!**

For preparative digests:

1. Use a reaction volume of 50 μl .
2. Digest of 2- 5 μg of DNA- depending on the size of the fragment you want. (You need to digest more plasmid DNA to isolate a smaller fragment.)
3. Use about 20 units of enzyme per digest.

4. Use the NEB catalogue to determine reaction conditions. For double digests (using two enzymes), check to see if you can do the reaction in the same buffer. If not you will have to do the digests in sequence. In between the digests you will need to get rid of the first enzyme, and change buffers.
5. Incubate reactions for about 1 hour.
6. ALWAYS RUN SIZE MARKERS ON YOUR GEL!