Restriction Digests

1. Thaw DNA sample, Lambda standard, buffer specific for the restriction enzyme being used, as well as BSA and RNAse if required. Keep RNAse on ice. Leave restriction enzyme in the freezer until the last minute, then keep cold at all times.

2. Add in a microfuge tube in this order:

1-3 μl of DNA (around 1 μg)
2 μl of 10X buffer specific for enzyme
(2 μl of 10X BSA if required for the restriction enzyme used)
__ μl sterile deionized water for a final volume of 20 μl
1 μl RNAse (required to see small fragments 0.1-2.5 kb)
0.5- 1 μl restriction enzyme (only 1 unit/μg DNA is required but more is OK)

20 μl total volume

3. Incubate at 37°C (or at a temperature specific for restriction enzyme) for 1 hour.

4. Add 2 μl 10X loading dye, heat at 65° for 5 min, cool on ice and load all 22 μl in the well of an agarose gel. (the heating will disrupt sticky overhangs from annealing after cut)

   Load a Lambda (or other) standard in an adjacent well to allow determination of DNA fragment sizes. You may also want to include an undigested sample control for comparison to see if the enzyme really cut the DNA sample.

Notes:

Always add the enzyme(s) to the reaction last, after the buffer and water has been added and mixed.

Use only a new (unused) sterile micropipette tip when obtaining samples of enzymes (they are very expensive and we don't want them contaminated with DNA or other enzymes).

RNAse is not required but will aid in visualizing small DNA fragments that might be obscured by the large amount of RNA in the sample.

Heating at 65° prior to loading will dissociate sticky ends that are cut but still associated by hydrogen bonding. This is **required** in order to see all the fragments (0.5 kb) of the Lambda *Hin*dIII standard. Placing the sample on ice following heating will prevent reassociation and will make the sample more dense and aid in loading into the gel wells.

10X Loading Dye: per ml add 500 μl glycerol, 200 μl 0.1 M EDTA, 60 μl 1% xylene cyanol, 60 μl 1% bromphenol blue, 180 μl 1 M Tris-Cl (pH 7.5)

Sizes for the Lambda *Hin*dIII standard are (base pairs):
23,130
9,416
6,557
4,361
2,322
2,027
564 (will be missing unless heated for several minutes at 65°C)
[125] (too small/low abundance to see)