Experiment 4: Bacterial genetics 3 (transposon mutagenesis)

Selection of the correct buffer for restriction enzyme double digests

As with any enzyme, restriction enzymes' activity depends on temperature, and salt concentration(s). In general, commercially available restriction enzymes are sold with an appropriate buffer for their usage. When digesting DNA with multiple restriction enzymes, it is important to select the optimal buffer that maximizes the activity of all the restriction enzymes being used (see Table 4.1) – if a suitable buffer cannot be identified, it may be necessary to carry out the digests sequentially.

Table 4.1 Restriction enzyme activity in NEB buffers.

Enzyme	**Buffer provided**	**% activity in NEBuffer r1.1**	**% activity in NEBuffer r2.1**	**% activity in NEBuffer r3.1**	**% activity in rCutSmart**
AflIII	NEBuffer r3.1	10	50	100	50
EcoRV-HF	rCutSmart	25	100	100	100
HindIII-HF	rCutSmart	10	100	10	100

Considering the information in Table 4.1, which are the optimal buffers to use for each of the following digests? Fill in Table 4.2 before coming to the practical.

Table 4.2. Restriction enzyme buffer selection for double digests.

Restriction enzymes	**Buffer**
AflIII-EcoRV	
AflIII-HindIII	
EcoRV-HindIII	
AflIII-EcoRV-HindIII	

Considering the plasmid DNA sample that you have been provided, how much DNA will you need to add to your digest in order to add 1 μg?

Initial DNA concentration: _____
Volume to add: _____
Final DNA amount: _____

Protocol 4.1: Restriction digest of plasmid DNA

Materials:
Pipette tips
100-1000µl pipette
10-100µl or 20-200µl pipette
1-10µl or 2-20µl pipette
1.5 ml microcentrifuge tubes (eppendorfs)
Marker pen to write on eppendorfs and plates
Eppendorf rack
Plasmid DNA
Restriction enzymes
Restriction enzyme buffers
Bovine serum albumin (BSA)
Ice buckets and ice

Protocol:

1. You will be setting up the following restriction digests:
    a. AflIII
    b. HindIII
    c. EcoRV
    d. AflIII-EcoRV
    e. AflIII-HindIII
    f. EcoRV-HindIII
    g. AflIII-EcoRV-HindIII

Table 4.3: General Schema for Restriction Enzyme Digests

Component	**Amount needed for 50 µL reaction**
DNA	1 µg
10X restriction enzyme buffer (see Table 4.2)	5 µL
Restriction enzyme(s)	1 µL each
Nuclease-free water	To 50 µL

Label each tube carefully and set up your digests according to table 4.3. Be sure to add the correct buffers and enzymes to each tube (it helps to organize your tubes sensibly).

Mix your restriction digests by pipetting the mixture gently several times.

2. Incubate your restriction digests at 37˚C for 30 min-1 hour. (Prepare your agarose gel while you are waiting.)

Protocol 4.2: Gel electrophoresis to analyse restriction digests

1. An agarose gel solution will have been prepared for you as follows: To prepare 1% agarose gel, add 1g of agarose to a glass flask. Add 100 ml 1X TAE buffer. Heat agarose in a microwave till agarose is completely dissolved in the buffer. Wait for agarose to cool down to about 50˚C.

2. Prepare a gel tray by taping both ends firmly, creating a solid seal, and inserting a comb in the appropriate position. Pour the agarose into the prepared gel tray to set.
3. Prepare a plan for the order in which you will load your samples on the gel. Be sure to record this order so that you will be able to identify the samples in your gel photo.
4. When the gel has set, remove the tape from your gel tray and place the tray in an electrophoresis tank. Add TAE buffer to cover the gel.
5. Mix 16 µl of your restriction digest with 4 µl of 5X loading buffer. Being careful to avoid bubbles and overspill into adjacent wells, load this into one well of the agarose gel. Repeat for each digest.
6. Add the DNA ladder to one well of the agarose gel.
7. Perform electrophoresis, 100 V, until the blue indicator front is about to reach the end of the gel.