Hands-on DNA: Bacterial Evolution

Starter Kit List and Preparation Instructions

Kit contents:

Store at -20˚C	*Store at room temperature*
• DNA samples: A 533µl B 533µl 1 133µl 2 133µl 3 133µl 4 133µl • DNA ruler 25µl	• EcoRI lyophilised, in 0.2ml tubes • 10x TBE 1 litre • 6x Loading dye 16x 2ml • Agarose 6g • Foam floaters 16 • Teeny tough tags for labelling 0.2ml tubes • Coloured 1.5ml tubes for aliquoting DNA • White 1.5ml tubes for loading dye, pipetting practice

Preparation instructions:

DNA samples

See next page for preparation instructions.

TBE

To prepare 1x TBE (used for diluting DNA ruler, making agarose gels and running gels), add 100ml 10x TBE to a 1L measuring cylinder and add water (distilled or deionised) to 1L.

1x TBE used for gel running can be reused - we recommend 5 uses before discarding.

DNA ruler

This needs to be diluted prior to use with 1x TBE and loading dye added.

Briefly spin down the DNA ruler tube, then add:

315µl 1x TBE

34µl 6x Loading dye

Mix well, spin down in a microcentrifuge and store @ -20°C.

You may wish to aliquot the prepared DNA ruler into smaller portions - use white 1.5ml tubes.

EcoRI

For convenience, cut the strips of tubes into single tubes on receipt of the kit. The individual tubes must be stored in the sealed foil pouch, complete with the desiccant sachet. *Do not* store the dried EcoRI in the fridge or the freezer as moisture will cause the enzyme to degrade.

Loading dye

You will need both concentrated (6x) and dilute (1x) loading dye for the workshop. To prepare 1x loading dye, add 150µl 6x loading dye to 750µl water (distilled or deionised) and mix well.

Please refer to the Bacterial Evolution Technician's Notes for full details of workshop setup, including instructions for preparing agarose gels and assigning DNA samples to each group.

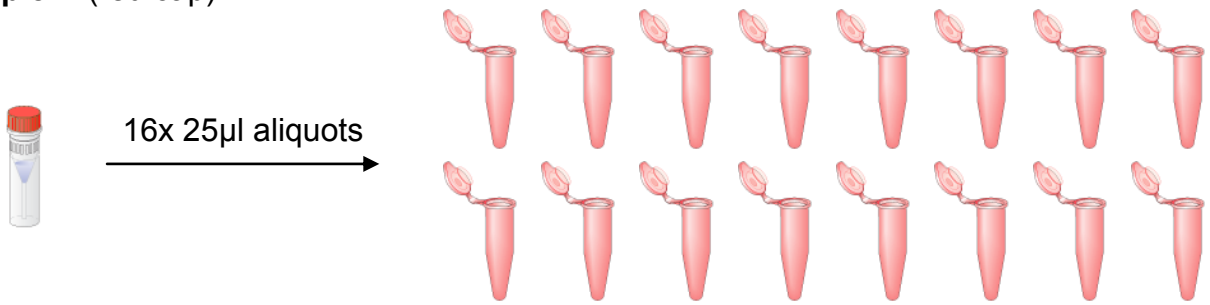
DNA samples

Before the workshop the DNA samples need to be aliquoted into single use tubes for each student group. It is easiest if you do this on receipt of the DNA, then you can store the individual aliquots in the freezer until needed.

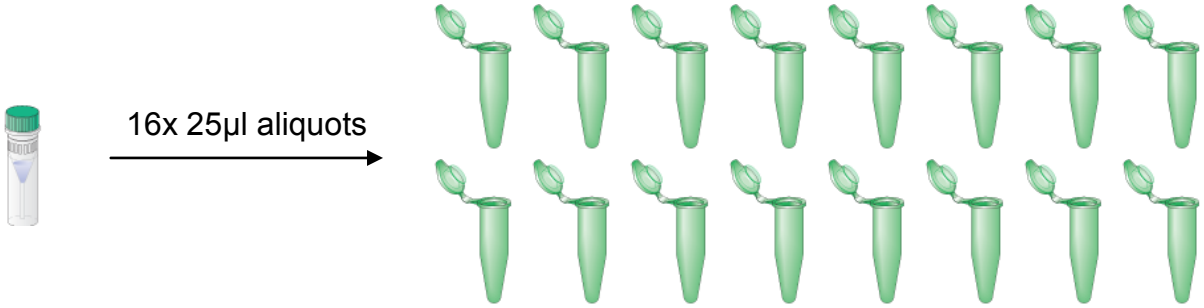
Use a micropipette to dispense DNA from the screw-capped tubes into labelled 1.5ml tubes of the matching colour. Each 1.5ml tube should contain 25µl DNA (students need 20µl).

Remember to use a clean tip for each different sample to prevent cross-contamination.

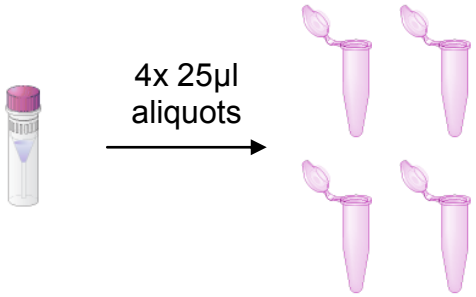
Sample A (red cap)



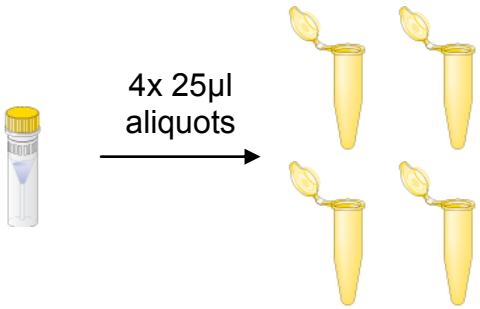
Sample B (green cap)



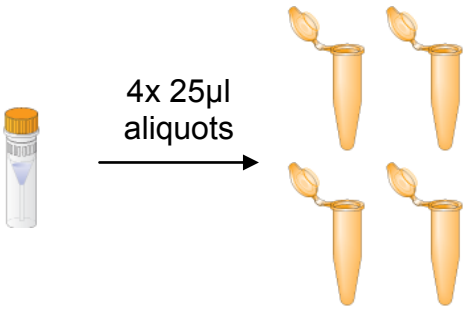
Sample 1 (pink cap)



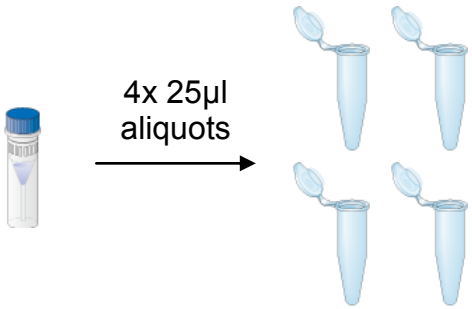
Sample 2 (yellow cap)



Sample 3 (orange cap)



Sample 4 (blue cap)



Please note that individual sample tubes stored at -20°C should be thawed in the fridge on the day of the workshop and spun down briefly before handing out to students on ice.