RE digestion of PCR products

Allows a more definitive identification of PCR amplicons

NOTE 1: Used to determine whether the PCR amplicon is of the appropriate region. A full sequence, or at least a rudimentary restriction map, of the expected amplicon will be required. It's preferable to employ a limited set of restriction enzymes (BamHI, EcoR1, EcoRV, HindIII or XbaI) since these are cheap efficient cutters and all cut well in *NEB buffer 2*.

BamHI	GGATCC	NEB	R0136S	20U/µl	NEB b^{o}2
EcoRI	GAATTC	NEB	R0101S	20U/µl	NEB b^{o}2
EcoRV	GATATC	NEB	R0195S	20U/µl	NEB b^{o}3 > 2
HindIII	AAGCTT	NEB	R0104S	20U/µl	NEB b^{o}2
XbaI	TCTAGA	NEB	R0145S	20U/µl	NEB b^{o}2

1. The usual thermostable enzyme used in the lab is *Denville TaqPro* (CB4050-8: final reaction conditions 16mM $(NH_4)_2SO_4$, 67mM Tris-HCl (pH 8.8 at 25°C), 0.01% Tween-20, 1.5–4mM $MgCl_2$). This enzyme generally gives a yield such that 5-10µl PCR product is easily detectable on a standard agarose gel.

2. If the product is of the expected size but you have reason to want to check further, restriction digestion is easy to perform and informative. Final reactions are 20µl, containing:

- 5µl PCR product
- 2µl 10 x RE buffer
- 0.2µl Restriction Enzyme (4U = in excess; see **NOTES 2 & 3**)
- 0.2µl BSA (10mg/ml)
- 12.1µl ddH_2O

Reactions are usually done in microfuge tubes or 96-well V-bottom plates: 37^{o}C, 60 minutes. Add loading dye and resolve on an Agarose / EtBr gel.

NOTE 2: If none of the recommended enzymes are available in your amplified sequence pick a cheap and cheerful alternative: something exotic is NOT recommended.

NOTE 3: You are obviously not going to be pipetting 0.2µl; multiple digestions are generally performed, so a digestion mix containing all but the PCR product is created and aliquots dispensed.

1X NEBuffer 2: pH 7.9 @ 25°C
- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM $MgCl_2$
- 1 mM Dithiothreitol

1X NEBuffer 3: pH 7.9 @ 25°C
- 100 mM NaCl
- 50 mM Tris-HCl
- 10 mM $MgCl_2$
- 1 mM Dithiothreitol