

## F. RESTRICTION ENZYME DIGESTION

Genotyping assays such as RFLP and dCAPS require restriction enzyme digestions. MacVector software is typically used to select restriction enzymes for RFLP assays. Selection of restriction enzymes for dCAPS genotyping procedures is based on the original computer application described by Neff et al. (Plant J. 1998 May; 14:387-92).

Restriction enzyme digestions are set up as 30  $\mu$ l reactions. In each digestion, a 7  $\mu$ l PCR reaction is used as a sample. The amount of restriction enzyme depends on whether the mutant or wild-type product is cleaved.

- If a mutant product is cleaved, 10 units of the restriction enzyme are used in each digestion reaction.
- If a wild-type product is cleaved, 20 units of the enzyme are used in each digestion reaction. An increased amount of restriction enzyme in these assays is to ensure that digestions are carried out to completion. Incompletely digested wild-type samples may be mistakenly regarded in these assays as heterozygous samples.

The volume of the restriction enzyme added to digestions should never exceed 10% of the total digestion volume (i.e. 3  $\mu$ l). This is to keep glycerol concentration at less than 5% in a reaction. Restriction enzymes are typically supplied in 50% glycerol to prevent freezing at  $-20^{\circ}\text{C}$ . Some restriction enzymes show reduced specificity, or increased star activity, when the glycerol concentration in the final reaction is higher than 5%.

Some restriction enzymes require specific reagents such as BSA (Bovine Serum Albumin) or SAM (S-adenosylmethionine) in their digestion reactions:

- BSA is used to stabilize an enzyme. It can protect restriction enzymes from proteases, non-specific adsorption and harmful environmental factors such as heat, surface tension and interfering substances.

Restriction enzyme digestions are prepared in a PCR plate. After digestions are assembled, the plates are covered with adhesive and incubated for six hours at the temperature specified by the restriction enzyme. Typically,  $37^{\circ}\text{C}$  and  $55^{\circ}\text{C}$  digestions are conducted in the incubators. Digestions at temperatures higher than  $55^{\circ}\text{C}$  are performed in thermal cyclers.

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