

DEPHOSPHORYLATION OF DNA

5' protruding ends

1. Digest DNA in restriction buffer.
2. Add 1 μ l SAP and incubate for 30' at 37°C.
3. Add another 1 μ l SAP and incubate for 30' at 37°C.
4. Heat inactivate enzyme at 65°C for 10'.

3' protruding ends

1. Digest DNA in restriction buffer.
2. Add 1 μ l SAP and incubate for 15' at 37°C. and then 15' at 56°C.
3. Add another 1 μ l SAP and incubate for 15' at 37°C. and then 15' at 56°C.
4. Heat inactivate enzyme at 65°C for 10'.