Typically 100µl of maxi-prep. DNA (1mg/ml) is used.

Cut the DNA in a total volume of 200µl with the appropriate restriction enzyme to produce the 5' end to be labelled.

Incubation at 37°C overnight.

Remove the 5' phosphate using 5 units (≈5µl) Calf Alkaline Phosphatase (CAP), incubate at 37°C for 60 minutes.

Phenol/chloroform extract the DNA to remove CAP activity.

Ethanol precipitate, wash and dry.

Resuspend the DNA pellet in 175µl of water.

Cut the DNA in a total volume of 200µl with the second restriction enzyme. Incubated the digest overnight at 37°C.

Ethanol precipitate, wash and dry.

Resuspend in 30µl 1x Blue.

Separate fragments on preparative acrylamide gel.

Isolate and purify promoter fragment using electroelution.

Resuspend in 50µl TE and check 1µl on an acrylamide gel.

END-LABELLING DNA FRAGMENTS WITH [γ^{32}P]ATP

This involves working with radio-isotopes, work in the "Hot" lab., wear gloves at all times, monitor all surfaces and equipment before and after use, dispose of "hot" waste safely in the bags provided.

Set up following labelling mix:

DNA fragment (phosphatased at one end)	16µl
10x Kinase Buffer	2µl
[γ^{32}P]ATP (Amersham Cat. No. PB10168)	1µl
T4 Polynucleotide kinase (5-10U/µl)	1µl

Incubate at 37°C for 30 minutes.

Remove unincorporated nucleotides by passing through a sephadex G-50 spin column.

NOTE: The column will contain a high level of [γ^{32}P]ATP it should be placed in a labelled "PIG" in the "Hot" freezer and transferred to the radio-active waste bag after several half-lives.

Typically 0.25-1µl of labelled fragment is used in each footprint.