# NAP-PCR and Gel

### 1. Why was the DNA recovery quantity low?

Buffer B with the incorrect ratio was added to the amplification reaction.

Verify that an equal volume of the Buffer B was added to the reaction.

The ethanol(96~100%) was not added to the W2 buffer.

The gel slice did not dissolve completely.

The gel slice was too big. If the column was overloaded, decrease the loading volume. If overloaded , separate into 2 columns. If the DNA fragments were more than 300mg, separate the gel slice into two microcentrifuge tubes.

Dissolved incompletely: Increase time for the Gel Extraction Step until the gel slice has completely dissolved. Use an equal volume of the Buffer B and/ or low-melting-point agarose gels.Incorrect elution conditions: Ensure that the Buffer E or dd$H_2O$ is added into the center of the PG Column.

The recovery buffer volume was too small. Increase the amount of the Buffer E to at least 50 µl for use.

### 2. Why was the Inhibition of downstream enzymatic reactions present?

Use the ethanol to precipitate the DNA, or repurify the DNA fragments and elute with the nuclease-free water.

Remove the EtOH in the hood briefly.

Following the Wash Step, dry the PG Column with additional centrifugation at 14~16,000 x g for 2 minutes.

### 3. What if the DNA passed through in the flow-through or wash fraction?

Check the loading volume. If overloaded , separate into two columns. Ensure that any buffer prepared in the laboratory was prepared according to the instructions.

### 4. Purified DNA floats out of wells while running in agarose gel

Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. Re-centrifuge if necessary.