Evaluating Yield, Purity and Size of the DNA in a Genomic Prep

Before proceeding further into costly and time-consuming manipulations it is critical to analyze, at least in a cursory way, the DNA prep.

Estimating DNA Concentration by A260

The UV absorbance spectrum exhibits an $A_{\text{max}}$ @ 260 nm based on the aromatic ring structures of the DNA bases. This is the most convenient way to estimate DNA concentration and determine yield, as long as the DNA preparation is relatively free of contaminants that absorb in the UV. Proteins, and residual phenol left from the isolation procedure, are typical contaminants leading to an overestimate DNA concentration.

An $A_{260} = 1.0$ indicates a $[\text{DNA}] = 50$ µg/ml, assuming the DNA is pure.

We routinely use the Nanodrop spectrophotometer for this determination:

Detecting protein contamination by $A_{260}/A_{280}$

Protein contaminants in a DNA prep also will absorb UV but their $A_{\text{max}} = 280$ nm. Therefore, the $A_{260}/A_{280}$ ratio can reveal the presence of gross amounts of protein contamination. Pure DNA has an $A_{260}/A_{280}$ ratio of 1.9-2.0. Lower ratios
indicate substantial protein contamination. A higher ratio generally may indicate RNA contamination.

Agarose Gel Electrophoresis

Gel electrophoresis can confirm that the DNA in your prep is HMW and will reveal if there is a large amount of RNA contamination.
Assignment:

Compare your DNA yield (based on the Nanodrop result) to the expected (theoretical) yield you calculated previously. It is easiest to present this as "% expected yield".

We will run the DNA preparations later, on the same gels that we use to analyze the PCR product.