

Axiom Genotyping DNA Guidelines.

Project Management.

- Once a project has been accepted a project code and a project coordinator will be assigned to it and queries will be dealt with by that individual.

Sample Preparation.

- No specific kit or protocol is specifically recommended. A protocol or kit which provides high quality DNA and sufficient yield are the main criteria. If required Edinburgh Genomics will provide in-house protocols.
- DNA should be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The gDNA extraction/purification method should render DNA that is generally salt-free because high concentrations of particular salts can also inhibit enzyme reactions.
- DNA purity is indicated by OD260/OD280 and OD260/OD230 ratios. The OD260/OD280 ratio should be between 1.8 and 2.0 and the OD260/OD230 ratio should be greater than 1.5.

DNA Quality.

- Edinburgh Genomics will assume DNA quality will have been checked before shipping.
- Acceptable and unacceptable DNA qualities are shown on figure 1.
- Greater than 90% of the DNA should be larger than 10 Kb in size.

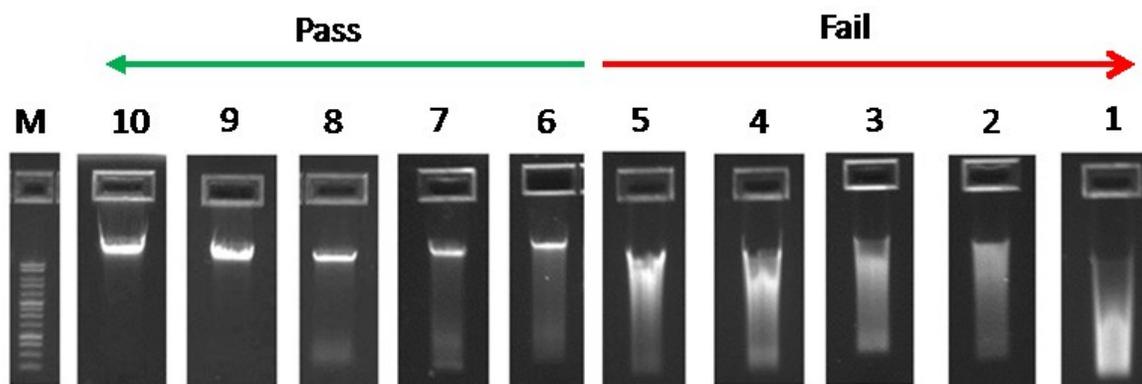


Figure 1: The marker is a 1Kb ladder (Pomega, G5711) with DNA fragments ranging from 250bp to 10Kbp. The gDNA samples range from very good quality HMW gDNA with very little degradation or RNA contamination (Image 9) to extremely degraded gDNA (Image 1). gDNA samples in images 9 to 6 would pass sample QC. gDNA samples in images 5 to 1 are degraded and would fail sample QC.



DNA Quantification.

- DNA samples should ideally be quantified by PicoGreen or Qubit assay. These assays have given consistent accurate quantitation required for good genotyping results.
- Quantification using other methods such as the Nanodrop and Spectrophotometers are less accurate leading to variable genotyping results. Please inform us if this is the only method available to you

DNA Requirements and Shipping.

- Dilute DNA to 50 ng/ μ l in water or 10mM Tris.
- A minimum volume of 20 μ l of each sample should be sent.
- Samples should be submitted in 96 well plates where possible
- Where multiple plates in a project are being studied consideration should be given to control samples to allow for plate to plate variation. There are two options:-
 - A single sample that is represented on each plate in at least one position
 - Or one sample from the first plate is repeated on the second and a different sample from the second plate is repeated on the third etc, ending with a sample from the last plate being repeated on the first plate
- When the Sample submission form 1 is complete Edinburgh Genomics administration team will send one of the following:
 - Samples sent in 96 well plates.
 - A Sample form. Fill in the requested details and return an electronic copy to Edinburgh Genomics. Also make a hard copy to return with your samples
 - A 96 well plate for each set of 96 samples or part thereof and self adhesive seals.
 - The sample form must be returned with your samples. Please could you return an electronic copy of the form in addition to a hard copy with the samples.
 - Wrap plates in bubble wrap and send to Edinburgh Genomics in an addressed jiffy bag by next day delivery at ambient temperature.

Return of Results.

- The results will be sent as a set of files. There will be a series of image processed files from the Affymetrix system, in the format of .ARR and .CEL files which are sufficient to recreate the analysis. We will also provide a file of genotypes from the data set produced using the Axiom Genomics Suite software or similar Affymetrix software using the default settings. A QC report of the results will also be prepared.