Sequenom Genotyping: Sample preparation guide

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This document details the requirements for submitting samples to our Sequenom Genotyping service. Before you send samples to us please ensure your have already completed an online quote request, received a formal quote from us, notified us of your acceptance the quote and provided us with purchase order. If you have not completed these steps, please do so, as this ensures that your samples can be registered by our sample tracking systems.

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1 Sample Preparation

1.1 Compatible DNA Extraction Methods

The SEQUENOM platform is compatible with most extraction methods that yield high molecular weight DNA of high purity. Cruder methods may work fine however you can expect lower pass rates and/or higher error rates. We have had reasonable success with Guthrie cards and buccal swabs.

Be cautious of:

- Highly degraded or very low concentration DNA (< 5ng/uL).
- EDTA levels in your samples. This can inhibit our chemistry. At 0.1mM EDTA we don't notice any problems.
- Tannins etc from plant based extractions.

1.2 Compatibility with Whole Genome Amplification Methods

Whole genome amplification methods can work reasonably well if amplification is performed on large enough quantities of starting material (>50 ng gDNA). This minimises introduction of allelic bias which can negatively affect genotyping performance. Please purify WGA material to remove enzymes and primers. DNA normalisation is also very important.

1.3 Amount of DNA

There are a number of factor that contribute to the amount of DNA required. As a rough guide we have provided some estimates below.

- For small projects (1 – 50 SNPs): Minimum of 100ng gDNA.
- For large projects (50-500 SNPS): Minimum of 500ng of gDNA.

Please discuss with us about the amount of DNA required for your project.

1.4 DNA concentration

Please supply DNA to us a **10ng/uL**.

We recommend that samples are normalised to the same concentration, as this can reduce variation in the data and yield better genotypes. This is particularly important for whole-genome amplified DNA.

1.5 Buffers

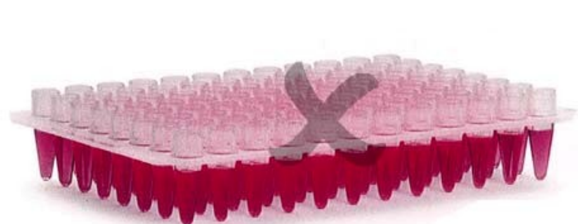
In general we recommend you submit to us in water.

However if you are submitting a large panel of samples that you intend on running several SNP studies on, over a reasonable period of time (i.e. greater than 6 months) then submitting in a buffer will help with sample stability. In this case we recommend you submit you DNA at a much higher concentration (i.e. 50-100 ng/uL) and use a suitable buffer. We will dilute aliquots of the samples in water when we process them.

1.6 Plate Format

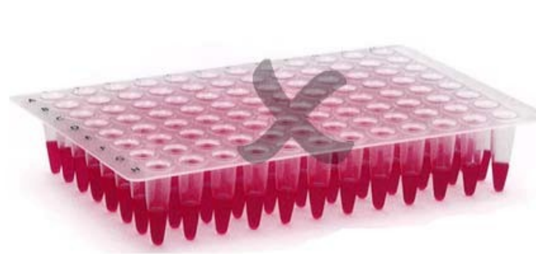
We require samples to be submitted to us in 96 well plates. Our preference is for V bottom half or full skirt plates. Pleas avoid the use of non-skirted or chimney stack plates.

A. Half Skirt 96 Well Plate



C. Chimney Stack 96 Well Plate

B. Full Skirt 96 Well Plate



D. Non-skirted 96 Well Plate

1.7 Plate Seals

Plates should be appropriately sealed. Well evaporation is quite common with poorer plate seals. We recommend a strong PCR film or tape. Effort should be spent ensuring the seal contains no bubbles and is firmly attached (i.e. pressed in using a pen or plate tool). There are a number of different grades of PCR films. Choose one that is thick and forms a strong bond with the plate. An example of a recommended seal is the ABgene Adhesive PCR Film: AB-0558. Heat-seals, alfoil seals, strip-caps may be used but these are not our preference, since they are more difficult to remove. Please ensure plates are enclosed in a resealable plastic bag.

1.8 Plate Labelling

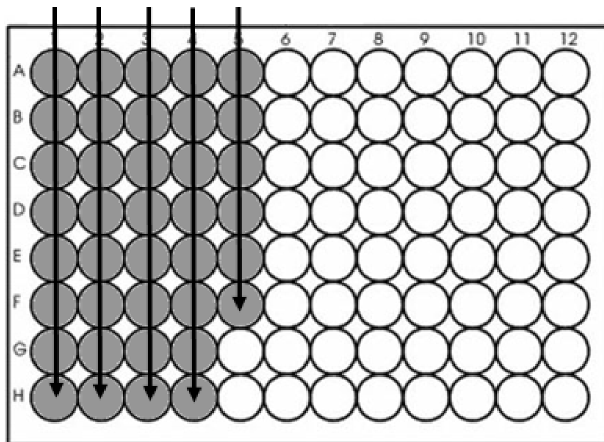
1. Please label your plates with the following:
2. A plate name / number
3. Date

Project Code (i.e. CAGRF1234). You can find this on the quote provided to you.

All plate labels should clearly match up with correctly labelled plates in electronic sample submission layouts. This will allow identification of your plates more readily should you require them at projects' completion. Plates should be labelled on the sides of the plates. Do not label on the top as this gets removed with the lid.

1.9 Sample Layout

Samples should be submitted in vertical format. That is, from A1 down to H1, then from A2 down to H2 and so on. **Blanks between samples will be charged at the normal rate**. Blanks after the last sample on your plate will not incur a charge. If you have already placed your samples in horizontal order and cannot change it, please contact the SNP team.



When submitting samples to AGRF an electronic sample submission file should be completed. Each sample well should be labelled as well as each submitted 96 well plate. This should be submitted as a Microsoft Excel spreadsheet prior to submitting samples. A sample submission template which outlines the correct format for submitting samples is available from the website.

1.10 Shipping DNA to us

The responsibility for ensuring samples reach AGRF in good condition remains the responsibility of the client. If samples arrive in poor condition we will notify the client of this as soon as the samples arrive. For domestic shipments (within Australia), AGRF recommend the use of a courier and shipping samples on ice. "Cold Packs" can be used instead of ice. Both TNT Express and DHL are commonly used for this. If samples have been dried down Australian Express Post may be used. For international shipments please ship DNA on 10kg of dry ice. A clearly marked air hole must be inserted for the dry ice to breathe so as not to explode the container.

agrf

AGRF Address for Sample Submission:

ATTN: SNP Team
Australian Genome Research Facility Ltd
Level 5 Gehrmann Laboratories Research Road
University of Queensland
St Lucia QLD 4072
Phone: +61 7 3346 9682

1.11 DNA Storage and Shipping DNA back to you

DNA is typically stored for 3 months after the completion of your project. After this time it is discarded unless specifically requested not to. We can also ship any DNA not used at the end of a project back to you. Please enquire if this is something you would like us to do.

2 Let AGRF extract and prepare your samples for you

To avoid the hassle of extracting DNA yourself and let AGRF do this step for you. Our extraction service works with a wide range of DNA sources and prepares DNA to the requirements of our service.

Please contact AGRF for a quote or assistance on your extraction.

PH: (08) 8313 7202.

3 Online Sample Submission

Once your samples are ready please fill out an online sample submission form so that we know they are coming. Details of how to fill out an online sample submission form can be found under the “Resources” section of our website.

4 I have more questions

If you have any further questions please contact us by phone or email.

PH: (07) 3365 4423

Email: SNPGroup@agrf.org.au