SERVICE GUIDE

Genotyping Agena SNP Service

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Service Guide:
Genotyping Agena SNP Service

1.0 Overview: Genotyping SNP Service at AGRF	3
2.0 Submitting Your Variants as RS Numbers	3
3.0 Submitting Your Variants Using Other Common Annotation Methods	3
4.0 Submitting Your Variants as Sequence	3
5.0 Proximal SNP Detection	4
6.0 GC Content	4
7.0 Duplicated Regions	4
8.0 Repetitive Regions	4
9.0 Sample Submission	5
10.0 Further Questions?	8

Service Guide:
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1.0 Overview

We know every project is unique, which is why we have a flexible approach to accommodate your research goal, with a broad range of applications and flexible solutions. You can expect proven assays and accuracy to ensure that projects are completed in accordance with our quality standards.

We offer support throughout the lifetime of your project whether it be experimental design, advice, or to answer any questions regarding sample status or analysis options.

This document details the requirements for submitting SNPs and/or variants to our Genotyping Service. This step is generally performed during initial discussions about your project and/or a quote request.

2.0 Submitting Your Variants as RS Numbers

The most common way to indicate which variant you want genotyped is to supply a dbSNP reference number (Reference SNP Number). You can supply these as a simple list within a quote or an email.

RS numbers are unique ID codes given to SNPs by the National Center for Biotechnology Information (NCBI) and stored in a database (dbSNP) (http://www.ncbi.nlm.nih.gov/SNP/)

We use the RS numbers to extract the SNP sequence information stored in the database, and these sequences form the basis of our designs. One of the advantages of dbSNP records is that proximal SNPs, which can interfere with our designs and are generally well annotated, can be modified to avoid them.

Not all valid SNPs have been assigned an RS number, and not all RS numbers refer to a valid SNP. However, for the majority of SNPs (and some variants) in human and many other model organisms dbSNP, provides an excellent resource.

IMPORTANT: Reported SNP Orientation

The SNP orientation (i.e. whether the SNP is reported in the + or – genomic strand) is dependent on sequence orientation provided by dbSNP. Unfortunately, this orientation can change between different builds. For A/G or C/T SNPs, orientation changes can be easily identified. However, for A/T or C/G SNPs orientation changes can go unnoticed. If you are concerned about a potential orientation change between projects completed at different times, we can provide the original sequences from which the designs were based. Comparisons with this information can reveal orientation changes between A/T and C/G SNPs.

3.0 Submitting Your Variants Using Other Common Annotation Methods

Generally we don't accept other SNP annotation methods. However, if there are only a few SNPs where you have a common annotation we will often try to help find the RS number or the relevant sequence.

4.0 Submitting Your Variants as a Sequence

For non-model organisms or SNPs which are not annotated in dbSNP, an RS number is sometimes not possible to obtain. In these cases, you can supply the sequence information directly by following the criteria below:

Service Guide:
Genotyping Agena SNP Service

1. Approximately 100 bases flanking each side of the SNP locus (minimum of 75 bp) for efficient assay design.
2. The sequence should not contain more than 1 ambiguous base ('N') within 25 bases of the SNP locus. If this is a limitation for a given SNP please discuss the options with us.
3. The flanking sequence itself must only contain A, G, C, T, N or IUPAC ambiguous codes.
4. The SNP should be bounded by square brackets as shown below:
   ACCAACACCA[A/C]CTTCCTGGGCTACACCAGGACGC
5. Insertions/deletions should be marked with a dash as follows:
   ACCAACACCA[AGCT/]CTTCCTGGGCTACACCAGGACGC
6. (Please note that the Agena platform can only deal with insertions or deletions up to around 10bp).
7. Trinucleotide polymorphisms should be marked as follows: ACGTCAGT[A/G/T]ACGTGCACCAC
   The sequence should contain no spaces.
8. The SNP ID for each SNP can be the RS number or any label you want to use. The SNP ID can be up to 12 characters long and must not contain any spaces or any of the following special characters (* ? / \ #. + : ;).
   An example of a formatted SNP list is shown below.

```
SNP_ID     Sequence
rs6504160  aacagaAGTAAACTTGGGAATGGCTGtgagtttt[A/C]aagttaaaagaaaaaatgaaagagttttaaATTC
rs11079820 TGATCCCTTGCAACagcgctgaggttctcattcc[G/T]caaaccttctGCCAGAAACTCTTCAGCATCtaaa
rs3826397  TAAATTGGGtgargggacagaggctggcatgag[C/T]ctctctggGGTATCTGGCTCCTGCTCATcctcct
rs2278868  GAATCTCTTCGCAGGtgggggccatccgtacac[C/T]gtagcccttAATGAGGAAGGTCCCTTTGGgctgc
rs12941830 TTCAGCATTGttactcacctcctagatttagtgt[C/T]ccttagtaaaataGAACTTGTCTCAGCTGCTCCc
rs1932618  TGTGAAGgaacgggcgaagaagtgagaagatgca[A/G]tgagcagcGATGCCCTGGTGAGAATGAAcccaaa
rs2296974  TCATGATTTGAGCATGCCcattaggaaacgaact[C/T]gaagaatctgcattgatGGGATCCTGACAGTTTC
rs913543   aaaGCAAAATGAATATTGGCATGGaaaccttgcc[A/G]catgaaaggctattGCACTACAAAGTAAACCCTG
rs2802699  CAACAAAAGTCattttctcaaaatatgtcacgct[G/T]tcttgcattattaaaCATAATCACAATCTCAAAT
rs2170508  GAAGAATTTGGTGgtataatctaagctgcactta[A/G]tatgttatataGCCAAGGGAAAAATAAAATGCta
rs495565   CCATCATTTCCTTCTGCaaaatgaaaaagacttc[A/G]ttttctCAACAGCTGCATCATTTTTttatgcata
rs3756772  taTCTCTCTTTTTAGGTGGTTCtttggagcaatc[A/G]gaagatcaGATGCAGAGAAACAACTATtatatty
```

Figure 1. 9. An example of a formatted SNP list. This information can be provided in a text file or Excel spreadsheet.

5.0 Proximal SNP Detection

Proximal SNPs (SNPs next to or near to a SNP of interest) can cause assay failure or incorrect results. At AGRF we perform automatic proximal SNP detection for human SNPs only. For other organisms where the locations of proximal SNPs is known within 100bp of the SNP of interest, it is recommend that these be marked on the submitted sequence using ambiguous DNA codes.

6.0 GC Content

GC content can affect the ability to develop a successful assay. The flanking sequence for the SNPs should have a GC content between 40% and 65% for ideal results.

7.0 Duplicated Regions

Duplicated regions of genomes are relatively common within mammalian organisms. Some species may contain substantial amounts of duplicated regions. In most cases, design in these regions is problematic and should be avoided. It is the submitter's responsibility to identify SNPs that fall in duplicated regions.

8.0 Repetitive Regions

SNP design in repetitive regions can be difficult or impossible. AGRF will attempt to design for SNPs in these regions but will fail them if the repeat regions cause significant problems.

9.0 Sample Submission

This section details the requirements for submitting samples to our Genotyping Service. Before you send samples to us please ensure you have already:

1. Completed an online quote request;
2. Received a formal quote from us;
3. Notified us of your acceptance of the quote, and
4. Provided us with a 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.

9.1 Sample Preparation

9.2 Compatible DNA Extraction Methods

The Agena 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.

9.3 Compatibility with Whole Genome Amplification Methods

Whole Genome Amplification (WGA) 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.

9.4 Amount of DNA

There are a number of factors 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 the amount of DNA required for your project with us.

9.5 DNA Concentration

Please supply DNA to us at 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.

9.6 Buffers

In general we recommend you submit your samples to us in water.
However, if you are submitting a large panel of samples that you intend to run several SNP studies on, over a reasonable period of time (i.e. more than 6 months) then submitting in a buffer will help with sample stability. In this case we recommend you submit your DNA to us 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.

9.7 Plate Format

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

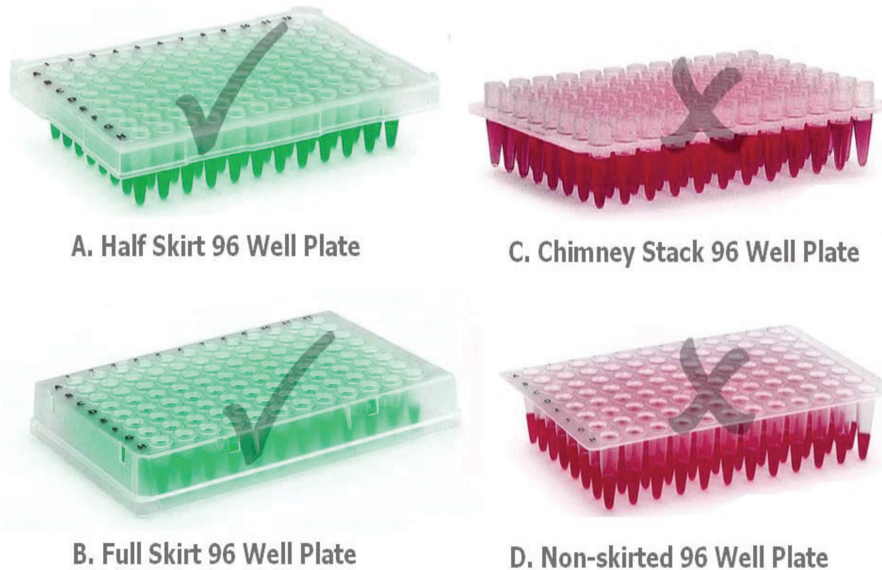

Figure 2. Our preference is for V-bottom half (A) or full-skirted plates (B) as seen above.

9.8 Plate Seals

Plates should be appropriately sealed since well evaporation is quite common with poorer plate seals. We recommend using 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. We suggest you 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. Alternatively, heat-seals, alfoil seals and 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.

9.9 Plate Labelling

Please label your plates with the following:

1. Plate name / number
2. Date
3. Project Code (e.g. 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 when the project is complete. Be sure to affix the label to the side of the plates. **Do not label on the top of plate as this is removed with the lid.**

9.10 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 (as you can see in Figure 3 below). Blanks between samples will be charged at the normal rate. However, 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 Genotyping SNP Team (genotyping@agrf.org.au)

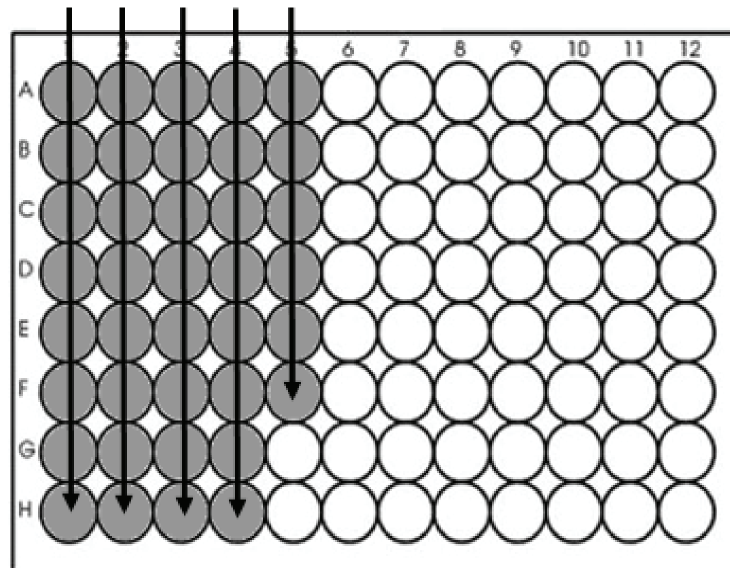

Figure 3. Sample layout format - submitted in vertical format, from A1 down to H1, then from A2 down to H2 and so on.

When submitting samples to AGRF, an electronic sample submission file should be completed. Each sample well should be labelled along with each submitted 96 well plate. This should be submitted as a Microsoft Excel spreadsheet prior to submitting samples.

9.11 Shipping DNA to us

The responsibility for ensuring samples reach AGRF in good condition remains with the client. If samples arrive in poor condition we will notify you as soon as the samples arrive. For domestic shipments (within Australia), we recommend you use a courier and ship samples on ice. Alternatively, 'Cold Packs' can be used instead of ice. Both TNT Express and DHL couriers are commonly used for this. If samples have been dried down, Australia Post Express may be used. For international shipments, please ship DNA to us on 10kg of dry ice with a clearly marked air hole for the dry ice to breathe. The air hole must be inserted to prevent the container from exploding. Please post/send/deliver samples to the address below:

Physical address:
AGRF BRISBANE
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

9.12 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 you have specifically requested for us not to do so. We can also ship any DNA not used at the end of a project back to you. Please enquire with us if this is something you would like us to do.

9.13 Let AGRF Extract and Prepare Your Samples for You

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 meet the requirements of our service.

Please contact AGRF for a quote or assistance with your extraction.
Phone: 1300 247 301
Email: CustomerCare@agrf.org.au

9.14 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.

10.0 Further Questions?

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

Phone: 1300 247 301
Email: CustomerCare@agrf.org.au