iPLEX & Uniplex Sample Submission Guidelines

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Please read these guidelines carefully. If samples are not submitted correctly, there may be additional charges per plate associated with the re-formatting of your samples.

I. DNA Mass Requirements

a. The mass of DNA required will depend on the scope of your project. The requirements of the individual assays run in the Core - which are the basis for the overall DNA requirements - are as shown here:

   i. **Two-step quality control** (QC#1 + QC#2) = **10 ng per 2-part QC**
   ii. **Uniplex** SNP genotyping (Taqman, Amplifluor), per call = **20 ng per assay**
   iii. **Agena** (iPLEX) multiplex genotyping, per 6-36 calls = **20 ng per assay**

b. The total mass required for a project includes overage to be used in re-analyses (if required), as well as to provide extra "dead-volume" for pipetting.

II. DNA Volume Requirements

a. The volume to be sent will depend on the mass required for your analyses and the concentration of your samples.

   i. First, calculate the amount of mass required for your analyses, adding a 50% overage for potential re-do runs (e.g., 10 ng for QC, 5 iPLEX plexes (for example) = 20 ng x 5 = 100 ng, 50% genotyping overage = 100 ng x 0.5 = 50 ng, TOTAL = 10 ng + 100 ng + 50 ng = 160 ng).
   ii. Next, based on the concentration of your samples, calculate the volume (e.g., if 10 ng/ul, 160 ng/10 ng/ul = 16 ul).

b. The minimum volume requirement for sample submission is 5 ul. If your DNA is so concentrated that the volume to be submitted is less than 5 ul, please dilute it prior to submission, such that the volume is at least 5 ul.

c. DNA may be shipped dried-down, if this is the only format in which it is available. If DNA is delivered dried down, **there will be a charge per plate to resuspend the DNA.**

III. Sample and Plate Requirements

a. Samples are to be delivered to the UMGC in **fully skirted, 96-well plates ONLY (Special charges: if DNA is received in an incorrect plate, sample transfer charges will apply).** Ideally, DNA should be provided at a uniform volume, mass and concentration for all samples (e.g., 200 ng DNA @ 20 ng/ul, 10 ul per well). If this is not possible, then users may instead provide a uniform, known mass (e.g.,

200 ng per well with varying volume and concentration). In the latter instance, you must ensure that the mass of all samples is adequate, according to the requirements described above in section I. Except under special circumstances; we do not accept plates that have varying mass and concentration.

b. Samples should be provided in standard 96-well plates that are properly sealed for shipping. We are not responsible for sample-to-sample cross-contamination that may occur during shipping.

c. **Special charges: if wet DNA is delivered at a fixed mass with varying volume and concentration, such that the plate requires drying down and resuspension before analysis, then there will be a charge per plate for this re-formatting to be carried out.**

IV. DNA Concentration Requirements

a. For wet DNA, the minimum concentration of DNA required for the individual assays we run is 5 ng/ul.

b. DNA should not be diluted by users; we prefer to receive DNA as concentrated as possible. The only circumstance in which users are encouraged to dilute their DNA samples in when they need to do so in order to increase the volume to assist in pipetting small masses. If users need to dilute their DNA prior to making an aliquot to send to us, it should be diluted with water only, not with buffers containing Tris or EDTA.

c. Variation in DNA concentration is typical of DNA sample plates. For Uniplex and Agena iPLEX assays, upwards variation in DNA concentration is generally not a problem. In other words, so long as the minimal DNA concentration on your plate is above the required concentration, more concentrated DNA (up to 10X more concentrated) will not cause the assay to fail

d. **Special charges**: **If DNA is delivered dried down, there will be a charge per plate to resuspend the DNA.**

V. Blanks and Replicates

a. Each 96-well plate must have two uniquely-located, empty (“Blank”) wells, to which nothing has been added. These blanks serve a dual purpose:

   i. They are negative (no-template) controls for genotyping assays
   ii. Their unique locations serve as a fingerprint to identify the plate and its orientation. Hence, the wells chosen must be different for each plate, and must be positioned asymmetrically, such that a 180-degree rotation of the plate results in a shift in the fingerprint. For example, wells A01 and G07 are acceptable, whereas wells A01 and H12 are not.

b. Sometimes, it is not possible for users to send plates with two uniquely-positioned blanks. Under those circumstances, we are able to create these blanks by either removing or repositioning samples. It is also good practice for users to include

sample replicates, which provide information on the reproducibility of genotype calls

c. **Special charges: if a plate does not contain uniquely located blanks, such that we must perform this re-formatting, there will be a charge per plate for this work to be carried out. If the Blanks are filled with water or buffer, there will be a charge to remove the solution and bleach these wells.**

VI. Quality Control

a. All plates of human DNA received in the BMGC undergo a two-step QC procedure, which involves two assays.

   i. QC#1 is a non-allelic quantitative-PCR analysis that measures the quantity of PCR-amplifiable DNA. It is a more relevant measure of DNA quantity than Picogreen fluorimetry, as it reflects the functional properties of the DNA samples (as opposed to the mere concentration). It is run against our own DNA standards, providing a measure of "functional DNA concentration."
   ii. QC#2 is an end-point reading from an Taqman allelic-discrimination (SNP) assay, and in addition to providing a second measure of the ability of PCR to amplify each sample, it is a sensitive indicator of sample-to-sample cross-contamination (which shows up as dispersed clusters).
   iii. Together, the two assays consume 10 ng of DNA.

b. Sample QC is performed prior to genotyping. Sample plates that perform well in QC tests are guaranteed to perform in subsequent Uniplex and iPLEX genotyping, whereas those that fail QC are not guaranteed to result in usable genotype data. After QC, has been performed, a QC report is generated and returned to users, with problematic samples flagged. Users may elect not to proceed with a project should QC suggest that a plate is of unacceptable quality.

c. In subsequent calculation of genotyping success rates, flagged samples will not be included. However, we do not, as a general rule, accept replacement samples for individual wells, as it is against our protocols to manipulate individual's wells, aside from the creation of blanks (see IV). Moreover, a "failure" in our QC tests does not mean that the samples will necessarily fail in genotyping. Our QC tests are stringent, hence "failed" samples may generate acceptable genotype information. We perform the QC analysis in order to provide a measure of the quality of an entire plate of samples, and in order to identify "poor" samples whose subsequent genotyping will be disregarded in measuring genotyping success rate. The results are interpreted against a backdrop of hundreds of other plates that have been assayed with these tests.

d. Under certain circumstances, users may elect to waive quality control, but do so with the understanding that all performance guarantees are therefore waived.

VII. Adjustment of Sample Concentrations (Normalization)

a. Normalization of individual samples is not a routine service of the BMGC, and we will not adjust samples that are determined to fail in QC analysis. If there are

many such samples, however, it is possible to have the Core carry out such work on a custom basis, for a fee. Alternatively, replacement plates may be provided, or the sample plates can be returned to users for adjustment and/or the replacement of individual samples.

b. **Special charges: the charge for sample normalization will depend on the extent of normalization required, and will be discussed prior to such work being carried out.**

VIII. Manifest and Shipping

a. Sample IDs should be provided on the form **Sample_Submission_Form.xls**, which includes instructions for completion. Plates should be shipped on dry ice, using overnight delivery.

IX. Frequently Asked Questions (FAQs)

a. *"Do you prefer DNA sent in wet or dry state?"*

   i. We prefer DNA sent in a wet state, frozen on dry ice. In fact, DNA sent dried down incurs a charge for resuspension (see section II).

b. *"Our DNA comes in a range of concentrations so I want to be sure to meet your minimum standards for all (or at least most) samples. How should I deal with this?"*

   i. There are three ways to deal with a range of concentrations in your samples:
      1. The first is to normalize the concentrations of DNA in your source plates prior to sending daughter plates to us. This will minimize the wastage of DNA, as none of the samples will be at an excess concentration, hence no DNA will be wasted.
      2. The second is for you to generate a daughter plate with different volumes for each well, such that you send us a fixed mass of DNA per well. If you send us such a wet plate of DNA with fixed masses but a range of concentrations and volumes, we will dry it down and resusupend it in a fixed volume and concentration (this will cost $100 per plate). Alternatively, you can dry the daughter plate down yourself, and send it to us dried, in which case we will simply resuspend it (this will cost $25 per plate). Lastly, you can dry down and resuspend the fixed mass daughter plate yourself at a fixed volume and concentration, in which case you will have normalized the daughter plate (there will be no charge).
      3. The third is for you to send us a wet DNA daughter plate with fixed volumes and the range of concentrations as they are, such that the concentration of the most dilute sample is equal to or greater than the minimum required concentration (5 ng/ul or 50 ng/ul, depending on the assay to be run). In this case, we will merely treat the plate as if it were all at the concentration of that most dilute sample (this should be the stated concentration on the sample submissions form).

c. *“There are a few (poor quality) DNA samples on my plates that are much less concentrated than the rest of the plate? Do these samples need to be at the minimum concentration? Does the stated concentration of my plate need to reflect these samples? Can’t you just disregard them?”*

    i. Yes, we can disregard them. If there are a few poor-quality samples on your plate, but you want to submit the plate without re-formatting, this is entirely up to you. Presumably, the poor-quality samples will be recognized as such in our QC tests. The stated concentration/mass of your plate does not have to reflect these samples, if you want them to be disregarded.