DNA Submission Guidelines
For Illumina Infinium and Golden Gate Genotyping Assays

OVERALL DNA QUALITY:

- All DNA** should be at a minimum concentration of 50ng/µl in TE (10mM Tris, 0.1mM EDTA), low conc. EDTA TE (10mM Tris, 0.1-.05mM EDTA) or Tris.
- Minimum volume of 15µl-20µl, for a total DNA quantity between 0.75-1.0 µg. Please note that concentrations between 50-100ng/µl are best suited for our genotyping assays.
- The 260/280 ratio from a UV spec. reading should be 1.65-2.1. Samples with 260/280 ratios below 1.65 will result in lower call rates. If the 260/280 ratio is low, the DNA should be re-extracted.
- CAG will run DNAs on an agarose gel to test for DNA quality and quantity. If the samples do not meet our specifications, CAG reserves the right to refrain from genotyping them.

PROJECT OVERVIEW:

- Samples should be gathered and submitted in the shortest time frame possible to avoid necessary Illumina chip version and reagent batch changes and other batch effects beyond our control.

Projects should be clearly identified with:

- Disease (project level)
- Cases, Cases and Controls, or Families (project level)
- affected or unaffected (sample level)
- If families are submitted, make sure the family ID and individual ID are clearly labeled and let us know your scheme for this (i.e. 122-1, 122-2 122-3 means family number 122, 1=mother, 2=father, 3=affected proband).
- Make sure the families submitted are complete trios. Most statistical test cannot make use of incomplete trios. Given knowledge of your family labeling, we will double check that complete trios are being submitted, if that is what is intended.

PLATING:

Don’ts:

- Do not leave any wells empty or fill with water/TE. The exception to this rule is the last plate of your samples. If wells are empty or filled with water/buffer, indicate it on the DNA manifest file.
- Do not duplicate samples. **REPEAT: DO NOT plate duplicates**
- Do not use an ELISA plate or any flat-bottom plate.

Do's:

- Plate DNA into a 96 well 0.2ml, V bottom, skirted plate (Fisher #AB-0800, USA Scientific #1402-9800, or World Wide Medical Products # 41081006)

Fill out a DNA manifest:

- *Plate Label*: Plate ID, make sure that manifest name matches what is written on the actual plate *Well*: Fill in the well locations.
- *Sample Label*: Unique sample ID without any patient identifiers (no initials, D.O.B., SS #)
- *Sex*: to best of your ability fill out the gender
- *Volume*: As stated above, the volume should be at least 15ul
- *Concentration*: at least 50ng/µl in TE (10mM Tris, 0.1mM EDTA)
- *Tissue Source***: What is the source of DNA: blood, tissue, cells?
- *Age*: Age of person at sample collection
- *Primary phenotype*: Chief diagnosis. Please indicate primary phenotype, ie. What disorder does this person have? Please note of the sample is a control
- *Family ID*: if applicable, if you do not have mother, fill with "0"
- *Paternal ID*: use 'Sample ID or family identifier + "-2"' of case study's father, if you do not have father, fill with "0"
- *Maternal ID*: Sample ID or family identifier + "-1"' Case study's mother, if you do not have mother, fill with "0"
- *Secondary phenotype*: any diagnosis other than primary phenotype
- *Co-variants 1 and Co-variants 2*: other available phenotype
- IS control: please leave this column filled with zeros

- Seal the plate with an adhesive seal (ABI, #4311971). This particular seal remains intact from room temperature through -80 degrees. Make sure the seal is secure by pressing down, with your finger, on each individual well. To submit samples or if you have any questions, please email Maria Lemma: GarrisM@email.chop.edu

** Whole Genome Amplified DNA must be a fresh amplification using Repli-G (Qiagen). Others kits have been shown (through collaborators) to amplify the water control. The concentration must be measured with picogreen. Older amplifications have been shown to give less than 96% call rates and will yield unusable results.

**We cannot use DNA extracted from fixed tissue for the Infinium assay, but can for the Golden Gate assay: The GoldenGate Assay can tolerate relatively short stretches of target DNA (> 200 bp) and can be quite tolerant of degraded FFPE samples. Internal experience with FFPE samples used with the GoldenGate Assay indicates that high-quality genotyping data can still be obtained by using FFPE samples with the GoldenGate Assay. Decreased call rates from FFPE samples compared to genomic DNA samples may be observed and the decrease in call rate depends on the level of sample degradation.

**If you need to concentrate the DNA, please use a speedvac and refrain from precipitating using NaOAC or other salts + ethanol

SHIPPING:

The following shipping guidelines were set up so that your DNA arrives safely. Please follow as closely as possible.

- Ship DNA overnight on dry ice Monday – Thursday (no one will be here to receive the package on weekends), in compliance with local, state, and federal regulatory commissions.

- Freeze the sealed plate in the -20 degree before placing on dry ice because placing the plate directly on dry ice, the plate will crack.

- Wrap plates in paper towels or bench pads. Do not cram plates into a small box, but use a larger box, and leave enough room for ample dry ice and padding. Ensure that the adhesive seals do not come in contact with other plates or sides of the box.

- Seal the 96 well plate with an appropriate adhesive seal that will withstand the shipping, and place in a water tight bag. If shipping more than one plate, stack them so there is padding between the plates. This padding prevents one plate from puncturing the seal of another. Further, provide enough padding to keep the plates from shifting inside the package during the shipping process.

Our shipping address:

Maria Lemma
The Center for Applied Genomics
Children's Hospital of Philadelphia
3615 Civic Center Blvd.
Abramson Research Center, 1215A
Philadelphia, PA 19104

Here are some things that we've received and do not want to see again:

- Frozen plates with holes in the foil seals from another plate sitting on top of it
- Frozen plates where the seal has lifted off of the plate completely and samples evaporated
- Frozen plates where the wells were completely cracked, broken off, or chipped into pieces
- Room temperature plates where the seals have lifted off and the DNAs seeped into other wells, contaminating other samples
- Room temperature plates with wells completely dried out
- ELISA/TC plates used with an adhesive seal + lid on top…most wells were evaporated.
- Manifest plate labels not matching what was written on the actual plate
- Manifests indicating an empty well when there was DNA
- Manifests indicating DNA and nothing is in the well (Even after elution, we found no DNA)
- Manifests with incorrect sample well locations and incorrect IDs