



Data2Bio, LLC
2052 Roy J. Carver Co-Laboratory
1111 WOI Road
Ames, IA 50011-1085
questions@data2bio.com

Sample Submission for tGBS projects

(Updated 12/13/2018)

If you will be submitting tissue samples for DNA isolation and tGBS genotyping, please refer to our tissue sample submission FAQ and then return here for additional tGBS-specific information.

Sample Requirements:

While the construction of tGBS libraries is relatively insensitive to DNA quality, low quality samples can interfere with our normalization process and result in greater variance in the number of sequence reads generated per sample and the overall quality of the project. For this reason, we request that you send us DNA samples that have been extracted using Qiagen kits (DNeasy kit or Magattract Core kit) or equivalent. For best results, we recommend employing a commercial high throughput DNA isolation kit such as Qiagen's DNeasy or Magattract Core lines.

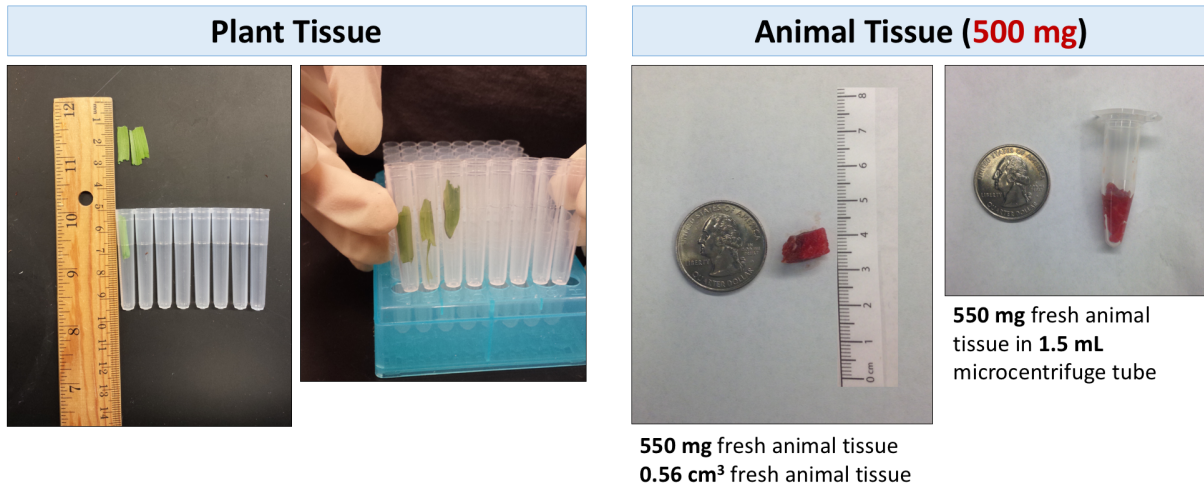
DNA quantification requirements¹:

- It is highly recommended that customers quantify their samples by fluorescent based methods (i.e. Qubit, PicoGreen Assay)
 - Recommended QC values based on **fluorescence**:
 - At least 5 ng/ μ L in 100 μ L volume = 0.5 μ g per sample
- In cases where customers are unable to quantify by fluorescence, we do accept QC values based on absorbance (i.e. NanoDrop)
 - Recommended QC values based on **absorbance**:
 - At least 75 ng/ μ L in 100 μ L volume = 7.5 μ g per sample
 - 260/280 ratio of each sample should be between 1.7-2.0

We will QC your sample upon their arrival, and let you know if any samples fall outside the expected range.

Please see pictures below for details about **preferred** and **maximum** tissue amounts required.

Tissue Amounts for tGBS Experiments



¹ When necessary we can often work with smaller sample quantities. Please contact us at questions@data2bio.com

What happens if some of my samples fail QC?

We will QC your samples upon their arrival, and let you know if any of them fall outside the expected range.

- If an entire plate of samples exhibits unacceptable quality (as is often seen with CTAB-extracted DNA) we may offer to re-purifying the entire plate at our facility which can often remove contaminants that influence DNA quality estimates and quantification. This will, however, incur an additional charge.
- **For logistical reasons, we are not able swap out (or re-array) individual samples that have failed to pass QC.**
- If samples do fail QC (e.g., because of insufficient DNA) and we cannot purify them, you are welcome to send an **entire plate** as a replacement. Please realize that this will delay of project completion while we wait for replacement samples (we won't proceed with the project until all samples have passed QC).



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- If you prefer, you can also submit lyophilized tissue samples and Data2Bio will isolate and quantify DNA. This will, however, incur an additional DNA extraction charge.

Naming Samples for Data2Bio's tGBS Analysis Pipeline:

Each unique genotype submitted for tGBS should have a unique ID consisting of only alpha-numeric characters and optionally underscores*. During analysis lowercase letters contained with sample identifiers will be converted to uppercase letters and all special characters will be replaced with underscores**. In addition, white space (blank) characters leading and/or trailing sample IDs will also be removed.

If you submit multiple plates of samples, each unique genotype should have an identifier that distinguishes not only from other genotypes on the same plate but across all submitted plates. In the event of projects requiring the use of the data generated from previous projects, please ensure that the new samples have unique identifiers relative to the previous project if samples are meant to be kept separate.

Our default analysis pipeline treats all samples having the same ID (regardless of case, within plate, or between multiple plates) as the same genotype and the generated reads from those sample wells will be pooled (combined) in downstream analyses. Sometimes clients intentionally replicate the same genotype among their samples. If you wish these replicates to be pooled during our post-sequencing analysis (this is our recommendation for parental controls) then you should use the identical sample ID for all the replicates (e.g., ABC, ABC etc.). If, on the other hand, you do not wish the replicates to be pooled during sequence analysis they should be given unique IDs (e.g., append an underscore followed by a number after the common ID shared by all replicates: ABC_1, ABC_2 etc).

* We realize some sample IDs contain dashes. Our analysis pipeline will automatically replace the dashes with underscores. Please alert us if that will be a problem.

** Please contact us if you have a special case or a more complicated situation.



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Preparing your samples for shipment:

Required Method:

- For tGBS all samples should be submitted in 96 well plates. **Samples submitted in individual tubes will incur additional handling fees.**
- Please send all your samples at the same time. **We will not proceed with library prep until all samples have arrived at our facility and have passed QC.**
- Please send frozen DNA samples eluted in EB buffer or water.
 - Do NOT send DNA eluted in solutions with high concentrations of EDTA (such as TE buffer).
 - In some circumstances with highly concentrated DNA, we can genotype samples sent in TE buffer, but only if you inquire and get approval from our QC team before mailing your samples.
- Carefully seal each plate using sealing caps (either rubber mat or plastic caps) and wrap each plate individually with bubble wrap before placing into Styrofoam container
 - Avoid foil because it be punctured during shipping
 - Avoid film because it can come loose from plate, especially if samples thaw
- Pack Styrofoam container with dry ice
- Use [this template form](#) to create your sample list and include a hard copy print out in the package.
- Seal the Styrofoam container with package tape to slow the sublimation of dry ice
- Ship via overnight express
- See further below about shipping

Some of our customers live in countries where overnight service to the United States is unreliable or not available. We have tested a number of protocols for shipping samples internationally, please contact us directly at questions@data2bio.com to discuss which method is most suited to your situation.



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Shipping:

When you are ready to mail your samples please send the tracking number and a copy of your sample list to: SampleSubmission@data2bio.com

Ship your samples to:

Data2Bio LLC
Attn: Lisa Coffey
2052 Roy J. Carver Co-Laboratory
1111 WOI Road
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It is important to time the shipment of your samples so they will arrive on a weekday rather than a Saturday, Sunday, or US Holiday. For an updated list of US holidays which may influence package delivery check this website:

<http://www.theholidayschedule.com/post-office-holidays.php>

Additional Notes:

Some projects rely on data provided by the client rather than (or in addition to) data generated by Data2Bio. Client-supplied data are expected to arrive at Data2Bio at project initiation (e.g., when samples are provided). Delays in supplying such data are likely to delay project completion.