Guidelines for GBS sample submission

DNA quality and quantity

High quality DNA is required to produce GBS libraries. We recommend Qiagen DNeasy miniprep kits for extraction of plant DNAs. DNA must be RNase treated. DNA should be suspended in TE buffer. Please also refer to our document *Guidelines for preparing gel images*.

RNAse-I treatment: 3? µL RNAse-I added to each gDNA sample.

DNA quality

DNA should be free of contaminants and symbionts, and should have low (less than 5%) organellar DNA content, since contaminating DNAs will be incorporated into GBS libraries along with the DNA of interest. When extracting DNA from animals and insects, the gut contents and other potential sources of non-target DNAs should be avoided.

Plant tissues, especially medicinal plants, may contain compounds that inhibit enzymatic activity. We recommend extracting plant DNAs from very young tissues as these have a higher DNA content and lower concentrations of compounds that inhibit enzyme activity. If extracting DNA from a medicinal plant (or if your samples do not cut well), add 2.8% polyvinylpyrrolidone-40 (PVP-40) to the extraction buffer before column purification (Wallace and Mitchell, 2017 doi: 10.1002/cppb.20042).

DNA must be of high molecular weight. Degraded samples (majority of fragments smaller than 20 Kb) are not acceptable. DNA samples are not acceptable if they contain RNA blobs, discrete low molecular weight bands, or migrate abnormally in agarose gels. We need to evaluate gel images of every sample (not cut by restriction enzyme) to be sure that they are not degraded. If we identify problems with any individual sample, you will have the chance to replace it or re-extract before we do our part. It is also good practice to see both uncut and cut so that we can compare them directly (see trial digestions below).

DNA quantity

We require 30µl of DNA at 50-100ng/µl concentration. DNA samples must be quantified using an intercalating dye such as PicoGreen. Nanodrop readings are not acceptable. We cannot guarantee results for samples at concentrations lower than 10ng/µl.

Provided sample concentrations are within the appropriate range, they do not need to be standardised across the plate. We will do this when the samples are received.

Each well must contain 30µl of DNA. We will not accept plates with different well volumes, even if you dry the DNAs prior to shipping.

Trial digestions

To ensure that DNA samples are of sufficient quality for library construction, trial digestions must be

performed prior to sample submission. We recommend the use of an inexpensive 6-base cutter, such as *Hin*dIII or *Eco*RI. Aliquots of cut and uncut DNA should be run on an agarose gel. Not all samples need to be tested, we recommend testing 10% of samples from each round of DNA extractions. Gel images must be sent to us for review.

Multiple species
Please avoid combining multiple, unrelated species in a single plate. The amount of adaptors required varies depending on genome size. Therefore, species with very different genome sizes cannot be genotyped on the same plate. In addition, mixing of species complicates data analysis.

Plate layout and controls
DNA samples must be submitted in half-skirted (semi-skirted) 96-well plates. An example is shown below. We will not accept full-skirted or non-skirted plates. Please do not use plates that have adhesive on the plate itself, as they stick to our liquid handling robots.

Please include only one set of DNAs per plate (eg. Each 16-plex or 32-plex set should be in a separate plate). All submitted plates must contain two empty wells. This allows us to include one negative control and one positive control. Thus, a 96-plex plate should contain 94 DNA samples and two empty wells, a 16-plex set should contain 14 DNA samples and two empty wells. We will provide a sample sheet indicating where the empty wells should be.

DNA samples in all plates are quantified when they arrive at the lab and the client will be notified if blank wells contain detectable levels of DNA. DNA in the blank wells can result if plates are not completely sealed and DNA samples leak during shipping, or if a DNA sample has been removed from a well to create the blank.

Plates must be arrayed in the order required
Please note that we do not re-array plates. DNA samples must be submitted in the required layout. We will not move DNA samples between wells, nor will we combine partial plates.

DNA samples for optimisation or validation
If we have not worked with your species before, we will need to perform an optimisation or validation

to determine the appropriate enzyme and adapter concentration. Please provide extra DNA for the optimisation/validation. This is in addition to your plated DNA samples. DNA from several individuals may be pooled for this step. We require at least 2µg of DNA dried down in a 200µl PCR tube (or multiple PCR tubes). Please let us know the original volume and concentration so that we can resuspend appropriately. There is a tab on the sample sheet for this information. Failure to send DNA for the optimisation/validation step or to provide information for resuspension will result in delays for the project.

Labelling plates and tubes

Please label plates with the LOE number and plate number. Please label optimisation/validation tubes with the LOE number and the tube number entered in the sample sheet.

Packaging and shipping

Do not ship your samples until you have received shipping instructions from us. Please do not pack your samples in polystyrene/ styrofoam for shipping.