ddRAD and Allegro genotyping

Sample preparation guidelines

Plant material (for plant DNA extraction service)

If a DNA extraction service is requested, leaf material must be provided in Qiagen collection microtubes and caps https://www.qiagen.com/us/products/discovery-and-translational-research/lab-essentials/plastics/collection-microtubes-and-caps/.

About 50mg of leaf material must be put in each tube during collection. After collection, the 96-racked plate must be snap-frozen (-80 °C is preferable) and then shipped using dry ice. Tubes must be racked in their container box. Sample coordinates and plate identifier must be recorded in the Sample Spreadsheet.

It is suggested, when possible, to send a replica plate in case extraction must be repeated for any reason.

DNA extraction and quantification suggestions

We recommend using column-based or beads-based isolation system, which provide more reproducible DNA quality and quantity across samples. Alternative protocols such as CTAB methods are suitable as well. However, customers should be aware that CTAB/phenol-chloroform extraction methods may leave contaminant residuals in the DNA, which can impair enzymatic reactions during library preparation. Moreover, CTAB leftover in the sample can cause over-estimation of DNA concentration when using absorbance-based instruments (Nanodrop). **We therefore encourage to estimate DNA concentration with dsDNA-specific intercalating agents** (e.g. Qubit, fluorometric plate reader).

For this pooled application **DNA normalization** is pivotal and DNA must be provided to same concentration for all samples (±20%). Failure in using normalized DNA will result in high sample-to-sample sequencing yield variation, hampering the quality of results.
This is the reason why we encourage our customers to provide accurate quantification of samples or to inquire for our internal quantification/normalization procedure.

Sample requirements

1. We require a minimum of **500 ng** of DNA per sample, with a concentration ranging from **20 to 50 ng/µL**. If DNA concentration has been measured using spectrophotometric methods, such as **Nanodrop**, we strongly recommend customer supply us with twice the required amount (min. 50ng/µL and 1µg total).
2. We require at least **30 µL** of volume per sample, in water or Tris-HCl (pH 8.0-8.5) or standard kit's elution buffers (no EDTA must be present in the solution – e.g. TE buffer).
3. Quality of the DNA should be **260/280 ≥ 1.7** and **1.6 ≤ 260/230 ≤ 2.2**.
4. Fragments must be >20Kbp (an 0.8% agarose gel can be used on few samples to check for this requirement)
5. **We only accept samples arranged in 96-wells plates indicated below.**
6. The excel file "Sample Spreadsheet" (reachable on our website, documents section) **must** be submitted via email, and **must** contain non-duplicated sample names, position in the plate, plate name, quantification.

If any of these conditions is not satisfied, IGATech will not take responsibility on poor quality of results (i.e., uneven coverage across samples or loci). IGATech will always perform a check on DNA concentrations and quality. If sample requirements are out of specifications, it will be communicated to the Customer and he will be responsible to proceed with the current conditions, enquire for additional normalization or custom clean-up or send replacement samples.

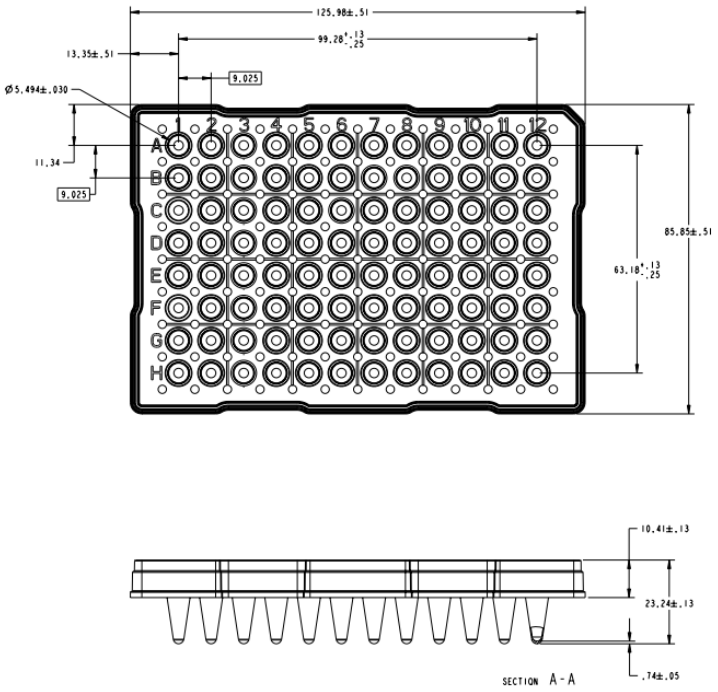
Dry ice shipment is the preferable method to avoid evaporation and DNA degradation. If the latter is not available, cold packs can be used instead if the duration of shipment does not take too long. Organize the packaging in a way to avoid damaging and dispersion during the shipment: place tubes and plates in smaller plastic bags or boxes. Put a separator between stacked plates to avoid perforations of adhesive foils and leakage. Wells must be **tightly sealed with cap-strips (preferable) or aluminum adhesive foils (plastic foils can often detach)**.

Include in the parcel a copy of the compiled **Samples Spreadsheet,** reporting the **quotation number**. Indicate in the notification mail the Tracking Number of your shipment.

*Quantification and normalization procedures can be applied at additional costs. Please inquire.

Plates must be one of the following or similar in their specification and sizes:

- *MicroAmp Optical 96-Well Reaction Plate' (Thermo Fisher™) REF N8010560*
- *Eppendorf 96-Well twin.tec™ PCR Plates (Eppendorf™) Order no: 0030 128.672, Cat. No: 951020460*
- *Sarstedt PCR plate half skirt, 96 well, transparent, High-Profile, 200 µl, PCR Performance Tested, PP; Order no: 72.1979*



Reference measures of accepted plates

- If you send in plates with different characteristics, please also send two empty plates to allow us setting-up liquid handlers properly.

Batch-effect

If samples have **different origin, such as tissue type, method of extraction, age of the specimen,** it is expected to have significant differences in concentration of samples and also different efficiencies in library preparation. IGATech will not take responsibility on variance caused by this factor and strongly encourage Customer to:

- For large projects: arrange the specimens that vary for quality/concentration in different plates (plates must be filled with 96 samples anyway).
- Indicate different sample origins (along with quality and concentrations) in the Sample Spreadsheet.

Population information (ddRAD only)

If customer provides any "population structure" information inside the Sample Spreadsheet, this will be used in the analysis to provide segmented output files on sub-population level and summary metrics of diversity and inbreeding.