# ddRAD-seq sample preparation guidelines and Quality Policy

**DNA extraction and quantification suggestions**

We recommend using column-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 inflate DNA concentration estimation when using absorbance-based instruments. **We therefore encourage to estimate DNA concentration with dsDNA-specific intercalating agents** (e.g. Qubit, fluorometric plate reader).

IGATech can perform normalization of plates in front of an additional fee. On a standard basis IGATech is not involved in DNA quantification/normalization. Normalization of DNA concentration is a crucial factor to obtain an even sequencing coverage across samples. 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 **1 µg** 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 (2 µg).
2. We require at least **50 µL** of volume per sample, in water or Tris-HCl (pH 8.0-8.5).
3. Quality of the DNA should be **260/280 ≥ 1.7** and **1.8 ≤ 260/230 ≤ 2.2**.
4. **We only accept samples arranged in 96-wells skirted plates and a minimum of 96 samples (with increases by multiples of 24).**
5. The excel file "Sample Spreadsheet" (reachable on our website, documents section) **must** be submitted via email, describing: sample names, position in the plate, quantification and absorbance ratios.

If any of these conditions is not satisfied IGATech may reject the preparation of samples.

Send DNA samples in a cold pack (*e.g.* blue ice) or dry ice. Do not ship plates without secondary containment as these may crack when placed directly on dry ice. Wells must be tightly sealed with cap-strips or adhesive foils. Include in the parcel a copy of the compiled Samples Spreadsheet, reporting the relative quote number. Indicate in the notification mail the Tracking Number of your shipment.

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.

**Quality policy**

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 IGATech will not take any responsibility on the outcome of sequencing yields in case it is agreed to proceed with the experiment.

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. We can only accept this situation if:

- Customer provides the specimens that vary for quality/concentration in separate plates.
- All different sample origins (along with quality and concentrations) must be documented in the Sample Spreadsheet.