ddRAD-seq sample preparation guidelines

DNA extraction and quantification suggestions

IGATech offers nucleic acids extraction service and we can set up a dedicated extraction workflow for your specific substrate. Please enquire.

We will accept samples only if arranged in 96-wells plates and sample location spreadsheet must be present (containing quantification and volumes, when provided by customer).

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 keep in mind that CTAB/phenol-chloroform extraction methods have the drawback of being more prone to leave contaminant residuals in the DNA, which can impair enzymatic reaction during library preparation, resulting in unbalanced representation of samples during sequencing. Moreover, CTAB leftover in the sample can dramatically inflate DNA concentration estimation when using absorbance-based instruments. We therefore encourage in such scenarios to estimate DNA concentration with dsDNA-specific intercalating agents (e.g Qubit or standard DNA electrophoresis with ethidium bromide). IGA Tech can perform estimation of DNA quantities with dsDNA-specific fluorimetry assay and perform normalization of plates in front of an additional fee. When IGATech is not involved in DNA quantification/normalization, IGATech is not responsible for the sequencing yield across samples. 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

- We require a minimum of **1ug** of DNA per sample, with a concentration ranging from **20 to 50 ng/uL**.
- Quality of the DNA should be 260/280 > 1.8 and 1.8 < *260/230* <2.0.
- Quantity of DNA should be estimated with dsDNA-specific assay if possible, especially when adopting CTAB-based extraction protocols. We always check few samples with dsDNA-specific assay. However, in case we notice the provided quantification is largely over-estimated and samples are not satisfying quality/quantity requirements for library preparation, we reserve to proceed in the experiment.
- We only accept samples arranged in 96-wells skirted plates, even if sample number is lower than 96.

- An excel spreadsheet must be submitted describing sample position in the plate (along with quantification when provided); *if customer provide any subpopulation information along the samples this will be included in the analysis to allow per-subpopulation genotyping ratio criteria and to provide segmented output files (plink, genepop) and summary metrics of diversity/inbreeding.*
- DNA concentration across samples should be even (+/-20% of average) within each plate unless a normalization procedure is requested as service (please inquire). In case internal normalization service is waived, IGAtech is not responsible for the quality of the data in term of per-sample coverage.

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.

Please, do not forget to send us the compiled **Samples Spreadsheet**, both with the shipped parcel and via e-mail. In order to be able to properly track and safeguard your samples we also ask you to send us the **Tracking Number** via e-mail.