PREPARING 96-WELL PLATES FOR ALLEGRO GENOTYPING

Quality of the DNA should be 260/280 > 1.8 and 260/230 >1.8.

Please note that fluorimetry-based quantification (e.g. Qubit, plate-reader) assays are more accurate methods than absorbance-based methods (e.g. Nanodrop) which might overestimate the quantity. So, the DNA quantifications should be made by a fluorometric method.

DNA must be re-suspended in 10mM Tris-HCl pH 8.5 (standard elution buffer of most commercial column-based extraction kits); water is accepted as an alternative (NO high concentration of EDTA must be present in the solution – e.g. no TE 1X buffer 1mM EDTA - but consider 10mM Tris-HCl as best buffer for DNA stability or 10mM TrisHCl + 0.1 mM EDTA).

1. Volume not less than 30µL possibly equal for all the wells of a plate;
2. Normalized concentration between 20 and 30 ng/µL;
3. Use 96-well plates 'MicroAmp Optical 96-Well Reaction Plate', cod. N8010560 from Thermo Fisher;
4. Place the name of the plate on the edge in a clear and long-lasting way (for example, use the serial progressive sticker placed on the edge of the plate by the manufacturer, but any UNIVOCAL CODE is fine; even for plates sent in multiple shipments use univocal codes);
5. Be careful to seal the plate very well with the adhesive aluminum foil;
6. Ship in dry ice to avoid evaporation of samples;
7. Be careful when stacking the plates. It is better to put a soft plastic separator or cardboard or insert each plate in a small bag, to avoid that during the transport the top plate wells, holes the wells of the underlying plate;
8. For each plate prepare an **Excel sheet** which shows:
    - ✓ Unique name of the plate (please use as characters ONLY Letters, Numbers, Underscore; DO NOT USE special characters or spaces between letters and numbers);
    - ✓ List the samples in plate **COLUMNS (from 1-12)**;
    - ✓ The Sample Name must be created as follow:

      IDxyz_PlateName_SampleName_WellPosition

      Please note that the IGATech Code will be assigned by IGATech when the plates are received. Use ONLY Letters, Numbers, Underscore; DO NOT USE special characters (%&$...) or spaces between letters and numbers;
    - ✓ Next to the column containing the name of the samples, the column must be placed with the corresponding concentration and a column with the volume of material sent;
    - ✓ Indicate in the Excel sheet also EMPTY WELLS, if they are eventually present.

Example of Excel sheet

Plate Name 10439924

Well Position	Sample Name	Conc. ng/uL	Vol. uL
	IGATechCode_PlateName_SampleName_WellPosition		
A01	IDxyz_10439924_GPE02816_A01	25	30
B01	IDxyz_10439924_GPE02817_B01	25	30
C01	IDxyz_10439924_GPE02818_C01	25	30
D01	IDxyz_10439924_GPE02819_D01	25	30
E01	IDxyz_10439924_GPE02820_E01	25	30
F01	IDxyz_10439924_GPE02821_F01	25	30
G01	IDxyz_10439924_GPE02822_G01	25	30
H01	IDxyz_10439924_GPE02823_H01	25	30
A02	IDxyz_10439924_GPE02824_A02	25	30
B02	IDxyz_10439924_GPE02825_B02	25	30
C02	IDxyz_10439924_GPE02826_C02	25	30
D02	IDxyz_10439924_GPE02826_D02	25	30
E02	IDxyz_10439924_GPE02828_E02	25	30
F02	IDxyz_10439924_GPE02829_F02	25	30
G02	IDxyz_10439924_GPE02830_G02	25	30
H02	IDxyz_10439924_GPE02831_H02	25	30

COLUMN 1

COLUMN 2