# Sample Requirements: DNA Barcoding & Sequencing

## First Things First

- Please download and complete the sample sheet from the GeneLethu website (www.genelethu.co.za)
- Email the completed sample sheet to services@genelethu.co.za
- Selected samples names should not be longer than 25 characters and may contain only underscores if need be.

## DNA Extractions

- If any field collections or sampling will take place prior to sending the samples to GeneLethu for DNA extraction, it would be best to consult with our Technical Team as the best method of storage for optimal results. However, for most taxa, standard collection and preservation methods are suitable.
- All tissue samples should be placed in individual containers (eppies or tubes) that are clearly labelled. If samples are suspended in ethanol, please place a small piece of paper inside the container with the sample/voucher number written on in pencil. Please ensure that tubes are sealed tightly, and ethanol cannot leak out.
- Herbarium/aged material present a significant disadvantage regarding DNA extraction due to age and preservation methods used (some preservation methods are not DNA friendly) therefore we require the following information:
    - Is the material fresh/aged? If aged, how old?
    - What is the preservation method used?
    - The weight of the tissue (more is best, though not less than 0.05g/50mg should be submitted)
    - If smaller organisms are to be submitted, please consult with the Technical Team.

## PCR Set-up

- All genomic DNA samples submitted should be placed in individual tubes that are clearly labelled.
- Please supply us with:

  - Information on the extraction/purification method (purity tests can be supplied by us for an additional cost which allows for better decisions;
  - A minimum of 20 µL need to be supplied;
  - Minimum concentration of 30 nh/ µL;
  - A gel picture;
  - (above services can be supplied by GeneLethu.
- 96 or more samples submitted should be sent through in a 96-well plate, if possible.
- You need to supply us with either the primer or the primer sequence (should we need to order the primer for you).
- Please supply the PCR run conditions if you have them available.

## Sanger Sequencing

- Template PCR submitted should be cleaned or Post-PCR clean-up should be requested. Please supply us with the following information:
  - A gel picture;
  - Concentration of cPCR product no less than 20 ng/ µL;
  - Minimum volume of 20 µL;
  - Supplied primers should have a volume of 10 µL with 10 µM concentration.
- Primer used for sequencing should have the following specifications: 5 µL per reaction at a concentration of 1.1 pmol/µL
- Please use below table for PCR optimisation of PCR product concentrations:
- We have the following Primers available:

| Primer name |
|---|
| matK-Kim3F |
| matK-Kim1R |
| matK-x-F |
| matK-MALPR1-R |
| matK-472-F |
| matK-1248-R |
| matK-390-F |
| matK-1326-R |
| matK-Gym-F-1A |
| matK-Gym-R-1A |
| psbA-3-F |
| trnH-f-R |
| ITS-101-F |
| ITS-102-R |
| ITS-2-R |
| ITS-3-F |
| ITS-NY90(or5)F |
| ITS-NY514-R |
| ITS-NY729-IR |
| ITS-NY728-IF |

| Primer name |
|---|
| trnL-c-F |
| trnL-f-R |
| trnL-d-R |
| trnL-e-F |
| CO1-F |
| CO1-R |

****Please contact us should you wish to confirm primer specifications.**

## DNA Barcoding: Data Submissions

- For BOLD sample submissions, please include the following:
  - Completed BOLD spreadsheet with all relevant data (i.e. GPS co-ordinates, collector's details etc.)
  - High-resolution specimen images