SAMPLE SUBMISSION GUIDELINES

- Create an order online in LIMS and mention the order number along with the samples.
- ***Timeline for Sequencing***

    1. Isolate Plasmid from E.Coli and sequence: 3-5 working days
    2. Purified PCR Products/Plasmid: 1-2 Working Days
    3. Unpurified PCR Products: 2-3 Working Days
    4. Primer Walking: 14-16 Working Days
    5. Amplification and Sequencing: 3 to 4 Weeks
    6. Microbial Identification: 3 to 4 weeks (Bacterial / Fungal / Algal)

- 260/280 ratio of all samples should be b/w 1.7 to 1.9
- GC Rich templates should be double the volume of normal template as described below.
- Kindly send samples in 1.5ml eppendorf tubes.

OUR SERVICES:

Plasmid DNA Sequencing

- Dissolve DNA in de-ionized (DI) water. Do not use TE to dilute or re-suspend the DNA as EDTA inhibits the cycle sequencing reaction.
- We recommend use of commercial kits for plasmid DNA isolation

Template Type	Sample Requirement	Primer Requirement:
Normal Samples	**Concentration** :125 ng/µl (Minimum) **Volume** : 25µl* (Min)	**Concentration** :60 ng/µl (Minimum) **Volume** : 20µl* (Minimum)

For each additional reaction provide 5µl each of primer and template at respective concentration.

PCR Product Sequencing

- DNA template should be free of contamination, unused primers and dNTPs.
- We recommend use of commercial PCR cleanup kits for post cleanup to ensure sequencing quality. ThisPCR purification can be provided at extra cost

Template type	Sample Requirement	Primer Requirement
PCR Product (Purified)	**Concentration** :10 ng/µl (Minimum) **Volume** : 25µl* (Min)	**Concentration** :60 ng/µl (Minimum) **Volume** : 20µl* (Min)
PCR Product (Unpurified)	**Concentration** :150 ng/µl (Minimum) **Volume** : 30µl* (Min)	

For each additional reaction provide 5µl each of primer and template at respective concentration.

Isolate Plasmid from E.coli and Sequence

- E.coli cultures are accepted as stab/plate/slant in LB media.
- Ampicillin and Kanamycin are the antibiotics available with us. Other antibiotics to be used will have to be providedfrom your end.

Microsatellite Analysis

- Minimum of 10ul of sample is required.
- Dyes compatible to our machine are- 6FAM, VIC/HEX, NED, PET/TET.
- Please provide samples in 96 well PCR plate if there are more than 48 samples.
- We can proceed for microsatellite analysis only if there are minimum of 48 samples. If there are less number ofsamples we will proceed only upon completion of 48 reactions in the well plate.

Amplification and Sequencing.

- Samples accepted as Genomic DNA

Template Type	Sample Requirement	Primer Requirement:
Genomic DNA	**Concentration** :250ng/µl (Min). **Volume** : 30ul (Min)	**Concentration** : 60ng/µl (Min) **Volume** :-20ul (Min)

- Provide primer sequence if primers are provided from your end.

Primer Walking

Template Type	Sample Requirement	Primer Requirement:
PCR Product	**Concentration** :50 ng/µl (Minimum) **Volume** : 40µl (Min)	**Concentration** :60 ng/µl (Minimum) **Volume** : 20ul (Min)
Plasmid	**Concentration** :200 ng/µl (Minimum) **Volume** : 40µl (Min)	
Genomic DNA	**Concentration**:200 ng/µl **Volume** : 25µl (Min)	

Microbial Identification

- Samples accepted as DNA or PCR products

Template Type	Sample Requirement	Primer Requirement:
PCR Product	**Concentration** :150ng/µl (Min). **Volume** : 30ul (Min	**N/A**
Genomic DNA	**Concentration** :250 ng/µl (Minimum) **Volume** : 25µl (Min)	

PREPARATION OF PRIMERS

- Primers should be provided in DI water
- Concentration of 10pmole = 73µg/ml
- Primers should be desalted or purified
- 18-25 bases length
- GC content 40%-60%
- Tm 55^{o}C- 60^{o}C
- Free of secondary priming sites, mismatches, salts, EDTA and other contaminants
- **RAPD** primers will not be accepted
- We are not accepting primers with degenerate bases.

Samples received in below format/condition will not be accepted.

- Broken tubes
- Improper tube labeling
- 96 well PCR plate without proper seal
- Samples in the form of gel slice.
- Samples having very low volume than the requirement.
- Contaminated and broken plates for Plasmid isolation.

Disclaimer

Any damage caused to the samples during transit will not be our responsibility.