PHILIPPINE GENOME CENTER

DNA SEQUENCING CORE FACILITY

SAMPLE SUBMISSION REQUIREMENTS

I. NUCLEIC ACID EXTRACTION

I.A Sample Requirements

- Bacterial, Fungal, or Whole Blood* samples are accepted (For bacterial/fungal isolates in broth tubes and whole blood samples, provide at least 2 mL)
- Bacterial and fungal samples should be pure isolates on agar plate/broth tubes.
- Samples should be properly labelled

*whole blood extraction is done exclusively on the KingFisher Flex Magnetic Particle Processor

Important Reminders!

- Do not exceed the specified size range and concentration range
- Ensure that there is no protein contamination in your sample
- If using TE buffer to dilute samples, keep salt concentration at a minimum: 10 mM Tris containing 125 mM KCl or lower for DNA and 10 mM Tris containing 1 mM EDTA for RNA

II. NUCLEIC ACID QUANTIFICATION

II.A QuBit 2.0 Fluorometry

II.A.1 Sample requirements

- Provide at least 3 µL of sample

II.A.2 Sample container formats accepted

- 0.2 mL PCR tube with cap
- 0.2 mL 8- or 12-tube PCR strip with cap

II.A.3 Submission

- Samples may be submitted from Monday to Friday until 3 PM.
- Clients may opt to be responsible for delivering their samples to DSCF. Otherwise, DSCF will charge an additional flat rate delivery fee of:

AREA	DELIVERY FEE (Php)
Metro Manila	300
Luzon	395
Visayas	480
Mindanao	570

II.A.4 Turnaround time

- Results will be given on the same day of submission.

II.B MultiNA Microchip Electrophoresis

II.B.1 Sample requirements

DNA

- Size must be from 25 bp to 2,500 bp only; no genomic DNA accepted
- Provide at least 10 µL sample with a concentration of 20-50 ng/µL
- Use nuclease-free water or TE buffer to dilute samples if necessary

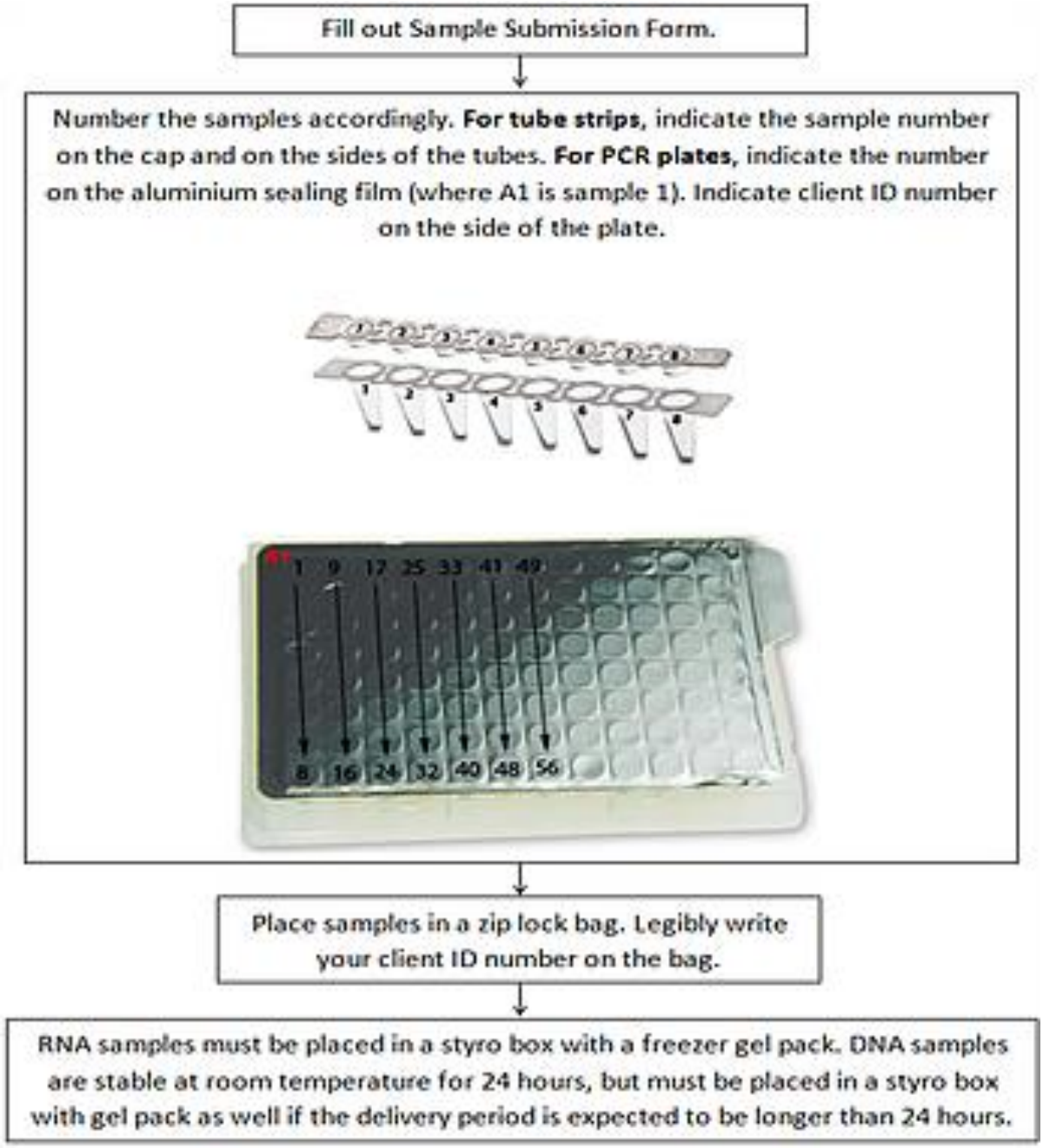
RNA

- Up to 28S rRNA (4.7 to 5.0 knt) accepted
- Provide at least 5 µL sample with a concentration of 50-250 ng/µL
- Use Invitrogen's THE RNA Storage Solution (recommended) or TE buffer to dilute samples if necessary

II.B.2 Sample container formats accepted

- 8- or 12-tube PCR strip with cap
- 96-well PCR plate with pierceable aluminium sealing film

II.B.3 Submission



- Samples may be submitted on Tuesdays and Wednesdays only. Samples that will be received on Thursdays and Fridays will be considered submissions for the following week.

- Clients may opt to be responsible for delivering their samples to DSCF. Otherwise, DSCF will charge an additional flat rate delivery fee of:

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II.B.4 Turnaround time

- Results will be given on Thursday of the same week of the submission.
- Results may be given sooner for 96-well plate submissions (at least 80 samples).

III. CAPILLARY SEQUENCING

III.A Sample requirements

TYPE OF DNA	CONCENTRATION AND VOLUME PER REACTION
Purified PCR product (90-500 bp)	20 ng/uL, min 20 uL per rxn in ddH_2O
Purified PCR product (>500 bp)	40 ng/uL, min 20 uL per rxn in ddH_2O
Purified plasmid	100 ng/uL, min 20 uL per rxn in ddH_2O
Primer*	10 uM or 10 pmol/uL in 10 uL per rxn in ddH_2O

Note:

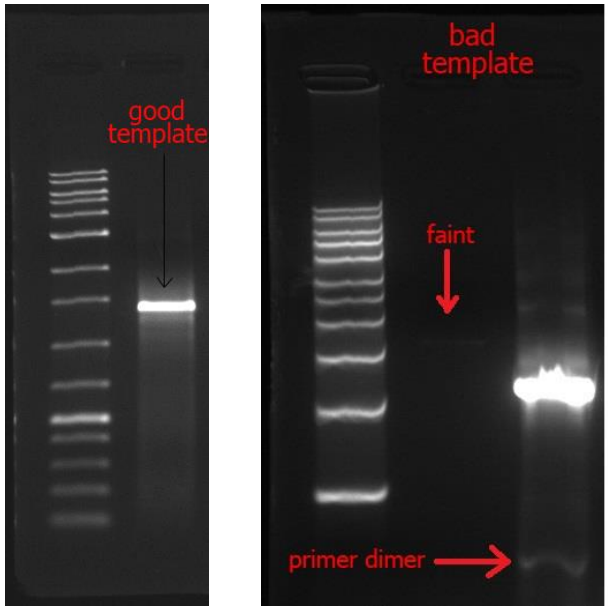
- We accept unpurified PCR products/plasmids for an additional fee
- For Extract2Seq 16s rRNA gene Bacterial Identification, provide pure bacterial isolates in agar plate/broth tubes (for broth cultures, provide at least 2mL of sample.

Important Reminders!

- Do not exceed the specified concentration ranges.
- Ensure that there are no leftover PCR reagents (dNTPs, primers) or protein contamination in your sample. As a guide, the A260/A280 ratio of DNA samples should be 1.7 to 1.9.
- Ensure that there is enough primer provided for the ordered reactions and any possible re-runs.
- If using TE buffer to dilute DNA samples, keep salt concentration at a minimum

III.B Sample Quality Requirements

Please refer to the following agarose gel image below:



A B

Figure 1. Agarose gel image of DNA template/samples with good *(A)* and bad *(B)* quality

Note:

- Samples should appear as a single, intense and sharp band on the gel.
- Samples should not contain primer dimers.

III.C Sample container formats accepted

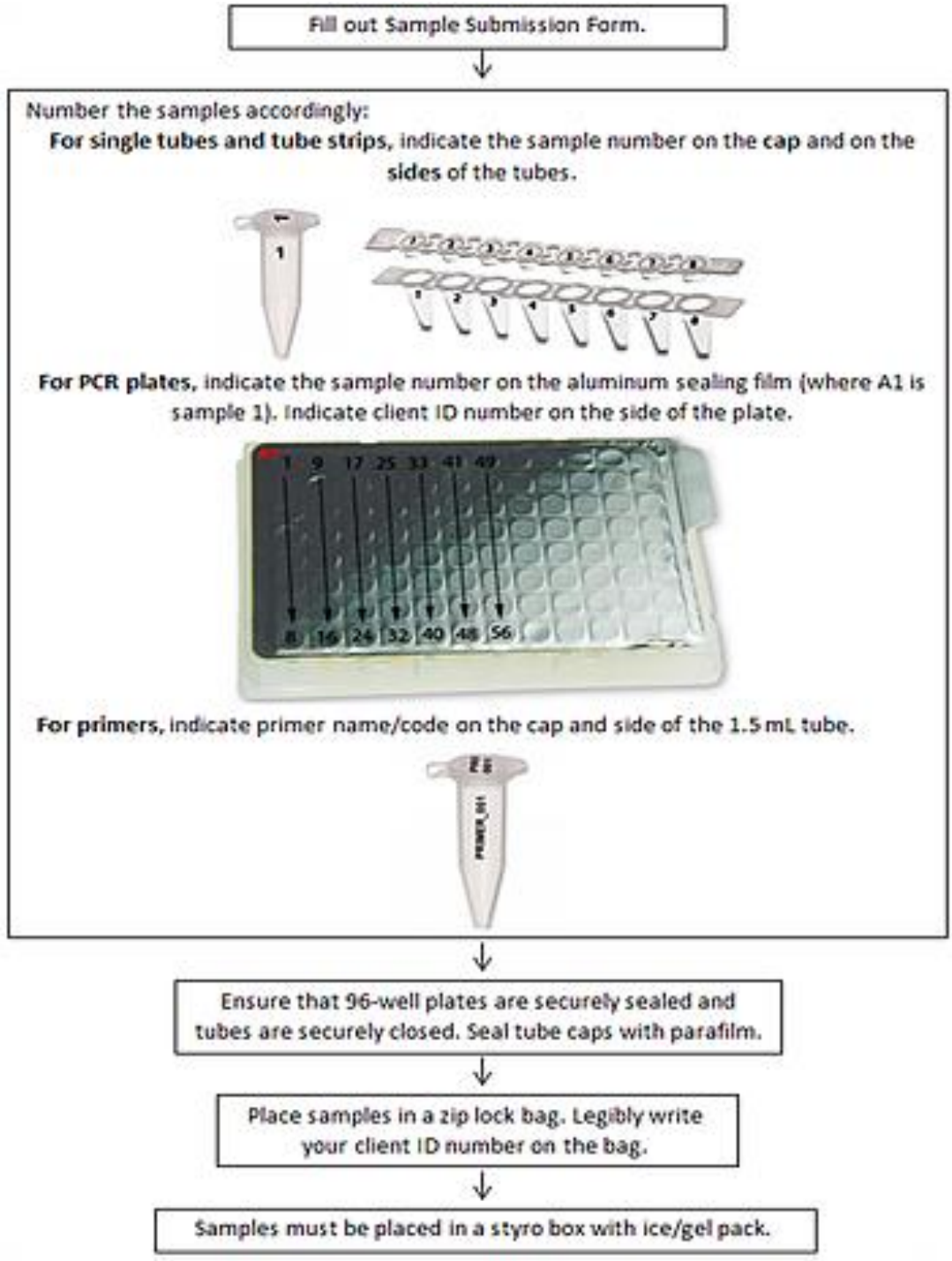
For primers:

- 1.5-mL microtubes

For samples:

- 1.5-mL microtubes
- 0.2-mL PCR tubes
- 8- or 12-tube PCR strip with cap
- 96-well PCR plate (ideally skirted) with pierceable aluminum sealing film

III.D Submission



- Samples may be submitted on Tuesdays and Wednesdays only. Samples that will be received on Thursdays and Fridays will be considered submissions for the following week.

- Clients may opt to be responsible for delivering their samples to DSCF. Otherwise, DSCF will charge an additional flat rate delivery fee of:

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III.E Turnaround time

- Results will be given on Friday of the same week of the submission.
- Results may be given sooner for 96-well plate submissions.

IV. NEXT GENERATION SEQUENCING

IV.A Whole Genome Sequencing Sample Requirements (Illumina MiSeq)

- Submit at least 80 uL of 50 ng/uL gDNA in water (concentration should be based on QuBit Fluorometer).
- A260/A280 ratio of DNA samples should be at ~1.8-2.0.
- Please refer to the agarose gel image below for sample quality guideline.

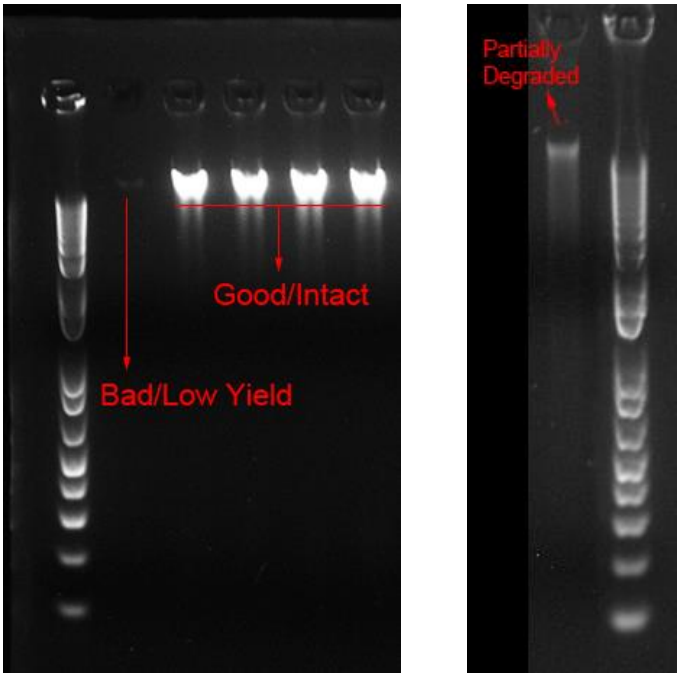


Figure 2. Agarose gel image of Intact *(A)* and Partially Degraded *(B)* gDNA input.

Note:

- Samples should appear as a single, intense and sharp band ~23 kb
- gDNA samples will be classified using the sample classification matrix

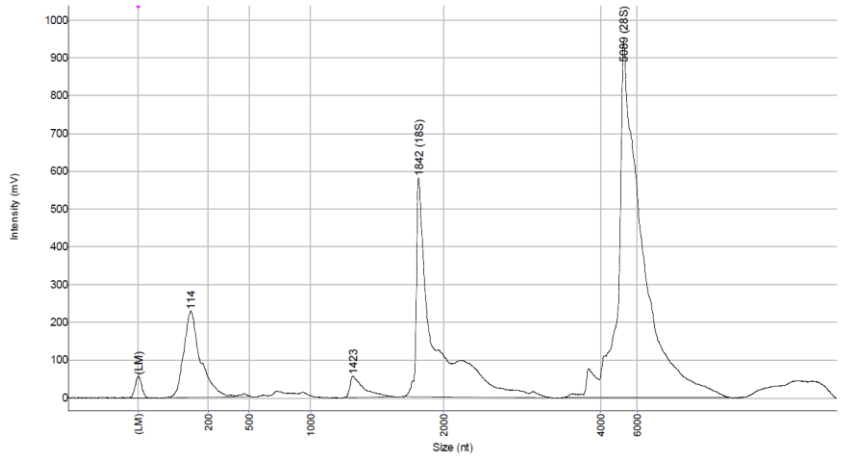
SAMPLE QUALITY CLASSIFICATION FOR GENOMIC DNA	
Level	**Description**
A	DNA is intact with no apparent contamination (single intense band at ~23 kb). DNA quantity is sufficient to prepare libraries more than once.
B	DNA is intact with no apparent contamination (single intense band at ~23 kb). DNA quantity is sufficient to prepare libraries only once.
C	DNA is partially degraded (intense band at ~23 kb with slight smearing). DNA quantity is sufficient to prepare libraries more than once.
D	DNA is partially degraded (intense band at ~23 kb with slight smearing). DNA quantity is sufficient to prepare libraries only once.
E	DNA is totally degraded (no distinct band or no band at all).

***note: Samples classified C and D are not guaranteed to produce successful sequencing run. Samples classified as E are definitely not recommended to proceed for sequencing.**

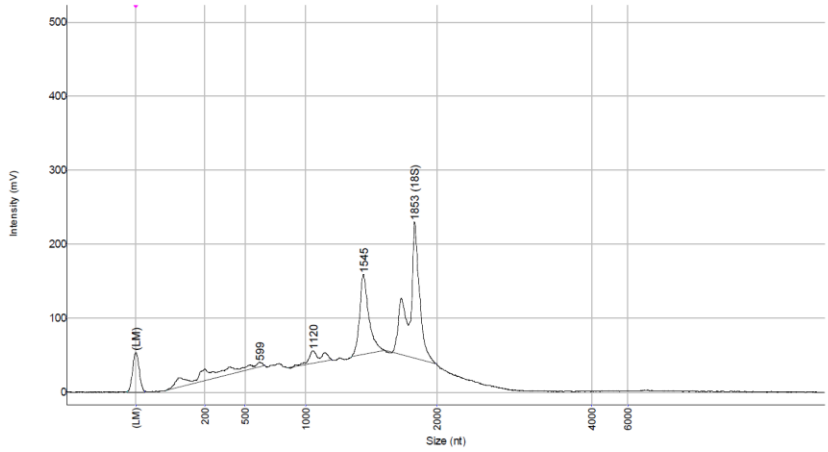
IV.B RNA Sequencing Sample Requirements (Illumina MiSeq)

- Submit at least 50 uL of 50 ng/uL Total RNA in water (concentration should be based on QuBit Fluorometer)
- DNase treatment during RNA isolation is recommended, especially if using a protocol that does not include a polyA purification step.
- Input RNA must not contain any trace of EDTA, Tween 20, ethanol, or phenol.
- Samples should have a Bioanalyzer RIN value of >8, an agarose gel profile showing two distinct bands corresponding to the 18S and 28S rRNA, or an MCE-202 MultiNA Trace profile indicative of a high-quality RNA *(refer to the Figure 3).*

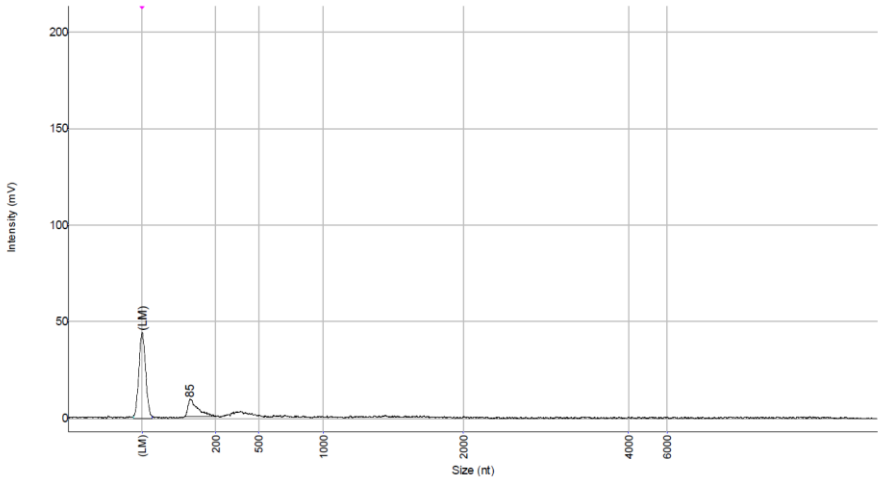
For further inquiries and other sample submission requirements for Next Generation Sequencing (Ion Torrent PGM, Ion Torrent Proton, or Illumina MiSeq), kindly contact us or send us an e-mail to dnasequencing@pgc.up.edu.ph.



A



B



C

Figure 3. MCE-202 MultiNA RNA 6000 analysis trace of high-quality RNA (A), partially degraded RNA (B), and fully degraded RNA (C)