PLEASE READ AND FOLLOW THESE INSTRUCTIONS

Preparing DNA samples in tubes for MicrobesNG

DNA preparation

- DNA should be eluted in EB buffer (i.e. 10 mM Tris-HCl pH 8.5) with no EDTA. Do not use TE for eluting the DNA as it contains EDTA, which inhibits our NGS library preparation.
- In the absence of a buffering agent, store samples at -25°C to -15°C to prevent degradation.

DNA concentration and volume required

- Please send between **30 – 100 uL** of genomic DNA at a concentration of **1 – 30 ng/uL** measured by Qubit/Picogreen (see below).
- If you use **NanoDrop** to quantify your DNA, the minimum concentration required is **10 ng/uL** (see below) in 30 – 100 uL.
- When sending more than one sample, it will facilitate the processing of your samples if you normalize them to the same concentration and volume.

DNA quantification

- DNA samples must be quantified before sending them to us, and the concentration recorded in the sample sheet that we will provide.
- For quantification we recommend the samples are quantified using a fluorescence based assay (i.e. Picogreen, Qubit, Quant-iT).
- You can also use a spectrophotometer assay e.g. NanoDrop. However, be aware that NanoDrop often overestimates DNA concentration, sometimes up to 10-fold!

DNA integrity control

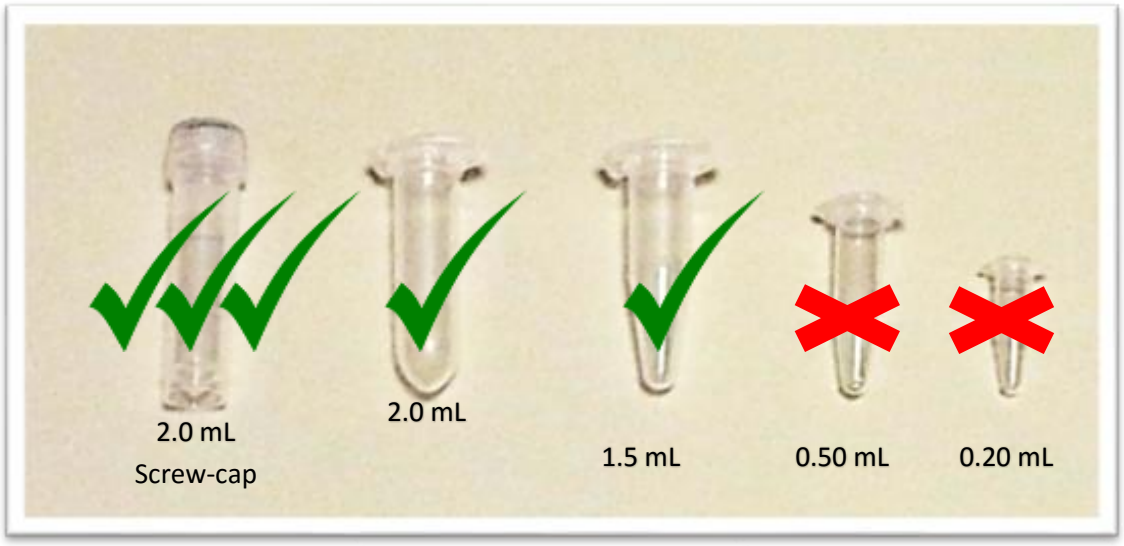
- We recommend that you check the DNA integrity before sending DNA to us.
- To check integrity we recommend the samples are run on an electrophoresis system like the Bioanalyzer HS DNA chip or Tapestation gDNA Screentape. Alternatively, you can run your genomic DNA samples on a 0.75% e-gel or agarose gel with a molecular weight ladder to verify the integrity of the DNA.
- Non-degraded genomic DNA will show a single band of High Molecular Weight (>40,000bp).
- A smear between 100 - 40,000bp indicates DNA degradation, which may jeopardize the success of our NGS library preparation.
- If you check integrity please send us your Bioanalyzer/Tapestation/gel results to assess if your samples will be OK for our NGS library method.

Document updated 16 March 2017

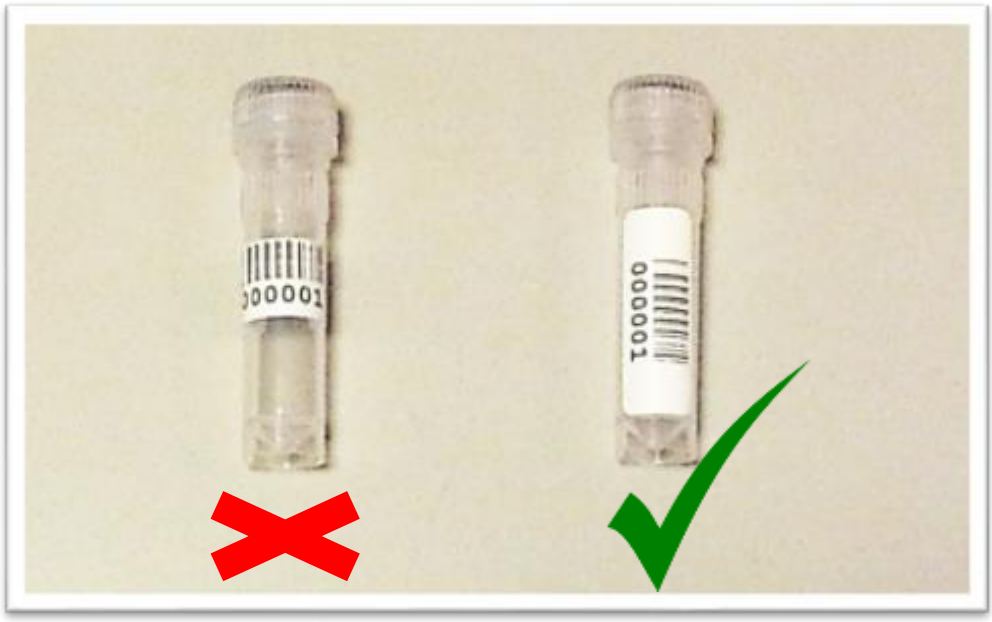
Preparing DNA tube for MicrobesNG

- DNA samples should be sent in **microfuge size** screw cap tubes. WE CAN ONLY PROCESS MICROFUGE SIZE TUBES (i.e. tubes that can fit in a standard benchtop centrifuge).
- If you don't have access to screw cap tubes, please put samples in 1.5ml microfuge tubes wrapped in parafilm to minimize the likelihood of samples opening in transit and delaying your order. Please do NOT send DNA in 0.5mL or 0.2mL tubes! (see **Picture 1**).
- Stick the provided barcode label(s) **lengthwise**, NOT AROUND the tube (see **Picture 2**). Barcodes cannot be read by the scanner if the labels are stuck around the tube!
- If you are sending >20 DNA samples, these should be sent in a 96-well PCR plate (contact us at info@microbesng.uk for more information).

PICTURE 1. TUBES TO USE TO SEND DNA



PICTURE 2. HOW TO STICK BARCODE ON TUBE



Preparing DNA plate for MicrobesNG

- Follow these instructions ONLY if we have agreed to receive your samples in a 96-well PCR plate.
- Load your samples in the plate by columns (rather than by rows) starting in well A1 (see **Picture 3**). Please only use the well locations that appear in your project web portal (samples received in wells not included in the project web portal will NOT be processed).
- Please seal the plate with a heat sealing foil or seal. If you don't have a heat sealer, you can use a PCR plate seal, incubated in a thermocycler for 10 minutes at 20C with heating lid on at 105-110°C.
- Alternatively you can use strip lids (make sure they are suitable for you 96-well PCR plate.
- Stick the provided barcode label in the front skirted edge of the plate (long side by row H).

PICTURE 3. HOW TO LOAD SAMPLES IN A 96-WELL PCR PLATE. Load by columns starting in well A1.