Updated 12/03/2018

Preparing stock tubes for MicrobesNG:

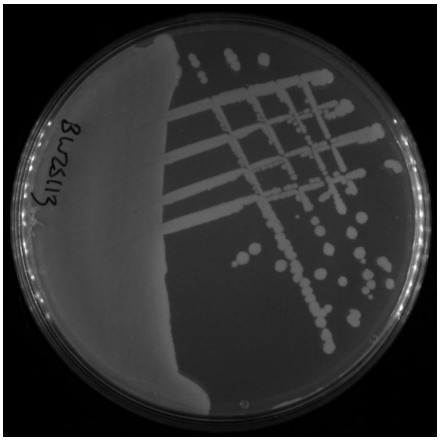
Enhanced Genome Service

To maximise the chance of successful DNA extraction from your strain it is important that you prepare your strain sample cryovial following the steps below. **We do not grow your samples for DNA extraction**. Instead, we extract it directly from the cells contained in the tube. It is important that enough cells have been collected and added to the cryovial as per our instructions, otherwise the DNA extraction will likely fail!

For the Enhanced Genome Service we require a **BETWEEN 300mg (MINIMUM) and 600mg (MAXIMUM)** well pelleted cells (with supernatant removed) harvested during the exponential growth phase. Please follow the procotol below to prepare your sample.

Protocol for Enhanced Genome Service: preparing a sample in liquid broth

1. Take a single colony of the strain to be sequenced, mix in 200 µl sterile buffer (for example 1xPBS).
2. Inoculate 25-50mL of sterile broth suitable for your strain to grow with 100 µl of buffer from step 1.
3. Streak out the remaining 100 µl from step 1 on a suitable agar plate to determine that the culture is pure (see right half side on the photo below).
    - Wait till colonies are clearly visible to the naked eye. Take a picture of the plate for QC[1]. Email us the picture(s) of plates indicating the sample barcode. Please include your project ID in the subject of the email.



[1] If your sequencing does not correspond to a pure culture we can use this picture to troubleshoot the source of the contamination.

4. Incubate the sample until the upper exponential phase (but BEFORE it reaches the stationary phase).
5. Pellet the bacteria in a centrifuge (~10 minutes at 500 x g). Discard the liquid without disturbing the pellet.
6. Weight the cell pellet and make sure that you have 300-600mg. You may need to repeat steps 1-5 until you get a combined wet weight of 300mg pelleted cells.
7. CHECK the agar plate prepared in Step 3 (once the colonies have grown) for contamination. IF the plate does not show signs of contamination with other microbes proceed to Step 8, otherwise repeat from Step 1.
8. Resuspend the pelleted cells in 500 uL of the cryopreservant liquid from the barcoded bead tube supplied by MicrobesNG (see picture below). Make sure that the pellet is fully resuspended (if required, mix by pipetting several times with a wide bore tip - a standard 1000uL tip with the end cut off will do). Transfer the resuspended cells into the RED CAPPED barcoded bead tube and close the tube.

9. Seal the tube with parafilm and send the tube at room temperature back to MicrobesNG.
10. Try to ensure package arrives in 2 days after making stock. If you anticipate delays please send the tube at 4℃. (DO NOT FREEZE.)