Updated 20/02/2018

Preparing stock tubes for MicrobesNG

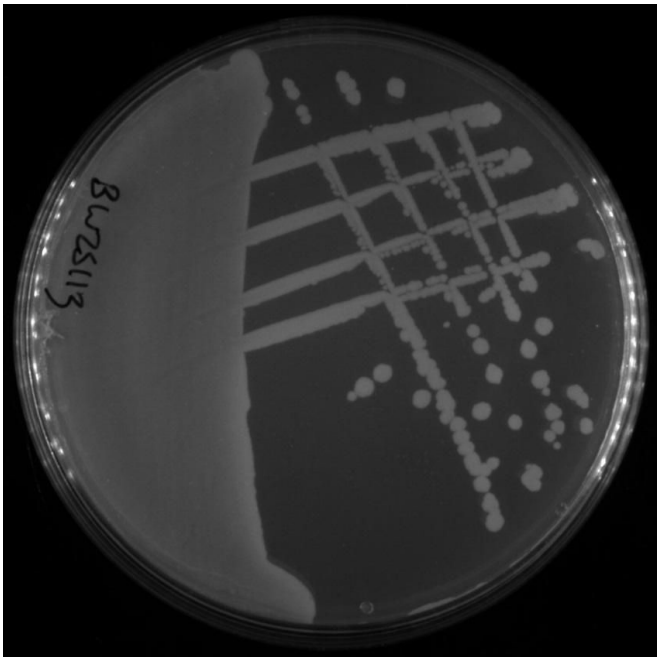
To maximise the chance of successful DNA extraction from your strain it is important that you prepare your strain sample cryovial following the steps below. **We do not grow your samples for DNA extraction**. Instead, we extract it directly from an aliquot taken from the tube. It is important that enough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

We support two protocols to grow strains for MicrobesNG. **Protocol A (Agar)** (preferred) is for strains that grow well on an agar plate. **Protocol B (Broth)** is for strains that multiply better in liquid broth. You should follow the protocol that works better with your strain.

Protocol A: preparing a sample on agar

1. Take a single colony of the strain to be sequenced, mix in 100 µl sterile buffer (for example 1xPBS).
2. Streak out your strain on a suitable agar plate from the 100 µl culture. Try to make around 1/3 of the plate a lawn of bacteria and then streak out to determine that the culture is pure (see photo). Leave the plate at the appropriate growing conditions until an abundant growth is observed (see photo).
    - (Optional) Take a picture of the plate for potential QC[1]. Email us the picture(s) of plates indicating the sample barcode. Please include your project ID in the subject of the email.
3. Using a large sterile loop take **all** bacterial culture off the plate and mix into the barcoded bead tube supplied by MicrobesNG. This includes the 1/3 plate lawn of bacteria.
4. Mix tube by inverting 10 times. Leave liquid in the tubes.
5. Seal and send the tube at room temperature back to MicrobesNG.
6. Try to ensure package arrives in 2 days after making stock.

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



Protocol B: preparing a sample on liquid broth

1. Take a single colony of the strain to be sequenced, mix in 200 µl sterile buffer (for example 1xPBS).
2. Inoculate 25mL of sterile broth suitable for your strain to grow with 100 µl of buffer from step 1. Incubate the inoculated broth to OD 0.25-0.5.
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 above).
    - (Optional) Take a picture of the plate for potential QC[2]. Email us the picture(s) of plates indicating the sample barcode.Please include your project ID in the subject of the email.
4. Pellet the bacteria in a centrifuge (~10 minutes at 4000 x g). Discard the liquid without disturbing the pellet.
5. Resuspend the pelleted cells in 500 uL of the cryopreservant liquid from the barcoded bead tube supplied by MicrobesNG. Transfer the resuspended cells into the barcoded bead tube. Mix tube by inverting 10 times.
6. Seal and send the tube at room temperature back to MicrobesNG.
7. Try to ensure package arrives in 2 days after making stock.

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