Preparing strains for MicrobesNG EGS in inactivation buffer

To maximise the chance of successful DNA extraction from your strain it is important that you prepare your strain sample 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 you send us. It is important that enough cells have been collected and added to the tube as per these instructions, otherwise the DNA extraction will likely fail!

These instructions are to prepare cells that will be submitted to MicrobesNG in DNA/ RNA Shield (Zymo Research). This buffer effectively inactivates pathogens, including tough-to-lyse microbes or viruses, without the need for additional steps (i.e. heat-treatment, homogenisation, or alcohol sterilisation).

In the UK DNA/RNA Shield can be purchased from Cambridge Biosciences (https://www.bioscience.co.uk/cpl/dna-rna-shield-reagent).

Outside the UK you can find your local distributor from the manufacturer's website (https://www.zymoresearch.com/pages/our-distributors).

Growing your cells

1. Take a single colony of the strain to be sequenced, mix in 200 µl of sterile buffer (for example 1xPBS). Cells can then be grown in (a) **liquid broth** or (b) **agar plate**.
    a. If you grow your cells in **liquid broth** we recommend that you use 100uL to inoculate the liquid broth and you streak out the remaining 100uL on a suitable agar plate to determine that the culture is pure.
    b. If you grow your cells in **agar plates** we recommend that you try to grow a lawn of bacteria in ⅓-½ of the plate and then streak out on the rest of the plate to determine that the culture is pure.

Collecting your cells

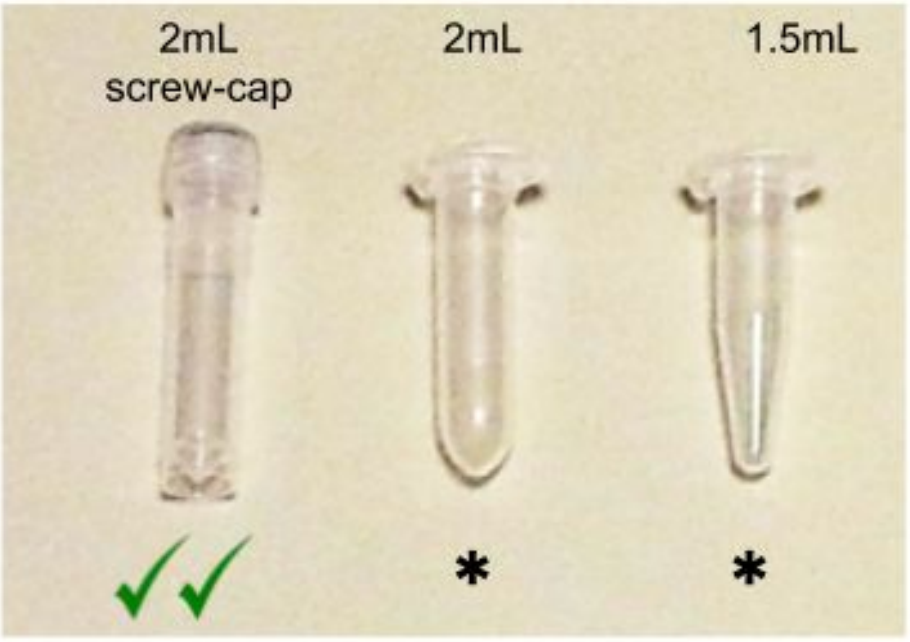
2. We require a **MINIMUM OF the equivalent of 10 OD600nm or 5x10^9 cells** (e.g. 10mL culture at 1.0 OD600nm). OD measures should only be considered as valid when they are between 0.5 and 1.5, and NEVER if they are 2.0 or above.
3. Cells should be harvested during the exponential growth phase.
4. Harvesting cells:
    a. **Liquid broth**: harvest the cells when they have reached an equivalent of 10 OD600nm or 5 x 10^9 cells. If cells are grown in bigger volume containers (e.g.

50mL Falcon tubes) you can do the first centrifugation in the container, resuspend them in 1mL PBS and transfer them to the microfuge tube for the second centrifugation.

b. **Agar plate**: using a large sterile loop take **<u>all</u>** bacterial culture off the plate and mix into a 1.5mL or 2mL microfuge tube containing 1mL PBS. Resuspend the cells in the PBS and check that you have an equivalent of 10 OD600nm or 5 x 10^9 cells. **You need to grow more cells if you have less than this value**. Once you have the right number of cells centrifuge to get a pellet.

5. The cells should be concentrated by centrifugation in a 1.5mL or 2mL microfuge tube, washed with 1mL PBS and pelleted again.
6. Resuspend the final pellet in **0.5mL** of **1x DNA/RNA Shield buffer** in a **screw cap** 1.5mL or 2mL microfuge tube (see Figure 1).

PICTURE 1. VALID TUBES TO SEND STRAINS TO MICROBESNG. Strains should be sent in 1.5mL or 2mL screw-cap microfuge tubes. *If you do not have screw-cap tubes you may use standard 1.5mL or 2mL microfuge tubes but **you must seal the tubes with parafilm to ensure they remain closed during shipment**.



7. Please check that the tube can fit in a standard micro centrifuge rotor for 1.5-2mL tubes - some tubes have a larger diameter and we cannot store/centrifuge these in our laboratory
8. **Label the strain tube(s)** with MicrobesNG barcode number (sample ID). The barcode number can be found in your project portal. Print your own label for the tube with the appropriate barcode number (please make sure the labels are printed and **NOT written by hand**), and attach the label to the DNA tube with transparent adhesive tape - i.e. Sellotape.
9. **Fill in the sample information in your project portal at this point.**

Shipping your strains

10. Strain samples in DNA/RNA later can be sent at room temperature for shipments up to 5-7 days, although shipping the samples at 4oC (blue ice) would be recommended if longer shipping times are expected.
11. Samples should be shipped inside a rigid package (e.g. polystyrene box, cardboard box) that will prevent them from breaking accidentally. We strongly recommend that you follow **packing instruction P650** (you can find this instruction in your project portal).
12. Attach the return label sent to you to the outside of your package. This will help our Laboratory Team with the identification of your project.
13. **VERY IMPORTANT: make sure that you have entered the sample information in your project portal before shipping your samples!**
14. Your samples are now ready to be shipped.