Preparing strains for MicrobesNG 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 (herein DRS) from 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).

MicrobesNG aims to provide customers with strain submission tubes containing DRS inactivation buffer. In the event that we are not able to provide these tubes you can also source the DRS buffer and use 2mL screw cap microfuge tubes to prepare your strains following the instructions below. (IMPORTANT: only use screw cap tubes with 11mm outer diameter, NOT storage tubes with 13mm outer diameter, these do not fit our standard centrifuge rotors!)

In the UK DNA/RNA Shield can be obtained 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).

IMPORTANT:

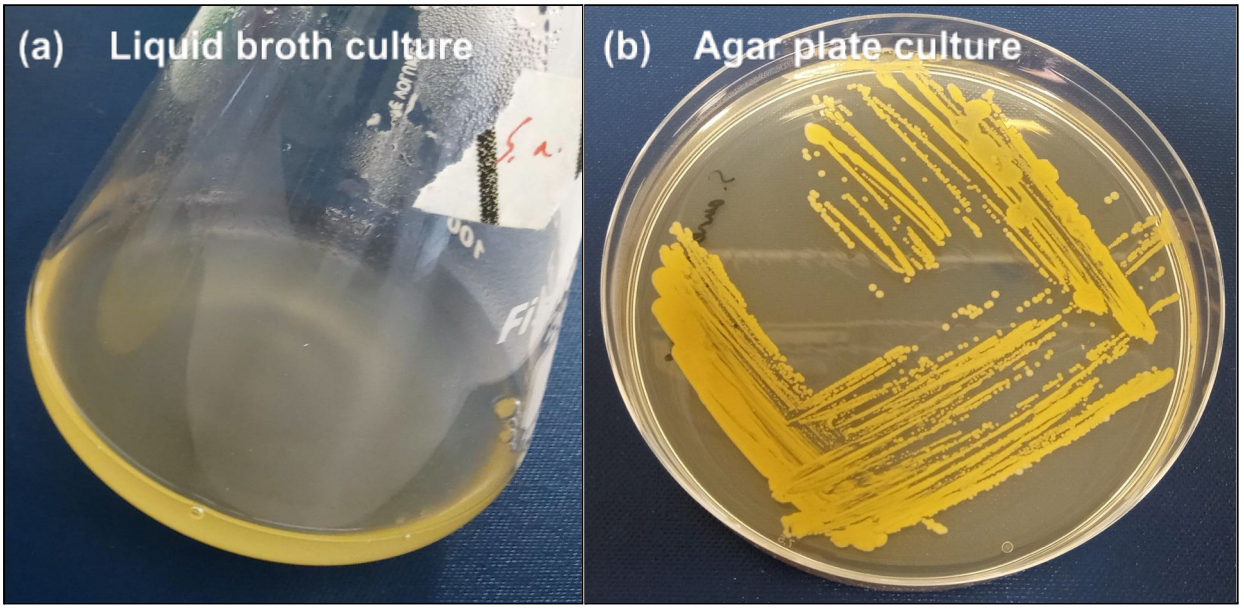
For Health and Safety reasons we advise that you read the manufacturer's Safety Data Sheet for DRS before handling the DRS tubes. A copy of the SDS can be obtained from the manufacturer's website:
https://files.zymoresearch.com/sds/dna_rna_shield_ce_eu.pdf (last accessed 5th May 2021)

MicrobesNG STRAIN PREPARATION PROTOCOL

(To see visual summary of the process see **Appendix A**)

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** (PICTURE 1a) or (b) **agar plate** (PICTURE 1b) .
    a. If you grow your cells in **liquid broth** we recommend that you use 100 µL to inoculate the liquid broth and you streak out the remaining 100 µL 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 *at least one plate* with a lawn of bacteria in ½-⅔ of the plate and then streak out on the rest of the plate to determine that the culture is pure. Depending on the species you may need to grow more than one plate per strain.

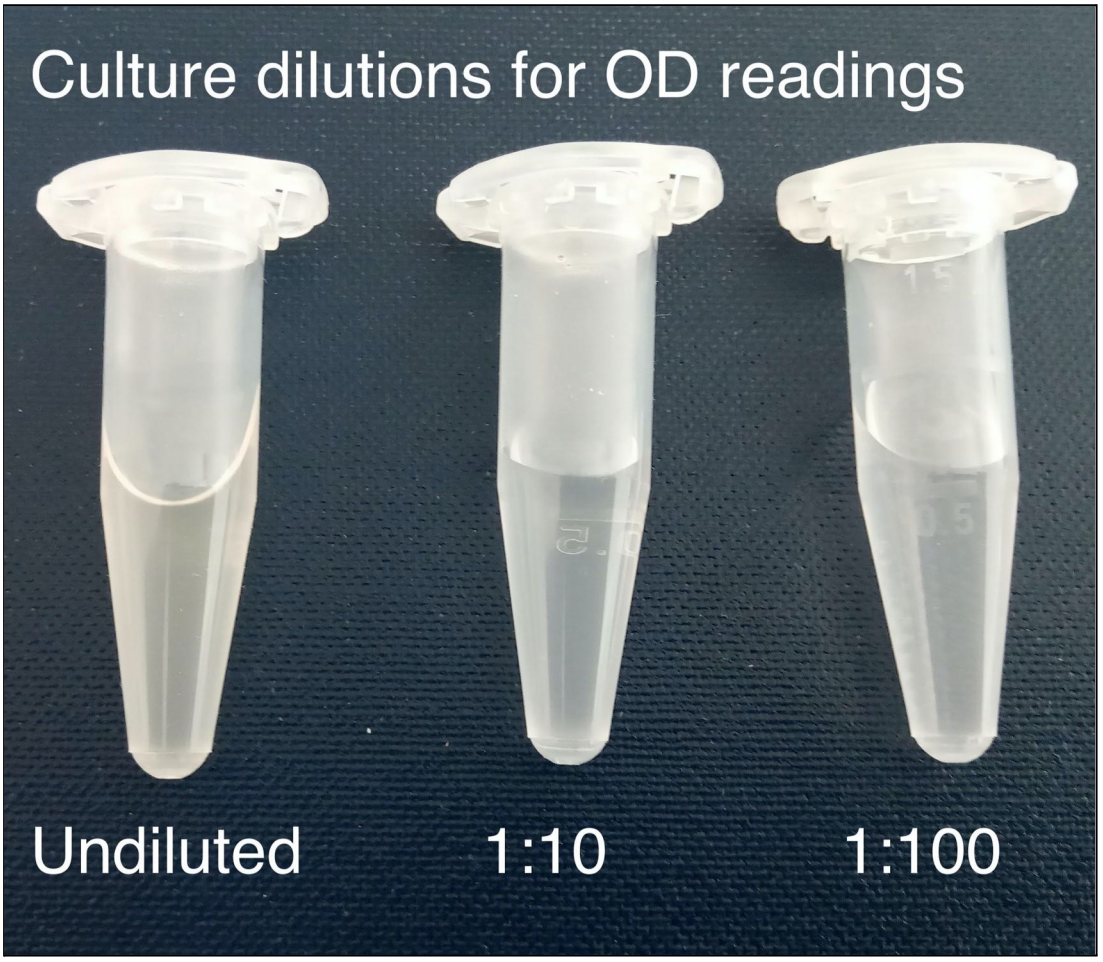


PICTURE 1. EXAMPLES OF CELL CULTURES. These images show growth examples of a *Staphylococcus aureus* strain. **(a)** Liquid broth culture at log-phase after 6 hours of growth; **(b)** Agar plate culture after overnight growth.

Collecting your cells

2. Measure the OD600nm of your liquid culture or your agar plate cells harvested and resuspended in PBS.
    a. **Liquid broth**: measure OD600nm to verify that cells are in the exponential growth phase. This is usually between 0.5 and 1.0 depending on the organism you are culturing.
    b. **Agar plate**: using a large sterile loop take **all** bacterial culture off the plate and mix into a 5mL or 10mL PBS. Measure the OD600nm of the resuspended cells.

    OD measures should only be considered as valid when they are below 1.0, so you may need to prepare a serial dilution of your culture when measuring the OD until one of the dilutions has an OD below 1.0 (PICTURE 2), and then calculate the OD of the stock culture by multiplying the measured value by the dilution factor. Cells should be harvested during the exponential growth phase.



PICTURE 2. SERIAL DILUTION TO MEASURE OD600nm. This image shows a serial dilution of *Staphylococcus aureus* liquid culture that has been prepared to measure OD600nm. Apart from the undiluted culture, two dilutions from the stock culture have been prepared (1:10 and 1:100). If the OD600nm of the undiluted culture is higher than 1.0 then the OD600nm reading of the first dilution that is below or equal to 1.0 will be used to estimate the OD600nm of the stock culture (multiplying the measured value of the dilution by the dilution factor).

3. Harvesting cells. We require **the equivalent of 8 - 12 OD600nm or 4 - 6 x 10^9 cells**[1] (e.g. 8 - 12 mL culture at 1.0 OD600nm)

   Harvest an equivalent OD600nm of 8 - 12, or 4 - 6 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.

KEEP THE AMOUNT OF CELLS HARVESTED WITHIN THIS RANGE:

MINIMUM: equivalent of **8 OD600nm** or **4 x 10^9 cells**
MAXIMUM: equivalent of **12 OD600nm** or **6 x 10^9 cells**

a. If you have harvested more cells than the upper limit **remove the excess**.

b. If you have not reached the lower limit **you need to grow more cells until you reach this value**. Once you have the right number of cells centrifuge to get a pellet.

c. We have optimised our DNA extraction for strain samples within this range. **Samples outside this range of cells will likely fail DNA extraction** and it is possible we will need to request you to send a DNA (not strain!) sample, which will delay your project.

What if you cannot measure OD600nm for your strain?

We strongly recommend that cell concentrations are estimated using OD600nm measurements. However some strains (e.g. Actinobacteria) grow forming cell aggregates that do not produce homogeneous cell suspensions, thus OD600nm measurements would not be valid to estimate cell concentrations.

[1] ***X equivalent OD600nm*** refers to the **amount of cells** that, if they were resuspended in **1mL** of liquid culture, would produce an OD600nm value of X.
For example, if you need an *equivalent OD600nm* of **10**, this means that you need the number of cells that would produce an OD600nm of 10 if they were suspended in 1mL of liquid. If you grow your cells in a plate, collect in 10mL of PBS and the OD600nm of the resuspended cells is **2.5** (you would have measured 0.25 OD600nm of a 1:10 dilution of the culture for a valid OD600nm reading), you will need to harvest 10 (*target* equivalent OD)/2.5 (*observed* OD) = 4 mL of culture. If you then pellet these 4mL, remove the liquid and resuspend the cells in 1 mL of liquid, the OD600nm of this new suspension would be OD600nm = 10.
REMEMBER: **equivalent OD600nm** relates to **amount of cells**, and it is used as a standardised way of knowing how much culture you need to pellet to obtain a desired number of cells.

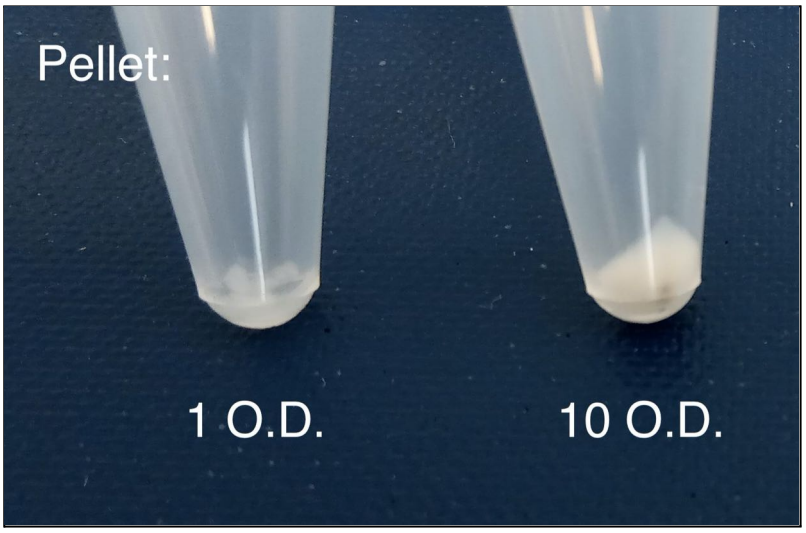
When OD600nm cannot be used as a proxy for cell concentration, or if you are absolutely unable to measure OD600nm, you can use the table below as a guideline for pellet size to prepare:

Pellet type	Pellet amount to harvest
Wet-compact, when most/all supernatant is removed the pellet is not disturbed (e.g. *E. coli* pellets)	12-18 mg
Wet-loose pellet, you need to leave some supernatant with the cells because it cannot be removed without disturbing the pellet, cells can be resuspended easily (e.g. *Streptococcus* sp. pellets)	30-50 mg (or 30-50 uL of wet pellet)

IMPORTANT! MAXIMUM CELL PELLET SIZE is 50 µL *or* 50 mg

Do NOT load more than these values in the DRS tube, otherwise there will be too many cells to be protected from degradation by the provided DRS buffer.

4. The cells should be concentrated by centrifugation in a 1.5mL or 2mL microfuge tube, washed with 1mL PBS and pelleted again (PICTURE 3).



PICTURE 3. CELL PELLETS. This picture shows two examples of harvested, PBS washed, pellets of *Staphylococcus aureus* cells. The tube on the right shows the cell pellet size for an equivalent OD600nm of 10, this is the amount of cells we would require you to resuspend in DRS buffer and send to us. The volume of the pellet is lower than 50 µL (here it is around 10-15 µL) Just for comparison, the pellet on the left is for an equivalent OD600nm of 1 - **this will NOT be enough for submission to MicrobesNG!**

5. Resuspend the final pellet in **0.5 mL** of **1x DNA/RNA Shield buffer** in the 2 mL **screw cap tube** provided.

6. (If your DRS tubes do not have a barcode label) **Label the strain tube(s)** with MicrobesNG barcode **number** (MicrobesNG's 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 lengthwise down the DRS tube with transparent adhesive tape - i.e. Sellotape.

7. **IMPORTANT: Fill in the sample information in your project portal at this point. We will not be able to accept your samples if you have not entered the sample information in your project portal.**

Shipping your strains

8. 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.

9. 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 or in https://microbesng.com/microbesng-faq/).

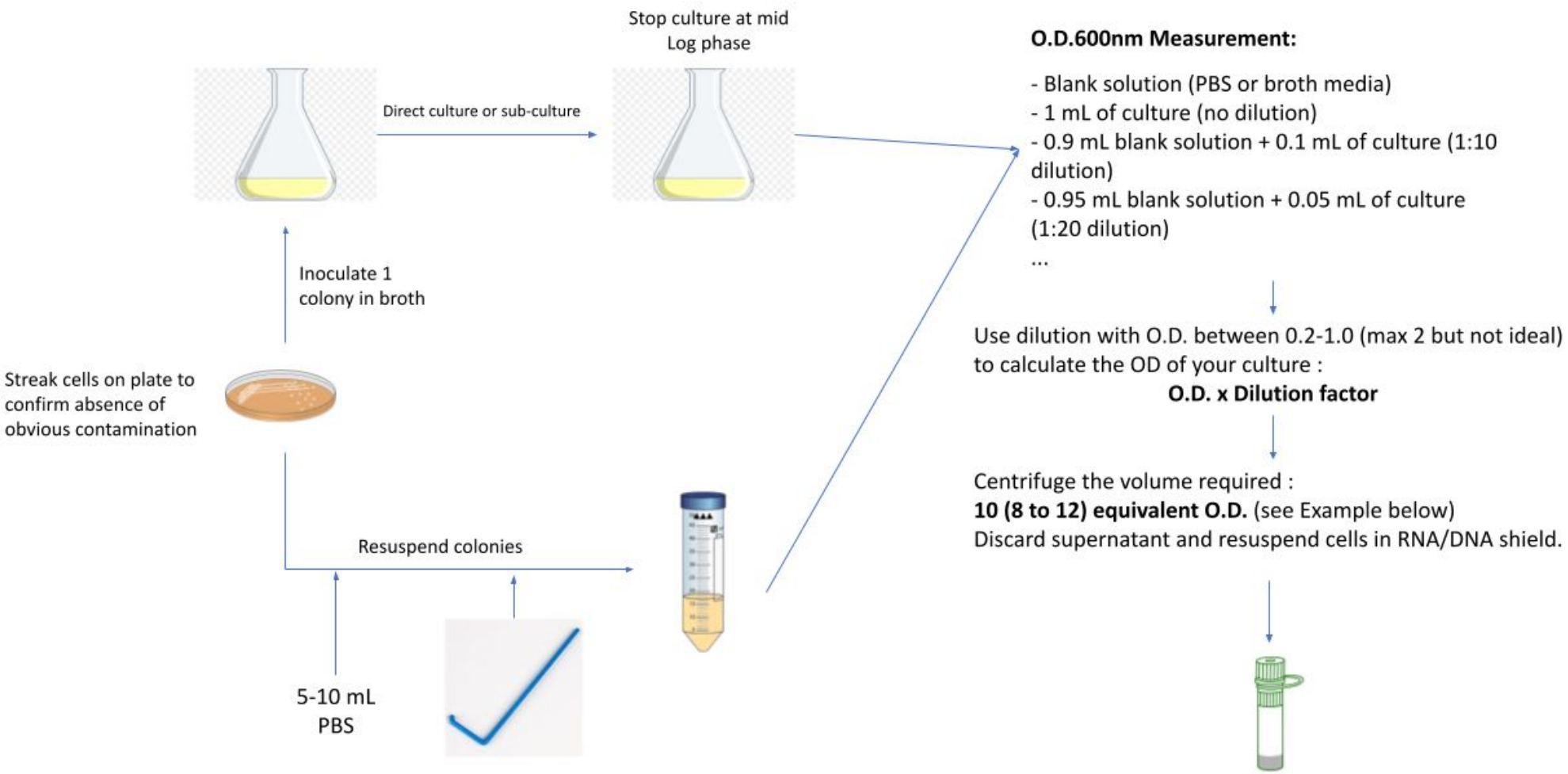
10. Attach the return label that you have received outside of your package. This will help our Laboratory Team with the identification of your project.

11. **VERY IMPORTANT: make sure that you have entered the sample information in your project portal before shipping your samples!**

    REMEMBER: We will NOT be able to accept your samples if you have not entered the sample information in your project portal.

12. Your samples are now ready to be shipped. Further information in shipping your samples to MicrobesNG can be found in https://microbesng.com/microbesng-faq/

APPENDIX A



Example: A 10 mL culture with an O.D.600nm of 0.25 after a 1 in 10 dilution has an OD of 2.5 (= 0.25 OD/mL x 10 [dilution factor]). This means that *1 mL* of this culture contains *the same number of cells* than *2.5 mL* of a *1.0 OD* culture, which is to say that our culture has an *equivalent OD of 2.5 per mL of culture*). We need to harvest **10 (8-12) equivalent OD**, that is the number of cells that would give an OD of 10.0 in 1mL of culture = **10 equivalent OD** [the number of cells we want to harvest to give 10.0 OD in 1 mL]] **/ 2.5** OD/mL [i.e. our culture's *equivalent OD per mL*]= **4 mL** (acceptable range 3.2-4.8 mL for equivalentOD 8-12) of culture to be pelleted, resuspended in DNA/RNA Shield and sent to us.