FlowChamber

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FlowChamber Protocol

To further ensure that the results we achieved with our designed FlowChamber are comparable, we decided to provide an instruction manual.
This protocol will establish procedures on how samples should be prepared for the measurements and how the measurements should be conducted.
The assembly FlowChamber assembly instructions can be found at the end of this document.

Preparation

To prepare your samples it is mandatory that they are grown on solid media with a height of four to five millimeters, with five millimeters being the critical upper limit. This upper limit is a result of the chamber's geometry and any sample higher than this will obstruct the flow, leading to wrong or falsified results as well as a destroyed sample.

We conducted tests in our lab and found, that roughly 25 to 30 mL of media will result in the needed media height of 4-5 mm in a petri dish with a diameter of 90 mm.

The overnight culture used for inoculation should have an OD600 of 2.0.
Write down the volume of culture you are using for the inoculation.

While preparing, try to spread the inoculation sample evenly across the media. This will have the highest chance of producing a homogenous biofilm, with homogenous structural properties.

When your sample is grown and you are ready to conduct a FlowChamber assay, cut a 2x3 cm rectangle out of your sample. If a special region piques your interest in terms of structural integrity, try to position it in the middle of the sample you want to cut out. This will subject the region of interest to the strongest surge forces, giving the most accurate results.

Measurements

To achieve comparable results, it is important to use a peristaltic pump with a selectable flow rate. The inner diameter tubing has to be 6 mm, in order to ensure a good fit.

Although there are connectors with an R ⅛" thread allowing a tube with a larger inner diameter, it is important to refrain from using larger diameters to prevent changes in fluid velocity and vortex formation, which could interfere with the comparability of the results.

To ensure that others can reproduce the results you have got, write down all important parameters like flow rate, duration of exposure, as well as the parameters necessary for creating the tested biofilm.

To create a reference, prepare a second flask containing the same volume of water as the flow chamber assay wastewater container.

Then inoculate the reference with the same volume of a fresh overnight culture (with an OD600 of 2.0) that was used for biofilm creation.

Gently stir the two vessels to ensure that the contents are mixed well.

Take four culture tubes and create the following cultures:

1. 3 ml of media and 10 µL of the water used for the flow chamber assay
2. 3 ml of media and 10 µL of the wastewater container
3. 3 ml of media and 10 µL of your reference sample
4. 3 ml of media and 10 µL of the overnight culture with an OD600 of 2.0

These will ensure a positive (4) and negative (1) control, as well as the sample (2) and a reference (3).

Incubate the tubes as you would with a normal over-night culture.

After incubation, measure the OD600 of all four cultures.

The negative control should ideally be clear, the positive control should have the OD600 expected from a normal overnight culture.

The OD600 of the reference culture should show how a normal inoculation with the same dilution would have grown.

The OD600 of the sample culture is supposed to show how many living cells were removed from the biofilm during the flow chamber assay.

You can calculate the quotient of sample to reference OD600 in order to make a statement about the relative number of living cells that were separated from the biofilm.

FlowChamber Assembly Instructions

Print the top and bottom halves of the chamber.

After printing you will find a 0.8mm deep and 2mm wide groove on the bottom half. This groove is intended for a seal.
You can either fill it with silicone, or cut a paper gasket to the right size.
After installation of the seal the bottom half is prepared and you can move on to the top half.

To prepare the top part you will need two hose nozzles with an outer thread of R ⅛".
Wrap a layer of thread seal tape around it and screw them in until they are hand tight.
Remember that the chamber is made out of plastic and the pressure inside is not very high, so there is no need to apply excessive force.

Now you are ready to insert a sample.

After doing so, seat the top half and fixate the both halves with M3 nuts and bolts in each corner.

Now you are ready to run your assay.

FlowChamber – What do you need?

- 3D printer
- Filament
- Silicone or a sheet of paper gasket
- 2x R ⅛" hose nozzles
- 4x M3 nuts and bolts

To perform an assay, you will also need hose with an inner diameter corresponding to the hose nozzles that you attached onto the chamber, and a peristaltic pump.