

# Bianca's 10-Day schedule

To make things easier, we are keeping cleaning and media preparation on a 10-day schedules.

## 10-Day requirements

Every 10-days the experiment has five two-day transfers and one 10-day transfers. For two-day transfers we use 60 flasks, on days where there is a 10-day transfer there is also a 2-day transfer, bringing the total number of flasks with media needed in 10 days up to **360**. In addition, we use 20 10mL tubes during each transfer, so every 10 days we need a minimum of 120 tubes.

## 1) Media Preparation

### Recipe

The recipe for the complex media created for Task 2 is **PYE with 0.2% glucose and 0.1% casamino acids**.

It's easier to make the media in 1.5 L batches in 2L media bottles. The adjusted amount you would need for 1.5L is as follows:

- 3 g bactopectone
- 1.5 g yeast extract
- 0.45 g  $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$
- 3 g dextrose
- 1.5 g casamino acids
- 1.5 L ePure  $\text{H}_2\text{O}$

Since you are autoclaving the allocated media in individual 50 mL flasks, you don't need to autoclave this.

Using the 10 mL pump, allocate 10 mL of the media into individual, clean 50 mL Erlenmeyer flasks and place a plastic beaker over each flask. Place the flasks in a large tray. Each tray holds 65 flasks 1.5L fills ~ 120 flasks, so you'll use a total of 4.5L of media each week. It's a good idea to fill an extra 60 flasks or so each week in case we need extra. You will also need to fill the glass tubes with 10 mL of media.

### Autoclave

Place ~ 3/4 in. of water in the bottom of each tray. Autoclave the flasks and/ or tubes for ~30 minutes in the autoclave and then run it on slow exhaust. Remove the flasks from the tray with water to a dry tray.

## **Setup**

**Note:** You don't need to label any of the tubes

## **Labelling**

We have a sticker-based labelling system. For each 10-day cycle you will need to print out stickers. Print out 5 sets of stickers for 2-day transfer and 1 set of stickers for 10-day transfers. The word documents for these stickers are on Box and the blank stickers are in a drawer by the flasks. To prep for the transfers, each sticker should be placed on the top of a flask with autoclaved media.

## **Layout**

We have 6 trays already labelled for set up. Five two-day trays and one 10-day tray. On the bottom of each tray is a sticker. Position each flask on the tray such that the sticker on the bottom matches the sticker on the top of the flask.

# **1) Washing Glassware**

## **Dumping used media**

We have two large flasks by the sink. Dump all used media into the flasks. When the flasks are full, autoclave them and dump the sterile waste down the sink.

## **Biofilm removal**

Biofilm builds up on glassware over time. To remove it, each flask and tube needs to be autoclaved with DI H<sub>2</sub>O in a tray with H<sub>2</sub>O on the bottom. When the flasks and tubes are done, dump the water down the sink and continue with washing.

## **Washing**

All flasks and tubes need to be hand washed until the dishwasher rack gets made. Once washed, let the tubes air dry.

## **Repeat 1-2 as necessary**

## **Emergency contact**

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