2-27 Bioscreen for knockouts

WEDNESDAY, 2019-10-09

Preparation for bioscreen to estimate effect of knockouts on MA lines. We have 264 lines to grow from the freezer.

- [x] Label 264 microcentrifuge tubes and 264 10 mL glass tubes according to this file:

line_codes_MA_KO.csv

- [x] Fill 10 mL glass tubes with 2 mL of liquid YPAD (you will need 528 mL of YPAD).
- [x] Perhaps make new YPAD for the assays? We need 150 µL $well^{-1}$ *400 wells day^{-1} * 8 days = **480 mL of YPAD**
- [x] Autoclave another litre of water for the dilutions for the assays.
- [x] Matt: check that we have sufficient space in the rotor or incubator for growing 264 samples.

Bryn & Matt: Try to make the sorting sheet. We do not have enough samples to fill the 400 wells, so we will need to reuse (400-264-1 blank) = 135 samples each day. How do we sort the samples? Do we have a sorting sheet such that after sampling the tube for the bioscreen that we move it immediately to a new position if it is to be reused. How do we make a sheet that is easy to follow? The file below has the order of the samples for each well and day (the samples are randomized with the restriction that each sample can only be reused once every day.

MA_KO_comparison_bioscreen.xlsx

FRIDAY, 2019-10-18

- [x] Take out and thaw the needed MA lines from aliquot 2 Matthew Stasiuk
- [x] Inoculate the 2 mL of YPAD with 10 µL of thawed culture.
- [x] Put MA lines back into freezer
- [x] Take out and thaw the needed KO lines from aliquot 1
- [x] Inoculate the 2 mL of YPAD with 10 µL of thawed culture.
- [x] Put KO lines back into freezer.
- [x] Grow all samples in rotor (?) at 30°C over the weekend.

MONDAY, 2019-10-21

- [x] Transfer 1 mL of each culture into its corresponding microcentrifuge tube.
- [x] Sort the tubes according to day 1 sheet: coming soon.
- [x] Follow the Bioscreen For KO and MA Comparison protocol. Edit: ran with shaking on Low.

TUESDAY, 2019-10-22

- [x] Bioscreen 1s lamp had went out. Linnea replaced light bulb.
- [x] Follow the Bioscreen For KO and MA Comparison protocol. Edit: We ran the machines on low with 300 µL of media rather than 150 µL.

WEDNESDAY, 2019-10-23

- [x] Follow the Bioscreen For KO and MA Comparison protocol.
- [x] Ran the machines on medium with 300 µL of media (changed the protocol accordingly).

THURSDAY, 2019-10-24

- [x] Follow the Bioscreen For KO and MA Comparison protocol. **Returned to 150 µL** because still settling in the 300 µL.

FRIDAY, 2019-10-25

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

MONDAY, 2019-10-28

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

TUESDAY, 2019-10-29

When Linnea came in the first Bioscreen was not doing any shaking or reading, but made tiny peeps. I tried to Abort the experiment, but it was stuck during a measurement reading and so wouldn't close the application. I tried using the Reset button on the machine which caused it to sound very loudly. Turned off the machine, but it did not move the plate, but left it at the reading position and once I started the machine it again made the loud noise, so I turned it off completely. Instead made a sheet for running only two plates in the other machine.

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

WEDNESDAY, 2019-10-30

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

THURSDAY, 2019-10-31

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

FRIDAY, 2019-11-01

- [x] Follow the Bioscreen For KO and MA Comparison protocol.

SATURDAY, 2019-11-02

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 11.

SUNDAY, 2019-11-03

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 12.

MONDAY, 2019-11-04

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 13.

TUESDAY, 2019-11-05

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 14.

WEDNESDAY, 2019-11-06

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 15.

THURSDAY, 2019-11-07

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 16.

FRIDAY, 2019-11-08

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 17.

SATURDAY, 2019-11-09

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 18.

SUNDAY, 2019-11-10

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 19.

MONDAY, 2019-11-11

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 20.

TUESDAY, 2019-11-12

- [x] Follow the Bioscreen For KO and MA Comparison protocol. Day 21.

WEDNESDAY, 2019-11-13

- [x] Measure OD of all undiluted samples, in Biotek.

Bioscreen for KO and MA comparison

Introduction

This protocol describes the prodedure of setting up a bioscreen from saturated culture.

Materials

- Bioscreen plates
- 0.2 mL PCR tubes, without lid
- ddH_20
- YPAD
- 200 µL pipette tips
- 2.5 mL Finntip stepper tip

Procedure

Dilution

- [ ] 1. Fill all 0.2 mL PCR tubes with 150 µL of ddH_2O using the finntip stepper (set on 3).
- [ ] 2. Vortex tube with culture.
- [ ] 3. Transfer 15 µL of saturated culture into a filled PCR tube. Pipette up and down until homogenized.

Prepare plate

- [ ] 4. Transfer 15 µL from diluted culture into assigned well in the Bioscreen plate (this can be done immediately after step 3, using the same pipette tip, and the PCR tube can be disposed of directly after use).
- [ ] 5. Repeat step 2-4 for all samples.
- [ ] 6. Fill all bioscreen wells with 150 µL of YPAD using Finntip stepper.

Run machine

- [ ] 7. Put bioscreen plate into machine (double check direction). Run for 1 day at 30°C, 200 samples, with Medium continuous shaking. Save in folder Linnea -> Dominance -> and label "dominance_KOMA_dayX_machineY"