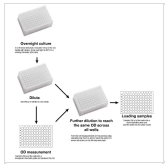

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Yeast growth and fluorescence 96-well plate reader experiment, set up from a 96 deep well plate

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ABSTRACT

This protocol is used to measure a time-series of growth and fluorescence output in yeast. We have used it on S288C-background *Saccharomyces cerevisiae* yeast expressing a range of fluorophores, from a fluorescent reporter gene or tagged protein. It can also be used to measure growth alone if no fluorophore is present.

Precisely, the protocol describes the measurement of yeast absorbance (OD600) and fluorescence output in a microtiter plate over 12-15 hours in a Tecan Infinity M200 series plate reader. We have also run essentially the same protocol on a fixed-wavelength F200 plate reader. The pre-culture of the cells was carried out on a 96 deep well plate. On the following day, the preparation and culturing of the cells on a black microtiter plate was carried out mostly according the protocol described in Lichten et al. (2014).

1. Lichten CA, White R, Clark IB, Swain PS. Unmixing of fluorescence spectra to resolve quantitative time-series measurements of gene expression in plate readers. BMC Biotechnol. 2014 Feb 3;14:11. doi: 10.1186/1472-6750-14-11.

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MATERIALS TEXT

Gas Permeable Adhesive Seals **Thermo Scientific Catalog #AB-0718**

96 well black microplate PS F-bottom (Chimney well) µCLEAR non-binding **greiner bio-one Catalog #655906**

Lid Ps low profile (6 mm) Clear sterile **greiner bio-one Catalog #656191**

Corning® 96-well Clear Flat Bottom Polystyrene Not Treated Microplate 20 per Bag with Lid Sterile **Corning Catalog #3370**

2.2 ml 96 Deep well Plate Square Wells with Conical Bottoms **StarLab Catalog #E2896-1810**

Reagent Reservoir 25 mL 100PK **Gilson Catalog #F267660**

Double-distilled or sterile water

BEFORE STARTING

Reserve a time slot for your plate reader experiment a few days before your experiment

The link to the plate reader booking system - (http://synthsys.bio.ed.ac.uk/biosched/ctrlpnl.php)

Growth of cells in a 96 deep well plate

1

Plan the plate set up for the experiment, both the 1) pre-culture plate and 2) the final 96-well black microtiter plate.

Tips for plate design:

- For the pre-culture plate, fill the plate, skipping every other row to allow a checkered pattern of loading
- Plan the final plate set up in blocks, where each block (labelled in black and red thick lines the final plate) is a technical replicate. This allows blocks to be set up in different ways, e.g. upside down.

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1) 96 well pre-culture plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	mod0 biorep 1	mod0 biorep 2	mod0 biorep 3		modC biorep 1	modC biorep 2	modC biorep 3		200bp biorep 1		200bp biorep 3	
B									POT biorep 1		POT biorep 3	
C	modA biorep 1	modA biorep 2	modA biorep 3		modD biorep 1	modD biorep 2	modD biorep 3		200bp biorep 2			
D									POT biorep 2			
E	modB biorep 1	modB biorep 2	modB biorep 3		modE biorep 1	modE biorep 2	modE biorep 3					
F												
G												
H												

2) Final 96 well plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	200bp biorep 3	mod0 biorep 1	modC biorep 2	mod0 biorep 3	modC biorep 1	mod0 biorep 2	modC biorep 3	mod0 biorep 1	modC biorep 2	mod0 biorep 3	200bp biorep 1	200bp biorep 2
C	POT biorep 3	modC biorep 1	mod0 biorep 2	modC biorep 3	mod0 biorep 1	modC biorep 2	mod0 biorep 3	modC biorep 1	mod0 biorep 2	modC biorep 3	POT biorep 1	POT biorep 2
D	200bp biorep 3	modA biorep 1	modD biorep 2	modA biorep 3	modD biorep 1	modA biorep 2	modD biorep 3	modA biorep 1	modD biorep 2	modA biorep 3	200bp biorep 2	200bp biorep 1
E	POT biorep 3	modD biorep 1	modA biorep 2	modD biorep 3	modA biorep 1	modD biorep 2	modA biorep 3	modD biorep 1	modA biorep 2	modD biorep 3	POT biorep 2	POT biorep 1
F	200bp biorep 3	modB biorep 1	modE biorep 2	modB biorep 3	modE biorep 1	modB biorep 2	modE biorep 3	modB biorep 1	modE biorep 2	modB biorep 3	200bp biorep 1	200bp biorep 2
G	POT biorep 3	modE biorep 1	modB biorep 2	modE biorep 3	modB biorep 1	modE biorep 2	modB biorep 3	modE biorep 1	modB biorep 2	modE biorep 3	POT biorep 1	POT biorep 2
H												

An example of the plate set up for the 1) pre-culture and 2) final plate. The pre-culture plate needs to be designed according to the final set up for easy pipetting.

In this example, strains mod0, modA, ..., modE were the treatment strains being compared, and POT and 200bp designated negative controls. You should name strains and lay out the plate according to the needs of your own experiment.

2 Aliquot **100 µl** of filter sterilised yeast media (with selection as needed) to each well to be used for the experiment according to the pre-culture plate plan.

This can be done by two methods:
1) Using a multi-channel pipette, with the media in a reagent reservoir.
2) Using a repeat pipette.

When dispensing this volume into each well, pipette to the bottom of the well to prevent any liquid from staying in the walls of the well.

3 Seal with a gas permeable seal. If the plate was designed with the outer wells empty, add **500 µl** of ddH2O.

4 Grow overnight in a incubator set at **30 °C** and shaking at 250 rpm.

Note: 250rpm is a minimum! Otherwise yeast cells will pellet instead of growing in suspension.

OD measurement

5 After 20 hours, carefully add **900 µl** of yeast media to each well containing yeast culture (this is a 1:10 dilution of the culture)

This can be done by two methods:
1) Using a P1000 multi-channel pipette, with the media on a reagent reservoir.
2) Using a repeat pipette.

Be careful not to cross contaminate. I would recommend changing tips after each well (if using a multi-channel

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pipette). If using a repeat pipette, pipette to the walls rather than straight to the liquid to prevent splashing. Adjust the dispensing speed as needed.

6 Using a P100 multi-channel pipette, resuspend the culture several times to mix then transfer **200 µl** of the culture to a clear 96 well microtiter plate.

7 Measure the OD600 using the plate reader.

Dilution calculation

8 Blank correct all the OD measurements by subtracting all the measured OD by the OD of the media.

On a Tecan M200 plate reader, this can be done in (a copy of) the excel spreadsheet that contains the output measurements.

9 Apply the following formula to each blank corrected OD measurement. The result of this will be the amount to add to the diluted pre-culture to normalise their OD

=800 * ((x - target OD) / target OD)

- 800 is for the remaining volume of the culture on the pre-culture deep well plate
- x is for the blank corrected OD value

We recommend a target OD of 0.2 to capture exponential growth of yeast.

Sometimes the lowest (blank corrected) OD of the overnight cultures can be below 0.2. In that case, it is convenient to adjust the target OD for the whole plate to the lowest (blank corrected) OD, so that all wells of the plate start at the same OD. For larger experiments comparing growth across multiple plates, if a value lower than 0.2 is used in the first plate, this new lower value may be used as the target OD for later plates.

10 Add the volumes of media as calculated from the previous step to their corresponding wells to normalise their ODs.

Do not round up or down! This can cause unwanted variations in your starting OD!

Plate loading

11 Using a multi-channel pipette, add **200 µl** of media to the (blank) media wells of the black microtiter plate.

12 Using a multichannel pipette, gently resuspend the diluted culture by pipetting up and down several times, before loading them on the next plate.

Cells may have sedimented during the dilution calculation.

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When resuspending, be careful to not to cross-contaminate! Gently resuspend!

13 Load **200 µl** of diluted culture to each well of the black microtiter plate, following the plate design. Load one biological replicate at a time and repeat 3x for technical replicates before moving to the next biological replicate.

An electronic repeat P1000 multi-channel pipette is convenient for pipetting 3 technical replicates.

14 Replace the lid and transfer the plate to the Tecan M200 plate reader.

15 On the Tecan plate reader program (Tecan icontrol), set up the following protocol:

- Select the plate you are using - (Corning flat bottom 96 black microtiter plate).
- Select the wells you would like to measure.
- Set the temperature at 29.9-30.5 °C.
- From the actions/functions on the left side of the screen, add a 'kinetic cycle' to the program.
  - Set this to 80 cycles, linear shaking, 6 mm amplitude at 200-220 rpm, for a duration of 550 seconds.
- Add an absorbance measurement to the kinetic cycle.
  - Set this to 595 nm with a maximum bandwidth of 9 nm with 15 reads.
- If needed, add a fluorescence measurement to the kinetic cycle
  - For mCherry fluorescence - set this to an excitation wavelength at 585 nm and an emission wavelength of 620 nm (excitation bandwidth of 9 nm and emission bandwidth of 20 nm) with the gain set at 100.
  - For mTurquoise2 fluorescence - set this to an excitation wavelength at 434 nm and an emission wavelength of 474 nm (excitation bandwidth of 9 nm and emission bandwidth of 20 nm) with the gain set at 60.

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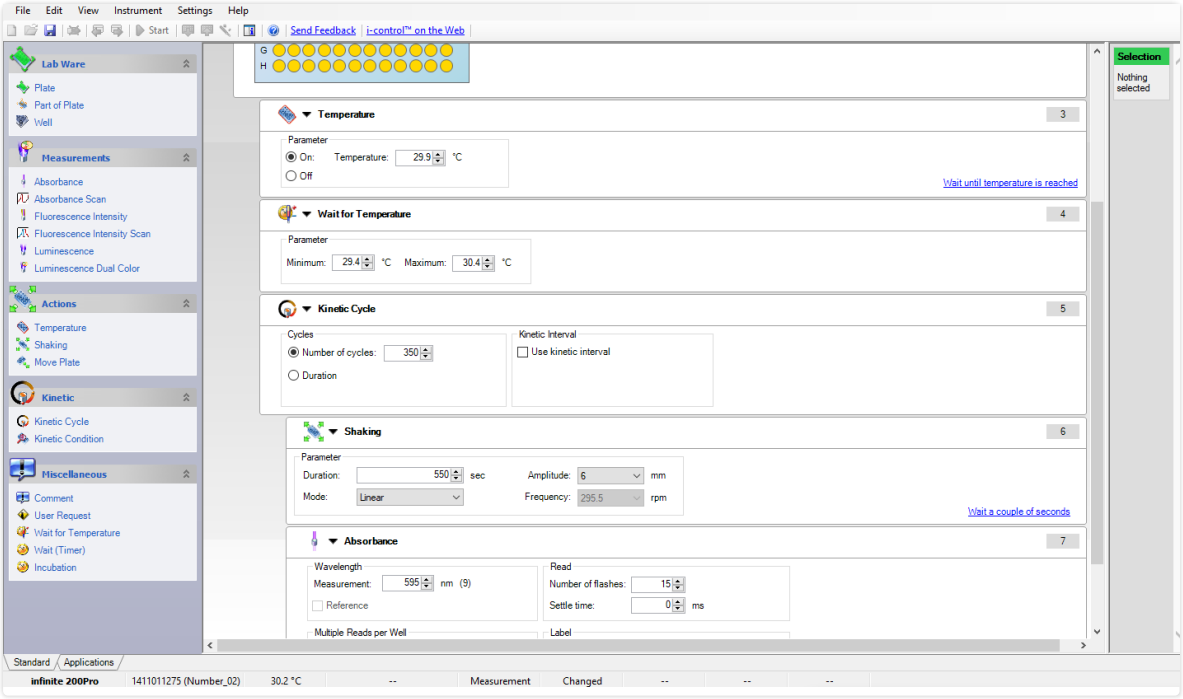

The steps included in the kinetic cycle are indented.

It helps to save this protocol for future use instead of setting it up afresh every time.

E.G. In the Wallace lab, a template is available on the computer for PR1/PR2 in Desktop > Wallace lab.

For different fluorophores and different levels of expression, you need to adjust the excitation and emission values and the gain. If in doubt run a fluorescence spectrum scan on your strains to find the best excitation and emission values.

For GFP fluorescence measurements, use 485/535 nm (ex/emm) - initial tests have shown overrflow values when using moderate to highly expressing promoters driving the expression of eGFP. Therefore it is worth testing lower gain values 50, 60 or 80

For mTurquoise fluorescence measurements, use 434/474 nm (ex/em) - initial tests have shown overrflow values when using moderate to highly expressing promoters driving the expression of mTurquoise. Therefore it is worth testing lower gain values 50, 60 or 80

**remember to take note of the Gain value for each setting through the 'Label'. For example, Label: GFP-80 for GFP measurements with the gain value set at 80.

16 Start the program. The plate reader should begin to heat up to ~30°C. After the temperature is reached, the plate

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reader should begin the shaking step. Wait until the first absorbance and fluorescence measurement is recorded to make sure the protocol is running as planned, before leaving the experiment to run.

17 When the experiment has finished running, save the raw data file from the Tecan icontrol software.

Give this data file a sensible name including the date of the experiment in a standard format (e.g. 2021-08-10). Backup the raw data file in the lab's shared storage space and make a copy for any data analysis, do not overwrite your raw data! Link to both the raw data and the analysis in your lab notebook entry.

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