Plating and imaging of yeast colonies for segmentation and classification with neural networks

Marc Bühler (✉ marc.buehler@fmi.ch)

Marc Bühler Lab, Switzerland   https://orcid.org/0000-0001-6661-9795

Lea Dümpelmann

Marc Bühler Lab, Switzerland   https://orcid.org/0000-0002-5775-5186

Sarah Carl

Marc Bühler Lab, Switzerland   https://orcid.org/0000-0003-3927-2559

Abstract

The yeast research community commonly makes use of a simple and powerful color reporter assay, based on adenine auxotrophy, to study genetic and epigenetic mechanisms. Manual classification and quantification of colony color in high numbers is tedious, immensely time consuming, can be erroneous and irreproducible. To overcome these problems, we have established an automated pipeline to quantify and classify yeast colonies from images of entire plates with high accuracy (98.6%). Having the same plating and imaging conditions as used for training of the network is key to this high accuracy. Here we provide a standardized protocol for plating of yeast and imaging within 5-7 days, compatible with the classification pipeline described in Carl *et al* (bioRxiv; doi: https://doi.org/10.1101/801845).

Introduction

Adenine auxotrophy is commonly used in yeast research to monitor genetic and epigenetic events. Mutation or silencing events interrupting the adenine synthesis pathway lead to the accumulation of a red pigment under adenine limiting conditions. Colonies with a functional adenine synthesis pathway appear white. An advantage of this non-selective reporter system is that a direct semi-quantitative comparison of expression levels between colonies can be made on the same plates. Furthermore, the degree and stability of expression (pink, variegating and red) can be determined by comparing the amount and distribution of accumulated red pigment within a colony. A disadvantage of this non-selective reporter is the very time-consuming and tedious color classification of colonies by hand. Further, if components of the adenine synthesis pathway are expressed at low level (pink phenotype), classification can vary between researchers and screens, potentially leading to erroneous and not fully reproducible results. To reduce these issues, we have developed a highly accurate and automated colony classification method based on neuronal networks with whole plate images as input (Carl *et al;* bioRxiv, doi: https://doi.org/10.1101/801845). In this protocol we describe in detail the procedures for plating and imaging as used for the pipeline training, which are crucial to achieve optimal results.

Reagents

Petri Dish (FALCON, #351029)

Difco™ Agar, Granulated (BD, #214530)

Bacto™ Yeast Extract (BD, #212750)

α-D-Glucose (Sigma-Aldrich, #158968)

Yeast strain

Equipment

Equipment:

Kaiser RS1 Copy Stand

Canon EOS 550D

Canon macro lens EF-S 60mm 1:2.8 USM

KAISER RB 5000 DL Lighting Unit

Software:

Project home page: https://github.com/fmi-basel/buehler-colonyclassification

Operating system(s): Platform independent

Other requirements: Python 3.6 or higher, fastai library v. 0.7

License: GNU GPL v3.0

Procedure

We present a detailed protocol describing: (i) Plating of no more than 200-500 colonies/ 10cm plate (1 hour/ 100 plates) (ii) Incubation of plates until colonies form (at 30°C for 5-7 days); (iii) Imaging of plates (30min/ 100 plates); and (iii) running the automated pipeline described in (Carl *et al;* bioRxiv, doi: https://doi.org/10.1101/801845) on the obtained images (20min/100 images). Although we established this protocol for *Schizosaccharomyces pombe*, implementation for other microbes should be straight-forward.

YE plate preparation (1l / 50 plates)

1. Mix 1.0 l ddH$_2$O, 5.0 g Yeast Extract, 30.0 g α-D-Glucose and 20.0 Agar with a magnet until all powder clumps disperse.

2. Autoclave at 121°C for 20 min.

3. Cool down under constant stirring to 50°C.

4.   If desired, add antibiotics and stir for 2 min to ensure a homogeneous mixture.

5.   Pure 20 ml of autoclaved agar media into each 10 cm plate.

6.   After solidification (c.a. 20 min at room temperature), transfer plates upside down into plastic bags and store in a cold room until use or up to two months.

Note: For consistent results keep autoclaving time the same for all plates within an experiment. Reduced autoclaving time can lead to slightly increased accumulation of the red pigment.

Note: When working with PMGc plates, low thiamine concentration reduces epigenetic ade6$^+$ silencing phenotypes in S. pombe.

Plating of yeast cells

From liquid culture:

1.   Measure optical density (OD).

2.   Dilute samples to 100 cells /ml.

3.   Pipette 1 ml onto a cover slip and recount under a microscope.

4.   Dilute to 500 cells / 100 ml (per plate).

5.   Homogeneously distribute 100 ml / plate with glass beads.

From plate:

1.   Transfer cells from a plate with a pipette tip into a 1 ml H_2O containing Eppendorf tube. Mix well by vortexing.

2.   Dilute 1:100.

3.   Pipette 1 ml onto a cover slip and count cells under the microscope.

4.   Dilute to 500 cells / 100 ml (per plate).

5.   Homogeneously distribute 100 ml / plate with glass beads.

Note: When working with mutants that impact viability or selecting for a fraction of cells that are resistant to antibiotics, estimate how many cells will survive beforehand and plate accordingly.

Incubation of yeast plates

Incubate plates upside down at 30°C for 5-7 days.

Note: If working with growth defective mutants or incubating plates at different temperatures, grow cells until colonies reach a diameter of at least 1 mm.

Note: If colonies are overgrown (e.g. 10 days at 30°C), i.e. the center protrudes and a ring forms around the center of the colony, many white colonies are misclassified as pink.

Note: Storing plates in the fridge enhances the red color. Treat all plates within an experiment the same.

Imaging of plates

1. Place plate on a dark black background.

2. Adjust camera height or zoom that the entire plate fits into one image.

Note: If plates were previously stored in the cold, let them warm up to room temperature before imaging to avoid classification artefacts caused by condensation.

Note: Different colored backgrounds can possibly be used, however a black background is recommended.

Note: Set color balance to manual and not automatic to avoid increased red in images with only white colonies.

Running the automatic classification

Run the classification pipeline as described in (Carl *et al;* bioRxiv, doi: https://doi.org/10.1101/801845):

1. Download and install the required python packages as detailed on the github page.

2. Download the classification pipeline script.

3. Place all images that you would like to classify into the same folder.

4. Run the pipeline as described on the github page.

github link: https://github.com/fmi-basel/buehler-colonyclassification

Troubleshooting

No colonies classified:

No colonies are classified when the neuronal network cannot distinguish colonies from background (segmentation). Change the background to a darker black. Bad quality of images (out of focus/ low pixel number) can reduce the number of classified colonies.

Few colonies classified:

When colonies are very dense (>500 colonies / plate), segmentation can frequently fail, leading to few classified colonies. Also colonies smaller than 20 x 20 pixels are not segmented. This can be easily resolved by decreasing plating density.

Misclassified colonies:

White colonies can be misclassified as pink, if plates were incubated too long (a ring forms around the center of the colony). Reduce incubation time of plates. To check classification of specific colonies, the classification pipeline can be run in "prediction-only" mode on previously-segmented images.

Overestimation of white colonies:

The accumulation of the red pigment decreases growth. If cells are plated too dense, red colonies do not reach the threshold size for segmentation, leading to a relative underestimation of red colonies in comparison to other colony phenotypes.

Further improvement:

If all those measures don't improve the output, one can re-train the neuronal network with own images (Carl *et al;* bioRxiv, doi: https://doi.org/10.1101/801845).

Time Taken

Estimated time requirements for 100 plates:

YE plate preparation: 40 min

Plating of cells: 1 hour

Incubation of yeast plates: 5-7 days

Imaging of plates: 30 min

Running the automatic classification: 15-20 min

Anticipated Results

The automatic classification pipeline generates a summary .csv file, containing the total number of classified colonies as well as the percentage of white and non-white colonies of each plate image .
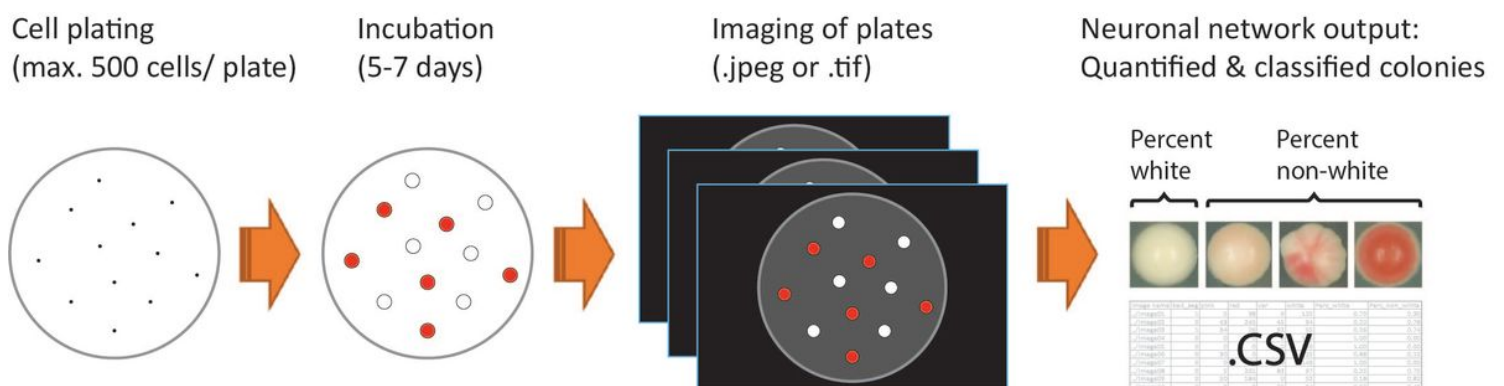
References

Carl S. H. et al. A fully automated deep learning pipeline for high-throughput colony segmentation and classification. bioRxiv, doi: https://doi.org/10.1101/801845

Acknowledgements

Figures

Figure 1

Workflow of colony plating, imaging, and quantification. 200-500 cells per 10 cm YE plate are evenly distributed with glass beads. Plates are incubated at 30°C for 5-7 days until colonies reach sufficient size. Subsequently, plate images are acquired on a black background. Finally, images are run through the

pipeline described in (Carl S. H. et al; bioRxiv, doi: https://doi.org/10.1101/801845) to obtain quantification and classification of yeast colonies.