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# Live cell Imaging of yeast cells expressing human PINK1-GFP and the TOM complex subunits

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

The PINK1-Parkin axis plays a major role in mitochondrial quality control and mutations have been closely associated with familial cases of Parkinson's disease [1]. To assess the correct functioning of mitochondria, PINK1 acts as a sensor by monitoring their import capabilities [2]. While proper import leads to degradation of PINK1, failed import causes PINK1 to form a complex with the translocon of the outer mitochondrial membrane, allowing it to be stabilized and activated by cross- phosphorylation [3]. Here we describe a protocol for live-cell imaging of Saccharomyces cerevisiae cells expressing human PINK1 with GFP tag at the C-terminus as well as all the human TOM complex subunits (TOMs 5, 6, 7, 20, 22, 40 and 70) immobilized using Concanavalin A coating. The aim of this experiment is to investigate if human PINK1 expressed in yeast cells is localized to the mitochondria. In this case, galactose was used to induce expression of the fluorescence-tagged protein-of interest and colocalization was tested using a respective mitochondrial tracker. The general workflow of this protocol is based on the one of [4].

## Attachments

PDF

1022-2635.pdf

351KB

# Materials

### Medium

Standard yeast medium you use for liquid culture- In this specific case:

- Yeast Synthetic Drop-out Medium Supplements without tryptophan **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y1876
  Yeast Nitrogen Base without Amino Acids Wickerham formula, classification of yeasts based on amino a **Becton Dickinson (BD) Catalog #**291940
- D(+)-Raffinose pentahydrate **Carl Roth Catalog #**5241.3

### Consumables and reagents

- Concanavalin A **Carl Roth Catalog #**7246.1
- MitoSpy™ Red CMXRos **BioLegend Catalog #**424801
- Cover glasses, thickness no. 1, 20 mm x 20 mm, square shape, pure white **BRAND Catalog #**470050
- Glass slides (Epredia, #AD00008432E01MN250)
- PBS pH 7.4 **Thermo Fisher Scientific Catalog #**10010023

### Instruments

- Leica DMi8 with HCPL APO 63x/1.40 lens
- Incubator (Thermo Fisher Scientific, #10519912)
- Tabletop centrifuge

### Software

- Leica Application Suite X, ImageJ

## Yeast Cell culture

1 Grow yeast cells Overnight in liquid medium.

Note

In our case, we picked a colony on a respective plate and inoculated 3 mL of liquid medium and let them incubate at 30 °C and 180 rpm Overnight.

2 On the next day, check $OD_{600}$ and dilute to $OD_{600}$=0.1.

Note

Here, we again aim for a total volume of 3 mL and continue the incubation.

3 Allow the yeast cells to regrow into early exponential phase until they reach approx. $OD_{600}$=0.3-0.6. At this state, we induce protein expression by addition of 2 % galactose into the medium. In our case, we were aiming for another incubation time of 03:00:00.

3h

4 For the staining of mitochondria, add [M] 500 nanomolar (nM) of the Mito-Tracker into the medium and let it incubate for 00:45:00 up to the completion of the incubation period.

45m

## Concanavalin A coating

5 Prepare a stock solution of 1 mg/ml Concanavalin A using sterile water.

6 Place the coverslips into a suitable container of choice (e.g., 6-well, petri-dish), add 500 µL of the stock-solution on each coverslip and let them incubate for

30m

00:30:00 at Room temperature .

7 Remove excessive Concanavalin A solution and wash 3x with sterile water for about 00:05:00 . 5m

Note

Note that you may want to collect and reuse the excessive Concanavalin A for future use. Storage is at -20 °C .

8 Leave the coverslips under the air at Room temperature until fully dry.

## Mounting of the cells for imaging

9 To remove excessive Mito-Tracker from the solution, spin down the yeast cells at 4000 x g , remove the supernatant and wash the pellet with 1x PBS. Repeat this step 2 more times.

Note

In our case, we took 2 mL of the liquid culture and washed the pellet with 1 mL of 1x PBS.

10 Finally, add 8 µL of the resuspension to the Concanavalin A-coated coverslip and place it onto the glass slide. This volume should be sufficient for an air-tight closure.

Note

Using the 2 mL of liquid culture, we typically resuspend the pellet in 60 µL prior to mounting.

## Imaging procedure

11 Place the sample in the microscope and navigate to a group of yeast cells for setting the correct imaging parameters.

> Note
>
> For navigation and identification, we recommend using differential interference contrast (DIC), as it has given us better contrast than simply brightfield.

12 Dial in the appropriate laser and filter set for the fluorophore of your target-of-interest and start adjusting the exposure parameters according to the resulting brightness of your image.

> Note
>
> Ideally, the signal should be completely reflected in your histogram without being too close on either the lower or upper end to avoid under- and overexposure respectively. Try to keep the laser intensity as low as possible and rather increase the exposure time, if possible.

13 Images were acquired in Z-stacks to enable later deconvolution and/or maximum intensity projection (MIP).

> Note
>
> We normally use a Z-range of 10 µm and a step-size of 0.5 µm for yeast, resulting in a stack of 20 frames per image.

## Basic image processing

14 For denoising the image stacks, we used the 'Small Volume Computational Clearing' in the Leica Application Suite X (LAS X) software.

Note

Here, we applied the adaptive strategy with a set reflective index of 1.33.

15 When adjusting the histogram, make sure not to over- or under-expose the signal in any region. Apply adjustments to each channel individually and combine them afterwards for a composite image.

Note

The various steps are color-coded according to the respective topic and labelled with respect to the relevant chapter number in the protocol:

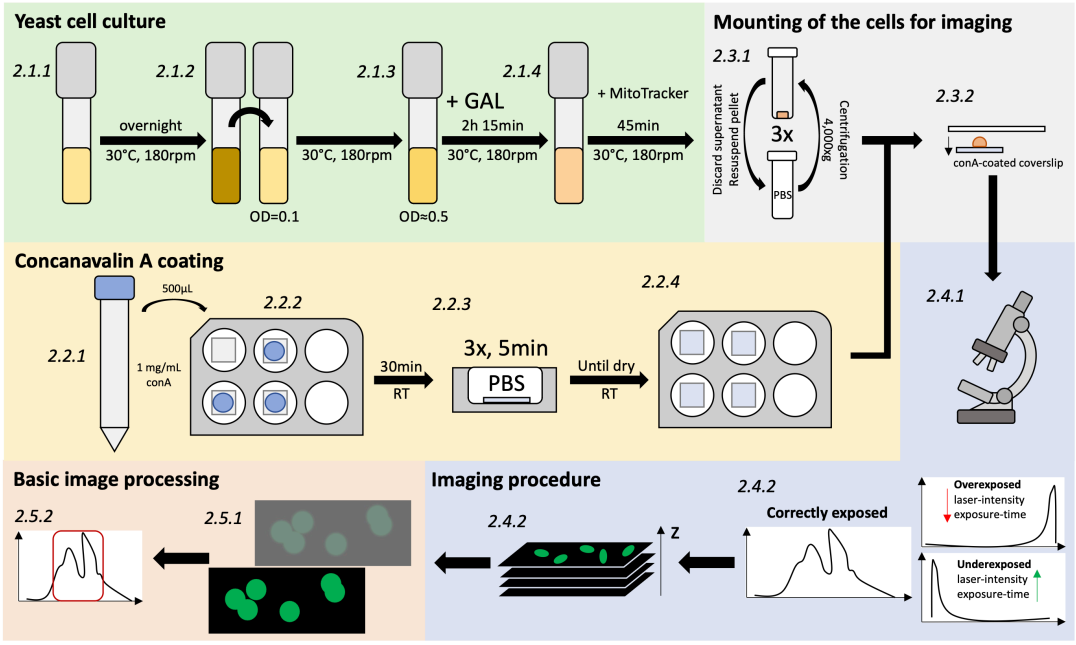


**Figure 1: Schematic representation of the entire workflow**

Note

This image shows a cropped and processed live-cell image of Saccharomyces cerevisiae. It shows colocalization of PINK1-GFP (green) with the MitoTracker (red) in the merged composite-image:

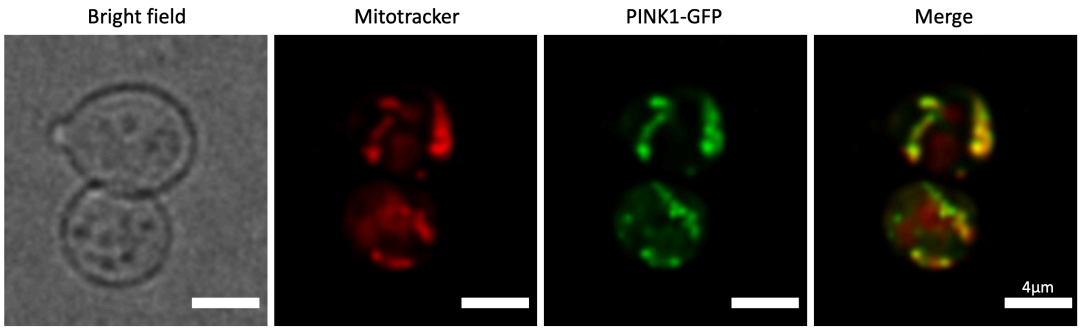


**Figure 2: Exemplary image following the protocol**

## Protocol references

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