**Supplementary Method: Budding Pattern Protocol**

# Yeast Strains

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| --- | --- | --- | --- |
| **Strain Name** | **Source** | **Budding Pattern** | **Filamentation** |
| S288c | ATCC® 26108™ | Axial | - |
| Σ1278b | NCYC 1391 | Bipolar | + |

# Reagents

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| --- | --- | --- | --- |
| **Chemicals** | **Abbreviation** | **Manufacturer** | **Catalog Number** |
| Yeast Extract  | YE | BD BactoTM | 212750 |
| Bacteriological peptone | Peptone | Sigma–Aldrich | 91249 |
| D-(+)-Glucose monohydrate | Glucose | Sigma–Aldrich | 49159 |
| Bacteriological agar | Agar |  |  |
| Yeast Nitrogen Base without amino acids and ammonium sulphate | YNB | BD DifcoTM | 233520 |
| Ammonium Sulphate | (NH4)2SO4 | Sigma–Aldrich | A4418 |
| Concanavalin A Type IV | Con-A | Sigma–Aldrich | C2272 |

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| --- | --- |
| **Material** | **Manufacturer (product code)** |
| TC-treated 12-well plates | VWR (734-2324) |
| Sterile Filter Q-Max 25 mm 0.22 µm CA | Frisenette (25CAGF022-500) |
| Neubauer Improved counting chamber | Assistant (40442) |

# Recipes

Yeast Extract Peptone Dextrose (YPD) broth

* 1 g YE
* 2 g peptone
* 2 g glucose
* 100 ml distilled water

The solution was stirred with heating until dissolved and autoclaved.

2% YPD agar plates

* 1 g YE
* 2 g peptone
* 2 g glucose
* 2 g agar
* 100 ml distilled water

The solution was stirred with heating until dissolved and autoclaved before pouring.

Synthetic Low Ammonium Dextrose (SLAD) broth

* 0.67 g YNB
* 2 g glucose
* 50 µl of ammonium sulphate solution (1M)
* 100 ml of distilled water

The solution was stirred with heating until dissolved. Solution was autoclaved and cooled to room temperature and sterile filtered before use.

Synthetic Ammonium Dextrose (SAD) broth

* 0.67 g YNB
* 2 g glucose
* 3.7 ml of ammonium sulphate solution (1M)
* 100 ml of distilled water

The solution was stirred with heating until dissolved. Solution was autoclaved and cooled to room temperature and sterile filtered before use.

Concanavalin A solution

* 1 mg Con -A
* 1ml MilliQ water water

Combined and sterile filtered.

# Equipment

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| --- | --- |
| **Equipment** | **Manufacturer** |
| oCelloScope | BioSense Solutions ApS |
| Centrifuge 5920 R | Eppendorf Nordic |

# Software

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| **Software** | **Version** | **Manufacturer** |
| UniExplorer | v. 8.1.0 | BioSense Solutions ApS |
| Minitab®  | 19.2020.1 (64-bit) | Minitab, LLC |
| MATLAB  | R202a | MathWorks Inc. |

# Procedure

## Image acquisition for budding pattern analysis

*Saccharomyces cerevisiae* strains were used in the experiment which linked budding pattern with physiological metabolite concentrations. Figure 3 represents a schematic of the overall experimental process used in this research. The experiment was carried out in both high (SAD) and low nitrogen (SLAD) liquid media with the same starting cell density of 103 cells/ml. The overnight culture was made by inoculating 5 ml of YPD broth in a 15 ml centrifuge tube with a single yeast colony grown on a 2% YPD agar plate. This was incubated overnight at 25°C with shaking. After overnight incubation, the cells were counted using a hemocytometer (Neubauer Improved counting chamber, Assistent), washed twice with the relevant media, and diluted to obtain a cell density of 1x103 cells/ml in the relevant media. Three 5 ml stock cultures were made in 15 ml centrifuge tubes with lids to produce biological replicates A, B and C for each condition. During the experiment these stock cultures grew at 25°C without shaking. Wells of the 12-well microtiter plate (VWR 734-2324 TC-treated 12-well plates) were prepared just before inoculation and imaging by coating the bottom of each well with 15 µl of Con A and allowing the wells to dry. 1 ml of each stock culture was pipetted into wells of the microtiter plate. After inoculation with the cell suspension the plate was placed in the oCelloScopeTM and allowed to rest for 30 minutes before starting image acquisition. This was to ensure that the cells were all immobilized on the bottom of the well before imaging to aid focusing. Preceding image acquisition, the focus was manually adjusted for each well through the UniExplorer software v. 8.1.0 program. The image distance was set to 9.8 µm and the illumination time to 2 ms. At each time point 90 images were collected of each well creating an 8010 µm scan area length. Subsequent two-dimensional images obtained with this technology have a magnitude comparable to that of a x200 magnification in a standard light microscope. Image acquisition was set to take place every 10 minutes for 10 hours with one scan area per well at 25°C.

After 10 hours of image acquisition, the plate was removed, and the media from each well was collected in 1.5 ml Eppendorf tubes. The media was spun down in a centrifuge at 4°C for 10 min at 4400 rcf (Centrifuge 5920 R, Eppendorf Nordic, Hørsholm, Denmark) and the supernatant collected and stored at -80° for 1H NMR analysis.

## Manual image analysis to obtain budding pattern

Since cells in our images were not immobilized in one plane it was necessary to determine the budding pattern of cells manually by eye rather than to develop an automated image analysis code.

Manual image analysis was carried out on the images acquired from the oCelloScopeTM to determine the budding patterns of individual cells. Images from each acquisition period were exported with the best-focus z-stack chosen manually. The scan areas were then cropped using a custom-made code in MATLAB (version R202a, The MathWorks Inc., Natick, MA, USA) such that thirty images were cropped from each scan area from the three biological replicate wells. Each cropped image was then analyzed by eye to determine the bud site sequence of new daughter cells over each period of image acquisition to obtain a bud site sequence for >200 cells and >300 bud sites for each condition. These values were chosen in order to ensure statistical significance. Samples of cropped images were randomly taken from the biological replicates A, B and C. This budding pattern categorization was done by observing new daughter cells and recording the positions of the first two or three bud sites (dependent on the number of times the cell budded within the time frame) to assign each cell with a bud site sequence. An example of this process on a representative cell can be found in Figure 2. These were then assigned their corresponding budding pattern classification (axial, bipolar or unipolar) according to Figure 1. The budding pattern assignments were then analyzed to determine the percentage value of each budding pattern for the different experimental conditions.

Statistical analyses were performed using Minitab® 19.2020.1 (64-bit). To determine statistically significant differences between conditions of budding pattern proportions, a 2x2 contingency table using Fisher’s Exact Test at a significance level of p=0.05 was applied.

# Custom Made MATLAB Codes

## Image Cropping Code

%%OCELLOSCOPE IMAGE CROP

clc;clear all;

%Define directory and folder

Origin = ['D:\Verification\_2/raw'];

A = dir(Origin);

Condition = {A.name}';

Condition = Condition(3:end);

firstsixth=[0,0,1600,1280];

secondsixth=[1600,0,1600,1280];

thirdsixth=[3200,0,1600,1280];

fourthsixth=[3200,1280,1600,1280];

fifthsixth=[1600,1280,1600,1280];

sixthsixth=[0,1280,1600,1280];

cropareas={firstsixth,secondsixth,thirdsixth,fourthsixth,fifthsixth,sixthsixth};

imagenumbers={1:5,6:10,11:15,16:20,21:25,26:30};

%%

for k=4

FolderName=cell2mat(Condition(k));

Directory = [Origin,'/',FolderName];

B = dir(Directory);

Names = {B.name};

Names = Names(3:end);

LastImage = cell2mat(Names(58)); %check last image number - should be rep 61=58

I1=imread(LastImage);

for z=1:6

 croparea=cell2mat(cropareas(z));

 imagenumber=cell2mat(imagenumbers(z));

 [Ja,recta]=imcrop(I1,croparea);

for j=imagenumber

number=num2str(j);

[Jb,rectb]=imcrop(Ja);

[Jc,rectc]=imcrop(Jb);

for i = 1:numel(Names)

file\_name\_cell = Names(i);

label=cell2mat(file\_name\_cell);

I2=imread(label);

Icrop1=imcrop(I2,croparea);

Icrop2=imcrop(Icrop1,rectb);

Icrop3=imcrop(Icrop2,rectc);

savename=[number,'\_',label];

location =['D:\Verification\_2/cropped','/',FolderName,'/',savename];

imwrite(Icrop3,location);

end

end

end

end

## Bud Site Sequence Code

%% Bud Site Number

clc; clear all;

Origin = ['C:\Users\mwinters\Documents\CPH Y1/BPA Data'];

O=dir(Origin);

Data = readtable('Sig\_SAD3.csv');

%Define variables

d=table2cell(Data(:,4));

bs1d=d(1:3:end);

bs2d=d(2:3:end);

bs3d=d(3:3:end);

D=string(table2cell(table(bs1d,bs2d,bs3d)));

newD=categorical(join(D));

%summary(newD)

gd=table2cell(Data(:,5));

bs1gd=gd(1:3:end);

bs2gd=gd(2:3:end);

bs3gd=gd(3:3:end);

GD=string(table2cell(table(bs1gd,bs2gd,bs3gd)));

newGD=categorical(join(GD));

%summary(newGD)

ggd=table2cell(Data(:,6));

bs1ggd=ggd(1:3:end);

bs2ggd=ggd(2:3:end);

bs3ggd=ggd(3:3:end);

GGD=string(table2cell(table(bs1ggd,bs2ggd,bs3ggd)));

newGGD=categorical(join(GGD));

%summary(newGGD)

%% ONLY FIRST BUD SITES

D1=string(table2cell(table(bs1d)));

GD1=string(table2cell(table(bs1gd)));

GGD1=string(table2cell(table(bs1ggd)));

BS1=categorical([D1;GD1;GGD1]);

summary(BS1)

%% Only First 2 bud sites

D12=string(table2cell(table(bs1d,bs2d)));

newD12=categorical(join(D12));

GD12=string(table2cell(table(bs1gd,bs2gd)));

newGD12=categorical(join(GD12));

GGD12=string(table2cell(table(bs1ggd,bs2ggd)));

newGGD12=categorical(join(GGD12));

BS1\_2=[newD12;newGD12;newGGD12];

summary(BS1\_2)

%% aLL bud sites

all=[newD;newGD;newGGD];

summary(all)