S. Dundon Protocols

Schizosaccharomyces pombe Growth and Sample Preparation for Imaging
Updated 3/28/18 by S. Dundon

Brief protocol

To freeze cells:

- ☐ Grow a patch of cells for two days at 25°C that covers 1/3-1/2 of a YE5S plate.
- ☐ Scrape cells off plate into cryo-tube with 1 mL YE5S + 25% glycerol, resuspend by vortexing.

To wake up cells:

- ☐ Using a sterile wooden applicator, scrape a small amount of frozen cells from the cryo-tube.
- ☐ Spread frozen cells in a small patch (~1 cm^2) on a YE5S plate.
- ☐ Grow at 25°C for 2 days.
- ☐ Seal plate with Parafilm to limit drying and store at room temperature for up to 1 week.

To grow for imaging:

Day 1:

- ☐ Towards the end of the day, using a sterile wooden applicator, collect a small amount of cells from a YE5S plate.
- ☐ Suspend cells into 5-10 mL YE5S in a 50 mL baffled flask.
- ☐ Grow cells in shaker at 150-200 rpm at 25°C.

Day 2:

- ☐ First thing in the morning, measure OD_{595} of culture.
- ☐ Dilute cells to OD_{595} ~0.1 in fresh YE5S.
- ☐ Return culture to shaking at 25°C for the remainder of the day.

- ☐ Before leaving at the end of the day, measure OD_{595} of culture.
- ☐ Dilute cells to OD_{595} ~0.05 in EMM5S synthetic imaging medium..
- ☐ Return culture to shaking at 25°C overnight.

Day 3:

- ☐ First thing in the morning, measure OD_{595} of culture.
  - Blank with EMM5S.
- ☐ Dilute culture throughout the day as necessary to maintain $0.3 < OD_{595} < 0.6$ for imaging.

- ☐ Transfer 1 mL of culture to sterile Eppendorf tube.
- ☐ Pellet cells at 5000 rpm (~2300 x g) for 30 seconds. Discard supernatant.
- ☐ Wash cells in 1 mL fresh EMM5S. Pellet at 5000 rpm for 30 seconds and discard supernatant.
- ☐ Re-suspend pellet in 10-20 µL EMM5S.
- ☐ Apply 1.5 µL cells to EMM5S agarose or gelatin pad.
- ☐ Apply 24 x 50 mm coverslip over top of the cells and agarose or gelatin pad.
- ☐ Seal edges of coverslip with VALAP.

To pour EMM5S pad for imaging:

Agarose:

- ☐ Make the agarose pads soon before imaging.
- ☐ Prepare molten solution of EMM5S + 2% agarose (w/v).

- ☐ Prepare glass slides with appropriate spacers to ensure pads of even thickness.
- ☐ Apply 70 µL molten EMM5S + 2% agarose to center of slide.
- ☐ Keep pads humid and discard at the end of the day.

Gelatin:

- ☐ Make gelatin pads the night before imaging or at least several hours ahead.
- ☐ Make a 25% gelatin solution in EMM5S in a 2 mL Eppendorf tube. Vortex immediately and place in a heat block at 65°C.
- ☐ Allow bubbles in solution to rise to the top for several minutes.
- ☐ Prepare glass slides with appropriate spacers to ensure pads of even thickness.
- ☐ Apply 70 µL molten EMM5S + 25% gelatin to center of slide.
- ☐ Apply a second slide to compress molten agarose between the two pieces of glass.
- ☐ Keep pads humid.

Detailed protocol

General notes:

- Use good sterile technique whenever working with cells and/or open containers of media. Turn on the flame, ethanol the benchtop around the flame, and work in this area only. Flame the tops of flasks and media bottles before and after opening. Do not leave tip boxes open that will be used for media.
- When imaging, **all** parameters must be recorded in your lab notebook. For example: microscope used, objective, z-spacing, number of slices, time intervals, number of XY coordinates, laser power(s), filter settings, exposure time(s).

To freeze cells:

- ☐ Grow a patch of cells for two days at 25°C that covers 1/3-1/2 of a YE5S plate.
- ☐ Scrape cells off plate into cryo-tube (Nalgene 5000-0020) with 1 mL YE5S + 25% glycerol, resuspend by vortexing.
    - Label tube with Strain ID, genotype, date, and your initials
- ☐ Store at -80°C.
    - Add the information for any new strains to the Pollard Lab Database Google sheet, which can be found at https://goo.gl/ahC1iz

To wake up cells:

- ☐ Using a sterile wooden applicator (Puritan REF 807), scrape a small amount of frozen cells from the cryo-tube.
- ☐ Spread frozen cells in a small patch (~1 cm^2) on a YE5S plate.
- ☐ Grow at 25°C for 2 days.
- ☐ Seal plate with Parafilm to limit drying and store at room temperature for up to 1 week.

To grow for imaging:

Day 1:

- ☐ Towards the end of the day, using a sterile wooden applicator (Puritan REF 807), collect a small amount of cells from a YE5S plate (<1 week old, stored at room temp).
- ☐ Suspend cells into 5-10 mL YE5S in a 50 mL baffled flask.
    - Do not exceed 20% of the flask volume with media.
    - Baffles increase aeration, use baffled flask if at all possible.
- ☐ Grow cells in shaker at 150-200 rpm (depending on the shaker) at 25°C.
    - Be sure to record which shaker was used, as the rpms vary between them at the same value.

Day 2:

- ☐ First thing in the morning, measure OD_{595} of culture.
  - Use a 1:10 dilution of culture in YE5S to measure OD_{595}. This reduces cells lost due to measurement and if the culture has exceeded 1.0 an accurate measurement can still be obtained.
  - Make sure cells are well mixed before taking a sample for dilution and before reading the OD_{595}. Yeast cells will settle by gravity.
  - Blank with YE5S from the same bottle that was used to grow the cells.
  - When pipetting YE5S for blank and dilutions, it is easier to keep a 100 mL bottle sterile than a 500 mL, as it is more possible to prevent the end of the pipette from entering the neck.
  - Record OD_{595} in lab notebook.
  - OD_{595} of culture should never exceed 1.0, or cells enter stationary phase. This will affect their metabolism rate and later division cycles, and increase autofluorescence.
- ☐ Dilute cells to OD_{595} ~0.1 in fresh YE5S.
  - Use calculation $\frac{Volume_{new} * OD_{new}}{OD_{old}} = Volume_{old}$, make $Volume_{old}$ up to $Volume_{new}$ with YE5S.
  - Make $Volume_{new}$ 1 mL larger than desired final volume, use this extra mL of diluted culture to measure the actual new OD_{595} after dilution.
  - Record calculations and new OD_{595} in lab notebook.
  - This OD_{595} works well for me when growing cells from 8:00 am to 6:00 pm before diluting again.
- ☐ Return culture to shaking at 25°C for the remainder of the day.

- ☐ Before leaving at the end of the day, measure OD_{595} of culture.
- ☐ Dilute cells to OD_{595} ~0.05 in EMM5S synthetic imaging medium..
  - Old EMM5S turns a very subtle shade of yellow that interferes with imaging by increasing background. Always store EMM5S in the dark to increase its longevity and check the bottle before using for dilution or making agarose/gelatin pads.
  - Some strains grow faster than WT (e.g. *wee1Δ*, or when growing at a higher temperature), so a lower OD_{595} may be desirable prior to growing these strains overnight.
  - Spin down $Volume_{old}$ in YE5S in microcentrifuge at ~2300 x g for 1 min. Discard supernatant and resuspend in complete $Volume_{new}$ EMM5S.
  - Blank with EMM5S from the same bottle used to dilute cells when measuring the new OD_{595}.
  - This OD_{595} works well for me when growing from 6:00 pm to 8:00 am before diluting again.
- ☐ Return culture to shaking at 25°C overnight.

Day 3:

- ☐ First thing in the morning, measure OD_{595} of culture.
  - Blank with EMM5S.
- ☐ Dilute culture throughout the day as necessary to maintain $0.3 < OD_{595} < 0.6$ for imaging.

- ☐ Transfer 1 mL of culture to sterile Eppendorf tube.
- ☐ Spin at ~2300 x g for 1 minute to pellet cells. Discard supernatant.

- ☐ Wash cells in 1 mL fresh EMM5S. Pellet at ~2300 x g for 1 min and discard supernant.
- ☐ Resuspend pellet in 10-20 µL EMM5S, depending on pellet size.
- ☐ Apply 1.5 µL concentrated cells to premade EMM5S agarose or gelatin pad (see below).
    - Spot 1.5 µL of cells in 3-4 drops around different areas of the pad to ensure good distribution.
- ☐ Apply 24 x 50 mm coverslip over top of the cells and agarose or gelatin pad.
    - A larger coverslip seems to increase the sample life before bubbles form on the pad.
- ☐ Seal edges of coverslip with VALAP using a 5 ¾" Pasteur pipette.

To pour EMM5S pad for imaging:

Agarose (higher melting temperature than gelatin, use whenever imaging ≥30°C, sets quickly):

- ☐ Make the agarose pads as closely as possible to the time of imaging. This seems to reduce the amount of bubbles that arise in the pad and move cells around during imaging.
- ☐ Prepare molten solution of EMM5S + 2% agarose (w/v).
    - I typically make 10 mL at a time in a 50 mL Corning bottle and melt it in the microwave using short bursts so that it does not boil off excessive water and concentrate the agarose. This 10 mL can be stored for a week or melted again 5-10 times, whichever comes first. If it will be stored, keep in the dark.
- ☐ Prepare glass slides with appropriate spacers to ensure pads of even thickness.
    - I use two pieces of lab tape on either side of the slide as spacers.
- ☐ Under sterile conditions near a Bunsen burner:
- ☐ Apply 70 µL molten EMM5S + 2% agarose to center of slide.
    - Do not press the plunger **all** the way down, as this increases the chance of bubbles in the final pad.
- ☐ As quickly as possible, take another glass slide and briefly wave it through the flame to warm it. Apply over the molten agarose and drop slowly, at an angle, to press molten agarose between the two pieces of glass.
    - Avoid bubbles as much as possible.
- ☐ Keep pads humid by covering the pouring apparatus (two glass slides and tape spacers) along with several wet Kimwipes with Saran wrap. Discard at the end of the day.

Gelatin (Sticker than agarose, so cells move around less, but takes a long time to set and melts ≥30°C):

- ☐ Make gelatin pads the night before imaging or at least several hours ahead, as they take quite a while to set.
- ☐ Make a 25% gelatin solution in EMM5S in a 2 mL Eppendorf tube. Vortex immediately and place in a heat block at 65°C.
- ☐ Allow bubbles in solution to rise to the top for several minutes before pouring pads.
- ☐ Prepare glass slides with appropriate spacers to ensure pads of even thickness.
    - I use two pieces of lab tape on either side of the slide as spacers.
- ☐ Under sterile conditions near a Bunsen burner:
- ☐ Apply 70 µL molten EMM5S + 25% gelatin to center of slide.
    - Do not press the plunger **all** the way down, as this increases the chance of bubbles in the final pad.
- ☐ As quickly as possible, take another glass slide and briefly wave it through the flame to warm it. Apply over the molten agarose and drop slowly, at an angle, to press molten agarose between the two pieces of glass.
    - Avoid bubbles as much as possible.

- ☐ Keeping the entire apparatus (two glass slides and tape spacers) together, store sealed in a small, light-shielded box with wet Kimwipes to maintain humidity.
    - Be sure to check for any contamination before using slides that have been stored.