



## Analysis of Cell Cycle Progression in the Budding Yeast *S. cerevisiae*

Deniz Pirincci Ercan and Frank Uhlmann

### Abstract

The cell cycle is an ordered series of events by which cells grow and divide to give rise to two daughter cells. In eukaryotes, cyclin–cyclin-dependent kinase (cyclin–Cdk) complexes act as master regulators of the cell division cycle by phosphorylating numerous substrates. Their activity and expression profiles are regulated in time. The budding yeast *S. cerevisiae* was one of the pioneering model organisms to study the cell cycle. Its genetic amenability continues to make it a favorite model to decipher the principles of how changes in cyclin–Cdk activity translate into the intricate sequence of substrate phosphorylation events that govern the cell cycle. In this chapter, we introduce robust and straightforward methods to analyze cell cycle progression in *S. cerevisiae*. These techniques can be utilized to describe cell cycle events and to address the effects of perturbations on accurate and timely cell cycle progression.

**Key words** Cell cycle, Cell synchronization, Time course experiment, Flow cytometry, Budding index, Western blotting, Immunofluorescence microscopy, *S. cerevisiae*

---

## 1 Introduction

The eukaryotic cell cycle is a highly regulated process that coordinates the doubling of all cellular content, especially genome duplication during DNA replication, with chromosome segregation and cell division. The timing of events and their unidirectional progression are controlled by the master cell cycle regulator, the cyclin–Cdk complexes. Cdk is a serine/threonine protein kinase that is activated by forming a complex with non-enzymatic cell cycle regulatory subunits, called cyclins. The cyclical expression of cell cycle stage-specific cyclins results in oscillations of cyclin–Cdk activity. This allows the step by step phosphorylation of many cyclin–Cdk substrates throughout the cell cycle [1].

The overall activity of cyclin–Cdk complexes increases from the time when cells are born in the G1 phase of the cell cycle until

metaphase, when cyclin-Cdk activity reaches its peak. Then, a ubiquitin ligase complex, the APC/C (anaphase promoting complex/cyclosome) is activated that ubiquitinates cyclins and thereby targets them for degradation. This causes cyclin-Cdk activity to fall, facilitating the dephosphorylation of cyclin-Cdk substrates by counteracting phosphatases and leading to completion of cell division and return of the cell cycle to G1. APC/C activation also results in degradation of securin, which liberates the protease separate to cleave the cohesive links between sister chromatids to trigger chromosome segregation.

The abovementioned events sketch out an overview of cell cycle control, however many crucial questions remain open. How are the phosphorylation and dephosphorylation timings of each cyclin-Cdk substrate controlled to coordinate the multitude of molecular events that are required for successful cell growth and division, and for maintaining the integrity of the genome? Here we report a series of techniques which allow the analysis of cell cycle progression in the unicellular model eukaryote *S. cerevisiae*. Human cells contain a similar set of G1, S phase, and mitotic cyclins to those found in this yeast. Studying the role of genes and pathways that are part of the cell cycle control network is of great importance to understand how cell cycle regulation goes wrong in human disease, notably in cancer.

In this chapter, we describe detailed protocols for cell synchronization and time course experiments for the analysis of the budding yeast cell cycle. We begin by describing cell cycle synchronization in a G1-like state using mating pheromone, from which cells can be released to synchronously progress through S phase and mitosis. We describe the use of flow cytometry to analyze DNA content. Together with the budding index, this is a useful marker to follow DNA replication and cell division. This is followed by a protocol for reliable protein extraction for Western blotting, used to visualize both protein abundance changes and electrophoretic mobility shifts that often go hand in hand with cyclin-Cdk phosphorylation. We then turn to the cytological observation of nuclei and the microtubule cytoskeleton, which yields additional information on the progression through mitosis.

---

## 2 Materials

### 2.1 Media and Chemicals

1. Yeast peptone (YP) media: weigh 20 g peptone, 10 g yeast extract and 0.05 g Adenine-HCl and make up to 1 L with water (water is Milli-Q ultrapure water unless otherwise stated). Autoclave before use.

2. Yeast peptone dextrose (YPD) media: YP is prepared as described above, dextrose is added at a final concentration 2% (w/v) from 20% (w/v) dextrose stock dissolved in water and sterilized by filtration.
3.  $\alpha$ -factor, stock: 0.2 mg/ml in methanol, stored at  $-20^{\circ}\text{C}$ .
4.  $\alpha$ -factor, stock: 5 mg/ml in methanol, stored at  $-20^{\circ}\text{C}$ .
5. Nocodazole (Sigma-Aldrich), stock: 2 mg/ml in DMSO, stored at  $-20^{\circ}\text{C}$ .
6. Flow cytometry buffer: 200 mM Tris-HCl pH 7.5, 210 mM NaCl, 78 mM  $\text{MgCl}_2$ .
7. Propidium iodide (Sigma-Aldrich), stock: 1 mg/ml in water, stored at  $4^{\circ}\text{C}$ .
8. 50 mM Tris-HCl pH 7.5.
9. RNase A (Sigma-Aldrich), stock: 10 mg/ml in 50% glycerol, 10 mM Tris-HCl pH 8.0, stored at  $-20^{\circ}\text{C}$ .
10. Fixation buffer: 100 mM  $\text{KH}_2\text{PO}_4$  pH 6.4, 0.5 mM  $\text{MgCl}_2$ , 3.7% formaldehyde (w/v, added freshly).
11. Wash buffer: 100 mM  $\text{KH}_2\text{PO}_4$  pH 7.4, 0.5 mM  $\text{MgCl}_2$ , 1.2 M Sorbitol.
12. 1 M Tris base, dissolved in water without pH adjustment.
13. Trichloroacetic acid (TCA), stock: 20%.
14. 70% Ethanol.
15. 37% Formaldehyde solution.
16. Methanol.
17. Acetone.
18. Poly-L-lysine (Sigma-Aldrich), stock: 0.1% (w/v) in water.
19. Lyticase (Sigma-Aldrich), stock: 20000 units/ml in 20% glycerol, 50 mM Tris-HCl pH 8.0, stored at  $-20^{\circ}\text{C}$ .
20.  $\beta$ -mercaptoethanol.
21. PBS: 2.7 mM KCl, 137 mM NaCl, 10 mM  $\text{Na}_2\text{HPO}_4$ , 1.8 mM  $\text{KH}_2\text{PO}_4$ .
22. Blocking buffer: 1% Bovine serum albumin (BSA) in PBS.
23. VECTASHIELD HardSet Antifade Mounting Medium containing 4',6-diamidino-2-phenylindole (DAPI).
24. Dithiothreitol (DTT), stock: 1 M in water stored at  $-20^{\circ}\text{C}$ .
25. SDS-PAGE loading buffer: 100 mM Tris-HCl pH 6.8, 4% SDS (w/v), 0.2% bromophenol blue (w/v), 20% glycerol (v/v), 200 mM DTT (added freshly).
26. Protein assay dye reagent concentrate (Bradford solution).

**2.2 Equipment**

1. Spectrophotometer.
2. Light microscope with 40× phase contrast objective and 10× eyepieces.
3. Flow cytometer.
4. Sonicator, MSE Soniprep 150.
5. Vortex.
6. Centrifuge for 50 ml centrifuge tubes.
7. Tabletop centrifuge for 1.5 ml microcentrifuge tubes and 2 ml screw-cap tubes.
8. 200 µl PCR tube.
9. Shaking water bath.
10. Vacuum filtration system.
11. FastPrep-24 cell breaker, MP Biomedicals.
12. Fluorescence microscope.

**2.3 Consumables**

1. 1.5 ml microcentrifuge tubes.
2. 2 ml screw-cap tubes.
3. 15 ml and 50 ml centrifuge tubes.
4. Mixed cellulose esters membrane filter, 1.2 µm pore size.
5. Flow cytometer tubes.
6. Acid-washed glass beads.
7. 23G needles.
8. Slides and coverslips.

---

**3 Methods**
**3.1 Cell Synchronization Methods in Budding Yeast**
**3.1.1 Pheromone-Induced Arrest of MATa Cells**

1. Culture cells overnight in YPD at 25 °C.
2. The following morning measure OD<sub>600</sub> (*see Note 1*).
3. When OD<sub>600</sub> reaches 0.2 (approximately 4 × 10<sup>5</sup> cells/ml) add α-factor to a final concentration 5 µg/ml and grow cells at 25 °C for 1 h (*see Note 2*).
4. Continue adding the same amount of α-factor to cultures every hour until 2.5 h after the initial α-factor addition.
5. Retrieve a small volume of the culture, briefly sonicate (*see Note 3*) and check the cells under a phase contrast microscope. More than 90% of cells are expected to have accumulated as unbudded cells with a mating projection tip called shmoo [2].

### 3.1.2 Pheromone-Induced Arrest of MAT $\alpha$ Cells

1. Follow **steps 1–5** in Subheading **3.1.1**. but use 0.02  $\mu\text{g}/\text{ml}$   $\alpha$ -factor instead of 5  $\mu\text{g}/\text{ml}$   $\alpha$ -factor (*see Note 4*).

### 3.1.3 Mitotic Arrest by Nocodazole Treatment

1. Arrest cells in G1 by following the steps in Subheading **3.1.1** or **3.1.2**.
2. Filter the culture and wash cells with 5 volumes of YP.
3. Resuspend the cells in a new flask containing the required amount of prewarmed YPD with nocodazole at a final concentration of 8  $\mu\text{g}/\text{ml}$ .
4. Incubate at 25 °C for 90 min to 2 h.
5. Observe the cells under microscope after incubation. More than 90% cells are expected to have accumulated as large budded cells and arrested in mitosis.

## 3.2 Analysis of Cell Cycle Progression in Time Course Experiments

### 3.2.1 Sample Collection

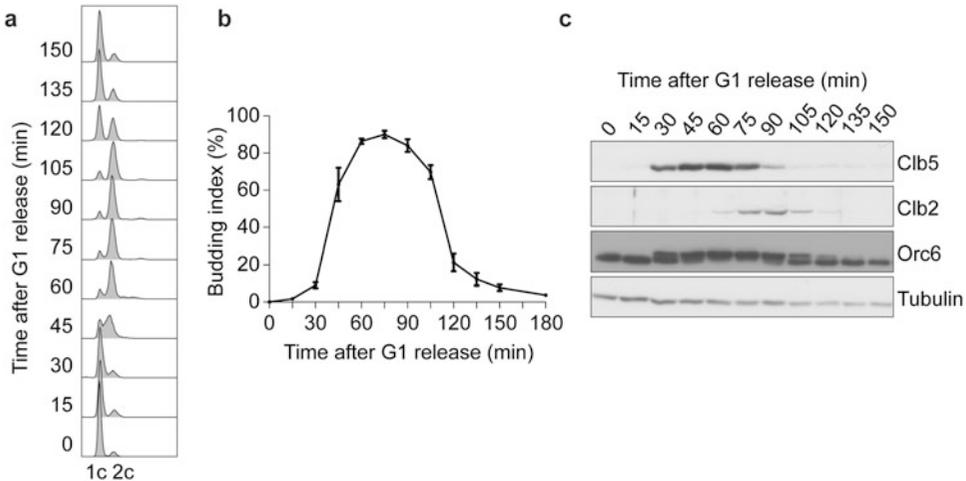
1. Grow and arrest cells using one of the methods described in Subheading **3.1**.  
Meanwhile, prepare the following:
  - Two 1.5 ml microcentrifuge tubes, for flow cytometry and fluorescence microscopy samples, one 15 ml centrifuge tube and one 2 ml screw-cap tube, for protein extraction, per time point (*see Note 5*).
  - 70% ethanol and 20% TCA stocks, kept on ice.
  - A new flask containing an equal culture volume of YPD placed in a shaking water bath that is set at 25 °C.
2. Collect 17 ml culture from the arrested culture using a 25 ml pipette and partition 1 ml each into the two microcentrifuge tubes and the remainder into a 15 ml centrifuge tube.
3. Spin down cells in microcentrifuge tubes at 6000 rpm ( $3430 \times g$ ), 1 min and the 15 ml centrifuge tube at 3500 rpm ( $14,130 \times g$ ) for 3 min at 4 °C.
4. Remove supernatant from all tubes by aspiration.
5. Resuspend the two small cell aliquots, to be analyzed by flow cytometry and fluorescence microscopy, in 1 ml 70% ethanol and 1 ml fixation buffer containing 3.7% formaldehyde, respectively.
6. Resuspend the large aliquot, to be used for protein extraction, in 1 ml 20% TCA and transfer to the 2 ml screw-cap tube.
7. Filter the culture and wash cells with 5 volumes of YP (*see Notes 6 and 7*).
8. Transfer the filter with the cells into the flask with fresh medium (containing nocodazole in case of a mitotic arrest), prepared prior to release (*see step 1*), and start timer.

9. Collect and process samples at regular intervals (e.g., every 15 min) following **steps 2–6** until the end of experiment.
10. Upon bud formation (approximately 60 min following the release)  $\alpha$ - or **a**-factor should be readded to the culture so that cells arrest again following complete passage through one cell cycle (this step is not required in mitotic arrest experiments using nocodazole).
11. End the experiment when all cells have returned to G1, or once all cells have reached mitosis in case of an experiment using nocodazole.
12. Sample processing at the end of the time course experiment:
  - Store flow cytometry samples in 70% ethanol for at least 2 h at room temperature or overnight at 4 °C.
  - Incubate fluorescence microscopy samples in fixation buffer for 2 h at 30 °C or overnight at 4 °C for fixation.
  - Leave Western blotting samples on ice in 20% TCA for at least 30 min (*see Note 8*).

### 3.2.2 Flow Cytometry: Analysis of DNA Content

Continue with the samples stored in 70% ethanol.

1. Pellet cells at 6000 rpm ( $3430 \times g$ ), 2 min and remove supernatant.
2. Resuspend samples in 1 ml 50 mM Tris–HCl pH 7.5, containing 0.1 mg/ml RNase A.
3. Incubate samples at 37 °C for at least 2 h or overnight.
4. Spin down again at 6000 rpm ( $3430 \times g$ ), 2 min and aspirate supernatant (*see Note 9*).
5. Resuspend pellets in 400  $\mu$ l flow cytometry buffer containing 50  $\mu$ g/ml propidium iodide.
6. Sonicate samples for 5 s to disrupt cell aggregates.
7. Transfer 50  $\mu$ l from cell suspension to flow cytometry tubes containing 400  $\mu$ l 50 mM Tris–HCl pH 7.5 and vortex briefly.
8. Use blue (488 nm) or yellow-green (561 nm) flow cytometer lasers to immediately read 10,000 events per sample prepared in **step 7**. A sample analysis that shows transition from 1c to 2c DNA content during DNA replication and regeneration of 1c daughter cells during cell division is shown in Fig. 1a.
9. Sonicated samples from **step 6** can be stored at 4 °C for further analysis, for example budding index counts.



**Fig. 1** Cell cycle analysis in budding yeast. **(a)** *MATa* cells were arrested by the addition of  $\alpha$ -factor with 1c DNA content and then released to progress through a synchronous cell cycle.  $\alpha$ -factor was re-added at 60 min (upon visible bud formation) to rearrest cells after completing one cell division cycle. Cell cycle progression was monitored by flow cytometry analysis of DNA content. **(b)** The fraction of visibly budded cells was counted in aliquots of the flow cytometry samples over time. One hundred cells were counted at each time point. The means of three independent experiments and the standard error are shown. **(c)** Protein extracts were prepared at the indicated times and analyzed by SDS-PAGE and Western blotting. Clb2, Clb5, and Orc6 were detected using specific antibodies (see **Note 13**). Tubulin served as a loading control and was detected using monoclonal antibody clone Tat1

### 3.2.3 Budding Index as a Marker of Cell Cycle Progression

1. Place 10  $\mu$ l of sonicated cell suspension (from **step 6** in Sub-heading 3.2.2) on a slide.
2. Cover with a coverslip and count the fraction of cells that display a visible bud, small or large, in a population of at least one hundred cells under the phase contrast microscope.
3. Plot the percentage of budded cells over time to obtain the budding index profile (Fig. 1b). Visible buds emerge at the time DNA replication.

### 3.2.4 Sample Preparation for Western Blotting and Detection of the Cell Cycle Marker Proteins

Continue with the Western blotting samples stored in 20% TCA.

1. Spin down samples at 13,000 rpm ( $16,060 \times g$ ), 1 min at 4 °C.
2. Wash cells with 1 ml 1 M Tris base (without pH adjustment).
3. Repeat **step 1** and resuspend the cells completely in 100  $\mu$ l SDS-PAGE loading buffer using a vortex mixer (see **Note 10**).
4. Measure 200  $\mu$ l of glass beads using a 200  $\mu$ l PCR tube and add to the screw-cap tubes.
5. Break cells in the Fast Prep 24 according to the manufacturer's instructions (see **Note 11**).
6. Boil samples at 95 °C for 5 min.

Meanwhile, prepare and label new microcentrifuge tubes for all the samples.

7. Release pressure in the screw-cap tubes and tighten the lids back.
8. Puncture the bottom of the screw-cap tubes twice with a 23 G needle and place them tightly onto new microcentrifuge tubes, prepared at **step 6**.
9. Loosen the screw caps slightly once again and place both the screw caps and the microcentrifuge tubes into a 50 ml centrifuge tube.
10. Spin at 1100 rpm ( $1400 \times g$ ) for 2 min (*see Note 12*).
11. Discard the screw-cap tubes, which should contain only glass beads, boil the microcentrifuge tubes containing the samples at  $95\text{ }^{\circ}\text{C}$  for 5 min.
12. Spin down samples at 13,000 rpm ( $16,060 \times g$ ), 3 min to sediment debris, then determine protein concentration using a Bradford protein assay.
13. Load 10  $\mu\text{g}$  protein per sample for analysis by typical SDS-PAGE methodology [3]. The remaining samples can be stored at  $-20\text{ }^{\circ}\text{C}$ .
14. Following protein transfer to a nitrocellulose membrane, probe against cell cycle regulated proteins, for example the S phase cyclin Clb5 and the mitotic cyclin Clb2, or proteins that show a cell cycle regulated mobility shift [4] (e.g., Orc6 [5]) (*see Note 13*). An example analysis is shown in Fig. 1c.

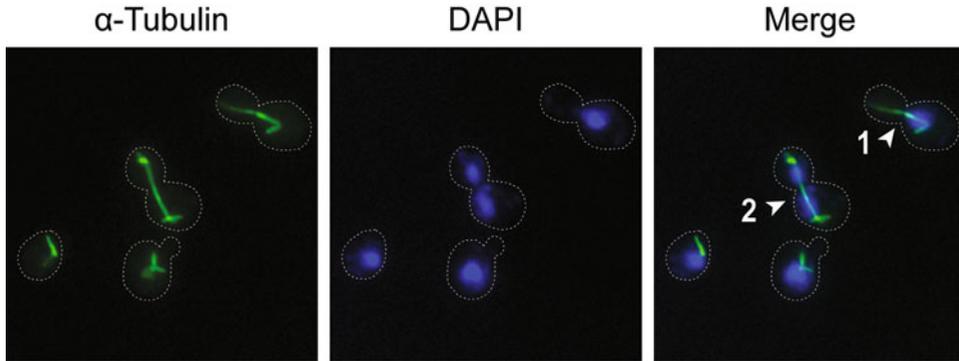
### 3.2.5 Fluorescence Microscopy to Determine Cell Cycle Stage by Spindle Staining

Continue with the samples stored in fixation buffer containing 3.7% formaldehyde.

1. Pellet cells at 6000 rpm ( $3430 \times g$ ), 2 min and remove supernatant.
2. Resuspend cells in 1 ml fixation buffer without formaldehyde.
3. Repeat **step 1** and resuspend pellets in 1 ml wash buffer.
4. Repeat **step 1** once again and resuspend cells in 200  $\mu\text{l}$  wash buffer containing 2  $\mu\text{l/ml}$   $\beta$ -mercaptoethanol and 2  $\mu\text{l/ml}$  lyticase solution to digest the cell wall (spheroplasting).
5. Incubate for 40 min at  $37\text{ }^{\circ}\text{C}$ .

Meanwhile, coat the wells of a 15-well slide with 10  $\mu\text{l}$  poly-L-lysine solution and leave it in a moist chamber for 30 min at room temperature. Next, wash the slide under flowing water briefly and dry by aspirating the remaining water drops. The slide should always be kept in a moist chamber from now on.

6. Check spheroplasts under the phase contrast light microscope. Spheroplasts appear dark, while intact cells remain bright (*see* **Notes 14** and **15**).
7. Centrifuge cells at 3500 rpm ( $1160 \times g$ ), 2 min and aspirate supernatant carefully.
8. Resuspend cells in 0.5 ml wash buffer and repeat **step 7**.
9. Add 200  $\mu$ l wash buffer to the pellets (*see* **Note 16**).
10. Carefully pipette up and down five times to mix and transfer 5 – 10  $\mu$ l cell suspension to a well on the 15-well slide. The remaining samples can be stored at  $-20^\circ\text{C}$ .
11. Let cells adhere to the slide for 30 min in a moist chamber at room temperature.
12. Aspirate the excess of cell suspension using a fine tip (*see* **Note 17**) and plunge the slide into  $-20^\circ\text{C}$  cold methanol for 3 min.
13. Transfer slide to  $-20^\circ\text{C}$  cold acetone for 10 s.
14. Let the acetone evaporate at room temperature quickly, then place the slide back into the moist chamber.
15. Add 10  $\mu$ l blocking buffer to the wells for at least 20 min.
16. Aspirate blocking buffer and add 8  $\mu$ l of primary antibody diluted in blocking buffer onto the wells (*see* **Note 18**).
17. Incubate for 1 h at room temperature.
18. Wash cells three times with 10  $\mu$ l blocking buffer.
19. Add 8  $\mu$ l of secondary antibody diluted in blocking buffer into the wells (*see* **Note 18**).
20. Repeat **step 17** in the dark, as well as **step 18**.
21. Aspirate as much as possible of the blocking buffer after the final wash and mount slides using 0.5  $\mu$ l mounting media containing DAPI.
22. Cover the slide with a coverslip and seal the edges with a nail polish.
23. Slides can be imaged immediately, or can be stored in the dark at  $-20^\circ\text{C}$  and imaged later.
24. Count the appearance of short ( $\leq 2 \mu\text{m}$ , metaphase) spindles, as well as long ( $> 2 \mu\text{m}$ , anaphase) spindles in a population of at least a hundred cells under a fluorescence microscope.
25. Plot the fraction of cells with metaphase and anaphase spindles over time to mark the onset of mitosis when short spindles form, anaphase when spindles elongate and mitotic exit when spindles disassemble (*see* **Fig. 2** for examples of cells with metaphase and anaphase spindles).



**Fig. 2** Immunofluorescence analysis of the microtubule cytoskeleton to identify mitotic stages. Asynchronously proliferating cells were fixed and stained using an anti- $\alpha$ -tubulin antibody. DNA was counterstained with DAPI. The cell outlines were marked with a dashed line. Examples of a metaphase cell with a short spindle (arrowhead 1) and an anaphase cell with an elongating spindle and separating nuclei (arrowhead 2) are highlighted (Adapted with permission from Borges et al. (2010) *Mol. Cell* 39:677–688 [8])

## 4 Notes

1. In the morning, if the culture is overgrown but cells are still budding, back dilute to  $OD_{600} = 0.1$  and allow one generation doubling to reach  $OD_{600} = 0.2$  before starting the experiment.
2. A lower concentration of  $\alpha$ -factor might be sufficient to achieve cell cycle arrest, depending on its purity. On the other hand, if most cells are still budded after 2.5 h in medium containing  $\alpha$ -factor, the concentration may need to be increased.
3. All the sonication steps in this chapter are performed using an exponential microprobe and the amplitude meter is set at 6 microns.
4. *MAT $\alpha$*  cells treated with  $\alpha$ -factor show less prominent shmoo formation compared to  $\alpha$ -factor treated *MAT $\alpha$*  cells [2].
5. Keep microcentrifuge tubes and screw-cap tubes on ice prior to release and during the experiment.
6. If the experiment requires filtration of more than one culture, membranes should be folded and kept on a sterile foil until filtration is completed for all cultures. Cells can then be simultaneously resuspended in new flasks.
7. If more than two cultures are analyzed, the use of more than one filtration system is recommended.
8. Western blotting samples can be stored as pellets at  $-80\text{ }^{\circ}\text{C}$  prior to protein extraction by simply spinning down the samples at 13,000 rpm ( $16,060 \times g$ ), 1 min and removing 20% TCA.

9. After RNase treatment, pellets become slightly translucent and adhere to the microcentrifuge wall less tightly. The supernatant should be carefully aspirated in order to prevent loss of the pellets.
10. If SDS-PAGE loading buffer turns yellow after mixing, this indicates an acidic pH due to the presence of residual TCA. In this case, samples can be neutralized by adding 1–2 drops of 1 M Tris base.
11. Running the manufacturer's *S. cerevisiae* program (6 m/s for 40 s) once is enough to break the cells.
12. It is possible to fit two screw-cap microcentrifuge tube combinations into one 50 ml centrifuge tube [6]. These 50 ml centrifuge tubes can be stored and reused.
13. Clb5 and Clb2 can be detected using specific primary antibodies (anti-Clb5, Santa Cruz sc20170, anti-Clb2 Santa Cruz sc9071, Fig. 1c) or by fusing small epitope tags (e.g., HA or Pk) to their C terminus [7]. Orc6 can be detected with monoclonal antibody clone SB49.
14. If a majority of the cells appear dark, continue with the next step. Otherwise, incubate cells longer at 37 °C.
15. Spheroplasts are fragile; therefore, subsequent centrifuge steps will be performed at a lower speed.
16. At this step, the samples can be stored longer term at –20 °C.
17. Always aspirate from the same side of the wells to reduce the risk of scratch marks on the slide.
18. For spindle staining, we recommend using an anti- $\alpha$ -tubulin monoclonal primary antibody clone YOL1/34, Abcam ab6161, diluted 1:200 in blocking buffer. Similarly, a secondary antibody conjugated to a fluorescent dye should be diluted in blocking buffer according to manufacturer's instructions before use.

---

## Acknowledgments

We would like to thank Florine Chrétien and Hon Wing Liu for their help. This work was supported by The Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001198), the UK Medical Research Council (FC001198), and the Wellcome Trust (FC001198). D.P.E. was supported by a Boehringer Ingelheim Fonds Ph.D. Fellowship.

## References

1. Morgan D (2007) *The cell cycle: principles of control*. New Science Press, London
2. O'Reilly N, Charbin A, Lopez-Serra L, Uhlmann F (2012) Facile synthesis of budding yeast a-factor and its use to synchronize cells of a mating type. *Yeast* 29:233–240
3. Sambrook J, Fritsch EF, Maniatis T (1989) Section 18.47-18.54. In: *molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York, NY, p 1659
4. Sambrook J, Fritsch EF, Maniatis T (1989) Section 18.64-18.66. In: *molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York, NY, p 1659
5. Nguyen VQ, Co C, Li JJ (2001) Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature* 411:1068–1073
6. Kakui Y, Uhlmann F (2019) Efficient depletion of fission yeast condensin by combined transcriptional repression and auxin-induced degradation. *Methods Mol Biol* 2004:25–33
7. Knop M, Siegers K, Pereira G, Zachariae W, Winsor B, Nasmyth K, Schiebel E (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast* 15(10B):963–972
8. Borges V, Lehane C, Lopez-Serra L, Flynn H, Skehel M, Rolef Ben-Shahar T, Uhlmann F (2010) Hos1 deacetylates Smc3 to close the cohesin acetylation cycle. *Mol Cell* 39 (5):677–688. <https://doi.org/10.1016/j.molcel.2010.08.009>

**Open Access** This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

