Published in final edited form as:

Methods Mol Biol. 2016 ; 1369: 125–136. doi:10.1007/978-1-4939-3145-3_10.

Isolation of Cytokinetic Actomyosin Rings from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

Junqi Huang[#], Mithilesh Mishra[#], Saravanan Palani, Ting Gang Chew, and Mohan K. Balasubramanian

[#] These authors contributed equally to this work.

Abstract

Cytokinesis is the final stage of cell division, through which cellular constituents of mother cells are partitioned into two daughter cells resulting in the increase in cell number. In animal and fungal cells cytokinesis is mediated by an actomyosin contractile ring, which is attached to the overlying cell membrane. Contraction of this ring after chromosome segregation physically severs the mother cell into two daughters. Here we describe methods for the isolation and partial purification of the actomyosin ring from the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae*, which can serve as in vitro systems to facilitate biochemical and ultrastructural analysis of cytokinesis in these genetically tractable model systems.

Keywords

Yeast; *Schizosaccharomyces pombe*; *Saccharomyces cerevisiae*; Actomyosin ring; Isolation; ATP-dependent contraction

1 Introduction

In most eukaryotes from yeast to human, cytokinesis is carried out by the regulated assembly and subsequent contraction of an actomyosin-based contractile ring, which leads to constriction of the plasma membrane at the division site. This ring is made up of a conserved set of proteins including actin, formin, myosin II, IQGAP, and F-BAR proteins [1, 2]. While genetic and cell biological analysis in the last couple of decades in yeasts, nematodes, fruit flies, and human cells have led to the identification of many conserved proteins involved in cytokinesis, the mechanism of ring formation, contraction, and disassembly remain mysterious [2–6]. For ring contraction, uncovering its mechanisms will

²*cdc25-22* background and the 36 °C shift up process are not essential for ring isolation.

¹⁶It is very important to choose proper rings for ATP perfusion movies (refer to Subheading 3.4, **step 5**) because it is unavoidable that structures of some isolated rings are altered after permeabilization.

¹⁷To search for proper rings under fluorescent microscopy, it is better to search by eyepiece (only if the fluorescent signal would not be bleached easily such as Rlc1p-3GFP). This is because the field of view through eyepiece is usually bigger than that through the camera.

¹⁸After washing away the unbound ghosts with reactivation buffer (Subheading 3.4, **step 4**), the number of remaining ghosts reduces. Depending on the purpose, it may be good to concentrate the ghosts from Subheading 3.2, **step 10** or try other ways to coat the coverslips with poly-L-lysine more efficiently.

require a definitive list of factors sufficient for ring contraction, as well as experimental tools enabling more detailed biochemical and ultrastructural examination of the process. Achieving these goals will demand a system of ring contraction in vitro.

Actomyosin contractile rings are highly complex assemblies of more than 130 different proteins [2]. As an important first step toward understanding this complex macromolecular structure, intact actomyosin rings were recently isolated/partially isolated from both budding yeast and fission yeast, respectively [7, 8]. Although it is still not clear whether rings isolated from budding yeast are fully contractile, cytokinetic rings isolated from fission yeast are functional and upon addition of ATP these rings undergo complete closure in vitro [7, 8]. Fission yeast ring contraction in vitro is myosin II-dependent but surprisingly is independent of actin turnover. Though functional actomyosin rings have been isolated from newt eggs and sea urchin embryos [9, 10], the genetic intractability of these organism has limited the progress in furthering these studies. On the other hand, yeasts are easy to manipulate genetically and have a short generation time. This allows for isolation of sufficient amounts of cellular material for cytokinesis studies.

The isolated contractile ring systems allow a combination of biochemical and genetic analysis that complement cell biological studies of the cytokinetic ring. Moreover, isolated rings would also be amenable to ultrastructural analysis. In summary, these approaches to the isolation of the cytokinetic apparatus and its analysis hold great potential in increasing our understanding of the important process of cytokinesis. The subsequent sections provide detailed step-by-step protocol for isolation and analysis of yeast actomyosin rings.

2 Materials

2.1 *S. pombe* Actomyosin Ring Isolation

1. Edinburgh minimal medium + Supplements (EMM4S) + 0.5 % Glucose (filter-sterilized, room temperature storage): 3 g/l potassium hydrogen phthalate, 2.2 g/l sodium phosphate dibasic, 5 g/l ammonium chloride, 5 g/l D-(+)-glucose (anhydrous), 1× salt solution, 1× minerals solution, 1× vitamins solution, 82.5 mg/l L-histidine, 82.5 mg/l L-LEUCINE, 112.5 mg/l adenine hemisulfate, 82.5 mg/l uracil.
2. 50× salt solution stock (filter-sterilized, 4 °C storage): 0.26 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 4.99 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.67 M KCl, 14.1 mM Na_2SO_4 .
3. 50× minerals solution stock (filter-sterilized, 4 °C storage): 80.9 mM boric acid, 23.7 mM MnSO_4 , 13.9 mM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 7.40 mM $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$, 2.47 mM molybdic acid, 6.02 mM KI, 1.60 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 47.6 mM citric acid.
4. 50× vitamins solution stock (filter-sterilized, 4 °C storage): 4.20 mM pantothenic acid, 81.2 mM nicotinic acid, 55.5 mM inositol, 40.8 μM biotin.
5. E-Buffer (filter-sterilized, room temperature storage): 50 mM sodium citrate-dihydrate, 100 mM sodium phosphate.

6. E-Buffer with 1.2 M sorbitol (filter-sterilized, room temperature storage): 50 mM sodium citrate-dihydrate, 100 mM sodium phosphate, 1.2 M D-sorbitol.
7. E-Buffer with 0.6 M sorbitol (filter-sterilized, room temperature storage): 50 mM sodium citrate-dihydrate, 100 mM sodium phosphate, 0.6 M D-sorbitol.
8. EMM4S+ 0.8 M Sorbitol (filter-sterilized, room temperature storage): 3 g/l potassium hydrogen phthalate, 2.2 g/l sodium phosphate dibasic, 5 g/l ammonium chloride, 20 g/l D-(+)-glucose (anhydrous), 1× salt solution, 1× minerals solution, 1× vitamins solution, 82.5 mg/l L-histidine, 82.5 mg/l L-LEUCINE, 112.5 mg/l adenine hemisulfate, 82.5 mg/l uracil, 0.8 M D-sorbitol.
9. Wash Buffer (pH ~7.0, no need to adjust pH, filter-sterilized, 4 °C storage): 0.8 M D-sorbitol, 2 mM EGTA, 5 mM MgCl₂, and 20 mM PIPES–NaOH (pH 7.0).
10. Isolation Buffer (pH ~7.0, no need to adjust pH, filter-sterilized, 4 °C storage): 0.16 M sucrose, 50 mM EGTA, 5 mM MgCl₂, 50 mM potassium acetate, 50 mM PIPES–NaOH (pH 7.0), 0.5 % NP-40 (Nonidet P40 substitute, from Fluka, added fresh every time before experiments), 10 µg /ml leupeptin hemisulfate (stock solution: 10 mg/ml), 10 µg /ml aprotinin from bovine lung (stock solution: 10 mg/ml), 400 µg/ml benzamidine hydrochloride hydrate (stock solution: 80 mg/ml), 1 mM phenylmethylsulfonyl fluoride (PMSF, stock solution: 0.1 M), and 1 mM dithiothreitol (DTT, stock solution: 1 M).
11. Reactivation Buffer (pH adjusted to ~7.5 with NaOH, filter-sterilized, 4 °C storage): 0.16 M sucrose, 5 mM MgCl₂, 50 mM potassium acetate, 20 mM MOPS–NaOH (pH 7.0), 10 µg /ml leupeptin hemisulfate (stock solution: 10 mg/ml), 10 µg /ml aprotinin from bovine lung (stock solution: 10 mg/ml), 400 µg/ml benzamidine hydrochloride hydrate (stock solution: 80 mg/ml), 1 mM phenylmethylsulfonyl fluoride (PMSF, stock solution: 0.1 M), and 1 mM dithiothreitol (DTT, stock solution: 1 M). For protease inhibitors (leupeptin hemisulfate, aprotinin from bovine lung, benzamidine hydrochloride hydrate, PMSF) and DTT, aliquot the stock solutions and store at –20 °C.
12. ATP, stock solution: 0.1 M (aliquot and storage at –20 °C).
13. Lysing enzymes from *Tricoderma harzianum* (Sigma, working concentration: 0.0125 g/5 ml).
14. Zymolyase (G-Biosciences, 1.5 units/µl).
15. Glass homogenizer (Corning Potter-Elvehjem Tissue Grinders, Thomas Scientific).

2.2 *S. cerevisiae* Ring Isolation

1. YPDA/YP-Raffinose media.
2. S-buffer: 100 mM potassium phosphate (pH 7.0), 1.33 M D-sorbitol, 40 mM β -mercaptoethanol.
3. Ring isolation buffer: 50 mM HEPES-KOH (pH 7.5), 10 mM MgOAc (pH 7.5), 60 mM potassium acetate (pH 7.5), 1 mM EDTA, 10 % glycerol, 1 mM DTT.
4. Lyticase (Sigma-Aldrich).
5. Protease Inhibitor (Roche, EDTA-free, complete cocktail).
6. 1 mM PMSF.
7. 0.5 % NP-40.
8. Strains used for the ring isolation (kindly provided by Dr. David G. Drubin, University of California, Berkeley): *Mat a mob1-77 MYO1-GFP::KANMX pep4 :: TRP1 MLC2-HPM (his9-precision2-myc9)::HIS3 ura3-52 trp1 1 his3 200 leu2*

3 Methods

3.1 *S. pombe* Protoplast Formation (Fig. 1)

1. Inoculate fresh (from -80°C freezer, 1 day on 24°C YES plate) *cdc25-22 S. pombe* cells (with fluorescent actomyosin ring marker such as Rlc1p-3GFP) into 20 ml liquid EMM4S + 0.5 % Glucose, and grow overnight at 24°C and shaking at 200 rpm (see Notes 1–3).
2. Next day morning, measure O.D.₅₉₅ (should be around 0.3; 3×10^6 cells/ml), shift up cells to 36°C for 3.5 h, while still shaking at 200 rpm (see Notes 4 and 5).
3. Spin down cells in a 50 ml Falcon tube at $450 \times g$ for 2 min at room temperature.
4. Wash cells once with E-buffer. Spin at $450 \times g$ for 2 min at room temperature.
5. Resuspend cells in E-buffer supplemented with 1.2 M sorbitol up to the volume of 5 ml in the 50 ml Falcon tube.

¹ It is very important that *S. pombe* cells are fresh. It may be helpful to dilute fresh cells 2 days before the isolation experiment and to sub-culture them on the following days.

³Low glucose medium (EMM4S + 0.5 % Glucose) may be good for protoplast formation.

⁴It is better to culture cells in a programmable shaker, as the whole fission yeast ring isolation process (including shift up) takes around 12–13 h. Inoculate a few flasks (each flask contains 20 ml EMM4S + 0.5 % Glucose medium, choose the best flask later for ring isolation) of fresh fission yeast cells at 9 a.m. 1 day before the experiments (24°C , 200 rpm). At 6 p.m., dilute the culture to O.D.₆₀₀ ~ 0.05 (6×10^5 cells/ml), keep the volume around 20 ml. Culture cells in a programmable shaker (setting: 200 rpm, 24°C for 10.5 h; then 200 rpm, 36°C for 3.5 h). The next day morning at 8 a.m. check O.D.₆₀₀, continue the steps from Subheading 3.1, **step 3**.

⁵Do not shift cells up at 36°C for more than 4 h.

6. Weigh 0.0125 g lysing enzymes from *Tricoderma harzianum* (Sigma, working concentration: 0.0125 g/5 ml) (*see* Note 6).
7. Mix lysing enzymes evenly with the cell suspension from **step 5**. Lay the Falcon tube flat on the air shaker. Digest cell walls at 36 °C, while shaking at 80 rpm for 1 h 20 min.
8. Monitor protoplast formation by phase-contrast microscopy. Take 5 µl cells from **step 7**, gently spread on a coverslip (24 × 40 mm), and observe cells directly without another coverslip on top. After **step 7**, more than 80 % cylindrical cells should form a bud-like extrusion at the tip(s)/cell middle (*see* Note 7).
9. Add 10 µl of zymolyase (G-Biosciences, 1.5 units/µl) into the cell suspension from **step 7**. Cells are kept at 36 °C, while shaking at 80 rpm for another 40 min (*see* Note 8).
10. Spin at 450 × *g* for 2 min at room temperature.
11. Wash once with E-buffer supplemented with 0.6 M sorbitol. Spin at 450 × *g* for 2 min at room temperature.
12. Resuspend protoplasts in 10 ml EMM4S + 0.8 M Sorbitol, 80 rpm at 24 °C for 5–6 h. Check actomyosin ring formation on the coverslip (similar to **step 8** without another coverslip) under wide-field fluorescence microscopy. Isolate rings when more than 30 % of the protoplasts form a full actomyosin ring.

3.2 *S. pombe* Actomyosin Ring Isolation

1. Cool down the bench-top centrifuge to 4 °C for ring isolation.
2. Prepare 2 ml isolation buffer (with 0.5 % NP-40) and 6 ml reactivation buffer by adding protease inhibitors (leupeptin, aprotinin, benzamidine, PMSF) and DTT every time before experiments. To make ATP- reactivation buffer, mix 995 µl reactivation buffer from above with 5 µl ATP (stock concentration: 100 mM. Final concentration after dilution: 0.5 mM) (*see* Note 9).
3. Spin down (450 × *g*, 2 min, room temperature, in a 50 ml tube) protoplasts after Subheading 3.1, **step 12**.
4. Resuspend protoplasts with 1 ml wash buffer in the 50 ml tube. Operate on ice and be gentle from this step on.

⁶Lysing enzymes activity may differ from batch to batch; one can either adjust the amount of lysing enzymes used in the experiment or adjust the lysing enzymes digestion time.

⁷When observing protoplasts directly on the coverslip under bench-top phase-contrast microscopy, do not observe cells on the edge or observe for too long. Protoplasts may shrink because of water evaporation.

⁸Adding Zymolyase may help improving the isolated ring constriction in the later steps.

⁹For NP-40, first use double-distilled water to dilute the 100 % NP-40 into 20 %, then used at the final working concentration of 0.5 %.

5. Transfer protoplasts to a 1.5 ml eppendorf tube by pouring (no pipetting). Spin at $450 \times g$ and at 4°C for 2 min. Decant the supernatant.
6. Add 1.5 ml isolation buffer and mix thoroughly by gently inverting and tapping.
7. Cells from **step 6** are poured into a 5 ml glass homogenizer (Corning Potter-Elvehjem Tissue Grinders, Thomas Scientific) pre-chilled on ice. Leave on ice for 5 min. Homogenize the sample (no need to rotate the pestle) for six times and avoid bubble formation. Leave on ice for another 5 min. Cells after this step are called ghosts as most of them are permeabilized (*see* Note 10).
8. Spin at $450 \times g$ in a centrifuge cooled to 4°C for 2 min.
9. Wash twice with 1 ml reactivation buffer. Spin at $450 \times g$ in a centrifuge cooled to 4°C for 2 min.
10. Resuspend ghosts in 250–800 μl (volume depends on the specific experiment planned) reactivation buffer. Isolated rings are preserved inside the ghosts.
11. Mix ghosts with equal volume ($\sim 8 \mu\text{l}$ each) of ATP-activation buffer (from **step 2**) to check ring contractility on the coverslip (similar to Subheading 3.1, **step 8**). Most isolated rings should be able to finish contraction and become a dot/cluster after 6 min (*see* Note 11).

3.3 Poly-L-lysine-Coated Coverslips for *S. pombe* Ring Constriction Movies

1. Add 40 μl poly-L-lysine (Sigma) onto the centre of the coverslips ($24 \times 40 \text{ mm}$).
2. Cut parafilm ($\sim 15 \times 15 \text{ mm}$) and place it on top of poly-lysine from **step 1**. Press the parafilm to spread the poly-lysine evenly.
3. Dry the coverslips from **step 2** in the laminar flow cabinets for 2–3 h. Peel off the parafilm.
4. Cut and paste two double-sided tapes (NICHIBAN, NW-5, 5 mm width) onto a poly-L-lysine-coated coverslip from **step 3**. The double-sided tapes should be parallel to each other and the space between them should be similar to the width (5 mm) of the NICHIBAN NW-5 double-sided tape for better perfusion efficiency (*see* Note 12).
5. To make sure the double-sided tapes stick tight to the coverslip, use the front end of a pipette to scratch them. Use a pair of fine point forceps to

¹⁰Homogenizer in Subheading 3.2, **step 7** may not be necessary. Protoplasts can be permeabilized with isolation buffer for 10 min on ice. Invert tubes 2–3 times every 2 min.

¹¹It is very important to mix equal volume of ATP-activation buffer with ghosts to check ring contractility. This is especially important when working with rings isolated from mutants that may affect ring constriction.

¹²The quality of the double-sided tape can influence the perfusion efficiency. NICHIBAN, NW-5 (5 mm width) is one of the best.

tear off the non-sticky side of the double-sided tape before perfusion experiments (Fig. 2).

3.4 ATP Perfusion for *S. pombe* Ring Constriction Movies

1. Add 14 μ l ghosts (from Subheading 3.2, **step 10**, ghosts are kept on ice before experiments) onto a poly-L-lysine-coated coverslip from Subheading 3.3.
2. To make a chamber for perfusion, place a small coverslip (18 $\times$ 18 mm) on top of the ghosts and the double-sided tapes from **step 1**. Use the front end of a pipette to press the sides of the small coverslip to make sure it sticks tight on the double-sided tapes.
3. Make sure the chamber is stable on the microscope stage (important) (*see* Note 13).
4. Add 40 μ l reactivation buffer (without ATP, this step is to wash away the unbound ghosts) on one side of the chamber, exchange buffer by sucking from the other side of the chamber using filter paper (Whatman, cut into a rectangular shape) (*see* Note 14).
5. Search for stable ghosts possessing a fully formed, unbroken ring. Optimal rings have a diameter slightly smaller than that of the ghost and are bright, even, round, and parallel to the coverslip (*see* Notes 15–19).
6. Start time-lapse movie acquisition (15 s interval time). Pause the movie after the first two time points (the first two time points are to show that there is no contraction before adding ATP).
7. Perfuse the chamber with 40 μ l of 0.5 mM ATP-reactivation buffer (from Subheading 3.2, **step 2**) similar to **step 4**.
8. Restart movie acquisition. The whole isolated ring contraction process typically finishes within 20 time points. Ring contraction should be obvious from the third or the fourth time point.

3.5 *S. cerevisiae* Ring Isolation (Modified from [7]) (Fig. 3)

1. Culture *S. cerevisiae* cells [using mitotic exit temperature-sensitive (ts) alleles, e.g., *mob1-77*] with Myo1-GFP (type-II myosin) actomyosin ring marker in YPDA/YP-Raffinose media at permissive temperature (23 $^{\circ}$ C/30 $^{\circ}$ C) until O.D.₆₀₀ $\sim$ 0.4 ($\sim 5 \times 10^6$ cells/ml) then shift cells to 37 $^{\circ}$ C for 4 h to arrest cells at late anaphase with fully formed rings. As an alternative to using *mob1-77* allele, galactose is added to overexpress

¹³Scotch tapes can be used to stabilize the chamber on the microscopy stage.

¹⁴It may be better to choose soft filter papers for perfusion experiment as the hard ones can move the chamber during buffer exchange.

¹⁵It is necessary to make a few time-lapse movies to check ring quality before other experiments such as drug treatment. Some solvents may affect ring constriction.

¹⁹ATP perfusion occasionally does not work well as quality differs from chamber to chamber. It is suggested to acquire more movies for quantitative analysis.

proteins to arrest cells at specific cell cycle stage (e.g., overexpression of non-degradable version of mitotic cyclin, Clb2 to arrest the cells in late anaphase) (see Note 20).

2. For the purpose of simplifying the protocol, the methods described below are detailed for a 100 ml culture. Cells are pelleted at $2000 \times g$ for 20 min in a rotor pre-warmed to 37 °C (in case of using *ts* allele) or 30 °C (see Note 21).
3. Wash pellets twice with 10 ml sterile PBS and once with 10 ml PBS + PMSF. Freeze cells using liquid nitrogen or by placing the pellets at -80 °C (see Note 22).
4. To isolate the cytokinetic rings, frozen cells (from **step 3**) are rapidly brought to 30 or 37 °C (in case of using *ts* alleles) by adding 0.5 ml pre-warmed sorbitol buffer (S-buffer).
5. Pre-incubate cells in a 37 °C water bath for 5–10 min before the addition of 25 µg Lyticase (recombinant *O. xanthineolytica* β -1,3-glucanase, either expressed and purified from *E. coli* (homemade, please refer to Scott and Schekman [11]) or obtained commercially (Sigma-Aldrich)). Incubate cells at 37 °C for 30 min (see Note 23).
6. Cells are centrifuged at $1000 \times g$ for 3 min. The cell pellets are then washed twice with 0.5 ml sorbitol buffer and osmotically lysed using 100 µl of ice-cold ring isolation buffer supplemented with EDTA-free protease cocktail inhibitor (Roche) and 1 mM PMSF.
7. NP-40 is added to a final concentration of 0.5 % and cells are incubated on ice for approximately 10 min.
8. Lysate is cleared of debris/unbroken cells by centrifugation at $300 \times g$ for 5–10 min at 4 °C (see Note 24).
9. Clarified lysate is spotted on a poly-L-lysine-coated coverslip (refer to Subheading 3.3 and Fig. 2), incubated for 10 min at room temperature and then washed twice with ring isolation buffer to clear the unattached rings and debris.
10. Rings attached on the poly-L-lysine-coated coverslips are visualized by fluorescence microscopy.
11. Actomyosin ring components from the clarified lysate are confirmed by immunoblotting using respective antibodies.

²⁰*S. cerevisiae* cells can be grown in YPDA medium when no external selection is required. Appropriate selection medium (with/without supplements) can be used for growing strains containing plasmids or genes with inducible promoters.

²¹Depending on the purpose of the experiment, the culture volume can be scaled up from 0.05 to 4 l.

²²Freshly frozen cells (refer to Subheading 3.5, **step 3**) yield better ring preps in comparison to ring preps from a few months old stock. Care is to be taken to minimize the storage time at permissive temperature prior to freezing.

²³Commercial lyticase may be better than homemade lyticase for cell wall digestion.

²⁴Isolated *S. cerevisiae* rings are stable in solution for a few hours (~2–4 h) on ice. Further lysate clarification and subcellular fractionation can be performed as described [7].

Acknowledgments

We thank Dr. David Drubin for kindly providing the *S. cerevisiae* strains. This work was supported by research funds from the Wellcome Trust, Royal Society, and the University of Warwick. M.M. was supported by funds from the Wellcome Trust India Alliance and the Tata Institute of Fundamental Research, Mumbai, India.

References

1. Wu JQ, Pollard TD. Counting cytokinesis proteins globally and locally in fission yeast. *Science*. 2005; 310(5746):310–314. DOI: 10.1126/science.1113230 [PubMed: 16224022]
2. Pollard TD, Wu JQ. Understanding cytokinesis: lessons from fission yeast. *Nat Rev Mol Cell Biol*. 2010; 11(2):149–155. DOI: 10.1038/nrm2834 [PubMed: 20094054]
3. Glotzer M. The molecular requirements for cytokinesis. *Science*. 2005; 307(5716):1735–1739. DOI: 10.1126/science.1096896 [PubMed: 15774750]
4. Huang J, Huang Y, Yu H, Subramanian D, Padmanabhan A, Thadani R, Tao Y, Tang X, Wedlich-Soldner R, Balasubramanian MK. Nonmedially assembled F-actin cables incorporate into the actomyosin ring in fission yeast. *J Cell Biol*. 2012; 199(5):831–847. DOI: 10.1083/jcb.201209044 [PubMed: 23185032]
5. D'Avino PP. How to scaffold the contractile ring for a safe cytokinesis - lessons from Anillin-related proteins. *J Cell Sci*. 2009; 122(Pt 8):1071–1079. DOI: 10.1242/jcs.034785 [PubMed: 19339546]
6. van den Heuvel S. The *C. elegans* cell cycle: overview of molecules and mechanisms. *Methods Mol Biol*. 2005; 296:51–67. [PubMed: 15576926]
7. Young BA, Buser C, Drubin DG. Isolation and partial purification of the *Saccharomyces cerevisiae* cytokinetic apparatus. *Cytoskeleton*. 2010; 67(1):13–22. DOI: 10.1002/cm.20412 [PubMed: 19790107]
8. Mishra M, Kashiwazaki J, Takagi T, Srinivasan R, Huang Y, Balasubramanian MK, Mabuchi I. In vitro contraction of cytokinetic ring depends on myosin II but not on actin dynamics. *Nat Cell Biol*. 2013; 15(7):853–859. DOI: 10.1038/ncb2781 [PubMed: 23770677]
9. Walker GR, Kane R, Burgess DR. Isolation and characterization of a sea urchin zygote cortex that supports in vitro contraction and reactivation of furrowing. *J Cell Sci*. 1994; 107(Pt 8):2239–2248. [PubMed: 7983183]
10. Fujimoto H, Mabuchi I. Isolation of cleavage furrows from eggs of regular sea urchins and identification of furrow-specific proteins. *J Biochem*. 1997; 122(3):518–524. [PubMed: 9348078]
11. Scott JH, Schekman R. Lyticase: endoglucanase and protease activities that act together in yeast cell lysis. *J Bacteriol*. 1980; 142(2):414–423. [PubMed: 6991473]

Fig. 1. *S. pombe* ring isolation. *Upper panel* shows the schematic of *S. pombe* ring isolation process. *Lower panel* shows an example of purified *S. pombe* ring constriction. Scale bar, 5 μm

Fig. 2.
Schematic of perfusion process for *S. pombe* ring constriction

Fig. 3.

S. cerevisiae ring isolation. **(a)** Schematic of *S. cerevisiae* ring isolation process. **(b)** Bright field and fluorescent images showing *S. cerevisiae* ring isolation process. Scale bar, 5 μ m. **(c)** Bright field, fluorescent, and Imaris 3D rendering images showing the purified *S. cerevisiae* rings on poly-L-lysine-coated coverslip