Protocol

Construction, Growth, and Harvesting of Fission Yeast Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) Strains

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Stable isotope labeling by amino acids in cell culture (SILAC) enables the relative quantification of protein amounts and posttranslational modifications in complex biological samples through the use of stable heavy isotope–labeled amino acids. Here we describe methods for the application of SILAC to fission yeast *Schizosaccharomyces pombe* using either labeled lysine or a combination of labeled lysine and labeled arginine. The latter approach is more complicated than the use of labeled lysine alone but may yield a more comprehensive (phospho)proteomic analysis. The protocol includes methods for construction of SILAC-compatible strains, growth of cultures in labeled medium, cell harvesting, and protein extraction.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

EMM2 <R> with appropriate supplements (plates containing solid medium)
EMM2-LowAmm <R> (liquid medium)

EMM2-LowAmm (low ammonium) is prepared in the same way as EMM2, but contains 6 mM ammonium chloride (0.32 g/L) instead of the normal 93.5 mM (5 g/L).

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Oligonucleotide primers for yeast colony polymerase chain reaction (PCR) as described in Protocol:
Colony Polymerase Chain Reaction with *Schizosaccharomyces pombe* (Murray et al. 2016)

aru1Δ reverse primer: 5'-GTTGGAGTCGTATCCTCCGTTG-3'

car1Δ reverse primer: 5'-CAATTCAATTCATGTGAAAAAG-3'

car2Δ reverse primer: 5'-CCAAAATTTTTATTTTAAC-3'

kanMX forward primer: 5'-GGTTGCATTCGATTCCTGTTTGTAATTG-3'

*These primers are used to verify gene deletions relating to arginine catabolism. The same forward primer is used for all three deletions, whereas each deletion requires a unique reverse primer. Primers are not needed if labeling is to be done only with lysine and the *lys1-131* or *lys3-37* mutant.*

Protein assay reagents

Stable isotope labeling by amino acids in cell culture (SILAC) amino acids <R> (see Discussion)

Prepare two or more of the following according to the provided recipe.

L-lysine·2HCl ("light" lysine)

L-Lysine-4,4,5,5-d₄·HCl ("heavy" lysine; lysine-4)

L-[¹³C₆]lysine·2HCl ("heavy" lysine; lysine-6)

L-[¹³C₆¹⁵N₂]lysine·HCl ("heavy" lysine; lysine-8)

L-arginine·HCl ("light" arginine)

L-[¹³C₆]arginine·HCl ("heavy" arginine; arginine-6)

Heavy isotope-labeled amino acids are available from Sigma-Aldrich (Isotec), Silantes, and Cambridge Isotopes (various distributors worldwide). Prices vary, and delivery times can sometimes be several weeks, so obtain prices from several suppliers and order well in advance of experiments. Because of bulk pricing, it is useful to buy reagents for multiple experiments at once.

Light and heavy arginines are not needed if labeling is to be done only with lysine. The decision about which version(s) of heavy lysine to use depends on the experiment. In double SILAC experiments (i.e., using two different isotopes), typical combinations are light lysine and either lysine-6 or lysine-8; in triple SILAC experiments, a combination of light lysine with lysine-4 and lysine-8 is preferred. In general, a mass difference between the labeled amino acids of 3 Da or higher will greatly facilitate quantification.

Yeast extract with supplements (YES) <R> (plates containing solid medium)

If desired, add 200 mg/L arginine to improve growth of arginine auxotrophic strains (see Step 2).

*If required, add 133 μg/mL of G418 to select for *kanMX6*.*

Yeast strains of interest, plus reagents for construction of the following mutant strains

lys1-131 or lys3-37

arg1-230 (for heavy arginine labeling)

car2Δ::kanMX, or aru1Δ::kanMX and car1Δ::kanMX (for heavy arginine labeling; see Discussion)

Equipment

Ball mill (Mixer Mill MM400 [Retsch] or similar)

Ball mill buckets (e.g., 50-mL) with metal ball (25-mm-diameter)]

Centrifuge

Centrifuge tubes (50-mL, screw-cap)

Cryotubes or Falcon tubes

Flasks

For all culture steps, use a flask that holds at least 10 times the amount of culture volume (i.e., a 100-mL flask for a 10-mL culture). We prefer baffled flasks.

Grinding jar tongs

Liquid nitrogen (LN₂) in an insulated container

In addition, prepare a small ice bucket containing LN₂ for Step 19.

Pipette tips (1000-mL)

Cut off 0.2 cm from the tip ends for pipetting cells in Step 19.

Shaking incubator (≥200 rpm)

Sieve
Spatula or laboratory spoon

METHOD

Constructing Strains for SILAC

If using heavy lysine only (and not heavy arginine), parts of the protocol can be ignored as indicated.

1. Introduce the following mutations into the yeast strains of interest to be compared.
 - *lys1-131* or *lys3-37*
 - *arg1-230*
 - either *car2Δ::kanMX*, or *car1Δ::kanMX* and *aru1Δ::kanMX* (see Discussion)

In addition, cross out any other auxotrophic markers.

*For heavy lysine labeling, either the *lys1-131* or the *lys3-37* mutation must be introduced; the other mutations are relevant only when heavy arginine is used.*

We have not determined the importance of crossing out other auxotrophic markers, but we routinely do this to minimize unknown factors that could potentially affect labeling and/or robust cell growth.

2. Germinate spores on plates containing YES solid medium.

*Lysine-auxotrophic strains grow well on YES, whereas *arg1-230* mutants sometimes grow slowly on YES, depending on the commercial source of the yeast extract. If in doubt, YES plates supplemented with 200 mg/L of arginine hydrochloride should be used.*

3. Check lysine and arginine auxotrophies independently by replica-plating on plates containing EMM2 solid medium with appropriate supplements.
4. Confirm *car2Δ::kanMX* or *car1Δ::kanMX* and *aru1Δ::kanMX* with the appropriate primers for yeast colony PCR as described in Protocol: Colony Polymerase Chain Reaction with *Schizosaccharomyces pombe* (Murray et al. 2016).

If multiple kanMX markers are present in a cross, gene conversion events can eliminate one kanMX allele. This is an important consideration when tetrad dissection is used for strain construction, because it will not always be sufficient to identify double mutants on the basis of nonparental ditype tetrads; this also applies to natMX or hphMX markers.

5. Freeze the validated autotrophic strain using standard methods (see Introduction: Growth and the Environment of *Schizosaccharomyces pombe* [Petersen and Russell 2016]). Store until ready to proceed with Step 6.

Growing Strains for SILAC

Day 0

6. Thaw the auxotrophic *S. pombe* strain on a plate containing YES solid medium with appropriate antibiotics. Incubate at the appropriate temperature overnight.

Day 1

7. Harvest a few cells from the plate with a pipette tip and resuspend them in 100 μ L of EMM2-LowAmm.
8. Add the cell solution to a flask containing 10 mL of EMM2-LowAmm supplied with light L-lysine and, if required, light L-arginine. Grow the cells overnight with shaking at ≥ 200 rpm.

The final concentration of amino acids in the medium should be 200 mg/L of lysine for labeling with lysine alone, or 60 mg/L of lysine and 80 mg/L of arginine for double labeling. Concentrations of 30 mg/L lysine and 40 mg/L arginine were used originally (Bicho et al. 2010) but result in an approximately twofold lower growth yield (measured by optical density at 595 nm during the stationary phase) and thus require larger culture volumes. Concentrations of lysine and arginine higher than the “60/80” formulation do not proportionally increase yields and are therefore less cost-effective.

Day 2

9. Pellet the culture and wash twice with 10 mL of EMM2-LowAmm (without L-lysine or L-arginine) to remove residual L-lysine (and L-arginine). Resuspend the cells in EMM2-LowAmm and determine cell concentration.
10. Inoculate a flask containing 10 mL of EMM2-LowAmm supplemented with light or heavy L-lysine and/or L-arginine using the same amino acid concentrations as in Step 8. Grow the cells for three to four cell doublings, such that cells are maintained in log-phase growth throughout culture (i.e., not exceeding $\sim 1\text{--}1.5 \times 10^7$ cells/mL).
11. From the culture in Step 10, inoculate a flask containing the desired culture volume of prewarmed EMM2-LowAmm supplemented with light or heavy L-lysine and/or L-arginine. Grow the cells to reach another four to six cell doublings.

In principle, growth in heavy medium for seven to eight generations should be sufficient to decrease the pool of light amino acids by at least 99% (e.g., $>200\text{-fold}$ if 8 generations = $1/2^8$). In practice, a few more generations of growth may be beneficial. Growing the labeled cultures in two stages ensures that the larger-volume culture is inoculated under optimal growth conditions. This can prevent problems if the strain being used does not grow well at very low cell densities.

12. Proceed to cell harvesting.

Cells should be harvested at a density no $>2.4 \times 10^7$ cells/mL. This density (late log phase) is higher than that typically used for conventional biochemical experiments and is motivated by the high cost of heavy-labeled amino acids. For some experiments, lower densities may be more appropriate. Cells grown in light medium should always be grown to the same density as cells grown in heavy medium. Cells grown in 30 mg/L of lysine and 40 mg/L of arginine should be harvested at a lower density of 1.2×10^7 cells/mL, because they reach the stationary phase at lower cell densities.

Harvesting Cells

The following steps can be performed at room temperature or in a cold room at 4°C, according to experimental needs. All reagents and materials (such as ddH₂O, centrifuge tubes, pipette tips, buckets, centrifuge, etc.) should be brought to the appropriate temperature before proceeding.

Methods other than centrifugation for harvesting cells (e.g., filtration) can be used depending on culture volume.

The highly viscous culture suspension required for dropping cells into LN₂ (Step 19) can be obtained easily in the conical bottom of a 50-mL screw-cap centrifuge tube; thus, we highly recommend switching to these tubes during the washing steps (Steps 15–18).

13. Count the cells to determine the volume of each sample that will later be required for mixing samples from different conditions at a 1:1(:1) ratio.

Mixing of samples from different conditions can be performed just before or just after protein extraction (see below).

14. Harvest the cells by centrifugation for 3–5 min at 3000g.

15. Decant the culture medium and wash the cells with ddH₂O to remove residual medium.

High salt concentration from residual medium may interfere with sample preparation (strong cation exchange [SCX] chromatography) for subsequent mass spectrometry.

16. Harvest the cells by centrifugation for 3–5 min at 3000g.

17. Wash twice more with ddH₂O as in Steps 15–16.

18. Decant the supernatant of the last washing step and resuspend the cell pellet in the residual liquid in the tube.

If needed, a very small volume of ddH₂O can be added to allow pipetting of the cells in the next step.

19. Take up the cell suspension with a 1000-μL pipette, using a tip with 0.2 cm removed. Do not take up more than 500 μL at once. Slowly, add cells dropwise to LN₂ in an ice bucket (Fig. 1A).

20. Recover the cell droplets by decanting the LN₂ through a sieve. Transfer the droplets to a 50-mL screw-cap tube precooled in LN₂. Store the cell droplets at –80°C as needed.

FIGURE 1. Preparation of protein extracts using a ball mill. (A) Cell suspension is dropped into liquid nitrogen. (B) Frozen cell droplets are transferred into a metal bucket (precooled in LN₂) that contains a metal ball to disrupt cells. (C) The bucket with cells is closed and again cooled in LN₂. (D) Cells within the bucket are placed into a ball mill and disrupted by rapid shaking of the bucket containing the metal ball. (E) Cell powder is obtained. (F) Cell powder is transferred to a tube for additional storage or further processing.

*The few residual cells remaining in the tube can be used for additional analyses, such as methanol/ethanol fixation and microscopy; see Protocol: **Immunofluorescence Microscopy of Schizosaccharomyces pombe Using Chemical Fixation** (Hagan 2016).*

Extracting Protein

Cell disruption should be performed under cryogenic conditions. Here we explain protein extraction using a ball mill grinder (Fig. 1; see online Movie 1 at cshprotocols.cshlp.org). The ball mill is easy to handle and makes it easy to maintain cryogenic conditions. However, any other method that efficiently lyses *S. pombe* cells without impairing protein integrity should work as well; see Protocol: **Large-Scale Immunoprecipitation from Fission Yeast Cell Extracts** (Grallert and Hagan 2016).

We typically mix the cell droplets of the different conditions 1:1(:1) (judged by cell number) before protein extraction to minimize differences in sample preparation. Alternatively, cells can be lysed first, and the protein concentration determined before mixing 1:1(:1).

We perform all of the following steps in a cold room. (Note that use of LN₂ in a closed room carries the risk of asphyxiation.) Precool all materials that come in direct contact with the cell droplets or the cell powder (ball mill bucket and ball, laboratory spoons, cryotubes or Falcon tubes, etc.) in LN₂.

21. Fill the cell droplets into the precooled ball mill bucket, include an appropriately sized ball, and close the bucket. Cool again before the first ball mill run (Fig. 1B,C).

22. Fix the bucket in the ball mill and run 3 min with 30 shakes/sec (Fig. 1D).
23. Remove the bucket and use precooled tongs to place in LN₂ in an insulated container until full cooling is achieved.
24. Repeat the ball mill run (Steps 22 and 23) two to three times.
25. Open the bucket and quickly transfer the cell powder to precooled cryotubes or Falcon tubes using a precooled laboratory spoon (Fig. 1E,F).
If this step takes too long, humidity from the air will freeze on the inside of the bucket and the cell powder will start to accumulate additional water in the form of ice, which reduces the overall protein concentration per volume. If the spoon used for transfer is too warm, the cell powder will melt and be difficult to transfer.
26. Take a small sample of cell powder to determine the protein concentration. Store the cell powder at −80°C until analysis (see Protocol: **Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC)-Based Quantitative Proteomics and Phosphoproteomics in Fission Yeast** [Carpy et al. 2016]).

DISCUSSION

One of the more salient technical issues associated with SILAC in *S. pombe* involves the use of heavy-labeled arginine. A key feature of the arginine-labeling protocol above is the use of a deletion mutant of predicted arginases *car1*⁺ and *aru1*⁺, or of predicted ornithine transaminase *car2*⁺, to minimize catabolic conversion of labeled arginine to other amino acids. This protocol was originally developed and validated for use with ¹³C₆-arginine (arginine-6), and ¹³C₆-lysine (lysine-6) (Bicho et al. 2010). Recently, 4,4,5,5-d₄-lysine (lysine-4), ¹³C₆, ¹⁵N₂-lysine (lysine-8), and ¹³C₆, ¹⁵N₄-arginine (arginine-10) have become more widely available. In our laboratory, we have encountered problems when the protocol is followed using arginine-10 rather than arginine-6; these are caused by degradation of the guanidinium group of ¹³C₆¹⁵N₄-arginine to ¹⁵N₁-ammonia, which is then used as a precursor in amino acid biosynthesis. We have independently developed two possible strategies to address this issue. First, a reduction of the urease activity by inactivation of the Ni²⁺ transporter *Nic1* enables full incorporation of ¹³C₆¹⁵N₄-arginine and compatibility of fission yeast with SILAC (Carpy et al. 2015). Alternatively, specific use of *car1Δ aru1Δ* double mutants instead of *car2Δ* single mutants for the inhibition of arginine conversion also addresses the problem (Borek et al. 2015). At present, for *S. pombe* strains described in this protocol, we strongly recommend the use of arginine-6 in experiments. We have not encountered comparable problems when lysine-8 is used in place of lysine-6, and thus lysine-6 and lysine-8 can be considered largely interchangeable. Lysine-4 is particularly useful for triple SILAC experiments (comparing three conditions), where it can be combined with unlabeled lysine and lysine-8.

When *arg1-230 lys3-37* cells are grown in conventional EMM2 containing arginine and lysine as supplements, inoculation of growing cultures into fresh medium at high dilution (i.e., low cell density) results in an extremely long lag phase (2–3 d!) before normal growth (Bicho et al. 2010). However, immediate robust growth upon dilution can be achieved simply by decreasing the concentration of ammonium chloride in the growth medium, from 96 mM (normal) to ~6–24 mM (Bicho et al. 2010). Remarkably, even at 6 mM ammonium chloride, good growth immediately ensues, indicating that normal formulations of EMM2 contain far more nitrogen source than is actually required for growth. The reason why growth of *arg1-230 lys3-37* mutants is improved by decreasing the ammonium chloride concentration has not been investigated in great detail, but there is evidence for two types of arginine permeases in fission yeast, one of which is competitively inhibited by basic amino acids (e.g., lysine), and the other of which is inhibited by ammonium ions (Fantes and Creanor 1984). Therefore, the decrease in ammonium chloride concentration likely allows successful uptake of exogenous arginine by one arginine permease in the face of lysine-dependent inhibition of the other arginine permease. Excellent results can also be obtained using EMMG, which contains 20–22 mM L-glutamic acid instead of ammonium chloride (Carpy et al. 2015); see Introduction: **Growth and the**

Environment of *Schizosaccharomyces pombe* (Petersen and Russell 2016). We have not yet tested this with *arg1-230 lys3-37* strains.

RECIPES

EMM2

Reagent	Quantity (for 1 L)	Final concentration
Ammonium chloride	5 g	93.5 mM
Potassium hydrogen phthalate	3 g	14.7 mM
Na ₂ HPO ₄	2.2 g	15.5 mM
Glucose	20 g	111 mM
Salt stock (50×)	20 mL	1×
Vitamins (1000×)	1 mL	1×
Minerals (10,000×)	0.1 mL	1×
H ₂ O	to 1 L	

Prepare 1 L of EMM2 by combining the reagents above. To prepare solid medium, include agar (20 g/L). Sterilize by autoclaving at 10 psi for 10 min (for liquid medium) or 15 min (for solid medium). (For liquid medium, low pressure and a short cycle is essential to avoid caramelization of glucose and breakdown of vitamins and minerals. For live cell imaging, filter-sterilized medium has lower background fluorescence than autoclaved medium. Use a 0.22-μm pore size for filtration.) Store at 4°C. If desired, add 12 μM thiamine (364 μL of a filter-sterilized stock solution of 10 mg/mL on H₂O) after autoclaving to fully repress expression from *nmt1*-derived promoters (Maundrell 1990).

EMM2-LowAmm

Reagent	Quantity (for 1 L)	Final concentration
Ammonium chloride	0.32 g	6 mM
Potassium hydrogen phthalate	3 g	14.7 mM
Na ₂ HPO ₄	2.2 g	15.5 mM
Glucose	20 g	111 mM
Salt stock (50×) <R>	20 mL	1×
Vitamins (1000×) <R>	1 mL	1×
Minerals (10,000×) <R>	0.1 mL	1×
H ₂ O	to 1 L	

Prepare 1 L of EMM2-LowAmm by combining the reagents above. Sterilize by autoclaving at 10 psi for 10 min. (Low pressure and a short cycle is essential to avoid caramelization of glucose and breakdown of vitamins and minerals.) Store at 4°C.

Minerals (10,000×)

Reagent	Quantity (for 1 L)	Final concentration
Boric acid	5 g	80.9 mM
MnSO ₄	4 g	23.7 mM
ZnSO ₄ · 7H ₂ O	4 g	13.9 mM
FeCl ₂ · 6H ₂ O	2 g	7.40 mM
Molybdic acid	0.4 g	2.47 mM
KI	1 g	6.02 mM
CuSO ₄ · 5H ₂ O	0.4 g	1.60 mM
Citric acid	10 g	47.6 mM

Store at 4°C.

Salt Stock (50×)

Reagent	Quantity (for 1 L)	Final concentration
MgCl ₂ · 6H ₂ O	52.5 g	0.26 M
CaCl ₂ · 2H ₂ O	0.735 g	4.99 mM
KCl	50 g	0.67 M
Na ₂ SO ₄	2 g	14.1 mM
Store at 4°C.		

SILAC Amino Acids

Dissolve amino acid powder in high-purity, deionized H₂O (i.e., Milli-Q) to make a 100× solution. After ensuring the amino acid has dissolved, filter the solution through a 0.22-μm filter. Store at 4°C.

Vitamins (1000×)

Reagent	Quantity (for 1 L)	Final concentration
Pantothenic acid	1 g	4.20 mM
Nicotinic acid	10 g	81.2 mM
Inositol	10 g	55.5 mM
Biotin	10 mg	40.8 μM
Store at 4°C.		

Yeast Extract with Supplements (YES)

Reagent	Amount to add (1 L)
Glucose	30 g
Yeast extract	5 g
Adenine	0.2 g
Uracil	0.2 g
Histidine	0.2 g
Leucine	0.2 g
Lysine	0.2 g
Agar (for solid medium only)	20 g
H ₂ O	to 1 L

Prepare 1 L of YES by combining the reagents listed above. Sterilize by autoclaving at 10 psi for 15 min. Store at 4°C. (Note that the yeast contains sufficient thiamine to repress *mtl1* promoters.)

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REFERENCES

- Bicho CC, de Lima Alves F, Chen ZA, Rappsilber J, Sawin KE. 2010. A genetic engineering solution to the “arginine conversion problem” in stable isotope labeling by amino acids in cell culture (SILAC). *Mol Cell Proteomics* 9: 1567–1577.

- Borek WE, Zou J, Rappsilber J, Sawin KE. 2015. Deletion of genes encoding arginase improves use of “heavy” isotope-labeled arginine for mass spectrometry in fission yeast. *PLoS One* 10: e0129548.
- Carpy A, Patel A, Tay YD, Hagan IM, Maček B. 2015. Nic1 inactivation enables stable isotope labeling with ¹³C⁶¹⁵N⁴-arginine in *Schizosaccharomyces pombe*. *Mol Cell Proteomics* 14: 243–250.
- Carpy A, Koch A, Bicho CC, Borek WE, Hauf S, Sawin KE, Maček B. 2016. Stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics and phosphoproteomics in fission yeast. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot091686.
- Fantes PA, Creanor J. 1984. Canavanine resistance and the mechanism of arginine uptake in the fission yeast *Schizosaccharomyces pombe*. *J Gen Microbiol* 130: 3265–3273.
- Grallert A, Hagan IM. 2016. Large-scale immunoprecipitation from fission yeast cell extracts. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot091595.
- Hagan IM. 2016. Immunofluorescence microscopy of *Schizosaccharomyces pombe* using chemical fixation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot091017.
- Maundrell K. 1990. *nmt1* of fission yeast. A highly transcribed gene completely repressed by thiamine. *J Biol Chem* 265: 10857–10864.
- Murray JM, Watson AT, Carr AM. 2016. Colony polymerase chain reaction with *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot090993.
- Petersen J, Russell P. 2016. Growth and the environment of *Schizosaccharomyces pombe*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top079764.

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