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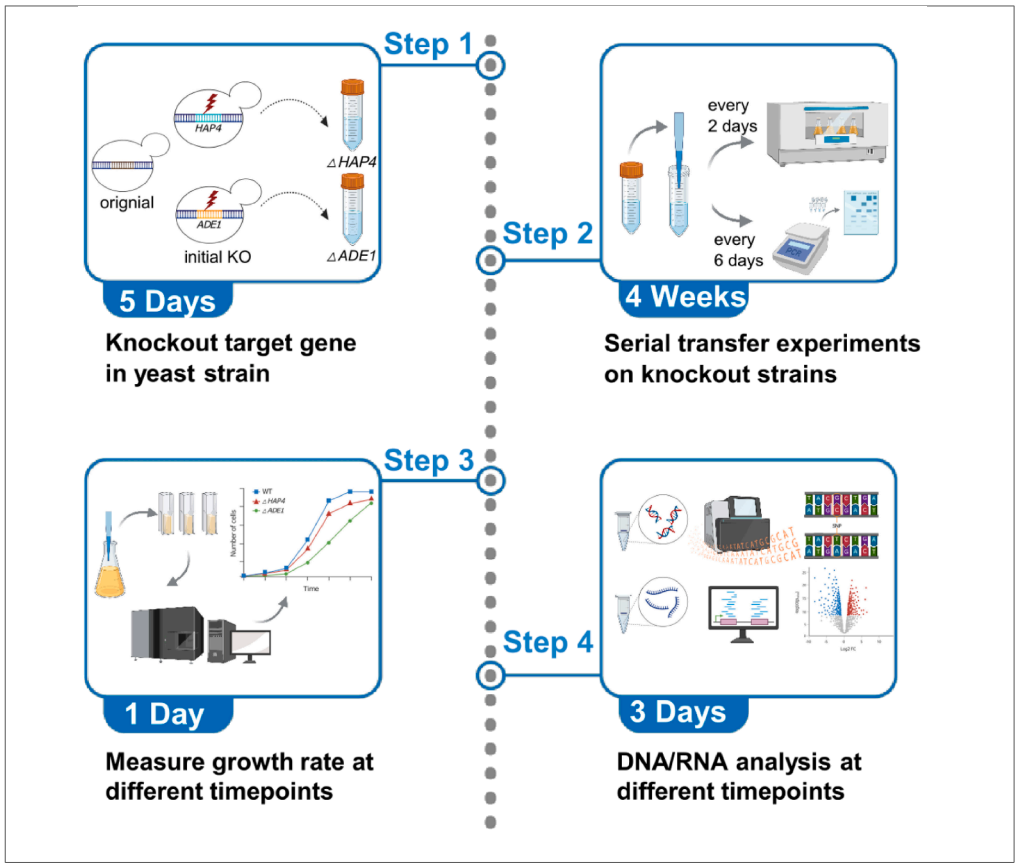
Protocol for adaptive laboratory evolution of *S. cerevisiae* by PEG/LiAc transformation and sequencing



Gene deletions perturb cellular homeostasis, affecting gene expression and phenotypes. Here, we present a protocol for serial transfer experiments in *Saccharomyces cerevisiae* strains following targeted gene knockouts. We describe steps for gene deletion, serial passage optimization, and growth kinetics analysis. We then detail procedures for genomic and transcriptomic profiling. This protocol enables the investigation of changes in yeast strains resulting from gene loss.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol examines yeast post-knockout using growth, genome, and RNA analysis

Track growth, gene expression, and mutations at various knockout time points

Evaluate continuous dynamic changes in growth and gene expression

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Protocol for adaptive laboratory evolution of *S. cerevisiae* by PEG/LiAc transformation and sequencing

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SUMMARY

Gene deletions perturb cellular homeostasis, affecting gene expression and phenotypes. Here, we present a protocol for serial transfer experiments in *Saccharomyces cerevisiae* strains following targeted gene knockouts. We describe steps for gene deletion, serial passage optimization, and growth kinetics analysis. We then detail procedures for genomic and transcriptomic profiling. This protocol enables the investigation of changes in yeast strains resulting from gene loss. For complete details on the use and execution of this protocol, please refer to Jiang et al.[1]

BEFORE YOU BEGIN

Gene knockout represents a fundamental approach for elucidating gene function across diverse organisms.[2] This technique, which involves targeted gene removal or inactivation, enables researchers to evaluate the gene's role in cellular processes and phenotypic manifestations. While providing valuable insights, gene deletion inevitably perturbs cellular homeostasis, triggering cascading alterations in gene expression networks and subsequent phenotypic traits, including growth dynamics.[3]

Cellular systems possess inherent adaptive mechanisms to restore equilibrium through epigenetic modifications and genetic variations, particularly evident during adaptive laboratory evolution (ALE).[4,5] ALE allows cells to adapt to new environments, often resulting in advantageous mutations that enhance fitness. Previous studies have demonstrated significant fitness improvements in populations of *Saccharomyces cerevisiae* (*S. cerevisiae*), highlighting the capacity of cells to undergo substantial changes over generations.[6]

This protocol presents a comprehensive methodology for conducting serial transfer experiments with post-knockout yeast strains. Our approach enables systematic investigation of progressive transcriptomic changes induced by gene deletion, integrating three key data streams: growth kinetics, whole-genome sequencing, and RNA sequencing analysis. Distinctively, we employ synthetic complete (SC) medium, which provides a defined yet nutrient-limited environment. This strategic choice of growth conditions drives evolutionary processes in yeast cells within a controlled laboratory setting, facilitating the study of adaptive responses.

Primer design

Timing: 2 h

1. Download Sequence.
   a. Retrieve the sequence of the target gene from Saccharomyces Genome Database (SGD, https://www.yeastgenome.org/).
   b. Include the 1000 bp upstream and downstream of the gene coding sequence.
2. Primer Design.
   a. Primers for Homologous Recombination: Design the left and right primers, each 60 bp in length.

   Note: These primers should consist of approximately 40 bp of sequence homologous to the target genomic region to facilitate precise integration through homologous recombination, and 20 bp of sequence complementary to the insertion gene, which serves as a selection marker, to enable efficient amplification of the desired DNA fragment. Take gene *ADE1* as example:

   ADE1-URA3-F.
   CAGTTGGTACTATTAAGAACAATCGAATCATAAGCATTGC*AGCGGAAGTGTATCGTA CAG*.
   ADE1-URA3-R.
   TCTGAGAACATTTATACATTAATACATACGGGTATGTATG*GGGAATAAGGGCGACA CGGA*.
   In the sequences above:
   The underlined regions represent sequences from the ADE1 gene used for homologous recombination.
   The italic regions correspond to sequences used to amplify the URA3 cassette as the selection marker using plasmid pYes2 as a template.
   b. Design the left and right primers with all 18-25 bp in length, for gene deletion verification (Figure S1). Take gene *ADE1* as example:
   ADE1-test-F.
   CAGTCTGACTCTTGCGAGAG.
   ADE1-test-R.
   GAACATCGTTGGACAGGAAC.
   URA3-test-R.
   CATCCAATGAAGCACACAAG.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
S. cerevisiae: strain BY4741	N/A	N/A
Chemicals, peptides, and recombinant proteins		
Bacteriological peptone	HuanKai Microbial	050170B
Yeast extract	Oxoid	LP0021
Tryptone	Oxoid	LP0042
SC complete medium	FunGenome	YGM003A-1
Ura minus media	FunGenome	YGM003A-3
NaCl	Tianjin Damao	7647-14-5

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
D-(+)-glucose	Sigma-Aldrich	G8270-1KG
EDTA	BioFroxx	1340
PEG3350	Sigma-Aldrich	P3640-100G
Agar powder	MYM	MA0451-500G
Lithium acetate dihydrate	Vetec	V900099-500G
67 mM potassium phosphate solution (sterile), pH 7.5	ECOTOP	ED-8681
Glycerol	Guangzhou Chemical Reagent Factory	HC05-AR-0.5L
NaOH	Aladdin	S111501-500g
KOD One PCR master mix, blue	TOYOBO	KMM-201
2×Taq PCR StarMix (dye)	GenStar	A012-100
Lyticase from *Arthrobacter luteus*	Sigma-Aldrich	L2524-25KU
Salmon sperm DNA solution (ssDNA)	Invitrogen	15632011
E.Z.N.A. gel extraction kit	Omega Bio-tek	D2500-02
Software and algorithms		
GATK (version 4.2.0.0)	GATK	https://github.com/broadinstitute/gatk/
SnpEff (version 4.3t)	SnpEff	https://pcingola.github.io/SnpEff/
STAR (version 2.7.8a)	STAR	Dobin et al.[7]
FASTX Toolkit (version 0.0.14)	FASTX Toolkit	http://hannonlab.cshl.edu/fastx_toolkit/
Trim galore (version 0.6.4_dev)	Trim Galore	https://github.com/FelixKrueger/TrimGalore
BWA (version 0.7.17-r1198-dirty)	BWA	Li and Durbin[8]
Picard tools	Picard tools	http://picard.sourceforge.net
SAMtools (version 1.10)	SAMtools	Li et al.[9]
Featurecounts (version 1.6.2)	Featurecounts	Liao et al.[10]
DESeq2	DESeq2	Love et al.[11]
R studio (R version 4.0.3)	RStudio	https://posit.co/downloads/
Other		
PCR apparatus	Bio-Rad	T100
Electrophoresis apparatus	Beijing Liuyi	DYY-8C
Oscillating metal bath	Bioer Technology	HB-202
Multi-functional ultra-pure water system	RSJ	Unique-R20
PH meter	METTLER TOLEDO	FiveEasy Plus FE20
Magnetic heating agitator	Thermos Fisher Scientific	Cimarec SP131320-33
Fully automatic gel imaging system	Bio-Rad	Gel Doc EZ
Low-temperature incubator	Yamato	IN612C
Temperature controlled shaker	Suzhou Jimei	IS-RDD3
Autoclave	TOMY	SX-700
Centrifuge	Eppendorf	5810R
Centrifuge	Eppendorf	5415R
Electronic balance	Saiduolisi	QUINTIX224-1CN
Water bath	Thermos Fisher Scientific Isotemp	FIS15-462-3Q
Clean bench	Airtech	BCM-1000A
Flake Icemachine	Coolium Instruments	FM50
Cell imaging system	Thermos Fisher Scientific	EVOS FL
Microspectrophotometer	Thermos Fisher Scientific NanoDrop	NanoDrop 2000

MATERIALS AND EQUIPMENT

[YPD liquid Media]		
Yeast Extract	1%	10 g
Bacteriological peptone	2%	20 g

D-glucose	2%	20 g
ddH_2O	N/A	To 1000 mL
Total	N/A	1000 mL

Storage up to six months at 4°C after autoclave sterilization.

[SC liquid Media]

Reagent	Final concentration	Amount
SC Complete medium	0.8%	8 g
D-glucose	2%	20 g
ddH_2O	N/A	To 1000 mL
Total	N/A	1000 mL

Storage up to six months at 4°C after autoclave sterilization.

[SC-Ura liquid Media]

Reagent	Final concentration	Amount
SC-Ura medium	0.8%	8 g
D-glucose	2%	20 g
ddH_2O	N/A	To 1000 mL
Total	N/A	1000 mL

Storage up to six months at 4°C after autoclave sterilization.

[60% Glycerol liquor]

Reagent	Final concentration	Amount
Glycerol (100%)	60%	60 mL
ddH_2O	N/A	40 mL
Total	N/A	100 mL

Storage up to six months at 4°C.

[1M LiAc liquor]

Reagent	Final concentration	Amount
LiAc powder	1 M	10.2 g
ddH_2O	N/A	To 100 mL
Total	N/A	100 mL

Storage up to six months at 4°C after autoclave sterilization.

[50% PEG]

Reagent	Final concentration	Amount
PEG3350 powder	50%	50 g
ddH_2O	N/A	To 100 mL
Total	N/A	100 mL

Storage up to six months at 4°C after autoclave sterilization.

[Lyticase mix]

Reagent	Final concentration	Amount
Lyticase from Arthrobacter luteus	0.2 U/μL	X g (100 U)
67 mM Phosphate Buffer	N/A	To 500 μL
Total	N/A	500 μL

Storage up to six months at −20°C.

[TE buffer]		
Reagent	Final concentration	Amount
Tris-HCl (pH 8.0)	10 mM	0.1576 g
EDTA	0.1 mM	0.0372 g
Total	N/A	100 mL

Storage up to six months at −4°C.

STEP-BY-STEP METHOD DETAILS

Knock out target genes in yeast strains

Timing: 5 days

This step is to knockout the interested genes in *S. cerevisiae* BY4741 yeast genome. Gene replacements can be achieved through homologous recombination using standard polyethylene glycol/lithium acetate (PEG/LiAc) transformation method.[12,13]

1. DNA Fragment Preparation.
   a. Amplify the homologous fragments by polymerase chain reaction (PCR) according to the following setup and cycling conditions.

PCR reaction master mix	
Reagent	Amount
DNA template	X μL(<50 ng)
KOD ONE Mix	25 μL
Primer #1 (10 pmol/μL)	1.5 μL
Primer #2 (10 pmol/μL)	1.5 μL
ddH_2O	To 50 μL

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	2 min	1
Denaturation	98°C	10 sec	30 – 35 cycles
Annealing	Tm-5°C	5 sec	
Extension	68°C	1 ~ 10 sec/kb	
Final extension	68°C	7 min	1

   b. Perform gel electrophoresis on the PCR products to verify the target band size, and excise the desired band from the gel.
   c. Recover the DNA from the gel using E.Z.N.A Gel Extraction Kit, following the manufacturer's instructions. Ensure the total amount of recovered DNA exceeds 2 μg, with a concentration >20 ng/μL.

Optional: For an alternative method of DNA gel recovery, follow steps below[14]:

   i. Grind the gel slice thoroughly using a metal rod and add 150 μL of TE buffer subsequently.
   ii. Freeze the gel mixture using dry ice or liquid nitrogen.
   iii. Incubate the mixture at 72°C for 3 min. Then centrifuge the tube at 10,000 × *g* for 30 s at 25°C. Transfer the solubilized gel solution into a new sterile 1.5 mL microcentrifuge tube.
   iv. Precipitate the DNA by adding 1/10 volume of sodium acetate (3 M, pH 5.2) and 2.5 volumes of cold ethanol (or alternatively, isopropanol).

v. Incubate the mixture at −20°C for at least 30 min (or 12 h for optimal yield).
vi. Centrifuge at 16,000 × *g* for 20 min at 25°C. Carefully remove and discard the supernatant without disturbing the DNA pellet.
vii. Wash the pellet with 600 μL of 70% ethanol. Centrifuge again at 16,000 × *g* for 5 min. Carefully remove and discard the ethanol wash.
viii. Air-dry the DNA pellet for 5–10 min (avoid over-drying, as it can make DNA hard to dissolve). Resuspend the DNA pellet in an appropriate volume (20–50 μL) of nuclease-free water or TE buffer.

2. Targeted Gene Deletion.
   a. Take an appropriate amount of glycerol stock stored at −80°C and streak it onto a YPD plate. Incubate at 30°C in an incubator for 2 days.
   b. Pick a single colony and inoculate it into YPD medium the night before transformation. Incubate at 30°C with shaking at 200 rpm 12–16 h until saturated.
   c. On the day of transformation, transfer the yeast cells from Step b into fresh YPD culture (1:100). Incubate until OD600 reaches 0.5–0.8 (approximately 4–6 h).

   Note: Use 20 ml of culture to transform 5 transformation samples; the following steps use 20 ml of culture.

   d. Prepare solutions fresh as needed.
      i. 0.1 M LiAc.
      ii. PLI solution which is transformation premix solution, consisting ddH_2O: 1 M LiAc: 50% PEG3300 in a 1:1:8 ratio, with 300 μL required per transformation.
   e. Centrifuge culture to remove supernatant at 1300 rcf for 4 min.
   f. Wash the pellet with water (gently pipette up and down). Centrifuge to remove supernatant. Repeat this step twice.
   g. Add 250 μL of 0.1 M LiAc to the pellet. Gently mix and let sit at 25°C for 10 min. The competent cells are now ready.

   Note: Prepare ssDNA: Heat in a metal bath at 95°C for 5 min. If there is a delay, heat again and place on ice.

   h. For each transformation sample, prepare the transformation mixture according to the following proportions: homologous DNA fragments (>2 μg), ssDNA (5 μL), competent cell (50 μL), and PLI (300 μL). Mix gently and incubate at 42°C for 25 min.
   i. Centrifuge to remove supernatant at 800 rcf for 2 min.
   j. Wash the pellet with water (gently pipette up and down). Centrifuge to remove supernatant. Repeat this step twice.
   k. Resuspend the cell pellet gently in 100–200 μL ddH_2O and plate onto synthetic complete medium lacking uracil (SC-URA) supplemented with 2% glucose.
   l. Incubate the plates at 30°C for 2 days.
   m. Obtain positive clones.
      i. For each gene deletion strain, select 10–20 independent clones and perform PCR to confirm positive clones.
      ii. Use 10 μL of lysis buffer containing lyticase (final concentration: 0.2 U) to lysis the clones.
      iii. Take 1-2 μL of the lysis product as the PCR template. Troubleshooting 3.

Lyticase mix

Reagent	Amount
0.2 U/μL Lyticase mix	10 μL
Yeast colony	N/A

Lyticase incubate conditions			
Steps	Temperature	Time	Cycles
Incubation	37°C	30 min	1
Heat deactivation	98°C	5 min	1

PCR reaction master mix	
Reagent	Amount
DNA template	X μL(<50 ng)
2 × Rapid Taq Master Mix	10 μL
Primer #1 (10 pmol/μL)	1 μL
Primer #2 (10 pmol/μL)	1 μL
ddH_2O	To 20 μL

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	30 cycles
Annealing	Tm-5°C	15 sec	
Extension	72°C	15 s/kb	
Final extension	72°C	5 min	1
Hold	4°C	forever	

n. Once confirmed, amplify positive clones in 5 mL SC medium at 15 mL tube at 30°C with shaking at 200 rpm for 1 day. Then we get the initial knockout strains (Initial KO). Store the saturated culture at −80°C as glycerol stocks (300 μL of 60% glycerol + 600 μL of saturated culture).

Serial transfer experiment on gene knockout strains

Timing: 4 weeks

This step shows the details of serial transfer experiments on positive clones.

3. Incubate samples in 50 mL conical tubes with screw caps containing 15 mL of synthetic SC medium.
4. Maintain cultures at 30°C with orbital shaking (200 rpm) in darkness.
5. Store the saturated culture at −80°C as glycerol stocks.
6. Transfer cultures to fresh SC medium every 48 h. Troubleshooting 4. The transfer ratio is $1:10^5$ dilution into 15 mL fresh SC medium. Troubleshooting 5.

Note: The serial transfer is carried out continuously for the designated number of days without interruption. The duration can be adjusted based on the experimental objective. Specifically in this study, if the growth rate stabilizes, the serial transfer experiment can be concluded.

7. Verify deletion loci by PCR every 6 days to monitor culture purity.

Measuring growth rate of strains at different time points

Timing: 1 day for each time point

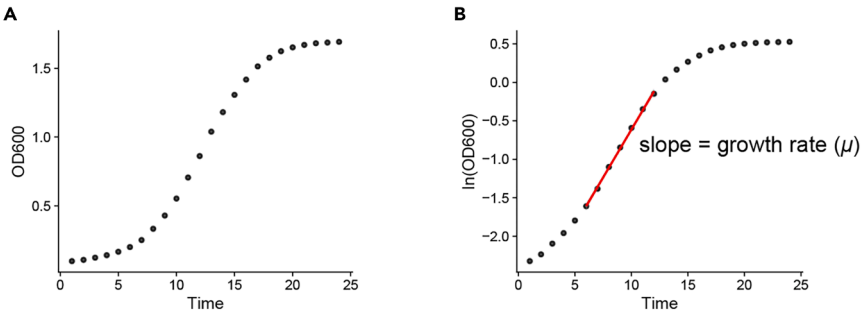

Figure 1. The schematic diagram for calculating growth rates from growth curves

(A) shows the change in OD600 over cultivation time.

(B) represents the calculation of the growth rate using ln(OD600) during the exponential growth phase.

The step describes how the protocol to measure the growth rate for yeast strains at different time points and the grow rate calculation.

8. Dilute saturated cultures 1:200 in fresh SC medium in 50 mL tubes to achieve an initial optical density (OD600) of 0.05.
9. Incubate cultures at 30°C with shaking (200 rpm).
10. Monitor growth by measuring OD600 at 2-h intervals until cultures reach stationary phase (typically when OD600 $\geq$ 1.0).
11. Calculate growth rate for each strain (Figure 1).
    a. Analyze the exponential growth phase data (OD600 between 0.1 and 0.8) by plotting the natural logarithm of OD600 values [ln(OD600)] against time (t).
    b. Calculate the specific growth rate (μ) for each strain from the slope of the linear regression of these plots.

Whole-genome profiles of strains from different time points

Timing: 3 days

The step describes how to extract whole genome of yeast strains collected from different time points, the sequencing strategy and data analysis (Figure 2). The sampling time points can be determined based on the research objectives, such as the starting point, intermediate stages, and the end of the experiment.

12. Revive glycerol stock samples from −80°C storage, transferring them into 5 mL fresh SC medium in 15 mL tubes and incubating 12–16 h at 30°C.
13. Extract genomic DNA from ~10^8 yeast cells using the Omega Yeast DNA kit (D3370-01) according to the instruction of supplier.
14. Submit the extracted genomic DNA for paired-end sequencing on an Illumina HiSeq platform through Genewiz or an equivalent service provider, targeting an average sequencing depth of 100× recommended.
15. Bioinformatics Analysis Pipeline.
    a. Trim the first 10 base pairs from the 5′-end using FASTX Toolkit (parameter: -f 11 -z).
    b. Clean the resulting reads using Trim Galore with minimum length requirement of 100 bp and quality threshold of 30 (parameters: –length 100 –quality 30).
    c. Align the processed reads to the S. cerevisiae S288C reference genome (version R64-1-1) using BWA with default settings, followed by removal of duplicate reads using Picard tools.
16. Variant Calling and Annotation.

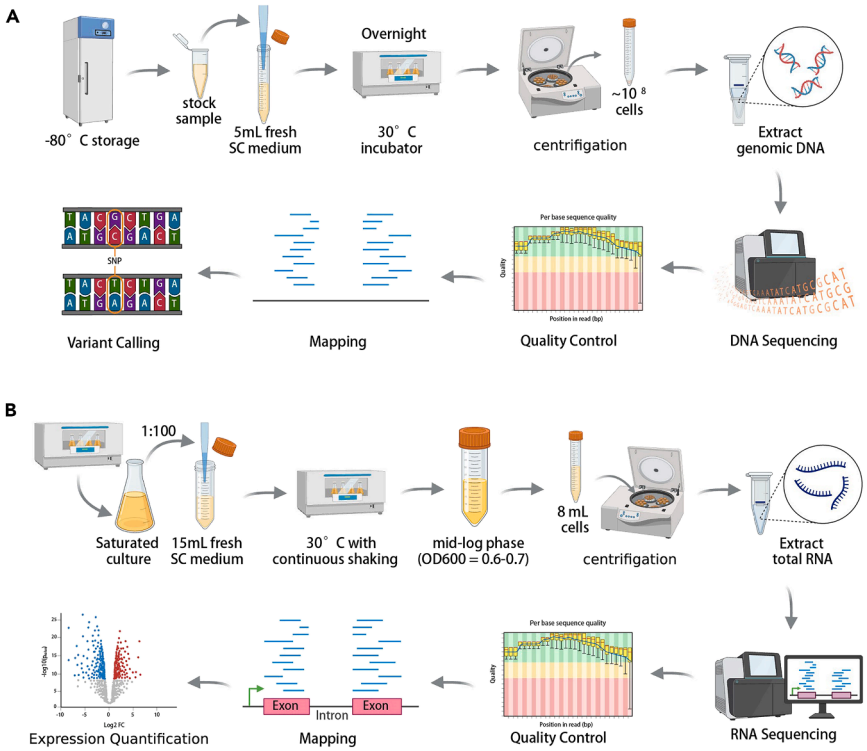

Figure 2. Workflow for Data Acquisition

(A) Schematic diagram illustrating the process for obtaining genomic data.

(B) Schematic diagram illustrating the process for transcriptomic data collection.

a. Perform variant calling for both single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels) using the Genome Analysis Toolkit (GATK) with default settings.

b. Annotate the identified variants and predict their effects using SnpEff software.

17. Variant Filtering.

a. Apply stringent filtering criteria to SNPs using GATK with the following quality parameters: minimum quality depth of 2.0, minimum QUAL score of 30.0, maximum Fisher Strand value of 60.0, minimum mapping quality of 40.0, maximum Symmetric Odds Ratio of 3.0, and specific thresholds for MQRankSum (−12.5) and ReadPosRankSum (−8.0). Additionally, implement cluster-based filtering with a window size of 10 and cluster size of 3.

b. For indels, apply separate filtering criteria including minimum quality depth of 2.0, maximum Fisher Strand value of 200.0, minimum QUAL score of 30.0, maximum Symmetric Odds Ratio of 10.0, and the same thresholds for MQRankSum and ReadPosRankSum as used for SNPs.

RNA extraction and sequencing profiles from different time points

Timing: 3 days

The step describes how the protocol to extract total RNA of yeast strains collected from different time points, the sequencing strategy and data analysis (Figure 2).

18. Dilute saturated yeast cultures 1:100 in 15 mL fresh SC medium, followed by incubation at 30°C with continuous shaking.

19. When cultures reach mid-log phase (OD600 = 0.6–0.7), harvest 8 mL of each culture for RNA extraction.

20. Extract total RNA using the QIAGEN RNeasy Plus mini kit (Cat No. 74136) according to the manufacturer's instructions.
21. Submit the purified RNA samples for paired-end sequencing on the Illumina HiSeq platform through Genewiz or an equivalent service provider, targeting a minimum data generation of 1G per sample.
22. Quality Control and Data Processing.
    a. Exclude any samples with fewer than 15 million total reads or showing signs of RNA degradation.
    b. Process the qualifying samples by trimming 15 base pairs from the 5′-end using FASTX Toolkit (parameter: -f 16 -z), followed by read cleaning using Trim Galore with specifications for minimum length and quality (parameters: –length 100 –quality 30).
23. Read Alignment and Filtering.
    a. Align the processed reads to the S. cerevisiae S288C reference genome using STAR aligner.
    b. Filter the resulting BAM files to retain only primary alignments using SAMtools with the parameter "-F 0x100", removing all secondary alignment records.
24. Expression Quantification. Quantify gene expression levels using Featurecounts with the following parameters: paired-end mode (-p), 20 threads (-T 20), multi-mapping read handling (-M), largest overlap mode (–largestOverlap), targeting exon features (-t exon), and using gene_id as the meta-feature (-g gene_id).
25. Special Considerations. Remove the features RDN37-1 and RDN37-2 from the GTF file before proceeding with downstream analysis, as these represent redundant annotations of rRNA transcripts (both pre-transcript and mature forms) in the original genome annotation.

Note: Use genome of S. cerevisiae strain S288C as the reference (version R64-1-1; http://www.yeastgenome.org) with genome annotation file (version R64-1-1.104, genome-date 2011-09, genome-build-accession GCA_000146045.2, genebuild-last-updated 2018-10).

EXPECTED OUTCOMES

Using this protocol, we successfully knocked out a specific gene from the yeast genome and conducted serial transfers of the knockout strain in the laboratory. Throughout the transfer period, we monitored growth rates as well as genomic and transcriptomic profiles to assess the effects of the gene deletion. The expected outcomes included distinct growth rate patterns between the knockout and wild-type strains, as well as genomic and transcriptomic profiles reflecting the absence of the target gene and potential compensatory changes in gene expression. For comprehensive details, please refer to Bei et al.[1]

All positive clones obtained from the gene deletion described in Step 'knock out target genes in yeast strains' were verified by PCR. Figure 3A displays the gel electrophoresis results for the ADE1-URA3 fragment, showing the expected target fragment of approximately 1335 bp, indicating successful insertion of URA3 into the *ADE1* region. Figure 3B illustrates the gel electrophoresis results for genotype verification during the serial transfer experiment. Using the same PCR reaction master mix, the target gene was detected only in wild-type (WT) strains, while URA3 was present in all replicates of the deletion strains. This confirms that the genotypes of the serial transfer samples remained uncontaminated throughout the experiment.

QUANTIFICATION AND STATISTICAL ANALYSIS

Identify differentially expressed genes using the negative binomial generalized linear models provided by DESeq2 software package. The analysis compares knockout strains to wild-type strains, as well as between knockout strains at adjacent time points. For effect size shrinkage, the apeglm method is recommended. Differentially expressed genes are determined based on established thresholds for $\log_2$ fold change ($\log_2$FC) and adjusted p-values.

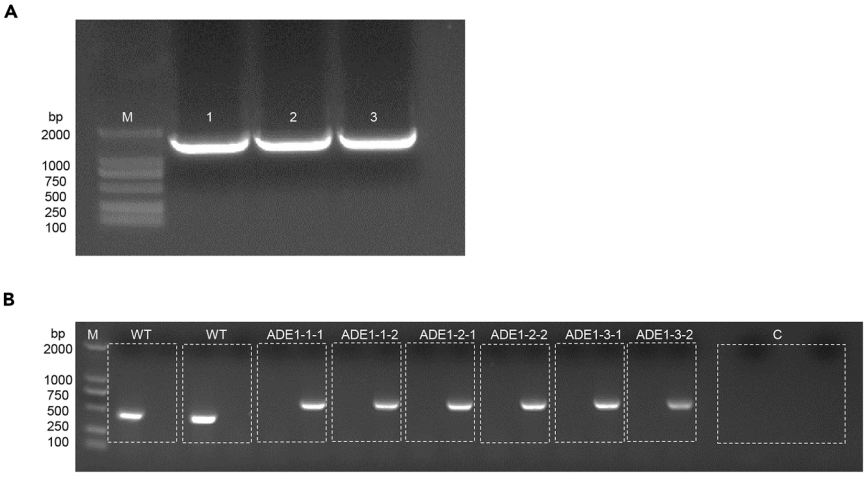

Figure 3. Gel electrophoresis from PCR of ADE1-URA3 fragment and Confirmation of the deleted loci of all samples in deletion lines

(A) shows the amplified ADE1-URA3 fragment with a length of approximately 1335 bp. *ADE1* is our target gene. M indicates marker 2000.

(B) displays the genotype check of serial transfer samples. The sample left lane amplified fragment is *ADE1* gene (length 439 bp). Right lane is URA3 (length 620 bp). C indicates negative control and M indicates marker 2000. WT represents the wild-type strain, while the subsequent lanes indicate different clones or parallel samples with ADE1 knocked out.

Further analysis of TPM (Transcripts Per Million) values for each gene is performed using RStudio. Specific examples of analyses include generating heatmaps and other visualizations (Figure 4). Dimensionality reduction is conducted using the UMAP algorithm from the R package umap, which projects the transcriptomes (log_2TPM) of all strains into two dimensions using default parameters.

LIMITATIONS

While we have developed a method that effectively highlights progressive transcriptomic changes resulting from specific gene loss, it is primarily suited for low-throughput analysis. To maintain optimal cell growth conditions, we employed a large culture system (15 mL), which unfortunately limits throughput. For other experimental purposes requiring more parallel experiments, using a plate shaker with smaller culture system (100–150 μL) may be a viable alternative.

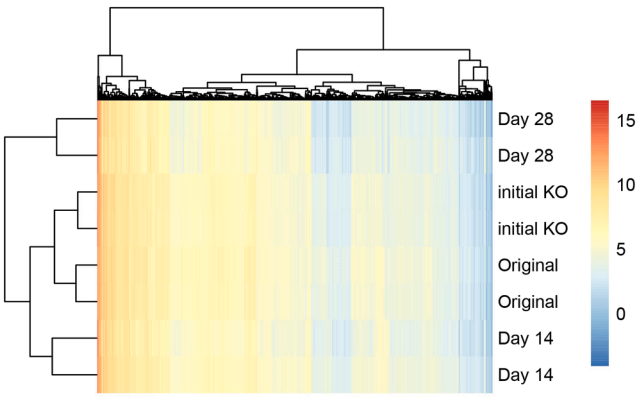

Figure 4. Heatmap of gene expression profiles for replicates of the original strain and ADE1 knockout strains at different cultivation times

Gene expression levels are estimated by log_2TPM.

TROUBLESHOOTING

Problem 1

Low transformation efficiency can lead to few or no positive clones (related to "Gene deletions: knock out target genes in yeast strains" section).

Potential solution

- Ensure that the yeast culture is at the appropriate density (OD600 of 0.5).
- Prepare fresh PEG/LiAc solutions and ensure all reagents are of high quality.
- Optimize the amounts of ssDNA and homologous fragments used in the transformation.
- Consider using a higher concentration of PEG or LiAc if transformation efficiency is low.

Problem 2

Transformed yeast may not grow on SC-URA plates if the selection pressure is too high or if the media is not prepared correctly (related to "Gene deletions: knock out target genes in yeast strains" section).

Potential solution

- Verify that the SC-URA plates are correctly prepared and fresh.
- Check the viability of the yeast strain used for transformation.

Problem 3

PCR may fail to amplify the target genes from the lysis product, leading to false negatives (related to step 2-i in "step-by-step method details").

Potential solution

- Optimize PCR conditions (e.g., annealing temperature, extension time).
- Ensure that the lysis buffer is effective in breaking down the cell wall and releasing DNA.
- Include positive controls in the PCR to validate the assay.

Problem 4

Interruptions in the serial transfer process could lead to loss of adapted phenotypes (related to step 6 in "step-by-step method details").

Potential solution

- Set reminders or use automated systems to ensure timely transfers every two days.
- Prepare fresh media in advance for each transfer to minimize delays.

Problem 5

Inaccurate dilution ratios can lead to either overdilution or underdilution, affecting culture density (related to step 6 in "step-by-step method details").

Potential solution

- Use calibrated pipettes and ensure accurate measurements during transfers.
- Double-check calculations for dilution ratios before each transfer.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact Li Liu: liul47@mail.sysu.edu.cn.

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contacts, Bei Jiang: kathryn.jiang@connect.polyu.hk.

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper does not report original code.

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AUTHOR CONTRIBUTIONS

L.L. and B.J. designed the protocol; B.J. and C.X. conducted the experiments and data analysis; L.L. and B.J. wrote the paper. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2025.103857.

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