Medium

LB

Materials

- 10 g/L tryptone
- 5 g/L yeast extract
- 5 g/L NaCl
- (15 g/L agar bacteriological agar type E)

Dissolve in demineralized water, stir and autoclave.

YEPD

Materials

- 10 g/L Yeast Extract
- 20g/L Peptone
- 20g/L Dextrose
- (20 g/L bacteriological agar type E)

Dissolve in demineralized water, stir and autoclave.

BG-11

Materials

Materials	**Concentration（g/L）**
NaNO3	1500
K2HPO4	40
MgSO4	36.6
CaCl2	27.2
Citric acid	6
Ammonium ferric citrate	6
EDTANa2	1
Na2CO3	20
H3BO3	2.86
MnCl2·4H2O	1.81
ZnSO4	0.22
Na2MoO4·2H2O	0.39
CuSO4·5H2O	0.08
CoCl2·6H2O	0.0409
HEPES	1
Total	1643.2

Methods of use

1. Take a beaker and add 1 L of deionized water. Turn on the magnetic stirring and add 1.64 g of culture medium until completely dissolved;
2. Adjust pH 7.3-7.5 with 1 M hydrochloric acid;
3. Sterilize under high pressure at 121 ℃ for 15 minutes and store at room temperature for future use.
4. If it is necessary to make a culture medium plate, agar (15g/L) should be added before sterilization

^{CoY}BG-11

Our co-culture medium was made with BG-11 medium to which we added

Materials

- 0.36g/L Yeast Nitrogen Base without animo acids
- 106mM NaCl
- 25mM HEPPSO
- 1mM KPO_3
- 40μM DCMU
- 190mM $Na_2S_2O_3$

Molecular biology 1

Gibson Assembly

Materials

- Linearized plasmid
- Primer extended insert
- Gibson HiFi DNA Assembly Master Mix (NEB)
- Deionized water
- 0.2 mL Eppendorf tubes
- Pipette and tips
- Incubator
- DNA fragments

Protocols

1 Dilute the linearized plasmid to a final concentration of 25ng/μL

2 On ice, assembly the following 10 μL-scale Gibson Reaction

i. 5μL Gibson Master mix
ii. 25ng plasmid DNA
iii. 5-6x molar excess of DNA fragment
iv. Up to 10 μL with deionized water.

3 Incubate the reactions for 60 minutes at 50 degrees, followed by storage at 4 degrees

4 use up to 4 μL of Gibson Assembly Plasmid for transformation.

Restriction Digestion

Materials

- Insert
- Plasmid
- Deionized water
- Pipette and tips
- rSAP enzyme
- Restriction enzymes (HindIII NheI)

Protocols

1. Combine the following components to get the digestion mixture

a) 1000 ng insert

b) 5µL CutSmart Buffer

c) 1µL Each Restriction Enzymes

d) Up to 5µL with deionized water

2. Incubate mixtures for 1 hour at 37 degrees
3. Determine the concentration of DNA using the NanaDrop Spectrophotometer
4. Store these DNA at 4 degrees

Ligation of Digestion Products

Materials

- Digested plasmid
- Digested insert
- T4 ligase
- Ligase buffer (10x)
- Deionized water
- Pipette and tips
- Bucket of ice

Protocols

1. Prepare DNA dilution to obtain a maximum of 20 ng/µL.
2. Mix the following. Also, make a negative control by combining everything except the
3. insert. (Ideal molar ratio is 3:1, insert: plasmid)

a) 20 ng digested plasmid

b) 20 ng digested insert

c) 2 µL ligase buffer

d) 1 µL T4-Ligase

e) Add up to 20 µL with deionized water

4. Let the mix incubate either at 16 °C for 1.5 hours or at 4 °C overnight. The temperature and time of the ligation might have to be optimized for each ligation.
5. Store ligation product in a -20 °C freezer.

Molecular biology 2

DNA PCR

Reagent:

Double distilled water, 2xPhanta(®) Max Master Mix (Dye Plus) P525 or Golden Mix(Green)(TSINGKE, production: TSE101), Template DNA(plasmid DNA), Forward primer(10μM), Reverse primer(10 μM), Ice

Device:

Pipette, Pipette tips, PCR tube, Icebox, PCR-Instrument, Centrifuge

Procedure:

1. Turn on the PCR instrument in advance.
2. Mix reagents on ice according to the table below in the PCR tube:
3. Mix the solution system evenly. Carry out rapid centrifugation if the droplets hang on the tube wall.
4. Put the PCR tube in the PCR-Instrument. Set the PCR program according to the table below.
5. Take the tube out and put it in the icebox containing ice.

Components / Volume

- *2 x Phanta® Max Master Mix (Dye Plus) P525 or Golden Mix(Green) / 25 μl*
- *Forward primer(10 μM) / 2 μl*
- *Reverse primer(10 μM) / 2 μl*
- *Template DNA(plasmid DNA) / xμl(10 pg-30 ng)*
- *Double distilled water / up to 50 μl*

Gel Preparation

Reagent:

1xTAE buffer, Agarose, 10000xNucleic acid dye

Device:

Pipette, Pipette tips, Measuring cylinder, Electronic balance, Medicine spoon, Microwave oven, Gel-making tank, Gel-making plate, Gel-making comb

Procedure(for a 1% nucleic acid electrophoresis gel):

1. Accurately weigh 0.3 g agarose and then add 30 ml of 1xTAE buffer.
2. Heat to boiling in the microwave oven at least 3 times to dissolve agarose completely.
3. Put a gel-making plate on the gel-making tank and put a gel-making comb on it.
4. Add 3 μl 10000xNucleic acid dye in the agarose solution when it's cooled to about 60℃.
5. Pour the agarose solution into the gel-making mold.
6. Wait about 20 minutes until the gel sets. Remove the comb.

Sample loading and Electrophoresis

Reagent:

Nucleic acid electrophoresis gel, DNA marker, PCR product, 1xTAE buffer

Device:

Pipette, Pipette tips, Electrophoresis apparatus, Electrophoresis tank for nucleic acid

Procedure:

1. Add 1xTAE buffer into the electrophoresis tank for nucleic acid. Put the gel into the tank and make sure it can be submerged by TAE buffer.
2. Make sure the loading pore is close to the negative side.
3. Add 10 μl DNA marker into the loading pore. Add 50 μl PCR product into the loading pore. Record the position of the sample added.
4. Open the electrophoresis apparatus and connect it with the electrophoresis tank for nucleic acid.
5. Set the voltage to 100 V. Run 30 minutes or as appropriate.

Gel Imaging

Reagent:

Post-electrophoresis nucleic acid gel

Device:

Gel imager(azure biosystems c150), Computer, Disposable gloves

Procedure:

1. Take out post-electrophoresis gel with disposable gloves on the hands.
2. Open the computer and gel imager. Open the software of the gel imager.
3. Put the gel in the imager. Set the exposure mode and capture the image of the gel.
4. Find the target bands and save the image.

Gel Recovery

Reagent:

Post-electrophoresis nucleic acid gel, Gel recovery kit(FastPure(®) Gel DNA Extraction Mini Kit DC301 from Vazynme), double distilled water

Device:

UV Transilluminator, Scalpel, 1.5ml EP tube, Metal bath heater, Pipette, Pipette tips, Electronic balance

Procedure:

1. Set the metal bath heater to 55℃ in advance.
2. Precisely cut the gel containing the target bands with a scalpel under the UV transilluminator.
3. Accurately weigh the pieces of gel. Put them into 1.5 ml EP tubes respectively.
4. According to the instructions in the kit recover the DNA fragment until the last step. Use double distilled water but not the buffer to dissolve the DNA fragment.

DNA concentration determination

Reagent:

Gel recovery product, double distilled water

Device:

NanoDrop one ultramicro ultraviolet spectrophotometer(Thermo SCIENTIFIC), Lens papers, Pipette, Pipette tips

Procedure:

1. Turn on the NanoDrop one in advance.

2. Select the "nucleic acid " tab and click the "double strain DNA" option on the home page of NanoDrop one.

3. Clean the instrument base with double distilled water. Wipe clean with lens papers.

4. Use 1 μl of double distilled water as a blank sample. After detecting, wipe clean with lens papers.

5. Add 1 μl gel recovery product to the base for detection. After detecting, record data and clean the instrument base with double distilled water. Wipe clean with lens papers.

6. Shut down the instrument after use.

Gibson Assembly

Reagent:

Gel recovery product, double distilled water, Exnase II, 5xCE II buffer, Ice

Device:

Pipette, Pipette tips, Icebox, PCR-Instrument, PCR tube

Procedure:

1. Open the PCR-instrument in advance.

2. Mix reagents on ice according to the table below in the PCR tube:

3. Mix the solution system evenly. Carry out rapid centrifugation if the droplets hang on the tube wall.

4. Put the PCR tube in the PCR-Instrument. Set the PCR program according to the table below.

5. Take the tube out and put it in the icebox containing ice.

Yeast protocol

Yeast Transformation 1

Materials

- Yeast cells
- YPD medium
- Flask 1 L
- 30°C incubator with agitation
- Table-top Centrifuge
- 1X TE/1X LiAc solution
- Template DNA
- Vortex
- PEG/LiAc solution
- DMSO
- 1X PEG solution
- 42°C water bath
- Selective YPD agar plate

Protocols

1. Inoculate 1 mL of YPD with several 2–3 mm diameter colonies
2. Vortex vigorously for 5 min to disperse clumps
3. Transfer into a flask containing 50 mL of YPD or the appropriate SD medium

4. Incubate at 30°C for 16–18 h with shaking at 250 rpm to reach stationary phase (OD600>1.5)
5. Transfer 30 mL of overnight culture to a flask containing 300 mL of YPD. Check the OD600 of the diluted culture and, if necessary, add more of the overnight culture to bring the OD600 up to 0.2–0.3
6. Incubate at 30°C for 3 hr with shaking (230 rpm). At this point, the OD600 should be 0.4–0.6
7. Place cells in 50-mL tubes and centrifuge at 1,000 g for 5 min at room temperature (20–21°C)
8. Discard the supernatant and thoroughly resuspend the cell pellets in sterile TE or distilled H2O. Pool the cells into one tube (final volume 25–50 mL)
9. Centrifuge at 1,000 g for 5 min at room temperature.
10. Decant the supernatant.
11. Resuspend the cell pellet in 1.5 mL of freshly prepared, sterile 1X TE/1X LiAc
12. Add 0.1 µg of plasmid DNA and 0.1 mg of carrier DNA to a fresh 1.5-mL tube and mix.

Notes:

a) For simultaneous cotransformation (using two different plasmids),use 0.1 µg of each plasmid (an approximately equal molar ratio),in addition to the 0.1 mg of carrier DNA.
b) For transformations to integrate a reporter vector, use at least 1 µg of linearized plasmid DNA in addition to the carrier DNA.

1. Mix 0.1 mL of yeast competent cells to each DNA tube and mix well by vortexing
2. Add 0.6 mL of sterile PEG/LiAc solution to each tube and vortex at high speed for 10 s to mix
3. Incubate at 30°C for 30 min with shaking at 200 rpm
4. Add 70 µL of DMSO, mix well by gentle inversion (do not vortex)
5. Heat shock for 15 min in a 42°C water bath
6. Chill cells on ice for 1–2 min
7. Centrifuge cells for 5 s at 14,000 rpm at room temperature. Remove the supernatant
8. Resuspend cells in 0.5 mL of sterile 1X TE buffer
9. Plate 100 µL on each YPD agar plate that will select for the desired transformants. To ensure that you will obtain a plate with well-separated colonies, also spread 100 µL of a 1:1000, 1:100, and 1:10 dilution on YPD agar plates. These will also serve as controls for cotransformation efficiency. Note: If you are performing a cotransformation, plate controls to check transformation efficiency and markers of each plasmid. On separate 100-mm plates, spread 1 µL (diluted in 100 µL H2O) on medium that will select for a single type of plasmid.
10. Incubate plates, upside-down, at 30°C until colonies appear (generally, 2–4 days).

Yeast Transformation 2

Reagent:

1. 10× Tris–EDTA (TE) buffer pH 7.5: 100 mM Tris–HCl pH 8.0, 10 mM EDTA. Set pH to 7.5 with acetic acid. Sterilize by autoclaving.
2. 10× lithium-acetate (LiAc) solution pH 7.5:10.2 g LiAc in 100 mL water, set pH to 7.5 with acetic acid. Filter sterilize.
3. 1× TE/LiAc buffer pH 7.5: prepare freshly by mixing equal amounts of 10× TE buffer and 10× LiAc solution. Add sterile water to obtain 1× working concentration for each component.
4. 50% (w/v) PEG 3350 solution; 50 g PEG 3350 in 100 mL water. Sterilize by autoclaving.
5. Carrier DNA: 10 mg/mL in water, salmon sperm DNA

Device:

Pipette, Sterile Pipette tips, Icebox, Metal bath heater, Ultra clean bench, Disposable spreader, 1.5ml EP tube

Procedure:

1. Incubate 50 µL of competent yeast cells at 30°C for 15 min.
2. Heat-denature 15 µL carrier DNA by incubation at 100°C for 10 min. Chill on ice.
3. Prepare transformation master mix by mixing 30 µL 10× TE pH 7.5 buffer, 30 µL 10× LiAc pH 7.5 solution, and 240 µL 50% (w/v) PEG 3350 solution.
4. Add the denatured carrier DNA to the transformation master mix and vortex immediately.
5. Add 50 µL competent yeast cells (from step 1) and vortex immediately.
6. Add 5 µg plasmid DNA (see Note 3) to be transformed and vortex immediately.
7. Incubate cells with plasmid DNA at 30°C for 30 min.
8. Incubate sample at 42°C for 20 min (heat shock).
9. Spin for 1 min in a microcentrifuge at full speed (about 16,000 × g).
10. Discard supernatant and re-suspend the pellet in 100–200 µL sterile water.
11. Spread on solid selective medium and incubate plates at 30°C for 3 days until transformed colonies appear

Co-culture protocol

Materials

- Synechococcus elongatus PCC7942 $CscB^{+}$
- Saccharomyces cerevisiae $W303^{clump}$
- ^{CoY}BG-11
- YEPD
- BG-11
- IPTG(1mM)

Protocol

1. characterization of S. elongatus growth and sucrose production

S. elongatus was cultured axenically in baffled flasks of ^{CoY}BG-11 and allowed to acclimate for ≥ 12 hours. Then cultures were adjusted to 25 mL with a final density of 0.5 OD750. IPTG (1 mM) was added, as appropriate. This was the start of the experiment and is referred to as time 0. Cultures were monitored at 24 hour intervals by withdrawal of 1 mL culture. OD750was measured via photospectrometer (ThermoScientific NonoDrop 2000c) and culture supernatant was analyzed for sucrose content via a colorimetric Glucose-Sucrose Assay.

2. prepare Saccharomyces cerevisiae $W303^{clump}$ with previous successfully imported plasmids

Single colonies were picked into YEPD media and grown until turbid at 30℃ before co-culture. Then Cells were diluted into the appropriate co-culture media +0.2% sucrose to acclimate to co-culture media, and maintained within log phase growth (OD600 < 0.70) before use in co-cultures. Acclimating cultures were grown at 35 °C, 150 rpm, 2% CO2, in light (PAR = ~80 µmol with 15 W Gro-Lux Sylvania fluorescent bulbs) within a Multitron Infors HT incubator. Yeast growth was measured by inoculating rinsed cells at 0.05 OD600 into fresh co-culture media at the indicated sucrose concentration. Data for growth rate was collected from 25 mL flask cultures while 96-well plates with

1 mL culture volumes were used to assay growth in a gradient of sucrose concentrations as well as growth in conditioned media; OD600 of plates were read on a BioTek Synergy Neo plate reader.
3.co-culture the prepared two strains
Flask co-cultures were completed in 25 mL volumes in baffled flasks. Co-cultures were grown at 35 °C, 150 rpm, 2% CO2, in light (15 W; Gro-Lux; Sylvania) within a Multitron Infors HT incubator. 1 mM IPTG was added when indicated. Inoculate cyanobacteria and yeast at OD_{750}=0.5 and OD_{750}=0.03 respectively.

Detection

HPLC

Sucrose

To analyze sucrose secretion, 1 mL of culture was collected and centrifuged at maximum speed for 5 minutes. The supernatant was collected and then filtered through a 0.2 μm filter. The sucrose secretion was quantified by HPLC with an Aminex HPX-87K Column This potassium-form, 300 x 7.8 mm column and mQ water as mobile phase. Column was kept at 70°C, flow rate 0.6 mL/min and analysis time 20 minutes.

Supporting Information of Molecular biology 1

Plasmids

1. p406GAL1: Vector with GAL1 promoter, Used as a backbone for the cloning of pSWI
2. https://www.addgene.org/17437/
3. pSP-G1: Used as a backbone for the cloning of pCYC
4. https://www.addgene.org/64736/
5. pLC41: Used as a backbone for the cloning of pCAF
6. https://www.novopro.cn/vector/Vg42tana

DNA Sequences

1. CPH: https://www.ncbi.nlm.nih.gov/nuccore/LC435389
2. H3H: https://www.ncbi.nlm.nih.gov/nuccore/LC435367
3. HispS: https://www.ncbi.nlm.nih.gov/nuccore/LC435355
4. NPGA: https://www.ncbi.nlm.nih.gov/nuccore/AF198117
5. Luz: https://www.ncbi.nlm.nih.gov/nuccore/LC435379
6. C3H: https://www.ncbi.nlm.nih.gov/nuccore/MF416092
7. TAL: https://www.ncbi.nlm.nih.gov/nuccore/LT629805
8. CPR1: https://www.ncbi.nlm.nih.gov/nuccore/EF104642

Primers

1.1-14 are used to Construct plasmids by Gibson Assembly
2.15-16 are used to add Restriction Site by PCR (HindIII and NheI)

Number	Sequences 5’-3’
1	AAGTGTTTGTGAACTATTGAATTCTTATTCAAACGAAAACGCTGGGG GTTCAG

2	CTGTTGCGGTTGGTGGAGTAGGTGAGGGCAAGAGTGTAAACGGAACTGACCCC
3	TCTACAAATGGCAGGAAGATATCTCCGTGATGTATGGTAACTCAAGGAGACTTTCG
4	GATCGAAGGGAGATAACTTAACGGAAGTTGTTGATTGCATAAAGGAGACTCTAGTTTAGC
5	CGGAGGCGTCTGAGGACAATCGATACTAGTGCGG
6	GAATTCTCAAACGATGCCATATGGATTACAAGGATGAC
7	TCGCTAGTGGCGGACACGAGATGAATTCCAGCAAGAATC
8	AAGCGGCCGCACTAGTATCGATTGTCCTCAGACGC
9	GGTTGGCACGGTCAATGAACATGGCGCCAATTTC
10	GGATTCTTGCTGGAATTCATCTCGTGTCCGCC
11	TAAGAAGTTCTAATTCTGCCGTCAACTTATGTAAACAGGT
12	GTTGAAGAAATTGGCGCCATGTTCATTGACCGTGC
13	TCGTCATCCTTGTAATCCATATGGCATCGTTTGAGA
14	ACCTGTTTACATAAGTTGACGGCAGAATTAGAACTTCTTAG
15	CCCAAGCTTATGACCCTCCAGTCCC
16	CTAGCTAGCCTAGGCGGGGGGG

3.Hint: All primers are designed with the help of SnapGene.

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[1]Li S, Sun T, Xu C, Chen L, Zhang W. 2018. Development and optimization of genetic toolboxes for a fast-growing cyanobacterium Synechococcus elongatus UTEX 2973. Metabolic Engineering. 48:163–174. doi:10.1016/j.ymben.2018.06.002.

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[3]Lin P-C, Zhang F, Pakrasi HB. 2020. Enhanced production of sucrose in the fast-growing cyanobacterium Synechococcus elongatus UTEX 2973. Scientific Reports. 10(1):390. doi:10.1038/s41598-019-57319-5.

[4] Hays SG, Yan LLW, Silver PA, Ducat DC. Synthetic photosynthetic consortia define interactions leading to robustness and photoproduction. J Biol Eng. 2017 Jan 23;11:4. doi: 10.1186/s13036-017-0048-5. PMID: 28127397; PMCID: PMC5259876.