Transformation of *P. pastoris*

Linearization of pPICZ α A Vector

Aim

Linearization of pPICZ α A vector by restriction digest with SacI.

Materials

- SacI restriction enzyme
- Vector DNA
- Nuclease-free water
- 10X digestion buffer
- Acetylated BSA (only for Promega enzymes)

Procedure

I. If using the FastDigest SacI enzyme from Thermo Fisher Scientific:

1. Thaw enzyme and 10X FastDigest Buffer on ice.
2. Add the following reagents in the following order:

Component	Amount
Water, Nuclease-Free	Adjust to a final volume of 50 µl
10x FastDigest Buffer	5 µl
Plasmid DNA	5 µg (volume depending on the DNA concentration)
FastDigest SacI	5 µl
Total Volume	50 µl

3. Mix gently and spin down.
4. Incubate at 37°C in a heat block for 30 minutes.
5. Check a small aliquot of your digest by agarose gel electrophoresis for complete linearization.
6. If the vector is completely linearized, heat inactivate at 65°C for 5 minutes (optional) and proceed with ethanol precipitation to purify and concentrate the DNA.

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II. If using the SacI enzyme from Promega:

1. Thaw enzyme, BSA and Buffer J on ice.
2. Add the following reagents in the following order:

Component	Amount
Water, Nuclease-Free	Adjust to a final volume of 50 µl
10x FastDigest Buffer	5 µl
Plasmid DNA	5 µg (volume depending on the DNA concentration)
Acetylated BSA	1 µl
SacI	2.5 µl
Total Volume	50 µl

3. Mix gently and spin down.
4. Incubate at 37°C in a heat block for 1-4 hours.
5. Check a small aliquot of your digest by agarose gel electrophoresis for complete linearization.
6. If the vector is completely linearized, proceed with ethanol precipitation to purify and concentrate the DNA.

Ethanol Precipitation

Aim

Concentrating and purifying nucleic acids (DNA or RNA) preparations in aqueous solution.

Materials

- DNA preparation to be precipitated
- 3M Sodium acetate pH 5.2
- Ethanol (95%-100%)

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- Ethanol 70%
- Distilled H_2O

Procedure

1. Add 1 volume of sodium acetate (3M, pH 5.2) and 2.5 volumes of ice-cold ethanol (95-100%) to the solution to be precipitated.
2. Incubate at -20°C overnight.
3. Centrifuge at 13,000 RPM at 4°C for 30 min and pipette off the supernatant.
4. Wash with 1 ml of 70% ethanol, making sure not to dislodge the pellet by pipetting against the opposite wall.
5. Decant the supernatant quickly, making sure not to discard the pellet.
6. Leave the tube to dry for 5 min at 37°C or until the liquid has evaporated. Do not let the pellet dry for too long as it will become more difficult to dissolve.
7. Add an appropriate volume of dH 2 O. Do not resuspend as the DNA will stick to the pipette tip.
8. Let the tube stand at room temperature for 30 min before quantifying.

Preparing Electrocompetent *P. pastoris* Cells

Aim

Preparing electrocompetent *Pichia pastoris* cells for transformation by electroporation.

Materials

- *P. pastoris* strain of choice
- YPD medium
- Sterile ice-cold (0°C) water
- Sterile ce-cold (0°C) 1M sorbitol

Procedure

1. Grow 20 mL of your *Pichia pastoris* strain in YPD in an erlenmeyer flask at 30°C overnight.
2. Inoculate 200 mL of fresh medium in a 1 liter flask with 25–100 µl of the overnight culture. It is recommended to inoculate several flasks with different concentrations to make sure that the right OD is reached. Grow overnight to an OD600 = 1.3–1.5.

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3. Centrifuge the cells at 900g for 5 minutes at 4°C. Resuspend the pellet with 200 ml of ice-cold (0°C), sterile water.
4. Centrifuge the cells as in Step 3, then resuspend the pellet with 100 ml of ice-cold (0°C), sterile water.
5. Centrifuge the cells as in Step 3, then resuspend the pellet in 8 ml of ice-cold (0°C) 1 M sorbitol.
6. Centrifuge the cells as in Step 3, then resuspend the pellet in 0.8mL of ice-cold 1 M sorbitol. Keep the cells on ice and use that day or snap freeze in liquid nitrogen for storage at -80°C.

Notes

Make sure to keep the cells on ice as much as possible during resuspension, as warming of the cells will reduce the efficiency of the procedure.

Transformation of *P. pastoris* by Electroporation

Aim

Preparing electrocompetent *Pichia pastoris* cells for transformation by electroporation.

Materials

- Electrocompetent *P. pastoris* cells
- 7-10µg of linearized pPICZ α DNA
- 0.2 cm electroporation cuvette
- Sterile ice-cold (0°C) 1M sorbitol
- Electroporation device
- YPDS + Zeocin plates

Procedure

1. Mix 80 µl of the cells from electrocompetent cells with 7–10 µg of linearized pPICZ α DNA (in 5–10 µl sterile water) and transfer them to an ice-cold (0°C) 0.2 cm electroporation cuvette.
2. Incubate the cuvette with the cells on ice for 5 minutes.

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3. Pulse the cells according to the parameters for yeast (Saccharomyces cerevisiae) as suggested by the manufacturer of the specific electroporation device being used or manually set the parameters to: 1.5 kV, 200 Ω, 25 µF.
4. Immediately add 1 ml of ice-cold 1 M sorbitol to the cuvette. Transfer the cuvette contents to a sterile Eppendorf tube.
5. Let the tube incubate at 30°C without shaking for 1 to 3 hours (longer incubation will increase transformation efficiency).
6. Spread 100, 200µl and the rest (spin down, decant supernatant and resuspend cells in remaining liquid) each on separate, labeled YPDS plates containing 100 µg/ml of Zeocin.
7. Incubate plates for 4 to 5 days at 30°C until colonies form.
8. Pick 10–20 colonies and purify (streak for single colonies) on fresh YPDS + Zeocin plates.

References

This protocol is a modified version of the protocols found in the pPICZ α A, B, and C manual (MAN0000035) from Invitrogen, as well as the the product manual of FastDigest SacI (Thermo Fisher Scientific) and Promega SacI.

The ethanol precipitation protocol was designed by Salla Koskela at the division of Glycoscience at KTH Royal Institute of Technology.