Yeast plasmid miniaturization kit

Project number: Y666144

Storage conditions: room temperature.

Products

component	/
Buffer P1	15ml
Buffer P2	15ml
Buffer N3	20ml
Buffer PS	15ml
Buffer PB	10ml
Buffer PW (concentrate)	10ml
Buffer EB	10ml
Glass Beads	2g
RNase A (10 mg/ml)	150 μl
Spin Columns DM with Collection Tubes	50

Products

This kit is improved on the basis of common alkaline lysis method, the glass beads can effectively break the yeast cell wall, the new silica matrix membrane and buffer system can efficiently and specifically bind the plasmid DNA, and at the same time can maximize the removal of proteins and other impurities, the whole process is convenient and fast, no need to use toxic and harmful reagents, and can be processed at the same time for multiple samples. In addition to yeast cells, it can also be used in E. coli. Plasmid DNA extracted with this kit can be used in various molecular biology experiments, such as ligation, transformation, sequencing and library screening.

Self-contained reagents: β-mercaptoethanol, anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. All components can be stably stored in dry, room temperature (15-30℃) environment for 1 year, the adsorption column can be stored at 2-8℃ for a longer period of time, and Buffer P1 with RNase A can be stably stored at 2-8℃ for 6 months.

2. Before the first use, add all the RNase A solution to Buffer P1, mix well, and store at 2-8℃.
3. Anhydrous ethanol should be added to Buffer PW before first use according to the instructions on the reagent bottle label.
4. Before use, please check whether Buffer P2 and Buffer N3 are crystallized or precipitated. If there is any crystallization or precipitation phenomenon, it can be clarified by taking a water bath at 37℃ for a few minutes to restore the clarity.
5. Be careful not to touch Buffer P2 and Buffer N3 directly, and tighten the lid immediately after use.
6. The amount of plasmid extracted is related to the yeast strain, plasmid copy number, culture conditions, etc. Usually, yeast plasmid copy number is very low, which is difficult to be detected by electrophoresis or spectrophotometer method.

Procedure

1. Take 1-5 ml of yeast culture (maximum 5×107 yeast cells, generally for Saccharomyces cerevisiae OD = 1.0, equivalent to 1-2×107 cells/ml) and add it to a centrifuge tube (self-provided), centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g), collect the bacterial precipitate, and aspirate as much as possible to discard the supernatant.
2. Add 250μl Buffer P1 to the bacterium (please check if RNase A has been added first) and resuspend the precipitate.
3. Add 40mg of Glass Beads to the above mixture and vortex and shake for 10 minutes.
4. Add 250 μl of Buffer P2 to the centrifuge tube, mix gently by turning up and down 6-8 times, and let stand at room temperature for 5-10 minutes, at which time the bacterial solution should become clear and viscous.
Note: Mix gently, do not shake violently, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid. If the solution does not become clear, it suggests that the amount of bacteria may be too large and the lysis is not complete, and the amount of bacteria should be reduced.
5. Add 350 μl of Buffer N3 to the centrifuge tube and immediately mix gently up and down 6-8 times, at which point a white flocculent precipitate appears, and centrifuge at 12,000 rpm for 20 minutes.
Note: Buffer N3 should be mixed immediately after addition to avoid localized precipitation.
6. Column Equilibration: Add 200 μl of Buffer PS to the Spin Columns DM in the collection tube, centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and place the column back into the collection tube.
7. Add the supernatant from step 5 to the adsorbent column that has been loaded into the collection tube, taking care not to aspirate the precipitate.
Note: The maximum volume of the adsorption column is 750 μl, and the solution is passed through the column in 2 times.
8. Centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube and place the adsorption column back into the collection tube.

9. Add 150 μl Buffer PB to the adsorbent column, centrifuge at 12,000 rpm for 1 min, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.
10. Add 750 μl Buffer PW to the adsorption column (please check that anhydrous ethanol has been added first), centrifuge at 12,000 rpm for 1 minute, and pour off the waste liquid in the collection tube.
11. Place the column back into the recovery collection tube and centrifuge at 12,000 rpm for 2 minutes, pouring off the waste liquid. Leave the column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
12. Place the adsorbent column in a new centrifuge tube, add 50-100 μl of Buffer EB to the center of the adsorbent membrane dropwise, let it stand at room temperature for a few minutes, centrifuge at 13,000 rpm for 1 minute, and collect the plasmid solution into the centrifuge tube. Store the plasmid at -20° C.
Attention:
1) To increase the recovery efficiency of the plasmid, the resulting solution can be reintroduced into the adsorbent column, left at room temperature for a few minutes, centrifuged at 13,000 rpm for 1 minute, and the plasmid solution collected into a centrifuge tube.
2) When the plasmid copy number is low or >10 kb, Buffer EB is preheated at 65-70° C in a water bath, which can increase the extraction efficiency.
3) Usually yeast plasmids have very low copy number and are difficult to detect by electrophoresis or spectrophotometry. If the extracted plasmid is to be used in the next step of the experiment, it is usually recommended to use 1-5μl of the plasmid as PCR template, and 5-10μl of the plasmid for transformation of E. coli.
4) Commercial high transformation efficiency receptor cells should be used for transformation of E. coli.