invitrogen
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MaV203 Competent Yeast Cells, Subcloning Scale

Catalog no. 11445-012

Size: 0.4 mL
Store at –80°C
(Do not store in liquid nitrogen)

Description

MaV203 Competent Yeast Cells, Subcloning Scale have been developed for the routine introduction of plasmid DNA into yeast cells for use with the ProQuest™ Two-Hybrid System or for other appropriate yeast-based applications. The high transformation efficiency of these cells (≥1 × 10^6 transformants per µg DNA) make them suitable for most of the applications in two-hybrid screening, including the assessment of protein-protein interactions by two known genes, optimization of 3-amino 1,2,4-triazole concentrations, and the reassessment of candidate interactions identified from library screens. For screening of cDNA libraries, MaV203 Competent Yeast Cells, Library Scale (Cat. no. 11281-011) are available.

MaV203 contains deletions in the endogenous *GAL4* and *GAL80* genes for use with most *GAL4*-based two-hybrid systems. This strain has been constructed with three *GAL4*-inducible reporter genes for identification of interacting fusion proteins: *GAL1*::*lacZ*, *HIS3*$_{UASGAL1}$::*HIS3* and the counterselectable *SPAL10*$_{UASGAL1}$::*URA3*.[1–3] MaV203 is also auxotrophic for leucine (*leu2*) and tryptophan (*trp1-901*), allowing for the selection of yeast transformed with *GAL4* DNA binding domain (DB) vectors and *GAL4* activation domain (AD) vectors (*e.g.*, pDBLeu and pPC86 from the ProQuest™ Two-Hybrid System). MaV203 also contains the recessive resistance alleles *cyh*2^R and *can*^{R} which are useful for plasmid shuffling.

Component	Amount per Vial
MaV203 Competent Yeast Cells	4 × 100 µL
PEG/Lithium Acetate (LiAc) Solution	2 × 1.5 mL
pMAB12 DNA, 20 ng/µL	10 µL

Genotype

MATα; leu2-3,112; trp1-901; his3Δ200; ade2-101; cyh2^R*; can1*^{R}*; gal4Δ; gal80Δ; GAL1::lacZ; HIS3*$_{UASGAL1}$*::HIS3@LYS2; SPAL10* $_{UASGAL1}$*::URA3*.

Note: While the genotype of MaV203 is *ade2*$^-$, the strain remains white upon starvation for adenine or amino acids, but retains an *ade*2 deficiency.

Transformation Procedure

1. Thaw the PEG/LiAc Solution in a beaker containing room temperature water before the assay. Mix the solution well before dispensing.
2. Thaw competent cells by placing in a 30°C water bath for no more than 90 seconds. Proceed immediately to step 3. Steps 3, 4, and 5 can be done at room temperature.
3. Once the cells are completely thawed, invert the cells several times and transfer 100 µL to a 1.5-mL autoclaved microcentrifuge tube (see Note 1). **Do not vortex the cells**.
4. To each 100 µL aliquot of cells, add 100 ng of vector DNA. Mix well by tapping the tube. As a control transformation, add 5 µL of pMAB12 DNA to another tube containing 100 µL aliquot of cells (see Note 2).
5. Add 600 µL of the PEG/LiAc Solution to each tube. Mix well by inverting the tubes until all of the components are homogeneous.
6. Incubate for 30 minutes in a 30°C water bath. Invert the tubes occasionally (every 10 minutes) to resuspend the components.
7. Add 35.5 µL of DMSO to each tube (see Note 3). Mix well by inversion.
8. Heat shock the cells for 20 minutes in a 42°C water bath. Invert the tubes occasionally.
9. Centrifuge tubes for 5 minutes at low speed (1800 rpm; 200–400 × *g*) in a microcentrifuge. Carefully discard the supernatant.
10. Suspend each pellet in 1 mL autoclaved saline (0.9% NaCl) by gentle pipetting.
11. Depending on the application, prepare the necessary dilutions, and plate each dilution onto the appropriate selective medium. To determine the transformation efficiency, remove 100 µL and dilute 1:100 and 1:1000 in autoclaved saline. Repeat for the control transformation and plate it on Synthetic Complete Medium minus Leucine. Incubate the plates at 30°C for 60–72 hours.

Notes

1. **Do not freeze/thaw.** Competent yeast can only be thawed once without dramatic loss in competency.
2. The addition of carrier DNA is not required.
3. For best results, use fresh DMSO from an unopened bottle. DMSO stored at –20°C can be used.
4. Number of colonies per transformation reaction = Colonies/plate × dilution factor(s)

   For example, if 30 colonies are counted when 100 µL of a 1:1000 dilution are plated, the calculation would be:

   $$30 \times \frac{1\ \text{mL}}{0.1\ \text{mL plated}} \times 10^3 = 3.0 \times 10^5\ \text{colonies/reaction}$$

   $$\text{Transformation efficiency per µg of DNA} = \frac{\text{number of colonies/ reaction}}{\text{µg of DNA}}$$

Control Plasmids

The control DNA pMAB12 (*LEU2*) provided with this kit is used to confirm the transformation efficiency of the cells and that the selection plates are functioning correctly. This plasmid does not contain the elements necessary to do a screen with the ProQuest™ Two-Hybrid System. A control transformation using 100 ng pMAB12 and 100 µL MaV203 Competent Yeast Cells should generate $\geq 1 \times 10^5$ colonies per reaction.

References

1. Vidal, M., Brachmann, R.K., Fattaey, A., Harlow, E., and Boeke, J.D. (1996) *Proc.Natl. Acad. Sci. 93*, 10315.
2. Vidal, M., Braun, P., Chen, E., Boeke, J.D., and Harlow, E. (1996) *Proc.Natl. Acad. Sci. 93*, 10321.
3. Vidal, M. (1997) The Reverse Two-Hybrid System in *The Two-Hybrid System* (Barel, P. and Fields, S., eds.), Oxford University Press, NY, 109.
4. Hill, J. K., Donald, A.I.G., and Griffiths, D.E. (1991) *Nucleic Acids Res. 19*, 5791.

Information for European Customers
The MaV203 yeast strain is genetically modified and contains three GAL4-inducible reporter genes stably integrated into the genome. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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