Aureobasidin A-Resistant Yeast Transformation System

Aureobasidin A..... Cat. #9000

pAUR101 DNA ..... Cat. #3600
(Chromosomal Integrating Shuttle Vector)

pAUR112 DNA ..... Cat. #3601
(Autonomously Replicating Shuttle Vector)

pAUR123 DNA ..... Cat. #3602
(Expression Shuttle Vector)

♦ Notice ♦

This product is for Aureobasidin A Resistant Yeast Transformation System. Please read the following provisions before use. Opening the package of the product means that you have consented to the provisions. When you do not consent to the provisions, do not open the package and contact us at :

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Provisions :

1. Limitation of use and prohibition of transfer

This product is for research use only. Do not use for other purposes.
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Aureobasidin A-Resistant Yeast Transformation System is a transformation system for *Saccharomyces cerevisiae* using an antibiotic Aureobasidin A (AbA) and a vector including a resistant gene AUR1-C. AUR1-C is a dominant gene and can make cells resistant to AbA when only a single or a few copies of plasmid are transformed into a cell. In addition, this system can provide transformed cells efficiently, even when the host is wild-type yeast (haploid and diploid) without auxotrophic mutation.

[Features]

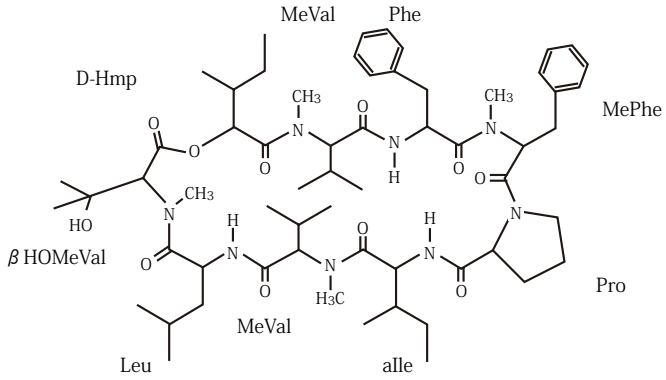
- Transformation into wild-type or industrial yeast (e.g. beer yeast or bread yeast) without auxotrophic mutation is achieved efficiently.
- Selective medium does not need a complicated operation of adding auxotrophic amino acids except Aureobasidin A into complete medium, such as YPD medium.
- It is extremely effective for screening of gene library with industrial yeast as a host.
- Transformation efficiency into *Saccharomyces cerevisiae* is equal to that obtained when auxotrophic selective marker is used (1 ～ 5 × 10^4 transformants/μg DNA by lithium acetate method).

1. Description :

(1) Aureobasidin A

Aureobasidin A (AbA) is a cyclic depsipeptide antibiotic with molecular weight of 1,100 isolated from *Aureobasidium pullulans* R106.

Aureobasidin A is toxic at a low concentration (0.5 μg/ml) against fungi (including yeast), such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, and some of the Aspergillus.



Structure of Aureobasidin A

Table1. Minimum Inhibitory Concentration(MIC) of Aureobasidin A against various Yeast Strains

Strain		MIC (μg/ml)
S. cerevisiae	ATCC9763 (diploid)	0.2 ～ 0.4
	SH3328 (haploid)	0.1
	Sake yeast (diploid)	0.1 ～ 0.2
	Shochu yeast (diploid)	0.1
	Beer yeast (triploid or tetraploid)	0.1
	Baker's yeast (diploid)	0.2 ～ 0.4
Schizo. pombe	JY-745 (monoploid)	0.1
C. albicans	TIMM-0136 (diploid)	0.04
C. tropicalis	TIMM-0324 (diploid)	0.08

(2) pAUR vectors

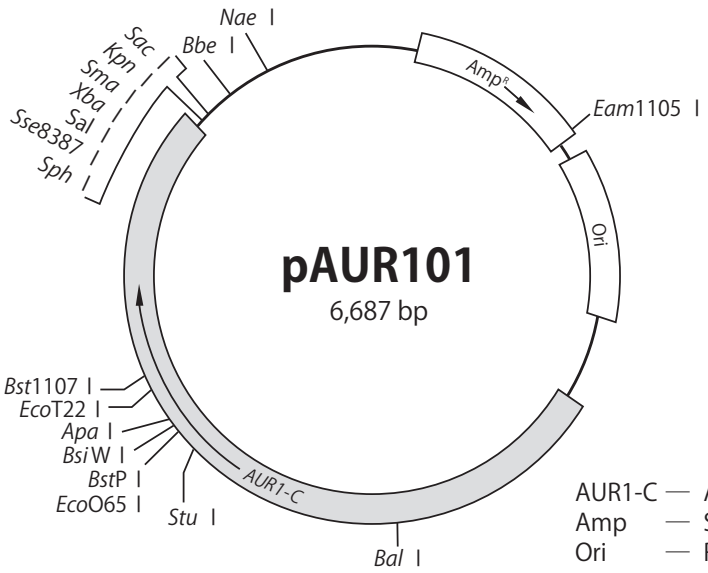
The pAUR vectors are *E.coli*-yeast shuttle vectors. These vectors have a mutant AUR1-C gene derived from *S. cerevisiae* as a selective marker for transformation of yeast. When *S. cerevisiae* is transformed with these vectors, transformants obtain resistance to antibiotic Aureobasidin A (AbA). The transformant can be selected on the selective medium including AbA.

pAUR101 is a chromosomal integrating vector to keep a target gene stable in transformant. pAUR101 does not replicate autonomously in yeast, and is maintained only when integrated into a chromosome by homologous *rec*ombination.

pAUR112 is a vector that can be kept as a plasmid in yeast cells. pAUR112 is a plasmid including CEN/ARS which can replicate autonomously in yeast, so is stable in cell division. Since this vector has CEN sequence, a copy number of this plasmid is low.

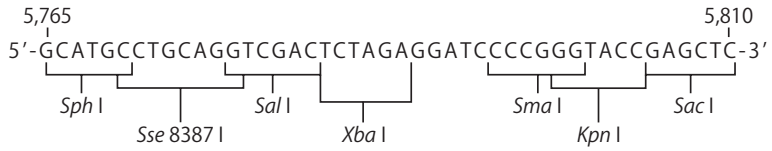
pAUR123 is a plasmid vector for protein expression derived from pAUR112. This vector includes a promoter of an alcohol dehydrogenase 1 (ADH1) gene as an expression promotor.

● **Restriction Enzyme Map of pAUR101 DNA**



AUR1-C — AbA-resistant gene in *S. cerevisiae*
Amp — Selective marker in *E. coli*
Ori — Replication origin in *E. coli*

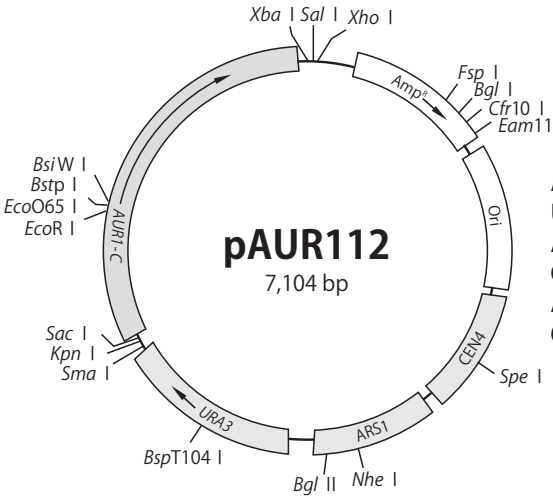
● **Cloning Sites of pAUR101 DNA**



Single cutting site in AUR1-C gene
*Bst*P I, *Eco*O65 I, *Bsi*W I, *Stu* I

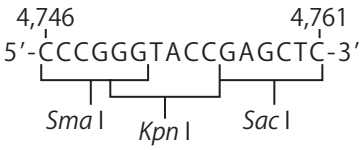
＊When transforming yeast, DNA should be cut at a single site using one of the above restriction enzymes. In the case of transformation into *E.coli*, no need to cut DNA by a restriction enzyme.

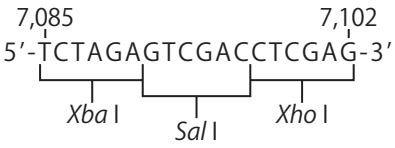
● Restriction Enzyme Map of pAUR112 DNA



AUR1-C — AbA-resistant gene in *S. cerevisiae*
URA3 — Uracil selective marker in *S. cerevisiae*
ARS — Replication origin in *S. cerevisiae*
CEN — Centromere in *S. cerevisiae*
Amp — Selective marker in *E. coli*
Ori — Replication origin in *E. coli*

● Cloning Sites of pAUR112

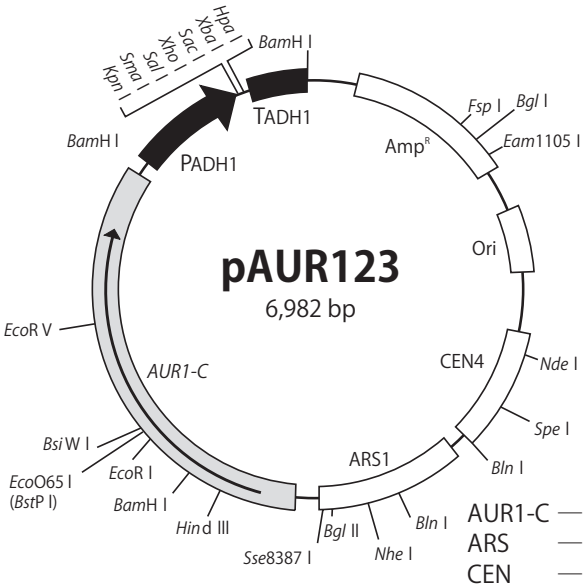




● Restriction Enzyme Map of pAUR123 DNA

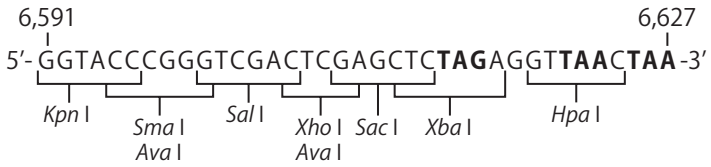
pAUR123 is a vector for protein expression derived from pAUR112. This vector includes a promoter of alcohol dehydrogenase 1 (ADH1) gene as an expression promoter [3]. Inserted into a cloning site of pAUR123, a target gene (including an initiation codon ATG)* will be expressed constitutively in yeast cells. The multicloning site includes 3 stop codons of three different reading frames.

* It is *rec*ommended that a target gene has a shorter 5'-noncoding sequence.



AUR1-C — AbA-resistant gene in *S. cerevisiae*
ARS — Replication origin in *S. cerevisiae*
CEN — Centromere in *S. cerevisiae*
Amp^r — Selective marker in *E. coli*
Ori — Replication origin in *E. coli*
PADH1 — Promotor of alcohol dehydrogenase 1 gene
TADH1 — Terminator of alcohol dehydrogenase 1 gene

● Cloning Sites of pAUR123 DNA



ADH1 promoter of pAUR123 is a promoter for consititutive expression. Aureobasidin A-resistant transformant will express a target protein, when it is grown in a complete medium(e.g YPD).

2. Protocol

2-1. Transformation of Yeast

Guide to Yeast Genetics and Molecular Biology (1991) and the Methods in Enzymology 194 (Academic Press) are *rec*ommended as references for the general treatment and the gene manipulation of yeast.

(1) Reagents :

- YPD medium : per liter
  Yeast extract..... 10 g
  Polypeptone ..... 20 g
  D-glucose..... 20 g

- YPD agar plate: Add 2 % agar to YPD medium.

- YPD selective medium :
  ＞ 0.5 μg/ml of Aureobasidin A
  ＊Sensitivity to Aureobasidin A may vary depending on the host strains used.

- Aureobasidin A stock solution
  Dissolve in ethanol or methanol to a concentration of 500 μg/ml, and store at 4 ℃. (A long-term storage is possible.)

- Carrier DNA
  After sonicating salmon testis DNA (10 mg/ml) into 3 ～ 15 kbp fragments, heat at 100 ℃ for 10 minutes, cool quickly, and use.

- Solution A : 100 mM Lithium acetate, 10 mM Tris-HCl pH 7.5, and 1mM EDTA.＊1
- Solution B : Dissolve 40 g of Polyethylene Glycol 4000 in 100 ml of Solution A. Prepare at the time of use.＊1, ＊2
  ＊1 : Solution A and B should be filter-sterilized.
  ＊2 : It is *rec*omended to prepare Solution B at the time of use to increase the transformation efficiency.

(2) Transformation of *S. cerevisiae* :

1. Add 0.5 ml of overnight-cultured yeast to 50 ml YPD medium (1:100 dilution).
2. Incubate at 30℃, for approximately 6 hours, to OD_{660} of 1 ～ 2. When using diploid, OD_{660} should be 2 ～ 4.
3. Centrifuge cells at 1000 × g for 5 minutes.
4. Resuspend the pellet in 10 ml Solution A, and centrifuge at 1000 × g for 5 minutes.
5. Resuspend the pellet in Solution A to the final OD_{660} of 150.
6. Dispense 100 μl cell suspension into microcentrifuge tubes, and incubate at 30 ℃ for 1 hour.
7. Add 5 μg vector (circular or linearized DNA), and 150 μg carrier DNA which was heated at 100 ℃ for 10 minutes and cooled quickly just before addition.
   ＊ In the case of pAUR101, linearized DNA should be used for transformation. Applying circular DNA strikingly lowers the transformation efficiency and no transformants might be obtained.
   In the case of pAUR112 and pAUR123, intact plasmid DNA should be used for transformation.

8. Add 850 μl Solution B, and mix gently.
9. Incubate at 30 ℃ for 30 minutes, and then incubate at 42 ℃ for 15 minutes.
10. Leave at room temperature for 10 minutes.
11. Centrifuge at 5,000 rpm for 1 minute, and resuspend the pellet in 5 ml of YPD medium.
12. Culture at 30 ℃ for 6 hours to overnight.
13. Centrifuge 5,000 ～ 10,000 rpm and resuspend the pellet in 1 ～ 10 ml of 0.9 % NaCl.
14. Plate out 100 μl of the cell suspension onto YPD selective medium plates. Transformants will appear in 3 ～ 4 days when cultured at 30 ℃.

2-2. Transformation into *E. coli*

Transformation into* *E. coli* HB101 or JM109 can be performed by a conventional method using shuttle vector pAUR101 or pAUR112 with DNA fragments inserted.

*It is possible to transform into *E.coli* containing *rec*A$^-$ as genotype.

3. Application example of transformation

Industrial yeasts (e.g. sake yeast, shochu yeast, baker's yeast, and beer yeast) was transformed with pAUR112, and transformation efficiency was examined. Transformation was carried out by following the above-mentioned protocol. Transformation efficiency is shown as colony number of transformant per 1 μg plasmid DNA. In case of absence of plasmid DNA, no colony was observed on a selective medium.

Table 2. Transformation efficiency of industrial yeasts using pAUR112

Strain	Conc. of Aureobasidin A (μg/ml)		
	0.5	1.0	2.0
Sake yeast	4.2×10^4	3.0×10^4	4.9×10^3
Shochu yeast	2.1×10^4	8.4×10^3	1.6×10^3
Baker's yeast	2.5×10^3	870	240
Beer yeast	2.0×10^3	1.3×10^3	350

4. Q & A

Q-1. Can transformant be selected on the medium containing 0.5 μg/ml Aureobasidin A in all strains of *S. cerevisiae*?

A-1. The Aureobasidin A concentration for selection depends on the sensitivity of a host cell.When minimum inhibitory concentration (MIC) of a host cell is 0.1 ～ 0.4 μg/ml, transformant can be selected at 0.5 μg/ml. When the MIC of a host cell is lower than 0.05 μg/ml or higher than 0.5 μg/ml, please adjust the concentration in the medium to 2 ～ 5 times of the MIC .

Q-2. When *S. cerevisiae* is transformed with chromosomal integrating vector pAUR101, what restriction enzymes can be used ?

A-2. Please use *Bst* PI, *Eco*O65 I, *Bsi*WI, or *Stu* I, which cuts only one site in the AUR1-C gene.

Q-3. For expression of a protein using pAUR123, is induction step necessary?

A-3. No need for induction. ADH1 promotor of pAUR123 is a promotor for a constitutive expression. When a AbA-resistant transformant is grown on a suitable medium (e.g. YPD), the transformant expresses a protein constitutively.

Q-4. How long is the maximum size of DNA that can be subcloned into pAUR vectors?
A-4. It was confirmed that about 3 kbp DNA was subcloned. Theoretically, it is possible to subclone 7 ～ 8 kbp DNA.

Q-5. Can pAUR vectors be used for yeasts other than *S.cerevisiae* ?
A-5. It was confirmed that pAUR101 can be used in *S. cerevisiae*, *Candida glabrata*, *Kluyveromyces marxianus*, and *K. lactis*. When these strains were transformed using linear pAUR101 which was cut in one site (e.g. cloning site), except the site of AUR1-C gene or a target gene, DNA was integrated into chromosome by non-homologous *rec*ombination. The transformants resistant to Aureobasidin A were obtained at the efficiency of 10 ～ 10^3/ μg DNA.
pAUR112 and pAUR123 can be kept as a plasmid only in *S.cerevisiae*. If these plasmids are used in transformation of yeasts except for *S.cerevisiae*, linearized vectors should be used.
Regarding *Schizosaccharomyces pombe*, *Pichia pastoris*, *Candida albicans*, pAUR vector cannot be used because AUR1-C gene is not functional.

Q-6. How does Aureobasidin A work to inhibit the growth of yeasts?
A-6. Aureobasidin A inhibits a yeast's enzyme, Inositol phosphoryl-ceramide (IPC) synthase which acts in synthetic pathway of IPC. The AUR1-C gene was constructed by introducing a dominant muta-tion into the *aur* 1 gene of *S. cerevisiae* which is considered to code IPC synthase [4].

Q-7. Can *S.cerevisiae*, which has no plasmid, become a resistance to Aureobasidin A?
A-7. The mutant of *S.cerevisiae* spontaneously resistant to Aureobasidin A has not been confirmed.
A spontaneous mutation conferring resistance to Aureobasidin A may hardly occur.

5. References

1) Takesako, K., Kuroda H., Inoue T., Haruna F., Yoshikawa Y., Kato I., Uchida K., Hiratani T. and Yamaguchi H. (1993) *J. Antibiot*. **46**, 1414-1420.
2) Hashida-Okado, T., Ogawa, A., Endo, M., Yasumoto, R., Takesako, K. and Kato, I. (1996) *Mol. Gen. Genet*, **251**, 236-244.
3) Mumberg D., Muller R. and Funk M. (1995) *Gene* **156**, 119-122.
4) M. M. Nagiec et al., (1997) *J. Biol. Chem*., **272**, 9809-9817.

6. Notes

1) This system is for research use only. Not for therapeutic or diagnostic purpose for humans or animals. Neither for foods, toiletries, nor house hold articles.
2) This product is sold for research used only. A license must be obtained for industrial use.

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