使用说明

黑曲霉菌株 ST31 是 orotidine-5'-phosphate decarboxylasedeficient mutant 需要在 complete medium with 5mM Uridine +10mM uracilr 或仅 10mM Uridine 中生长. ST31 带上质粒 ANEP2_SP2 后，可以在 minimal medium 上生长，AMA1 是可以让 ANEp2 在 Aspergillus 中自主复制的 sequence. ANEp2 上有 AP/pUC sequence, 可以让这个质粒在 E. coli (ampicillin resistance)中扩增. ANEP2-_SP 的克隆位点是 Asc I 和 Fse I. 这个载体不能用 gateway technology 进行克隆. PyrG 是选择标记.

下面是一段 ANEP2-_SP 的构建方法:

For the construction of the vector ANEp2 with the signal peptide of *A. niger* glucoamylase (ANEp2_SP), ANEp2_AscI DNA was digested with *Nhe*I and *Asc* I and ligated with two complementary oligonucleotides, 5'-CTAGCAATGTCGTTCC GATCTCTACTCGCCCTGAGCGGCCTCGTCTGCACAGGGTTGGCAAATGTG ATTTCCAGG-3' and 5'-CGCGCCTGGAAATCACATTTGCCAACCCTGTGCAGACGAG GCCGCTCAGGGCGAGTAGAGATCGGAACGACATTG-3', which encode the first 25 amino acids of glucoamylase with *Nhe*I at the 5' end and *Asc*I at the 3' end.

ASPERGILLUS MEDIA

Minimal medium(MM): for 1 liter of solid medium use:

(A)	Agar	12.5g; Steam in 750 ml of demineralized or distilled water
(B)	Salts	50ml of "20x Salt mix-$MgSO_4$"
	$MgSO_4$	0.52g of $MgSO_4$ solution
	C-source	10g of Glucose
	TE	1ml of Trace element stock solution (see next page)
	Water	

Mix (A)with (B) , dispense,supplement as needed, autoclave, and pour plates, or store at room temperature until needed.

Complete medium (CM); for 1 liter of solid CM)

To(B) of minimal medium , add the following(before agar):

Difco-Bacto Peptone	2g
Difco-Bacto yeast Extract	1g
Casamino Acids	1g
Vitamin Solution (see next page)	1ml
Water	

Mix with (A) of MM, dispense to flasks, autoclave and pour as needed.

Acetate Medium: for 1 liter of solid medium

Ammonium acetate	12g
NaCl	2g
$MgSO_4.7H_2O$	0.5g
KH_2PO_4	3g
Trace Element	1ml
Agar	12.5g
Water	up to 1 liter

Adjust Ph to 6 by 4N-HCl ,and autoclave

Salt Mix-MgSO4(20X stock)

To make up 1 liter, dissolve in 800 ml distilled water

$NaNO_3$(sodium nitrate)	120.0g
KCl(potassium chloride)	10.4g
KH_2PO_4(potassium monobasic)	16.3g
K_2HPO4(phosphate dibasic)	20.9g

Hutner's Trace Elemnet Solution : use 1 ml per liter of medium

To make up 100ml, dissolve in 80ml distilled water(in sequence, one at a time)

$ZnSO_4.7H_2O$(Zinc sulfate)	2.2g
H_3BO_3(boric acid)	1.1g
$MnCl_2.4H_2O$(Manganous chloride)	0.5g
$FeSO_4.7H_2O$(Ferrous sulfate)	0.5g
$CoCl_{l2}.6H_2O$(cobaltous chloride)	0.16g
$CuSO_4.5H2O$(Cupric sulfate)	0.16g
$(NH4)6Mo_7O_{24}.4H_2O$(Ammonium molybdate)	0.11g
EDTA, tetrasodium salt	6.5g
EDTA, disodium salt	0.77g

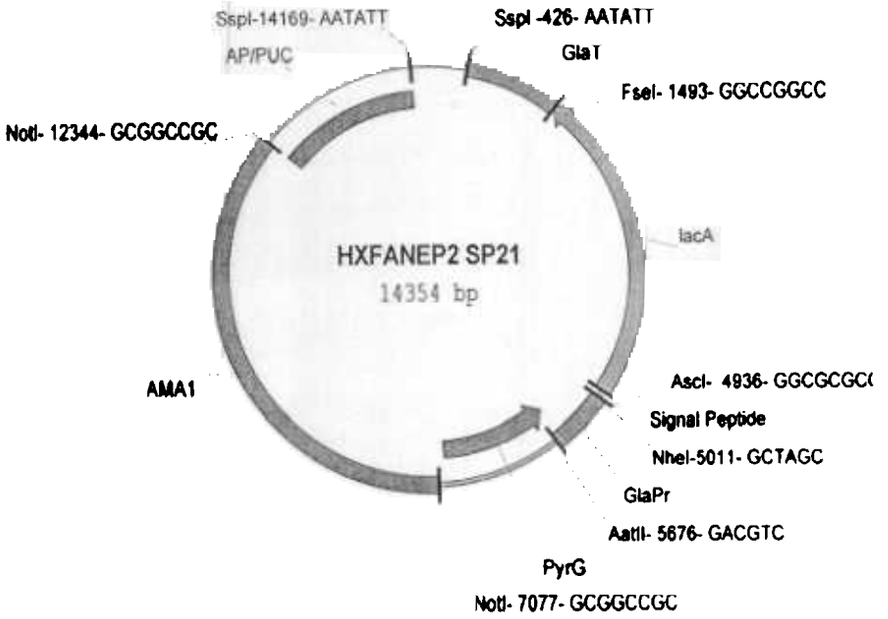
Fill up to 100ml. Autoclave and srore at 4℃; unadjusted Ph will be 6.5

Vitamin Solution : use 1 ml per liter of medium

To make up 200ml dissolve in 160ml distilled water

Pyridoxin-HCl	0.02g
Thiamin-HCl	0.03g
p-aminobenzoic acid	0.15g
Nicotinic acid	0.5g
Riboflavin	0.5g
Chohne-HCl	4.0g
Biotin(use conc.stock:50mg/100ml)	10ml

ANEP2_SP2 质粒图谱



AP/pUC 可以让载体在 E. coli (ampicillinresistance) 中扩增的序列

AMA1 基因，是可以让载体在 Aspergillus 中自主复制的序列

PryG 是编码 orotidinean-5'-phosphate decarboxylase（尿苷合成的必需成分）的基因

GlaPr 是 A. niger 的 glucoamylase 这个基因上的启动子

Signal Peptide 是 A. niger 的 glucoamylase 这个基因上的信号肽

GlaT 是 A. niger 的 glucoamylase 基因上的终止子

AscI 和 **FseI** 为可克隆位点