pPIC9K 载体

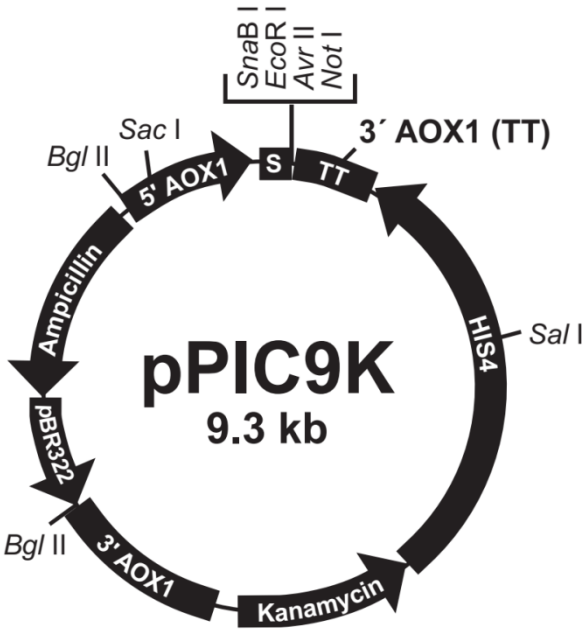
载体基本信息

出品公司: Invitrogen
载体名称: pPIC9K, pPIC 9K
质粒类型: 毕赤酵母蛋白表达载体
表达水平: 高拷贝
启动子: AOX1
克隆方法: 多克隆位点，限制性内切酶
载体大小: 9276 bp
5'测序引物及序列: 5′ AOX1:5′-GACTGGTTCCAATTGACAAGC-3′； alpha-Factor-F: 5′-TACTATTGCCAGCATTGCTGC-3′
3'测序引物及序列: 3′ AOX1:5′-GCAAATGGCATTCTGACATCC-3′
载体标签: N-alpha factor
载体抗性: 氨苄和卡那
筛选标记: HIS4
备注: 利用 alpha 分泌因子，分泌表达蛋白。

载体质粒图谱和多克隆位点信息

Comments for pPIC9K:
9276 nucleotides

5′ *AOX1* promoter fragment: bases 1-948
5′ *AOX1* primer site: bases 855-875
α-Factor secretion signal(s): bases 949-1218
α-Factor primer site: bases 1152-1172
Multiple Cloning Site: bases 1192-1241
3′ *AOX1* primer site: bases 1327-1347
3′ *AOX1* transcription
termination (TT): bases 1253-1586
HIS4 ORF: bases 4514-1980
Kanamycin resistance gene: bases 5743-4928
3′ *AOX1* fragment: bases 6122-6879
pBR322 origin: bases 7961-7288
Ampicillin resistance gene: bases 8966-8106



载体简介

pPIC9K 载体中存在卡那抗性基因，允许利用卡那抗性在酵母体内筛选多克隆拷贝，载体其余部分与 pPIC9 载体完全一样。pPIC9K 载体能够使用的毕赤酵母宿主菌是 KM71 和 GS115。
pPIC9K 载体详细信息如下：
1. pPIC9 载体大小 9276 bp，是融合表达载体。
2. 载体构建过程中，可供使用的单一限制性内切酶位点为 SnaB I, EcoR I, Avr II, Not I.
3. 利用 alpha 因子分泌信号肽，分泌表达蛋白基因。
4. 在载体构建过程中，你的基因必须保证与信号肽的起始密码子的读码框一致。
5. 毕赤酵母中利用 HIS4 进行筛选。
6. 为了将基因插入 GS115 或 KM71 的 AOX 区，使用 SacI 限制性内切酶线性化质粒(GS115 中产生 His+Mut+基因型，KM71 中产生 His+ MutS 基因型)
7. 为了将基因插入 GS115 或 KM71 的 His4 区，使用 Sal I 或者 Stu I 限制性内切酶线性化质粒(GS115 中产生 His+ Mut+基因型，KM71 中产生 His+ MutS 基因型)

8. 将基因插入 GS115 的 AOX1 区域，需要使用 Bgl II 线性化质粒 (产生 His+MutS 基因型)。

载体序列：

AGATCTAACATCCAAAGACGAAAGGTTGAATGAAACCTTTTTGCCATCCGACATCCAC
AGGTCCATTCTCACACATAAGTGCCAAACGCAACAGGAGGGGATACACTAGCAGCAG
ACCGTTGCAAACGCAGGACCTCCACTCCTCTTCTCCTCAACACCCACTTTTGCCATCGA
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