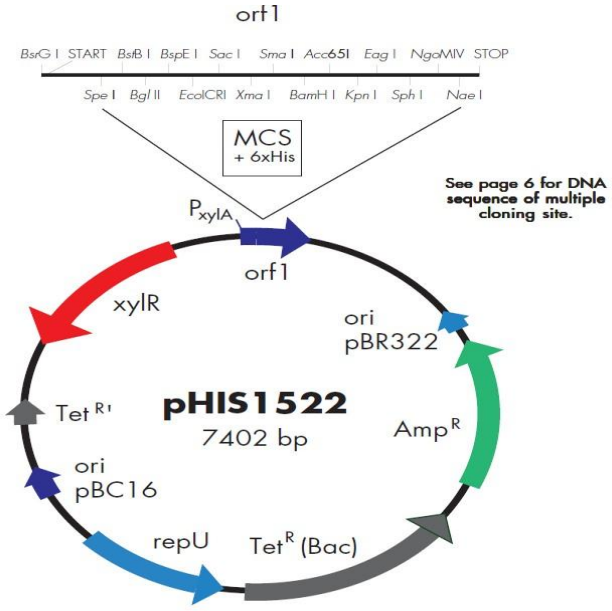
载体基本信息

平台编号:	bio-106970
载体名称:	pHIS1525
质粒类型:	巨大芽孢杆菌表达载体
高拷贝/低拷贝:	--
启动子:	--
克隆方法:	多克隆位点，限制性内切酶
载体大小:	7402 bp
5' 测序引物及序列:	--
3' 测序引物及序列:	--
载体标签:	6xHis
载体抗性:	氨苄青霉素和四环素
筛选标记:	四环素
备注:	--
产品目录号:	--
稳定性:	--
组成型:	--
病毒/非病毒:	--

载体参考质粒图谱和多克隆位点信息



载体简介

Plasmids pHIS1522/pHIS1525 both contain the strong xylA promoter (PxylA) originating from Bacillus megaterium. Transcription from this promoter is xylose inducible (after xylose addition, the xylose repressor coded by the xylR gene on the plasmids is released from PxylA and transcription is initiated). The most

convenient cloning sites for insertion of DNA fragments carrying heterologous genes are located in an open reading frame (orf1) under control of the xyloseinducible promoter PxylA (15 unique restriction sites; see sequence). Therefore, any protein can be expressed using one out of three functionally different fusion strategies.

A transcriptional fusion requires that the gene of interest carries its own ribosome binding sequence (RBS) and translation initiation codon. Such a DNA fragment can be fused into any of the available restriction sites within orf1. Whether the resulting transcriptional fusion leads to expression of the gene of interest, which is independent from orf1 expression, depends on the location of the created orf1 stop codon with respect to the start codon of the gene of interest. lf these are close together, translational coupling may occur, in which the ribosomes translating the orf1 reading frame would terminate at its stop codon, creating a locally high concentration of ribosomes, so that translation initiation at the new start codon would be more efficient compared to a construct in which the orf1 translation terminates farther away from the start codon.

On the other hand, if the orf1 reading frame continues for a long distance into the reading frame of the gene of interest, the ribosomes translating the created orf1 fusion protein might inhibit initiation of translation of the protein of interest. Therefore, it is advisable to pay attention to placement of a stop codon when constructing the gene fusion. Taken together, although a transcriptional or operon fusion is constructed, the efficient translation of the orf1 reading frame, and any fusion thereof created by the insertion, is likely to, positively or negatively, influence the translation efficiency of the gene of interest.

Alternatively, a truncated version of the gene of interest, lacking its own start codon, may be fused in frame to the orf1 reading frame on pHIS1522/pHIS1525 to create a translational or protein fusion. This will result in expression of a chimeric protein consisting of up to 18 amino acids specified by the orf1 encoding sequence, followed by the sequence encoded by the gene of interest.

Using the BsrGI restriction site directly before the ATG start codon enables cloning without changing the N-terminus of the protein of interest. pHIS1525 contains a sequence encoding the B. subtilis extracellular esterase (LipA) and allows cloning of target genes directly after the signal peptidase restriction site using the Kas I, Nar I, or SfoI restriction sites.

It is important to note that the multiple cloning site and its reading frame are identical in pHIS1522 and pHIS1525 starting from BglII. Hence, a parallel cloning strategy of the gene of interest in pHIS1522 for intracellular and in pHIS1525 for extracellular production is possible. The 6xHis sequence upstream the MCS allows convenient purification and detection of the expressed Histagged target proteins. For expression of target proteins without a 6xHis tag, suitable plasmid constructs (pMM1522 & pMM1525) are also available.