牛肠激酶轻链基因的克隆及其在大肠杆菌中的融合表达Cloning and Fusion Express
Light Chain Gene in Escherichia Coli

摘要为克隆表达牛肠激酶（enterokinase）轻链(EKL)编码基因，以期应用于融合蛋白的切割与纯化
组织中提取总RNA,以RT-PCR方法扩增其cDNA片段，将此片段克隆于pUCmT载体中，经过特异性限制
后，进行全序列分析。结果表明,克隆的cDNA与GenBank上的序列相比完全一致，得到了编码正确的牛
后，将目的基因片段插入pET39b中，构建了融合型表达载体pET39b-EKL，转化大肠杆菌BL21(DE3)，
ET39b-EKL经过酶切鉴定和测序，证实其插入方向、读码框架正确，所表达重组蛋白经SDS-PAGE分析
达28%，通过镍亲和层析纯化得到融合蛋白DsbA-rEKL的单一条带。该粗酶经脱盐后在适宜的缓冲体系
高的自催化切割活性，为进一步进行重组牛肠激酶活性的研究及应用奠定了基础。

Abstract:The objective of the study was to obtain the gene of bovine enterokinase light chain，which wo
d purification of fusion proteins．The fragment of bovine enterokinase light chain cDNA was obtained by RT
denal mucosa， then cloned into the pUCmT cloning vector and sequenced.Compared with the sequence depo
d gene sequence is correct.Then the interested gene fragment was inserted into the pET39b expression plasmi
39b-EKL was transformed into E.coli BL21(DE3) and induced by IPTG.It was confirmed that the nucleotide
onjunction site between the recombinant DNA 5′terminal multi-cloning site and recombinant fragment after the
uence.SDS-PAGE analysis indicated that target product was about 65 kDa which occupied 28% of the total p
s obtained by nickel chelating chromatogram using His*Binding Resin.After desalting and changing buffer,the
t 21℃ overnight and demonstrated a high autocatalytic cleavage activity.This investigation would be able to la
e activity research and farther application of expression products on a large scale.