Expression and Purification of pancreatic lipase-like protein

Gene and Expression Vector

1.1 Gene coding for the target protein

The cDNA coding for the target protein was chemically-synthesized with optimization for expression in *E.coli* and described as below.

> 16_pancreatic_lipase-like_protein-1445bp

atgggtcaccatcaccatcaccatatgtcggactcagaagtcaatcaagaagctaagccagaggtca
agccagaagtcaagcctgagactcacatcaatttaaaggtgtccgatggatcttcagagatcttcttcaagat
caaaaagaccactcctttaagaaggctgatggaagcgttcgctaaaagacagggtaaggaaatggactcc
ttaagattcttgtacgacggtattagaattcaagctgatcagaccctgaagatttggacatggaggataac
gatattattgaggctcacagagaacagattggtggccaaggatccATGGCCGAGGTGTGCTACGG
TGACCTGGGCTGCTTCACCAACATTGCGCCGTTTTGGGGCCTGAACCGTCCGCTGA
GCAGCCTGCCGGACCCGCCGGATGTGGTTGGTACCAAGTTCTACCTGTATACCCGTG
CGAACCCGACGATGGCGCTGCGTGAGCGTCTGGTGGCGGATAGCATCGCGACCCTG
AGCGCGAGCCACTTCAGCAGCAGCAAGCCGACCCGTATGGTTATCCACGGTTTTAC
CGGTAGCGCGGAACATGCGTGGGTGCAGACCATTGTTGACGAGCTGCTGCTGAAAG
AAGATATCAACGTGATTACCGTTGACTGGGCGGATGGCGCGAGCATTGGTGGCAGC
TACGGTCAAGCGACCGCGAACAGCCGTGTGGTTGGTGCGGAAGTGGCGAAGATTG
TTAACTATATGAGCGCGCAAACCGGTGCGAACGCGCGTAACTTCCACCTGATTGGTC

ACAGCCTGGGTTGCCATGTGGCGGGTTATGCGGGTGACATCCTGGGTAACGTTGGC

CGTATTACCGCGCTGGATGCGAGCGAACCGTATTTTGACGGTATGGATGCGATCGTG

AAACTGGACCCGACCGATGCGCTGTTCGTGGACGTTATCCACAGCGATGGTAGCCCG

TTTATTGGTACCCTGGGTATGGGTACCAGCCTGCCGACCGGTCATGTGGACTTCTACC

CGAACGGTGGCGAGTATCAGCCGGGTTGCCACGATAACTTCGTTAGCAGCGTGGTT

AGCACCGGTTTTGGTCTGCTGACCGAGGGTTATGATGGTGCGGAAGCGGCGGCGGC

GTGCAGCCACCTGCGTGCGATCGATTATTTCACCGAGAGCATTAACAGCGAATGCCC

GTTTACCGCGTACCCGTGCGAAAGCTATGACAAGTTCAAAGATGGTTTTTGCATGAG

CTGCGCGGGTAGCACCTGCAGCCAAATGGGTTACCGTGCGCGTGACCACTATGGTG

CGCGTGGCAAACTGTACCTGACCACCACCGATGGTACCCAGCCGGGTGGCAAGTTC

TGCGCGTACCACTATAAAGTGCAAGTTACCAGCAGCACCCGTATGGAGGAAACCGC

GGGTCGTATCTACGTTACCTTTCAGGGTCCGAGCGGCATTACCGACACGATGGAAGT

GACCGACGGCGATGTTATGGATCTGAAGCCGGGTGTGACCTATCAAAAACTGGTTAG

CAGCGCGAGCAACATCGGTGACGTGAGCCGTGTTGGCCTGCGTTTCGAACGTAGCA

GCAGCATTTGGGATTTCTTTGCGGCGCAGTACTATACCGTGGGCAAGGTTACCATCA

CCGCGGGCGAGACCCAAACCAGCTACAAATTTTGCGGCAGCGACACCCCGGTGAA

CAACGGTGAAACCCTGCAGCTGACCCAAGTTGGTGGCACCTGCtgaCTCGAG

1.2 Expression vector

The cDNA sequence described was cloned in pATX-sumo expression vector.

> 16_pancreatic_lipase-like_protein (581AAs,62.98kDa, pI:5.05)

MGHHHHHHMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRR
LMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGQGSMAE
VCYGDLGCFTNIAPFWGLNRPLSSLPDPPDVVGTKFYLYTRANPTMALRERLVADSIAT
LSASHFSSSKPTRMVIHGFTGSAEHAWVQTIVDELLLKEDINVITVDWADGASIGGSYG
QATANSRVVGAEVAKIVNYMSAQTGANARNFHLIGHSLGCHVAGYAGDILGNVGRIT
ALDASEPYFDGMDAIVKLDPTDALFVDVIHSDGSPFIGTLGMGTSLPTGHVDFYPNGG
EYQPGCHDNFVSSVVSTGFGLLTEGYDGAEAAAACSHLRAIDYFTESINSECPFTAYPC
ESYDKFKDGFCMSCAGSTCSQMGYRARDHYGARGKLYLTTTDGTQPGGKFCAYHYK
VQVTSSTRMEETAGRIYVTFQGPSGITDTMEVTDGDVMDLKPGVTYQKLVSSASNIG
DVSRVGLRFERSSSIWDFFAAQYYTVGKVTITAGETQTSYKFCGSDTPVNNGETLQLTQ
VGGTC

Features: SUMO-tag:[1:109]

Subcloning

2.1 Primer

9408p-4-F gaacagattggtggccaaggatccATGGCCGAGGTGTGCTACGGTGAC
9408p-4-R tcagtggtggtggtggtggtgctcgagtcaGCAGGTGCCACCAACTTGGGTCAGCTG

PCR reaction system	50ul
2x PrimeSTAR MAX	25ul
Forward Primer: 10uM	2ul
Reverse Primer:10uM	2ul
Template:	0.5ul
ddH2O	to 50ul

PCR profile	
Denaturation	95℃,10s
Annealing	55℃,5s
Extension	72℃, 15s
Cycles	30 times
Storage	16℃,hold

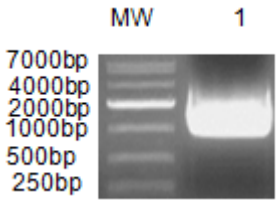

Figure 1. PCR analysis for target gene fragment. Goldview staining.

Target gene was obtained. Subcloning test was done, the gene was collected with pATX-sumo vector and the positive clones were identified with PCR. The results were showed as below.

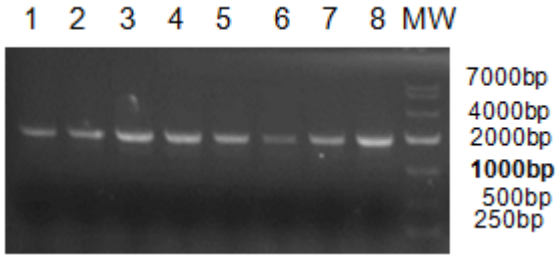

Figure 2. PCR analysis for positive clones. *Goldview staining.*

2.2 Conclusion of the test

Sequencing results showed that positive clone was obtained.

Expression Tests

3.1 Aim

-Determination of optimal conditions for protein expression by evaluation of:

*Induction strategy

*Temperature and time for induction

*_E.coli_ strain used for protein production

-Identification of best extraction condition: native or denatured

3.2 Short protocol description

-Culture:

*Bacteria starter obtained by incubation at 37°C

*IPTG induction with different temperatures and incubation times (see details in §3.3).

-Protein samples preparation (for each expression condition tested):

*Bacteria harvested by centrifugation

*Lysis with native buffer

*Clarification (centrifugation C1): The supernatant is the native protein extract (NPE)

*Solubilization of the pellet from C1 with a denaturing buffer (Urea 8M)

*Clarification (centrifugation): The supernatant is the denatured protein extract (DPE)

*PAGE analysis of NPE and DPE.

3.3 Results of the expression tests

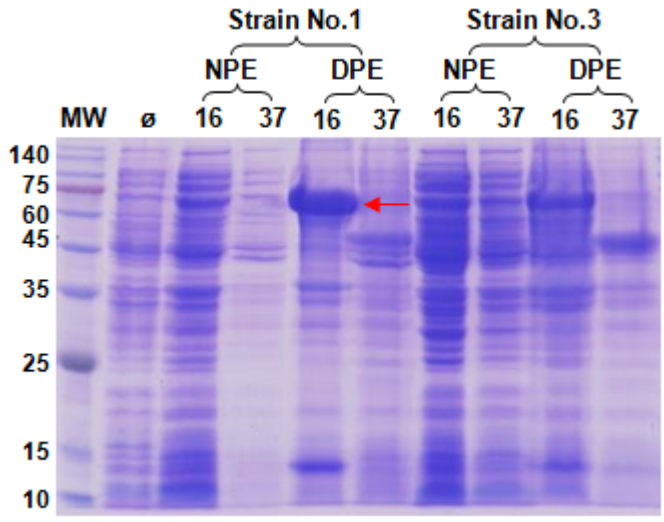

Figure 3. Expression tests of the target protein. Reducing SDS-PAGE. Analysis of NPE and DPE prepared as described in §3.2.

MW. Molecular weight marker. **Ø.** Non-induced bacteria culture (negative control).

16 and 37. Incubation temperature (°C) during induction with IPTG.

Induction with IPTG 1mM during 16h at 16°C, or during 4h for other temperatures.

3.4 Conclusion of the expression tests

Note: *E.coli strains tested are intended to overcome one or several expression issues such as (but not limited to) expression level, solubility or toxicity.*

Target proteins had expressed, but most were in denatured condition.

Optimal expression condition was described as below.

	Strain No.	Temperature	Induction Time
16 pancreatic lipase-like protein (DPE)	No.1	37°C	4h

Table1. Optimal denatured expression conditions of target protein.

1L scale-up purification tests

4.1 Aim

1L scale up purification tests in denatured conditions for target protein. Starting material: DPE prepared after production in optimal denatured expression conditions described in the Table 1.

4.2 Short protocol description

-Lysis buffer: PBS, pH7.5, 10% Glycerol

-Washing buffer: PBS, pH7.5., 1% Triton X 100, 5 mM EDTA

-Denaturing buffer: PBS, pH7.5, 2% Sarcosyl

-Dialysis buffer: PBS, pH7.5, 0.02% Sarcosyl

-Analysis by SDS-PAGE of fractions of interest

-Final sample QC: qualitative by SDS-PAGE, quantitative by Bradford method.

4.3 Results of the purification tests

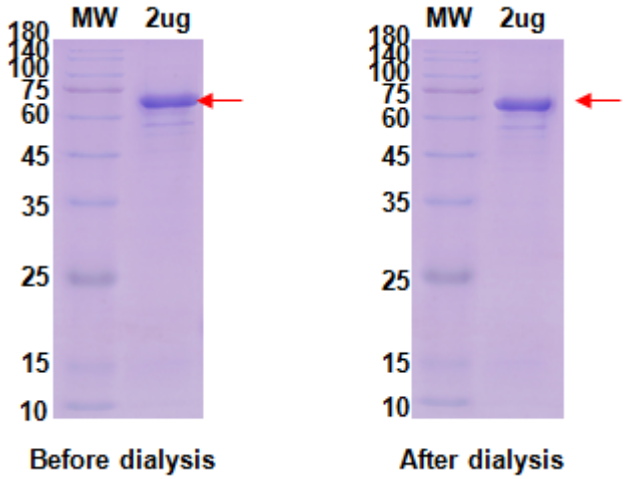

Figure 4. Final sample QC of denatured condition. *Coomassie blue staining.*

Reducing-PAGE analysis. 2μg of sample loaded.

4.4 Conclusion of the purification tests

Target proteins could be obtained from DPE.