

# S1a sequences

## Six-histidine (6His)-tagged esterase (gi: 29893336)

### Origin

HHHHHH

1 atg**caccatcatcaccatcac**gatcaat ctaaaca<sup>aaa</sup> tcaaaca<sup>aat</sup> caacattctg tagctgaaag tgcacaactg  
61 aaaagtgatg agacagcaaa tcagccaaaa gtagaagaag aaagctcagt aaaacaagac  
121 gtccaaccgt ctaaaaatgt aatcaacaa gacgtagcta ctcaatcaaa tgagagagaa  
181 aataatgaca taaaagg<sup>tga</sup> aagccaaact ttaaaggcga gcaatcaaca tactcagagt  
241 tctaacagtc ataatcaatc aataggaaca aaagatagcg actcagaaga actcgatcaa  
301 ccattagtga aattacaaaa gccgtctaat gattctacat atcaaacaca atcaaaagca  
361 aaacaagata gctctaaaca actccctcaa gaaaaaaca<sup>aaa</sup> caaaacatca aattca<sup>aaaca</sup>  
421 actgaaaatg aacagacaac taacgttgat tctaaaaaag ctaatgacac tcaagatgct  
481 agacaacata ctcaagagcc taaaatgat acatcaacat cacaaaaaa<sup>aaa</sup> tcatcatcaa  
541 gtagctacaa aagaacaatc taatagaagt acaacaaggg agacacaaaa gcaatcagca  
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661 gtatttgtcc atggtttcct aggctttaca ggtgataatc aatttagttt agctccaaaa  
721 tattgggg<sup>tg</sup> gtacaaaata caatattgac agaaatttaa ctaatgaggg atacaatgta  
781 catgaagcaa atacgggtgc ttttagtagt aactatg<sup>atc</sup> gagcagtaga attgtattac  
841 tatgtcaaag gaggacgtgt tgattacggc gcagcgc<sup>atg</sup> cagctaaata tgg<sup>tcatcat</sup>  
901 cgctatgg<sup>tc</sup> gaacatacaa ggg<sup>tatcatg</sup> cgtgattggg aacctgg<sup>caa</sup> aaaaattcat

961 tttataggtc acagtatggg tggtaaacc attcgtcaaa tggaagagtt cttaagaaat  
1021 ggtaaccaag aagaaataga atatcaacgt caacatgggg gtactatata cgatttatt  
1081 acaggtggta aagataatat ggttgcttca attactacac ttggcacacc acataatggt  
1141 acaccggcgg cagataaaat tggcacacgt aaacttgtaa aagaaacgat taatcgatt  
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1441 acaggacctc ttgtaatga attacctaat tctagtgaaa ttctttgtt gaacttaacc  
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1561 gttatatcat cacaacatcc ttctaaccaa accttaaaa aagttgatga tcataacca  
1621 gctactgata aaggagttg gcaagtgaga cccgtcaac atgattggga ccatttagat  
1681 ttagtaggta tggatgcatt tgatttaaca catacaggta gagaattagg tcaattctat  
1741 ctaggtatta tggataatat catgagaatc gaagaagcag acggtattac aaataaataa

### Translation map

mHHHHHdqsktnqnnqhsvaesqlksdetanqpkveessvkqdvqpsknvnqqdvatqsnerenndikgesqtl  
kasnqhtqssnshnqsigtksdseeldqplvklqkpsndstyqtqskakqdsqkqlpkekttkhqiqtteneqttnvdskkand  
tqdarqhtqepkndtstsqknhhqvatkeqsnrstretqkqsansqnqhsthaqfknqypvfvhgflgftgdnqfslapky  
wggtkynidrnltnegynvheanigafssnydravelyyyvkgrvdygaahaakyghhrygrtykgimrdwepgkkihfi  
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vdidlgfsqwglkqqpnesyidaervskskiwntedqavndlttqgaekinqqtslnpni vytytgtsathtgplgnelpnssei  
llnltsciigkdankeirpndgvvpvissqhpsnqtfkkvddhtpatdkgvwqvrpvqhdwdhldlvgmdafdlthtgrellgq  
fylgimdnimri eeadgitnk

## six-histidine (6His)-tagged mutant (M326L)

### Origin

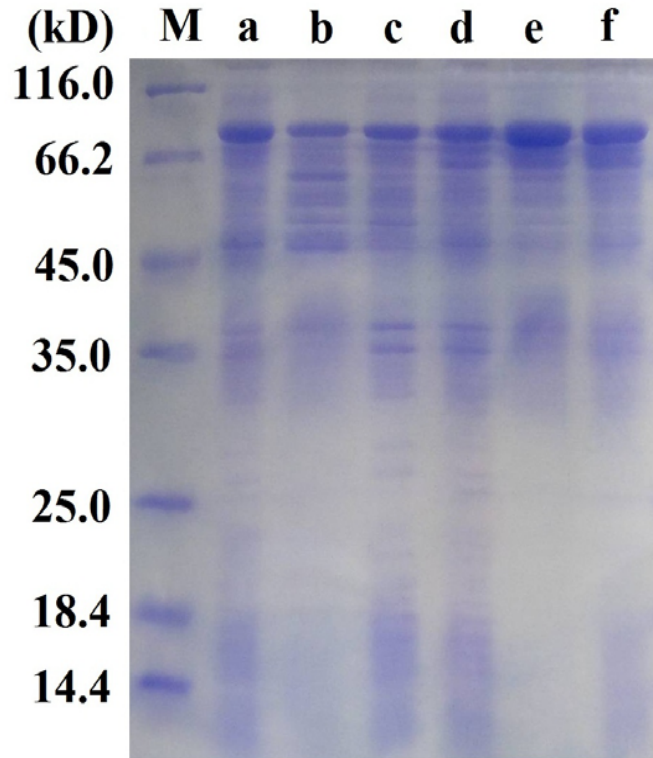
1 atg**caccatcatcaccatcac**gatcaat ctaaacaaca tcaaacaat caacattctg tagctgaaag tgcacaactg  
61 aaaagtgatg agacagcaaa tcagccaaaa gtagaagaag aaagctcagt aaaacaagac  
121 gtccaaccgt ctaaaaatgt aatcaacaa gacgtagcta ctcaatcaaa tgagagagaa  
181 aataatgaca taaaaggtga aagccaaact ttaaaggcga gcaatcaaca tactcagagt  
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841 tatgtcaaag gaggaegtgt tgattacggc gcagcgcagc cagctaaata tggatcatc  
901 cgctatggtc gaacatacaa gggatcatg cgtgattggg aacctggcaa aaaaattcat  
961 tttataggtc acagtat**ctg** tggcacaacc attcgtcaaa tggaagagtt ctaagaaat  
1021 ggtaaccaag aagaaataga atatcaacgt caacatgggg gtactatc cgattattt

1081 acagtggtta aagataatat gggtgcttca attactacac ttggcacacc acataatggt  
1141 acaccggcgg cagataaaat tggcacacgt aaacttgtaa aagaaacgat taatcgtatt  
1201 ggtagattaa gtggtggtaa agatgtagat atagatttag gttttctca atggggatta  
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1321 tggaaactg aagatcaagc tgtaaatgat ctgacaacgc aaggtgctga aaaaattaat  
1381 caacaacaa gtctaaatcc taatattgtc tacactactt atacggggtc tgcgacgcac  
1441 acaggacctc ttgtaatga attacctaata tctagtgaaa ttctttgtt gaacttaacc  
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1621 gctactgata aaggagttg gcaagtgaga cccgttcaac atgattggga ccatttagat  
1681 ttagtaggta tggatgcatt tgatttaaca catacaggta gagaattagg tcaattctat  
1741 ctaggtatta tggataatat catgagaatc gaagaagcag acggtattac aaataaataa

## Translation map

mHHHHHdqsktnqnnqhsvaesaqlksdetanqpkveeessvkqdvqpsknvnqqdvatqsnerenndikgesqt  
lksnqhtqssnshnqsigtkdsdseeldqplvklqkpsndstyqtqskakqdsskqlpkekttkhqiqtteneqttnvdsakkan  
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ntedqavndlttqgaekinqtslnpnivytytgsathtgplgnelpnsseillnltsciigkdankeirpndgvvpvissqhpsn  
qtfkkvddhtpatdkgvwqvrpvqhdwdhldlvgm dafdlhtgrelgqfy lgimdnimri eadgitnk

## S1b Fig s1b



- a. the tagged esterase in sample lysate after induced expression in 250 mL medium
- b. the sample lysate of a after affinity chromatography through Ni-NTA
- c. the sample lysate of a after chromatography through Sepharose 6B
- d. the tagged mutant (M326L) in sample lysate after induced expression in 250 mL medium
- e. the sample lysate of d after affinity chromatography through Ni-NTA
- f. the sample lysate of d after chromatography through Sepharose 6B

After lysis of cells via sonication treatment as described in context, the supernatant after centrifugation at 10,000×g for 20 min at 4 0C was passed through Sepharose 6B equilibrated and eluted with the lysis buffer. The portion with the highest apparent specific activity (the esterase appeared as a large aggregate) was collected and lympholized. After dissolution in water, the partially-purified enzyme was loaded onto Sepharose 6B again, and the portion of the highest specific activity was collected. The esterase and its mutants after twice steps exhibited increases of about 80% of apparent specific activity and were slightly enriched as analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis PAGE (SDS-PAGE).

For affinity chromatography, Ni-NTA column was equilibrated with 20 mM Tris-HCl at pH 7.4 plus 0.40 M NaCl; supernatant after centrifugation of sample lysate was loaded and washed with 20 mM Tris-HCl at pH 7.4 plus 0.40 M NaCl and 20 mM imimidazole. Finally, the bound enzyme was eluted out with 20 mM Tris-HCl at pH 7.4 plus 0.10 M imimidazole. Enzyme activity was determined after dialysis against 20 mM Tris-HCl at pH 7.4 at 4 0C for 4 h with several changes of the buffer.

# S1c 4ml medium

## 4 ml Culture medium

six-histidine (6His)-tagged esterase, maximum specific activity ~ 150 kU/g gi: 29893336

Protein cocentration (g/L)	Apparent specific activity (kU/g)	Target protein (g/L)	Target protein (percentage)
7.2	4.4	0.2	0.03
7.3	7.0	0.3	0.05
6.9	5.7	0.3	0.04
8.7	6.6	0.4	0.04
7.2	4.1	0.2	0.03
8.0	3.5	0.2	0.02
11.2	7.3	0.5	0.05
6.1	8.0	0.3	0.05
5.7	4.2	0.2	0.03
6.2	5.3	0.2	0.04
2.9	6.7	0.1	0.04
6.1	6.1	0.2	0.04
6.1	7.3	0.3	0.05
5.9	6.2	0.2	0.04
5.7	4.3	0.2	0.03
6.2	6.7	0.3	0.04
6.5	7.1	0.3	0.05
6.0	6.2	0.2	0.04
5.7	6.5	0.2	0.04
6.2	8.5	0.4	0.06
6.2	8.5	0.4	0.06
5.5	8.3	0.3	0.06

## 4 ml Culture medium

six-histidine (6His)-tagged mutant (M326L), maximum specific activity ~ 50 kU/g

Protein cocentration (g/L)	Apparent specific activity (kU/g)	Target protein (g/L)	Target protein (percentage)
6.4	1.7	0.2	0.03
6.7	2.0	0.3	0.04
7.3	2.1	0.3	0.04
4.1	1.1	0.1	0.02
6.1	2.6	0.3	0.05
6.0	1.5	0.2	0.03
6.1	1.5	0.2	0.03
6.1	2.2	0.3	0.04
6.2	1.9	0.2	0.04
6.5	2.8	0.4	0.06
6.2	1.8	0.2	0.04
6.2	3.0	0.4	0.06
6.5	2.9	0.4	0.06
6.4	2.4	0.3	0.05
6.2	1.9	0.2	0.04
6.3	2.3	0.3	0.05
6.4	2.2	0.3	0.04
6.8	2.6	0.4	0.05
6.4	2.2	0.3	0.04
6.4	2.4	0.3	0.05
5.9	1.4	0.2	0.03
4.8	3.1	0.3	0.06

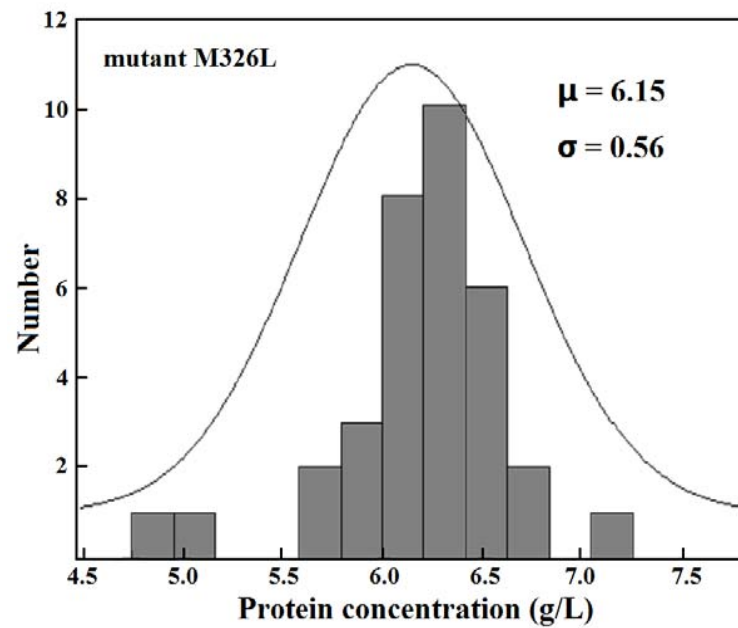
6.1	5.9	0.2	0.04		5.1	4.8	0.5	0.10
6.0	8.5	0.3	0.06		5.6	3.4	0.4	0.07
4.2	7.4	0.2	0.05		6.5	2.2	0.3	0.04
5.7	10.5	0.4	0.07		5.7	1.3	0.1	0.03
6.2	6.0	0.2	0.04		5.9	2.0	0.2	0.04
6.5	5.4	0.2	0.04		6.1	2.2	0.3	0.04
5.9	8.0	0.3	0.05		6.0	2.4	0.3	0.05
6.0	9.5	0.4	0.06		6.3	2.3	0.3	0.05
5.7	6.4	0.2	0.04		6.3	1.5	0.2	0.03
5.7	6.5	0.2	0.04		6.5	2.1	0.3	0.04
5.8	7.8	0.3	0.05		6.5	1.5	0.2	0.03
5.8	10.9	0.4	0.07		6.4	1.6	0.2	0.03
6.1	7.6	0.3	0.05		6.4	1.6	0.2	0.03
5.8	7.6	0.3	0.05					
5.6	7.6	0.3	0.05	Average	6.1	2.2	0.3	0.04
7.1	7.0	0.3	0.05	SD	0.6	0.7	0.1	0.01
5.9	6.4	0.3	0.04	CV	9.1%	32.1%	29.2%	32.1%
5.8	9.5	0.4	0.06					
6.9	10.7	0.5	0.07					
7.9	12.7	0.7	0.08	See S1e for their distributions				
5.6	8.9	0.3	0.06					
5.6	5.4	0.2	0.04					
7.4	4.7	0.2	0.03					
8.4	8.1	0.5	0.05					
5.4	6.7	0.2	0.04					
3.8	7.8	0.2	0.05					
5.3	9.7	0.3	0.06		ratio of apparent specific acti	3.2	SD	1.28
4.9	7.1	0.2	0.05				CV	0.41
7.0	3.7	0.2	0.02					
6.8	5.1	0.2	0.03		<i>F</i> -test	<i>F</i> = 5.90		
6.0	4.5	0.2	0.03			<i>F</i> <sub>0.005</sub> = 1.43		
							Two groups of data have	
5.7	4.9	0.2	0.03			<i>F</i> > <i>F</i> <sub>0.005</sub>	significant difference	
5.5	5.5	0.2	0.04			<i>P</i> < 0.005		



5.3	5.6	0.2	0.04
5.2	5.3	0.2	0.04
5.7	7.6	0.3	0.05
4.6	6.9	0.2	0.05
4.5	7.3	0.2	0.05
4.8	4.5	0.1	0.03
4.5	6.2	0.2	0.04
9.9	7.9	0.5	0.05
8.2	7.3	0.4	0.05
6.4	8.0	0.3	0.05
6.5	8.5	0.4	0.06
7.6	7.3	0.4	0.05
7.2	9.1	0.4	0.06
6.4	7.0	0.3	0.05
6.8	4.8	0.2	0.03
5.2	4.0	0.1	0.03
5.9	6.0	0.2	0.04
7.8	5.9	0.3	0.04
4.2	5.9	0.2	0.04
5.4	9.8	0.4	0.07
6.2	7.8	0.3	0.05
5.2	8.4	0.3	0.06
5.5	8.5	0.3	0.06
4.8	8.2	0.3	0.05
5.3	8.6	0.3	0.06
8.3	6.5	0.4	0.04
8.2	5.0	0.3	0.03
6.3	8.0	0.3	0.05
7.3	7.9	0.4	0.05
6.5	7.9	0.3	0.05
5.7	8.7	0.3	0.06
8.7	8.5	0.5	0.06
8.9	6.9	0.4	0.05
7.0	7.0	0.3	0.05
7.6	7.8	0.4	0.05

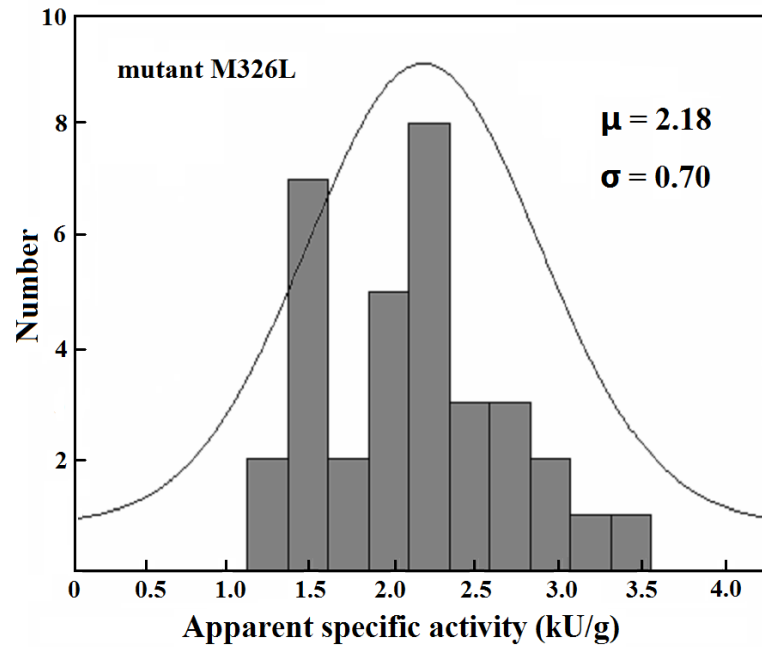
6.7	8.0	0.4	0.05	
6.6	7.8	0.3	0.05	
4.9	7.4	0.2	0.05	
5.5	7.1	0.3	0.05	
6.1	6.3	0.3	0.04	
4.8	6.1	0.2	0.04	
5.3	7.6	0.3	0.05	
5.0	5.5	0.2	0.04	
6.0	5.7	0.2	0.04	
5.9	6.5	0.3	0.04	
6.5	4.9	0.2	0.03	
6.5	5.9	0.3	0.04	
6.0	7.4	0.3	0.05	
5.9	8.8	0.3	0.06	
6.2	4.0	0.2	0.03	
6.4	4.2	0.2	0.03	
7.3	3.3	0.2	0.02	
7.3	2.4	0.1	0.02	
6.6	7.0	0.3	0.05	
7.1	6.8	0.3	0.05	
9.5	6.0	0.4	0.04	
7.9	7.3	0.4	0.05	
6.1	8.0	0.3	0.05	
6.3	8.6	0.4	0.06	
7.3	7.1	0.3	0.05	
6.9	9.2	0.4	0.06	
6.1	7.3	0.3	0.05	
6.6	4.8	0.2	0.03	
5.0	4.1	0.1	0.03	
5.7	6.1	0.2	0.04	
7.5	6.0	0.3	0.04	
4.1	8.0	0.2	0.05	
Average	6.3	6.9	0.3	0.05
SD	1.2	1.7	0.1	0.01
CV	19.7%	25.3%	32.6%	25.3%

**distribution of protein concentrations and apparent specific activities after induced expression under HTP mode**



checked with function in Matlab 6.5 supported normal distribution

**distribution of protein concentrations and apparent specific activities after induced expression under HTP mode**



checked with function in Matlab 6.5 supported normal distribution

**response of initial rates to quantities of total proteins  
from artificial lysates with an apparent specific  
activity of 5.4 kU/g**

<b>artificial lysate 1</b>	<b>5.4 kU/g</b>							
<b>total prtoeins</b>	<b>8</b>	<b>16</b>	<b>32</b>	<b>48</b>	<b>64</b>	<b>96</b>	<b>128</b>	<b>160</b>
<b>absorbance change in 30 min</b>	<b>-0.002</b>	<b>-0.001</b>	<b>0.006</b>	<b>0.021</b>	<b>0.037</b>	<b>0.056</b>	<b>0.075</b>	<b>0.085</b>

<b>artificial lysate 2</b>	<b>5.4 kU/g</b>							
<b>total prtoeins</b>	<b>8</b>	<b>16</b>	<b>32</b>	<b>48</b>	<b>64</b>	<b>96</b>	<b>128</b>	<b>160</b>
<b>absorbance change in 30 min</b>	<b>-0.00867</b>	<b>-0.00067</b>	<b>0.009</b>	<b>0.0163333</b>	<b>0.010333</b>	<b>0.009667</b>	<b>0.032666667</b>	<b>0.033</b>



# S1e M326L in 4 ml medium

4 ml Culture medium      inapplicable

six-histidine (6His)-tagged mutant (M326L), maximum  
specific activity ~ 50 kU/g

First sample: apparent specific activity 3.2 kU/g

Quantity of total protein (µg)	43	64	85	128	256
V	0.0324	0.0511	0.0378	0.0347	0.0229

The change of absorbance in 30min is much lower than 0.09, So it can't predict  
the end point accurately.

Second sample: apparent specific activity 5.8 kU/g

Quantity of total protein (µg)	43	64	85	128	256
V	0.0537	0.0638	0.0590	0.0529	0.0408

The change of absorbance in 30 min is much lower than 0.09, So it can't  
predict the maxium activity accurately.