

CX2-128USP1_ST25
SEQUENCE LISTING

<110> Codexis, Inc.
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SMITH, Derek
YANG, Hong
YEO, Wan Lin
MOORE, Jeffrey C.

<120> ENGINEERED BIOCATALYSTS USEFUL FOR CARBAPENEM SYNTHESIS

<130> 2-128USP1

<160> 120

<170> PatentIn version 3.5

<210> 1

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

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<210> 2

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

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Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg
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Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val
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<210> 3

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

CX2-128USP1_ST25

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<210> 4
<211> 489
<212> PRT
<213> Artificial Sequence

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<220>
<223> Engineered variant of B. subtilis pNB esterase

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35     40     45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50     55     60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65     70     75     80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85     90     95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100    105    110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
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Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
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His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
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Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
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Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180    185    190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
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Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210    215    220
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Val	Arg	Ser	Gln	305	Glu	Thr	Leu	310	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly	
Lys	Pro	Leu	Ala	325	Glu	Lys	Ala	330	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu		
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Leu	Asp	Ser	Glu	450	Ile	Thr	Ile	455	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg	
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<210> 5

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

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<210> 6
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

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 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Ser Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
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 Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
 165 170 175
 Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
 180 185 190
 Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
 195 200 205
 Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
 210 215 220
 Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
 225 230 235 240
 Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
 245 250 255
 Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
 260 265 270
 Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
 275 280 285
 Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
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 Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
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 Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
 325 330 335
 Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
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 Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
 355 360 365
 Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
 370 375 380
 Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
 385 390 395 400
 Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
 405 410 415
 Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
 420 425 430
 Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
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Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
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 Gln Lys Leu Phe Pro Ser Lys Gly Glu
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<210> 7
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

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 <223> Engineered variant of B. subtilis pNB esterase

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<210> 8
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 <213> Artificial Sequence

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 <223> Engineered variant of B. subtilis pNB esterase

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 35 40 45
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 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met

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130	His	Leu	Ser	Ser	Phe	Asp	135	Glu	Ala	Tyr	Ser	Asp	140	Asn	Leu	Gly	Leu	Leu
145	Asp	Gln	Ala	Ala	Ala	Leu	150	Lys	Trp	Val	Arg	Glu	155	Asn	Ile	Ser	Ala	Phe
	Gly	Gly	Asp	Pro	Asp	Asn	165	Val	Thr	Val	Phe	Gly	170	Glu	Ser	Ala	Gly	Gly
	Ala	Ser	Ile	Ala	Ala	Leu	180	Leu	Ala	Met	Pro	Ala	185	Ala	Lys	Gly	Leu	Phe
	Gln	Lys	Ala	Ile	Met	Glu	195	Ser	Gly	Ala	Ser	Arg	200	Thr	Met	Thr	Lys	Glu
	Gln	Ala	Ala	Ser	Thr	Ala	210	Ala	Ala	Phe	Leu	Gln	215	Val	Leu	Gly	Ile	Asn
225	Glu	Ser	Gln	Leu	Asp	Arg	230	Leu	His	Thr	Val	Ala	235	Ala	Glu	Asp	Leu	Leu
	Lys	Ala	Ala	Asp	Gln	Leu	245	Arg	Ile	Ala	Glu	Lys	250	Glu	Asn	Ile	Phe	Gln
	Leu	Phe	Phe	Gln	Pro	Ala	260	Leu	Asp	Pro	Lys	Thr	265	Leu	Pro	Glu	Glu	Pro
	Glu	Lys	Ser	Ile	Ala	Glu	275	Gly	Ala	Ala	Ser	Gly	280	Ile	Pro	Leu	Leu	Ile
	Gly	Thr	Thr	Arg	Asp	Glu	290	Gly	Tyr	Leu	Phe	Phe	295	Thr	Ser	Asp	Ser	Asp
305	Val	Arg	Ser	Gln	Glu	Thr	310	Leu	Asp	Ala	Ala	Leu	315	Glu	Tyr	Ser	Leu	Gly
	Lys	Pro	Leu	Ala	Glu	Lys	325	Ala	Ala	Asp	Leu	Tyr	330	Pro	Arg	Ser	Leu	Glu
	Ser	Gln	Ile	His	Met	Val	340	Thr	Asp	Leu	Leu	Phe	345	Trp	Arg	Pro	Ala	Val
	Ala	Phe	Ala	Ser	Ala	Gln	355	Ser	His	Tyr	Ala	Pro	360	Val	Trp	Met	Tyr	Arg
	Phe	Asp	Trp	His	Pro	Glu	370	Lys	Pro	Pro	Tyr	Asn	375	Lys	Ala	Phe	His	Ala
385	Leu	Glu	Leu	Pro	Phe	Val	385	Phe	Gly	Asn	Leu	Asp	390	Gly	Leu	Glu	Arg	Met
	Ala	Lys	Ala	Glu	Ile	Thr	395	Asp	Glu	Val	Lys	Gln	400	Leu	Ser	His	Thr	Ile
	Gln	Ser	Ala	Trp	Ile	Thr	405	Phe	Ala	Lys	Thr	Gly	410	Asn	Pro	Ser	Thr	Glu
	Ala	Val	Asn	Trp	Pro	Ala	420	Tyr	His	Glu	Glu	Thr	425	Arg	Glu	Thr	Val	Ile
	Leu	Asp	Ser	Glu	Ile	Thr	435	Ile	Glu	Asn	Asp	Pro	440	Glu	Ser	Glu	Lys	Arg
450	Gln	Lys	Leu	Phe	Pro	Ser	455	Lys	Gly	Glu			460					
465							470						475					480
							485											

<210> 9

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 9

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gcgccggaac	cgccggaggt	ctggggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgctcgacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
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gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgcg	gcattgccgc	gctgtgggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagccgtacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720

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caactgcgta	tcgcgagaaa	agagaatatc	ttccagctgt	tcttccagcc	tgacttgat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
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gttcgtagcc	aggaacacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgtttggca	1020
gagaaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	agggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttgca	1320
aagaccggca	acccgagcac	cgaggcggtg	aactggccgg	cttatcatga	agaaacccgt	1380
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cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 10
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 10

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Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75				80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Trp	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Ala	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Arg	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		

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Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
 355 360 365
 Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
 370 375 380
 Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
 385 390 395 400
 Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
 405 410 415
 Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
 420 425 430
 Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
 450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
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<210> 11
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 11
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgccgcg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcggtgc gttttacctg ggtgcccgtg gccagccgct gtacgatggc 360
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 ttcggtttca tgacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atggggttcgc gagaacatca gcgcgtttgg tggcgacccg 540
 gataatgtga cgggtgttgg tgaaagcgct ggtggcgcg gatttgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtc aagccgtacc 660
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 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatata ttccagctgt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcmg aaggtgcmg aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaacactt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gtttcgattg gcacccggag aaaccgcctg acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagacaa gggtgaa 1467

<210> 12
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 12
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro

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Pro	Val	Gly	20	Gln	Trp	Arg	Phe	Lys	25	Ala	Pro	Glu	Pro	30	Glu	Val	Trp
Glu	Asp	Val	35	Leu	Asp	Ala	Thr	Ala	40	Tyr	Gly	Pro	Val	45	Cys	Pro	Gln
Ser	Asp	Leu	50	Leu	Ser	Leu	Ser	Tyr	55	Thr	Glu	Leu	Pro	60	Arg	Gln	Ser
65	Asp	Cys	Leu	Tyr	85	Val	Asn	Val	70	Phe	Ala	Pro	Asp	75	Thr	Pro	Ser
Leu	Pro	Val	100	Met	Val	Trp	Ile	His	90	Gly	Gly	Ala	Phe	110	Tyr	Leu	Gly
Gly	Ser	Gln	115	Pro	Leu	Tyr	Asp	Gly	120	Ser	Lys	Leu	Ala	125	Ala	Gln	Gly
Val	Ile	Val	130	Val	Thr	Leu	Asn	Tyr	135	Arg	Leu	Gly	Pro	140	Phe	Gly	Phe
His	Leu	Ser	145	Ser	Phe	Asp	Glu	Ala	150	Tyr	Ser	Asp	Asn	155	Leu	Gly	Leu
Asp	Gln	Ala	165	Ala	Leu	Lys	Trp	Val	170	Arg	Glu	Asn	Ile	175	Ser	Ala	Phe
Gly	Gly	Asp	180	Pro	Asn	Val	Thr	Val	185	Phe	Gly	Glu	Ser	190	Ala	Gly	Gly
Ala	Ser	Ile	195	Ala	Ala	Leu	Leu	Ala	200	Met	Pro	Ala	Ala	205	Lys	Gly	Leu
Gln	Lys	Ala	210	Ile	Met	Glu	Ser	Gly	215	Ala	Ser	Arg	Thr	220	Met	Thr	Lys
Gln	Ala	Ala	225	Ser	Thr	Ala	Ala	Phe	230	Leu	Gln	Val	Leu	235	Gly	Ile	Asn
Glu	Ser	Gln	245	Leu	Asp	Arg	Leu	His	250	Thr	Val	Ala	Ala	255	Glu	Asp	Leu
Lys	Ala	Ala	260	Gln	Leu	Arg	Ile	Ala	265	Glu	Lys	Glu	Asn	270	Ile	Phe	Gln
Leu	Phe	Phe	275	Gln	Pro	Ala	Leu	Asp	280	Pro	Lys	Thr	Leu	285	Pro	Glu	Pro
Glu	Lys	Ser	290	Ile	Ala	Glu	Gly	Ala	295	Ala	Ser	Gly	Ile	300	Pro	Leu	Leu
Gly	Thr	Thr	305	Arg	Asp	Glu	Gly	Tyr	310	Leu	Phe	Phe	Thr	315	Ser	Asp	Ser
Val	Arg	Ser	325	Gln	Glu	Thr	Leu	Asp	330	Ala	Ala	Leu	Glu	335	Tyr	Ser	Leu
Lys	Pro	Leu	340	Ala	Glu	Lys	Ala	Ala	345	Asp	Leu	Tyr	Pro	350	Arg	Ser	Leu
Ser	Gln	Ile	355	His	Met	Val	Thr	Asp	360	Leu	Leu	Phe	Trp	365	Pro	Ala	Val
Ala	Phe	Ala	370	Ser	Ala	Gln	Ser	His	375	Tyr	Ala	Pro	Val	380	Trp	Met	Tyr
Phe	Asp	Trp	385	His	Pro	Glu	Lys	Pro	390	Pro	Tyr	Asn	Lys	395	Ala	Phe	His
Leu	Glu	Leu	405	Pro	Phe	Val	Phe	Gly	410	Asn	Leu	Asp	Gly	415	Leu	Glu	Arg
Ala	Lys	Ala	420	Glu	Ile	Thr	Asp	Glu	425	Val	Lys	Gln	Leu	430	Ser	His	Thr
Gln	Ser	Ala	435	Trp	Ile	Thr	Phe	Ala	440	Lys	Thr	Gly	Asn	445	Pro	Ser	Thr
Ala	Val	Asn	450	Trp	Pro	Ala	Tyr	His	455	Glu	Glu	Thr	Arg	460	Glu	Thr	Val
Leu	Asp	Ser	465	Glu	Ile	Thr	Ile	Glu	470	Asn	Asp	Pro	Glu	475	Ser	Glu	Lys
Gln	Lys	Leu	485	Phe	Pro	Ser	Lys	Gly		Glu							Arg

<210> 13

<211> 1467

<212> DNA

<213> Artificial sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

CX2-128USP1_ST25

<400> 13

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gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
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ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgtg 480
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atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccctg 1380
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cagaagctgt tcccagacaa gggtgaa 1467

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<210> 14

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 14

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      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180      185      190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195      200      205
Gln Lys Ala Ile Met Gly Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240

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Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 15

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 15

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gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgcccgtg	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cggtgtttgg	tgaaagcgct	ggtggcgcg	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagcgatacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccagctgt	tcttccagcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaacattt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgtttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttga	1320
aagaccggca	acccgagcac	cgaggcgggt	aactggccgg	cttatcatga	agaaaccctg	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagacaa	gggtgaa				1467

<210> 16
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 16
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160
 Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
 165 170 175
 Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
 180 185 190
 Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
 195 200 205
 Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Asp Thr Met Thr Lys Glu
 210 215 220
 Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
 225 230 235 240
 Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
 245 250 255
 Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
 260 265 270
 Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
 275 280 285
 Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
 290 295 300
 Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
 305 310 315 320
 Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
 325 330 335
 Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
 340 345 350
 Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
 355 360 365
 Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
 370 375 380
 Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
 385 390 395 400
 Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
 405 410 415
 Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
 420 425 430
 Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile

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450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
 485

<210> 17
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 17
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcgggtg gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt ggggtctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgaccgc 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgca gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcctgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
 gagagccagc tggatcggtt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaacactt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcccccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagagaa gggtgaa 1467

<210> 18
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 18
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125

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Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
130						135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Ala	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Leu	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 19

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 19

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gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgcgg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
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ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cgggtgttgg	tgaaagcgct	ggtggcgcgga	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagccgtacc	660

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atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcggtt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggaaga agagaatatc ttccaggagt tcttcagacc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccctg 1380
gaaaccgtga tcctggactc tgagattacc atcagaaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 20

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 20

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260      265      270
Glu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu

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Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70				75					80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
			85						90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Ala	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Arg	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	His	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 23

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of *B. subtilis* pNB esterase

<400> 23

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gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgcgccg gataccccg tccaaaatct gccggttatg 300
gtttggattc acggcggtgc gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
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ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
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gataatgtga cgggtgtttg tgaaagcgct ggtggcgcg gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcggtgc aagccgtacc 660
tgaccaagg aacaagcggc gagcactcgc gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttactcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaacacct ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaaaggc cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggtatgtatc gtttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgct 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagagca gggtgaa 1467

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<210> 24

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of *B. subtilis* pNB esterase

<400> 24

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn

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225	Glu	Ser	Gln	Leu	Asp	230	Arg	Leu	His	Thr	Val	235	Ala	Ala	Glu	Asp	Leu	240	Leu
					245							250					255		
	Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln			
				260															
	Leu	Phe	Phe	Thr	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro			
			275					280											
	Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile			
		290					295					300							
	Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp			
		305				310					315								
	Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly			
				325						330									
	Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu			
			340						345										
	Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val			
			355					360											
	Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg			
		370					375					380							
	Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala			
		385				390					395								
	Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met			
				405						410									
	Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile			
			420						425										
	Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu			
			435					440					445						
	Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile			
		450					455					460							
	Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg			
		465				470					475								
	Gln	Lys	Leu	Phe	Pro	Lys	Gly	Glu											
				485															

<210> 25

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 25

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgcgg	gatacccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgcg	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaa	gcgattatgg	agagcggtgc	aagccgtacc	660
tgaccaaggg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccagctgt	tcttcctgcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggcaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaacacct	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttcacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttga	1320
aagaccggca	acccgagcac	cgaggcggtg	aactggccgg	cttaccatga	agaaaccggt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcagagaatg	accctgagag	cgagaagcgt	1440

cagaagctgt tcccgagcaa ggggtgaa

1467

<210> 26

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 26

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260      265      270
Leu Phe Phe Leu Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340      345      350
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355      360      365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370      375      380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385      390      395      400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405      410      415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420      425      430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
435      440      445

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Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
 450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
 485

<210> 27
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 27
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 gcgcccgaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggtatc acggcggtgc gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcact gtcgtcacc tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgaccgc 540
 gataatgtga cgggtgtttgg tgaaagcgct ggtggcgcg gatttgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagccgtacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccatcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 ttgatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaacccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagagca gggtgaa 1467

<210> 28
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 28
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Ala Phe Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu

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Val	Thr	115	Val	Val	Thr	Leu	Asn	120	Tyr	Arg	Leu	Gly	Pro	125	Phe	Gly	Phe	Met
His	Leu	130	Ser	Ser	Phe	Asp	Glu	135	Ala	Tyr	Ser	Asp	Asn	140	Leu	Gly	Leu	Leu
145	Asp	Gln	Ala	Ala	Ala	150	Leu	Lys	Trp	Val	Arg	Glu	155	Asn	Ile	Ser	Ala	Phe
Gly	Gly	Asp	Pro	165	Asn	Val	Thr	170	Val	Phe	Gly	Glu	175	Ser	Ala	Gly	Gly	
Ala	Ser	Ile	Ala	180	Ala	Leu	Leu	185	Ala	Met	Pro	Ala	190	Lys	Gly	Leu	Phe	
Gln	Lys	195	Ala	Ile	Met	Glu	Ser	200	Gly	Ala	Ser	Arg	205	Thr	Met	Thr	Lys	Glu
210	Gln	Ala	Ala	Ser	Thr	Ala	215	Ala	Phe	Leu	Gln	220	Val	Leu	Gly	Ile	Asn	
225	Glu	Ser	Gln	Leu	Asp	Arg	230	Leu	His	Thr	Val	235	Ala	Ala	Glu	Asp	Leu	Leu
Lys	Ala	Ala	Asp	245	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	250	Asn	Ile	Phe	Gln	
Leu	Phe	Phe	His	260	Pro	Ala	Leu	Asp	265	Pro	Lys	Thr	270	Leu	Pro	Glu	Glu	Pro
Glu	Lys	Ser	Ile	275	Ala	Glu	Gly	280	Ala	Ala	Ser	Gly	285	Ile	Pro	Leu	Leu	Ile
290	Gly	Thr	Thr	Arg	Asp	Glu	Gly	295	Tyr	Leu	Phe	Phe	300	Thr	Ser	Asp	Ser	Asp
305	Val	Arg	Ser	Gln	Glu	310	Thr	Leu	Asp	Ala	Ala	Leu	315	Glu	Tyr	Ser	Leu	Gly
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	325	Asp	Leu	Tyr	Pro	330	Arg	Ser	Leu	Glu	
Ser	Gln	Ile	His	340	Met	Val	Thr	345	Leu	Leu	Phe	Trp	350	Pro	Ala	Val		
Ala	Phe	Ala	Ser	355	Ala	Gln	Ser	360	His	Tyr	Ala	Pro	365	Val	Trp	Met	Tyr	Arg
370	Phe	Asp	Trp	His	Pro	Glu	Lys	375	Pro	Pro	Tyr	Asn	380	Lys	Ala	Phe	His	Ala
385	Leu	Glu	Leu	Pro	Phe	Val	Phe	390	Gly	Asn	Leu	Asp	395	Gly	Leu	Glu	Arg	Met
Ala	Lys	Ala	Glu	405	Ile	Thr	Asp	Glu	410	Val	Lys	Gln	415	Leu	Ser	His	Thr	Ile
Gln	Ser	Ala	Trp	420	Ile	Thr	Phe	425	Ala	Lys	Thr	Gly	430	Asn	Pro	Ser	Thr	Glu
Ala	Val	Asn	Trp	435	Pro	Ala	Tyr	440	His	Glu	Glu	Thr	445	Arg	Glu	Thr	Val	Ile
450	Leu	Asp	Ser	Glu	Ile	Thr	Ile	455	Glu	Asn	Asp	Pro	460	Glu	Ser	Glu	Lys	Arg
465	Gln	Lys	Leu	Phe	Pro	Ser	Lys	470	Gly	Glu			475					
				485														

<210> 29

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 29

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgccacacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcgcga	gcattgccgc	gctgctggcc	600

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atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
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ctggattttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggtg 1140
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aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccctg 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 30
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

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<400> 30
Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
      260      265      270
Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
      275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335

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Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Asp	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 31
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 31
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcgggtg gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggccc 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgtg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgcg gatttgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagccgtacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtt gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgtcttttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggt 1140
 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagcaaa gggtgaa 1467

<210> 32
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 32
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1	Thr	Glu	Asn	Gly	5	Val	His	Lys	Trp	10	Gly	Ile	Pro	Tyr	15	Ala	Lys	Pro
			20							25						30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Pro	Glu	Val	Trp		
		35					40						45					
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro			
	50					55					60							
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu			
65					70					75					80			
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn			
			85						90					95				
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala			
		100						105					110					
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu			
		115					120					125						
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met			
	130					135					140							
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu			
145					150					155					160			
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			165						170					175				
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly			
		180						185					190					
Ala	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe			
		195					200					205						
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Arg	Thr	Met	Thr	Lys	Glu			
	210					215					220							
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn			
225					230					235					240			
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu			
			245						250					255				
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln			
		260						265					270					
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro			
		275					280					285						
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile			
	290					295					300							
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp			
305					310					315					320			
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly			
			325						330					335				
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu			
			340					345					350					
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ser	Phe	Trp	Arg	Pro	Ala	Val			
		355					360					365						
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg			
	370					375				380								
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala			
385					390					395					400			
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met			
			405						410					415				
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile			
		420						425					430					
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu			
		435					440					445						
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile			
	450					455					460							
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg			
465					470					475					480			
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu										
			485															

<210> 33
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

CX2-128USP1_ST25

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 33

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gcgccggaac cgccggagggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
gtttggattc acggcgggtgc gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
tccaagctgg ctgcaagcag tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
gataatgtga cgggtgtttg tgaaagcgct ggtggcgcgga gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcggtgc aagccgtacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgaggagaa agagaatatc ttccagctgt tcttcagacc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgttacc aggaacaccc ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
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atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgtg 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 34

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 34

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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210     215     220

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Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Gln	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 35

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 35

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgccacacgc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaaatgt	ttttgcgcgg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
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ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaatct	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgaga	gcattgccgc	gctgtgtggc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagccgtacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcggtt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccagctgt	tcttcacgac	tgacttggtg	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
cgcgtgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
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ctgctgtttt	ggcgcgccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttcacgccc	1200
ctggagctgc	cgtttggttt	cggtaacctc	gatggcttgg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320
aagaccggca	accgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaaccggt	1380

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gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtagaa 1467

<210> 36
<211> 489
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered variant of B. subtilis pNB esterase

<400> 36
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20 25 30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35 40 45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50 55 60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65 70 75 80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85 90 95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100 105 110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115 120 125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130 135 140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145 150 155 160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165 170 175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180 185 190
Glu Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195 200 205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210 215 220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225 230 235 240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245 250 255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260 265 270
Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275 280 285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290 295 300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305 310 315 320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325 330 335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340 345 350
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355 360 365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370 375 380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385 390 395 400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405 410 415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420 425 430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu

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<210> 37
<211> 1467
<212> DNA
<213> Artificial Sequence
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<220>
<223> Engineered variant of B. subtilis pNB esterase

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ttcggtttca	tgcacctgag	cagcttcgat	gaggcgata	gcgacaattt	gggtctgctg	480	
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540	
gataatgtga	cggtgttttg	tgaaagcgct	gggtggcgata	gcattgccgc	gctgtctggcg	600	
atgccggctg	cgaaggcct	gtttcaaaag	gcgattattg	agagcgggtg	aagccgtacc	660	
atgaccaagg	aacaagcggc	gagcactcgc	gcagcttttc	tgcaagtgct	gggtatcaat	720	
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caactgcgta	tcgcggagaa	agagaatatc	ttccagctgt	tcttccagcc	tgacttgat	840	
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aagggtgcgg	aagcgggtatt	900	
ccgctgtttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgct	tgatagcgac	960	
gttcgtagcc	aggaaacctt	ggacgttcgc	ctggagtaca	gccttgggcaa	gccgtttggca	1020	
gcgaaagcgg	cggactttga	cccgcgacgc	ctggaattccc	aaatccacat	ggtgaccgat	1080	
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	gcgaccggtg	1140	
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200	
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260	
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttga	1320	
aagaccggca	acccgagcac	cgaggcgggt	aactggccgg	cttatcatga	agaaacccgt	1380	
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440	
caagaagctgt	tcccgaagcaa	gggtgaa				1467	

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<210> 38
<211> 489
<212> PRT
<213> Artificial Sequence
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<220>
<223> Engineered variant of B. subtilis pNB esterase

<400>	38														
Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1				5					10					15	
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		

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Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Arg	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 39

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 39

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gttcataagt	ggaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggctctgtc	180
tgccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgcgg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gctgtacctg	ggtgcccgtg	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgacctgag	cagcttcgat	gaggcgata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540

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gataatgtga cgggtgttttg tgaaagcgct ggtggcgctca gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtgc aagccgtacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaaagcg cgacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggatgtatc gtttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 40

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 40

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly

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Lys	Pro	Leu	Ala	325	Glu	Lys	Ala	Ala	Asp	330	Leu	Tyr	Pro	Arg	Ser	335	Leu	Glu
Ser	Gln	Ile	His	340	Met	Val	Thr	Asp	345	Leu	Leu	Phe	Trp	Arg	Pro	350	Ala	Val
Ala	Phe	Ala	Ser	355	Ala	Gln	Ser	360	His	Tyr	Ala	Pro	Val	Trp	Met	365	Tyr	Arg
Phe	Asp	Trp	His	370	Pro	Glu	Lys	375	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala	
Leu	Glu	Leu	Pro	385	Phe	Val	Phe	390	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met	
Ala	Lys	Ala	Glu	405	Ile	Thr	Asp	410	Gly	Val	Lys	Gln	Leu	Ser	His	415	Thr	Ile
Gln	Ser	Ala	Trp	420	Ile	Thr	Phe	425	Ala	Lys	Thr	Gly	Asn	Pro	Ser	430	Thr	Glu
Ala	Val	Asn	Trp	435	Pro	Ala	Tyr	440	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile	
Leu	Asp	Ser	Glu	450	Ile	Thr	Ile	455	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg	
Gln	Lys	Leu	Phe	465	Pro	Ser	Lys	470	Gly	Glu								
				485														

<210> 41
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 41
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 gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgccgctg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcgggtg gttttacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggccc 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgctc gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagccgtacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggttt tcttcgcgcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa gggttacctg ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggaagtaca gcctgggcaa gccgttggca 1020
 gagaaaagcgg cgacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaacccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagacaa gggtgaa 1467

<210> 42
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 42

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Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1				5					10					15	
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Arg	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Val	Phe	Phe	Ala	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 43

<211> 1467

<212> DNA

<213> Artificial sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 43

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gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
tgccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
gtttggattc acggcgggtg gttttacctg ggtgccggta gcgagccgct gtacgatggc 360
tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccc tgaactatcg tctgggcccg 420
ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgtg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgacccg 540
gataatgtga cgggtgtttg tgaaagcgct ggtggcgta gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
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gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccaggttt tcttcgcgcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctggtgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggtatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcggtg aactggccgg cttatcatga agaaacccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 44

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 44

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Ala Phe Tyr Leu Gly Ala
      100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180     185     190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu

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CX2-128USP1_ST25

210	Gln	Ala	Ala	Ser	Thr	Ala	215	Ala	Ala	Phe	Leu	Gln	220	Val	Leu	Gly	Ile	Asn
225	Glu	Ser	Gln	Leu	Asp	Arg	230	Leu	His	Thr	Val	235	Ala	Ala	Glu	Asp	Leu	Leu
					245	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln	
	Lys	Ala	Ala	Asp	260	Pro	Ala	Leu	Asp	265	Lys	Thr	Leu	Pro	Glu	Glu	Pro	
	Val	Phe	Phe	Ala	275	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile		
	Glu	Lys	Ser	Ile	Ala	Glu	290	Gly	Tyr	Leu	Phe	Phe	300	Thr	Ser	Asp	Ser	Asp
	Gly	Thr	Thr	Arg	Asp	Glu	310	Gly	Leu	Asp	Ala	Ala	315	Glu	Tyr	Ser	Leu	Gly
	Val	Arg	Ser	Gln	Glu	325	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu	
	Lys	Pro	Leu	Ala	Glu	340	Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Val	Phe	Trp
	Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Val	Phe	Trp	Arg	Pro	Ala	Val		
	Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg		
	Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala		
	Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met		
	Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile		
	Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu		
	Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile		
	Leu	Asp	Ser	Glu	Ile	Thr	455	Ile	Glu	Asn	Asp	Pro	460	Glu	Ser	Glu	Lys	Arg
	465	Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu			475					
					485													

<210> 45
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 45

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gctttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaaagctg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgcca	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaaag	gcgattatgg	agagcgggtg	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggtatcggt	gcacaccgtc	gcggcgagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccagggtt	tcttccagcc	tgactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgtttggc	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggtatgtat	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggtttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttgc	1320

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aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccggt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

<210> 46
<211> 489
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered variant of B. subtilis pNB esterase

<400> 46
Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1 5 10 15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20 25 30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35 40 45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50 55 60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65 70 75 80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85 90 95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala
100 105 110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115 120 125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130 135 140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145 150 155 160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165 170 175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180 185 190
Ala Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195 200 205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
210 215 220
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225 230 235 240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245 250 255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260 265 270
Val Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275 280 285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290 295 300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305 310 315 320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325 330 335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340 345 350
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355 360 365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370 375 380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385 390 395 400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405 410 415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420 425 430

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Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
 450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
 485

<210> 47
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 47
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 gttcataagt ggaaaggcat cccgtatgcg aagccgccag tggggccagt gcgtttcaaa 120
 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttgattc acggcgggtg gctgtacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgacccg 540
 gataatgtga cgggtgttgg tgaaagcgct ggtggcatga gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtt gggatatcaat 720
 gagagccagc tggaccgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagggtt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccctg 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaacgct 1440
 cagaagctgt tcccagacaa gggtgaa 1467

<210> 48
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 48
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala

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Gly	Ser	Glu	100	Leu	Tyr	Asp	Gly	105	Ser	Lys	Leu	Ala	Ala	110	Gln	Gly	Glu
Val	Ile	Val	115	Val	Thr	Leu	Asn	120	Tyr	Arg	Leu	Gly	Pro	125	Phe	Gly	Met
His	130	Leu	Ser	Ser	Phe	Asp	135	Glu	Ala	Tyr	Ser	Asp	140	Asn	Leu	Gly	Leu
145	Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	155	Ile	Ser	Ala	160
				165	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	175	Ala	Gly	Gly
			180	Pro	Asp	Asn	Val	Thr	Val	185	Phe	Gly	Glu	Ser	190	Ala	Gly
Met	Ser	Ile	195	Ala	Ala	Leu	Leu	Ala	200	Met	Pro	Ala	Ala	Lys	205	Gly	Leu
Gln	Lys	210	Ala	Ile	Met	Glu	Ser	215	Gly	Ala	Ser	Val	Thr	220	Met	Thr	Lys
Gln	225	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn	240
Glu	Ser	Gln	Leu	Asp	245	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu	255
Lys	Ala	Ala	Asp	260	Gln	Leu	Arg	Ile	Ala	265	Glu	Lys	Glu	Asn	Ile	Phe	Gln
Val	Phe	Phe	Gln	Pro	Ala	Leu	Asp	280	Pro	Lys	Thr	Leu	Pro	285	Glu	Glu	Pro
Glu	Lys	290	Ser	Ile	Ala	Glu	Gly	295	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
Gly	305	Thr	Thr	Arg	Asp	Glu	310	Gly	Tyr	Leu	Phe	Phe	315	Thr	Ser	Asp	Ser
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	335	Leu	Gly	320
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	345	Leu	Tyr	Pro	Arg	350	Ser	Leu	Glu
Ser	Gln	Ile	355	His	Met	Val	Thr	Asp	360	Leu	Leu	Phe	Trp	Arg	365	Pro	Ala
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	375	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg	380
Phe	385	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala	400
Leu	Glu	Leu	Pro	Phe	405	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met	415
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	445	Ser	Thr	Glu	
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	460	Thr	Val	Ile	
Leu	465	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg	480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu									

<210> 49

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 49

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480

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gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgacccg 540
gataatgtga cggtgtttgg tgaaagcgct ggtggcgctca gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagccgtacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgctga tcgcggagaa agagaatatc ttccagggtt tcttcgcgcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctggcgtttt ggcccccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggtg 1140
tggtatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagcaa gggtgaa 1467

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<210> 50

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 50

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190     195
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
200     205     210
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
215     220     225
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Val Phe Phe Ala Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320

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Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ala	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 51
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 51

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gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
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tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tgccgacccg	540
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gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgctga	tcgcggagaa	agagaatatc	ttccaggttt	tcttccagcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
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gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctggtgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320
aagaccggca	acccgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaacccgt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	ttccgagcaa	gggtgaa				1467

<210> 52
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

CX2-128USP1_ST25

<400> 52

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Ala Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Val Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325     330     335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340     345     350
Ser Gln Ile His Met Val Thr Asp Leu Val Phe Trp Arg Pro Ala Val
355     360     365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370     375     380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385     390     395     400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405     410     415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420     425     430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
435     440     445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
450     455     460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465     470     475     480
Gln Lys Leu Phe Pro Ser Lys Gly Glu
485

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<210> 53

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 53

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gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
gcgccggaac cgccggagggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
tgccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgccgag gataccccgt cccaaaatct gccggttatg 300
gtttggattc acggcgggtgc gttttacctg ggtgcccgtg gcgagccgct gtacgatggc 360
tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccc 420
ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
gataatgtga cgggtgtttgg tgaaagcgct ggtggcgctc gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtc aagcgtgacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcggtg aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcagaaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa ggtgtaa 1467

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<210> 54

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 54

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
      65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
      145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205

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Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
210	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn	
225					230				235					240	
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 55
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 55

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gttcataagt	ggaagagcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgcgggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gagcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggtcgaccc	540
gataatgtga	cggtgtttgg	tgaaagcgct	ggtggcgtca	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaagccct	gtttcaaaag	gcgattatgg	agagcggtgc	aagccgtacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggtatcgtt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttcgcgcc	tgactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gctcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aatccacat	ggtgaccgat	1080
ctggcggttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggtatgtatc	gcttcgattg	gcaccggag	aaaccgccgt	acaacaaagc	cttcacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260

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atcacggacg aggttaagca actgtccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcggg aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

<210> 56
<211> 489
<212> PRT
<213> Artificial Sequence

<220>
<223> Engineered variant of B. subtilis pNB esterase

<400> 56
Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20 25 30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35 40 45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50 55 60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65 70 75 80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85 90 95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100 105 110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115 120 125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130 135 140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145 150 155 160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165 170 175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180 185 190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195 200 205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210 215 220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225 230 235 240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245 250 255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260 265 270
Ala Phe Phe Ala Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275 280 285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290 295 300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305 310 315 320
Ala Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325 330 335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340 345 350
Ser Gln Ile His Met Val Thr Asp Leu Ala Phe Trp Arg Pro Ala Val
355 360 365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370 375 380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385 390 395 400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405 410 415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile

CX2-128USP1_ST25

<220>
<223> Engineered variant of B. subtilis pNB esterase

<400>	57						
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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180	
tgcccacagc	cgtcggagct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240	
gattgtctgt	acgttaactgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300	
gtttggattc	acggcggtgc	gttttactcg	ggtgcccgta	gcgagccgct	gtacgatggc	360	
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	gcgactatcg	tctgggcccg	420	
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgata	gcgacaattt	gggtctgctg	480	
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gataatgtga	cgggtgtttg	tgaagcgct	ggtggcgcta	gcattgcccg	gctgctggcc	600	
atgccggctg	cgaaggcct	gtttcaaaag	gcgattatgg	agagcggctg	aagcgcgacc	660	
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggatcaat	720	
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780	
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttcgcgcc	tgcactggat	840	
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900	
ccgctgtttg	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgct	tgatagcgac	960	
gttcgtagcc	aggaaaactt	ggacgtctgc	ctggagtaca	gcctgggcaa	gccgtttgga	1020	
gagaaagcgg	ggcacttgta	cccgcgacgc	ctggaattcc	aaatccacat	ggtgaccgat	1080	
ctggtgtttt	cgcgcccagc	cgtggcattc	gcgctctcac	aaagccatta	cgcaccgggtg	1140	
tggtatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200	
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260	
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320	
aagaccggca	accctgagc	cgaggcgttg	aactggccgg	cttatcatga	agaaaccctg	1380	
gaaaccgtga	tccctgactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440	
cagaagctgt	tcccgaqcaa	qqgtqaa				1467	

<220>
<223> Engineered variant of B. subtilis pNB esterase

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1				5					10					15	
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	

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Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Ala	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Ala	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Val	Phe	Trp	Arg	Ser	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Val	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 59

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 59

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgccacacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gctgtacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420

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ttcggttttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgaccgg 540
gataatgtga cgggtgtttg tgaaagcgct ggtggcatga gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtg gggatatcaat 720
gagagccagc tggatcgitt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccagttat tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctggcgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggtg 1140
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atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccgtg 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 60

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 60

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20     25     30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35     40     45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50     55     60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65     70     75     80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85     90     95
Leu Pro Val Met Val Trp Ile His Gly Ala Leu Tyr Leu Gly Ala
100    105    110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115    120    125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130    135    140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145    150    155    160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165    170    175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180    185    190
Met Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195    200    205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
210    215    220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225    230    235    240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245    250    255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260    265    270
Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275    280    285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290    295    300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp

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305	Val	Arg	Ser	Gln	Glu	310	Thr	Leu	Asp	Ala	Ala	315	Leu	Glu	Tyr	Ser	Leu	320	Gly
					325							330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu				
			340					345					350						
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ala	Phe	Trp	Arg	Pro	Ala	Val				
		355					360					365							
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg				
	370					375					380								
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala				
385					390					395					400				
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met				
				405					410					415					
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile				
			420					425						430					
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu				
		435				440							445						
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile				
	450					455					460								
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg				
465					470				475						480				
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu											
				485															

<210> 61
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 61
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
 tgccacacag cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcggtgc gctgtacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc cccaactatc tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggttttgg tgaaagcgct ggtggcatga gcattgccgc gctgtggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagttat tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctggtgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
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 ctggagctgc cgtttgtttt cgtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
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 cagaagctgt tcccagagca gggtgaa 1467

<210> 62
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

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<400> 62

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Met Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
      210      215      220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
      260      265      270
Leu Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
      275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
      290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
      340      345      350
Ser Gln Ile His Met Val Thr Asp Leu Val Phe Trp Arg Pro Ala Val
      355      360      365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
      370      375      380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385      390      395      400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
      405      410      415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
      420      425      430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
      435      440      445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
      450      455      460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465      470      475      480
Gln Lys Leu Phe Pro Ser Lys Gly Glu
      485

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<210> 63

<211> 1467

CX2-128USP1_ST25

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 63

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gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
gcgccggaac cgccggagggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
gtttggattc acggcgggtgc gctgtacctg ggtgcccgga gcgagccgct gtacgatggc 360
tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
ttcggtttca tgcacctgag cagcttcgat gaggcgata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
gataatgtga cgggtgtttg tgaaagcgct ggtggcgta gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagccgtacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtt gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcgagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
ccgaaaactt tggcgaaga gccggaataa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctggcgtttt ggcgccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggtg 1140
tggatgtatc gcttcgattg gcacccggag aaaccgcgct acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgtt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagagca gggtgaa 1467

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<210> 64

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 64

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala
      100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180     185     190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe

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Gln	Lys	195	Ala	Ile	Met	Glu	Ser	200	Gly	Ala	Ser	Arg	Thr	205	Met	Thr	Lys	Glu
210	Ala	Ala	Ser	Thr	Ala	Ala	Ala	215	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn		
225	Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu		
				245						250					255			
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln			
			260															
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro			
			275															
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile			
			290															
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp			
305					310										320			
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly			
				325											335			
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu			
			340															
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ala	Phe	Trp	Arg	Pro	Ala	Val			
			355															
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg			
			370															
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala			
385					390										400			
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met			
				405											415			
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile			
				420											430			
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu			
			435												445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile			
			450															
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg			
465					470										480			
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu										
				485														

<210> 65

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 65

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gcgccggaac	cgccggagggt	ctgggaggat	gttctggacg	caaccgcgta	tggctctgtc	180
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gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
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gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cggtgtttgg	tgaaagcgct	ggtggcgta	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtg	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcgagaa	agagaatatc	ttccaggctt	tcttcgcgcc	tgactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgtta	cccgcgcagc	ctggaatccc	aatccacat	ggtgaccgat	1080
ctggcgtttt	ggcgcccagc	cgtggcattc	cgctctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttcacgcc	1200

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ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagagca gggtgaa 1467

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<210> 66
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 66

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1			5					10					15		
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
		20						25				30			
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65				70					75					80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
			85					90					95		
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
		100						105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145				150					155					160	
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
			165					170					175		
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
		180						185					190		
Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225				230					235					240	
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
			245					250					255		
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
		260						265					270		
Ala	Phe	Phe	Ala	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305				310					315					320	
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
			325					330					335		
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
		340						345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ala	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385				390					395					400	
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
			405					410					415		

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Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 67
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 67

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	ccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tgccgacccg	540
gataatgtga	cggtgtttgg	tgaaagcgct	ggtggcgta	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gagattatgg	agagcggtgc	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggtatcggt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgacttggtg	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtat	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtatgcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctggggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgggtgttt	ggcgcggcag	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggtatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttggttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
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aagaccggca	acccgagcac	cgaggcgggt	aactggccgg	cttatcatga	agaaaccggt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	ttccgagcaa	gggtgaa				1467

<210> 68
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 68

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1				5					10					15	
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn

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Leu	Pro	Val	Met	85	Val	Trp	Ile	His	Gly	90	Gly	Ala	Phe	Tyr	Leu	95	Gly	Ala
Gly	Ser	Glu	Pro	100	Leu	Tyr	Asp	Gly	105	Ser	Lys	Leu	Ala	Ala	Gln	110	Gly	Glu
Val	Ile	Val	Val	115	Thr	Leu	Asn	Tyr	120	Arg	Leu	Gly	Pro	Phe	Gly	125	Phe	Met
His	Leu	Ser	Ser	130	Phe	Asp	Glu	Ala	135	Tyr	Ser	Asp	Asn	Leu	Gly	140	Leu	Leu
Asp	Gln	Ala	Ala	145	Ala	Leu	Lys	Trp	150	Val	Arg	Glu	Asn	Ile	Ser	155	Ala	Phe
Gly	Gly	Asp	Pro	165	Asp	Asn	Val	Thr	170	Val	Phe	Gly	Glu	Ser	Ala	175	Gly	Gly
Val	Ser	Ile	Ala	180	Ala	Leu	Leu	Ala	185	Met	Pro	Ala	Ala	Lys	190	Gly	Leu	Phe
Gln	Lys	Ala	Ile	195	Met	Glu	Ser	Gly	200	Ala	Ser	Val	Thr	Met	205	Thr	Lys	Glu
Gln	Ala	Ala	Ser	210	Thr	Ala	Ala	Ala	215	Phe	Leu	Gln	Val	Leu	Gly	220	Ile	Asn
Glu	Ser	Gln	Leu	225	Asp	Arg	Leu	His	230	Thr	Val	Ala	Ala	Glu	Asp	235	Leu	Leu
Lys	Ala	Ala	Asp	245	Gln	Leu	Arg	Ile	250	Glu	Lys	Glu	Asn	Ile	Phe	255	Gln	
Ala	Phe	Phe	Gln	260	Pro	Ala	Leu	Asp	265	Pro	Lys	Thr	Leu	Pro	Glu	270	Glu	Pro
Glu	Lys	Ser	Ile	275	Ala	Glu	Gly	Ala	280	Ala	Ser	Gly	Ile	Pro	Leu	285	Leu	Ile
Gly	Thr	Thr	Arg	290	Asp	Glu	Gly	Tyr	295	Leu	Phe	Phe	Thr	Ser	Asp	300	Ser	Asp
Val	Arg	Ser	Gln	305	Glu	Thr	Leu	Asp	310	Ala	Ala	Leu	Glu	Tyr	Ser	315	Leu	Gly
Lys	Pro	Leu	Ala	325	Glu	Lys	Ala	Ala	330	Asp	Leu	Tyr	Pro	Arg	Ser	335	Leu	Glu
Ser	Gln	Ile	His	340	Met	Val	Thr	Asp	345	Leu	Val	Phe	Trp	Arg	Pro	350	Ala	Val
Ala	Phe	Ala	Ser	355	Ala	Gln	Ser	His	360	Tyr	Ala	Pro	Val	Trp	Met	365	Tyr	Arg
Phe	Asp	Trp	His	370	Pro	Glu	Lys	Pro	375	Pro	Tyr	Asn	Lys	Ala	Phe	380	His	Ala
Leu	Glu	Leu	Pro	385	Phe	Val	Phe	Gly	390	Asn	Leu	Asp	Gly	Leu	Glu	395	Arg	Met
Ala	Lys	Ala	Glu	405	Ile	Thr	Asp	Glu	410	Val	Lys	Gln	Leu	Ser	His	415	Thr	Ile
Gln	Ser	Ala	Trp	420	Ile	Thr	Phe	Ala	425	Lys	Thr	Gly	Asn	Pro	Ser	430	Thr	Glu
Ala	Val	Asn	Trp	435	Pro	Ala	Tyr	His	440	Glu	Glu	Thr	Arg	Glu	Thr	445	Val	Ile
Leu	Asp	Ser	Glu	450	Ile	Thr	Ile	Glu	455	Asn	Asp	Pro	Glu	Ser	Glu	460	Lys	Arg
Gln	Lys	Leu	Phe	465	Pro	Ser	Lys	Gly	470	Glu						475		480
				485														

<210> 69

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 69

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgctcgacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgcgg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gttttacctg	ggtgccggta	gcgagccgct	gtacgatggc	360

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tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggcccc 420
ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgaccgg 540
gataatgtga cgggtgttgg tgaaagcgct ggtggcgctca gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtgc aagccgtacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccaggttt tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcggattt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccgggtg 1140
tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggcctgg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaacccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467
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<210> 70

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 70

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20     25     30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35     40     45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50     55     60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65     70     75     80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85     90     95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
100    105    110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115    120    125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130    135    140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145    150    155    160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165    170    175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180    185    190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195    200    205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Arg Thr Met Thr Lys Glu
210    215    220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225    230    235    240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245    250    255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260    265    270
Val Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275    280    285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290    295    300
```

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Gly 305	Thr	Thr	Arg	Asp	Glu 310	Gly	Tyr	Leu	Phe	Phe 315	Thr	Ser	Asp	Ser	Asp 320
Val	Arg	Ser	Gln	Glu 325	Thr	Leu	Asp	Ala	Ala 330	Leu	Glu	Tyr	Ser	Leu 335	Gly
Lys	Pro	Leu	Ala 340	Glu	Lys	Ala	Ala	Asp 345	Leu	Tyr	Pro	Arg	Ser 350	Leu	Glu
Ser	Gln	Ile 355	His	Met	Val	Thr	Asp 360	Leu	Leu	Phe	Trp	Arg 365	Pro	Ala	Val
Ala	Phe 370	Ala	Ser	Ala	Gln	Ser 375	His	Tyr	Ala	Pro	Val 380	Trp	Met	Tyr	Arg
Phe 385	Asp	Trp	His	Pro	Glu 390	Lys	Pro	Pro	Tyr	Asn 395	Lys	Ala	Phe	His	Ala 400
Leu	Glu	Leu	Pro	Phe 405	Val	Phe	Gly	Asn	Leu	Asp 410	Gly	Leu	Glu	Arg 415	Met
Ala	Lys	Ala	Glu 420	Ile	Thr	Asp	Glu	Val 425	Lys	Gln	Leu	Ser	His 430	Thr	Ile
Gln	Ser	Ala 435	Trp	Ile	Thr	Phe	Ala 440	Lys	Thr	Gly	Asn	Pro 445	Ser	Thr	Glu
Ala	Val 450	Asn	Trp	Pro	Ala	Tyr 455	His	Glu	Glu	Thr	Arg 460	Glu	Thr	Val	Ile
Leu 465	Asp	Ser	Glu	Ile	Thr 470	Ile	Glu	Asn	Asp	Pro 475	Glu	Ser	Glu	Lys	Arg 480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							

<210> 71
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 71

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gctgtacctg	gggtgccggt	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gagcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgtttgg	tggcgacccg	540
gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcgctc	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaaag	gcgattatgg	agagcggtgc	aagcgcgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tgatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgacttgat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcggatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgag	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaaagcg	cggacttgta	cccgcgcagc	ctggaatccc	aatccacat	ggtgaccgat	1080
ctggcgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggtgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320
aagaccggca	acccgagcac	cgaggcggtg	aactggccgg	cttatcatga	agaaaccggt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcagagaat	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagacaa	gggtgaa				1467

<210> 72
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Engineered variant of *B. subtilis* pNB esterase

<400> 72

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Leu Tyr Leu Gly Ala
      100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Ala Thr Met Thr Lys Glu
      210      215      220
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
      260      265      270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
      275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
      290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
      340      345      350
Ser Gln Ile His Met Val Thr Asp Leu Ala Phe Trp Arg Pro Ala Val
      355      360      365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
      370      375      380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385      390      395      400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
      405      410      415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
      420      425      430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
      435      440      445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
      450      455      460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465      470      475      480
Gln Lys Leu Phe Pro Ser Lys Gly Glu
      485

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<210> 73

<211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 73
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcgggtg gttttacctg ggtgccggta gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcga cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgta gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtt gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggttt tcttcgcgcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaaagcg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgggtgtttt ggcccccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggttaacct gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaacccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagacaa gggtgaa 1467

<210> 74
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 74
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160
 Asp Gln Ala Thr Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
 165 170 175
 Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
 180 185 190

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Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Val	Phe	Phe	Ala	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Val	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 75

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 75

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gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gtattacctg	ggtgccggtg	gccagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcggtc	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgtttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgcaatttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140

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tggaatgatac	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttgca	1320
aagaccggca	acccgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaaccctg	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	ccccgagcaa	gggtgaa				1467

<210> 76

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 76

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1			5					10					15		
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
			85					90						95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Gln	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
			165					170						175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
		180					185						190		
Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
			245						250				255		
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
			325					330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
		340						345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Gln	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met

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Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Phe	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
	145				150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Val	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
	225				230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Leu	Phe	Phe	Leu	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
	305				310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Gln	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
	385				390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
	465				470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 79

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 79

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaatct	gccggttatg	300

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gtttggattc acggcgggtgc gtattacctg ggtgccggtg gccagccgct gtacgatggc 360
tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
ttcggtttca tgcacctgag cagcttcgat gaggcgata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
gataatgtga cgggtgtttgg tgaaagcgct ggtggcgctc gcattgccgc gctgctggcc 600
atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcttgacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgctga tcgcggagaa agagaatatc ttccaggcat tcttccaacc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
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atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccctg 1380
gaaaccgtga tcctggactc tgagattacc atcagaaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 80

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 80

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      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
      65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
      100      105      110
Gly Ser Gln Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
      130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
      145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Leu Thr Met Thr Lys Glu
      210      215      220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
      225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
      260      265      270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
      275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile

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290		295		300
Gly Thr Thr Arg Asp Glu	Gly Tyr Leu Phe Phe	Thr Ser Asp Ser Asp		
305 Val Arg Ser Gln Glu Thr	Leu Asp Ala Ala Leu	Glu Tyr Ser Leu Gly		
Lys Pro Leu Ala Glu	Lys Ala Ala Asp Leu	Tyr Pro Arg Ser Leu	Glu	
Ser Gln Ile His Met Val	Thr Asp Leu Gln Phe	Trp Arg Pro Ala Val		
Ala Phe Ala Ser Ala Gln	Ser His Tyr Ala Pro	Val Trp Met Tyr Arg		
Phe Asp Trp His Pro Glu	Lys Pro Pro Tyr Asn	Lys Ala Phe His Ala		
385 Leu Glu Leu Pro Phe Val	Phe Gly Asn Leu Asp	Gly Leu Glu Arg Met		
Ala Lys Ala Glu Ile Thr	Asp Glu Val Lys Gln	Leu Ser His Thr Ile		
Gln Ser Ala Trp Ile Thr	Phe Ala Lys Thr Gly	Asn Pro Ser Thr Glu		
Ala Val Asn Trp Pro Ala	Tyr His Glu Glu Thr	Arg Glu Thr Val Ile		
Leu Asp Ser Glu Ile Thr	Ile Glu Asn Asp Pro	Glu Ser Glu Lys Arg		
465 Gln Lys Leu Phe Pro	Ser Lys Gly Glu			

<210> 81
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 81

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gcgccggaac	cgccggagggt	ctgggaggat	gttctggacg	caaccgcgta	tggctctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccc	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gtattacctg	ggtgcccgga	gccagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgata	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaaag	gcgattatgg	agagcgggtg	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tgatcggtt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcggcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccgggtg	1140
tgatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320
aagaccggca	acccgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaaccctg	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 82
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 82

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
100     105     110
Gly Ser Gln Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Asp Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325     330     335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340     345
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355     360     365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370     375     380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385     390     395     400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405     410     415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420     425     430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
435     440     445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
450     455     460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465     470     475     480
Gln Lys Leu Phe Pro Ser Lys Gly Glu
485

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<210> 83
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 83
 atgacccatc aaatcgctac cactcagtac ggcaaagtca aaggtacgac ggagaacggt 60
 gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccgccg cccaaaatct gccggttatg 300
 gtttgatttc acggcgggtgc gtattacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgata gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaaag gcgattatgg agagcgggtg aagcgatacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttcttgcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa gggtacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaagcggc cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcccccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gtttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcggg aactggccgg cttatcatga agaaaccggt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagagca gggtgaa 1467

<210> 84
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 84
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160
 Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
 165 170 175
 Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly

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Asp	Ser	Ile	180	Ala	Ala	Leu	Leu	Ala	185	Met	Pro	Ala	Ala	Lys	190	Gly	Leu	Phe
Gln	Lys	195	Ala	Ile	Met	Glu	Ser	200	Gly	Ala	Ser	Asp	Thr	205	Met	Thr	Lys	Glu
Gln	210	Ala	Ala	Ser	Thr	Ala	215	Ala	Phe	Leu	Gln	220	Val	Leu	Gly	Ile	Asn	240
225	Glu	Ser	Gln	Leu	230	Arg	Leu	His	Thr	235	Val	Ala	Ala	Glu	Asp	Leu	Leu	255
Lys	Ala	Ala	245	Asp	Gln	Leu	Arg	Ile	Ala	250	Glu	Lys	Glu	Asn	Ile	Phe	Gln	270
Leu	Phe	260	Phe	Leu	Pro	Ala	Leu	265	Asp	Pro	Lys	Thr	Leu	270	Pro	Glu	Glu	Pro
Glu	275	Lys	Ser	Ile	Ala	Glu	Gly	280	Ala	Ala	Ser	Gly	Ile	285	Pro	Leu	Leu	Ile
Gly	290	Thr	Thr	Arg	Asp	Glu	Gly	295	Tyr	Leu	Phe	Phe	Thr	300	Ser	Asp	Ser	Asp
305	Val	Arg	Ser	Gln	310	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly	320
Lys	Pro	Leu	315	Ala	Glu	Lys	Ala	Ala	320	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu	335
Ser	Gln	Ile	325	His	Met	Val	Thr	330	Asp	Leu	Leu	Phe	Trp	335	Arg	Pro	Ala	Val
Ala	340	Phe	Ala	Ser	Ala	Gln	Ser	345	His	Tyr	Ala	Pro	Val	350	Trp	Met	Tyr	Arg
Phe	355	Asp	Trp	His	Pro	Glu	Lys	360	Pro	Pro	Tyr	Asn	Lys	365	Ala	Phe	His	Ala
385	Leu	Glu	Leu	Pro	Phe	Val	Phe	390	Gly	Asn	Leu	Asp	Gly	395	Leu	Glu	Arg	Met
Ala	Lys	Ala	405	Glu	Ile	Thr	Asp	410	Glu	Val	Lys	Gln	Leu	415	Ser	His	Thr	Ile
Gln	Ser	Ala	420	Trp	Ile	Thr	Phe	425	Ala	Lys	Thr	Gly	Asn	430	Pro	Ser	Thr	Glu
Ala	435	Val	Asn	Trp	Pro	Ala	Tyr	440	His	Glu	Glu	Thr	Arg	445	Glu	Thr	Val	Ile
Leu	450	Asp	Ser	Glu	Ile	Thr	Ile	455	Glu	Asn	Asp	Pro	Glu	460	Ser	Glu	Lys	Arg
465	Gln	Lys	Leu	Phe	470	Pro	Ser	Lys	Gly	Glu	475			480				
				485														

<210> 85

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 85

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atgacccatc aaatcgtcac cactcagtac ggcaaagtca aaggtacgac ggagaacggt 60
gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
gcgccggaac cgccggagggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
gattgtctgt acgttaatgt ttttgcgccg gatacccccgt cccaaaatct gccggttatg 300
gtttggattc acggcgggtgc gtattacctg ggtgccggta gcgagccgct gtacgatggc 360
tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc tgaactatcg tctgggcccg 420
ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
gataatgtga cgggtgtttg tgaaagcgct ggtggcgaaa gcattgccgc gctgctggcc 600
atgcccgtg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtc aagcgatacc 660
atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtc gggatatcaat 720
gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
gttcgtagcc aggaacactt ggacgctgcg ctggagtaca gcctgggcaa gccgttgga 1020
gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080

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ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaacccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 86

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 86

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20     25     30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35     40     45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50     55     60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65     70     75     80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85     90     95
Leu Pro Val Met Val Trp Ile His Gly Ala Tyr Tyr Leu Gly Ala
100    105    110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115    120    125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130    135    140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145    150    155    160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165    170    175    180
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
185    190    195
Glu Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
200    205    210
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Asp Thr Met Thr Lys Glu
215    220    225
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
230    235    240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245    250    255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260    265    270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275    280    285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290    295    300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305    310    315    320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325    330    335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340    345    350
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355    360    365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370    375    380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385    390    395    400

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Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
 405 410 415
 Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
 420 425 430
 Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
 450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
 485

<210> 87
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 87
 atgacccatc aaatcgtcac cactcagtac ggcaaagtca aaggtagcac ggagaacggt 60
 gttcataagt ggaaaggcat cccgtatgcg aagccgccag tgggccagtg gcgtttcaaa 120
 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgccacacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcggtgc gtattacctg ggtgccggtg gcgagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt ggggtctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgaccgc 540
 gataatgtga cgggtgtttgg tgaaagcgct ggtggcgata gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcggatatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
 tggatgtatc gtttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcggtg aactggccgg cttatcatga agaaacccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagagca gggtagaa 1467

<210> 88
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 88
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
 1 5 10 15
 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu

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65	Asp	Cys	Leu	Tyr	Val	70	Asn	Val	Phe	Ala	Pro	75	Asp	Thr	Pro	Ser	Gln	80	Asn
					85							90					95		
	Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala			
			100						105							110			
	Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu			
			115					120											
	Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met			
			130				135					140							
	His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu			
					150						155								
	Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe			
					165					170									
	Gly	Gly	Asp	Pro	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly				
			180					185											
	Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe			
			195					200					205						
	Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu			
			210				215					220							
	Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn			
					230						235								
	Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu			
					245					250									
	Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln			
				260					265										
	Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro			
			275					280											
	Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile			
			290				295					300							
	Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp			
					310						315								
	Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly			
					325					330									
	Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu			
				340					345										
	Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val			
				355				360					365						
	Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg			
							375					380							
	Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala			
					390						395								
	Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met			
					405						410								
	Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile			
				420					425										
	Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu			
				435				440					445						
	Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile			
				450			455					460							
	Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg			
					470						475								
	Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu										
					485														

<210> 89

<211> 1467

<212> DNA

<213> Artificial sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 89

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gcgccggaac	cgccggagggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240

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gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gtattacctg	ggtgccggta	gccagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
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atgccggctg	cgaagggcct	gtttcaaaag	gcgattatgg	agagcgggtg	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
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ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
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gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgtttggca	1020
gagaaagcgg	cggacttcta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgtcatttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
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<210> 90

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 90

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			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
			35				40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
			50			55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65				70					75					80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Gln	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
			115				120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
			130			135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145				150					155					160	
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
			195				200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
			210			215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn	
225				230					235					240	
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
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Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
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Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ser	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 91

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 91

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gtattacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
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gataatgtga	cggtgtttgg	tgaaagcgct	ggtggcgctca	gcattgccgc	gctgctggcc	600
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atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
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gttcgtagcc	aggaacacct	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
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aagaccggca	acccgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaacccgt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 92

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 92

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Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325     330     335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340     345     350
Ser Gln Ile His Met Val Thr Asp Leu Leu Phe Trp Arg Pro Ala Val
355     360     365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370     375     380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385     390     395     400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405     410     415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420     425     430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
435     440     445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
450     455     460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465     470     475     480
Gln Lys Leu Phe Pro Ser Lys Gly Glu
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<210> 93
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

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 gcgccggaac cgccggaggt ctgggaggtt gttctggacg caaccgcgta tggctctgtc 180
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 gattgtctgt acgttaatgt ttttgccggt gataccccgt cccaaaatct gccggttatg 300
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 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
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 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaaccgt 1380
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 cagaagctgt tcccagacaa gggtgaa 1467

<210> 94
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 94
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 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
 100 105 110
 Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160
 Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
 165 170 175

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Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
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Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Ser	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440				445				
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 95

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 95

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gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020

CX2-128USP1_ST25

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gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
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tggatgtatc gcttcgattg gcaccggag aaaccgccgt acaacaaagc cttccacgcc 1200
ctggagctgc gttttgtttt cggtaacctg gatggccttg aacgtatggc caaagccgaa 1260
atcacggacg aggttaagca actgtcccac acgattcaga ggccttgat tacgtttgca 1320
aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccggt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 96

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 96

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
      20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
      35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
      50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
      85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
      100      105      110
Gly Ser Gln Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
      115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
      165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
      180      185      190
Asp Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
      195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Leu Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
      245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
      260      265      270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
      275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
      325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
      340      345      350
Ser Gln Ile His Met Val Thr Asp Leu Ser Phe Trp Arg Pro Ala Val
355      360      365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370      375      380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala

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Ser 65	Asp	Leu	Leu	Ser 70	Leu	Ser	Tyr	Thr	Glu	Leu 75	Pro	Arg	Gln	Ser	Glu 80
Asp	Cys	Leu	Tyr	Val 85	Asn	Val	Phe	Ala	Pro 90	Asp	Thr	Pro	Ser	Gln 95	Asn
Leu	Pro	Val	Met 100	Val	Trp	Ile	His	Gly 105	Gly	Ala	Tyr	Tyr	Leu 110	Gly	Ala
Gly	Ser	Gln 115	Pro	Leu	Tyr	Asp	Gly 120	Ser	Lys	Leu	Ala	Ala 125	Gln	Gly	Glu
Val 130	Ile	Val	Val	Thr	Leu	Asn 135	Tyr	Arg	Leu	Gly	Pro 140	Phe	Gly	Phe	Met
His 145	Leu	Ser	Ser	Phe	Asp 150	Glu	Ala	Tyr	Ser	Asp 155	Asn	Leu	Gly	Leu	Leu 160
Asp	Gln	Ala	Ala 165	Leu	Lys	Trp	Val	Arg 170	Glu	Asn	Ile	Ser	Ala 175	Phe	
Gly	Gly	Asp	Pro 180	Asp	Asn	Val	Thr	Val 185	Phe	Gly	Glu	Ser	Ala 190	Gly	Gly
Asp	Ser	Ile 195	Ala	Ala	Leu	Leu	Ala 200	Met	Pro	Ala	Ala	Lys 205	Gly	Leu	Phe
Gln	Lys 210	Ala	Ile	Met	Glu	Ser 215	Gly	Ala	Ser	Val	Thr 220	Met	Thr	Lys	Glu
Gln 225	Ala	Ala	Ser	Thr	Ala 230	Ala	Ala	Phe	Leu	Gln 235	Val	Leu	Gly	Ile	Asn 240
Glu	Ser	Gln	Leu	Asp 245	Arg	Leu	His	Thr	Val 250	Ala	Ala	Glu	Asp	Leu 255	Leu
Lys	Ala	Ala	Asp 260	Gln	Leu	Arg	Ile	Ala 265	Glu	Lys	Glu	Asn	Ile 270	Phe	Gln
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp 280	Pro	Lys	Thr	Leu	Pro 285	Glu	Glu	Pro
Glu	Lys 290	Ser	Ile	Ala	Glu	Gly 295	Ala	Ala	Ser	Gly	Ile 300	Pro	Leu	Leu	Ile
Gly 305	Thr	Thr	Arg	Asp	Glu 310	Gly	Tyr	Leu	Phe	Phe 315	Thr	Ser	Asp	Ser	Asp 320
Val	Arg	Ser	Gln	Glu 325	Thr	Leu	Asp	Ala	Ala 330	Leu	Glu	Tyr	Ser	Leu 335	Gly
Lys	Pro	Leu	Ala 340	Glu	Lys	Ala	Ala	Asp 345	Leu	Tyr	Pro	Arg	Ser 350	Leu	Glu
Ser	Gln	Ile 355	His	Met	Val	Thr	Asp 360	Leu	Leu	Phe	Trp	Arg 365	Pro	Ala	Val
Ala	Phe	Ala	Ser	Ala	Gln	Ser 375	His	Tyr	Ala	Pro	Val 380	Trp	Met	Tyr	Arg
Phe 385	Asp	Trp	His	Pro	Glu 390	Lys	Pro	Pro	Tyr	Asn 395	Lys	Ala	Phe	His	Ala 400
Leu	Glu	Leu	Pro	Phe 405	Val	Phe	Gly	Asn	Leu 410	Asp	Gly	Leu	Glu	Arg 415	Met
Ala	Lys	Ala	Glu 420	Ile	Thr	Asp	Glu	Val 425	Lys	Gln	Leu	Ser	His 430	Thr	Ile
Gln	Ser	Ala 435	Trp	Ile	Thr	Phe	Ala 440	Lys	Thr	Gly	Asn	Pro 445	Ser	Thr	Glu
Ala 450	Val	Asn	Trp	Pro	Ala	Tyr 455	His	Glu	Glu	Thr	Arg 460	Glu	Thr	Val	Ile
Leu 465	Asp	Ser	Glu	Ile	Thr 470	Ile	Glu	Asn	Asp	Pro 475	Glu	Ser	Glu	Lys	Arg 480
Gln	Lys	Leu	Phe	Pro 485	Ser	Lys	Gly	Glu							

<210> 99

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 99

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gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180

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tgccacagc	cgctcgacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gtattacctg	ggtgccggta	gctggccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggcccg	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
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gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcgata	gcattgccgc	gctgctggcc	600
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gttcgtagcc	aggaacacct	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttgcca	1020
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tggtatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttcacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
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cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 100
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 100

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		20						25				30			
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50				55					60					
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65				70					75					80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
			85					90						95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala
		100						105					110		
Gly	Ser	Trp	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145				150					155					160	
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
			165					170						175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
		180					185						190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Leu	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225				230						235				240	
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
			245					250						255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
		260					265						270		
Ala	Phe	Phe	Leu	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro

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Glu	Lys	275	Ser	Ile	Ala	Glu	Gly	280	Ala	Ala	Ser	Gly	Ile	285	Pro	Leu	Leu	Ile
	290						295						300					
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp			
305					310					315					320			
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly			
				325					330						335			
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu			
			340					345						350				
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Gln	Phe	Trp	Arg	Pro	Ala	Val			
			355				360						365					
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg			
	370					375					380							
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala			
385					390					395					400			
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met			
				405				410						415				
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile			
			420					425						430				
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu			
		435					440					445						
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile			
	450					455					460							
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg			
465					470					475					480			
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu										
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<210> 101
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 101
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggctctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttgatttc acggcgggtgc gttttacctg ggtgccggtg gccagccgct gtacgatggc 360
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 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
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 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcggtgc aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa gggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
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 cagaagctgt tcccagagca gggtgaa 1467

<210> 102
 <211> 489
 <212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 102

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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
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Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
55      60      65      70      75      80
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
85      90      95      100
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
105      110      115      120
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
125      130      135      140
Leu Pro Val Met Val Trp Ile His Gly Ala Phe Tyr Leu Gly Ala
145      150      155      160
Gly Ser Gln Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
165      170      175      180
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
185      190      195      200
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
205      210      215      220
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
225      230      235      240
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
245      250      255      260
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
265      270      275      280
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
285      290      295      300
Gln Ala Ala Ser Thr Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
305      310      315      320
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
325      330      335      340
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
345      350      355      360
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
365      370      375      380
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
385      390      395      400
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
405      410      415      420
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
425      430      435      440
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
445      450      455      460
Ser Gln Ile His Met Val Thr Asp Leu Gln Phe Trp Arg Pro Ala Val
465      470      475      480
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
485      490      495      500
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
505      510      515      520
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
525      530      535      540
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
545      550      555      560
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
565      570      575      580
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
585      590      595      600
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
605      610      615      620
Gln Lys Leu Phe Pro Ser Lys Gly Glu
625      630      635      640

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<210> 103
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 103
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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
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 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcacc cgaactatcg tctgggcccg 420
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 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgtttgg tggcgacctg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgata gcattgccgc gctgctggcc 600
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 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgtt gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccagctgt tcttccaacc tgcactggat 840
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 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
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 aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaacccgt 1380
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 cagaagctgt tcccagacaa ggggtgaa 1467

<210> 104
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 104
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 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
 100 105 110
 Gly Ser Trp Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160
 Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe

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Gly	Gly	Asp	Pro	165	Asn	Val	Thr	Val	170	Phe	Gly	Glu	Ser	Ala	175	Gly	Gly
Asp	Ser	Ile	Ala	180	Ala	Leu	Leu	Ala	185	Met	Pro	Ala	Ala	Lys	190	Gly	Leu
Gln	Lys	Ala	Ile	195	Met	Glu	Ser	Gly	200	Ala	Ser	Leu	Thr	Met	205	Thr	Lys
Gln	Ala	Ala	Ser	210	Thr	Ala	Ala	Phe	215	Leu	Gln	Val	Leu	Gly	220	Ile	Asn
225	Glu	Ser	Gln	230	Leu	Asp	Arg	Leu	235	His	Thr	Val	Ala	Ala	Glu	Asp	Leu
Lys	Ala	Ala	Asp	245	Gln	Leu	Arg	Ile	250	Ala	Glu	Lys	Glu	Asn	255	Ile	Phe
Leu	Phe	Phe	Gln	260	Pro	Ala	Leu	Asp	265	Pro	Lys	Thr	Leu	Pro	270	Glu	Glu
Glu	Lys	Ser	Ile	275	Ala	Glu	Gly	Ala	280	Ala	Ser	Gly	Ile	Pro	285	Leu	Leu
Gly	Thr	Thr	Arg	290	Asp	Glu	Gly	Tyr	295	Leu	Phe	Phe	Thr	Ser	300	Asp	Ser
305	Val	Arg	Ser	310	Gln	Glu	Thr	Leu	315	Asp	Ala	Ala	Glu	Tyr	320	Ser	Leu
Lys	Pro	Leu	Ala	325	Glu	Lys	Ala	Ala	330	Leu	Tyr	Pro	Arg	Ser	335	Leu	Glu
Ser	Gln	Ile	His	340	Met	Val	Thr	Asp	345	Leu	Leu	Phe	Trp	Arg	350	Pro	Ala
Ala	Phe	Ala	Ser	355	Ala	Gln	Ser	His	360	Tyr	Ala	Pro	Val	Trp	365	Met	Tyr
Phe	Asp	Trp	His	370	Pro	Glu	Lys	Pro	375	Pro	Tyr	Asn	Lys	Ala	380	Phe	His
385	Leu	Glu	Leu	390	Phe	Val	Phe	Gly	395	Asn	Leu	Asp	Gly	Leu	400	Glu	Arg
Ala	Lys	Ala	Glu	405	Ile	Thr	Asp	Glu	410	Val	Lys	Gln	Leu	Ser	415	His	Thr
Gln	Ser	Ala	Trp	420	Ile	Thr	Phe	Ala	425	Lys	Thr	Gly	Asn	Pro	430	Ser	Thr
Ala	Val	Asn	Trp	435	Pro	Ala	Tyr	His	440	Glu	Glu	Thr	Arg	Glu	445	Thr	Val
Leu	Asp	Ser	Glu	450	Ile	Thr	Ile	Glu	455	Asn	Asp	Pro	Glu	Ser	460	Glu	Lys
465	Gln	Lys	Leu	470	Pro	Ser	Lys	Gly	475	Glu					480	Arg	
				485													

<210> 105

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 105

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gtattacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcaccc	tgaactatcg	tctgggcccg	420
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gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcgata	gcattgccgc	gctgctggcc	600
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atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgacttggtg	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960

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gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tgatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggccttg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgtttgca	1320
aagaccggca	acccgagcac	cgaggcgggtg	aactggcccg	cttatcatga	agaaacccgt	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 106

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 106

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
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Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
			20					25					30		
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
		35					40					45			
Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65				70					75					80	
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala
		100						105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
145					150					155					160
Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
			165						170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
		180						185					190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
			245						250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
			325					330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				

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Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
 385 390 400
 Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
 405 410 415
 Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
 420 425 430
 Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
 435 440 445
 Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
 450 455 460
 Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
 465 470 475 480
 Gln Lys Leu Phe Pro Ser Lys Gly Glu
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<210> 107

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 107

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 gcgccggaac cgccggaggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
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 gtttggattc acggcgggtg gttttacctg ggtgcccgtg gccagccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccc tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggctctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgct ggtggcgata gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaag gcgattatgg agagcgggtg aagcttgacc 660
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 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttcttgcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
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 tggatgtatc gcttcgattg gcacccggag aaaccgccgt acaacaaagc cttccacgcc 1200
 ctggagctgc cgtttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcggat aactggccgg cttatcatga agaaaccggt 1380
 gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tccccagcaa gggtgaa 1467

<210> 108

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 108

Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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 Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro

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50	Ser	Asp	Leu	Leu	Ser	Leu	55	Ser	Tyr	Thr	Glu	Leu	60	Pro	Arg	Gln	Ser	Glu
65	Asp	Cys	Leu	Tyr	Val	Asn	70	Val	Phe	Ala	Pro	Asp	75	Thr	Pro	Ser	Gln	Asn
	Leu	Pro	Val	Met	Val	Trp	85	Ile	His	Gly	Gly	Ala	90	Phe	Tyr	Leu	Gly	Ala
	Gly	Ser	Gln	Pro	Leu	Tyr	100	Asp	Gly	Ser	Lys	Leu	105	Ala	Ala	Gln	Gly	Glu
	Val	Ile	Val	Val	Thr	Leu	115	Asn	Tyr	Arg	Leu	Gly	120	Pro	Phe	Gly	Phe	Met
	His	Leu	Ser	Ser	Phe	Asp	130	Glu	Ala	Tyr	Ser	Asp	135	Asn	Leu	Gly	Leu	Leu
145	Asp	Gln	Ala	Ala	Ala	Leu	150	Lys	Trp	Val	Arg	Glu	155	Asn	Ile	Ser	Ala	Phe
	Gly	Gly	Asp	Pro	Asp	Asn	165	Val	Thr	Val	Phe	Gly	170	Glu	Ser	Ala	Gly	Gly
	Asp	Ser	Ile	Ala	Ala	Leu	180	Leu	Ala	Met	Pro	Ala	185	Ala	Lys	Gly	Leu	Phe
	Gln	Lys	Ala	Ile	Met	Glu	195	Ser	Gly	Ala	Ser	Leu	200	Thr	Met	Thr	Lys	Glu
	Gln	Ala	Ala	Ser	Thr	Ala	210	Ala	Ala	Phe	Leu	Gln	215	Val	Leu	Gly	Ile	Asn
225	Glu	Ser	Gln	Leu	Asp	Arg	230	Leu	His	Thr	Val	Ala	235	Ala	Glu	Asp	Leu	Leu
	Lys	Ala	Ala	Asp	Gln	Leu	245	Arg	Ile	Ala	Glu	Lys	250	Glu	Asn	Ile	Phe	Gln
	Ala	Phe	Phe	Leu	Pro	Ala	260	Leu	Asp	Pro	Lys	Thr	265	Leu	Pro	Glu	Glu	Pro
	Glu	Lys	Ser	Ile	Ala	Glu	275	Gly	Ala	Ala	Ser	Gly	280	Ile	Pro	Leu	Leu	Ile
	Gly	Thr	Thr	Arg	Asp	Glu	290	Gly	Tyr	Leu	Phe	Phe	300	Thr	Ser	Asp	Ser	Asp
305	Val	Arg	Ser	Gln	Glu	Thr	310	Leu	Asp	Ala	Ala	Leu	315	Glu	Tyr	Ser	Leu	Gly
	Lys	Pro	Leu	Ala	Glu	Lys	325	Ala	Ala	Asp	Leu	Tyr	330	Pro	Arg	Ser	Leu	Glu
	Ser	Gln	Ile	His	Met	Val	340	Thr	Asp	Leu	Asp	Phe	345	Trp	Arg	Pro	Ala	Val
	Ala	Phe	Ala	Ser	Ala	Gln	355	Ser	His	Tyr	Ala	Pro	360	Val	Trp	Met	Tyr	Arg
	Phe	Asp	Trp	His	Pro	Glu	370	Lys	Pro	Pro	Tyr	Asn	375	Lys	Ala	Phe	His	Ala
385	Leu	Glu	Leu	Pro	Phe	Val	390	Phe	Gly	Asn	Leu	Asp	395	Gly	Leu	Glu	Arg	Met
	Ala	Lys	Ala	Glu	Ile	Thr	405	Asp	Glu	Val	Lys	Gln	410	Leu	Ser	His	Thr	Ile
	Gln	Ser	Ala	Trp	Ile	Thr	420	Phe	Ala	Lys	Thr	Gly	425	Asn	Pro	Ser	Thr	Glu
	Ala	Val	Asn	Trp	Pro	Ala	435	Tyr	His	Glu	Glu	Thr	440	Arg	Glu	Thr	Val	Ile
	Leu	Asp	Ser	Glu	Ile	Thr	450	Ile	Glu	Asn	Asp	Pro	455	Glu	Ser	Glu	Lys	Arg
465	Gln	Lys	Leu	Phe	Pro	Ser	470	Lys	Gly	Glu			475					
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<210> 109

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 109

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aagaccggca acccgagcac cgaggcgggtg aactggccgg cttatcatga agaaaccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa gggtgaa 1467

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<210> 110

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 110

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20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Asp Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Leu Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270

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	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315				320	
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325					330					335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Gln	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405					410					415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440				445				
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 111

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 111

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gcgccggaac	cgccggaggt	ctgggaggtat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgctggacct	gttgagcctg	agctatacgg	aaactgccacg	tcagagcgaa	240
gattgtctgt	acgttaattgt	ttttgccccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gtattacctg	ggtgccccgt	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
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gataatgtga	cggtgttttg	tgaaagcgct	ggtggcgctca	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaag	gcgattatgg	agagcggtgc	aagcgtgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgacttggtg	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgtgtttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctgggcaa	gccgttggca	1020
gagaaagcgg	cggacttgta	cccgcgcagc	ctggaatccc	aaatccacat	ggtgaccgat	1080
ctgcaatttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
tggtatgtatc	gcttcgattg	gcacccggag	aaaccgccgt	acaacaaagc	cttccacgcc	1200
ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
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aagaccggca	acccgagcac	cgaggcgggtg	aactggccgg	cttatcatga	agaaaccgtg	1380
gaaaccgtga	tcctggactc	tgagattacc	atcagagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	ttccgagcaa	gggtgaa				1467

<210> 112

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 112

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
1      5      10      15
Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
100      105      110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115      120      125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130      135      140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145      150      155      160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165      170      175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180      185      190
Val Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195      200      205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Val Thr Met Thr Lys Glu
210      215      220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225      230      235      240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245      250      255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260      265      270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275      280      285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290      295      300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305      310      315      320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325      330      335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340      345      350
Ser Gln Ile His Met Val Thr Asp Leu Gln Phe Trp Arg Pro Ala Val
355      360      365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg
370      375      380
Phe Asp Trp His Pro Glu Lys Pro Pro Tyr Asn Lys Ala Phe His Ala
385      390      395      400
Leu Glu Leu Pro Phe Val Phe Gly Asn Leu Asp Gly Leu Glu Arg Met
405      410      415
Ala Lys Ala Glu Ile Thr Asp Glu Val Lys Gln Leu Ser His Thr Ile
420      425      430
Gln Ser Ala Trp Ile Thr Phe Ala Lys Thr Gly Asn Pro Ser Thr Glu
435      440      445
Ala Val Asn Trp Pro Ala Tyr His Glu Glu Thr Arg Glu Thr Val Ile
450      455      460
Leu Asp Ser Glu Ile Thr Ile Glu Asn Asp Pro Glu Ser Glu Lys Arg
465      470      475      480
Gln Lys Leu Phe Pro Ser Lys Gly Glu

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<210> 113
 <211> 1467
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 113
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 gcgccggaac cgccggagggt ctgggaggat gttctggacg caaccgcgta tggtcctgtc 180
 tgcccacagc cgtcggacct gttgagcctg agctatacgg aactgccacg tcagagcgaa 240
 gattgtctgt acgttaatgt ttttgcgccg gataccccgt cccaaaatct gccggttatg 300
 gtttggattc acggcggtgc gttttacctg ggtgccggtg gctggccgct gtacgatggc 360
 tccaagctgg ctgcgcaagg tgaggtcatt gtcgtcaccg tgaactatcg tctgggcccg 420
 ttcggtttca tgcacctgag cagcttcgat gaggcgtata gcgacaattt gggtctgctg 480
 gaccaagcgg cagccctgaa atgggttcgc gagaacatca gcgcgttttg tggcgacccg 540
 gataatgtga cgggtgtttg tgaaagcgcg ggtggcgata gcattgccgc gctgctggcc 600
 atgccggctg cgaaaggcct gtttcaaaaag gcgattatgg agagcgggtc aagcgtgacc 660
 atgaccaagg aacaagcggc gagcactgcg gcagcttttc tgcaagtgct gggatatcaat 720
 gagagccagc tggatcgttt gcacaccgtc gcagcggagg acctgctgaa ggcagctgac 780
 caactgcgta tcgcggagaa agagaatatc ttccaggctt tcttccagcc tgcactggat 840
 ccgaaaactt tgccggaaga gccggaaaaa tccattgcgg aaggtgcggc aagcgggtatt 900
 ccgctgttga ttggtaccac gcgcgacgaa ggttacctgt ttttcacgtc tgatagcgac 960
 gttcgtagcc aggaaacctt ggacgctgcg ctggagtaca gcctgggcaa gccgttggca 1020
 gagaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
 ctgctgtttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
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 ctggagctgc gttttgtttt cggtaacctg gatggcttgg aacgtatggc caaagccgaa 1260
 atcacggacg aggttaagca actgtcccac acgattcaga gcgcctggat tacgtttgca 1320
 aagaccggca acccgagcac cgaggcggtg aactggccgg cttatcatga agaaaccgt 1380
 gaaaccgtga tcctggactc tgagattacc atcgaagaatg accctgagag cgagaagcgt 1440
 cagaagctgt tcccagacaa ggtgaa 1467

<210> 114
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Engineered variant of B. subtilis pNB esterase

<400> 114
 Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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 20 25 30
 Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Glu Val Trp
 35 40 45
 Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
 50 55 60
 Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
 65 70 75 80
 Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
 85 90 95
 Leu Pro Val Met Val Trp Ile His Gly Gly Ala Phe Tyr Leu Gly Ala
 100 105 110
 Gly Ser Trp Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
 115 120 125
 Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
 130 135 140
 His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
 145 150 155 160

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Asp	Gln	Ala	Ala	Ala	Leu	Lys	Trp	Val	Arg	Glu	Asn	Ile	Ser	Ala	Phe
				165					170					175	
Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
			180					185					190		
Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
		195					200					205			
Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Val	Thr	Met	Thr	Lys	Glu
	210					215					220				
Gln	Ala	Ala	Ser	Thr	Ala	Ala	Ala	Phe	Leu	Gln	Val	Leu	Gly	Ile	Asn
225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
				245					250					255	
Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
			260					265					270		
Ala	Phe	Phe	Gln	Pro	Ala	Leu	Asp	Pro	Lys	Thr	Leu	Pro	Glu	Glu	Pro
		275					280					285			
Glu	Lys	Ser	Ile	Ala	Glu	Gly	Ala	Ala	Ser	Gly	Ile	Pro	Leu	Leu	Ile
	290					295					300				
Gly	Thr	Thr	Arg	Asp	Glu	Gly	Tyr	Leu	Phe	Phe	Thr	Ser	Asp	Ser	Asp
305					310					315					320
Val	Arg	Ser	Gln	Glu	Thr	Leu	Asp	Ala	Ala	Leu	Glu	Tyr	Ser	Leu	Gly
				325				330						335	
Lys	Pro	Leu	Ala	Glu	Lys	Ala	Ala	Asp	Leu	Tyr	Pro	Arg	Ser	Leu	Glu
			340					345					350		
Ser	Gln	Ile	His	Met	Val	Thr	Asp	Leu	Leu	Phe	Trp	Arg	Pro	Ala	Val
		355					360					365			
Ala	Phe	Ala	Ser	Ala	Gln	Ser	His	Tyr	Ala	Pro	Val	Trp	Met	Tyr	Arg
	370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His	Ala
385					390					395					400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg	Met
				405				410						415	
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
			420					425					430		
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
	450					455					460				
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
465					470					475					480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu							
				485											

<210> 115

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 115

atgacccatc	aaatcgtcac	cactcagtac	ggcaaagtca	aaggtacgac	ggagaacggt	60
gttcataagt	ggaaaggcat	cccgtatgcg	aagccgccag	tgggccagtg	gcgtttcaaa	120
gcgccggaac	cgccggagggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcgggtg	gtattacctg	ggcgccggta	gcgagccgct	gtacgatggc	360
tccaagctgg	ctgcgcaagg	tgaggtcatt	gtcgtcacc	tgaactatcg	tctgggcccc	420
ttcggtttca	tgcacctgag	cagcttcgat	gaggcgatata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
gataatgtga	cgggtgtttg	tgaaagcgct	ggtggcgata	gcattgccgc	gctgctggcc	600
atgccggctg	cgaaaggcct	gtttcaaaaag	gcgattatgg	agagcgggtg	aagcttgacc	660
atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgct	gggtatcaat	720
gagagccagc	tggtatcggtt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttcagacc	tgactctggt	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900

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ccgctgttga ttggtaccac gcgcgacgaa gggtacctgt ttttcacgtc tgatagcgac 960
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gagaaaagcgg cggacttgta cccgcgcagc ctggaatccc aaatccacat ggtgaccgat 1080
ctgtcatttt ggcgcccagc cgtggcattc gcgtctgcac agagccatta cgcaccggtg 1140
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aagaccggca acccgagcac cgaggcgggt aactggccgg cttatcatga agaaacccgt 1380
gaaaccgtga tcctggactc tgagattacc atcgagaatg accctgagag cgagaagcgt 1440
cagaagctgt tcccagacaa ggggtgaa                                     1467

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<210> 116

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 116

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Met Thr His Gln Ile Val Thr Thr Gln Tyr Gly Lys Val Lys Gly Thr
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Thr Glu Asn Gly Val His Lys Trp Lys Gly Ile Pro Tyr Ala Lys Pro
20      25      30
Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35      40      45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50      55      60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65      70      75      80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85      90      95
Leu Pro Val Met Val Trp Ile His Gly Gly Ala Tyr Tyr Leu Gly Ala
100     105     110
Gly Ser Glu Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115     120     125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
130     135     140
His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
145     150     155     160
Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165     170     175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180     185     190
Asp Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195     200     205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Leu Thr Met Thr Lys Glu
210     215     220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225     230     235     240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245     250     255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln
260     265     270
Ala Phe Phe Gln Pro Ala Leu Asp Pro Lys Thr Leu Pro Glu Glu Pro
275     280     285
Glu Lys Ser Ile Ala Glu Gly Ala Ala Ser Gly Ile Pro Leu Leu Ile
290     295     300
Gly Thr Thr Arg Asp Glu Gly Tyr Leu Phe Phe Thr Ser Asp Ser Asp
305     310     315     320
Val Arg Ser Gln Glu Thr Leu Asp Ala Ala Leu Glu Tyr Ser Leu Gly
325     330     335
Lys Pro Leu Ala Glu Lys Ala Ala Asp Leu Tyr Pro Arg Ser Leu Glu
340     345     350
Ser Gln Ile His Met Val Thr Asp Leu Ser Phe Trp Arg Pro Ala Val
355     360     365
Ala Phe Ala Ser Ala Gln Ser His Tyr Ala Pro Val Trp Met Tyr Arg

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370					375					380				
Phe	Asp	Trp	His	Pro	Glu	Lys	Pro	Pro	Tyr	Asn	Lys	Ala	Phe	His
385					390					395				400
Leu	Glu	Leu	Pro	Phe	Val	Phe	Gly	Asn	Leu	Asp	Gly	Leu	Glu	Arg
				405					410					415
Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr
			420					425					430	
Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr
		435					440					445		
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val
	450					455					460			
Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys
465					470					475				480
Gln	Lys	Leu	Phe	Pro	Ser	Lys	Gly	Glu						
				485										

<210> 117

<211> 1467

<212> DNA

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 117

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gcgccggaac	cgccggaggt	ctgggaggat	gttctggacg	caaccgcgta	tggtcctgtc	180
tgcccacagc	cgtcggacct	gttgagcctg	agctatacgg	aactgccacg	tcagagcgaa	240
gattgtctgt	acgttaatgt	ttttgcgccg	gataccccgt	cccaaaatct	gccggttatg	300
gtttggattc	acggcggtgc	gtattacctg	ggtgccggta	gcgagccgct	gtacgatggc	360
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ttcggtttca	tgcacctgag	cagcttcgat	gaggcgtata	gcgacaattt	gggtctgctg	480
gaccaagcgg	cagccctgaa	atgggttcgc	gagaacatca	gcgcgttttg	tggcgacccg	540
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atgaccaagg	aacaagcggc	gagcactgcg	gcagcttttc	tgcaagtgtc	gggtatcaat	720
gagagccagc	tggatcgttt	gcacaccgtc	gcagcggagg	acctgctgaa	ggcagctgac	780
caactgcgta	tcgcggagaa	agagaatatc	ttccaggctt	tcttccagcc	tgcactggat	840
ccgaaaactt	tgccggaaga	gccggaaaaa	tccattgcgg	aaggtgcggc	aagcgggtatt	900
ccgctgttga	ttggtaccac	gcgcgacgaa	ggttacctgt	ttttcacgtc	tgatagcgac	960
gttcgtagcc	aggaaacctt	ggacgctgcg	ctggagtaca	gcctggggcaa	gccgtttggca	1020
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ctgctgtttt	ggcgcccagc	cgtggcattc	gcgtctgcac	agagccatta	cgcaccggtg	1140
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ctggagctgc	cgtttgtttt	cggtaacctg	gatggcttgg	aacgtatggc	caaagccgaa	1260
atcacggacg	aggttaagca	actgtcccac	acgattcaga	gcgcctggat	tacgttttga	1320
aagaccggga	acccgagcac	cgaggcggtg	aactggccgg	cttatcatga	agaaaccctg	1380
gaaaccgtga	tcctggactc	tgagattacc	atcgagaatg	accctgagag	cgagaagcgt	1440
cagaagctgt	tcccagagcaa	gggtgaa				1467

<210> 118

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 118

Met	Thr	His	Gln	Ile	Val	Thr	Thr	Gln	Tyr	Gly	Lys	Val	Lys	Gly	Thr
1			5					10					15		
Thr	Glu	Asn	Gly	Val	His	Lys	Trp	Lys	Gly	Ile	Pro	Tyr	Ala	Lys	Pro
		20					25					30			
Pro	Val	Gly	Gln	Trp	Arg	Phe	Lys	Ala	Pro	Glu	Pro	Pro	Glu	Val	Trp
	35						40				45				

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Glu	Asp	Val	Leu	Asp	Ala	Thr	Ala	Tyr	Gly	Pro	Val	Cys	Pro	Gln	Pro
50	50					55					60				
Ser	Asp	Leu	Leu	Ser	Leu	Ser	Tyr	Thr	Glu	Leu	Pro	Arg	Gln	Ser	Glu
65					70					75					80
Asp	Cys	Leu	Tyr	Val	Asn	Val	Phe	Ala	Pro	Asp	Thr	Pro	Ser	Gln	Asn
				85					90					95	
Leu	Pro	Val	Met	Val	Trp	Ile	His	Gly	Gly	Ala	Tyr	Tyr	Leu	Gly	Ala
			100					105					110		
Gly	Ser	Glu	Pro	Leu	Tyr	Asp	Gly	Ser	Lys	Leu	Ala	Ala	Gln	Gly	Glu
		115					120					125			
Val	Ile	Val	Val	Thr	Leu	Asn	Tyr	Arg	Leu	Gly	Pro	Phe	Gly	Phe	Met
	130					135					140				
His	Leu	Ser	Ser	Phe	Asp	Glu	Ala	Tyr	Ser	Asp	Asn	Leu	Gly	Leu	Leu
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Gly	Gly	Asp	Pro	Asp	Asn	Val	Thr	Val	Phe	Gly	Glu	Ser	Ala	Gly	Gly
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Asp	Ser	Ile	Ala	Ala	Leu	Leu	Ala	Met	Pro	Ala	Ala	Lys	Gly	Leu	Phe
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Gln	Lys	Ala	Ile	Met	Glu	Ser	Gly	Ala	Ser	Leu	Thr	Met	Thr	Lys	Glu
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225					230					235					240
Glu	Ser	Gln	Leu	Asp	Arg	Leu	His	Thr	Val	Ala	Ala	Glu	Asp	Leu	Leu
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Lys	Ala	Ala	Asp	Gln	Leu	Arg	Ile	Ala	Glu	Lys	Glu	Asn	Ile	Phe	Gln
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Ala	Lys	Ala	Glu	Ile	Thr	Asp	Glu	Val	Lys	Gln	Leu	Ser	His	Thr	Ile
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Gln	Ser	Ala	Trp	Ile	Thr	Phe	Ala	Lys	Thr	Gly	Asn	Pro	Ser	Thr	Glu
		435					440					445			
Ala	Val	Asn	Trp	Pro	Ala	Tyr	His	Glu	Glu	Thr	Arg	Glu	Thr	Val	Ile
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Leu	Asp	Ser	Glu	Ile	Thr	Ile	Glu	Asn	Asp	Pro	Glu	Ser	Glu	Lys	Arg
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<210> 119

<211> 1467

<212> DNA

<213> Artificial sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 119

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<210> 120

<211> 489

<212> PRT

<213> Artificial Sequence

<220>

<223> Engineered variant of B. subtilis pNB esterase

<400> 120

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Pro Val Gly Gln Trp Arg Phe Lys Ala Pro Glu Pro Pro Glu Val Trp
35     40     45
Glu Asp Val Leu Asp Ala Thr Ala Tyr Gly Pro Val Cys Pro Gln Pro
50     55     60
Ser Asp Leu Leu Ser Leu Ser Tyr Thr Glu Leu Pro Arg Gln Ser Glu
65     70     75     80
Asp Cys Leu Tyr Val Asn Val Phe Ala Pro Asp Thr Pro Ser Gln Asn
85     90     95
Leu Pro Val Met Val Trp Ile His Gly Ala Tyr Tyr Leu Gly Ala
100    105    110
Gly Ser Gln Pro Leu Tyr Asp Gly Ser Lys Leu Ala Ala Gln Gly Glu
115    120    125
Val Ile Val Val Thr Leu Asn Tyr Arg Leu Gly Pro Phe Gly Phe Met
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His Leu Ser Ser Phe Asp Glu Ala Tyr Ser Asp Asn Leu Gly Leu Leu
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Asp Gln Ala Ala Ala Leu Lys Trp Val Arg Glu Asn Ile Ser Ala Phe
165    170    175
Gly Gly Asp Pro Asp Asn Val Thr Val Phe Gly Glu Ser Ala Gly Gly
180    185    190
Asp Ser Ile Ala Ala Leu Leu Ala Met Pro Ala Ala Lys Gly Leu Phe
195    200    205
Gln Lys Ala Ile Met Glu Ser Gly Ala Ser Leu Thr Met Thr Lys Glu
210    215    220
Gln Ala Ala Ser Thr Ala Ala Ala Phe Leu Gln Val Leu Gly Ile Asn
225    230    235    240
Glu Ser Gln Leu Asp Arg Leu His Thr Val Ala Ala Glu Asp Leu Leu
245    250    255
Lys Ala Ala Asp Gln Leu Arg Ile Ala Glu Lys Glu Asn Ile Phe Gln

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Glu	Lys	Ser	275	Ile	Ala	Glu	Gly	280	Ala	Ala	Ser	Gly	Ile	Pro	285	Leu	Leu	Ile
Gly	Thr	Thr	290	Arg	Asp	Glu	Gly	295	Tyr	Leu	Phe	Phe	Thr	Ser	300	Asp	Ser	Asp
Val	Arg	Ser	305	Gln	Glu	Thr	Leu	310	Asp	Ala	Ala	Leu	Glu	Tyr	315	Ser	Leu	Gly
Lys	Pro	Leu	325	Ala	Glu	Lys	Ala	330	Ala	Asp	Leu	Tyr	Pro	Arg	335	Ser	Leu	Glu
Ser	Gln	Ile	340	His	Met	Val	Thr	345	Asp	Leu	Leu	Phe	Trp	Arg	350	Pro	Ala	Val
Ala	Phe	Ala	355	Ser	Ala	Gln	Ser	360	His	Tyr	Ala	Pro	Val	Trp	365	Met	Tyr	Arg
Phe	Asp	Trp	370	His	Pro	Glu	Lys	375	Pro	Pro	Tyr	Asn	Lys	Ala	380	Phe	His	Ala
Leu	Glu	Leu	385	Pro	Phe	Val	Phe	390	Gly	Asn	Leu	Asp	Gly	Leu	395	Glu	Arg	Met
Ala	Lys	Ala	405	Glu	Ile	Thr	Asp	410	Glu	Val	Lys	Gln	Leu	Ser	415	His	Thr	Ile
Gln	Ser	Ala	420	Trp	Ile	Thr	Phe	425	Ala	Lys	Thr	Gly	Asn	Pro	430	Ser	Thr	Glu
Ala	Val	Asn	435	Trp	Pro	Ala	Tyr	440	His	Glu	Glu	Thr	Arg	Glu	445	Thr	Val	Ile
Leu	Asp	Ser	450	Glu	Ile	Thr	Ile	455	Glu	Asn	Asp	Pro	Glu	Ser	460	Glu	Lys	Arg
Gln	Lys	Leu	465	Phe	Pro	Ser	Lys	470	Gly	Glu					475			
			485															