

Additional file 1

Tailoring the properties of (catalytically)-active inclusion bodies

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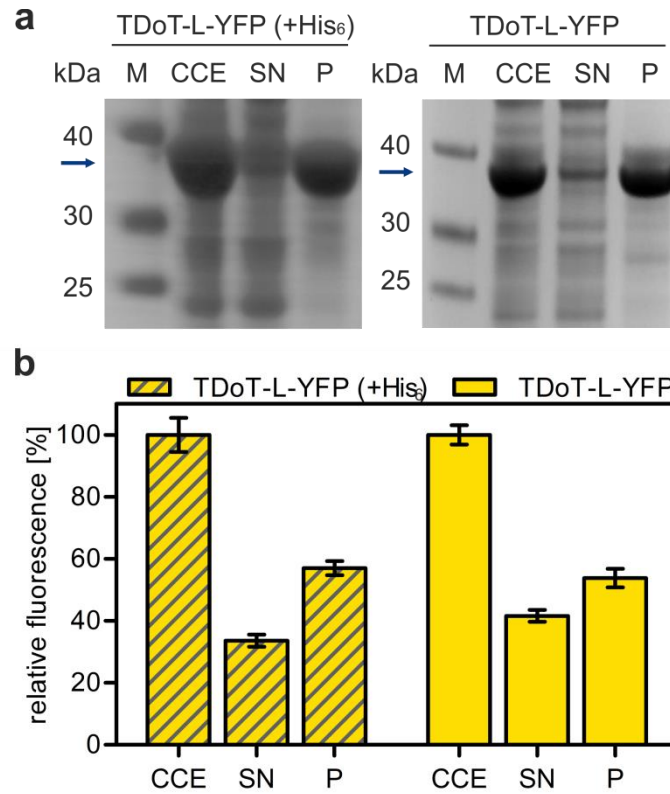


Figure S1: Improving the CatIB strategy by deleting the Hexahistidine-tag (His₆-tag) from the original vector [1]. Evaluation by (a) SDS-PAGE analysis and (b) fluorescence distribution for TDoT-L-YFP (+His) (36.8 kDa) and TDoT-L-YFP (34.6). After cell disruption, the crude cell extract (CCE) was separated by centrifugation into the soluble protein containing supernatant (SN) and the insoluble IB containing pellet (P) fraction. (a) SDS-PAGE analysis of the respective protein fractions: CCE, SN, and P. The molecular mass of the respective fusion protein is indicated by arrows. The protein content in the SN was measured according to Bradford [2]. (b) Normalized fluorescence in the CCE, SN, and P fractions of the respective proteins. Note, that the P fraction was washed once with water and centrifuged again before the fluorescence measurement. The fluorescence in CCE, SN, and P fractions was calculated relative to the activity in the CCE, which was set to 100%. Error bars correspond to the standard deviation of the mean derived from at least three technical replicates.

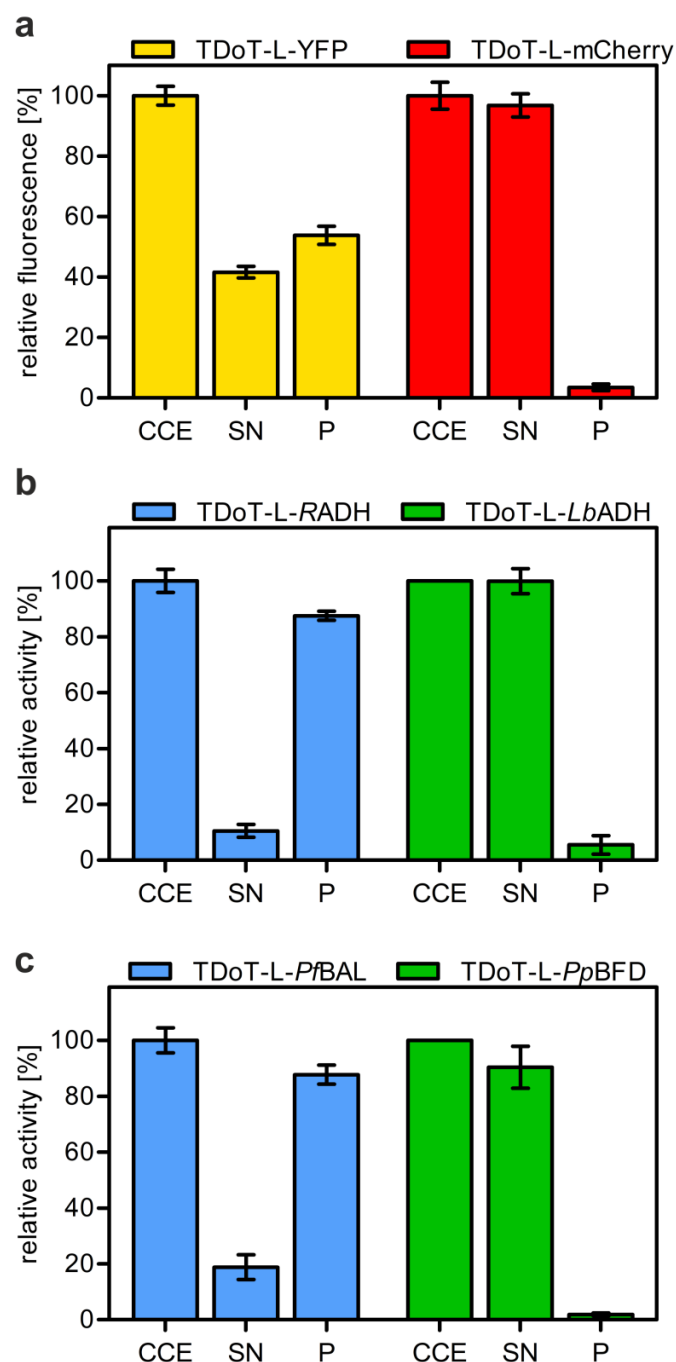


Figure S2: The fluorescence/activity distribution of (a) TDoT-L-YFP-, TDoT-L-mCherry-, (b) TDoT-L-RADH-, TDoT-L-LbADH-, (c) TDoT-L-PfBAL- and TDoT-L-PpBFD-CatIB. Sample preparation was done as in Figure S1. Initial rate activities were measured by carbonylation of 3,5-dimethoxybenzaldehyde (DMBA) to the respective benzoin catalyzed by TDoT-L-PfBAL-CatIBs and decarboxylation of benzoylformate to benzaldehyde by TDoT-L-PpBFD-CatIBs, which was followed by the reduction to benzyl alcohol by horse liver HL-ADH under the oxidation of NADPH. Initial rate activities of the ADH enzymes were measured by reduction of 1-phenylethanol (TDoT-L-LbADH) or cyclohexanone (TDoT-L-RADH) under the consumption of NADPH. For reaction equations see Figure S13. Error bars correspond to the standard deviation of the mean derived from at least three biological replicates. Controls expressing the corresponding soluble versions of YFP, mCherry, RADH, and PfBAL (with TDoT tag) have been presented before [3].

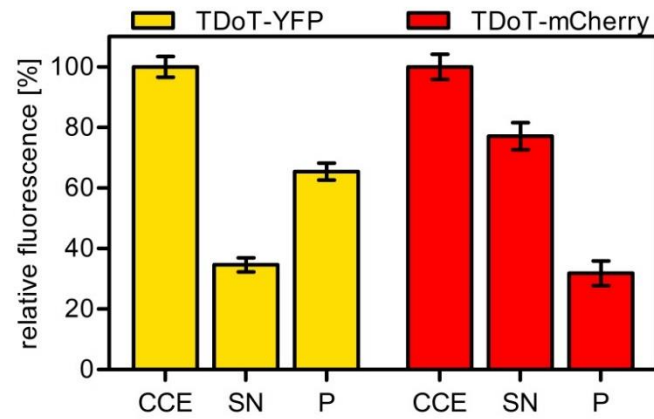


Figure S3: The fluorescence distribution of the linker-free variants TDoT-YFP- and TDoT-mCherry-FIBs. Sample preparation was done as in Figure S1. Error bars correspond to the standard deviation of the mean derived from at least three biological replicates.

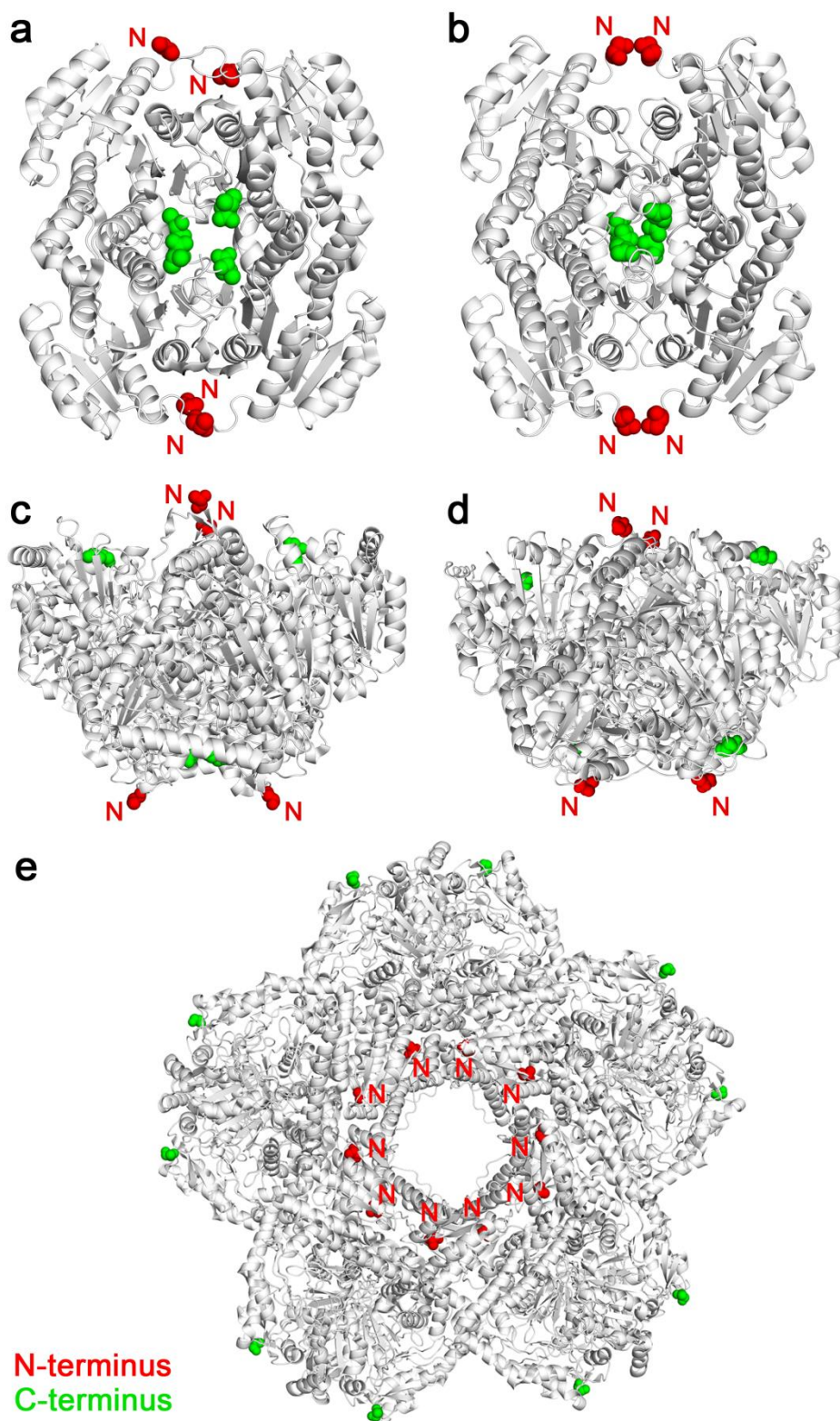


Figure S4: Structures of the multimeric target enzymes of this study: (a) RADH (PDB: 4BMN), (b) *Lb*ADH (PDB: 1ZK4), (c) *Pβ*BAL (PDB: 2UZ1), (d) *Pp*BFD (PDB: 5DEI) and (e) *Ec*LDC (PDB: 5FKZ). All subunits are shown in grey cartoon representation, while the N- and C-terminal amino acids are shown as spheres in red (N-terminus) and green (C-terminus). For clarity, only the N-terminus is labelled in each panel. The N-terminus is accessible in RADH, *Lb*ADH, *Pβ*BAL, and *Pp*BFD. In contrast, it is buried within the decameric structure in the N-terminal wing domain of the *Ec*LDC multimer, whereas the C-terminus is located at the outer part in the C-terminal domain [4] and hence should be accessible for tag fusion.

a



b



Figure S5: Schematic illustration of the N-terminal (a) and C-terminal (b) fusion strategy employed for the generation of CatIBs/FIBs. All expression vectors/gene fusions are constructed modularly, so that every element can be easily exchanged or deleted, by the use of the depicted restriction sites. Gene fusions consist of coding sequences for: the respective target protein (blue), a coiled-coil domain (green), for which we used the TDoT coiled-coil domain (52 amino acids) of the cell-surface protein tetrabrachion of *Staphylothermus marinus* [5] or the 3HAMP coiled coil (172 amino acids) of the soluble oxygen sensor Aer2 of *Pseudomonas aeruginosa* [6]. Whenever mentioned gene fusions additionally contained a linker region, comprising a cleavage site of the Factor Xa protease (orange, 4 amino acids) and a (GGGS)₃-motif (red, 12 amino acids). The gene fusion design shown in (b) was chosen only for the enzyme *EcLDC* [4].

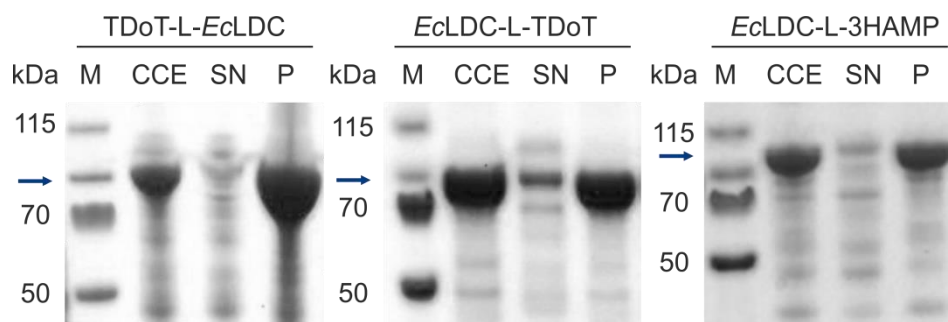


Figure S6: SDS-PAGE analysis of the respective protein fractions of TDoT-L-*EcLDC*-CatIBs (90.3 kDa), *EcLDC*-L-TDoT-CatIBs (87.5 kDa) and *EcLDC*-L-3HAMP-CatIBs (100.5 kDa): crude cell extract (CCE), supernatant (SN), and pellet (P). The target protein is indicated by arrows. Sample preparation was done as in Figure S1.

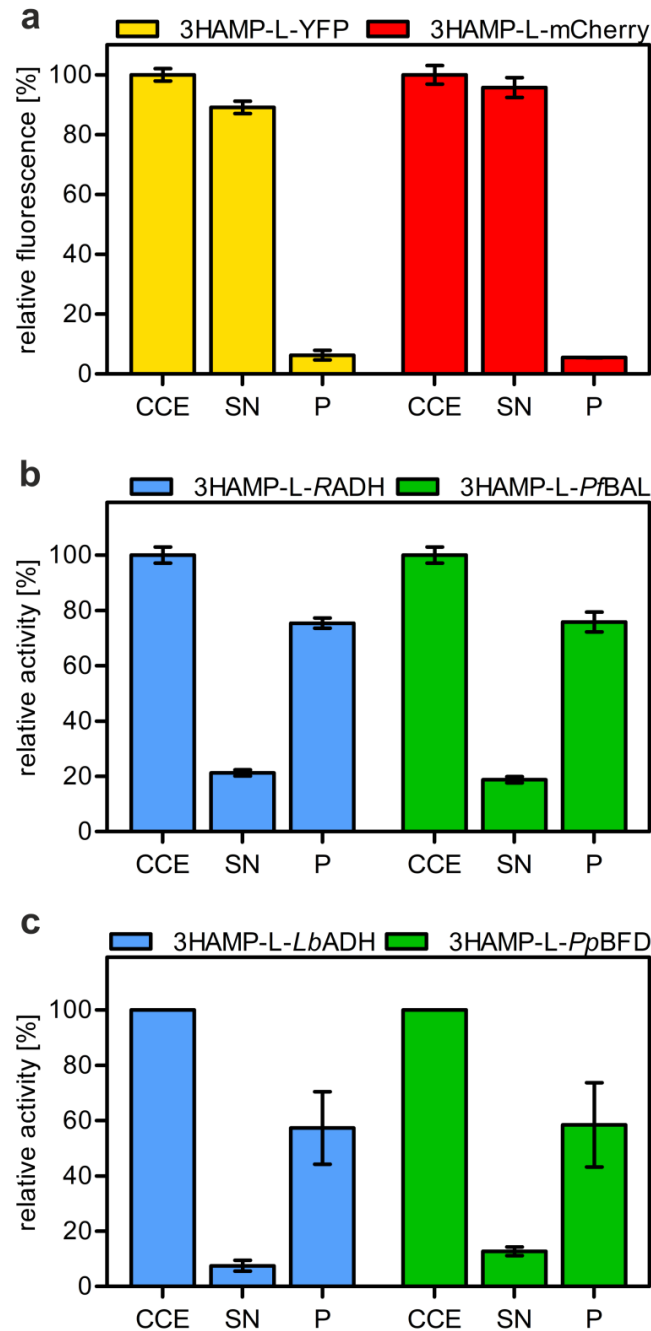


Figure S7: The fluorescence/activity distribution of (a) 3HAMP-L-YFP-, 3HAMP-L-mCherry-, (b) 3HAMP-L-RADH-, 3HAMP-L-PfBAL-, (c) 3HAMP-L-LbADH-, and 3HAMP-L-PpBFD-CatIBs.. Sample preparation was done as in Figure S1. Initial rate activities were measured by carbonylation of 3,5-dimethoxybenzaldehyde (DMBA) to the respective benzoin catalyzed by 3HAMP-L-PfBAL-CatIBs and decarboxylation of benzoylformate to benzaldehyde by 3HAMP-L-PpBFD-CatIBs, which was followed by the reduction to benzyl alcohol by horse liver HL-ADH under the oxidation of NADH. The initial rate activities of the ADH enzymes were measured by reduction of 1-phenylethanol (3HAMP-L-LbADH) or cyclohexanone (3HAMP-L-RADH) under the consumption of NADPH. For reaction equations see Figure S13. Error bars correspond to the standard deviation of the mean derived from at least three biological replicates.

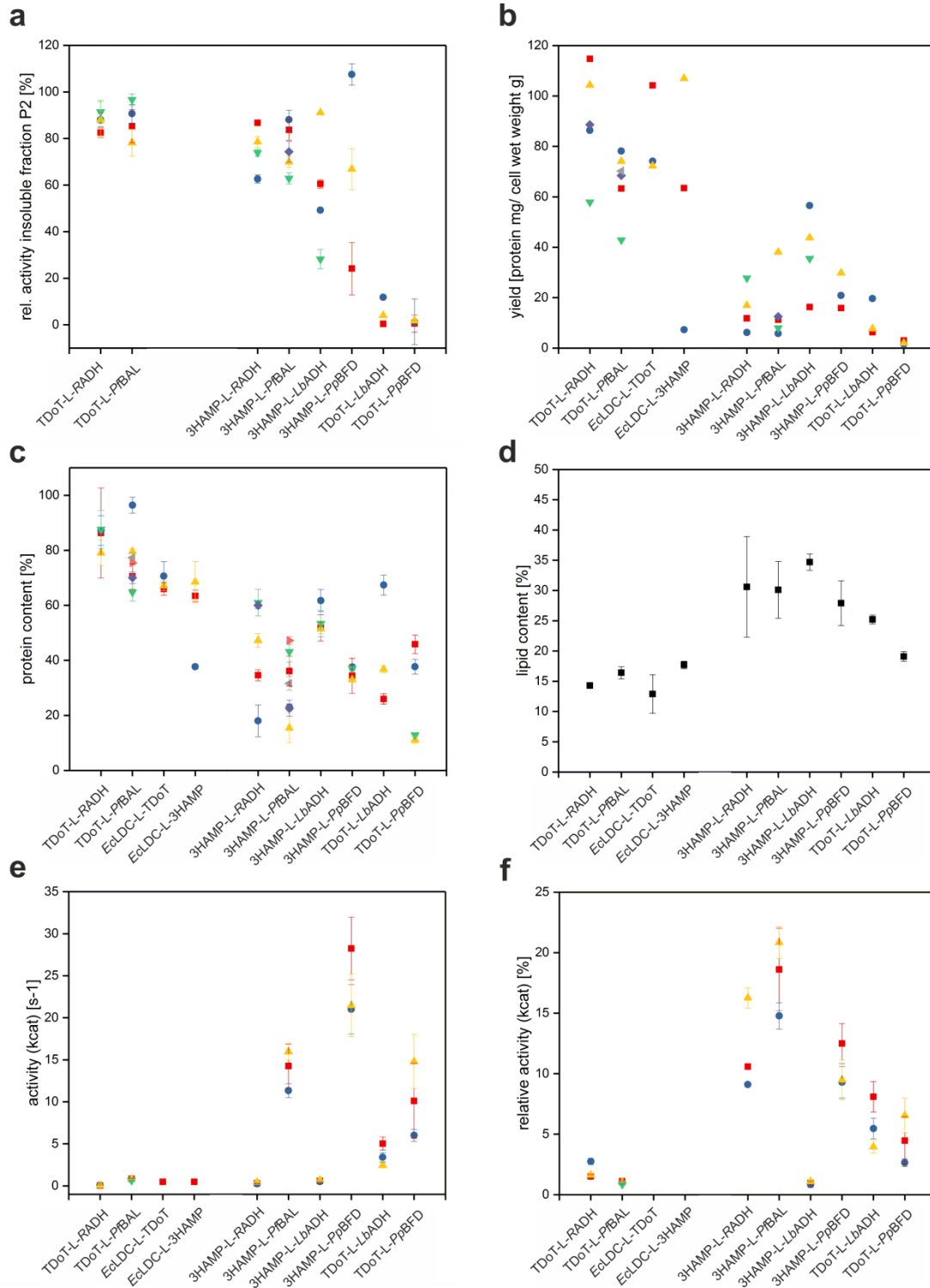


Figure S8: Mean values for each biological replicate with standard deviation (A) for the activity in the pellet compared to CCE, (B) yield (mg protein for 1 g wet cell weight), (C) protein content, and (D) lipid content, based on the dry weight of lyophilized CatIBs, (E) initial rate activity (k_{cat}) and (F) residual activity compared to the soluble enzyme.

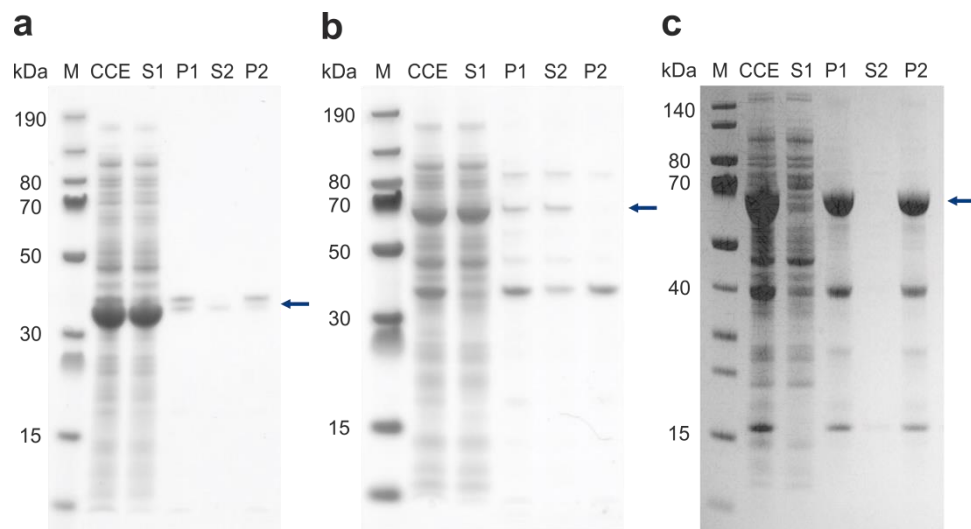


Figure S9: Evaluation of the CatIB strategy by SDS-PAGE analysis of the respective protein fractions of (a) TDoT-L-*LbADH* (34.3 kDa), (b) TDoT-L-*PpBFD* (64.2 kDa) and (c) TDoT-L-*P/BAL* CatIBs (66.5 kDa); crude cell extract (CCE), supernatant (SN), and pellet (P). The target protein is indicated by arrows. Sample preparation was done as in Figure S1.

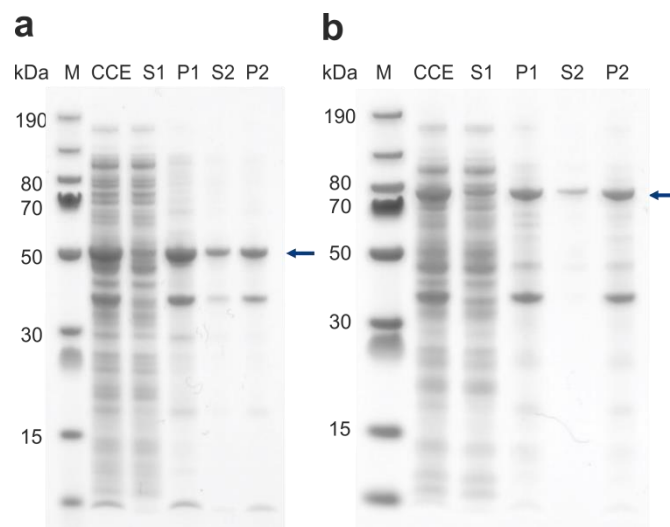


Figure S10: Evaluation of the CatIB strategy by SDS-PAGE analysis of the respective protein fractions of (a) 3HAMP-L-*LbADH*- (47.1 kDa), (b) 3HAMP-L-*PpBFD*-CatIBs (77.0 kDa); crude cell extract (CCE), supernatant (SN), and pellet (P). The target protein is indicated by arrows. Sample preparation was done as in Figure S1.

Table S1: Sequence-based aggregation-propensity prediction for each target protein and the corresponding TDoT fusion. All predictions were carried out using the AGGRESCAN web server (<http://bioinf.uab.es/aggrescan>) [7]. Displayed are the average aggregation-propensity values per amino acid (a^4v) normalized to a 100 residue protein (Na^4vSS) and the relative change in Na^4vSS (ΔNa^4vSS) due to fusion of the TDoT domain.

target protein	Na^4vSS	TDoT-target-protein fusion	Na^4vSS	ΔNa^4vSS^*
YFP	-7.9	TDoT-L-YFP	-6.5	17.7
mCherry	-16.6	TDoT-L-mCherry	-12.6	24.1
BsLA	0.8	TDoT-L-BsLA	-1.4	-275.0
AtHNL	3.9	TDoT-L-AtHNL	1.9	-51.3
EcMenD	-3.2	TDoT-L-EcMenD	-3.1	3.1
RADH	-1.7	TDoT-L-RADH	-1.5	11.8
LbADH	-4.5	TDoT-L-LbADH	-3.8	15.6
PfBAL	3.6	TDoT-L-PfBAL	2.9	-19.4
PpBFD	-1.3	TDoT-L-PpBFD	-1.4	-7.7

*calculated according to: $\Delta Na^4vSS = \left(\frac{(Na^4vSS_{fusion} - Na^4vSS_{target})}{|Na^4vSS_{target}|} \right) \times 100$ [7].

Table S2: Surface properties of the target proteins used in this study. The presence/absence of large hydrophobic surface patches for the corresponding target protein structures was quantified using the hpatch tool implemented in Rosetta [8-10]. Surface areas were quantified using Pymol 1.7.0.0 (Schrödinger, LCC, New York, NY, USA).

target protein	PDB-ID	comments	hydrophobic patch area [Å ²]	solvent accessible surface area (SASA) [Å ²]	hydrophob. patch area / overall SASA [%]
YFP	1YFP	asu, monomer, chain A and B	1000	9467	10.6
YFP*	1YFP	1 st PISA dimer	1085	16914	6.4
mCherry*	2H5Q	asu, monomer	0	9493	0
BsLA*	1ISP	asu, monomer	972	7867	12.4
AtHNL*	3DQZ	asu, dimer, chain A and B	1692	18662	9.1
EcMenD	2JLC	asu, dimer	1566	34022	4.6
EcMenD*	2JLC	1 st PISA tetramer	3797	54278	7.0
RADH	4BMN	tetramer	1183	27318	4.3
RADH*	4BMN	1 st PISA dimer	2068	17423	11.9
LbADH	1ZK4	1 st PISA tetramer	2748	27590	9.9
LbADH*	1ZK4	2 nd PSIA dimer	874	18316	4.8
PfBAL*	2UZ1	asu, tetramer	5194	52235	9.9
PpBFD*	5DEI	asu, tetramer	185	69766	0.27

* final data used in Figure 6c; main paper; asu: the pdb coordinates present in the asymmetric unit were used for surface calculations.

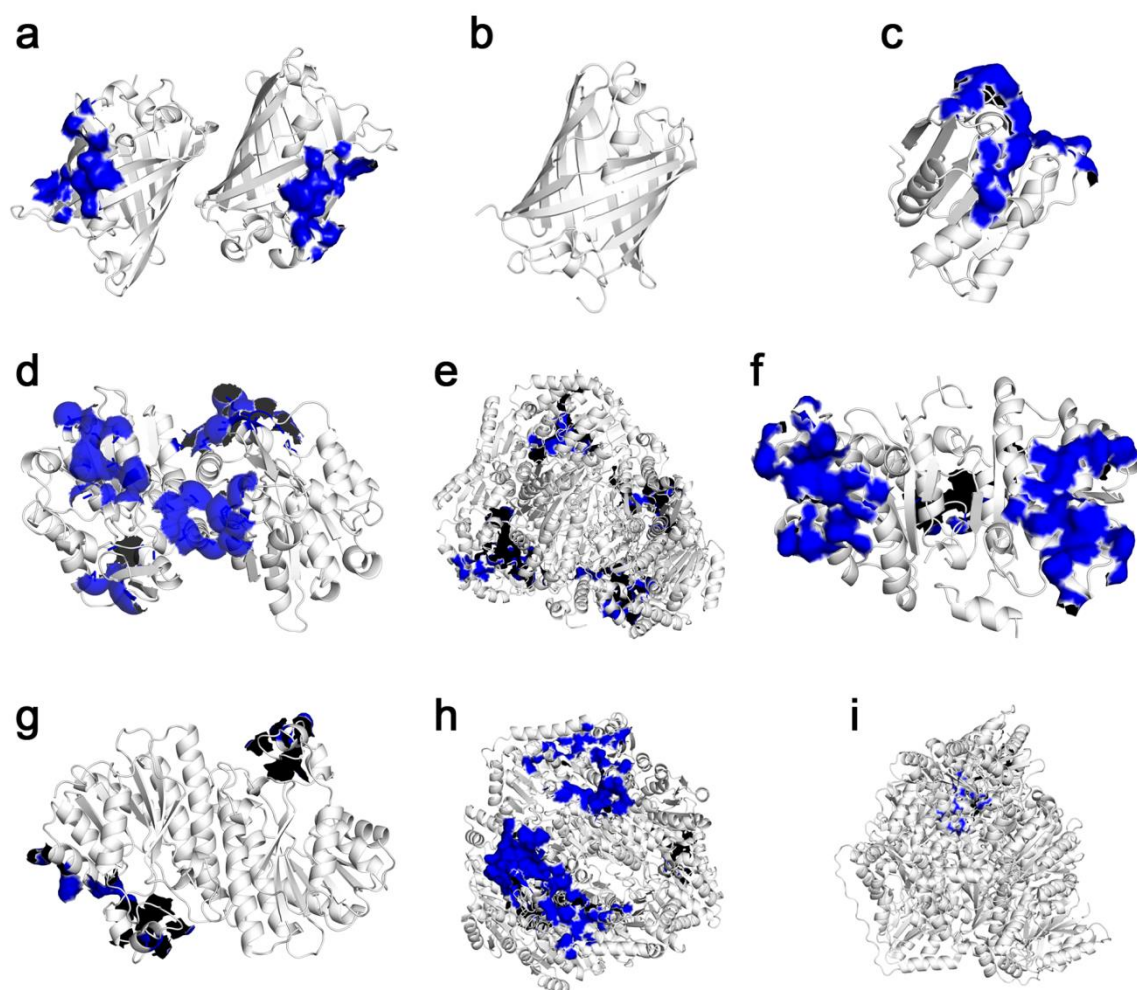


Figure S11: Hydrophobic patch analysis of (a) YFP, (b) mCherry, (c) *BsLA*, (d) *AtHNL*, (e) *EcMenD*, (f) *RADH*, (g) *LbADH*, (h) *PfBAL*, and (i) *PpBFD*. Structures are shown in cartoon representation in grey with the Rossetta-identified hydrophobic surface patches shown as blue surfaces [9, 10]. PDB-IDs were as follows YFP (1YFP), mCherry (2H5Q), *BsLA* (1ISP), *AtHNL* (3DWZ), *EcMenD* (2JLC), *RADH* (4BMN), *LbADH* (1ZK4), *PfBAL* (2UZ1) and *PpBFD* (5DEI).

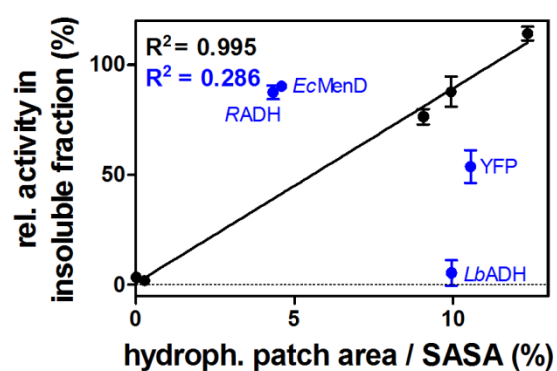


Figure S12: Computational analysis of the structural determinants of CatIB formation. The presence/absence of large hydrophobic surface patches for the corresponding target protein structures was quantified using the hpatch tool implemented in Rosetta [8-10]. Surface areas were quantified using Pymol 1.7.0.0 (Schrödinger, LCC, New York, NY, USA). CatIB-formation was plotted as the relative activity in the insoluble fraction (Table S6). Coefficient of determination (R^2) values are always given excluding the blue-highlighted outliers (black) and including the outliers (blue).

Table S3: Plasmids used in this study. DNA and amino acid sequences of all gene fusions are given in the annex.

Vector	Genotyp	Description
pET28a	<i>ColE1 lacZ' Kan^R P_{T7} P_{lac}</i>	Merck (Darmstadt, Germany)
pTDoT-Linker-YFP	pET-28a, P _{T7} , gene fusion [<i>His-tag-tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-yfp</i>]	[11]
pTDoT-L-YFP	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-yfp</i>]	pTDoT-Linker-YFP derivative, insertion of a 40 bp <i>XbaI/NdeI</i> RBS containing fragment of pET28a in pTDoT-Linker-YFP; without the 99 bp fragment containing RBS and <i>His-tag</i> [3]
pTDoT-YFP	pET-28a, P _{T7} , gene fusion [<i>tdot-yfp</i>]	pTDoT-L-YFP derivative, insertion of a 155 bp <i>NdeI/BamHI</i> <i>tdot</i> -containing fragment in pTDoT-L-YFP; without the 215 bp fragment containing <i>tdot</i> , factor Xa recognition site and (<i>GGS</i>) ₃ <i>linker</i>
p3HAMP-L-YFP	pET-28a, P _{T7} , gene fusion [<i>3hamp-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-yfp</i>]	pTDoT-L-YFP derivative, insertion of a 518 bp <i>NdeI/SpeI</i> <i>3hamp</i> -containing fragment in pTDoT-L-YFP; without the 155 bp fragment containing <i>tdot</i> ,
pTDoT-L-mCherry	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-mcherry</i>]	pTDoT-L-YFP derivative, insertion of 717 bp PCR-amplified <i>BamHI/SalI</i> <i>mcherry</i> fragment in pTDoT-L-YFP; without the 726 bp fragment containing <i>yfp</i> [3]
pTDoT-mCherry	pET-28a, P _{T7} , gene fusion [<i>tdot-mcherry</i>]	pTDoT-L-mCherry derivative, insertion of 155 bp <i>NdeI/BamHI</i> <i>tdot</i> -containing fragment in pTDoT-L-mCherry; without the 215 bp fragment containing <i>tdot</i> , factor Xa recognition site and (<i>GGS</i>) ₃ <i>linker</i> [3]
p3HAMP-L-mCherry	pET-28a, P _{T7} , gene fusion [<i>3hamp-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-mcherry</i>]	pTDoT-L-mCherry derivative, insertion of a 518 bp <i>NdeI/SpeI</i> <i>3hamp</i> -containing fragment in pTDoT-L-mCherry; without the 155 bp fragment containing <i>tdot</i> ,
pET22b-RADH	pET22b, P _{T7} , <i>radh</i> gene	[12]
pTDoT-L-RADH	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-radh</i>]	pTDoT-L-YFP derivative, insertion of 756 bp PCR-amplified <i>BamHI/SalI</i> <i>radh</i> fragment in pTDoT-L-YFP; without the 726 bp fragment containing <i>yfp</i> [3]
p3HAMP-L-RADH	pET-28a, P _{T7} , gene fusion [<i>3hamp-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-radh</i>]	pTDoT-L-RADH derivative, insertion of a 518 bp <i>NdeI/SpeI</i> <i>3hamp</i> -containing fragment in pTDoT-L-RADH; without the 155 bp fragment containing <i>tdot</i>
pET21a-LbADH	pET21a, P _{T7} , <i>lbadh</i> gene	[13]
pTDoT-L-LbADH	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-lbadh</i>]	pTDoT-L-YFP derivative, insertion of 766 bp PCR-amplified <i>BamHI/SalI</i> <i>lbadh</i> fragment in pTDoT-L-YFP; without the 726 bp fragment containing <i>yfp</i>
p3HAMP-L-LbADH	pET-28a, P _{T7} , gene fusion [<i>3hamp-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-lbadh</i>]	p3HAMP-L-RADH derivative, insertion of 766 bp PCR-amplified <i>BamHI/SalI</i> <i>lbadh</i> fragment in p3HAMP-L-RADH; without the 756 bp fragment containing <i>radh</i>
pKK233_2-PfBAL-His	pKK233_2 P _{trc} , gene fusion [<i>pfbal</i> , <i>His-tag</i>]	[14]
pTDoT-L-PfBAL	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-pfbal</i>]	pTDoT-L-YFP derivative, insertion of 1699 bp PCR-amplified <i>BamHI/NotI</i> <i>pfbal</i> fragment in pTDoT-L-YFP; without the 739 bp fragment containing <i>yfp</i> [3, 15]
p3HAMP-L-PfBAL	pET-28a, P _{T7} , gene fusion [<i>3hamp-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-pfbal</i>]	pTDoT-L-PfBAL derivative, insertion of a 518 bp <i>NdeI/SpeI</i> <i>3hamp</i> -containing fragment in pTDoT-L-PfBAL; without the 155 bp fragment containing <i>tdot</i> [15]
pKK233_2-PpBFD L476Q-His	pKK233_2 P _{trc} , gene fusion [<i>ppbfd</i> , <i>His-tag</i>]	[16]
pTDoT-L-PpBFD L476Q	pET-28a, P _{T7} , gene fusion [<i>tdot-factor</i> Xa recognition site-(<i>GGS</i>) ₃ <i>linker-ppbfd</i>]	pTDoT-L-YFP derivative, insertion of 1600 bp PCR-amplified <i>BamHI/NotI</i> <i>ppbfd</i> fragment in pTDoT-L-YFP; without the 739 bp fragment containing <i>yfp</i>

p3HAMP-L-PpBFD L476Q	pET-28a, P _{T7} , gene fusion [<i>3hamp</i> -factor Xa recognition site-(GGGS) ₃ linker- <i>ppbfd</i>]	p3HAMP-L-PfBAL derivative, insertion of 766 bp PCR-amplified <i>Bam</i> HI/ <i>Not</i> I <i>ppbfd</i> fragment in p3HAMP-L-PfBAL; without the 1699 bp fragment containing <i>pfbal</i>
pTDoT-L-EcLDC	pET-28a, P _{T7} , gene fusion [<i>tdot</i> -factor Xa recognition site-(GGGS) ₃ linker- <i>ecldc</i>]	pTDoT-L-YFP derivative, insertion of 2148 bp PCR-amplified <i>Bam</i> HI/ <i>Sal</i> I <i>ecldc</i> fragment in pTDoT-L-YFP; without the 726 bp fragment containing <i>yfp</i>
pEcLDC-L-TDoT	pET-28a, P _{T7} , gene fusion [<i>ecldc</i> -(GGGS) ₃ linker- <i>tdot</i>]	pTDoT-L-PfBAL derivative, i) insertion of 2144 bp PCR-amplified <i>Nde</i> I/ <i>Nhe</i> I <i>ecld</i> fragment in pTDoT-L-PfBAL; without the 173 bp fragment containing <i>tdot-Xa</i> , ii) insertion of 160 bp PCR-amplified <i>Bam</i> HI/ <i>Not</i> I <i>tdot</i> fragment in pEcLDC-L-PfBAL, without the 1699 bp fragment containing <i>bfbal</i> [17]
pEcLDC-L-3HAMP	pET-28a, P _{T7} , gene fusion [<i>ecldc</i> -(GGGS) ₃ linker- <i>3hamp</i>]	pEcLDC-L-TDoT derivative, insertion of 526 bp PCR-amplified <i>Bam</i> HI/ <i>Not</i> I <i>3hamp</i> fragment in pEcLDC-L-TDoT; without the 160 bp fragment containing <i>tdot</i>

Table S4: Oligonucleotide primers used in this study. Restriction endonuclease cleavage sites are underlined.

Name	Sequence (5' - 3')	Application
<i>Bam</i> HI_YFP_fw	ATATAT <u>GGATCC</u> ATGGTGAGCAAGGG CGAG	PCR amplification of <i>yfp</i>
YFP_ <i>Sal</i> I_rev	ATATATGTCGACTTACTTGTACAGCTC GTCCATG	
<i>Bam</i> HI_mCherry_fw	ATATAT <u>GGATCC</u> ATGGTGAGCAAGGG CGAGGAGG	PCR amplification of <i>mcherry</i>
mCherry_ <i>Sal</i> I_rev	ATATATGTCGACTTACTTGTACAGCTC GTCCATGCCGC	
<i>Bam</i> HI_PfBAL_fw	ATATAT <u>GGATCC</u> ATGGCGATGATTACA GGCGGCGAAC	PCR amplification of <i>pfbal</i>
PfBAL_ <i>Not</i> I_rev	ATATATGCGGCCGCTTATGCGAAGGG GTCCATG	
<i>Bam</i> HI_RADH_fw	ATATAT <u>GGATCC</u> ATGTATCGTCTGCTG AATAAACCCG	PCR amplification of <i>radh</i>
RADH_ <i>Sal</i> I_rev	ATATATGTCGACTTAAACCTGGGTCAG ACCACCATC	
<i>Nde</i> I_TDoT_fw	ATATATCATATGATCATTAACGAAACT GCCGATGAC	Removal of factor Xa recognition site and (GGGS) ₃ linker
TDoT_ <i>Bam</i> HI_rev	TATATAGGATCCAATGCTCGCGAGAAT GGTG	
RBS_Oligo_fw	CTAGAAATAATTTTGTTTAACTTTAAG AAGGAGATATACA	Removal of His-Tag
RBS_Oligo_rev	TATGTATATCTCCTTCTTAAAGTTAAA CAAAATTATTT	
<i>Bam</i> HI_LbADH_fw	ATATATGGATCCATGTCTAACCGTTTG GATGGTAAGGTAG	PCR amplification of <i>lbadh</i>
LbADH_ <i>Sal</i> I_rev	ATATATGTCGACTCTATTGAGCAGTGT AGCCACCG	
<i>Bam</i> HI_BFD_fw	ATATATGGATCCATGGCTTCGGTACAC GGCACCAC	PCR amplification of <i>ppbfd</i>
BFD_ <i>Not</i> I_rev	ATATATGCGGCCGCTTAAGATCTCTTC ACCGGGCTTAC	
<i>Bam</i> HI_LDC_fwd	ATATATGGATCCATGAACATCATCGCT ATCATGGGCCC	PCR amplification of <i>ecldc</i>
LDC_ <i>Sal</i> I_rev	ATATATGTCGACTTAGCCTGCCATCTT AAGGACGCG	
<i>Nde</i> I_LDC_fw	ATATATCATATGATGAACATCATCGCT ATCATGGGCCC	PCR amplification of <i>ecldc</i>
LDC_ <i>Nhe</i> I_rev	ATATATGCTAGCGCCTGCCATCTTAAG GACG	
<i>Bam</i> HI_TDot_fw	ATATATGGATCCATCATTAACGAAACT GCCGATGACATCG	PCR amplification of <i>tdot</i>
TDot_ <i>Not</i> I_rev	ATATATGCGGCCGCTTAAATGCTCGCG AGAATG	
<i>Bam</i> HI_3HAMP_fw	ACGTATGGATCCATGGGCCTGTTTAAC GCCCATGCAGTTG	PCR amplification of <i>3hamp</i>
3HAMP_ <i>Not</i> I_rev	ACATATGCGGCCGCTTAATTGTAGGCG GCTGTGGCCAGC	

Table S5: The used strains are given with genotype and reference or source.

strains	genotype	reference or source
<i>E. coli</i> BL21 (DE3)	<i>F⁻ ompT hsdSB(rB⁻ mB⁻) gal dcm (λts857ind1 Sam7 nin5 lacUV5-T7 gene1)</i>	[18] Invitrogen (Carlsbad, USA)
<i>E. coli</i> DH5α	<i>supE44 ΔlacU169 (Φ80lacZDM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Invitrogen (Carlsbad, USA)
<i>E. coli</i> SG 13009	<i>F⁻ ompT hsdS_B (rB⁻ mB⁻) dcm gal (DE3)</i>	Qiagen (Hilden Germany)

Table S6: Extinction coefficient and molecular weight of all used enzymes and proteins in soluble and CatIB form calculated with ExPASy ProtParam Tool (<http://expasy.org/tools/protparam> [19]).

Protein	Extinction coefficient [L·mol ⁻¹ ·cm ⁻¹]	Molecular weight [kDa]
TDoT-L-YFP-CatIBs	26 485	34.6
TDoT-YFP-CatIBs	26 485	33.1
3HAMP-L-YFP-CatIBs	34 965	47.4
TDoT-L-mCherry-CatIBs	37 360	34.3
TDoT-mCherry-CatIBs	37 360	32.7
3HAMP-L-mCherry-CatIBs	45 840	47.1
soluble RADH	14 440	26.7
TDoT-L-RADH-CatIBs	17 420	34.3
3HAMP-L-RADH-CatIBs	25 900	47.1
soluble <i>Lb</i> ADH	19 940	26.8
TDoT-L- <i>Lb</i> ADH-CatIBs	22 920	34.3
3HAMP-L- <i>Lb</i> ADH-CatIBs	31 400	47.1
soluble <i>Pf</i> BAL	52 160	60.0
TDoT-L- <i>Pf</i> BAL-CatIBs	55 140	66.5
3HAMP-L- <i>Pf</i> BAL-CatIBs	63 620	79.3
soluble <i>Pp</i> BFD	62 340	57.4
TDoT-L- <i>Pp</i> BFD-CatIBs	65 320	64.2
3HAMP-L- <i>Pp</i> BFD-CatIBs	73 800	77.0
TDoT-L- <i>Ec</i> LDC-CatIBs	109 210	90.3
<i>Ec</i> LDC-L-TDoT-CatIBs	109 210	87.5
<i>Ec</i> LDC-L-3HAMP-CatIBs	117 690	100.5

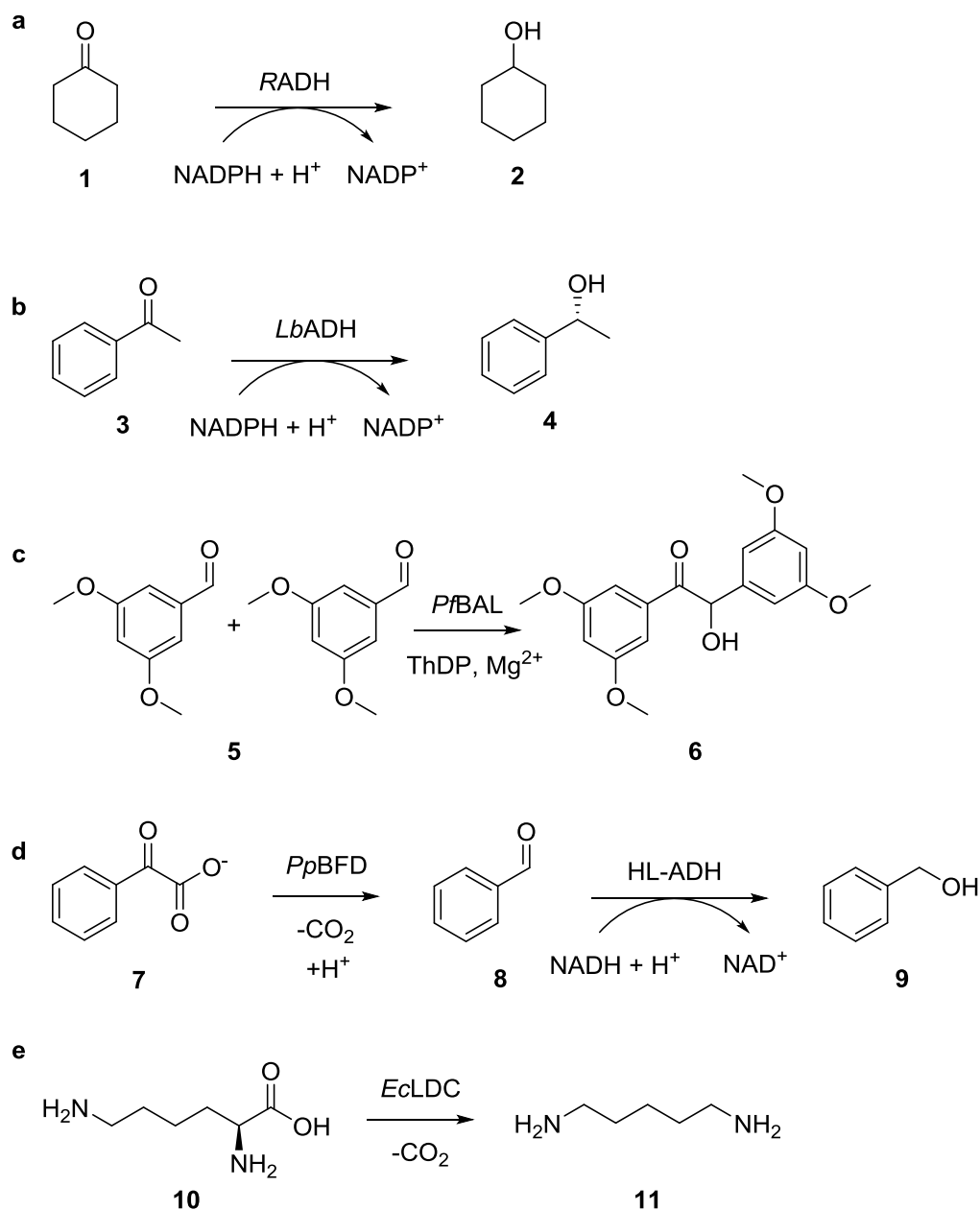


Figure S13: Reactions to measure the initial rate activities of the enzymes used in this study. (a) RADH-CatIBs catalyze the reduction of cyclohexanone **1** to cyclohexanol **2** under the consumption of NADPH. (b) LbADH-CatIBs catalyze the reduction of acetophenone **3** to (*R*)-1-phenylethanol **4** under the consumption of NADPH. (c) PfBAL-CatIBs catalyze the carboligation of 3,5-dimethoxybenzaldehyde (DMBA, **5**) to the respective benzoin (*R*)-(3,3',5,5')-tetramethoxybenzoin (TMBZ, **6**). (d) PpBFD-CatIBs catalyze the decarboxylation of benzoylformate **7** to benzaldehyde **8** followed by the reduction to benzyl alcohol **9** by horse liver (HL-)ADH under the oxidation of NADH. (e) EcLDC-CatIBs catalyze the decarboxylation of L-lysine **10** to cadaverine (1,5-diaminopentane, **11**).

DNA and amino acid sequence of the fusion constructs

The here employed variant of the yellow fluorescent protein (YFP) was derived from introducing the A206K mutation into enhanced YFP (eYFP). In contrast to mYFP [20] this variant lacks the Q69K mutation, which renders YFP less pH-sensitive in the neutral pH range [21]. The here used *PpBFD* variant L476Q was derived from error-prone PCR and is described elsewhere [16].

TDot-L-YFP

```

M I I N E T A D D I V Y R L T V I I D D R Y E S
CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
NdeI      TDoT
L K N L I T L R A D R L E M I I N D N V S T I L A
CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG

S I T S I E G R A S G G G S G G S G G S G G S G S M
AGCATTACTAGTATTGAAGGCCGTGCTAGCGGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCTGGGATCCATG
SpeI      Xa      NheI      3xGGGS-Linker      BamHI
V S K G E E L F T G V V P I L V E L D G D V N G H
GTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGCGACGTAAACGGCCAC
YFP
K F S V S G E G E G D A T Y G K L T L K F I C T T
AAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACC

G K L P V P W P T L V T T F G Y G L Q C F A R Y P
GGCAAGCTGCCCCTGCCCTGGCCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCCGTACCCC

D H M K Q H D F F K S A M P E G Y V Q E R T I F F
GACCACATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGCACCATCTTCTTC

K D D G N Y K T R A E V K F E G D T L V N R I E L
AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG

K G I D F K E D G N I L G H K L E Y N Y N S H N V
AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCCACAACGTC

Y I M A D K Q K N G I K V N F K I R H N I E D G S
TATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAGTTCAGATCCGCCACAACATCGAGGACGGCAGC

V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y
GTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTAC

L S Y Q S K L S K D P N E K R D H M V L L E F V T
CTGAGCTACCAGTCCAACTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTCTGCTGGAGTTCGTGACC

A A G I T L G M D E L Y K *
GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAGTCGACAAGCTTGCGGGCCGCACTCGAG
SalI      NotI      XhoI
```

TDot-YFP

```

M I I N E T A D D I V Y R L T V I I D D R Y E S
CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
NdeI      TDoT
L K N L I T L R A D R L E M I I N D N V S T I L A
CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG

S I G S M V S K G E E L F T G V V P I L V E L D G
AGCATTGGATCCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGC
```


GTGCCCTGGCCACCCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCCTACCCCGACCACATGAAG
Q H D F F K S A M P E G Y V Q E R T I F F K D D G
CAGCACGACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGC
N Y K T R A E V K F E G D T L V N R I E L K G I D
AACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGAC
F K E D G N I L G H K L E Y N Y N S H N V Y I M A
TTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCC
D K Q K N G I K V N F K I R H N I E D G S V Q L A
GACAAGCAGAAGAACGGCATCAAGGTGAAGTTCGAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC
D H Y Q Q N T P I G D G P V L L P D N H Y L S Y Q
GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCACAACCACTACCTGAGCTACCAG
S K L S K D P N E K R D H M V L L E F V T A A G I
TCCAACTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCCGGGGATC
T L G M D E L Y K *
ACTCTCGGCATGGACGAGCTGTACAAGTAAGTCGACAAGCTTGCGGCCGCACTCGAG
SalI *NotI* *XhoI*

TDoT-L-mCherry

M I I N E T A D D I V Y R L T V I I D D R Y E S
CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
NdeI **TDoT**
L K N L I T L R A D R L E M I I N D N V S T I L A
CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG
S I T S I E G R A S G G G S G G G S G G G S G S M
AGCATTACTAGTATTGAAGGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCTGGGATCCATG
SpeI *Xa* *NheI* **3xGGGS-Linker** *BamHI*
V S K G E E D N M A I I K E F M R F K V H M E G S
GTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCC
mCherry
V N G H E F E I E G E G E G R P Y E G T Q T A K L
GTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGCCCCCTACGAGGGCACCCAGACCGCCAAGCTG
K V T K G G P L P F A W D I L S P Q F M Y G S K A
AAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCTCAGTTCATGTACGGCTCCAAGGCC
Y V K H P A D I P D Y L K L S F P E G F K W E R V
TACGTGAAGCACCCCGCGACATCCCCGACTACTTGAAGCTGTCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTG
M N F E D G G V V T V T Q D S S L Q D G E F I Y K
ATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCTTGCAGGACGGCGAGTTCATCTACAAG
V K L R G T N F P S D G P V M Q K K T M G W E A S
GTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCCTCC
S E R M Y P E D G A L K G E I K Q R L K L K D G G
TCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGC
H Y D A E V K T T Y K A K K P V Q L P G A Y N V N
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I K L D I T S H N E D Y T I V E Q Y E R A E G R H
ATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCAC
S T G G M D E L Y K *
TCCACGGCGGCATGGACGAGCTGTACAAGTAAGTCGACAAGCTTGCGGCCGCACTCGAG
SalI *NotI* *XhoI*

TDoT-mCherry

M I I N E T A D D I V Y R L T V I I D D R Y E S
CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
NdeI TDoT
L K N L I T L R A D R L E M I I N D N V S T I L A
CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG
S I G S M V S K G E E D N M A I I K E F M R F K V
AGCATTGGATCCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTG
BamHI mCherry
H M E G S V N G H E F E I E G E G E G R P Y E G T
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Q T A K L K V T K G G P L P F A W D I L S P Q F M
CAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCTCAGTTCATG
Y G S K A Y V K H P A D I P D Y L K L S F P E G F
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K W E R V M N F E D G G V V T V T Q D S S L Q D G
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E F I Y K V K L R G T N F P S D G P V M Q K K T M
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G W E A S S E R M Y P E D G A L K G E I K Q R L K
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L K D G G H Y D A E V K T T Y K A K K P V Q L P G
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A Y N V N I K L D I T S H N E D Y T I V E Q Y E R
GCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGC
A E G R H S T G G M D E L Y K *
GCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGTCGACAAGCTTGCGGCCGCACTCGAG
SalI NotI XhoI

3HAMP-L-mCherry

M G L F N A H A V A Q Q R A D R I A T L L Q S F
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NdeI 3HAMP
A D G Q L D T A V G E A P A P G Y E R L Y D S L R
GCGGATGGTCAGTTGGACACCGCCGTGGGTGAAGCGCCAGCACCTGGTTACGAACGCCTGTATGACTCGCTTCGC
A L Q R Q L R E Q R A E L Q Q V E S L E A G L A E
GCCCTTCAGCGCCAACCTGCGCGAACAACGTGCGGAGTTACAACAGGTTGAGAGCCTGGAAGCAGGCTTGGCTGAA
M S R Q H E A G W I D Q T I P A E R L E G R A A R
ATGAGTCGGCAGCATGAAGCAGGGTGGATTGACCAGACGATTCCGGCTGAACGGTTAGAGGGCCCGTGCAGCACGT
I A K G V N E L V A A H I A V K M K V V S V V T A
ATCGCCAAAGGCGTGAATGAGCTGGTTGCTGCGCACATTGCGGTGAAAATGAAAGTCGTGAGCGTAGTCACCGCG
Y G Q G N F E P L M D R L P G K K A Q I T E A I D
TATGGCCAAGGGAACCTTCGAACCGCTCATGGATCGCCTGCCGGGTAAAGAAAGCCAGATCACGGAGGCCATTGAT
G V R E R L R G A A E A T S A Q L A T A A Y N T S

GGCGTACGTGAACGCCTGCGTGGAGCTGCTGAAGCGACCTCTGCGCAGCTGGCCACAGCCGCCTACAATACTAGT
SpeI
I E G R A S G G G S G G G S G G G S G S M V S K G
ATTGAAGGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCGGGATCCATGGTGAGCAAGGGC
Xa NheI **3xGGGS-Linker** BamHI **mCherry**
E E D N M A I I K E F M R F K V H M E G S V N G H
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E F E I E G E G E G R P Y E G T Q T A K L K V T K
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G G P L P F A W D I L S P Q F M Y G S K A Y V K H
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P A D I P D Y L K L S F P E G F K W E R V M N F E
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D G G V V T V T Q D S S L Q D G E F I Y K V K L R
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G T N F P S D G P V M Q K K T M G W E A S S E R M
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Y P E D G A L K G E I K Q R L K L K D G G H Y D A
TACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCT

E V K T T Y K A K K P V Q L P G A Y N V N I K L D
GAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGACGTGCCCCGGCGCCTACAACGTCAACATCAAGTTGGAC

I T S H N E D Y T I V E Q Y E R A E G R H S T G G
ATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGC

M D E L Y K *
ATGGACGAGCTGTACAAGTAAGTCGACAAGCTTGCGGCCGCACTCGAG
SalI
NotI
XhoI

RADH

catatgtatcgtctgctgaataaaaaccgcagttattaccggtggtaatagcggatttgggt
H M Y R L L N K T A V I T G G N S G I G
NdeI
ctggcaaccgcaaaacggttttgttgccgaaggtgcctatgtttttattggttggtcgctcgt
L A T A K R F V A E G A Y V F I V G R R

cgtaaagaactggaacaggcagcagcagaaaattggtcgtaatgttaccgcagttaaagcc
R K E L E Q A A A E I G R N V T A V K A

gatgttaccaaaactggaagatctggatcgtctgtatgcaattgttcgtgaacagcgtgggt
D V T K L E D L D R L Y A I V R E Q R G

agcattgatgttctgttttgcaaatagcgggtgccattgaacagaaaaccctggaagaaatt
S I D V L F A N S G A I E Q K T L E E I

acaccggaacattatgatcgcacctttgatgttaatgtgcgtgggtctgatttttaccggt
T P E H Y D R T F D V N V R G L I F T V

cagaaagcactgccgctgctgcgtgatgggtggtagcgttattctgaccagcagcgttgcc
Q K A L P L L R D G G S V I L T S S V A

ggtgttctgggtctgcaggcacatgatacctatagcgcagcaaaagcagcagttcgtagc
G V L G L Q A H D T Y S A A K A A V R S

ctggcacgtacctggaccaccgaactgaaaggctcgtagcattcgtgttaatgcagttagt
 L A R T W T T E L K G R S I R V N A V S

ccgggtgcaattgatacccccgattattgaaaatcagggttagcaccaggaagaagcagac
 P G A I D T P I I E N Q V S T Q E E A D

gaactgcgcgcaaaatttgcagcagcaacaccgctgggtcgtgttggtcgtccggaagaa
 E L R A K F A A A T P L G R V G R P E E

ctggcagcagccgttctgtttctggcaagtgatgatagcagctatgttgcaggtattgaa
 L A A A V L F L A S D D S S Y V A G I E

ctgtttgttgatggtggtctgacccagggtttaataactcgag
 L F V D G G L T Q V - - L E
XhoI

TDot-L-RADH

M I I N E T A D D I V Y R L T V I I D D R Y E S
 CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
NdeI *TDot*

L K N L I T L R A D R L E M I I N D N V S T I L A
 CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG

S I T S I E G R A S G G G S G G G S G G G S G S M
 AGCATTACTAGTATTGAAGGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCTGGGATCCATG
SpeI *Xa* *NheI* *3xGGGS-Linker* *BamHI*

Y R L L N K T A V I T G G N S G I G L A T A K R F
 TATCGTCTGCTGAATAAAACCGCAGTTATTACCGGTGGTAATAGCGGTATTGGTCTGGCAACCGCAAAACGTTTT
 RADH

V A E G A Y V F I V G R R R K E L E Q A A A E I G
 GTTGCCGAAGGTGCCTATGTTTTTATTGTTGGTCTGCTCGTAAAGAACTGGAACAGGCAGCAGCAGAAATTGGT

R N V T A V K A D V T K L E D L D R L Y A I V R E
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Q R G S I D V L F A N S G A I E Q K T L E E I T P
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E H Y D R T F D V N V R G L I F T V Q K A L P L L
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R D G G S V I L T S S V A G V L G L Q A H D T Y S
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A A K A A V R S L A R T W T T E L K G R S I R V N
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A V S P G A I D T P I I E N Q V S T Q E E A D E L
 GCAGTTAGTCCGGGTGCAATTGATACCCCGATTATTGAAAATCAGGTTAGCACCCAGGAAGAAGCAGACGAACTG

R A K F A A A T P L G R V G R P E E L A A A V L F
 CGCGCAAAATTTGCAGCAGCAACACCGCTGGGTCTGTGGTCTGCCGAAGAACTGGCAGCAGCCGTTCTGTTT

L A S D D S S Y V A G I E L F V D G G L T Q V *
 CTGGCAAGTGATGATAGCAGCTATGTTGCAGGTATTGAACTGTTTGTGATGGTGGTCTGACCCAGGTTTAAAGTC
SalI

GACAAGCTTTCGGCCGCACTCGAG
NotI *XhoI*

3HAMP-L-RADH

M G L F N A H A V A Q Q R A D R I A T L L Q S F
CATATGGGCCTGTTTAACGCCCATGCAGTTGCGCAGCAACGCGCGGATCGCATTGCGACTCTCCTGCAGTCCTTT
NdeI 3Hamp
A D G Q L D T A V G E A P A P G Y E R L Y D S L R
GCGGATGGTCAGTTGGACACCGCCGTGGGTGAAGCGCCAGCACCTGGTTACGAACGCCTGTATGACTCGCTTCGC

A L Q R Q L R E Q R A E L Q Q V E S L E A G L A E
GCCCTTCAGCGCCAACTGCGCGAACAACGTGCGGAGTTACAACAGGTTGAGAGCCTGGAAGCAGGCTTGGCTGAA

M S R Q H E A G W I D Q T I P A E R L E G R A A R
ATGAGTCGGCAGCATGAAGCAGGTTGATTGACCAGACGATTCCGGCTGAACGGTTAGAGGGCCGTGCAGCACGT

I A K G V N E L V A A H I A V K M K V V S V V T A
ATCGCCAAAGGCGTGAATGAGCTGGTTGCTGCGCACATTGCGGTGAAAATGAAAGTCGTGAGCGTAGTCACCGCG

Y G Q G N F E P L M D R L P G K K A Q I T E A I D
TATGGCCAAGGGAACCTCGAACCGCTCATGGATCGCCTGCCGGTAAGAAAGCCAGATCACGGAGGCCATTGAT

G V R E R L R G A A E A T S A Q L A T A A Y N T S
GGCGTACGTGAACGCCTGCGTGGAGCTGCTGAAGCGACCTCTGCGCAGCTGGCCACAGCCGCTACAATACTAGT
SpeI
I E G R A S G G G S G G S G G S G S M Y R L L
ATTGAAGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCCGGATCCATGTATCGTCTGCTG
Xa NheI 3xGGGS-Linker BamHI
N K T A V I T G G N S G I G L A T A K R F V A E G
AATAAAACCGCAGTTATTACCGGTGGTAATAGCGGTATTGGTCTGGCAACCGCAAAACGTTTTTGTGCGGAAGGT
RADH
A Y V F I V G R R R K E L E Q A A A E I G R N V T
GCCTATGTTTTTATTGTTGGTCTGTCGTCGTAAAGAACTGGAACAGGCAGCAGCAGAAATTGGTCTGAATGTTACC

A V K A D V T K L E D L D R L Y A I V R E Q R G S
GCAGTTAAAGCCGATGTTACCAAACCTGGAAGATCTGGATCGTCTGTATGCAATTGTTCTGTAACAGCGTGGTAGC

I D V L F A N S G A I E Q K T L E E I T P E H Y D
ATTGATGTTCTGTTTGCAAATAGCGGTGCCATTGAACAGAAAAACCTGGAAGAAATTACACCGGAACATTATGAT

R T F D V N V R G L I F T V Q K A L P L L R D G G
CGCACCTTTGATGTTAATGTGCGTGGTCTGATTTTTTACCGTTCAGAAAGCACTGCCGCTGCTGCGTGATGGTGGT

S V I L T S S V A G V L G L Q A H D T Y S A A K A
AGCGTTATTCTGACCAGCAGCGTTGCCGGTGTCTGGGTCTGCAGGCACATGATACCTATAGCGCAGCAAAAGCA

A V R S L A R T W T T E L K G R S I R V N A V S P
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G A I D T P I I E N Q V S T Q E E A D E L R A K F
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A A A T P L G R V G R P E E L A A A V L F L A S D
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D S S Y V A G I E L F V D G G L T Q V *
GATAGCAGCTATGTTGCAGGTATTGAACTGTTTGTGATGGTGGTCTGACCCAGGTTTAAGTCGACAAGCTTGCG
SaI
GCCGCACTCGAG
NotI XhoI

LbADH

CATATGTCTAACCGTTTGGATGGTAAGGTAGCAATCATTACAGGTGGTACGTTGGGTATCGGTTTAGCTATCGCC
M S N R L D G K V A I I T G G T L G I G L A I A
NdeI
ACGAAGTTCGTTGAAGAAGGGGCTAAGGTCATGATTACCGGCCGGCACAGCGATGTTGGTGAAAAAGCAGCTAAG
T K F V E E G A K V M I T G R H S D V G E K A A K
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S V G T P D Q I Q F F Q H D S S D E D G W T K L F
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D A T E K A F G P V S T L V N N A G I A V N K S V
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E E T T T A E W R K L L A V N L D G V F F G T R L
GGGATTCAACGGATGAAGAACAAAGGCTTAGGGGCTTCCATCATCAACATGTCTTCGATCGAAGGCTTTGTGGGT
G I Q R M K N K G L G A S I I N M S S I E G F V G
GATCCTAGCTTAGGGGCTTACAACGCATCTAAAGGGGCCGTACGGATTATGTCCAAGTCAGCTGCCTTAGATTGT
D P S L G A Y N A S K G A V R I M S K S A A L D C
GCCCTAAAGGACTACGATGTTTCGGGTAAACACTGTTACCCCTGGCTACATCAAGACACCATTGGTTGATGACCTA
A L K D Y D V R V N T V H P G Y I K T P L V D D L
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P G A E E A M S Q R T K T P M G H I G E P N D I A
TACATCTGTGTTTACTTGGCTTCTAACGAATCTAAATTTGCAACGGGTTCTGAATTCGTAGTTGACGGTGGCTAC
Y I C V Y L A S N E S K F A T G S E F V V D G G Y
ACTGCTCAATAGTAGCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGC
T A Q * * R G S E F E L R R Q A C G R
BamHI SalI NotI

TDoT-L-LbADH

CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
M I I N E T A D D I V Y R L T V I I D D R Y E S
NdeI TDoT
CTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGGAGATGATCATCAATGACAATGTGTCCACCATTCTCGCG
L K N L I T L R A D R L E M I I N D N V S T I L A
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S I T S I E G R A S G G G S G G G S G G G S G S M
SpeI Xa NheI 3xGGGS-Linker BamHI
TCTAACCGTTTGGATGGTAAGGTAGCAATCATTACAGGTGGTACGTTGGGTATCGGTTTAGCTATCGCCACGAAG
S N R L D G K V A I I T G G T L G I G L A I A T K
LbADH
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F V E E G A K V M I T G R H S D V G E K A A K S V
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G T P D Q I Q F F Q H D S S D E D G W T K L F D A
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T E K A F G P V S T L V N N A G I A V N K S V E E
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T T T A E W R K L L A V N L D G V F F G T R L G I
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 Q R M K N K G L G A S I I N M S S I E G F V G D P
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 S L G A Y N A S K G A V R I M S K S A A L D C A L
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 A E E A M S Q R T K T P M G H I G E P N D I A Y I
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 C V Y L A S N E S K F A T G S E F V V D G G Y T A
 CAATAGAGTCGACAAGCTTGCGGCCGCACTCGA
 Q * S R Q A C G R T R
 SalI NotI

3HAMP-L-LbADH

CATATGGGCCTGTTTAAACGCCCATGCAGTTGCGCAGCAACGCGCGGATCGCATTGCGACTCTCCTGCAGTCCTTT
 M G L F N A H A V A Q Q R A D R I A T L L Q S F
 NdeI 3HAMP
 GCGGATGGTCAGTTGGACACCGCCGTGGGTGAAGCGCCAGCACCTGGTTACGAACGCCTGTATGACTCGCTTCGC
 A D G Q L D T A V G E A P A P G Y E R L Y D S L R
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 A L Q R Q L R E Q R A E L Q Q V E S L E A G L A E
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 M S R Q H E A G W I D Q T I P A E R L E G R A A R
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 I A K G V N E L V A A H I A V K M K V V S V V T A
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 Y G Q G N F E P L M D R L P G K K A Q I T E A I D
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 G V R E R L R G A A E A T S A Q L A T A A Y N T S
 SpeI
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 I E G R A S G G G S G G G S G G S G S M S N R L
 Xa NheI 3xGGGS-Linker BamHI LbADH
 GATGGTAAGGTAGCAATCATTACAGGTGGTACGTTGGGTATCGGTTTAGCTATCGCCACGAAGTTCGTTGAAGAA
 D G K V A I I T G G T L G I G L A I A T K F V E E
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 Q I Q F F Q H D S S D E D G W T K L F D A T E K A
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 F G P V S T L V N N A G I A V N K S V E E T T T A
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 A S N E S K F A T G S E F V V D G G Y T A Q * S R
 CAAGCTTGCGGCCGCACTCGA
 Q A C G R T R
 NotI

PfBAL-His₆

ATGGCGATGATTACAGGCGGCGAACTGGTTGTTTCGCACCCTAATAAAGGCTGGGGTCGAACATCTGTTTCGGCCTG
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 A A A G H A A E G Y A R A G A K L G V A L V T A G
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 G G F T N A V T P I A N A W L D R T P V L F L T G
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 S G A L R D D E T N T L Q A G I D Q V A M A A P I
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 L G A R F G L N T G H G S G Q L I P H S A Q V I Q
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 P E E L I L I G M D P F G S H H H H H H * A S
His-tag
HindIII

TDot-L-PfBAL

M I I N E T A D D I V Y R L T V I I D D R Y E S
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NdeI TDot
 L K N L I T L R A D R L E M I I N D N V S T I L A
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 S I T S I E G R A S G G G S G G G S G G G S G S M
 AGCATTACTAGTATTGAAGGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTGCGGATCCATG
SpeI *Xa* *NheI* 3xGGGS-Linker *BamHI*
 A M I T G G E L V V R T L I K A G V E H L F G L H
 GCGATGATTACAGGCGGCGAACTGGTTGTTTCGCACCCTAATAAAGGCTGGGGTGAACATCTGTTTCGGCCTGCAC
PfBAL
 G A H I D T I F Q A C L D H D V P I I D T R H E A
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 G F T N A V T P I A N A W L D R T P V L F L T G S
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 G A L R D D E T N T L Q A G I D Q V A M A A P I T
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 K W A H R V M A T E H I P R L V M Q A I R A A L S
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 F V A A T G V P V F A D Y E G L S M L S G L P D A
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 M R G G L V Q N L Y S F A K A D A A P D L V L M L
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 G A R F G L N T G H G S G Q L I P H S A Q V I Q V
 GGGGCGCGCTTTGGCCTTAACACCGGGCATGGATCTGGGCAGTTGATCCCCCATAGCGCGCAGGTCATTCAGGTC
 D P D A C E L G R L Q G I A L G I V A D V G G T I
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 E A L A Q A T A Q D A A W P D R G D W C A K V T D
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 A L A Q A L A H N R P A C I N V A V A L D P I P P
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 E E L I L I G M D P F A *
 GAAGAACTCATTCTGATCGGCATGGACCCCTTCGCATAAGCGGCCGCACTCGAG
NotI XhoI

3HAMP-L-PfBAL

M G L F N A H A V A Q Q R A D R I A T L L Q S F
 CATATGGGCCTGTTTAACGCCCATGCAGTTGCGCAGCAACGCGCGGATCGCATTGCGACTCTCCTGCAGTCCTTT
NdeI 3HAMP
 A D G Q L D T A V G E A P A P G Y E R L Y D S L R
 GCGGATGGTCAGTTGGACACCGCCGTGGGTGAAGCGCCAGCACCTGGTTACGAACGCCTGTATGACTCGCTTCGC
 A L Q R Q L R E Q R A E L Q Q V E S L E A G L A E
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 Y G Q G N F E P L M D R L P G K K A Q I T E A I D
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 G V R E R L R G A A E A T S A Q L A T A A Y N T S
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 I E G R A S G G G S G G G S G G G S G S M A M I T *SpeI*
 ATTGAAGGCCGTGCTAGCGGCGGTGGGTCTGGAGGCGGCTCAGGTGGTGGGTCGGGATCCATGGCGATGATTACA
 Xa *NheI* 3xGGGS-Linker *BamHI* *PfBAL*
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 G L N T G H G S G Q L I P H S A Q V I Q V D P D A
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 C E L G R L Q G I A L G I V A D V G G T I E A L A
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 Q A T A Q D A A W P D R G D W C A K V T D L A Q E
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 R Y A S I A A K S S S E H A L H P F H A S Q V I A
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 K H V D A G V T V V A D G A L T Y L W L S E V M S
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 R V K P G G F L C H G Y L G S M G V G F G T A L G
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 A Q V A D L E A G R R T I L V T G D G S V G Y S I
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 A L A H N R P A C I N V A V A L D P I P P E E L I
 GCGCTCGCCCATAAATCGCCCCGCCTGCATCAATGTGCGGTCGCGCTCGATCCGATCCCGCCGAAGAACTCATT

 L I G M D P F A *
 CTGATCGGCATGGACCCCTTCGCATAAGCGGCCGCACTCGAG
NotI XhoI

PpBFD L476Q –His₆

ATGGCTTCGGTACACGGCACCACATACGAACTCTTGCGACGTCAAGGCATCGATACGGTCTTCGGCAATCCTGGC
 M A S V H G T T Y E L L R R Q G I D T V F G N P G

 TCGAACGAGCTCCCGTTTTTTGAAGGACTTTCCAGAGGACTTTTCGATACATCCTGGCTTTGCAGGAAGCGTGTGTG
 S N E L P F L K D F P E D F R Y I L A L Q E A C V

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 V G I A D G Y A Q A S R K P A F I N L H S A A G T

 GGCAATGCTATGGGTGCACTCAGTAACGCCTGGAACACATTCCCCGCTGATCGTCACTGCCGGCCAGCAGACC
 G N A M G A L S N A W N S H S P L I V T A G Q Q T

 AGGGCGATGATTGGCGTTGAAGCTCTGCTGACCAACGTCGATGCCGCCAACCTGCCACGACCACTTGTCAAATGG
 R A M I G V E A L L T N V D A A N L P R P L V K W

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 S Y E P A S A A E V P H A M S R A I H M A S M A P

 CAAGGCCCTGTCTATCTTTTCGGTGCCATATGACGATTGGGATAAGGATGCTGATCCTCAGTCCCACCACCTTTTT
 Q G P V Y L S V P Y D D W D K D A D P Q S H H L F

 GATCGCCATGTCAAGTTCATCAGTACGCCTGAACGACCAGGATCTCGATATTCTGGTGAAAGCTCTCAACAGCGCA
 D R H V S S S V R L N D Q D L D I L V K A L N S A

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 S N P A I V L G P D V D A A N A N A D C V M L A E

 CGCCTCAAAGCTCCGGTTTGGGTTGCGCCATCCGCTCCACGCTGCCCATTCCTACCCGTCATCCTTGCTTCCGT
 R L K A P V W V A P S A P R C P F P T R H P C F R

 GGATTGATGCCAGCTGGCATCGCAGCGATTTCTCAGCTGCTCGAAGGTCACGATGTGGTTTTGGTAATCGGCGCT
 G L M P A G I A A I S Q L L E G H D V V L V I G A

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 P V F R Y H Q Y D P G Q Y L K P G T R L I S V T C

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 D P L E A A R A P M G D A I V A D I G A M A S A L

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 G G L G F A L P A A I G V Q L A E P E R Q V I A V
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 I G D G S A N Y S I S A L W T A A Q Y N I P T I F
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 V I M N N G T Y G A L R W F A G V L E A E N V P G
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 Q D V P G I D F R A L A K G Y G V Q A L K A D N L
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 E Q L K G S L Q E A L S A K G P V L I E V S T V S
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 P V K R S H H H H H H * A S R G S
HindIII

TDot-L-PpBFD L476Q

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NdeI *TDot*
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 L K N L I T L R A D R L E M I I N D N V S T I L A
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 S I T S I E G R A S G G G S G G G S G G S G S M
SpeI *Xa* *NheI* *3xGGGS-Linker* *BamHI*
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 A S V H G T T Y E L L R R Q G I D T V F G N P G S
PpBFD L476Q
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 N E L P F L K D F P E D F R Y I L A L Q E A C V V
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 G I A D G Y A Q A S R K P A F I N L H S A A G T G
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 A M I G V E A L L T N V D A A N L P R P L V K W S
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 Y E P A S A A E V P H A M S R A I H M A S M A P Q
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 G P V Y L S V P Y D D W D K D A D P Q S H H L F D
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 R H V S S S V R L N D Q D L D I L V K A L N S A S
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 L K A P V W V A P S A P R C P F P T R H P C F R G
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 L M P A G I A A I S Q L L E G H D V V L V I G A P
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 V F R Y H Q Y D P G Q Y L K P G T R L I S V T C D
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 G L G F A L P A A I G V Q L A E P E R Q V I A V I
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 D V P G I D F R A L A K G Y G V Q A L K A D N L E
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 V K R S * A A A L E
 NotI

3HAMP-L-PpBFD L476Q

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 M G L F N A H A V A Q Q R A D R I A T L L Q S F
 NdeI 3HAMP
 GCGGATGGTCAGTTGGACACCGCCGTGGGTGAAGCGCCAGCACCTGGTTACGAACGCTGTATGACTCGCTTCGC
 A D G Q L D T A V G E A P A P G Y E R L Y D S L R
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 I E G R A S G G G S G G G S G G G S G S M A S V H
 Xa NheI 3xGGGS-Linker BamHI PpBFD
 L476Q
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 F L K D F P E D F R Y I L A L Q E A C V V G I A D
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 S S V R L N D Q D L D I L V K A L N S A S N P A I
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 I D F R A L A K G Y G V Q A L K A D N L E Q L K G
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 S L Q E A L S A K G P V L I E V S T V S P V K R S
 TAAGCGGCCGCACTCGAG
 * A A A L E
 NotI

TDoT-L-EcLDC

CATATGATCATTAAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGATCGCTACGAATCG
 M I I N E T A D D I V Y R L T V I I D D R Y E S
 NdeI TDoT
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 L K N L I T L R A D R L E M I I N D N V S T I L A
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 S I T S I E G R A S G G G S G G G S G G G S G S M
 SpeI Xa NheI 3xGGGS-Linker BamHI
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 N I I A I M G P H G V F Y K D E P I K E L E S A L
 EcLDC
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 V A Q G F Q I I W P Q N S V D L L K F I E H N P R
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 I C G V I F D W D E Y S L D L C S D I N Q L N E Y
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 L P L Y A F I N T H S T M D V S V Q D M R M A L W
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 F F E Y A L G Q A E D I A I R M R Q Y T D E Y L D
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 N I T P P F T K A L F T Y V K E R K Y T F C T P G
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 H M G G T A Y Q K S P V G C L F Y D F F G G N T L
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 E Y I A R T F G A E Q S Y I V T N G T S T S N K I
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 R R E F T R D S I E E K V A A T T Q A Q W P V H A
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 S Y P I V A S V E T A A A M L R G N P G K R L I N
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 L P G L M L R A F D T L P E M I M T P H Q A W Q R
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 Q I K G E V E T I A L E Q L V G R V S A N M I L P
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 Y P P G V P L L M P G E M L T K E S R T V L D F L
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 L M L C S V G Q H Y P G F E T D I H G A K Q D E D
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 G V Y R V R V L K M A G * V D K L A A A L E
SalI NotI

EcLDC-L-TDoT

CATATGATGAACATCATCGCTATCATGGGCCCTCACGGTGTCTTCTACAAGGATGAGCCAATCAAGGAGCTGGAA
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NdeI EcLDC
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 S A L V A Q G F Q I I W P Q N S V D L L K F I E H
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N P R I C G V I F D W D E Y S L D L C S D I N Q L
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N E Y L P L Y A F I N T H S T M D V S V Q D M R M
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Q A S L I H I K G E Y D E E A F N E A F M M H T T
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T S P S Y P I V A S V E T A A A M L R G N P G K R
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L I N R S V E R A L H F R K E V Q R L R E E S D G
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W F F D I W Q P P Q V D E A E C W P V A P G E Q W
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H G F N D A D A D H M F L D P V K V T I L T P G M
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D E Q G N M S E E G I P A A L V A K F L D E R G I
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V V E K T G P Y N L L F L F S I G I D K T K A M G
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L L R G L T E F K R S Y D L N L R I K N M L P D L
TACGCTGAAGATCCTGATTTCTACCGCAACATGCGCATCCAGGACCTCGCACAGGGCATCCACAAGCTCATTCGC

Y A E D P D F Y R N M R I Q D L A Q G I H K L I R
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 K H D L P G L M L R A F D T L P E M I M T P H Q A
 TGGCAGCGCCAGATCAAGGGCGAGGTGGAAACCATCGCACTGGAGCAGCTGGTTGGTCGTGTCTCCGCCAACATG
 W Q R Q I K G E V E T I A L E Q L V G R V S A N M
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 I L P Y P P G V P L L M P G E M L T K E S R T V L
 GACTTCCTTCTCATGTTGTGCTCTGTTGGCCAGCACTACCCAGGCTTCGAGACCGACATCCACGGCGCTAAGCAA
 D F L L M L C S V G Q H Y P G F E T D I H G A K Q
 GATGAAGACGGCGTTTACCGCGTTCGCGTCCTTAAGATGGCAGGCGCTAGCGGCGGTGGGTCTGGAGGCGGCTCA
 D E D G V Y R V R V L K M A G **A S** G G G S G G G S
NheI **3xGGGS-Linker**
 GGTGGTGGGTGCGGATCCATCATTAACGAAACTGCCGATGACATCGTTTATCGCCTGACAGTCATTATCGATGAT
 G G G S **G S** I I N E T A D D I V Y R L T V I I D D
BamHI *TDot*
 CGCTACGAATCGCTGAAAAACCTGATTACCTTACGTGCAGATCGCTTGGAGATGATCATCAATGACAATGTGTCC
 R Y E S L K N L I T L R A D R L E M I I N D N V S
 ACCATTCTCGCGAGCATTTAAGCGGCCGCACTCGAG
 T I L A S I * **A A A L E**
NotI

EcLDC-L-3HAMP

CATATGATGAACATCATCGCTATCATGGGCCCTCACGGTGTCTTCTACAAGGATGAGCCAATCAAGGAGCTGGAA
 H M M N I I A I M G P H G V F Y K D E P I K E L E
NdeI *EcLDC*
 TCCGCACTAGTTGCACAGGGCTTTTCAGATCATCTGGCCCCAGAACTCCGTTGACCTTCTCAAATTCATCGAGCAC
 S A L V A Q G F Q I I W P Q N S V D L L K F I E H
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 A L W F F E Y A L G Q A E D I A I R M R Q Y T D E
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 Y L D N I T P P F T K A L F T Y V K E R K Y T F C
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 T P G H M G G T A Y Q K S P V G C L F Y D F F G G
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 N K I V G M Y A A P S G S T L L I D R N C H K S L
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A H L L M M N D V V P V W L K P T R N A L G I L G
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V P S I H F D S A W V P Y T H F H P I Y Q G K S G
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M S G E R V A G K V I F E T Q S T H K M L A A L S
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Q A S L I H I K G E Y D E E A F N E A F M M H T T
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I L P Y P P G V P L L M P G E M L T K E S R T V L
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D F L L M L C S V G Q H Y P G F E T D I H G A K Q
GATGAAGACGGCGTTTACCGCGTTTCGCGTCCTTAAGATGGCAGGCGCTAGCGGCGGTGGGTCTGGAGGCGGCTCA
D E D G V Y R V R V L K M A G **A S** G G G S G G G S
GGTGGTGGGTGCGGATCCATGGGCCTGTTTAACGCCCATGCGAGTTGCGCAGCAACGCGCGGATCGCATTGCGACT
G G G S **G S** M G L F N A H A V A Q Q R A D R I A T
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L L Q S F A D G Q L D T A V G E A P A P G Y E R L
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Y D S L R A L Q R Q L R E Q R A E L Q Q V E S L E
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 A G L A E M S R Q H E A G W I D Q T I P A E R L E
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 T E A I D G V R E R L R G A A E A T S A Q L A T A
 GCCTACAATTAAGCGGCCGCACTCGAG
 A Y N * A A A L E
 NotI

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