molecules

MDPI

Supporting Information for

Simple Enzyme Immobilization for Flow Chemistry? An Assessment of Available Strategies for an Acetaldehyde-Dependent Aldolase

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SDS-PAGE

SDS-PAGE samples have been prepared by diluting *ad* 60 µL deionized water according to empirical equation

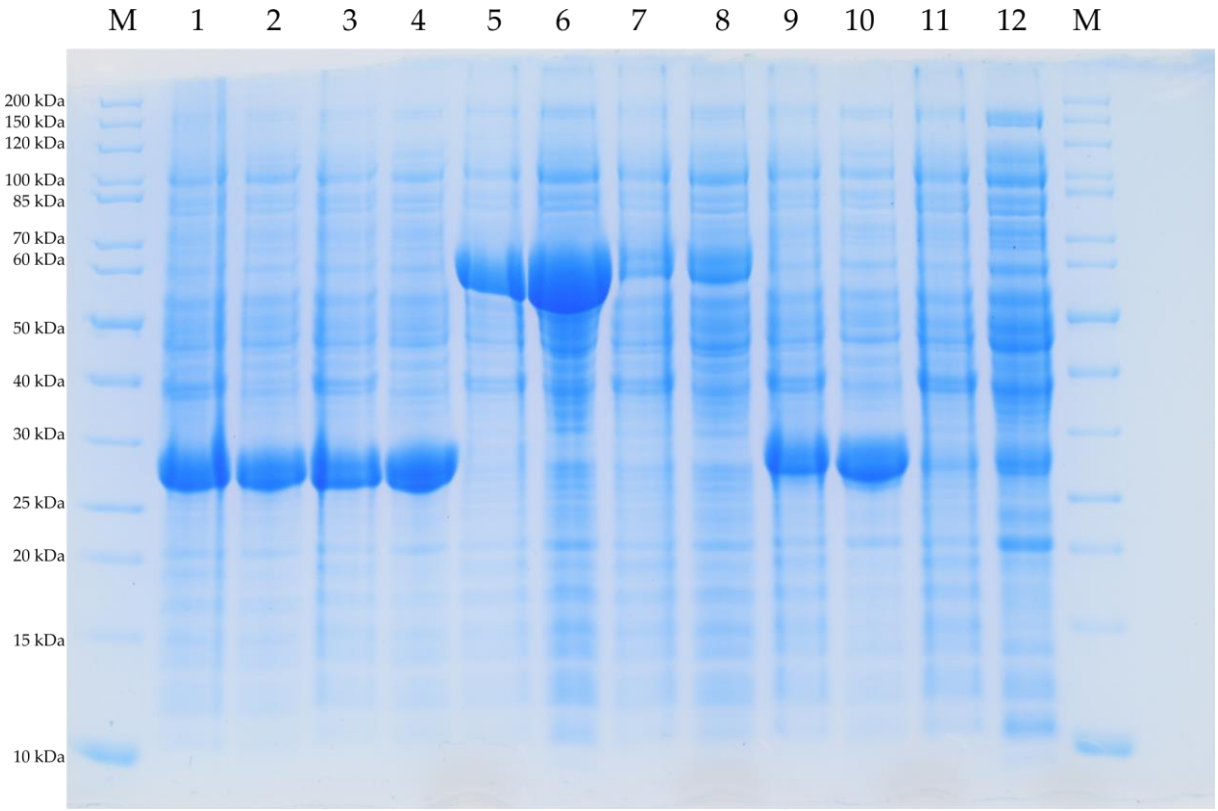
$$V_{sample} = \frac{375\ \mu L}{OD_{600}} \quad (1)$$

for cell containing samples, or equation

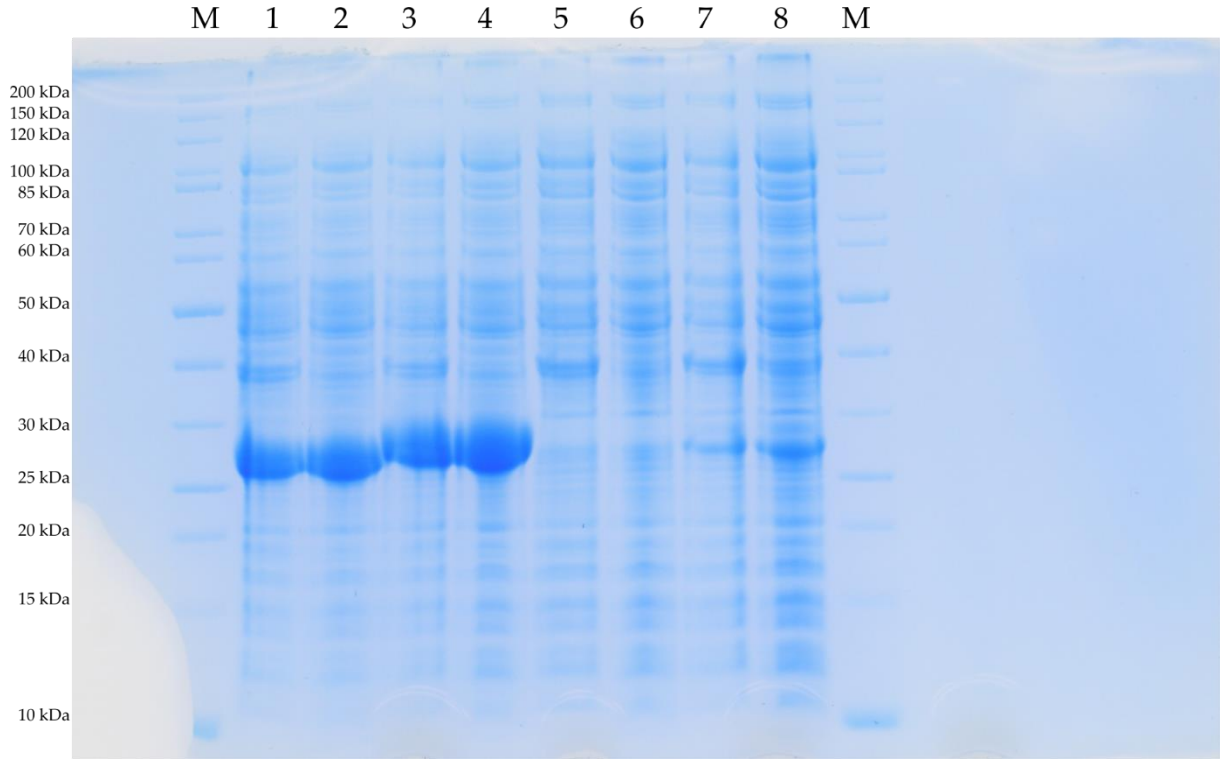
$$V_{sample} = \frac{72\ mg\ mL^{-1}}{protein\ amount\ in\ [mg\ mL^{-1}]} \quad (2)$$

for protein solutions. 15 µL 5x SDS-PAGE sample buffer (10 % w/v SDS, 40 % v/v glycerol, 0.5 % w/v bromophenol blue, 500 mM dithiothreitol in 500 mM Tris/HCl buffer, pH 6.5) were added and the mixture was vortexed. Before application on gel, the samples were heated for 10 min at 95 °C.

0.75 mm gels were prepared with 4 % collection phase and 10 % separation phase. Electrophoresis was carried out at 190 V, 3 Amax for 40 min driven by a *PowerPac HC* (*BioRad*) according to *Schägger et al.* [1]. Staining was conducted as described by *Kang et al* [2]. The result was documented with an *EOS 1000D* (*Canon*).

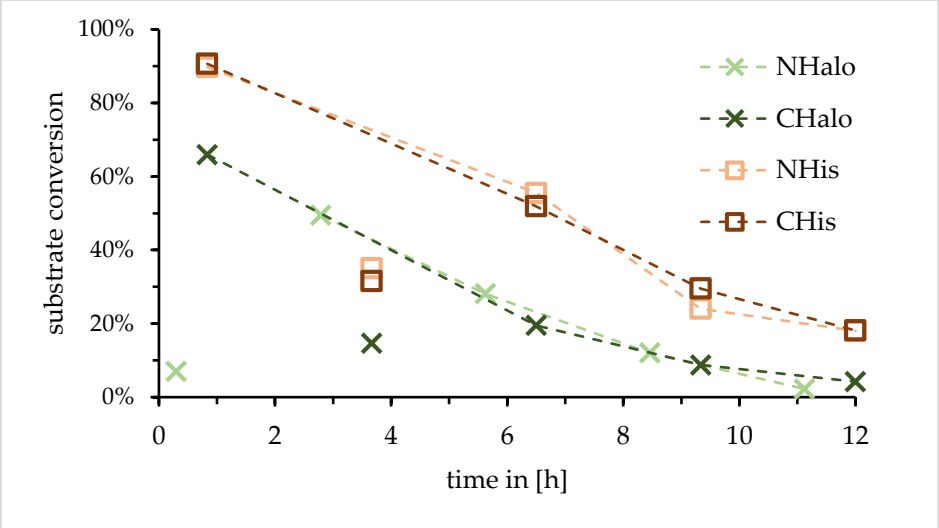


Supplementary Figure S1. SDS-PAGE for protein expression control. **M:** 5 µL Protein Marker: PageRuler™ unstained protein ladder (ThermoScientific) contains Proteins of size: 200 kDa, 150 kDa, 120 kDa, 100 kDa, 85 kDa, 70 kDa; 60 kDa, 50 kDa, 40 kDa, 30 kDa, 25 kDa, 20 kDa, 15 kDa, 10 kDa. **1:** 15 µL sample from deoC-EC-DM-CHis expression (pellet resuspension), calculated molecular weight (MW_c): 28.9 kDa. **2:** 15 µL sample from DeoC-EC-DM-CHis preparation (clear cell-free extract), MW_c: 28.9 kDa. **3:** 15 µL sample from deoC-EC-DM-NHis expression (pellet resuspension), MW_c: 28.9 kDa. **4:** 15 µL sample from DeoC-EC-DM-NHis preparation (clear cell-free extract), MW_c: 28.9 kDa. **5:** 15 µL sample from deoC-EC-DM-CHalo expression (pellet resuspension), MW_c: 63.6 kDa **6:** 15 µL sample from DeoC-EC-DM-CHalo preparation (clear cell-free extract), MW_c: 63.6 kDa. **7:** 15 µL sample from deoC-EC-DM-NHalo expression (pellet resuspension), MW_c: 63.8 kDa. **8:** 15 µL sample from DeoC-EC-DM-NHalo preparation (clear cell-free extract), MW_c: 63.8 kDa. **9:** 15 µL sample from deoC-EC-DM-CHisNu expression (pellet resuspension), MW_c: 29.2 kDa. **10:** 15 µL sample from DeoC-EC-DM-NNuHis preparation (clear cell-free extract), MW_c: 29.2 kDa. **11:** 15 µL sample from deoC-EC-DM-NNuHis expression (pellet resuspension), MW_c: 29.2 kDa. **12:** 15 µL sample from DeoC-EC-DM-NNuHis preparation (clear cell-free extract), MW_c: 29.2 kDa.



Supplementary Figure S2. SDS-PAGE for protein expression control. **M:** 5 µL Protein Marker: PageRuler™ unstained protein ladder (ThermoScientific) contains Proteins of size: 200 kDa, 150 kDa, 120 kDa, 100 kDa, 85 kDa, 70 kDa; 60 kDa, 50 kDa, 40 kDa, 30 kDa, 25 kDa, 20 kDa, 15 kDa, 10 kDa. **1:** 15 µL sample from deoC-EC-DM-CNu expression (pellet resuspension), calculated molecular weight (MW_c): 28.4 kDa. **2:** 15 µL sample from DeoC-EC-DM-CNu preparation (clear cell-free extract), MW_c: 28.4 kDa. **3:** 15 µL sample from deoC-EC-DM-CStrep expression (pellet resuspension), MW_c: 29.3 kDa. **4:** 15 µL sample from DeoC-EC-DM-CStrep preparation (clear cell-free extract), MW_c: 29.3 kDa. **5:** 15 µL sample from deoC-EC-DM-NNu expression (pellet resuspension), MW_c: 28.4 kDa **6:** 15 µL sample from DeoC-EC-DM-NNu preparation (clear cell-free extract), MW_c: 28.4 kDa. **7:** 15 µL sample from deoC-EC-DM-NStrep expression (pellet resuspension), MW_c: 29.3 kDa. **8:** 15 µL sample from DeoC-EC-DM-NStrep preparation (clear cell-free extract), MW_c: 29.3 kDa.

Reactor stability - full data



Supplementary Figure S3. Change in substrate conversion over time by continuous usage for the reaction of hexanal (**1**) and acetaldehyde (**2**) towards 3-hydroxyoctanal (3) catalyzed by the DERA reactors. (hexanal (1) as observed substrate). Light-green cross: reactor with N-terminal HaloTag®-DERA (NHalo). Dark-green cross: reactor with C-terminal HaloTag®-DERA (CHalo). Light brown square: reactor with N-terminal HisTag-DERA (NHis). Dark-brown square: reactor with C-terminal HisTag-DERA (CHis).

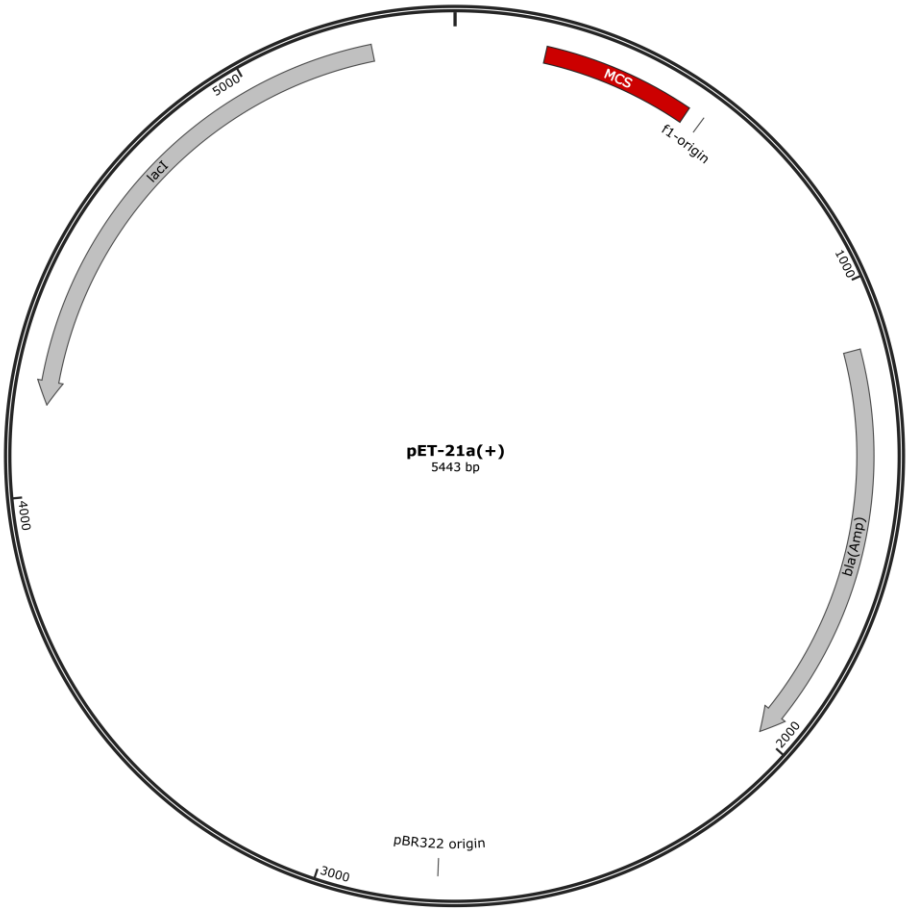
Fit parameters

Supplementary Table S1. DERA-CHalo-Link: DeoC$_{EC}$-C47M-A95C with C-terminal HaloTag® immobilized on HaloLink®-resin. CStrep-Tactin: DeoC$_{EC}$-C47M-A95C with C-terminal StrepTag immobilized on StrepTactin™-Sepharose®. CHis-Ni-S6FF: DeoC$_{EC}$-C47M-A95C with C-terminal HisTag immobilized on IMAC Sepharose® 6 FastFlow tethered by Nickel ions. CHisNu-Ni-S6FF: DeoC$_{EC}$-C47M-A95C with C-terminal HisTag with additional cysteins immobilized on IMAC Sepharose® 6 FastFlow tethered by nickel ions. CHis-Immobead: DeoC$_{EC}$-C47M-A95C with C-terminal HisTag immobilized on Immobead150P. Reactor size: ~350 µL.

	Fit-parameters			Fit-Quality	
	limit t→∞ y0 [µmol/10 min]	initial t=0 A_1 [µmol/10 min]	decay rate factor t_1 [x10 min]	R^2	corr. $R^{2\,‡}$
DERA-CHalo-Link	5.6 ± 0.6	62.4 ± 7.6	30.9 ± 3.4	0.97	0.97
DERA-CStrep-Tactin	0.0 ± 0.1	96.1 ± 14.9	9.6 ± 0.7	0.99	0.99
DERA-CHis-Ni^{2+}-S6FF	15.7 ± 28.5	33.1 ± 25.8	230.9 ± 308.3	0.85	0.83
DERA-CHisNu-Ni^{2+}-S6FF	0 ± 174	35.4 ± 172.5	776.9 ± 4392	0.81	0.78
DERA-CHis-Immobead	0.9 ± 0.2	6.1 ± 0.8	61.6 ± 10.4	0.99	0.98

‡: corrected R^2 considers degrees of freedom of fit. Please refer to *OriginLab*-manual for detailed calculation [3].

Sequences



Supplementary Figure S4. Plasmid map of pET-21a(+)

pET21a(+)-MCS

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GATC          Plasmid features

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                           T7-Promoter          lac-Operator
  81 TGTTTAACTT TAAGAAGGAG ATATACATAT GGCTAGCATG ACTGGTGGAC AGCAAATGGG TCGCGGATCC GAATTCGAGC
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 161 TCCGTCGACA AGCTTGCGGC CGCACTCGAG CACCACCACC ACCACCACTG AGATCCGGCT GCTAACAAAG CCCGAAAGGA
                                      His-Tag
 241 AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT
                                  T7-terminator
 321 TG
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DeoC reference sequence

UniProt accession number: P0A6L0 [4]

pET21a-deoC-EC-DM-NHis_MCS-frame

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GATC         Plasmid features
GATC         deoC-gene
GATC         gene modifications
GATC         HisTag

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                           T7-Promoter         lac-Operator
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 161 GTGCACTGAA ATTGATGGAC CTGACCACCC TGAATGACGA CGACACCGAC GAGAAAGTGA TCGCCCTGTG TCATCAGGCC
 241 AAAACTCCGG TCGGCAATAC CGCCGCTATC ATGATCTATC CTCGCTTTAT CCCGATTGCT CGCAAAACTC TGAAAGAGCA
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 321 GGGCACCCCG GAAATCCGTA TCGCTACGGT AACCAACTTC CCACACGGTA ACGACGACAT CGACATCGCG CTGGCAGAAA
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 801 CGGTGCTGAC TGGGCAGATG CGCGTCACTA CCGCTTTGGC GCTTCCAGCC TGCTGGCAAG CCTGCTGAAA GCGCTGGGTC
 881 ACGGCGACGG TAAGAGCGCC AGCAGCTACG GATCCTGACT CGAGCACCAC CACCACCACC ACTGAGATCC GGCTGCTAAC
                                 C-flank          His-Tag
 961 AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT
                                                 T7-Terminator
1041 CTTGAGGGGT TTTTTG
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DeoC-EC-DM-NHis AA-sequence *"NHis"*

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 161 KASEISIKAG ADFIKTSTGK VAVNATPESA RIMMEVIRDM GVEKTVGFKP AGGVRTAEDA QKYLAIADEL FGADWADARH
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```

pET21a-deoC-EC-DM-NHalo_MCS-frame

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GATC         deoC-gene
GATC         gene modifications
GATC         linker-sequence
GATC         HaloTag

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                           T7-Promoter         lac-Operator
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DeoC-EC-DM-NHalo AA-sequence "NHalo"

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pET21a-deoC-EC-DM-CHis_MCS-frame

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GATC         deoC-gene
GATC         gene modifications
GATC         HisTag

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pET21a-deoC-EC-DM-CHisNu_MCS-frame

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GATC         deoC-gene
GATC         gene modifications
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* His8 instead of His6 due to misaligned primer. No consequence for gene.
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 161 IKAGADFIKT STGKVAVNAT PESARIMMEV IRDMGVEKTV GFKPAGGVRT AEDAQKYLAI ADELFGADWA DARHYRFGAS
 241 SLLASLLKAL GHGDGKSASS YGSHHHHHHG GCC
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pET21a-deoC-EC-DM-CStrep_MCS-frame

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GATC         deoC-gene
GATC         gene modifications
GATC         StrepTag

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 161 ACCTGACCAC CCTGAATGAC GACGACACCG ACGAGAAAGT GATCGCCCTG TGTCATCAGG CCAAAACTCC GGTCGGCAAT
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                  C47M
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                                                                                      A95C
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 881 CCAGCAGCTA CGGATCCTCT GCGTGGTCAC ATCCTCAGTT CGAGAAATGA CTCGAGCACC ACCACCACCA CCACTGAGAT
                C-flank StrepTag                                    His-Tag
 961 CCGGCTGCTA ACAAAGCCCG AAAGGAAGCT GAGTTGGCTG CTGCCACCGC TGAGCAATAA CTAGCATAAC CCCTTGGGGC
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 241 SLLASLLKAL GHGDGKSASS YGSSAWSHPQFEK
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pET21a-deoC-EC-DM-CHalo_MCS-frame

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GATC         Plasmid features
GATC         deoC-gene
GATC         gene modifications
GATC         linker-sequence
GATC         HaloTag

   1 AGATCTCGAT CCCGCGAAAT TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GAAATAATTT
                           T7-Promoter         lac-Operator
  81 TGTTTAACTT TAAGAAGGAG ATATACATAT GGCTAGCACT GATCTGAAAG CAAGCAGCCT GCGTGCACTG AAATTGATGG
                                      N-flank
 161 ACCTGACCAC CCTGAATGAC GACGACACCG ACGAGAAAGT GATCGCCCTG TGTCATCAGG CCAAAACTCC GGTCGGCAAT
 241 ACCGCCGCTA TCATGATCTA TCCTCGCTTT ATCCCGATTG CTCGCAAAAC TCTGAAAGAG CAGGGCACCC CGGAAATCCG
                 C47M
 321 TATCGCTACG GTAACCAACT TCCCACACGG TAACGACGAC ATCGACATCG CGCTGGCAGA AACCCGTGCG GCAATCTGCT
                                                                                      A95C
 401 ACGGTGCTGA TGAAGTTGAC GTTGTGTTCC CGTACCGCGC GCTGATGGCG GGTAACGAGC AGGTTGGTTT TGACCTGGTG
 481 AAAGCCTGTA AAGAGGCTTG CGCGGCAGCG AATGTACTGC TGAAAGTGAT CATCGAAACC GGCGAACTGA AAGACGAAGC
 561 GCTGATCCGT AAAGCGTCTG AAATCTCCAT CAAAGCGGGT GCGGACTTCA TCAAAACCTC TACCGGTAAA GTGGCTGTGA
 641 ACGCGACGCC GGAAAGCGCG CGCATCATGA TGGAAGTGAT CCGTGATATG GGCGTAGAAA AAACCGTTGG TTTCAAACCG
 721 GCGGGCGGCG TGCGTACTGC GGAAGATGCG CAGAAATATC TCGCCATTGC AGATGAACTG TTCGGTGCTG ACTGGGCAGA
 801 TGCGCGTCAC TACCGCTTTG GCGCTTCCAG CCTGCTGGCA AGCCTGCTGA AAGCGCTGGG TCACGGCGAC GGTAAGAGCG
 881 CCAGCAGCTA CGGATCCGAA GCAGCGGCCA AAGAAGCTGC GGCCAAAGAG GCAGCCGCGA AAGAAGCAGC GGCGAAAGCA
               C-flank 4x EAAAK-linker
 961 GAAATTGGTA CGGGATTTCC GTTTGACCCG CATTATGTGG AGGTTCTGGG TGAACGCATG CACTACGTGG ATGTTGGTCC
1041 GCGCGATGGC ACACCGGTGC TGTTTCTGCA TGGTAATCCG ACCTCCAGCT ATGTTTGGCG CAACATTATT CCGCATGTCG
1121 CCCCAACGCA TCGCTGTATT GCCCCAGATC TCATTGGCAT GGGCAAAAGC GACAAACCGG ATTTGGGCTA CTTCTTCGAC
1201 GATCACGTAC GGTTTATGGA CGCCTTTATC GAGGCTCTGG GACTGGAGGA AGTAGTGCTG GTTATTCATG ACTGGGGCTC
                                                 C-->G (silent)
1281 TGCATTAGGC TTTCACTGGG CTAAACGGAA CCCAGAACGC GTCAAGGGGA TTGCCTTCAT GGAGTTCATC CGTCCGATTC
1361 CGACCTGGGA TGAATGGCCC GAATTTGCCC GTGAAACCTT TCAGGCGTTT CGTACCACGG ATGTTGGCCG TAAGCTCATC
1441 ATCGACCAAA ACGTGTTCAT TGAGGGCACT CTTCCCATGG GAGTAGTGCG TCCTTTAACC GAAGTCGAGA TGGACCACTA
1521 TCGCGAACCC TTCCTGAATC CGGTTGATCG CGAACCGCTG TGGCGCTTCC CGAATGAGCT GCCTATTGCT GGTGAACCGG
1601 CGAATATCGT GGCACTTGTG GAAGAATACA TGGATTGGCT GCATCAGAGT CCAGTCCCTA AGCTGTTGTT TTGGGGTACA
1681 CCTGGCGTGT TGATTCCGCC TGCAGAAGCT GCTCGCTTAG CGAAAAGCTT GCCCAACTGC AAAGCGGTCG ATATTGGGCC
1761 AGGTCTGAAC CTGTTACAGG AGGATAACCC GGATCTGATC GGGAGTGAAA TCGCGCGTTG GCTGTCAACT CTGGAAATCT
1841 CGGGTCTTGC ATCCTGACTC GAGCACCACC ACCACCACCA CTGAGATCCG GCTGCTAACA AAGCCCGAAA GGAAGCTGAG
                             His-Tag
1921 TTGGCTGCTG CCACCGCTGA GCAATAACTA GCATAACCCC TTGGGGCCTC TAAACGGGTC TTGAGGGGTT TTTTG
                                   T7-Terminator
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DeoC-EC-DM-CHalo AA-sequence *"CHalo"*

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   1 MASTDLKASS LRALKLMDLT TLNDDDTDEK VIALCHQAKT PVGNTAAIMI YPRFIPIARK TLKEQGTPEI RIATVTNFPH
  81 GNDDIDIALA ETRAAICYGA DEVDVVFPYR ALMAGNEQVG FDLVKACKEA CAAANVLLKV IIETGELKDE ALIRKASEIS
 161 IKAGADFIKT STGKVAVNAT PESARIMMEV IRDMGVEKTV GFKPAGGVRT AEDAQKYLAI ADELFGADWA DARHYRFGAS
 241 SLLASLLKAL GHGDGKSASS YGSEAAAKEA AAKEAAAKEA AAKAEIGTGF PFDPHYVEVL GERMHYVDVG PRDGTPVLFL
 321 HGNPTSSYVW RNIIPHVAPT HRCIAPDLIG MGKSDKPDLG YFFDDHVRFM DAFIEALGLE EVVLVIHDWG SALGFHWAKR
 401 NPERVKGIAF MEFIRPIPTW DEWPEFARET FQAFRTTDVG RKLIIDQNVF IEGTLPMGVV RPLTEVEMDH YREPFLNPVD
 481 REPLWRFPNE LPIAGEPANI VALVEEYMDW LHQSPVPKLL FWGTPGVLIP PAEAARLAKS LPNCKAVDIG PGLNLLQEDN
 561 PDLIGSEIAR WLSTLEISGL AS
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Supplementary References

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