

Supplementary data for article:

Molloy, S.; Nikodinović-Runić, J.; Martin, L. B.; Hartmann, H.; Solano, F.; Decker, H.; O'Connor, K. E. Engineering of a Bacterial Tyrosinase for Improved Catalytic Efficiency towards D-Tyrosine Using Random and Site Directed Mutagenesis Approaches.

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Supplemental Table I. List of strains, plasmids and primers used in this study

A list of strains used in this study

Strain	Relevant characteristics	Source or reference
<i>Ralstonia solanacearum</i>	Wild type, tyrosinase activity	(21)
<i>Escherichia coli</i> BL21(DE3)	F ⁻ , <i>ompT</i> , high level expression of genes regulated by T7 promoter	Novagen
Recombinant <i>E.coli</i>		
WT	Expressing pRSET-tyrR0	This work
RVC10	Expressing pRSET-tyrR1-1	This work
RV145	Expressing pRSET-tyrR1-2	This work
145_Y119F	Expressing pRSET-tyr-F119	This work
145_Y119T	Expressing pRSET-tyr-T119	This work
145_Y119W	Expressing pRSET-tyr-W119	This work
145_Y119H	Expressing pRSET-tyr-H119	This work
145_V153A	Expressing pRSET-tyr-A153	This work
145_V153T	Expressing pRSET-tyr-T153	This work
145_V153L	Expressing pRSET-tyr-L153	This work
145_V153H	Expressing pRSET-tyr-H153	This work
C10_T183I	Expressing pRSET-tyr I183	This work
C10_T183G	Expressing pRSET-tyr-G183	This work
C10_T183R	Expressing pRSET-tyr-R183	This work
C10_T183S	Expressing pRSET-tyr-S183	This work
C10_F185Y	Expressing pRSET-tyr-Y185	This work
145_D317Y	Expressing pRSET-tyr-Y317	This work
C10_N322S	Expressing pRSET-tyr-S322	This work
C10_N322K	Expressing pRSET-tyr-K322	This work
C10_N322D	Expressing pRSET-tyr-D322	This work
C10_N322Q	Expressing pREST-tyr-Q322	This work
145_L330V	Expressing pRSET-tyr-V330	This work
C10_T359M	Expressing pRSET-tyr-M359	This work
C10_Rev1	Expressing pRSET-tyr-I183S322	This work
C10_Rev2	Expressing pRSET-tyr-Y185M359	This work

Each strain was named according to the corresponding plasmid which was expressed in *E.coli* BL21(DE3) cells

A list of plasmids used in this study.

Plasmid	Relevant characteristics	Source or reference
pRSETb	expression under T7 promoter, amp ^R	Invitrogen
pRSET-tyrR0	1.5 kbp <i>tyr</i> fragment in pRSETb, amp ^R	This work
pRSET-tyrR1-1	pRSET-tyrR0 derivative, positive first clone from mutagenesis, amp ^R	This work
pRSET-tyrR1-2	pRSET-tyrRM0 derivative, positive second clone from mutagenesis, amp ^R	This work
pRSET-tyrR2	pRSET-tyrR1-2 derivative, positive clone from mutagenesis, amp ^R	This work
pRSET-tyr-F119	pRSET-tyrR0 derivative, mutation Y119F introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-T119	pRSET-tyrR0 derivative, mutation Y119T introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-W119	pRSET-tyrR0 derivative, mutation Y119W introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-H119	pRSET-tyrR0 derivative, mutation Y119H introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-A153	pRSET-tyrR0 derivative, mutation V153A introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-T153	pRSET-tyrR0 derivative, mutation V153T introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-L153	pRSET-tyrR0 derivative, mutation V153L introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-H153	pRSET-tyrR0 derivative, mutation V153H introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr I183	pRSET-tyrR0 derivative, mutation T183I introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-G183	pRSET-tyrR0 derivative, mutation T183G introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-G183	pRSET-tyrR0 derivative, mutation T183I introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-R183	pRSET-tyrR0 derivative, mutation T183R introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-S183	pRSET-tyrR0 derivative, mutation T183S introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-Y185	pRSET-tyrR0 derivative, mutation F185Y introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-Y317	pRSET-tyrR0 derivative, mutation D317Y introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-S322	pRSET-tyrR0 derivative, mutation N322S introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-K322	pRSET-tyrR0 derivative, mutation N322K introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-D322	pRSET-tyrR0 derivative, mutation N322D introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-Q322	pRSET-tyrR0 derivative, mutation N322Q introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-V330	pRSET-tyrR0 derivative, mutation L330V introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-M359	pRSET-tyrR0 derivative, mutation T359M introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-I183S322	pRSET-tyr-I183Y185S322 derivative, mutation Y185F introduced by site directed mutagenesis, amp ^R	This work
pRSET-tyr-Y185M359	pRSET-tyr-Y185S322M359 derivative, mutation S322N introduced by site directed mutagenesis, amp ^R	This work

A list of primers used in this study

Primer	Sequence(5'-3')	Application
Ralst_Tyr_XhoI (F)	AACTAT <u>CTCGAGGGT</u> CGTGCGTAGAACGGT	cloning, epPCR, sequencing
Ralst_Tyr_EcoRI (R)	AACTAT <u>GAATTCT</u> CAAATGACGGCGACCTCG	cloning, epPCR, sequencing
Tyr_middle (F)	AATATCGACCGGGTATGGG	sequencing
Tyr_middle (R)	CCCATACCCGGTCGATATT	sequencing
Tyr_middle (F2)	ACCGCCTGCTGCCGAAG	sequencing
Tyr_middle (R2)	TACCGTGCTGGTTGGCA	sequencing
Tyr_middle (F3)	CCTCGATGTGCCGAAAC	sequencing
Tyr_middle (F4)	TGCCAACAGCACGGTA	sequencing
Tyr_SDM_Y119F (F)	CGCTCACCGGCTTCAAGACCTTCGC	SDM
Tyr_SDM_Y119F (R)	GCGAAGGTCTTGAAGCCGGTGAGCG	SDM
Tyr_SDM_Y119W (F)	CGCTCACCGGCTGGAAGACCTTCGC	SDM
Tyr_SDM_Y119W (R)	GCGAAGGTCTTCCAGCCGGTGAGCG	SDM
Tyr_SDM_Y119T (F)	CGCTCACCGGCACCAAGACCTTCGC	SDM
Tyr_SDM_Y119T (R)	GCGAAGGTCTTGGTGCCGGTGAGCG	SDM
Tyr_SDM_Y119H (F)	CGCTCACCGGCCACAAGACCTTCG	SDM
Tyr_SDM_Y119H (R)	GCGAAGGTCTTGTGGCCGGTGAGCG	SDM
Tyr_SDM_V153A (F)	AACCCGCTCTACGCGCCCAACCGGAAT	SDM
Tyr_SDM_V153A (R)	ATTCCGGTTGGGCGCGTAGAGCGGGTT	SDM
Tyr_SDM_V153L (F)	AACCCGCTCTACTTGCCCAACCGGAAT	SDM
Tyr_SDM_V153L (R)	ATTCCGGTTGGGCAAGTAGAGCGGGTT	SDM
Tyr_SDM_V153T (F)	AACCCGCTCTACGCGCCCAACCGGAAT	SDM
Tyr_SDM_V153T (R)	ATTCCGGTTGGGCGTGTAGAGCGGGTT	SDM
Tyr_SDM_V153H (F)	AACCCGCTCTACCATCCCAACCGGAAT	SDM
Tyr_SDM_V153H (R)	ATTCCGGTTGGGATGGTAGAGCGGGTT	SDM
Tyr_SDM_T183I (F)	CAAGATCTATGCCGAAATCAACTTCGAAGTCTTCG	SDM
Tyr_SDM_T183I (R)	CGAAGACTTCGAAGTTGATTTCCGGCATAGATCTTG	SDM
Tyr_SDM_T183G (F)	CAAGATCTATGCCGAAAGGCAACTTCGAAGTCTTCG	SDM
Tyr_SDM_T183G (R)	CGAAGACTTCGAAGTTGCCTTCGGCATAGATCTTG	SDM
Tyr_SDM_T183R (F)	CAAGATCTATGCCGAAAGCAACTTCGAAGTCTTCG	SDM
Tyr_SDM_T183R (R)	CGAAGACTTCGAAGTTGCCTTCGGCATAGATCTTG	SDM
Tyr_SDM_T183S (F)	CAAGATCTATGCCGAAAGCAACTTCGAAGTCTTCG	SDM
Tyr_SDM_T183S (R)	CGAAGACTTCGAAGTTGCTTTCGGCATAGATCTTG	SDM
Tyr_SDM_F185Y (F)	ATGCCGAAACCAACTTCGAAGTCTTCGGCAC	SDM
Tyr_SDM_F185Y (R)	GTGCCGAAGACTTCGAAGTTGGTTTCGGCAT	SDM
Tyr_SDM_D317Y (F)	CATGCCGCGCGCCTACAACAAGGTGGT	SDM
Tyr_SDM_D317Y (R)	ACCACCTTGTTGTAGGCGCGCGGCATG	SDM
Tyr_SDM_N322S(F)	ACAACAAGGTGGTGAGCAACGCCCGTGCCGA	SDM
Tyr_SDM_N322S(R)	TCGGCACGGGCGTTGCTCACCACCTTGTTGT	SDM
Tyr_SDM_N322K(F)	ACAACAAGGTGGTGAAAAACGCCCGTGCCGA	SDM
Tyr_SDM_N322K(R)	TCGGCACGGGCGTTTTTACCACCTTGTTGT	SDM
Tyr_SDM_N322D(F)	ACAACAAGGTGGTGACAACGCCCGTGCCGA	SDM
Tyr_SDM_N322D(R)	TCGGCACGGGCGTTGTCCACCACCTTGTTGT	SDM
Tyr_SDM_N322Q(F)	ACAACAAGGTGGTGAGAACGCCCGTGCCGA	SDM
Tyr_SDM_N322Q(R)	TCGGCACGGGCGTTCTGCACCACCTTGTTGT	SDM
Tyr_SDM_L330V (F)	GCCGAGCATCTGGTGCCCTGTTCAA	SDM
Tyr_SDM_L330V (R)	TTGAACAGGGCCACCAGATGCTCGGC	SDM
Tyr_SDM_T359M (F)	AACATCCGGTTGCCATGGCGGTGAAACCGCT	SDM
Tyr_SDM_T359M (F)	AGCGGTTCCACCGCCATGGCAACCGGATGTT	SDM

epPCR = error prone PCR, SDM=Site Directed Mutagenesis. Underlining indicates restriction nuclease recognition sites