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. *Biotechnology and Bioengineering* **2013**, *110* (7), 1849–1857.
https://doi.org/10.1002/bit.24859

Directed evolution
library of mutants

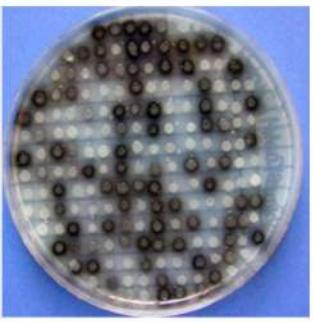
E. coli BL21(DE3) cells
transformed with library of mutants
in liquid LB medium

Transformants incubated on LB agar plates
with 50 µg/ml carbenicillin at 37°C for 18 h

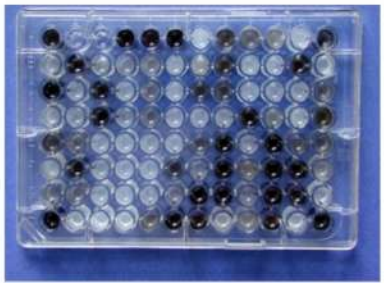
B) M9 agar plate
supplemented with 1mM
D-tyrosine and
50 µg/ml carbenicillin

Transformed colonies
simultaneously transferred
with a sterile toothpick
to a single well of a micrtotitre
plate (A) and onto an M9 agar
plate (B) supplemented with 1 mM
D-tyrosine and carbenicillin

A) Microtitre plate containing LB
broth and 50 µg/ml carbenicillin
incubated at 37°C shaking for 18 h



C) After 18 h incubation,
cells from microtitre plate (A)
transferred to M9 agar plate
supplemented with 1mM D-tyrosine and
50 µg/ml carbenicillin



D) Cells from (A) were transferred to another MT plate
and grown for 24 h in LB and 50 µg/ml carbenicillin.
Harvested by centrifugation (3000 x g), washed once in
50 mM phosphate buffer and resuspended in 50 mM
phosphate buffer. Biotransformation of 1 mM D-tyrosine in
50 mM phosphate buffer was carried out for 1-4 h.