

## Preparation and application of micro-crystalline tyrosine for high throughput screening of tyrosine phenol-lyase

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Rapid assay of enzyme is a primary requirement for successful application of directed evolution technology. Halo generation on a turbid plate would be very choiceable for high throughput screening of enzymes in that context. Here we report a new method to prepare turbid plates, by controlling the crystallization of tyrosine to form needle-like particles. In the presence of tyrosine phenol-lyase (TPL), the needle-like tyrosine crystals were converted to soluble phenol faster than the usual rectangular tyrosine crystals. When an error-prone PCR library of TPL was spread on the turbid plate, approximately 10% of the colonies displayed halo surrounding the colony after 36 hours of incubation at 37°C. Selected positives were moved to 96-wellplates and examined about the enzyme activity by a phenol-detection method. The determined activity was proportional in quantitative manner to the size of halo. In consequent two mutants of 50%-enhanced in the activity were obtained through the high throughput screening method.

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