A robust bacterial high-throughput screening assay to identify pharmacological chaperones targeting human homogentisate 1,2-dioxygenase missense variants in alkaptonuria

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Specific Research Group or Service

In Vitro Toxicology and Dermato-Cosmetology (IVTD)

Country Belgium

Geographical Area Brussels Region

Partners and collaborations

RWTH Aachen

SCOPE OF THE METHOD

The Method relates to	Human health

The Method is situated in	Translational - Applied Research
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

Homogentisate dioxygenase

Escherichia coli-based expression system

Maleylacetoacetate quantification

Assay validation

Primary screening platform

Enzyme activity assay

High-throughput screening

Variant ranking

Compound screening

Scientific area keywords

Tyrosine degradation pathway

Tyrosine inherited metabolic disorders

Orphan disease

Drug repurposing

Protein misfolding

Enzyme stabilization

Personalized medicine

Alkaptonuria

Missense variants

Pharmacological chaperones

Genotype-phenotype correlations

Method description

We developed a high-throughput screening (HTS) assay to identify small molecules that stabilize mutant homogentisate 1,2-dioxygenase (HGD), the enzyme deficient in alkaptonuria (AKU). The method uses Escherichia coli cells expressing human HGD variants and measures the conversion of homogentisic acid to maleylacetoacetic acid. Product formation is monitored over time by spectrophotometry, allowing quantitative assessment of enzyme activity. The assay was optimized for reproducibility and robustness, with a Z'-value greater than 0.4 and a signal window above 2, confirming its suitability for screening. Using this screening platform, we tested 2,320 FDA-approved drugs and identified 30 compounds that increased the activity of the common HGD-G161R variant by at least threefold. One compound showed a clear dose-dependent effect, doubling activity at 100 µM and 250 µM. Molecular docking suggested that this compound binds at multiple regions of the enzyme, stabilizing its structure before substrate and cofactor binding. This assay provides a reliable tool for assessing functional recovery of HGD variants and supports the identification of existing compounds with potential for drug repurposing and personalizd treatment of AKU.

Lab equipment

- Biosafety cabinet
- Microplate reader
- Incubator shaker
- Microplate incubator
- Automated liquid handling system (optional)

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Enables high-throughput, quantitative screening of HGD activity across multiple variants and compound simultaneously.
- Uses E. coli as an efficient, low-cost expression system, avoiding the need for complex eukaryotic expression.
- Simplified workflow without enzyme purification maintains throughput while retaining sufficient assay sensitivity.
- Demonstrates robust performance with Z' values > 0.4 and signal window > 2, confirming assay reliability.
- Supports drug repurposing efforts by identifying existing compounds that enhance HGD activity.
- Facilitates variant-specific functional assessment, contributing to personalized medicine approaches for AKU.
- Provides a scalable and adaptable platform that can be expanded to other protein-misfolding disorders.

Challenges

- Cannot model compound heterozygosity or hetero-oligomer formation of HGD variants.
- The use of E. coli limits physiological relevance, as bacterial cells lack the endoplasmic reticulum where chaperone action occurs in humans.
- The non-purifiifed enzyme preparation leads to higher apparent Km values and may reduce sensitivity to weak binders.
- Stabilizing effects observed from bacterial lysates require confirmation in human cell-based systems and further validation in *in vivo* models.
- Hit reproducibility may vary between primary and dose-response screens, requiring confirmation and optimization.

Modifications

The method could be further optimized in several ways. For example, adaptation to mammalian or human cell-based systems could provide a more physiologically relevant environment for assessing HGD stabilization. The use of partially purified enzyme preparations might enhance kinetic accuracy and enable clearere interpretation of compound effects. Additionally, further miniaturization to higher-density plate formats and increased automation could further improve throughput and reproducibility.

Future & Other applications

This method could serve as a primary screening platform for identifying stabilizing compounds in other inborn errors of metabolism (IEMs) caused by enzyme misfolding or instability. The overall workflow provides a useful basis for adaptation to other target enzymes, but would require further optimization and validation to account for differences in enzyme structure, cofactors, and assay conditions. it could also be used for variant functional studies to assess residual activity and support genotype-phenotype correlation analyses. In addition, this approach may contribute to drug repurposing and precision medicine research by enabling rapid identification of candidate compounds for follow-up in more physiologically relevant systems.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

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Links

Published method

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