Technology Commercialization Opportunity

AOH Select: Selection Marker for Plasmid Development

Technology Description:
Plasmid vectors are circular pieces of DNA that are used to facilitate the cloning of genetic material. The over-expression of two plant enzymes (**AOH1** and **AOH2*** from *Arabidopsis thaliana*) in *E. coli* leads to cell death due to the activity of the enzymes on a substrate that is necessary for the synthesis of two essential bacterial metabolites. However, repression of the enzymes in the bacteria leads to cell viability. To this end, we propose that this system can be further developed for use as a novel selection marker to facilitate the development of novel plasmids/vectors that can be used in industry or academia for cloning purposes.

This system is unique from current technologies in that all the cloning can be done in one *E. coli* strain rather than having to propagate the plasmid in a unique strain (DB3.1) that is resistant to the toxin produced by the ccdB gene.

AOH Select holds the promise to reduce production time because the cloning can be done in the same *E. coli* strain rather than having to propagate the selection plasmid in a different strain such as the ccdB system. It also is a tight system because both pathways are absolutely essential so there will be zero background leading to false positives.

Keywords: *E. coli*, cloning, plasmid vector development, essential gene, plant enzyme, selection marker

Technology Readiness:
AOH Select is currently in the conceptual phase. We have shown that this is a legitimate selection marker.

Idea	Concept	Prototype	Alpha Version	Beta Version	Released

Intellectual Property (IP):
Presently the IP is a trade secret of Rochester Institute of Technology.

Applications:
The development of a plasmid/vector using this technology can facilitate the cloning process using *E. coli.* The main idea of the technology is that interruption of AOH1 and or AOH2 expression facilitated by insertion of DNA recombinant means will lead to *E. coli* cell viability. However, if AOH1 and or AOH2 are uninterrupted, then the over-expression of the AOH enzymes will lead to cell death.

**AOH1/AOH2 are confidential names of enzymes that catalyze a reaction in plants*

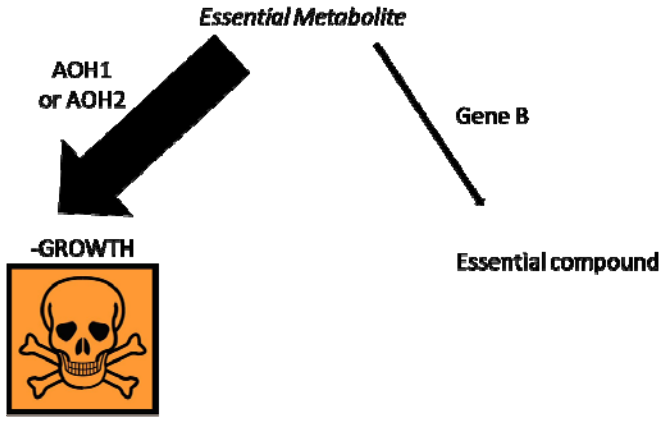

Figure 1. The schematic shows the conceptual idea that the over-expression of AOH1 and or AOH2 in *E. coli* causes the depletion of an essential metabolite for *E. coli* growth. This is due to the fact both AOH enzymes are competing with Gene B for the same substrate.

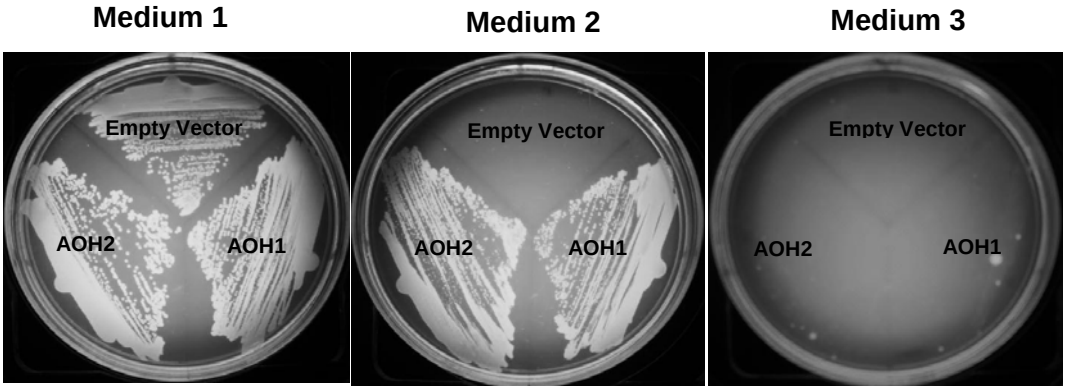

Figure 2 The first observation of this unique phenotype was from an *E. coli* mutant that was auxotrophic for a particular amino acid. The complementation screen was done by transforming empty vector along with the vector harboring the cDNAs from AOH1 and AOH2. The bacteria were plated on three different media. Media 1 contained the essential amino acid (therefore all three bacteria grew) Media 3 did not contain the essential amino acid but it was supplemented with a compound to induce AOH1 and AOH2 production. Media 2 lacked the essential amino acid but it was supplemented with a compound capable of repressing AOH1 and AOH2 production

Target Customers:

- Biotechnology companies
- Academic institutions

Opportunity:

RIT's Intellectual Property Management Office (IPMO) is interested in working with those parties who are qualified and interested in the commercialization of this AOH Select intellectual property by creating a plasmid with a cassette that includes a inducible promoter fused to AOH1 or AOH 2 which is a poly-linker to facilitate cloning. Arrangement types include licensing the application to existing organizations or new organizations that have expertise in the field or related fields. The inventor of the AOH Select technology is available to work with licensees, to finalize the development of plasmid vectors to AOH1 or AOH2 which is a poly-linker to facilitate cloning.

Contact:

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