GFP-Amplification Mutagenesis Assay (GMA): Quantitative, Scalable Detection of Chemical Mutagenesis

Tech ID: 22023 / UC Case 2010-252-0

BACKGROUND

Genotoxic (DNA damage-inducing) chemicals often increase the risk of cancer. Genotoxicity testing is mandatory for approval of new drugs as an indicator of potential carcinogenicity and is therefore generally undesired. The Ames test is the most widely-used genotoxicity assay and is required by regulatory agencies for the registration and approval of new chemicals. This test uses Salmonella and E. coli as model organisms because of the exquisite conservation of DNA damage and repair mechanisms across kingdoms. The Ames Test is a mutation reversion test; the assay can be performed on solid plates (Ames plate incorporation test) or in a liquid version (Ames fluctuation assay). The primary difficulty is the large amount of compound required, and even the miniaturized versions of Ames (solid, mini-Ames), requires >10mg of test compound. Thus, the use of this assay to detect mutagenic activity during early stages of drug discovery (when typically, little amount of compound is available), or in complex mixtures of compounds (where the active compound may be a small fraction of the total), is limited. The high sample amount also limits the ability for biomonitoring of environmental mutagens in typical environmental mixtures.

UCSC researchers have developed technology based on the same principles as the Ames test, but is far more sensitive and provides quantitative information. The inventors have developed a novel method for the detection of chemical mutagenesis that requires only a fraction of the sample used in Ames, at minimum 100-fold less. The information provided in a 96-well plate for the liquid Ames test could be obtained in one well, dramatically reducing the amount of test sample required, and the method can be used for both drug discovery and environmental biomonitoring. Also, it may be used to screen high-yield natural products libraries for compounds targeting DNA as potential antimicrobial, anti-inflammatory or antitumor agents.

TECHNOLOGY DESCRIPTION

UCSC's invention embodies a technique for the quantitative detection of mutations that incorporates elements of the liquid Ames tests. However, the invention features a quantitative readout. This readout is made possible by two amplification steps, one occurring at the level of individual cells and the other occurring at the level of culture. Combined, these two amplification steps magnify a fluorescent signal originating in a very rare event. The final signal integrates a multiplicity of single, random events occurring in liquid culture. Using a panel of error-prone polymerases demonstrates that fluorescence correlates with mutation error and that the assay can detect mutation frequencies as low as 1 in 10^{-7}. Easily adaptable to a microplate format, this assay significantly saves on sample,

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OTHER INFORMATION

KEYWORDS

Ames test, early detection, high throughput, quality control, screening, carcinogenesis, genotoxicity, directed evolution, drug development, drug discovery, antitumor, antimicrobial, mutagenesis reversion assay, mutagenesis, point mutations, frameshifts, Cat2

CATEGORIZED AS

- **Biotechnology**
  - Bioinformatics
  - Genomics
  - Other
- **Research Tools**
  - Other
  - Screening Assays

RELATED CASES

2010-252-0

reagents and labor.

APPLICATIONS

Because of the savings in test sample and the ease with which this assay can be adapted to a 96-well format, this proprietary mutagenesis assay is ideally suited for three types of applications:

- **High-throughput screening of mutagens:**

Examples of classes of mutagens that can be screened for include:

1. Identification of DNA-targeting compounds from chemical or natural product libraries as potential antimicrobial or antitumor agents

2. Identification of compounds that decrease fidelity of replication as potential antiviral and antitumor agents

3. Identification of mutagenic nucleotide analogs for the generation of random mutant libraries for protein engineering purposes

- **Early detection of carcinogenic potential:**

Remove agents from the drug development pipeline early on, representing a significant cost-savings opportunity

- **Quality control for random mutant libraries:**

Reporter can be used to monitor the quality of random mutant libraries to ensure adequate genetic diversity

ADVANTAGES

- Preserves the exquisite specificity of the Ames test
- Quantitative readout dramatically decreases the amount of sample and of reagents necessary to carry out the test
- Significant cost-savings for compounds in early stages of development

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	9,068,972	06/30/2015	2010-252
Patent Cooperation Treaty	Published Application	WO 2011/053944	05/05/2011	2010-252

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- System For Continuous Mutagenesis In Vivo To Facilitate Directed Evolution

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