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**Cambareri et al.**

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(54) **METHODS FOR IN VIVO DIVERSIFICATION OF SINGLE GENES**

FOREIGN PATENT DOCUMENTS

WO WO 99/27072 6/1999

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(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 593 days.

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(57) **ABSTRACT**

**Related U.S. Application Data**

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Methods and compositions are provided for the efficient in vivo diversification of gene-products in filamentous fungi, starting from (but not limited to) two or more copies of a single gene constituent. The diversification involve use of the Repeat-Induced Point mutation (RIP) process in *N. crassa*, and other fungi that have analogous mutational processes. The invention takes advantage of the induction of G:C to A:T transition mutations specific to duplicated DNA sequences during the premeiotic dikaryon phase of the life cycle of the fungus. The methods and compositions of the invention can be utilized to generate diversity in target genes, and are proposed for the purpose of altering and generating novel forms and activities of gene-products thus encoded. Duplicated genes may be introduced into the organism and are present either in tandem, or at separate ectopic locations within the genome of the fungus. After crossing the resulting transformant(s), sexual progeny can then be selected which contain the mutated gene, and subsequently screened for a desired product.

(51) **Int. Cl.**  
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(52) **U.S. Cl.** ..... 435/6; 435/7.2; 435/7.31; 435/252.3; 536/23.1

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

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**6 Claims, 3 Drawing Sheets**