



US006268140B1

(12) **United States Patent**
Stuart

(10) **Patent No.:** **US 6,268,140 B1**
(45) **Date of Patent:** **Jul. 31, 2001**

- (54) **COMBINATORIAL METABOLIC LIBRARIES**
- (75) **Inventor:** **W. Dorsey Stuart**, San Francisco, CA (US)
- (73) **Assignee:** **Neugenesis**, San Carlos, CA (US)
- (*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.
- (21) **Appl. No.:** **09/310,398**
- (22) **Filed:** **May 12, 1999**

Related U.S. Application Data

- (63) Continuation-in-part of application No. 09/214,699, filed on Jan. 8, 1999, which is a continuation-in-part of application No. 08/678,462, filed on Jul. 9, 1996, now Pat. No. 5,683,899, which is a continuation-in-part of application No. 08/191,337, filed on Feb. 3, 1994, now Pat. No. 5,643,745.

- (51) **Int. Cl.**⁷ **C12Q 1/68**; C12N 1/15
- (52) **U.S. Cl.** **435/6**; 435/254.11; 435/254.21; 435/254.3; 435/254.4; 435/254.5; 435/320.1; 435/440; 435/171
- (58) **Field of Search** 435/6, 254.11, 435/254.21, 254.3, 254.4, 254.5, 320.1, 440, 171

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,486,533	12/1984	Lambowitz .	
4,816,405	3/1989	Yelton et al. .	
4,885,249	12/1989	Buxton et al. .	
4,935,340	* 6/1990	Baltz et al.	435/6
4,935,349	6/1990	McKnight et al. .	
5,643,763	* 7/1997	Dunn et al.	435/91.1
5,712,146	1/1998	Khosla et al. .	

FOREIGN PATENT DOCUMENTS

0409156	1/1991	(EP) .
0812914	12/1997	(EP) .
WO 95/21263	8/1995	(WO) .
WO 96/34112	10/1996	(WO) .
WO 96/40968	12/1996	(WO) .
WO 97/35966	10/1997	(WO) .
WO 98/01534	1/1998	(WO) .

OTHER PUBLICATIONS

Arnaise, S. et al., Heterologous Expression Of Mating-Type Genes In Filamentous Fungi, Proc. Natl. Acad. Sci. USA (1993) 90:6616-6620.

Bartel, P. L. et al., Biosynthesis Of Anthraquinones By Interspecies Cloning Of Actinorhodin Biosynthesis Genes In Streptomyces: Clarification Of Actinorhodin Gene Functions, J. Bacteriol. (1990) 172 (9):4816-4826.

Buczynski, S. et al., Expression And Secretion Of Human Antibody Light And Heavy Chains In Neurospora Crassa, Fungal Genetics Newsletter-Abstracts From the 18th Fungal Genetics Conference (1995 Suppl.) 42A:83.

Carattoli, A. et al., A Chimeric Light-Regulated Amino Acid Transport System Allows The Isolation Of Blue Light Regulator (BLR) Mutants Of Neurospora Crassa, Proc. Nat. Acad. Sci. USA (1995) 92:6612-6616.

Carú, M. et al., Molecular Cloning And Expression In Saccharomyces Cerevisiae And Neurospora Crassa Of The Invertase Gene From Neurospora Crassa, J. Appl. Bacteriol. (1989) 67:401-410.

Connerton, I.F. et al., Comparison and Cross-Species Expression Of The Acetyl-CoA Synthetase Genes Of The Ascomycete Fungi, Aspergillus Nidulans and Neurospora Crassa, Molecular Microbiology (1990) 4(3):451-460.

Dalbey, R. E. et al., Signal Peptidases In Prokaryotes and Eukaryotes—A New Protease Family, TIBS (1992) 17:474-478.

Davis, R. H. et al., Genetic And Microbiological Research Techniques For Neurospora Crassa, Methods in Enzymology (1970) 17A:79-143.

Haas, F. et al., A Series Of Histidineless Mutants Of Neurospora Crassa, Genetics (1952) 37:217-26.

Hiatt, K.L. et al., Induced Expression Of The Aspergillus Nidulans QUTE Gene Introduced By Transformation Into Neurospora Crassa, Mol. Gen. Genet. (1990) 222:201-205.

Hopwood, D.A. et al., Production Of 'Hybrid' Antibiotics By Genetic Engineering, Nature (1985) 314(18):642-644.

Khosla, C. et al., Genetic Construction And Functional Analysis Of Hybrid Polyketide Synthases Containing Heterologous Acyl Carrier Proteins, J. Bacteriol. (1993) 175(8):2197-2204.

Koo, K. et al., Sequence And Structure Of mtr, An Amino Acid Transport Gene Of Neurospora Crassa, Genome (1991) 34:644-651.

Lambowitz, A. M. et al., Mitochondrial Ribosome Assembly In Neurospora, J. Cell Biology (1979) 82:17-31.

MacKenzie, D.A. et al., Regulation Of Secreted Protein Production By Filamentous Fungi: Recent Developments And Perspectives, J. Gen. Microbiol. (1993) 139:2295-2307.

Malpartida, F. et al., Molecular Cloning Of The Whole Biosynthetic Pathway Of A Streptomyces Antibiotic And Its Expression In A Heterologous Host, Nature (1984) 309(31):462-464.

Nakano, E. T. et al., Expression Vectors For Neurospora Crassa And Expression Of A Bovine Preprochymosin cDNA, Fungal Genetics Newsletter (1993) 40:54-56.

(List continued on next page.)

Primary Examiner—James Ketter
(74) *Attorney, Agent, or Firm*—Morrison & Foerster LLP

(57) **ABSTRACT**

The present invention provides methods and compositions for producing and screening combinatorial metabolic libraries of multimeric proteins. The present invention relies on the use of filamentous fungal heterokaryons that are produced using two or more parent strains into which a population of heterologous nucleic acids encoding components of a metabolic pathway have been introduced.

22 Claims, No Drawings