



US005580726A

United States Patent [19]

[11] **Patent Number:** **5,580,726**

Villeponteau et al.

[45] **Date of Patent:** **Dec. 3, 1996**

[54] **METHOD AND KIT FOR ENHANCED DIFFERENTIAL DISPLAY**

[75] Inventors: **Bryant Villeponteau; Junli Feng**, both of San Carlos; **Walter Funk**, Union City; **Maarten H. K. Linskens**, Palo Alto, all of Calif.

[73] Assignee: **Geron Corporation**, Menlo Park, Calif.

[21] Appl. No.: **235,180**

[22] Filed: **Apr. 29, 1994**

[51] Int. Cl.⁶ **C12Q 1/68; C12P 19/34**

[52] U.S. Cl. **435/6; 435/91.2**

[58] Field of Search **435/6, 92.1; 935/77, 935/78**

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,066,584	11/1991	Gyllensten et al.	435/91.2
5,262,311	11/1993	Pardee et al.	435/91.2
5,489,508	2/1996	West et al.	435/6

FOREIGN PATENT DOCUMENTS

9318176 9/1993 WIPO .

OTHER PUBLICATIONS

Ito et al., "Fluorescent differential display: arbitrarily primed RT-PCR fingerprinting on an automated DNA sequencers", *FEBS Letters* 351:231-236 (1994).

Joseph et al., "Molecular Cloning of a Novel mRNA (Neuronatin) That Is Highly Expressed in Neonatal Mammalian Brain", *Biochem. Biophys. Res. Comm.* 201:1227-1234 (1994).

Kumar et al., "Expression of interleukin 1-inducible genes and production of interleukin 1 by aging human fibroblasts", *Proc. Nat'l Acad. Sci.* 89:4683-4687 (1992).

Linskens et al., "Cataloging altered gene expression in young and senescent cells using enhanced differential display", *Nucleic Acid Research* 23:3244-3251 (1995).

Mou et al., "Improvements to the Differential Display Method For Gene Analysis", *Biochem. Biophys. Res. Comm.* 199:564-569 (1994).

West et al., "Replicative Senescence of Human Skin Fibroblasts Correlates with a Loss of Regulation and Overexpression of Collagenase Activity", *Experimental Cell Research* 184:138-147 (1989).

Zimmerman and Schultz, "Analysis of gene expression in the preimplantation mouse embryo: Use of mRNA differential display", *Proc. Nat'l Acad. Sci.* 91:5456-5460 (1994).

Liang, et al., "Distribution and cloning of eukaryotic mRNAs by means of differential display: refinements and optimization", *21 Nucl. Acids Res.* 3269, 1993.

Torres et al *Enferm. Infecc. Microbiol. Clin.* 10:345-8 (1992).

PNAS 85:8998-9002 (1988).

Clontech Catalog (1993)-Quick -Clone p. 38.

Primary Examiner-W. Gary Jones

Assistant Examiner-Eggerton Campbell

Attorney, Agent, or Firm-Kevin Kaster; Richard J. Warburg; Amy S. Hellenkamp

[57] **ABSTRACT**

An improved method for detecting and isolating differentially expressed mRNAs which comprises using first oligonucleotide primers for reverse transcription of mRNAs and both the first oligonucleotide primers and second oligonucleotide primers for amplification of the resultant cDNAs. The improvement of this method comprises providing first and second oligonucleotide primers with a length of at least 21 oligonucleotides. The method further comprises using a two-step PCR amplification, wherein non-stringent conditions are used for the first 1 to 4 cycles, and stringent conditions are used for the next 16 to 22 cycles. This highly reproducible method will permit the preparation of comprehensive catalogs of gene expression for any given cell type.

14 Claims, 4 Drawing Sheets