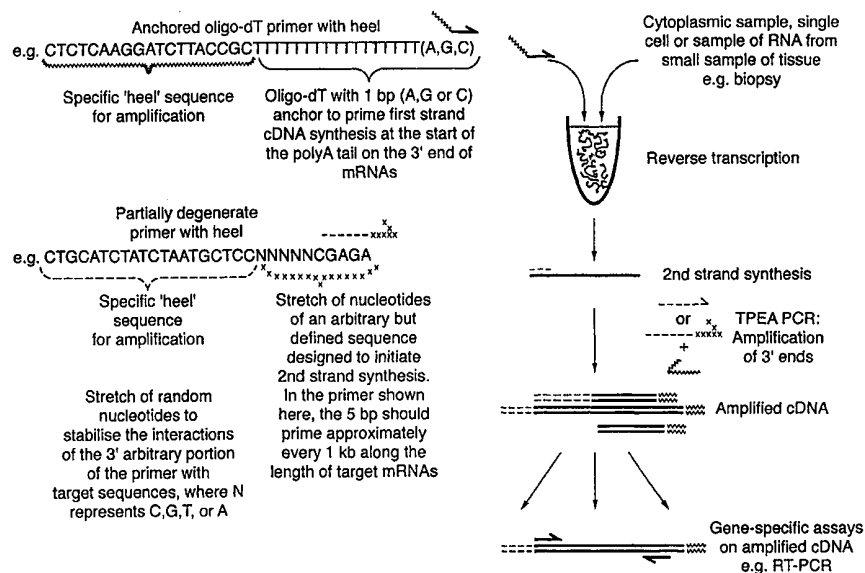




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b>  <b>C12Q 1/68</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/08208</b>  <b>(43) International Publication Date:</b> 17 February 2000 (17.02.00)
<b>(21) International Application Number:</b> PCT/GB99/02579  <b>(22) International Filing Date:</b> 5 August 1999 (05.08.99)  <b>(30) Priority Data:</b> 9817055.8      5 August 1998 (05.08.98)      GB  <b>(71) Applicant (for all designated States except US):</b> MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1 4AL (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> FREEMAN, Thomas, Charles [GB/GB]; 80 Whitehill Road, Cambridge CB5 8LX (GB). RICHARDSON, Peter, John [GB/GB]; Hillcrest Cottage, Bartlow Road, Hadstock CB1 6PF (GB). DIXON, Alistair, K. [GB/GB]; 108 Gwydir Street, Cambridge CB1 2LL (GB).  <b>(74) Agent:</b> HEPWORTH LAWRENCE BRYER & BIZLEY; Merlin House, Falconry Court, Bakers Lane, Epping, Essex CM16 5DQ (GB).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 11 May 2000 (11.05.00)

**(54) Title:** REVERSE TRANSCRIPTION AND AMPLIFICATION PROCESSES AND PRIMERS THEREFOR**(57) Abstract**

A method is provided for the expression profiling of single cells. The method employs a first heeled primer for reverse transcription of mRNA in a sample to provide first strand cDNA species, and then a second heeled primer population to generate second strand cDNAs. The non-heeled portion of the second heeled primers are capable of hybridizing to the reverse transcribed first strands of cDNA species, at least one along the lengths thereof. Due to the presence of random and preselected sequences in the second primers a qualitatively more uniform and therefore representative cDNA profile is produced from cellular mRNAs.

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# INTERNATIONAL SEARCH REPORT

In: International Application No

PCT/GB 99/02579

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHAO ET AL.: "3'-END cDNA POOL SUITABLE FOR DIFFERENTIAL DISPLAY FROM A SMALL NUMBER OF CELLS" BIOTECHNIQUES, vol. 24, May 1998 (1998-05), pages 842-852, XP002128314 the whole document	1-48
Y	WO 98 08973 A (HAMPSON LYNNE ;CANCER RES CAMPAIGN TECH (GB); HAMPSON IAN NOEL (GB) 5 March 1998 (1998-03-05) the whole document	1-48

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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# INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>FROUSSARD: "A RANDOM-PCR METHOD (rPCR) TO CONSTRUCT WHOLE cDNA LIBRARY FROM LOW AMOUNTS OF RNA"</p> <p>NUCLEIC ACIDS RESEARCH, vol. 20, no. 11, 1992, page 2900 XP002128315 the whole document</p> <p style="text-align: center;">---</p>	1-48
Y	<p>ZHAO ET AL.: "NEW PRIMER STRATEGY IMPROVES PRECISION OF DIFFERENTIAL DISPLAY"</p> <p>BIOTECHNIQUES, vol. 18, no. 5, 1995, pages 842-850, XP002128267 the whole document</p> <p style="text-align: center;">---</p>	1-48
Y	<p>CHEUNG AND NELSON: "WHOLE GENOME AMPLIFICATION USING A DEGENERATE OLIGONUCLEOTIDE PRIMER ALLOWS HUNDREDS OF GENOTYPES TO BE PERFORMED ON LESS THAN ONE NANOGRAM OF GENOMIC DNA"</p> <p>PNAS, vol. 93, 1996, pages 14676-14679, XP002128316 the whole document</p> <p style="text-align: center;">---</p>	1-48
Y	<p>TELENIUS H ET AL: "DEGENERATE OLIGONUCLEOTIDE-PRIMED PCR: GENERAL AMPLIFICATION OF TARGET DNA BY A SINGLE DEGENERATE PRIMER"</p> <p>GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 13, 1992, page 718-725 XP000199116 ISSN: 0888-7543 the whole document</p> <p style="text-align: center;">---</p>	1-48
A	<p>WO 91 15601 A (US ARMY) 17 October 1991 (1991-10-17) the whole document</p> <p style="text-align: center;">---</p>	
P,X	<p>DIXON ET AL.: "EXPRESSION PROFILING OF SINGLE CELLS USING 3 PRIME END AMPLIFICATION (TPEA) PCR"</p> <p>NUCLEIC ACIDS RESEARCH, vol. 26, no. 19, October 1998 (1998-10), pages 4426-4431, XP002128317 the whole document</p> <p style="text-align: center;">-----</p>	1-48

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/02579

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WO 9808973 A	05-03-1998	CA 2233228 A EP 0864001 A	05-03-1998 16-09-1998
WO 9115601 A	17-10-1991	NONE	