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(54) Titre : UTILISATION D'ARNM LOCALISES DANS LES NEURITES EN VUE DE PROCEDES MEDICAUX
THERAPEUTIQUES ET DIAGNOSTIQUES
(54) Title: USE OF NEURITE LOCALIZED mRNAs FOR MEDICAL DIAGNOSIS AND THERAPEUTICS

(57) **Abrégé/Abstract:**

A method of identifying neurite cDNA clones by determining and comparing mRNA expression in selected neurites is provided. cDNA clones identified by this method are also provided. In addition, methods of profiling mRNA expression and diagnosing and treating conditions associated with a pattern of mRNA expression by determining an mRNA expression profile are provided. The figure illustrates a differential display pattern obtained in neurites using the oligonucleotide OP-5 as the 3' primer.



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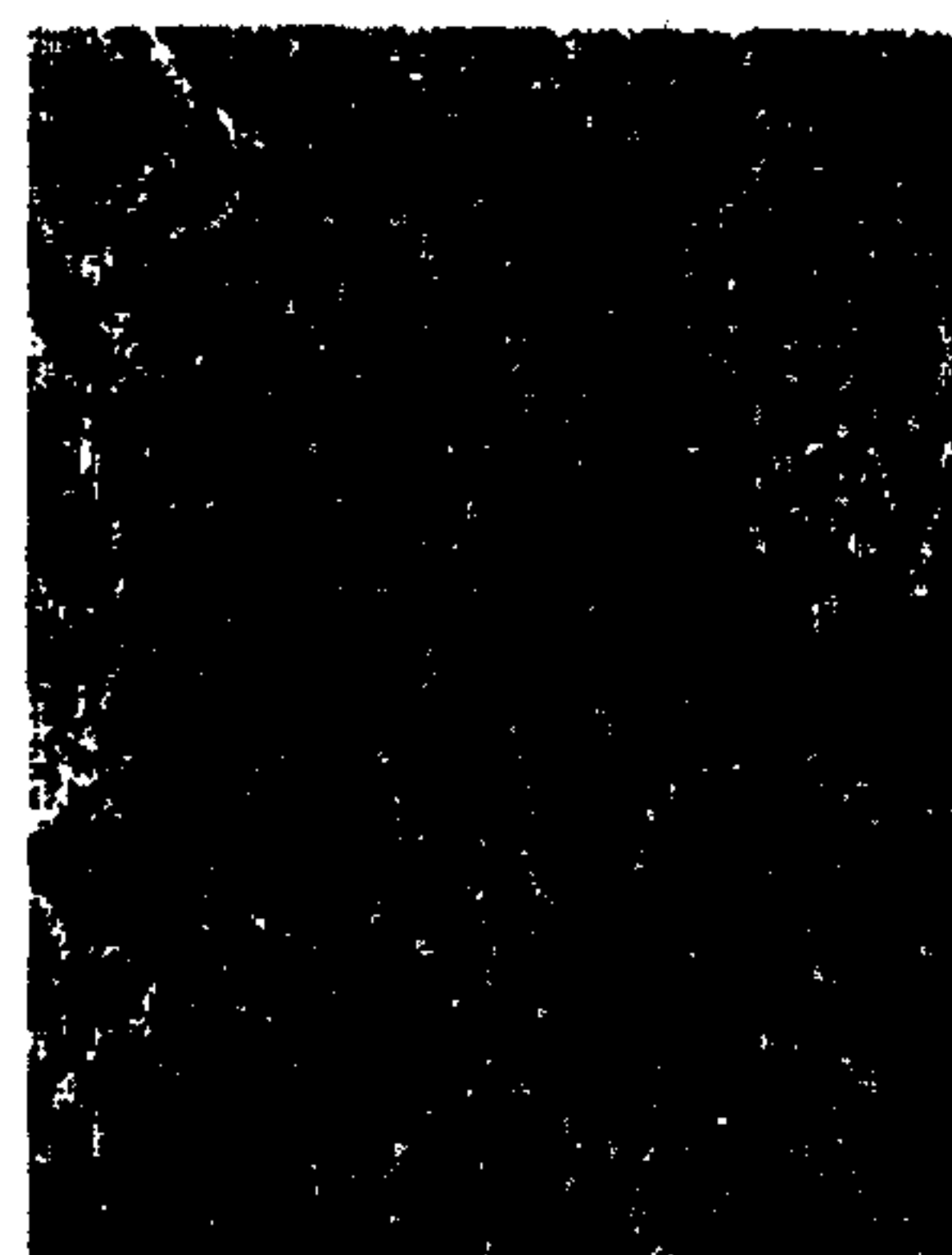
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(21) International Application Number: PCT/US95/14792 (22) International Filing Date: 3 November 1995 (03.11.95) (30) Priority Data: 08/334,254 3 November 1994 (03.11.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/334,254 (CON) Filed on 3 November 1994 (03.11.94) (71) Applicant (for all designated States except US): THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; Center for Technology Transfer, Suite 300, 3700 Market Street, Philadelphia, PA 19104-3147 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): EBERWINE, James [US/US]; 3918 Henry Street, Philadelphia, PA 19129 (US). DICHTER, Marc [US/US]; 311 Myrtle Lane, Penn Valley, PA 19072 (US). MIYASHIRO, Kevin [US/US]; Apartment 215, 338 Lombard Street, Philadelphia, PA 19147 (US).		(74) Agents: LICATA, Jane, Massey et al.; Law Offices of Jane Massey Licata, Woodland Falls Corporate Park, Suite 201, 210 Lake Drive East, Cherry Hill, NJ 08002 (US). (81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: USE OF NEURITE LOCALIZED mRNAs FOR MEDICAL DIAGNOSIS AND THERAPEUTICS**(57) Abstract**

A method of identifying neurite cDNA clones by determining and comparing mRNA expression in selected neurites is provided. cDNA clones identified by this method are also provided. In addition, methods of profiling mRNA expression and diagnosing and treating conditions associated with a pattern of mRNA expression by determining an mRNA expression profile are provided. The figure illustrates a differential display pattern obtained in neurites using the oligonucleotide OP-5 as the 3' primer.

5' PRIMER	OPA-5		
	A	B	C
3' PRIMER	799	799	799
CELL	999	999	999



250 bp

100 bp

LANE 123 456 789

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**USE OF NEURITE LOCALIZED mRNAs FOR
MEDICAL DIAGNOSIS AND THERAPEUTICS**

Introduction

This invention was made in the course of research
5 sponsored by the National Institutes of Health. The U.S.
Government may have certain rights in this invention.

Background of the Invention

In recent years there has been great progress in the
understanding of the genetic basis of neuropsychiatric
10 disorders including those associated with mental retardation.
While the genetic defects of such disorders are disparate and
each mechanism appears to be novel, the unifying theme behind
the development of these diseases is brain development and
maintenance of neurological networks.

15 Neurological networks are made up of individual neurons,
each neuron being a separate structural and functional cellular
unit. Neurons have special features for the reception of nerve
impulses from other neurons, the effect of which may be either
excitation or inhibition, and conduction of nerve impulses.
20 Neurons commonly have long cytoplasmic processes known as
neurites which end in close apposition to the surfaces of other
cells. The ends of these neurites are called synaptic
terminals and the cell-to-cell contacts they make are known as
synapses. The neurites in higher animals are usually
25 specialized to form dendrites and axons which conduct impulses
toward and away from the cell body, respectively. The arrival
of an impulse at a terminal triggers the process of synaptic

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transmission. This event usually involves the release of a chemical compound from the neuronal cytoplasm invoking a response in the postsynaptic cell. Neurons of the central nervous system consist of discrete segments including the cell
5 body, the dendrites and the axon. While most nerve cells conform to this basic structure, there is a wide range of structural diversity based upon the specific function of the cell within the body.

It has been shown that these polarized cells contain a
10 variety of cytoplasmic and membrane-bound proteins differentially distributed throughout the axon, dendrites, and cell body of the neuron. It is believed that neurons of the central nervous synthesize proteins locally, at or near postsynaptic sites which are independent of the cell body.
15 Ultrastructural studies have revealed that polyribosomes are preferentially located either beneath post-synaptic sites or occasionally associated with membrane specializations on dendrites. It has been suggested that these anatomical structures represent the protein synthetic machinery necessary
20 to translate and post-translationally modify different classes of protein in neurons. An energy-dependent mechanism for the selective transport of RNA in neurons has also been shown. The nature and distribution of the RNAs present in these cells, however, is poorly understood.

25 *In situ* hybridization (ISH) studies have been successful in identifying very few mRNAs in neuronal processes. Studies using *in situ* hybridization and Northern blot analyses of synaptosomal RNA fractions with the AMPA-GluR1, -GluR2, GluR3 and GluR-4 and kainate-sensitive GluR5 and GluR6 receptor
30 subunits failed to reveal mRNAs at dendritic locations. (Craig, A. et al., *Neuron* 1993, 10, 1055-1068; Chicurel, M. et al. *J. Neurosci* 1993, 13, 4054-4063).

Microdissection of individual neurites has now revealed a large number of mRNAs, including members of the glutamate
35 receptor family, second messenger components, and components of the translational control apparatus, present in hippocampal neurites. It has now been found that the profiles of expressed

mRNAs from discrete segments of the same neurons have different characteristics. These differences in expressed mRNA can be used as a means to specifically target discrete segments of the neuron and to identify and diagnose genetic neurological disorders at the molecular level.

Summary of the Invention

The invention seeks to provide a method of identifying neurite cDNA clones which comprises determining the mRNA expression in selected neurites, comparing the relative levels of mRNA expression, and identifying neurite cDNA clones based on the level of mRNA expression.

The invention also seeks to provide neurite cDNA clones.

The invention also provides a method for determining neuron types comprising:

- (a) determining mRNA expression in selected neurons;
- (b) comparing levels of mRNA expression in the selected neurons with levels in known neurons; and
- (c) identifying a neuron type based on the level of mRNA expression.

The present invention further seeks to provide a method of profiling mRNA expression in a selected neurite which comprises converting an mRNA population in a soma or process of a selected neurite into cDNA, making the cDNA double stranded, linearly amplifying the double stranded cDNA into aRNA, and using the aRNA as a probe in reverse phase Northern analysis to produce an mRNA expression profile.

The present invention additionally seeks to provide a method of diagnosing a condition associated with an mRNA expression pattern which comprises determining the relative levels of mRNA expression in selected cells associated with a selected condition, comparing the relative levels

determined with established controls, and diagnosing a condition based upon the comparison of the mRNA expression levels.

The present invention also seeks to provide a method of treating a condition associated with an mRNA expression pattern which comprises determining the relative levels of mRNA expression in selected cells associated with a selected condition, and altering the relative levels with an effective amount of an agent capable of altering mRNA expression.

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Brief Description of the Figures

Figure 1 is a differential display of three representative cells. A single oligonucleotide, OPA-5, served as the 3'-primer. In combination with three different modified
5 oligo-dT₁₁ primers (oligo A, 5'-T₁₁AC-3'; oligo B, 5'-T₁₁CA-3'; oligo C, 5'-T₁₁GC-3'), differential display reactions were carried out on separate neurites (HP3-7 and HP3-8) (panel a shown in Figure 1A), proximal (HP2-10) and distal (HP2-12) segments of the same process as well as another separate
10 process (HP2-11) (panel b shown in Figure 1B), and distal branch points (HP3-5 and HP3-6) of the same process (panel c shown in Figure 1C) from single hippocampal cells. Each of these neurites were isolated as described in Example 1. In panels a and b (Figures 1A and 1B, the corresponding soma (HP
15 3-9 in panel a; and HP2-13 in panel b) were isolated. In addition to the large number of mRNAs expressed in some neurites (for example, lane 5 in panel a shown in Figure 1A), there are some commonly shared PCR products between neurites or neurite segments (closed arrowheads) and between neurites and
20 their cell bodies (open arrowheads). These data were reproduced a minimum of three times. In each panel, a phase-contrast photomicrograph of the cell and the neurites of interest prior to isolation are shown. Dark bars perpendicular to each process represent the approximate transection point.

25 Detailed Description of the Invention

Different regions of the central nervous system are populated by functionally and anatomically distinct synaptic connections. During synapse construction, maintenance and remodeling, it is believed that proteins are selectively
30 transported to these distinct connections. As a result, the specificity of the nervous system is established and modified, at least in part, by these protein targeting mechanisms. It has now been found that mRNA expression profiles are specific for messages seen in neurites. A method has now been developed
35 for identifying neurite cDNA clones by determining the mRNA expression in selected neurites, comparing the relative levels

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of mRNA expression, and identifying neurite cDNA clones based on the level of mRNA expression.

Isolated hippocampal cells free of overlapping processes from neighboring cells were identified in low density cultures. Under these conditions, neurons grow as isolated cells or in small 2-4 cell groups, either directly on the substrate or on glial cells. Neurons are identified by morphological criteria involving synaptic interactions. Individual proximal and distal neurites were harvested by transecting them at varying distances from the cell body and aspirating them into a micropipette containing the reagents necessary for the first step in the antisense RNA (aRNA) amplification procedure. In a number of cases, multiple processes were isolated from a single cell followed by the aspiration of the cell body. Individual neurites or cell bodies were processed through the following aRNA amplification procedure to produce an mRNA expression profile. The mRNA population in the cell soma or cell process was converted into a complementary DNA (cDNA) using an oligo-dT-T7 primer. After the cDNA was made double-stranded, it was linearly amplified into aRNA using T7 RNA polymerase. For reverse Northern analysis, aRNAs served as a probe for mRNA expression profiles. In subsequent experiments, aRNAs were made into double-stranded cDNAs and used as templates for experiments using the polymerase chain reaction (PCR).

The population of the mRNAs in neurites was assessed initially by mRNA expression profiling. Southern blots containing cloned cDNAs encoding members of the ionotropic glutamate receptor family were probed with radiolabeled aRNA from individual neurites or cell bodies. Glutamate receptors, classified into N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA; GluR1-4), and kainate (GluR5-7) subtypes are the primary mediators of excitatory synaptic transmission in the brain. These receptors also play a role in the biochemical events associated with excitotoxicity and long-term potentiation (LTP), a specialized form of synaptic plasticity. The qualitative mRNA expression of

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multiple members of the glutamate receptor family was examined. All neurites expressed GluR1, GluR2, GluR4 and NMDAR1 mRNA. In a majority of neurites, mRNA expression of GluR3 (15/19), GluR5 (14/19), was observed; while expression for GluR6 mRNA was
5 detectable in approximately one half the neurites studied. The presence of these subunits was confirmed by subunit specific PCR. In contrast, only one neurite showed detectable hybridization signals for NR2a and NR2c mRNA. Thus, neurite cDNA clones may be identified by determining the mRNA
10 expression for glutamate receptors. Examples of preferred glutamate receptors include, but are not limited to, GluR1, GluR2, GluR3, GluR4, GluR5, GluR6, GluR7, NR2a and NR2b.

A number of neurite cDNA clones have now been identified in accordance with the methods of the present invention. Two
15 of these clones have been found to correspond to farnesyl diphosphate (FPP) synthase mRNA identified in two separate distal processes. As part of the isoprene biosynthetic pathway, FPP synthase generates the farnesyl moiety ultimately transferred to the COOH-terminal CaaX motif of mammalian ras
20 proteins. Two other cDNAs have been found to have sequence similarity with mRNAs for the γ -subunit of the interleukin-2 receptor and the tumor necrosis factor inducible protein A20. The remaining cDNA clones have little sequence similarity with any published gene sequences. By sequencing the cDNA clones
25 corresponding to full-length RNAs, it is possible to identify the role the primary sequence and secondary structural characteristics of the mRNA play as recognition elements in targeting and transport of the mRNA. The cDNA clones of the present invention are identified in Table 1 as SEQ ID NO: 1
30 through SEQ ID NO: 28.

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TABLE 1

CLONE	SEQ ID NO:
57-3	SEQ ID NO: 1
59-3	SEQ ID NO: 2
60-3	SEQ ID NO: 3
63-3	SEQ ID NO: 4
64-3	SEQ ID NO: 5
65-3	SEQ ID NO: 6
66-3	SEQ ID NO: 7
67-3	SEQ ID NO: 8
68-3	SEQ ID NO: 9
70-3	SEQ ID NO: 10
71-3	SEQ ID NO: 11
72-3	SEQ ID NO: 12
73-3	SEQ ID NO: 13
76-3	SEQ ID NO: 14
78-3	SEQ ID NO: 15
60-7	SEQ ID NO: 16
63-7	SEQ ID NO: 17
64-7	SEQ ID NO: 18
66-7	SEQ ID NO: 19
67-7	SEQ ID NO: 20
68-7	SEQ ID NO: 21
69-7	SEQ ID NO: 22
70-7	SEQ ID NO: 23
71-7	SEQ ID NO: 24
72-7	SEQ ID NO: 25
74-7	SEQ ID NO: 26
78-7	SEQ ID NO: 27
100-7	SEQ ID NO: 28

The cDNA clones of the present invention can be used in the diagnosis of neuropsychiatric diseases. Human genes

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containing unstable triplet repeats are associated with several neuropsychiatric diseases including, but not limited to, Huntington's disease, spinal and bulbar muscular atrophy and spinocerebellar ataxia type 1. These diseases show a variety of clinical symptoms making them difficult to diagnose. However, an understanding of these diseases at the molecular level provides the diagnostic laboratory with the capability to test directly whether an individual's DNA contains the disease-causing mutation, either as a confirmation of a clinical diagnosis or prior to any symptoms. For example, fragile X chromosomes, associated with mental retardation in most males that have it and, to a lesser degree, some of the females heterozygous for it, can be identified using cDNA clones of the present invention having SEQ ID NO: 1 through 15. The cDNA clones of the present invention can also be used in prenatal screening.

Distinct relative variations in mRNA expression have also been identified using reverse Northern blot analysis. Differences in the relative levels of glutamate receptor mRNAs expressed between a neuronal process or processes from the same cell and its cell body have been observed. In a number of cells, the qualitative expression patterns were very similar, yet the relative intensity of the hybridization signal was more profound for specific subunits. For example, relative levels of NMDAR1 and GluR5 mRNA were clearly elevated in HP9a, the apical neurite, versus HP9b, the basal neurite, or the soma. This tendency was complimented in other cells which displayed a more differentiated qualitative pattern of glutamate receptor mRNA expression. This differentiated pattern of mRNA expression contributes to the physiological function of a synapse. These differences in the relative levels of mRNA expression result in the occurrence of different neuronal processes in the same cell. In addition to glutamate receptors, the expression of several other cDNAs has been assessed with reverse Northern blot analysis. Previous reports have demonstrated the dendritic localization of the α -subunits of the Ca_2+ /calmodulin-dependent protein kinase (CaMK II) mRNA.

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It has now been found that mRNA expression in distal and proximal segments of isolated neurites is strongly positive for CaMK II. Thus, specific segments and processes of a neuron may be targeted by determining the profile of mRNA expression involved in the process, identifying an mRNA with a high level of expression and targeting an agent to that mRNA.

Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease and epilepsy are all examples of disorders which involve the degeneration of specific groups of neurons in discrete regions of the nervous system. It appears that different regions of the nervous systems, and specifically, different types of neurons possess different sensitivities to agents. Therefore, to assess the neurotoxicity of an agent, the agent should be assessed in neurons isolated from different regions since it appears that some neuron types are more sensitive than others. It has now been found, however, that neuron types can be identified by determining the mRNA expression in selected neurons, comparing levels of mRNA expression in the selected neurons with levels in known neurons, and identifying the neuron type based on the level of mRNA expression. Thus, the neurotoxicity of an agent for a specific neuron type may be assessed in a culture of mixed neuron types. In this method, it is preferred that mRNA expression is determined by an aRNA amplification procedure.

The complexity of mRNA expression in neurons was investigated further using a PCR-based assay, differential display developed by Liang P. et al., *Science* 1993, 257, 967-970. In these experiments, a single 10 mer (OPA-5;5'-AGGGGTCTTG-3', SEQ ID NO: 29) which serves as the 5'-primer and a modified polythymidine primer containing a two base extension were used to amplify specific populations of the polyadenylated RNA pool. In each of these reactions, banding patterns unique to the soma or process and the combination of primers used were observed. Differential display (DD) of mRNAs from three sets of cells are shown in Figure 1. The complement of DD profile of neurites exhibited a large number of mRNA species. These patterns of PCR products indicate that mRNAs are differentially

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distributed. For example, in a typical cell in which two neurites are transected and isolated, there are transcripts that migrate at similar molecular sizes between individual neurites. This circumstance is repeatedly seen in proximal and
5 distal segments of the same process and in distal branch points of a single process. While there are some products found concomitantly in one or more neurites and between neurites and their corresponding cell bodies, there are numerous transcripts that are unique to individual neurites or segments of
10 individual neurites. Samples of media and tRNA did not display any banding pattern using differential display.

The contamination of neurites with surrounding glia or astroglial processes was assessed by glial fibrillary acidic protein (GFAP) mRNA. Neurite and soma preparations used in
15 these experiments were determined to be free of GFAP mRNA. The existence of multiple mRNAs in neuronal processes suggests that mRNA transport and local protein synthesis plays a role in the regulation of neuronal physiology, development and regeneration.

20 In the present invention, mRNA expression patterns can be used to diagnose conditions related to the presence or absence of synthesized proteins. In recent years, several human neurological diseases have been identified resulting from the expansion of trinucleotide repeats. These triplet
25 repeats are normally polymorphic and exonic, though not always coding. In disease states, they become markedly unstable and may expand moderately or by thousands of repeats in a single generation thereby altering gene expression, message stability or protein structure. Thus, in a diseased state, alterations
30 in normal mRNA expression patterns or profiles would be expected. Methods related to diagnosing a condition associated with an mRNA expression pattern comprise determining the relative levels of mRNA expression in selected cells associated with a selected condition; comparing the relative levels
35 determined with established controls; and diagnosing a condition based upon the comparison of the mRNA expression

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levels. In this method, it is preferred that the selected cells are neurons.

Measuring of mRNA expression patterns as disclosed in the present invention can also be used in methods for treating a condition associated with an mRNA expression pattern. Levels of mRNA expression in selected cells associated with a selected condition are determined and compared to normal levels in the same type of cells. If the level of mRNA expression is abnormal, an effective amount of an agent capable of altering mRNA expression is administered. In this method, it is preferred that the mRNA expression levels be measured in neurons. Examples of agents capable of altering mRNA expression include, but are not limited to, antisense oligonucleotides and pharmacological agents such as dopamine, serotonin and cyclic AMP. Such agents are administered in an effective amount either alone or in conjunction with a suitable pharmaceutically acceptable carrier. By "effective amount" it is meant a concentration of an agent to be administered which is sufficient to modulate expression of mRNA. Such concentrations can be routinely determined by those of skill in the art upon this disclosure and will depend upon the age, weight and condition of a patient. Suitable pharmaceutically acceptable carriers are well known in the art and are described for example in Gennaro, Alfonso, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition 1990. Mack Publishing Co., Easton, PA, a standard reference text in this field. Pharmaceutical carriers may be selected in accordance with the intended route of administration and the standard pharmaceutical practice.

The following nonlimiting examples are provided for illustrative purposes.

EXAMPLES

Example 1: Preparation of neurites

Hippocampi were dissected from ED20-21 rat fetuses, dissociated in trypsin and plated at 30,000-100,000 viable cells/ml of media onto poly-lysine covered glass coverslips

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held in 35 mm tissue culture petri dishes. One day after plating, 0.5 ml of media was replaced with media containing 20 mM potassium. The cultures were subsequently fed once a week with a drop of the high K⁺ media. Experiments were performed
5 after 21-28 days in culture. Cell bodies and their neurites were taken from cells cultured from different animals on three different days under similar conditions. During each of these sessions, a sample of culture media was also aspirated and processed through aRNA processing to assess the possible
10 presence of mRNAs in the culture media from dying cells.

Example 2: Determination of mRNA Band Patterns

Reactions were carried out in 25 μ l volumes using the Hot Start^{*} technique (Perkin Elmer, product #N808-0100) with an upper-lower ratio of 1.5 to 1. Reactions contained 200 μ M of
15 dATP, dGTP and TTP, 4 μ M dCTP, 5 μ Ci of ³³P-dCTP or 4 μ Ci ³²P-dCTP or 18.75 μ Ci of ³⁵S-dATP (NEN/Dupont), 0.4 μ M of OPA-5 or other 10 mers (Operon Technologies), 0.6 μ M of oligo A, B or C, 2.5 mM MgCl₂, 1.25 units of AmpliTaq^{*} polymerase (Perkin Elmer), and 1 μ l of a 1:10 dilution of DNA previously processed through
20 a single round of aRNA amplification. Under these conditions, the primers are in vast excess to the amount of template used for the reaction. Reactions were cycled 35 rounds at 94°C for 30 seconds, 40°C for 90 seconds and 72°C for 45 seconds, followed with a final 5 minute elongation at 72°C in a
25 Bioscyler^{*} thermocycler. Approximately 5 μ l of the reaction was loaded onto a 6% acrylamide/7 M urea gel. Gels were vacuum dried and apposed to XAR film. Gels shown in Figure 1 used ³³P-dCTP. The reactions exhibited a banding pattern unique to a sample.

30 Example 3: cDNA synthesis

Micropipettes containing reagents for first strand cDNA synthesis also contained 5 μ M dithiothreitol and RNAsin (Promega, Madison, WI) at 0.5 units/ μ l. The efficiency of amplification, as based upon trichloroacetic acid precipitated
35 counts and Northern blot analysis to assess the size

*Trade-mark

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distribution of mRNAs, did not differ with or without digitonin. Antisense RNA processed through two rounds of amplification was added to Southern blots containing equal amounts (500 ng) of glutamate receptor cDNAs linearized with
5 the appropriate restriction enzyme and applied by vacuum onto a slot blot apparatus. A random-primed vector cDNA clone, pBluescript SK (Stratagene, La Jolla, CA) was added to stripped blots to demonstrate the presence of DNA in each slot. Scanning densitometry analysis was performed on autoradiographs
10 using a Scanning Laser Densitometer (Molecular Dynamics, Sunnyvale, CA). Hybridization signals were normalized to ribosomal RNA for each neurite or soma studied.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: USE OF NEURITE LOCALIZED mRNAs FOR
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(iii) NUMBER OF SEQUENCES: 28

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 200

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CCTGGNACNC AAGCATCTTN CACCGAACCN TCCNAAAGTN CTGGAATTAN 50
AGTATTNAAG TACATCTAAG TAATGNACTG TAAANTNAAA ATTAANTTAA 100
ANTTTCANTN ATTATNAANA TGAGCTTCGT GCATGTAATA TTGTCGNAAC 150
AAAAGGTGCT TNATGGNANC CTTCTAANGT ATAGTCTCTA AAGNCCTGTT 200

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 434

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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AGGGGTCTTG CCAACANTNN AATTCCTTCG GNAGAATGTA AGCNCCATAA 50
GGGCAGGGGC CACATCTACC CACTNACCTN ATGTCCCCAG CGCTTAGCCT 100
AGTGTNTGGA ACATTNAAGG TGCTCAACCC TTTTNTAGAA TNAATAAATN 150
AATGAAGGCA CACAACGTGC CGAANATTTA AANGTATTGG AGATCTTNTN 200
TTTAANATGG NNAAATAGAG AGCCCAAGTAT TATTTAAAAT GTCAGCAATG 250
GGCAAGGCTT CAACCCCCAG TCTTCTGGCT TTTGCCATCC AATACATCCC 300

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NCTACTTCCC ATCTANAATN ATGCCTTCCT TTGTNTAGTN ATCTTGCTTT 350
ACCTTTGCNA TTTACCTCGC TCAAGTTCAA CTTTTCAGTN GCAAGCCTTG 400
GCCCNCAAGN CCTGCCGCGG NTCAAAGCCC CCTG 434

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 404
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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GGGTAGAAAT GTCANTTCCC ATGAAGGGCA GGGGCCACAT CTACCCACTC 50
ACCTNATGTC CCCAGCGCTT AGCCTAGTGT CTGGNACATT GAAGGTGCTC 100
AACCCTTTTG TAGAATGAAT AAATGAATGA NGGCACACAA CGTGCCGAAC 150
ATTTNAANAT ATTGGAGATC TTGTTTTTAA NATGGGAAAA TAGAGAGCCC 200
AGTATTATTT AAAATGTCAG CAATGGGCAA GGCTTCAACC CCCAGTNTTC 250
TGGCTTTTGC CATCCAATAC ATCCCACTAC TTCCCATCTA AAATNATGCC 300
TTCCTTTGTA AAGTNATCTT GCTTTTACCT TTGCNATTAC CTCGCTCAAG 350
TTCACTTTTT AGTAGCAAGC CTGGGCCCAC AAGGCCTGCC NCGGTCAANA 400
CCCT 404

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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AGGGGTCTTG GAACAAGAAA TGNNNNNTNNN NNNNNATCTG CAGGCTGGAA 50
GTCCCAGAGC ACAGTGTCTG CAGGGTNGGN TTCTCCCAAG GCCTCTNTCC 100
TTGCNTTGTA GACGGCCACC TGTNCTCTGT GTCCTCTNAC AGTNNTCCNT 150
CTGTNTNTNT CTNNNTAATA ATCTCCCCTT CATAAAAGGA CACCAGTCAC 200
TCTGGATTAG GGCTCACTCT AGTAGCCTNN TCTAACATCC NTNACCTCTT 250
TNAAGACCCC T 261

```

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 141
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
AGGGGTCTTN CAGAGGAACA AAGGAATCNC NCTACAGGNC TCTTTNCTNA 50
NTNANGGACA ACNNNAAACA AGTCCTTTAN GCAGGCTAAG GTCTACATGC 100
NTCTNTCCAT GCAAATCCNG AATATGGCTC CCAAGACCCC T 141
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 141
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
AGGGGTCTTG CAGAGGAACA AAGGAATCGC TCTACAGGTC TCTGTACTGA 50
GTGAAGGACA ACTTCAGACA AGTNCTTTNA GCAGGCTAAG GTCTACATGC 100
ATCTATCCAT GCAAATNCAG AATATGGCTC CCAAGACCCC T 141
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 489
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```

AGGGGTCTTG TCTTGGAGCT GTGTTAATAC AGCAAGCACA GTATGTATAT 50
TGCATGTNAC AGATCAATAA ACATGGTAAG ACTTTCTAAA AATNCTTTTA 100
ATTCACACTT TACTTAAGAT TAAGAAACCT CAAAAACACC AAAGTGTGGT 150
AGGGGTGTAG CAGGGGAGAC ACAAAGAAG AGACAGGAAG GGGCTGAGAC 200
CCTAAGCTCC AGAAGAGGTA TGTNATAAAA TGAGTGGGNT AATAAATTCC 250
TTGGTGAAGT ATGTTTTTNA NCAACAAAAA AATTGAAGAT GAATGTTTAT 300
CCTAGCATGG TAAAATGTGT GGTATGAAGG CAGCACCCAC TGGTTTTAAG 350
AGTCTATTAG TCTGTGAATA TCTGNTCTCA CTCAATTATA ACTAAAGGAA 400
TAATTTCCCTA GTNTTCAGGA ATTTGNAAAT TTCCNCAAAT GTGCTTTNNG 450
GNCCAAGGNT TTTTTCAGC CNACNCCNA AGCCCCNTG 489

```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 422
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```

AAAACAGGTN AGGACNCTCN AAAAATGCCA NNTNAATTCA CACTTTACTT 50
AAGANTATGA AACCNCAAAA NCACCAAAGT NTNGTAGGGG TGTAGCAGGG 100
GNGACACAAA CGAAGAGNNA GGAAGGGGCT GNGACCCNAA GCTCCAGAAG 150
AGGTATNTNA TAAAATTAGT GGGATAATAA ATNCCNNGT GAAGTATGTT 200
TTTAAACAAC AAAAAAATNG ANGATGNATG TTTANCCTAG CATGNCNAAN 250
TGTGTGGTAT GANGGCAGCA CCCNCTGGTT TTAAGAGCCT ANTAGGCGTG 300
TNGATANCTG ATCTCACTCA NTNNTAGCNN GNGAAGTACN TCCTNGNNGT 350
NCNGAACTTG TAAATTTTCN GAAAGGTGTT TNTGGTNACA GGTTNTTTNA 400
AGCCAATCNC TCAGGNCCCC TG 422

```

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(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 136
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```

AGGGGTCTTG TNAGTNAATT GTNACTTTAA TCATTTGGAA ATAACCTTCT 50
TTNCTNTNAA TATTTNAGGT ATCTNTTTTG TAAACAGCAT ACACAGTAGT 100
CCCCACTTAG CAGAGGGGGA TAGTTCCAAG ACCCCT 136

```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 427
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```

AGGGGTCTTG AGATATAATT CAGGTGCCAC ACAATTTACC ATTTAANGTG 50
TACAATTCAA TGGTTTTNGG TATATTACAT TATTTACTTT TNAAGCNGTG 100
GTAAAATATA CATAACATAA AATTNGCCAT TTNAACATTT TAAATGTGCA 150
ATTCAGTGGC ATTAGTNGTA TTCACAATGT TATGCAACCA TCACCAGTAT 200
TTNNNTAACT TTTNATCACC CCAAACAAAA ATTCTGTAA CATTAAAGCAG 250
TAACTNTNTN CTCCTCTNGT CCTGTCTTTG ATAACCTCTA ATCTGTTTTN 300
NGTCTCTATG AATTNGCCCA TTCTNCTCTG GGGATTTACT AGACATTTNG 350
TATGAGTGGA ATTATCTAAN ANTGGTNCNT TGCTGTTTGA CTTATTTTAC 400
CCAGTATAGN ATGTNCAANG CCCCTG 427

```

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(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 286
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```

CATCATGTAA TGACGNNGAA GNAGTGCACA GATTGGA AAA CAGATNAACC 50
GCAGTCGCCA ATCTNTGACC CTTATTGACT GTNTGATCTT AAGCAAGTTG 100
CTTAACCTTT CAGAATCTTT NTNCTTTTAA NTNAAATAGG AGAATAGAGT 150
ACCTAACTGA TAGGTTTNTN GTNAGGGCTT ANTGAGAGAA TGTATGTATG 200
GTAGCCATTT TCCAAATAAA GNAGCTATTC TCCAATAATG GCTCCCCAGT 250
AAGCTTTACC TCCTGGTATT CACACCCAAG ACCCCT 286

```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```

NNGNNTCTTG NTACTAATTA NTAGCAGCAT GAATGCGTAG AGAGAGCACA 50
GATTGGA AAA CAGNTAACCG CAGTCCCAAT CTCTNACCCT TATTNACTGT 100
GTGATCTTAA GCAAGTTGCT TAACCTTTCA GAATCTTTNT TCTTNTAAAT 150
GAAATAGGAG AATAGAGTAC CTAACCTGATA GGNTTGTGNT GAGGGCTTAA 200
NTAGAGANTN TATGTATGGT AGCCATTTTC CAAATAAAGC AGCTATTCTC 250
CAATAATGNC TCCCCAGTAA GCTTTACCTC CTGGTATTCA CACCCAAGNC 300
CCCTT 305

```

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(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 136
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```

NNGGGTCTTG TTAGTGAATT GTGACTTTNA TCATTTGGAA ATAGCCTTCT 50
TTTCTNTTAA TATTTTAGGT ATCTNTTTTG TAAACAGCAT ACACAGTAGT 100
CCCCACTTAG CAGAGGGGGA TAGTTCCAAG ACCCCT 136

```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```

AGGGGTCTTG GCCTCACATA CAGCAGGTGT CTATAAATNT TTNTTTAATA 50
AATGATTTAT ACTAGTGCAG TTTCACTATC ACAGTTACTT ACCTTTNTNA 100
GTGTGACAAA CACAGTCACT GAAAACCATA CATCAGGACC CCT 143

```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 511
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```

AGGGGTCTTG CAGTGATGGT TATNCTACTG ACACCTGGTG ACAAGAATGG 50
GAATTCTCTG ANGTAATCTC AAGTTAAATG TTACCTCATT TTNTTCTATA 100
GGTAATGGAA GCATAACATC ATTTGATTAG CAGATAGGAC AATATTTCTG 150
CAATTNTCAT CATGGTGGCA CAAGCATCAC ATTTTNTNTN GCCATTGGTA 200
TTTNGATAAA GTTTTCAAAA GTTACTGCAA TTNGTTATCA GAACACTTGG 250
GTACTGTGTT TGCNGATCAG ACAGANGACT ATTAANGCCA AAAGTATTAA 300
NGAGCTAACA AGCAAAGCCA TCCAATACAA GGCATGTTTT NACAANTNAT 350
ATATCTNGTA GGCAGCTTCA AAATTAATAG TTGAAAGTCC AGAAATCACC 400
ACAGGNTATC ATTTGAGGCC TTA AACATA NCTGGAAATN TTTNTTGAGN 450
ATTAATNCAA AANCCTAAGC NAGGCNATGT TANNNCCTTA GCNTTATTTT 500
NCAGGATGTT T                                     511

```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 370
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```

ACCAGGGNTC TNGNTCGTGG CCGGGCTTGT GGGTCCAGGC TTGCTACTGN 50
AANGTTGAAC TNGAGCGAGG TAATAGCATA GGTAAGCAA GATGNCTTGA 100
CAACGGAGGC ATCATTTNAG ATGGGANGTA GTGGGCTGTA TTGGATGGCA 150
AAAGCCAGAA GACTGGGGGT TGAAGCCTTG CCCATTGCTG ACATTTTAAA 200
TAATACTGGG NTCTCTATTT TCCCATCTGA AAAACAAGAT CTCCNGTATC 250
TTAAAAATGT TCGGCACGTT GTNTGCCTNC ATTCATTTAA TTCATTCTAC 300
AAAAGGGTTN ANGCNCCTTC CANTGTGACC CNACACTTGG GATAAGNGNN 350
TGGGGACATN AGGTAAAGTA                                     370

```

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(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```

AGGGGTNTTG AAAGAGGTAA TGGATGTNAG ATGAGGCTAC TAGAGTGAGC 50
CCTAATCCAG AGTGACTGGT GTCCTTTTAT GAAGGGGAGA TTATAACAAA 100
GACACAGACA GAGGGATGAC TGTNAGAGGA CACAGAGAAC AGGTGGCCGT 150
CTACAAGGCA AGGAGAGAGG CCTTGGGAGA AACCAACCCT GCAGACACTG 200
TGCTCTGGGA CTTCCAGCCT NCAGATATGT GAGAAAATAC ATTTNTTGTT 250
CCAAGACCCC T                                     261

```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 169
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```

AGGGGNCTTG GNAGCCATAT TCTNCATTTT CNTGGANAGA TGCATGTNGA 50
CCTTAGCCTG CTAAAAGCAC TTGTTTNCGG NGNTCCNTNA CTNAGTNCAG 100
NGNCCGGTNG NGCGNTTCCT GGNCCCCTTT GNAAGACCCN TGGGCTAGAG 150
CGGACGAAAT NGTGTNTNA                               169

```

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(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 386
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```

AGGGGTCTTG AGGTGATTGA CTTGCAAATA ATCTTGTGCT CTTAAAGCAC 50
ATTTTGGTA AATTTTCAA TTTCTGAATA CTAGAAATNA TTTCTTTAGT 100
TATAATTGAG TGAGATCAGA TATTCACAGA CTAATAGACT CTCAAAACCA 150
GTGGGTGCNG CCTTCATACC ACACATTTNA CCATGCTAGG NTAAACATTC 200
ATCTTCAATT TTNTNGTTGT TAAAAACAT ACTTCACCAA GGNATTTATT 250
ATCCCACTCA TTTTATCACA TACCTCTNCT GGNGCTTAGG GTCTCAGGCC 300
CTTCCTGTCT TCTNCTTTGG TGNCTCCCCT GCTAAANCCC TACCACACTT 350
TGGNGTTTTT NAGGGTTTCT NAACCTTAAG TAAAGT 386

```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 395
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```

AGGGGTCTTG AGGTGATTGA NTTGCAAATA ATCTTGTGCT CTTAAAGCAC 50
ATTTTGGTA AATTTTCAA TTTCTGAATA CTAGAAATTA TTTCTTTAGT 100
TATAATTGAG TGAGATCAGA TATTCACAGA CTAATAGACT CTCAAAACCA 150
GTGGGTGCTG CCTTCATACC ACACATTTTA CCATGCTAGG NTAAACATTC 200
ATCTTCAATN TTTTNGTTGT TAAAAACAT ACTTCACCAA GGAATTTATT 250
ATCCCACTCA TTTTATCACA TACCTCTTCT GGAGCTTAGG GTCTCAGNCC 300
CTTCCTGTCT CTNCTTTGGT GTCTCCCCTG CTACANCCNT ACCACACTTT 350
GGNGTTTTTG NGGGTTCTTA ATCTTAAGTA AAGTGTGAAT AAAAA 395

```

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(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 136
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```

AGGGGTCTTG GAACTATCCC CCTCTGCTAA GTGGGGACTA CTGTGTATGC 50
TGTTTACAAA ACAGATACCT AAAATATTAA CAGAAAAGAA GGNTATTTCC 100
AAATGATAAA AGTCACAATT CACTAACAAG ACCCCT 136

```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 237
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```

AGGGGNCTTG GAACAAAAAA GACTATAAGA TCAGAGGTAA TGAGGTTGGG 50
ATAGAGATAT GTGGATGAAC CTATGAACAC AAAATATAAA GATCTCATGT 100
TTAATGCTCA TATTAATACT CACCAGAAAG CGTAGAATAA CATTGGCTGA 150
GTATGGTGGC TCTTTGAAAG GCTGAGGTGG GAGGNTCACT TGATGCCAGT 200
AGTTTAAGAC CAGCTTGGGC AACATAGCAA GACCCCT 237

```

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(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 383
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```

AACTGNNGCT GCANAATTAA GTCCAAAACA GNAAAAGGAA CAAAATATTA 50
AGCTAATTCC ACTCATACAA AATGTCTAGT AAATTCCTCAG AGGNGAATGG 100
GCAAATTCAT AGAGACAGAA AACAGATTAG AAGTTATCAA AGACAGGACA 150
AGAGGAGAAC AGAGTTACTG CTTAATGGTT ACAGAATTTT TGTTTGGGGT 200
GATAAAAAGT TATAAANTA CTGGTGATGG TTGCATAACA TTGTGAATAC 250
AACTAATGCC ACTGAATTGC ACATTTAAAA TGTTAAATGG CAAATTTTAT 300
GTTATGTATA TTTTCCACAG CTTAAAAAGT AATAATGTAA TATNCCAAAA 350
CCCATTTGAAT TGTNCACTTT AAATGGGTAA TTT 383

```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```

AGGGGNCTTG GGTGTGAATA CCAGGAGGTA AAGCTTACTG GGGAGCCATT 50
ATTGGAGAAT AGCTGCTTTA TTTGGAAAAT GGCTACCATA CATACTTCT 100
CTCATTAAGC CCTCACAACA ATCCTATCAG TTAGGTACTC TATTCTCCTA 150
TTTCATTTAA AAGAACAAAG ATTCTGAAAG GTTAAGCAAC TTGCTTAAGA 200
TCACACAGTC AATAAGGGTC AGAGATTGGG ACCTGCGGTT ATCTGTTTCC 250
AAATCTGTGC TCTCTCTACG NATTCATGCT GCTATTAATT AGTATCAAGA 300
CCCCT 305

```

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(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```

AGGGGTCTTG GGTGTGAATA CCAGGAGGTA AAGCTTACTG GGGAGCCATT 50
ATTGGAGAAT AGCTGCTTTA TTTGGAAAAT GGCTACCATA CATAATTCT 100
CTCATTAAGC CCTCACAACA ATCCTATCAG TTAGGTACTC TATNCTCCTA 150
TTTCATTTAA AAGANCAAAG ATTCTGAAAG GTTAAGCAAC TTGCTTAAGA 200
TCACACAGTC AATAAGGGTC AGAGATTGGG ACCTGCGGTT ATCTGTTTCC 250
AAATCTGTGC TCTCTCTACG CATTCATGCT GCTATTAATT AGTATCAAGA 300
CCCCTG                                     306

```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 446
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```

ANGGGGNAC TTNACTGTAT GGGTTTNNAG NGACCTNTTT TTTNNNACAC 50
TCAGAAANGG TAAGNAANTG TNATAGNNAA ACTNCNCTAG GATAATCNAT 100
TTTNTTAAAC AAACATTTNT NGACNCCNNN NTGTTNTNTN AGGCCAAGAC 150
CCNCTGGGGT NAGANGTGGG CCCCNACCCG GGGGNGGGGG GCNCCCACCT 200
TTTTTTTNNC CCCNTTTAAN NGNGGGGGGG TTNAATTGCC CCNGTTTTTG 250
GGGGTNAANN NAATNGGGNC CANAAGGANT GTTTTTTCCC NCNGGGGGGA 300
AAAANATTGT TTTTACCCNC GNTTNCNAAA AAATTTCCCT ANNTNATATA 350
TNNNTTGNGG GGTGCCNCNN GAAGNGNNTA TTAAAGAGTT GTTANANNAC 400
CNCTTNGNGG GGGCGCCCN AATTGGGGGG GGNGGTCNTA CACCTN      446

```

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(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 396
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

AGGGGTCTTG	GGCCCAGACA	GACATGGGTT	CAATTTATAG	CACTGATAAT	50
GTATAGCTGT	GGGNCCTCGT	GCAGTGATTT	AACCTCTGAA	AGTTTTCTCA	100
CCTTTAAATG	GTGAGGAAAA	TACTGATGTG	AAAAATATAC	AAAAGNAAAC	150
ACGCAAAGCA	CCTAGCCTTG	CTGGAAACAT	CAGTTACTCA	TGATGNTGAT	200
CATGATGATG	CCAATGATAA	TNNTGATAAT	GGNGTTAATN	NTGGTGATGA	250
AGACTGATCA	CAGNCTGCCC	TTCTTTTTTG	NGGAATTTGG	GAAAATGAAA	300
TCTCTTTGAT	TCCCACTAAT	GCNTTTAAGC	TGTGTCANGC	AACNGATGGG	350
TTGGGGAGGT	GGATNGGGGT	GACTCAATAT	TTAGGTNCCT	GCNCTT	396

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(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 136

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

AGGGGTCTTG	GAACATATCCC	CCTCTGCTAA	GTGGGGACTA	CTGTGTATGC	50
TGTTTACAAA	ACAGATACCT	AAAATATTAA	CAGAAAAGAA	GGNTATTTCC	100
AAATGATAAA	AGTCACAATT	CACTAACAAG	ACCCCT		136

- 30 -

CLAIMS:

1. A method of identifying neurite cDNA clones comprising:
 - (a) isolating selected neurites by transecting at varying distances from the cell body;
 - (b) converting an mRNA population in selected neurites into cDNA;
 - (c) determining the pattern of mRNA expression of the mRNA population in selected neurites;
 - (d) comparing the relative pattern of mRNA expression to the pattern in a control; and
 - (e) identifying neurite cDNA clones based on the pattern of mRNA expression.
2. The method of claim 1 wherein the mRNA expression is determined by an aRNA amplification procedure.
3. A method of profiling mRNA expression in a selected neurite, the method comprising:
 - (a) isolating selected neurites by transecting at varying distances from the cell body;
 - (b) converting an mRNA population in selected neurites into cDNA;
 - (c) making the cDNA double stranded;
 - (d) linearly amplifying the double stranded cDNA into aRNA; and
 - (e) using the aRNA as a probe in reverse phase Northern analysis to produce an mRNA expression profile.
4. The method of claim 3 wherein the mRNA population is converted into cDNA using an oligo-dT-T7 primer.

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5. The method of claim 3 wherein the double stranded cDNA is linearly amplified into aRNA using T7 RNA polymerase.

6. A method of diagnosing a neurological condition associated with mRNA expression, the method comprising:

(a) isolating selected neurites by transecting at varying distances from the cell body;

(b) determining the pattern of mRNA expression in neurites associated with the disease;

(c) comparing the determined pattern of mRNA expression with the pattern of mRNA expression of established controls; and

(d) diagnosing the neurological condition based upon the comparison of the mRNA expression pattern.

7. The method of claim 6 wherein the condition is a neuropsychiatric disorder.

8. The method of claim 7 wherein the condition is fragile X syndrome.

1 / 3

5' PRIMER	OPA-5								
3' PRIMER	A			B			C		
	7	8	9	7	8	9	7	8	9
	3	3	3	3	3	3	3	3	3
CELL	0	0	0	0	0	0	0	0	0
	1	1	1	1	1	1	1	1	1



250 bp

100 bp

FIG. 1A

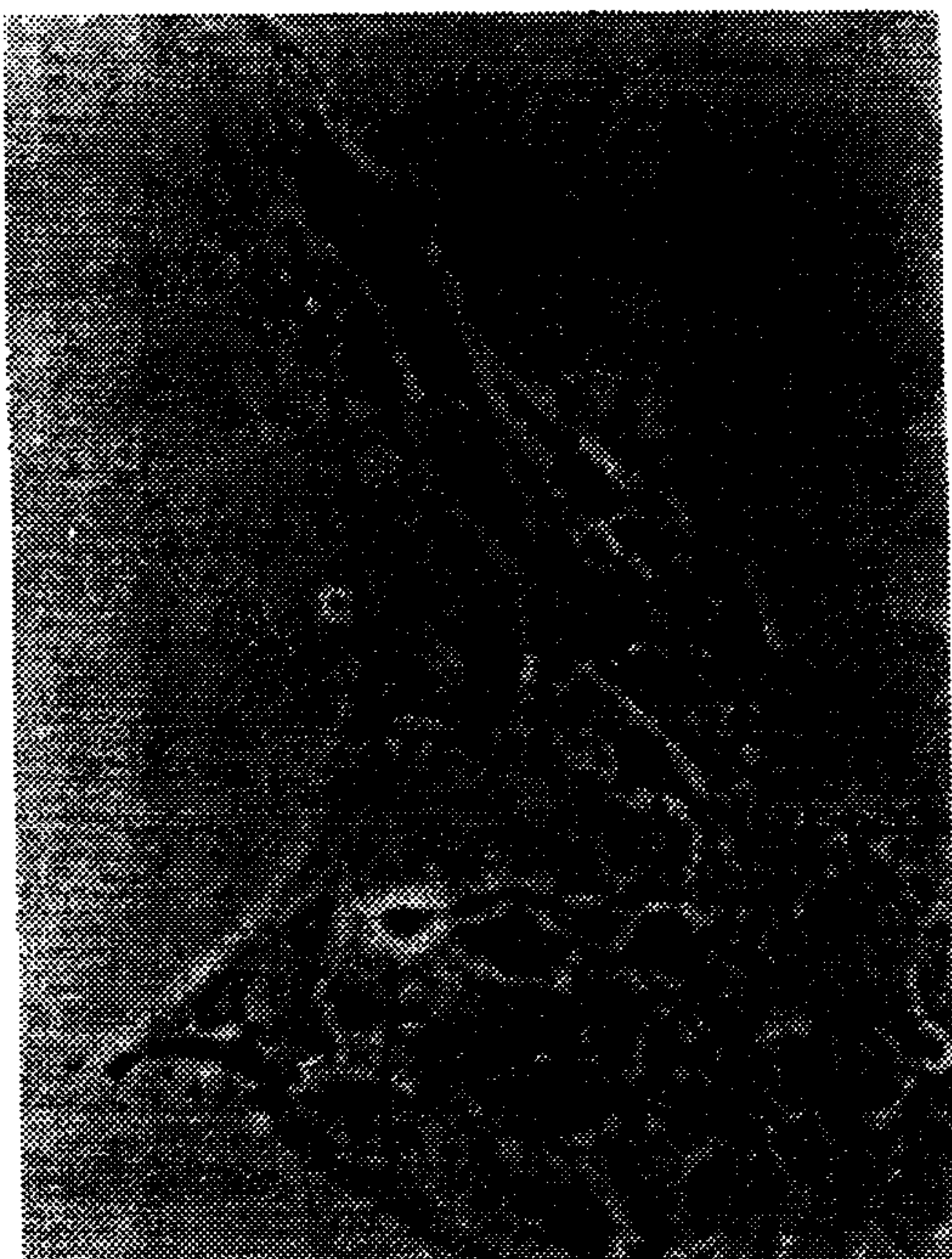
LANE 1 2 3 4 5 6 7 8 9

WO 96/14435

PCT/US95/14792

2 / 3

5' PRIMER	OPA-5								
3' PRIMER	A			B			C		
	0	1	2	0	1	2	0	1	2
	1	2	3	1	2	3	1	2	3
	2	3	4	2	3	4	2	3	4
CELL	0	0	0	0	0	0	0	0	0
	1	1	1	1	1	1	1	1	1



250 bp

100 bp

FIG. 1B

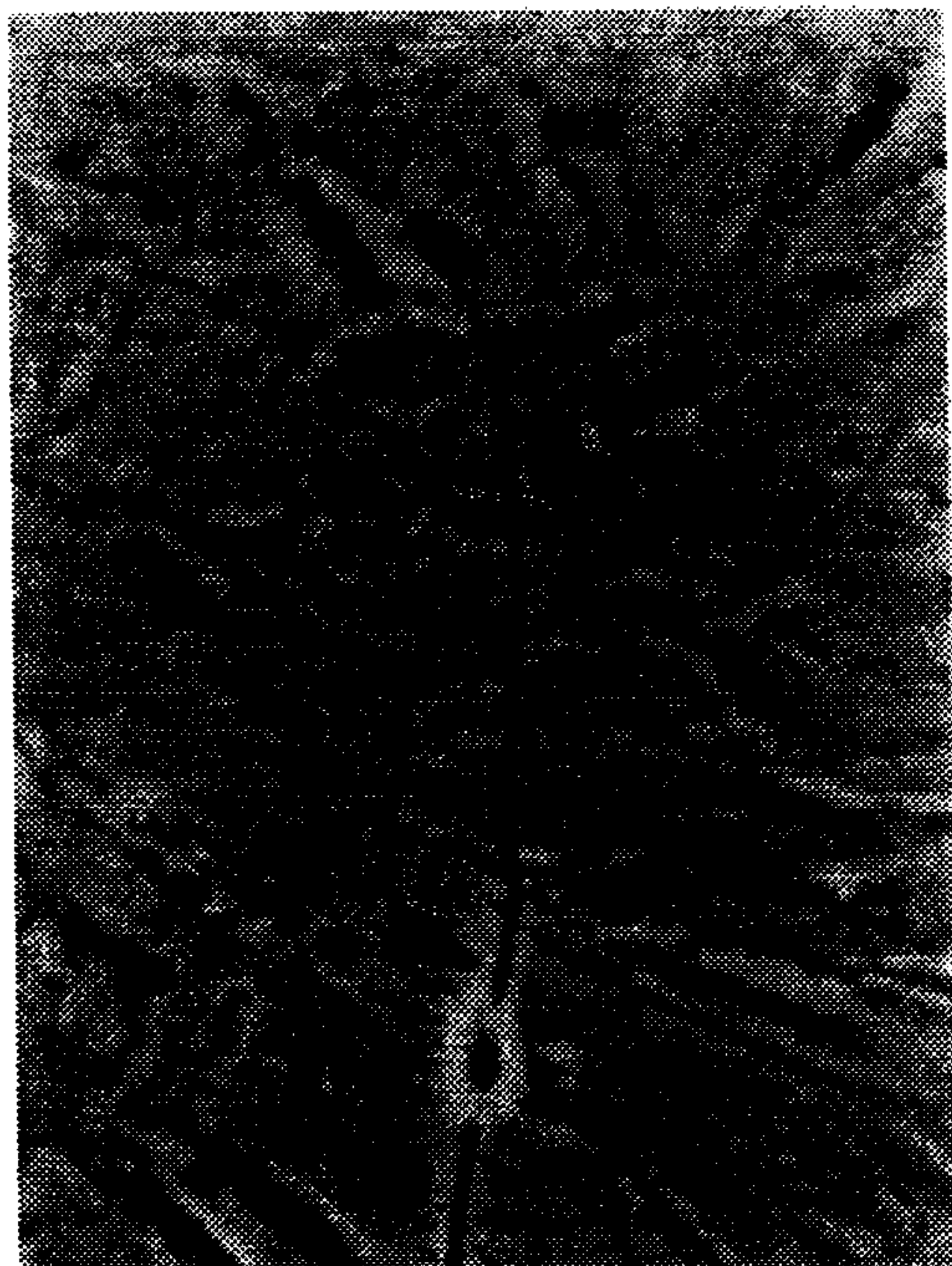
LANE 1 2 3 4 5 6 7 8 9 10 11 12

WO 96/14435

PCT/US95/14792

3 / 3

5' PRIMER	OPA-5		
3' PRIMER	A	B	C
	5' 3'	5' 3'	5' 3'
	3' 5'	3' 5'	3' 5'
CELL	1 2	1 2	1 2
	1 1	1 1	1 1



250 bp

100 bp

FIG. 1C

LANE 1 2 3 4 5 6