US010370717B2

(12) **United States Patent**
Maheshwari et al.

(10) **Patent No.: US 10,370,717 B2**
(45) **Date of Patent: Aug. 6, 2019**

(54) **METHODS FOR THE MEASUREMENT OF POST-TRAUMATIC STRESS DISORDER MICRORNA MARKERS**

(71) Applicant: **The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.**, Bethesda, MD (US)

(72) Inventors: **Radha K. Maheshwari**, Rockville, MD (US); **Nagaraja S. Balakathiresan**, Clarksburg, MD (US); **Manish Bhomia**, Rockville, MD (US); **Raghavendar Chandran**, Rockville, MD (US)

(73) Assignee: **The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.**, Bethesda, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 93 days.

(21) Appl. No.: **15/306,178**

(22) PCT Filed: **Apr. 21, 2015**

(86) PCT No.: **PCT/US2015/026956**
§ 371 (c)(1),
(2) Date: **Oct. 24, 2016**

(87) PCT Pub. No.: **WO2015/164431**
PCT Pub. Date: **Oct. 29, 2015**

(65) **Prior Publication Data**

US 2017/0044613 A1 Feb. 16, 2017

Related U.S. Application Data

(60) Provisional application No. 61/982,651, filed on Apr. 22, 2014.

(51) **Int. Cl.**
C12Q 1/68 (2018.01)
C12Q 1/6883 (2018.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6883*** (2013.01); *C12Q 2600/158* (2013.01); *C12Q 2600/178* (2013.01)

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

(56) **References Cited**

U.S. PATENT DOCUMENTS

2014/0073516	A1	3/2014	Hood et al.	
2014/0256562	A1 *	9/2014	Umansky	C12Q 1/6883 506/2

FOREIGN PATENT DOCUMENTS

WO	2006/033020	A2	3/2006
WO	2013/018060	A2	2/2013

OTHER PUBLICATIONS

Free Dictionary definition of "Measuring" available via url: < thefreedictionary.com/measuring>, printed on Sep. 20, 2017.*

Balakathiresan et al J Neurotrauma. May 2012. 29: 1379-1387 and Supplementary Data.*

MiRBase entry for MI0000074 / hsa-mir-19b-1, printed on Jul. 2, 2018, available via URL: <mirbase.org/cgi-bin/mirna_entry.pl?acc= MI0000074>.*

MiRBase entry for MI0000298 / has-mir-221, printed on Jul. 2, 2018, available via URL: <mirbase.org/cgi-bin/mirna_entry.pl?acc= MI0000298>.*

MiRBase entry for MI0000300 / has-mir-223 (printed on Jul. 2, 2018, available via URL: < mirbase.org/cgi-bin/mirna_entry.pl?acc= MI0000300>.*

Alderfer et al J Head Trauma. 2005. 20(6): 544-562.*

Redell et al J of Neurotrauma. Dec. 2010. 27: Supplemental Table 2.*

Pasinetti et al Am J Neurodegener Disease. 2012. 1(1): 88-98.*

International Search Report and Written Opinion issued in the corresponding International Application No. PCT/US2015/026956 dated Mar. 30, 2016.

Lei et al., "Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury," Brain Research, doi:10.1016/j.brainres.2009.05.074 (2009).

Mondello et al., "Blood-based diagnostics of traumatic brain injuries," Expert Rev. Mol. Diagn.,11(1): 65-78 (2011).

Balakathiresan et al., "MicroRNA Let-7i Is a Promising Serum Biomarker for Blast-Induced Traumatic Brain Injury," J. Neurotrauma, 29(7): 1379-1387 (2012).

Hu et al. "Expression of miRNAs and their cooperative regulation of the pathophysiology in traumatic brain injury," PLoS ONE, 7(6):e39357 (2012).

Redell et al., "Traumatic Brain Injury Alters Expression of Hippocampal MicroRNAs: Potential Regulators of Multiple Pathophysiological Processes," Journal of Neuroscience Research, 87:1435-1448 (2009).

Guo et al., "The Fate of miRNA* Strand through Evolutionary Analysis: Implication for Degradation as Merely Carrier Strand or Potential Regulatory Molecule?" PLoS ONE, 5(6): e11387 (2010).

TaqMan Array MicroRNA Cards, TaqMan OpenArray MicroRNA Plates and Megaplex Primer Pools (Date: Mar. 18, 2014) [online] [retrieved on Sep. 23, 2015] Retrieved from Thermofisher.com, 60 pages <https://www.thermofisher.com/order/catalog/product/4398967> + TaqMan Rodent MicroRNA A Array v2.0 [online], [retrieved on Sep. 23, 2015] Retrieved from Thermofisher.com, 2 pages <https://www.thermofisher.com/order/catalog/product/4398967>.

(Continued)

Primary Examiner — Carla J Myers

(74) *Attorney, Agent, or Firm* — Morgan, Lewis & Bockius LLP

(57) **ABSTRACT**

The present invention provides for methods of measuring levels of micro RNAs for the diagnosis, treatment and/or monitoring the progression of post-traumatic stress disorder (PTSD) or traumatic brain injury (TBI) in a subject having or suspected of having PTSD and/or TBI. The methods, in general comprise measuring levels of at least one of miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* is a sample from a subject suffering from or suspected of having PTSD and/or TBI.

7 Claims, 15 Drawing Sheets

Specification includes a Sequence Listing.

(56) **References Cited**

OTHER PUBLICATIONS

Balakathiresan et al., "Serum and amygdala microRNA signatures of posttraumatic stress: fear correlation and biomarker potential," J. Psychiatr. Res., 57:65-73 (2014).
Cho et al. (2014) Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder, PNAS, 111:3188-3193.
Lei et al. (2009) Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury, Brain Res., 1284:191-201.
Redell et al. (2010) Human traumatic brain injury alters plasma microRNA levels, J. Neurotrauma, 27:2147-2156.
Schmidt et al. (2013) Therapeutic action of fluoxetine is associated with a reduction in prefontal cortical MiR-1971 expression levels in a mouse model of posttraumatic stress disorder, Front Psychiatry, 4:66.

* cited by examiner

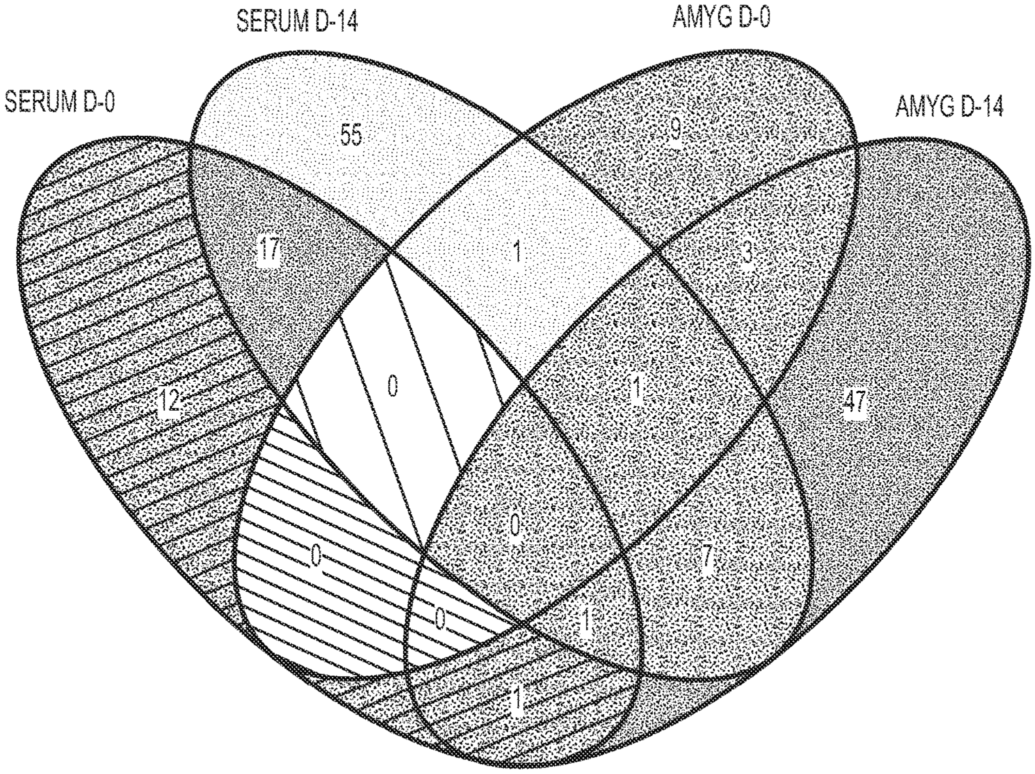

FIG. 1

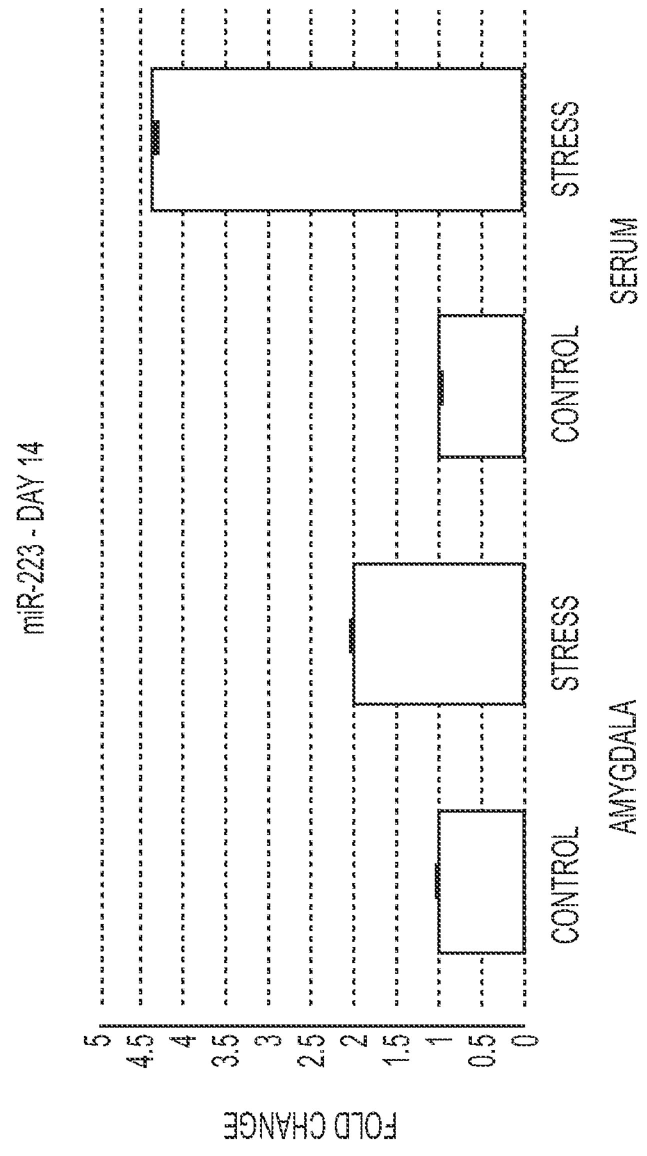
miR-223 - DAY 14
FOLD CHANGE
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
STRESS
AMYGDALA
CONTROL
STRESS
SERUM

FIG. 2

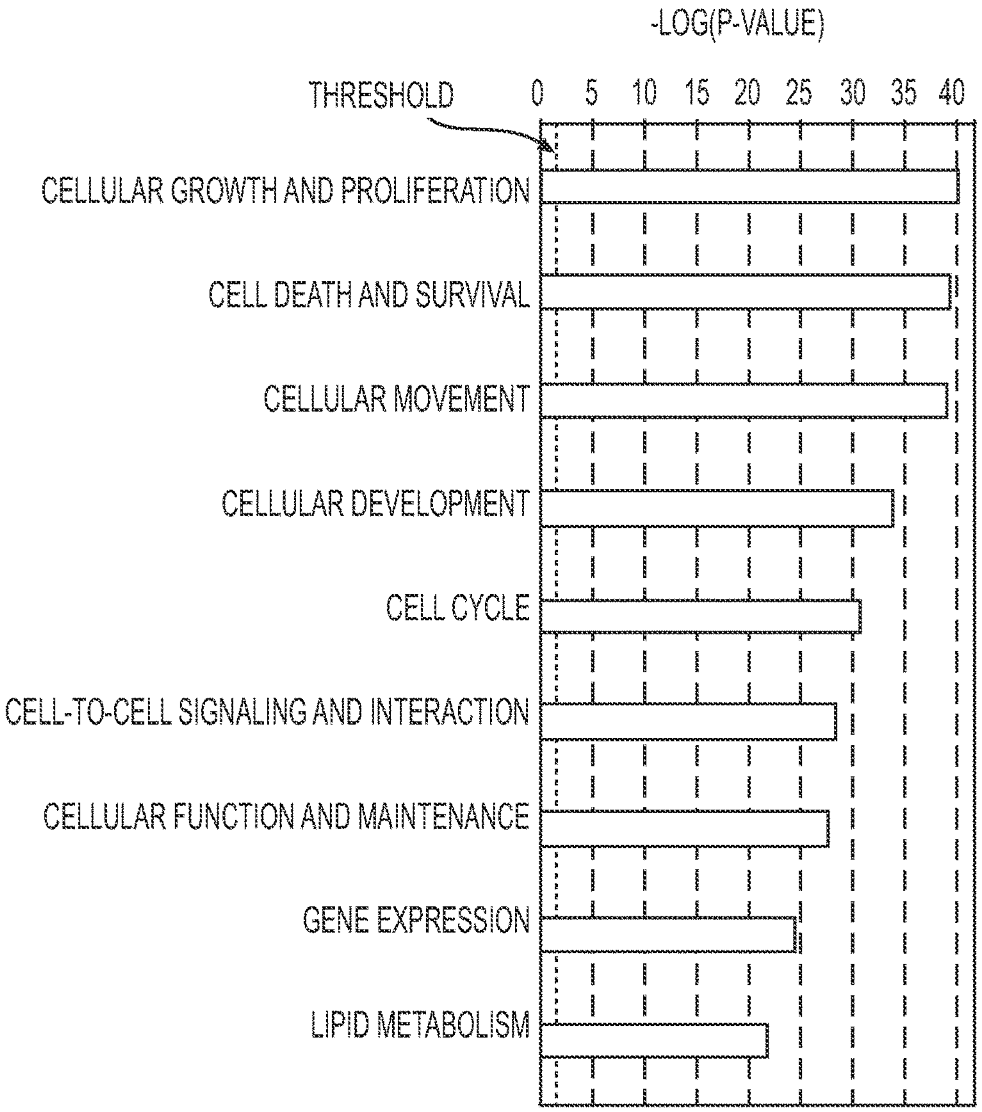
-LOG(P-VALUE)
THRESHOLD
0
5
10
15
20
25
30
35
40
CELLULAR GROWTH AND PROLIFERATION
CELL DEATH AND SURVIVAL
CELLULAR MOVEMENT
CELLULAR DEVELOPMENT
CELL CYCLE
CELL-TO-CELL SIGNALING AND INTERACTION
CELLULAR FUNCTION AND MAINTENANCE
GENE EXPRESSION
LIPID METABOLISM

FIG. 3A

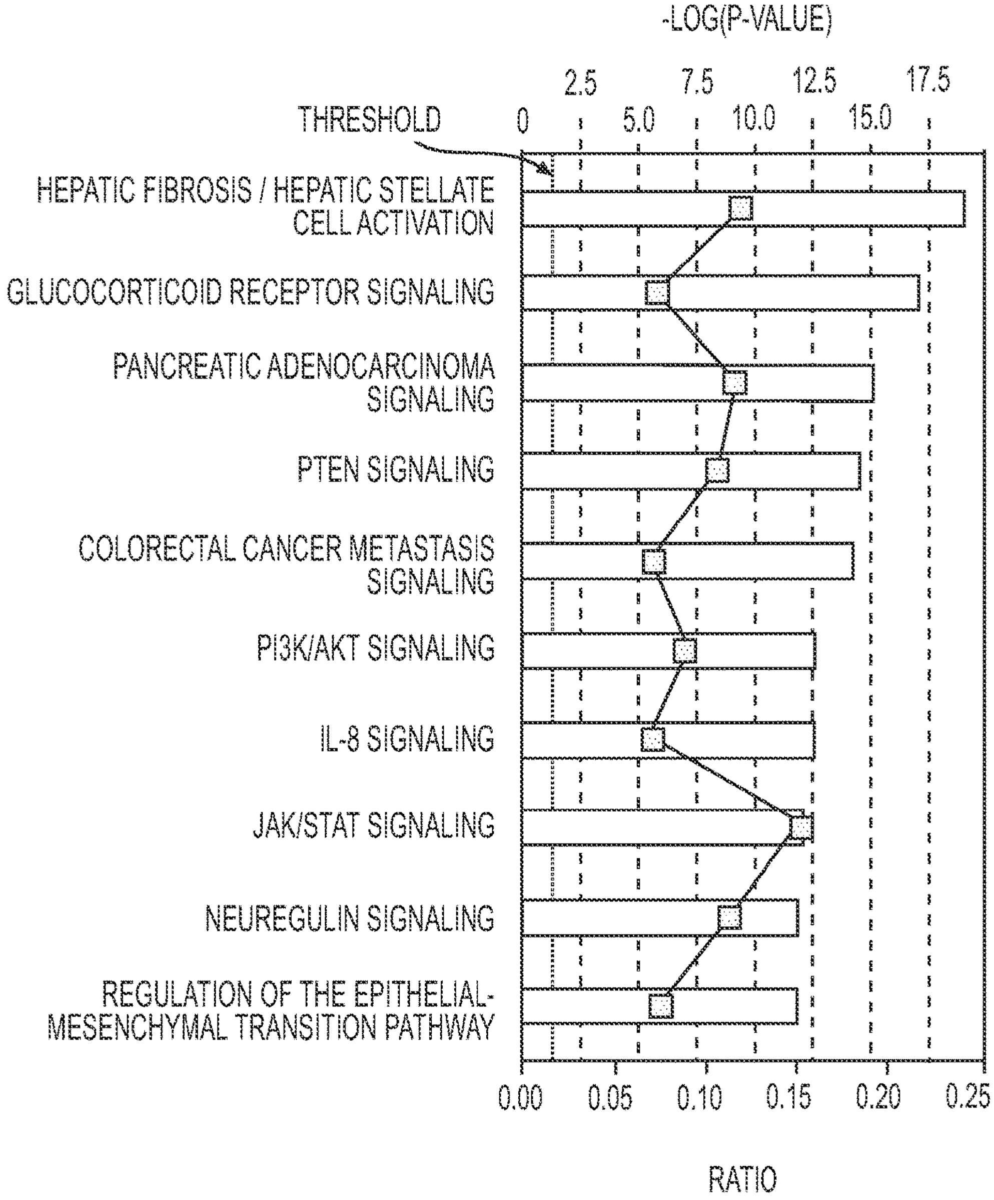
-LOG(P-VALUE)
THRESHOLD
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
HEPATIC FIBROSIS / HEPATIC STELLATE CELL ACTIVATION
GLUCOCORTICOID RECEPTOR SIGNALING
PANCREATIC ADENOCARCINOMA SIGNALING
PTEN SIGNALING
COLORECTAL CANCER METASTASIS SIGNALING
PI3K/AKT SIGNALING
IL-8 SIGNALING
JAK/STAT SIGNALING
NEUREGULIN SIGNALING
REGULATION OF THE EPITHELIAL-MESENCHYMAL TRANSITION PATHWAY
0.00
0.05
0.10
0.15
0.20
0.25
RATIO

FIG. 3B

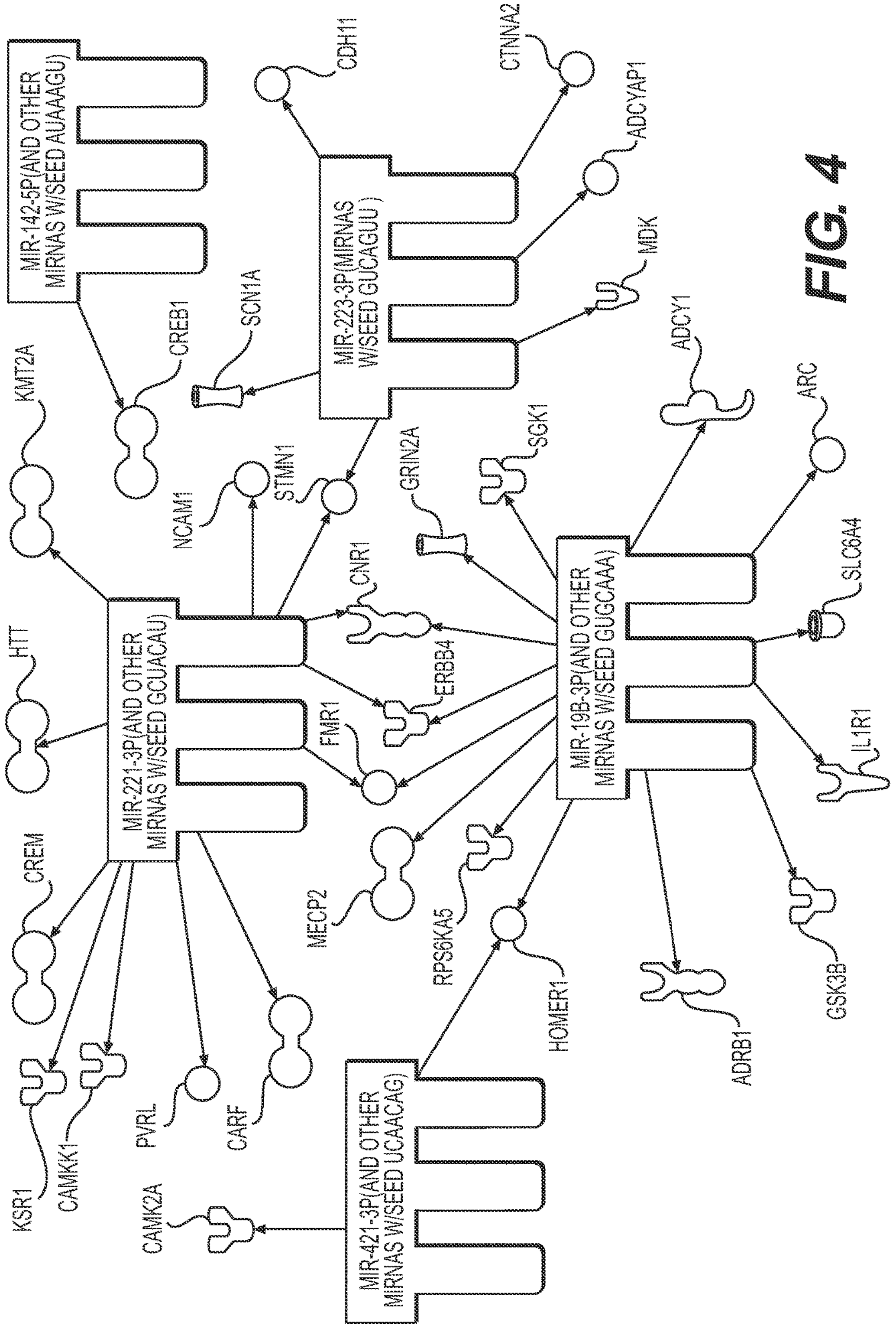
MIR-142-5P(AND OTHER MIRNAS W/SEED AUAAAGU)
MIR-223-3P(MIRNAS W/SEED GUCAGUU)
MIR-221-3P(AND OTHER MIRNAS W/SEED GCUACAU)
MIR-19B-3P(AND OTHER MIRNAS W/SEED GUGCAAA)
MIR-421-3P(AND OTHER MIRNAS W/SEED UCAACAG)
CDH11
CTNNA2
ADCYAP1
MDK
SCN1A
CREB1
KMT2A
STMN1
NCAM1
GRIN2A
SGK1
ADCY1
ARC
SLC6A4
IL1R1
CNR1
ERBB4
FMR1
HTT
CREM
MECP2
RPS6KA5
HOMER1
GSK3B
ADRB1
KSR1
CAMKK1
PVRL
CARF
CAMK2A

FIG. 4

FIG.5	FIG.5 CONT. 2
FIG.5 CONT. 1	FIG.5 CONT. 3
FIG.5 CONT. 4	FIG.5 CONT. 6
FIG.5 CONT. 5	FIG.5 CONT. 7

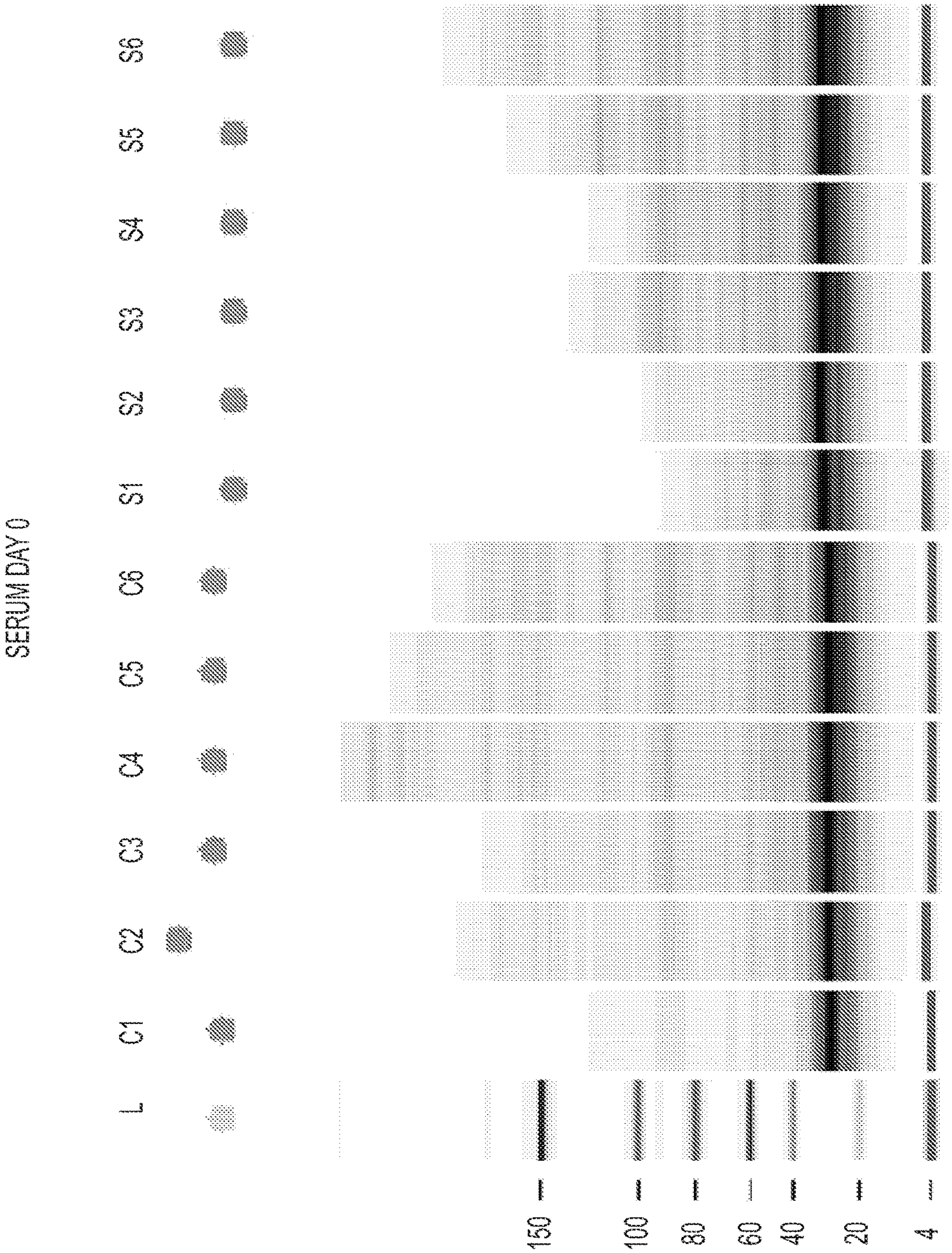

FIG. 5

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

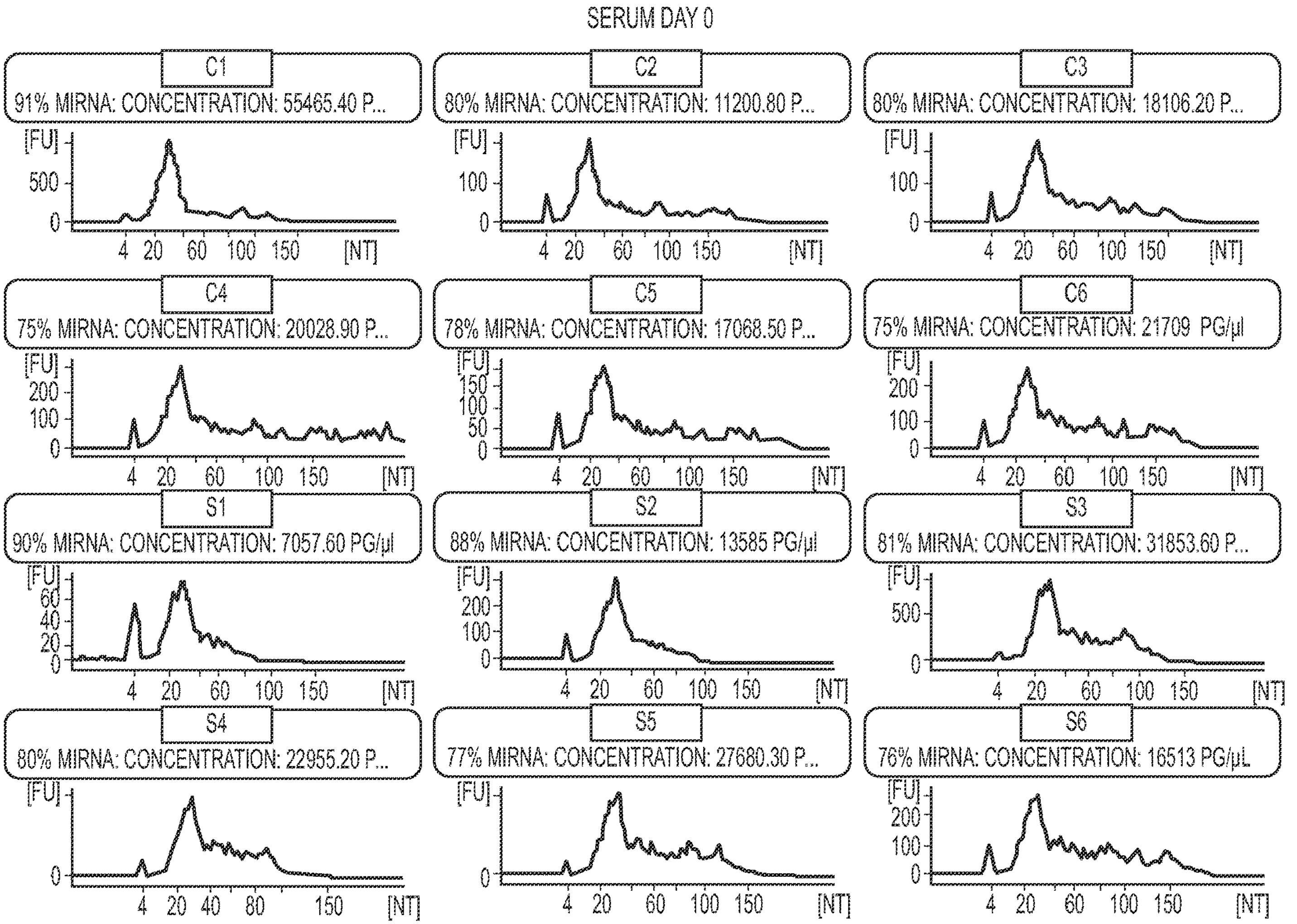

FIG. 5
CONT. 1

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

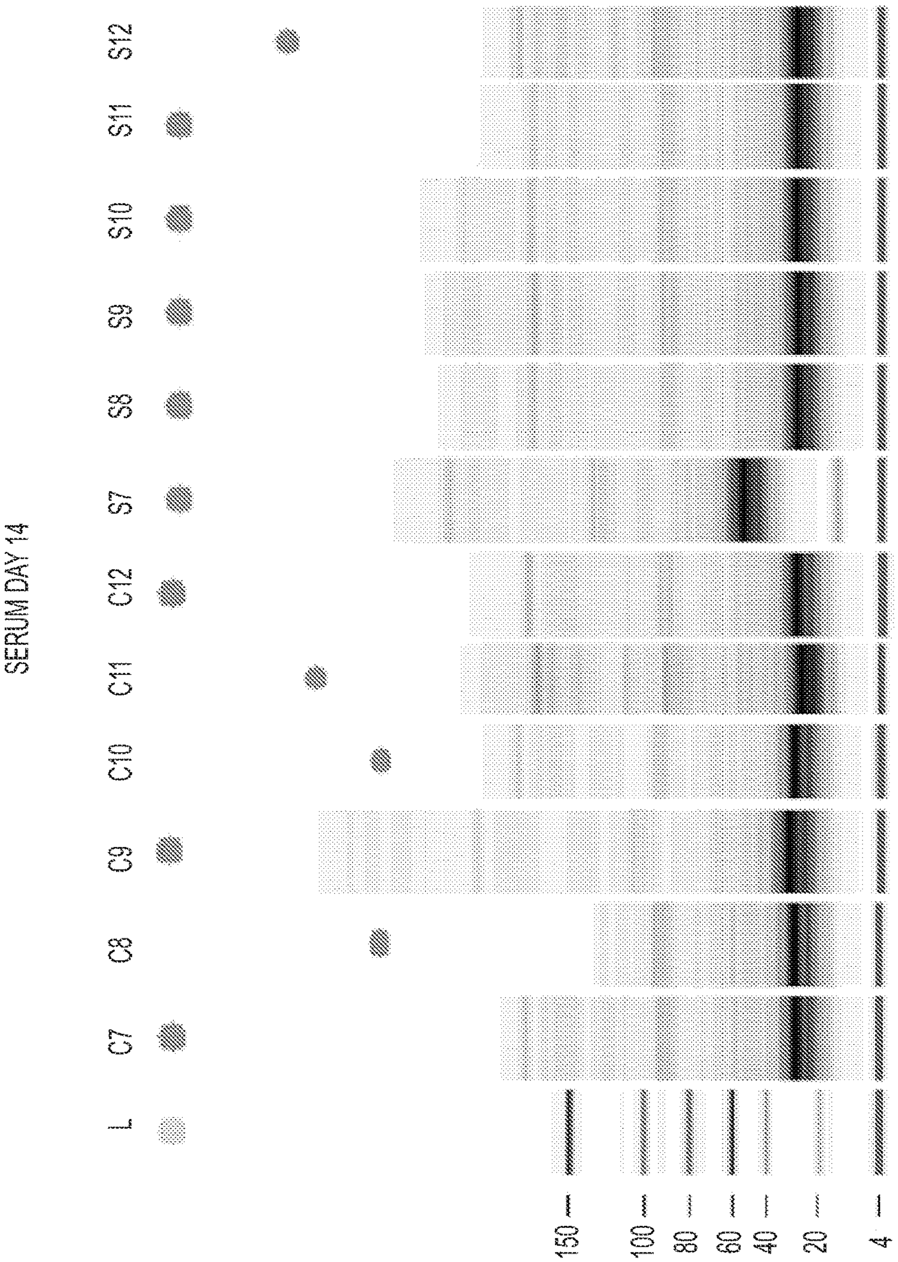

FIG. 5 CONT. 2

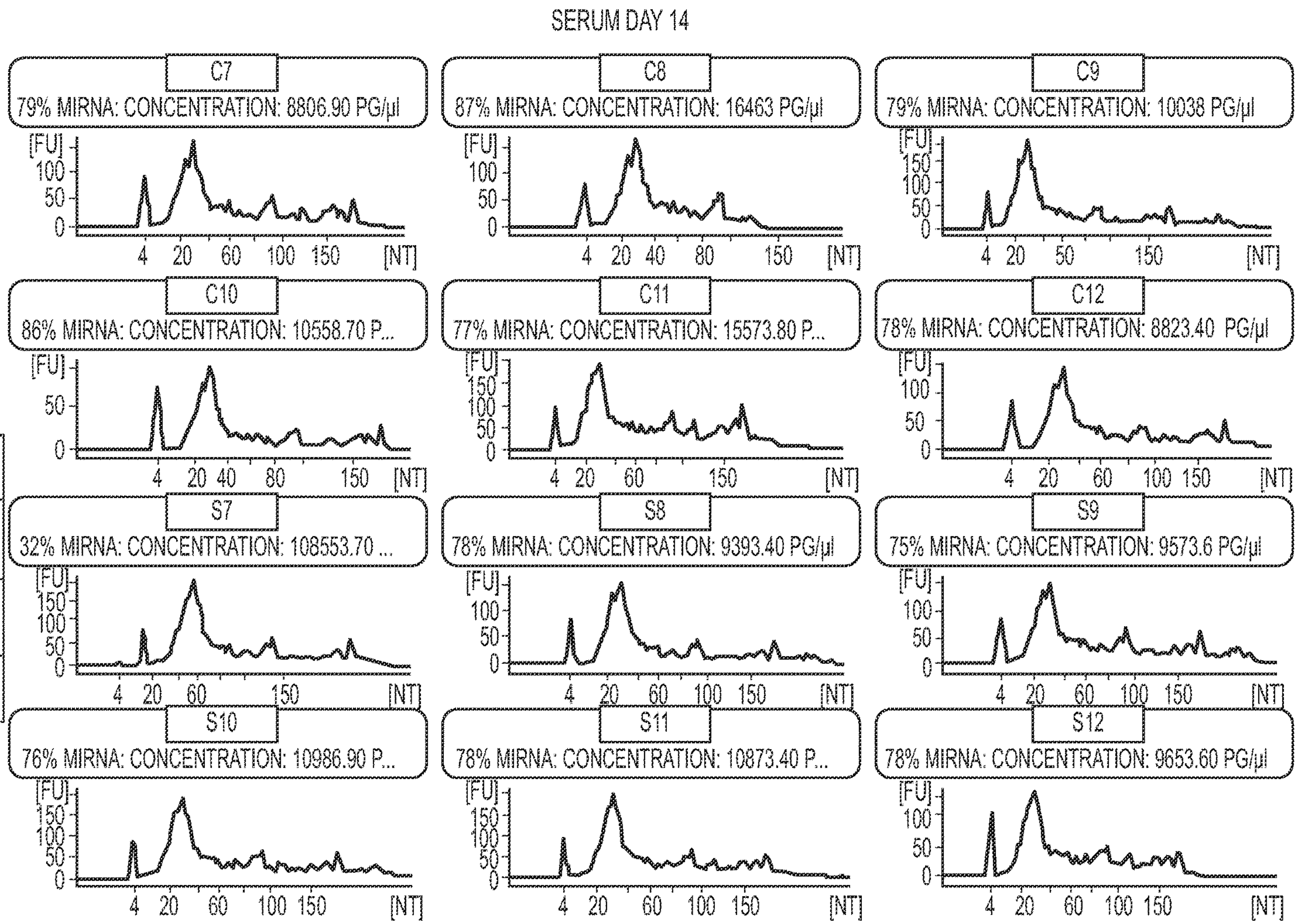

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

FIG. 5
CONT. 3

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

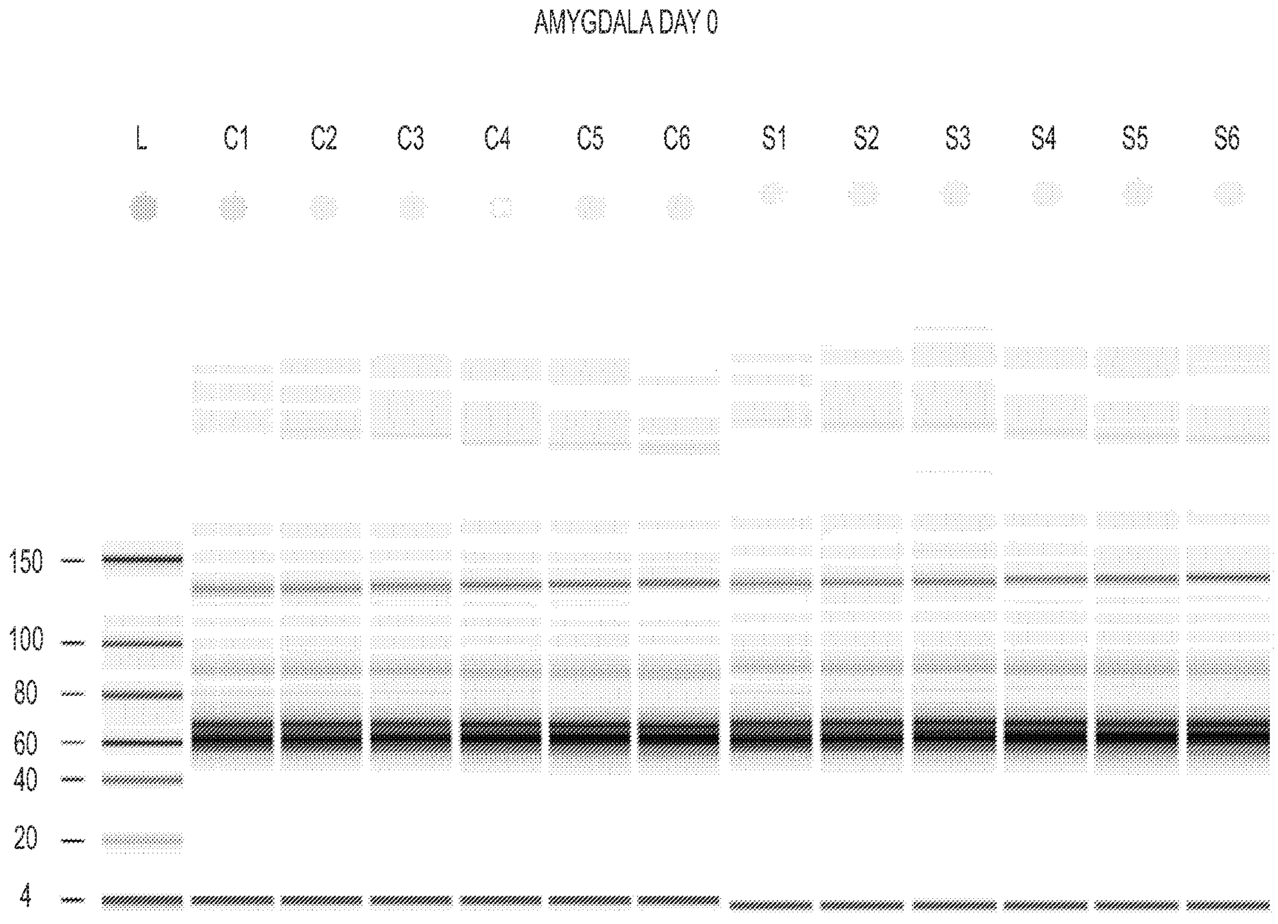

FIG. 5
CONT. 4

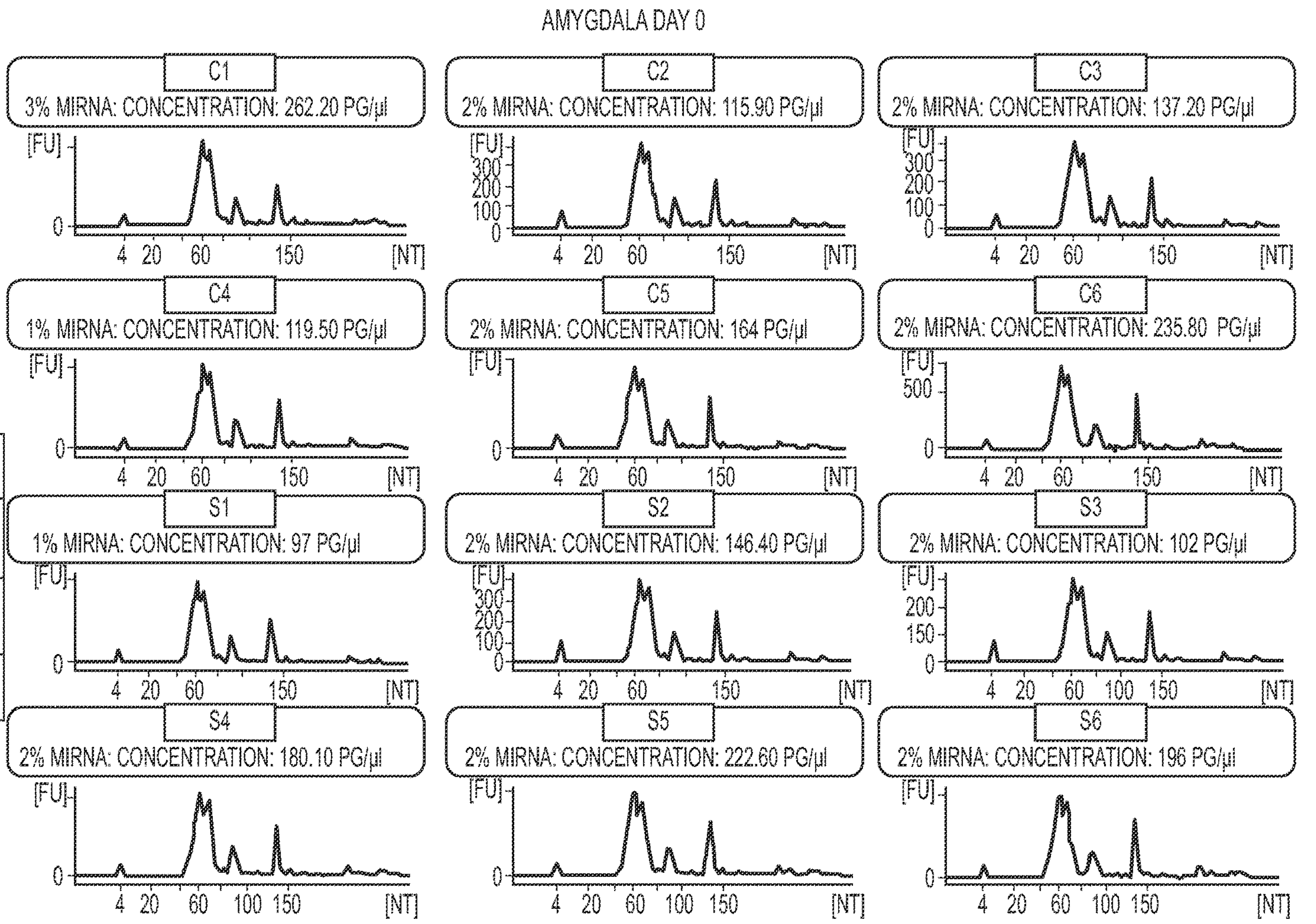

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

FIG. 5
CONT. 5

FIG.5	FIG.5 CONT. 2
FIG.5 CONT. 1	FIG.5 CONT. 3
FIG.5 CONT. 4	FIG.5 CONT. 6
FIG.5 CONT. 5	FIG.5 CONT. 7

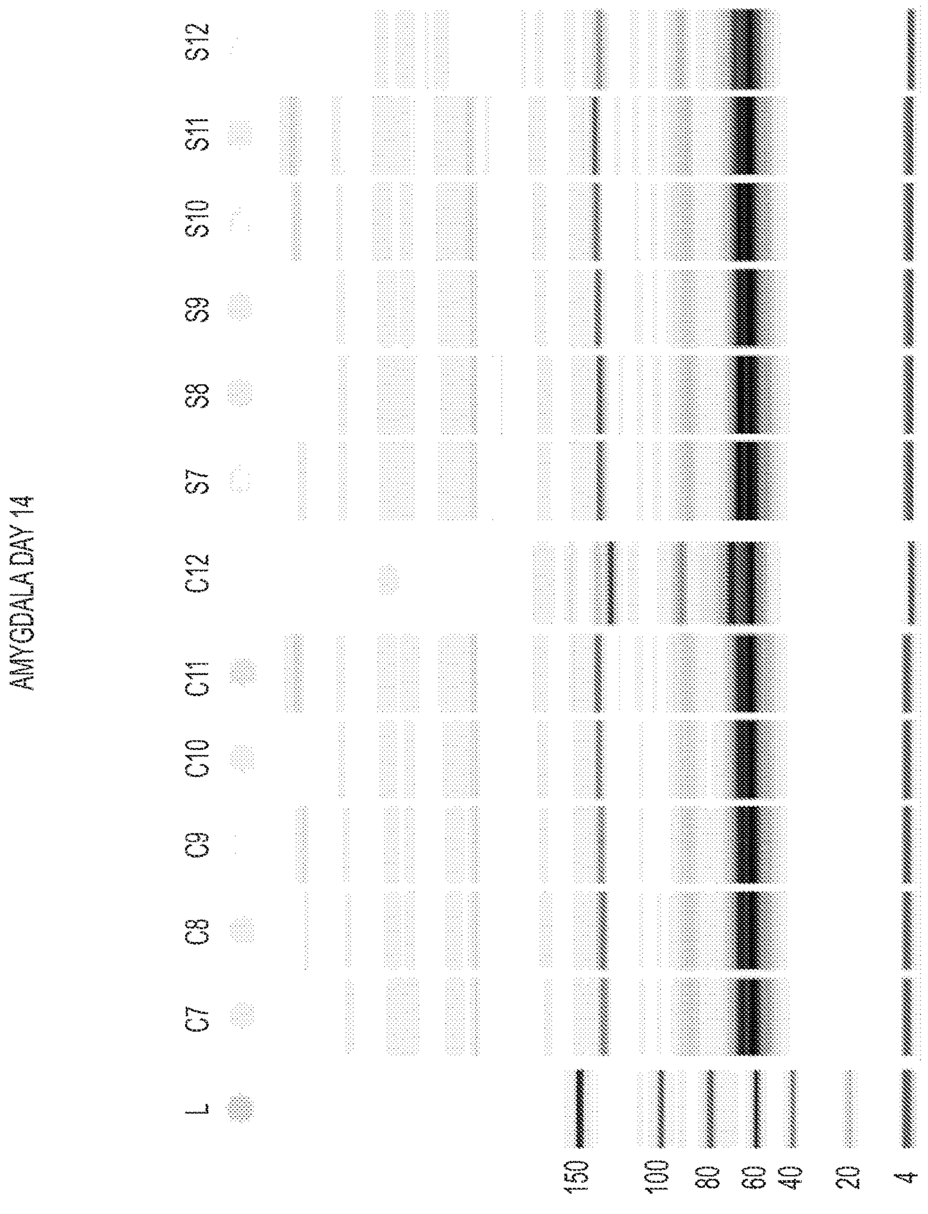
AMYGDALA DAY 14

FIG. 5
CONT. 6

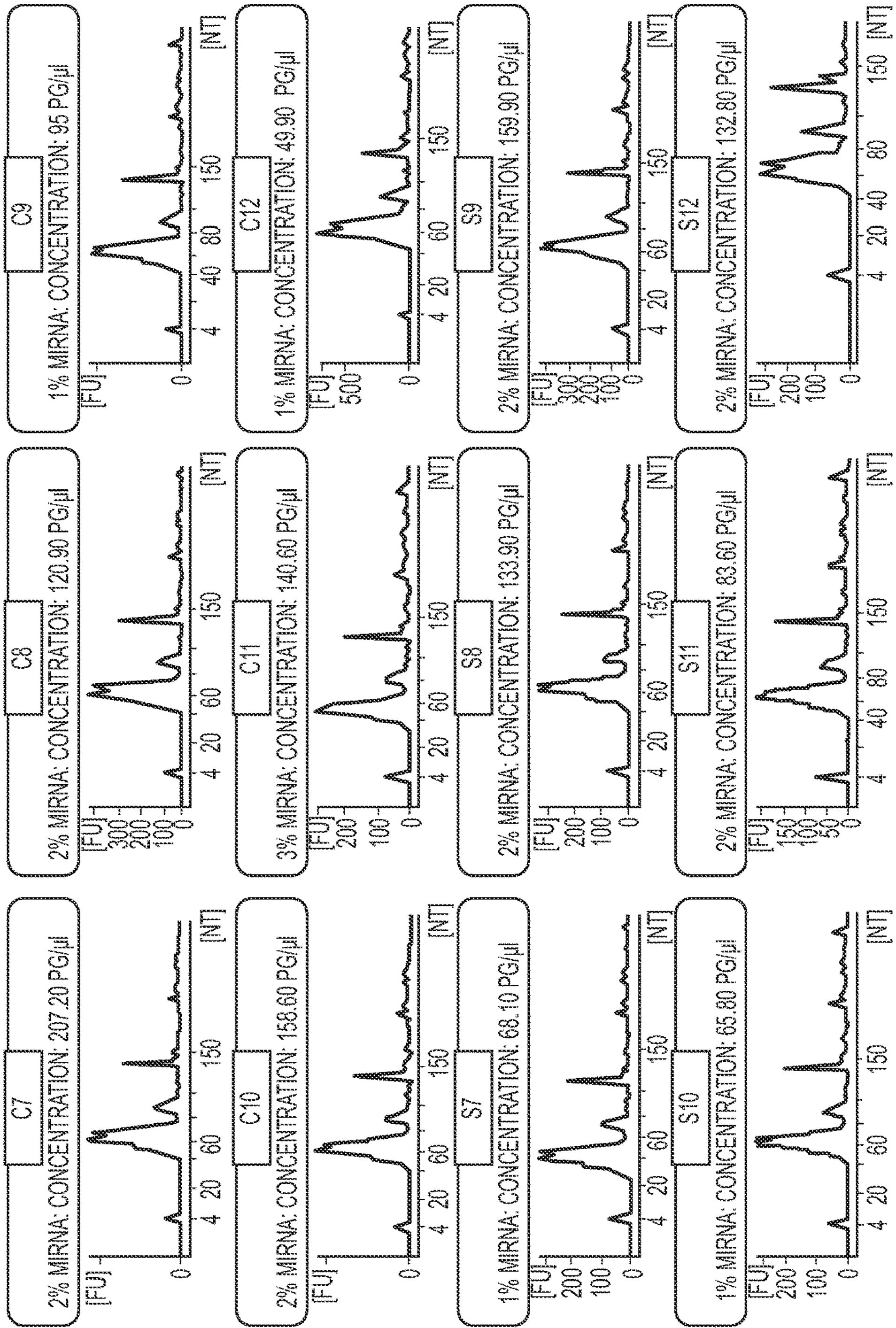

FIG. 5	FIG. 5 CONT. 2
FIG. 5 CONT. 1	FIG. 5 CONT. 3
FIG. 5 CONT. 4	FIG. 5 CONT. 6
FIG. 5 CONT. 5	FIG. 5 CONT. 7

FIG. 5 CONT. 7

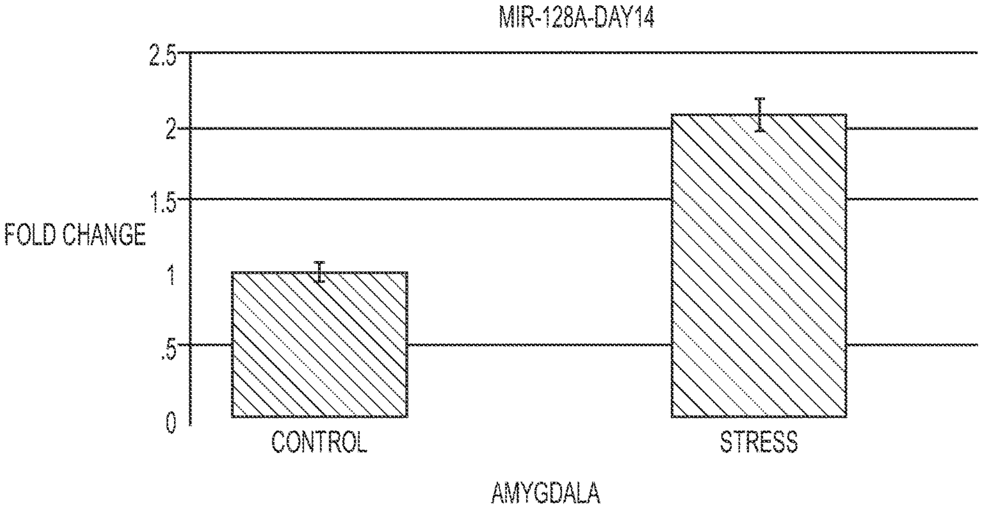
MIR-128A-DAY14
2.5
2
1.5
FOLD CHANGE
1
.5
0
CONTROL
STRESS
AMYGDALA

FIG. 6

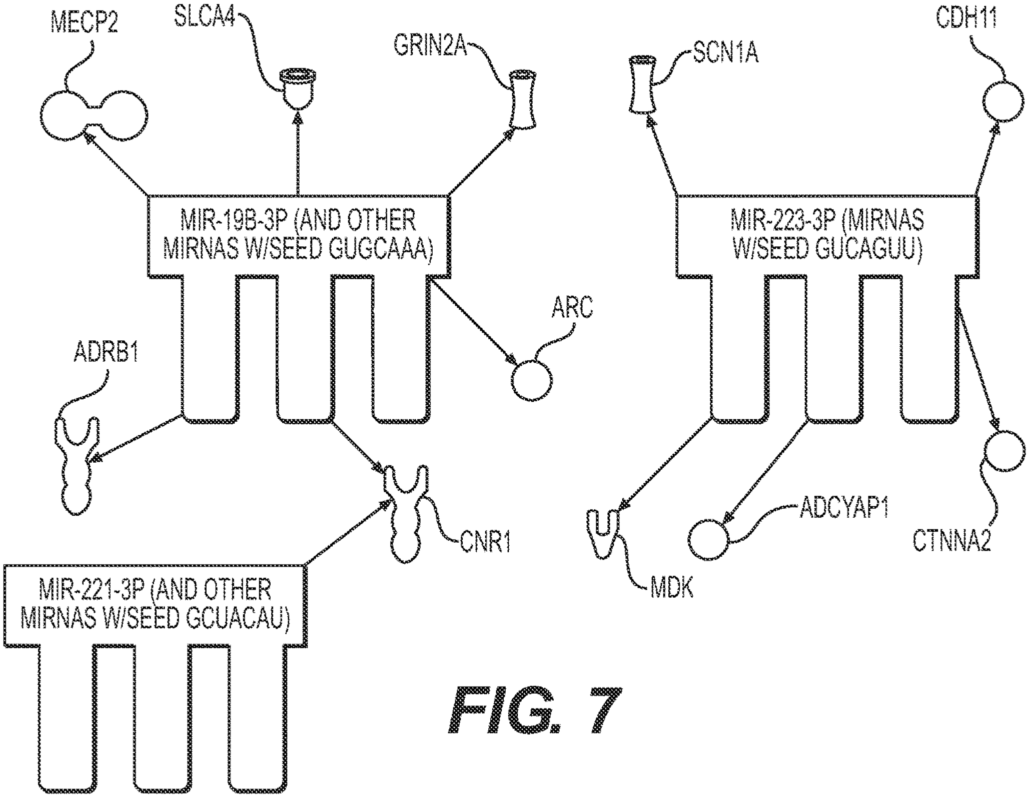
MECP2
SLCA4
GRIN2A
SCN1A
CDH11
MIR-19B-3P (AND OTHER MIRNAS W/SEED GUGCAAA)
MIR-223-3P (MIRNAS W/SEED GUCAGUU)
ARC
ADRB1
CNR1
MDK
ADCYAP1
CTNNA2
MIR-221-3P (AND OTHER MIRNAS W/SEED GCUACAU)

FIG. 7

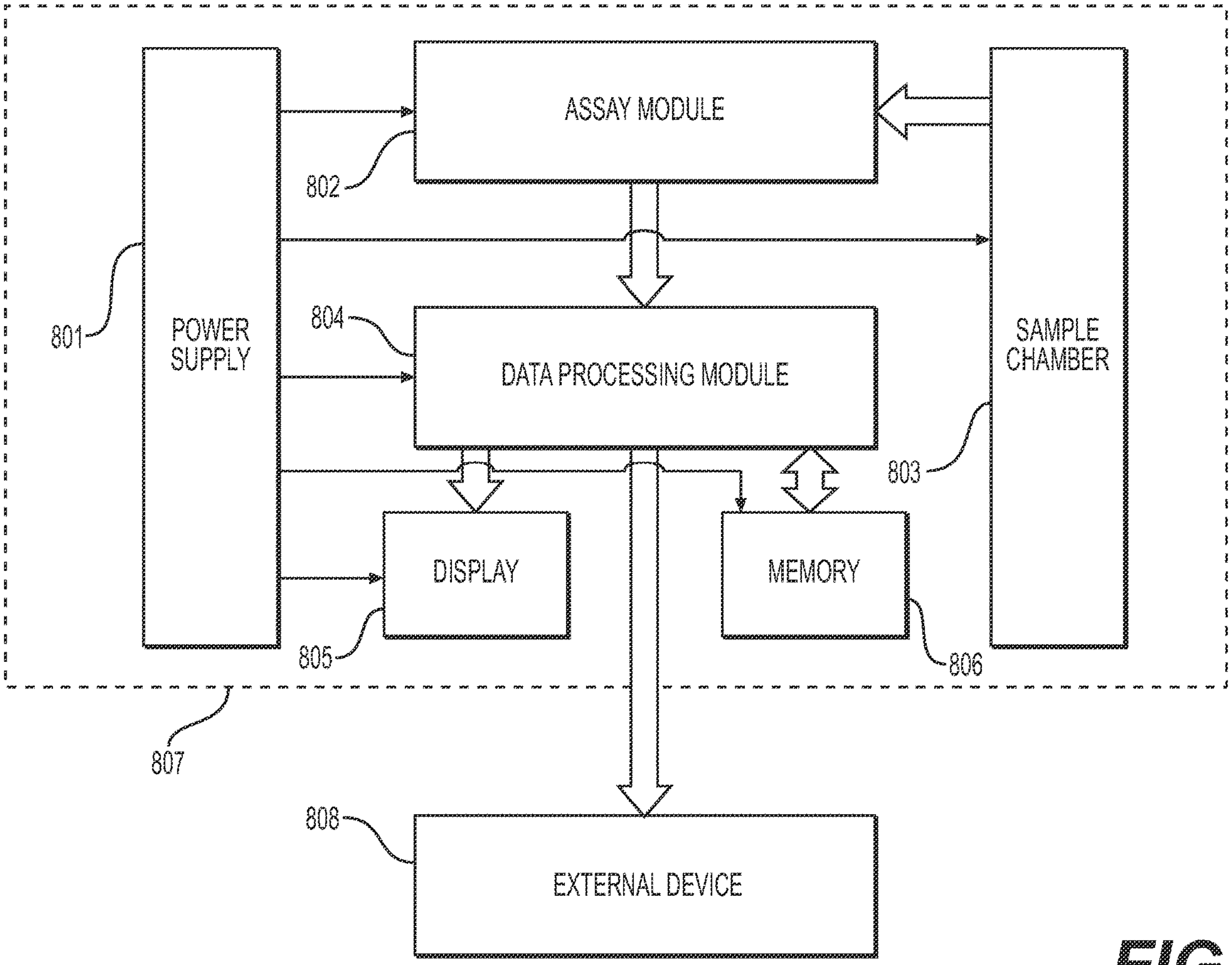
POWER SUPPLY
801
ASSAY MODULE
802
SAMPLE CHAMBER
803
DATA PROCESSING MODULE
804
DISPLAY
805
MEMORY
806
807
EXTERNAL DEVICE
808

FIG. 8

METHODS FOR THE MEASUREMENT OF POST-TRAUMATIC STRESS DISORDER MICRORNA MARKERS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority of U.S. Provisional patent application No. 61/982,651 filed on Apr. 22, 2014, the entire contents of which are incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was made with government support under HT9404-13-1-0003 awarded by the Uniformed Services University of the Health Sciences. The government has certain rights in the invention

SEQUENCE LISTING SUBMISSION VIA EFS-WEB

A computer readable text file, entitled "044508-5050-SequenceListing.txt," created on or about 24 Oct. 2016 with a file size of about 2.4 kb contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates in general to the reliable detection and identification of biomarkers produced in subjects suffering from post-traumatic stress disorder (PTSD). Inventive markers include DNA, RNA, or microRNA (μRNA) that may play a role in central nervous system function and therapy. In particular the invention relates to processes and kits for the detection and measurement of PTSD, μRNA biomarkers and administration of therapeutics for patients suffering from the disorder. In addition, the invention provides for an in vitro diagnostic device which enables the reliable detection and identification of biomarkers, important for the diagnosis and prognosis of PTSD and to serve as objective surrogate endpoints for therapy.

BACKGROUND OF THE INVENTION

Post-Traumatic Stress Disorder (PTSD) affects 7-8% of the general population of the United States and approximately 15% of veterans returning from combat. The symptoms can persist for months or decades. Unfortunately, PTSD is often misdiagnosed and left untreated in affected civilian and military individuals, disrupting the quality of their lives, their families and children, as well as our healthcare system.

PTSD is a severely disabling anxiety disorder which can occur after mild traumatic brain injury (TBI), a subject has seen or experienced a traumatic event that involved the threat of injury or death and which can be found clinically in acute or chronic forms. Relevant traumatic experiences include experiencing or witnessing childhood abuse, vehicle accidents, medical complications, physical assaults, natural disasters, jail, or war. The symptoms of PTSD include, but are not limited to, intrusion of recurrent nightmares or daytime flashbacks, characterized by high anxiety, hyperarousal, which is a constant jumpy preparation for fight or flight and avoidance of contact with anything or anyone that might remind the patient of the trauma. Acute PTSD may

resolve within 3-6 months, whereas chronic PTSD is a waxing and waning disorder that can persist for months, years, or decades. PTSD is often co-morbid with other psychiatric disorders, such as, but not limited to, depression, substance abuse, and suicidal thoughts.

Current diagnosis of PTSD is established on the basis of clinical history and subjective mental status examination, using a clinically structured interview, symptom checklists, or patient self-reports. These subjective tests, however, make it difficult to distinguish PTSD from other psychiatric disorders, resulting in difficult treatment decisions as to both treatment interventions and a more definitive understanding of the etiology. The existing limitations of current clinical assessment would benefit substantially from a more objective means to enhance the ability to identify PTSD in a patient and thus enabling the ability to differentiate PTSD from other psychiatric disorders in patients.

Treatments for PTSD include but are not limited to psychotherapy, such as but not limited to Cognitive Behavioral Therapy, pharmacotherapy, such as but not limited to serotonin-specific reuptake inhibitor (SSRI's). Many different pharmacological approaches have been investigated. For example, it is believed that Major Depressive Disorder (MDD) and PTSD have much in common, thus antidepressants, such as but not limited to the SSRI drugs fluoxitine (Prozac) and paroxatine (Paxil) are widely considered effective at treating some symptoms of PTSD. Other commonly administered SSRI antidepressants have included venlafaxine (Effexor) and sertraline (Zoloft).

Other commonly administered antipsychotics used to treat PTSD include but are not limited to mirtazapine (Remeron), olanzapine (Zyprexa) and quetiapione (Seroquel). The beta blocker propranolol has also been used to try to block memory formation in PTSD patients. Prazosin, an α_1-selective adrenoceptor antagonist, has been reported to reduce trauma-related nightmares and sleep disturbances associated with PTSD.

Because PTSD can only be diagnosed through a personal interview of a patient, where the patient may be cognizant to give answers they know to be correct, the current methods leave it difficult to diagnose subjects suffering from these disorders. As a result, a majority of PTSD cases are often missed, misdiagnosed or left untreated in thousands of affected individuals. To date there are no clinical methods for diagnosing PTSD because of the lack of reliability, specificity and cost efficacy.

Biomarkers are increasingly used to diagnose diseases promptly and accurately, and to identify individuals at high risk for certain conditions and tendencies even before clinical manifestations arise. There are presently no biomarkers to validate the diagnosis or to serve as objective surrogate endpoints for therapy for PTSD.

Micro RNAs (μRNAs) are small (~22 nucleotides) non-coding RNAs that can be posttranscriptional gene regulators for diverse biological processes. In circulation, μRNAs are considered as good biomarkers because they are highly stable in serum. Currently, however, there are no reports on the use of circulatory μRNAs as non-invasive biomarkers for the diagnosis of PTSD.

Despite today's technology with biomarker analysis, there remains an unmet need for prognostic indicators that can aid in the objective detection PTSD. In addition, there exists a need for a method of diagnosing PTSD, a need to monitor PTSD progression, a need for detecting PTSD prior to the onset of detectable symptoms and a need for clinical intervention with therapeutics. Finally, there remains for an unmet need for an in vitro diagnostic device to identify neurochemical markers to detect and/or diagnose PTSD.

SUMMARY OF THE INVENTION

A process for measuring for an amount of μRNA biomarkers is provided for the clinical evaluation of the levels of biomarkers of at least one of miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674*. In at least one embodiment, the invention is directed to clinical evaluation of the levels of biomarkers of mir-19b-3p, mir-223-3p and mir-421-3p. In one embodiment, the methods include obtaining at least one biological sample from a subject suspecting of having or being at risk of suffering from post-traumatic stress disorder (PTSD) or traumatic brain injury (TBI). In another embodiment, assessing levels of biomarker comprises the use of agents that specifically hybridize to each μRNA for quantitative PCR using amplification, hybridization, and/or sequencing methods.

The inventive process utilizes biological samples obtained at least thirteen days after a subject has been exposed to traumatic event likely to cause PTSD or TBI. In at least one embodiment a biological sample is obtained within one week after the subject presents with clinical symptoms of PTSD or TBI. In at least one embodiment, the biological samples are obtained within 24 hours after the subject presents with clinical symptoms of PTSD or TBI. In another embodiment, the biological samples are obtained within 24 hours after the subject experiences a traumatic episode. In at least one embodiment, exemplar biological samples include whole blood, plasma, serum, CSF, urine, saliva, sweat, prefrontal cortex tissue, hippocampus tissue, or ipsilateral cortex tissue.

In some embodiments of the inventive process, a therapeutic agent is administered to a subject and an additional biological sample is obtained some time after the therapeutic has been administered and the biological sample is measured for the RNA biomarkers. In some embodiments, the therapeutic may be administered if the quantities of the measured biomarkers are modulated with respect to an amount present in a normal control, or modulated with respect to the amounts measured in a historical biological sample of the subject, where the historical biological sample was taken from the subject at some time prior to the subject experiencing the traumatic event or receiving the traumatic brain injury. In at least one embodiment the therapeutic is an antidepressant, an antipsychotic, or combinations thereof. Exemplar antidepressant and antipsychotic therapeutics include fluoxitine (Prozac) and paroxatine (Paxil), venlafaxine (Effexor), sertraline (Zoloft), mirtazapine (Remeron), olanzapine (Zyprexa) and quetiapione (Seroquel), propranolol, or an α_1-selective adrenoceptor antagonist (Prazosin), or combinations thereof. In other embodiments, the administered therapeutic agent may be a therapeutically effective amount of a pharmaceutical composition including a pharmaceutically acceptable salt or ester for the treatment of PTSD or TBI, which may further include an anti-depressant or an antipsychotic.

In at least one embodiment successive biological samples are collected as a function of time, i.e., a sample is obtained at a second, third, fourth, etc. time point, and the biomarkers are measured in each sample to monitor for a change in the amount of the biomarkers present in the subject over time.

Other embodiments include a process of determining the presence of a post-traumatic stress disorder (PTSD) or traumatic brain injury (TBI) in a subject. These embodiments include collecting a biological sample from an affected subject suspected of suspected of having PTSD or TBI or presenting with clinical symptoms of PTSD or TBI, and measuring levels of at least one micro RNA (biomarker) selected from miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463*, miR-674*, and combinations thereof, and comparing the amount of the biomarker with a normal levels of the at least one micro RNA

Once the levels of the one or more micro RNAs have been determined, this determination can then be compared to normal levels or baseline levels of the one or more micro RNAs. “Normal levels” of the micro RNA may be assessed by measuring levels of the micro RNA in a known healthy subject, including the same subject that is later screened or being diagnosed. Normal levels may also be assessed over a population sample, where a population sample is intended to mean either multiple samples from a single subject or at least one sample from a multitude of subjects. The samples used to generate the population can be taken from previously harvested tissues that, for example, may be stored in paraffin or cryogenically stored. The population of samples can continually grow as additional samples are added to the population to gain statistical confidence in the data. Normal levels of the micro RNA, in terms of a population of samples, may or may not be categorized according to characteristics of the population including, but not limited to, sex, age, weight, ethnicity, geographic location, fasting state, state of pregnancy or post-pregnancy, menstrual cycle, general health of the subject, alcohol or drug consumption, caffeine or nicotine intake and circadian rhythms.

The invention is not limited by the means by which the biomarker micro RNAs are assessed. The assessment of the levels of the individual biomarkers can be expressed as absolute or relative values, such as but not limited to a concentration, and may or may not be expressed in relation to another component, such as a standard an internal standard or another molecule of compound known to be in the sample, such as but not limited to a ratio. If the levels are assessed as relative to a standard or internal standard, the standard may be added to the test sample prior to, during or after sample processing.

Of course, measurements of the individual biomarkers, e.g., concentration, can fall within a range of values, and values that do not fall within this “normal range” are said to be outside the normal range. These measurements may or may not be converted to a value, number, factor or score as compared to measurements in the “normal range.” For example, a measurement for a specific micro RNA that is below the normal range, may be assigned a value or −1, −2, −3, etc., depending on the scoring system devised.

In one embodiment, the collection of micro RNAs can be used to generate a “micro RNA profile value.” The profile can be a single value, number, factor or score given as an overall collective value to the individual components of the profile. For example, if each of the components is assigned a value, such as above, the profile value may simply be the overall score of each individual value. For example, if 9 components are used to generate the micro RNA profile and five of the components are assigned values of “−2” and four are assigned values of “−1,” the micro RNA profile value in this example would be −14, with a normal value being “0.” In this manner, the micro RNA profile value could be useful single number or score, the actual value or magnitude of which could be an indication of the actual risk of PTSD, e.g., the “more negative” the value, the greater the risk of suffering from PTSD.

Some embodiments include a kit to assist with the process of obtaining a biological sample and measuring for the amounts of the RNA biomarkers described herein. In at least one embodiment, the kit includes a substrate for holding a biological sample isolated from a human subject and an agent which specifically hybridizes to the micro RNA. In at least one embodiment the micro RNA the kit also includes printed instructions for reacting the agent with the sample or a portion of the sample to detect the presence or amount of the biomarker. In at least one embodiment, the amount of the measured biomarker is used for diagnosing the PTSD or TBI in the subject from whom the biological sample was obtained. In at least one embodiment the level of a micro RNA is detected using amplification, hybridization, and/or sequencing methods (e.g., quantitative PCR).

Further embodiments include an in vitro diagnostic device for measuring certain biomarkers. In some embodiments the in vitro diagnostic device is used for detecting PTSD or TBI in a subject. The inventive in vitro diagnostic devices include at least one sample chamber for holding a biological sample collected from the subject and an assay module in fluid communication with the sample chamber. In at least one embodiment the in vitro diagnostic device includes a power supply. In at least one embodiment an inventive in vitro diagnostic device includes a data processing module in operable communication with the power supply and the assay module where the assay module analyzes the biological sample to detect at least one of the biomarkers associated with PTSD or TBI present in the biological sample and electronically communicates a presence of the biomarker detected in the first biological sample to said data processing module to be displayed on an output. In at least one embodiment the data processing module has an output that relates to detecting the PTSD or TBI in the subject, the output being the amount of the biomarker measured, the presence or absence of PTSD or TBI, or the severity of PTSD or TBI. In some embodiments the output is a display in electrical communication with the data processing module communicating the output as an amount of the PTSD or TBI biomarker measured, a comparison between the amount of PTSD or TBI and a normal control or historical control, the presence of PTSD or TBI, or the severity of PTSD or TBI. In some embodiments, the in vitro diagnostic device includes a transmitter for communicating the output to a remote location. In an alternative embodiment, the in vitro diagnostic device includes a handheld sample chamber for holding a biological sample from the subject, an assay module in fluid communication with said sample chamber, and a dye providing a colorimetric change in response to at least one measured PTSD or TBI biomarker present in the biological sample.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates the validation of miR-223 expression in amygdala and serum samples of day 14. The levels of micro RNA were normalized by the level of MammU6 endogenous control RNA, and all reactions were performed in triplicate.

FIG. **2** illustrates overlapping micro RNAs data analysis for the modulated micro RNAs among the four traumatic stress groups was done using the online Venn diagram generation tool.

FIG. **3**A illustrates the top 10 functional pathways of posttraumatic stress altered day 14 serum and amygdala common micro RNAs and their validated targets from miR Walk database using Ingenuity pathway analysis program. While FIG. **3**B illustrates the top 10 canonical pathways of posttraumatic stress altered day 14 serum and amygdala common micro RNAs and their validated targets from miR Walk database using Ingenuity pathway analysis program.

FIG. **4** illustrates network analysis of posttraumatic stress altered day 14 serum and amygdala common micro RNAs and their fear related gene targets based on published literatures and available in Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems). The network correlation between micro RNAs and their targets relevant to fear response were custom-built using “my pathway” tool in IPA. Molecular functional network suggests that miR-223, miR-1928 (miR-221) may have direct role in STMN1 regulation.

FIG. **5** shows electronic gel and electropherogram images of small RNA quality checking before performing micro RNA expression experiments from the serum and amygdala samples of control and posttraumatic stress.

FIG. **6** illustrates validation of miR-128, expression in serum and amygdala samples of day 14. The levels of µRNA were normalized by the level of MammU6 endogenous control RNA, and all reactions were performed in triplicate.

FIG. **7** illustrates network analysis of posttraumatic stress altered day 14 serum and amygdala common micro RNAs and their fear related gene targets based on published literatures and available in ingenuity pathway analysis (IPA) which identifies the relationship of µRNAs towards a specific pathway by predicting the binding affinity of a µRNA with the proteins of the pathway. In addition, it also used the current literature to identify the role of micro RNAs in a specific pathway. Three micro RNAs had a direct interaction with genes regulating the stress and fear response. These micro RNAs were identified as mir-19b-3p, mir-223-3p and mir-221-3p. Mir-19b and mir-223 regulate proteins involved in regulation of both fear and stress response

FIG. **8** is a schematic view of the in vitro diagnostic device.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention has utility as processes, devices and biomarkers neural injuries, disorders and psychiatric or behavioral disorders such as PTSD and TBI thereby allowing for clinical intervention. The invention may further be used to detect neural injuries or neuronal disorders which the provided neural protein biomarkers may be comorbid.

The following detailed description is merely exemplary in nature and is in no way intended to limit the scope of the invention, its application, or uses, which may vary. The invention is described with relation to the non-limiting definitions and terminology included herein. These definitions and terminology are not designed to function as a limitation on the scope or practice of the invention, but are presented for illustrative and descriptive purposes only. Various terms used throughout the specification and claims are defined as set forth below as it may be helpful to an understanding of the invention.

“Marker” in the context of the present invention refers to Nucleic acids including micro RNA (µRNA), protein or breakdown product (BDP) or an antibody to one of the aforementioned that thereof is differentially present in a sample taken from patients having neural injury and/or psychiatric disorders as compared to a comparable sample taken from control subjects (e.g., a person with a negative diagnosis, normal or healthy subject) or from a historical value of the marker for the patient.

A "breakdown product" is defined as a fragment of a micro RNA or protein that is detectable and of sufficient size to correlate to the base micro RNA or protein.

The phrase "psychiatric disorder" is used herein in the broadest sense, and indicates a mental disorder that interferes with the way a person behaves, interacts with others, and functions in daily life. The Diagnostic and Statistical Manual (DSM) of Mental Disorders, published by the American Psychiatric Association, classifies psychiatric disorders such as PTSD, MDD, BP and SCZ.

The term "traumatic episode" refers to, with or without temporary or permanent injury, a near death experience, criminal assault, rape, natural disasters, serious accidents, combat exposure, child physical or sexual abuse or severe neglect, witnessing the death or destruction of someone or something, being exposed to events causing elevated levels of fear for a person's life or the lives of others, imprisonment/hostage/displacement as refugees, torture, or the sudden unexpected death of loved ones.

The terms "patient," "individual" or "subject" are used interchangeably herein, and is meant a mammalian subject to be treated, with human patients being one specific embodiment. In some cases, the processes of the invention find use in experimental animals, in veterinary application, and in the development of vertebrate models for disease, including, but not limited to, rodents including mice, rats, and hamsters; birds, fish reptiles, and primates.

The term "normal subject" refers to a mammalian subject, such as a human patient, that is not or has not suffered from neural injury manifest in psychiatric terms and does not have a history of past neural injuries or any psychiatric disorders.

The term "normal amount" refers to the amount of biomarkers measured from a normal subject.

The term "historical sample" refers to a biological sample taken from a subject prior to the exposure to a traumatic event, or prior to the manifestation of clinical symptoms of neural injuries or any psychiatric disorders.

The term "historical amount" refers to the amount of biomarkers measured from a historical sample.

"Biological Sample" is used herein includes polynucleotides, polypeptides, peptides, antibodies fragments and correlatable breakdown products and is a bodily fluid, a soluble fraction of a cell preparation, or media in which cells are grown; a chromosome, an organelle, or membrane isolated or extracted from a cell; genomic DNA, RNA, or cDNA, polypeptides, or peptides in solution or bound to a substrate; a cell; a tissue; a tissue print; a fingerprint; skin; or hair; and fragments of the aforementioned.

"Substrate" refers to any rigid or semi-rigid support to which nucleic acid molecules or proteins are bound and includes membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, capillaries or other tubing, plates, polymers, and microparticles with a variety of surface forms including wells, trenches, pins, channels and pores.

"Immunoassay" is an assay that uses an antibody to specifically bind an antigen or an antigen to bind an antibody (e.g., a marker). The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen. It should be appreciated that many immunoassays exist and could be used interchangeably with this invention.

As used herein, the term "Traumatic Brain Injury" or "TBI" is art recognized and is intended to include the condition in which, a traumatic blow to the head causes damage to the brain, often without penetrating the skull. Usually, the initial trauma can result in expanding hematoma, subarachnoid hemorrhage, cerebral edema, raised intracranial pressure (ICP), and cerebral hypoxia, which can, in turn, lead to severe secondary events due to low cerebral blood flow (CBF). Depending upon severity, TBI may also be classified as severe, mild or moderate.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised against marker NF-200 from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with marker NF-200 and not with other proteins, except for polymorphic variants and alleles of marker NF-200. This selection may be achieved by subtracting out antibodies that cross-react with marker NF-200 molecules from other species. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

As used herein, the term "in vitro diagnostic" means any form of diagnostic test product or test service, including but not limited to a FDA approved, or cleared, In Vitro Diagnostic (IVD), Laboratory Developed Test (LDT), or Direct-to-Consumer (DTC), that may be used to assay a sample and detect or indicate the presence of, the predisposition to, or the risk of, diseases, disorders, conditions, infections and/or therapeutic responses. In one embodiment, an in vitro diagnostic may be used in a laboratory or other health professional setting. In another embodiment, an in vitro diagnostic may be used by a consumer at home. In vitro diagnostic test comprise those reagents, instruments, and systems intended for use in the in vitro diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. In one embodiment in vitro diagnostic products may be intended for use in the collection, preparation, and examination of specimens taken from the human body. In certain embodiments, in vitro diagnostic tests and products may comprise one or more laboratory tests such as one or more in vitro diagnostic tests. As used herein, the term "laboratory test" means one or more medical or laboratory procedures that involve testing samples of blood, serum, plasma, CSF, sweat, saliva or urine, buccal sample or other human tissues or substances.

A nucleic acid probe or primer able to hybridize to a target biomarker micro RNA or is used for detecting and/or quantifying micro RNA encoding a biomarker protein for PTSD. A nucleic acid probe can be an oligonucleotide of at least 10, 15, 30, 50 or 100 nucleotides in length and sufficient to specifically hybridize under stringent conditions to the biomarker protein micro RNA or complementary sequence thereof. A nucleic acid primer can be an oligonucleotide of at least 10, 15 or 20 nucleotides in length and sufficient to specifically hybridize under stringent conditions to the micro RNA, or complementary sequence thereof.

"Complement" and "complementary" refers to Watson-Crick base pairing between nucleotides and specifically refers to nucleotides hydrogen bonded to one another with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds. In general, a nucleic acid includes a nucleotide sequence described as having a "percent complementarity" to a specified second nucleotide sequence. For example, a nucleotide sequence may have 80%, 90%, or 100% complementarity to a specified second nucleotide sequence, indicating that 8 of 10, 9 of 10 or 10 of 10 nucleotides of a sequence are complementary to the specified second nucleotide sequence. For instance, the nucleotide sequence 3'-TCGA-5' is 100% complementary to the nucleotide sequence 5'-AGCT-3'. Further, the nucleotide sequence 3'-TCGA- is 100% complementary to a region of the nucleotide sequence 5'-TTAGCTGG-3'.

"Hybridization" and "hybridizes" refer to pairing and binding of complementary nucleic acids. Hybridization occurs to varying extents between two nucleic acids depending on factors such as the degree of complementarity of the nucleic acids, the melting temperature, Tm, of the nucleic acids and the stringency of hybridization conditions, as is well known in the art.

"Stringency of hybridization conditions" refers to conditions of temperature, ionic strength, and composition of a hybridization medium with respect to particular common additives such as formamide and Denhardt's solution. Determination of particular hybridization conditions relating to a specified nucleic acid is routine and is well known in the art, for instance, as described in J. Sambrook and D. W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; and P. M. Ausubel, Ed., Short Protocols in Molecular Biology, Current Protocols; 5th Ed., 2002. High stringency hybridization conditions are those which only allow hybridization of substantially complementary nucleic acids. Typically, nucleic acids having about 85-100% complementarity are considered highly complementary and hybridize under high stringency conditions. Intermediate stringency conditions are exemplified by conditions under which nucleic acids having intermediate complementarity, about 50-84% complementarity, as well as those having a high degree of complementarity, hybridize. In contrast, low stringency hybridization conditions are those in which nucleic acids having a low degree of complementarity hybridize.

"Specific hybridization" and "specifically hybridizes" refer to hybridization of a particular nucleic acid to a target nucleic acid without substantial hybridization to nucleic acids other than the target nucleic acid in a sample.

Stringency of hybridization and washing conditions depends on several factors, including the Tm of the probe and target and ionic strength of the hybridization and wash conditions, as is well-known to the skilled artisan. Hybridization and conditions to achieve a desired hybridization stringency are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2001; and Ausubel, F. et al., (Eds.), Short Protocols in Molecular Biology, Wiley, 2002.

An example of high stringency hybridization conditions is hybridization of nucleic acids over about 100 nucleotides in length in a solution containing Denhardt's solution and related chemistry such as 30% formamide incubated at 37° C. overnight followed by conventional washing.

PTSD and TBI Biomarker Processes

A process for measuring for an amount of micro RNA biomarkers is provided for the simultaneous clinical evaluation of the levels of biomarkers of miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674*. It should be appreciated that additional markers selected from Tables 1-3, illustrated herein, are also suitable micro RNA candidates and may be used in addition to, or as a substitution of any of the aforementioned micro RNAs. In at least one embodiment simultaneous clinical evaluation of the levels of biomarkers of miR-19b-3p, miR-223-3p and miR-221-3p is provided. In another embodiment measuring for the quantity of at least one micro RNA biomarker selected from miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* is provided. In some embodiments the process includes the administration of a therapeutic agent after a biological sample is obtained from a subject presenting with symptoms of PTSD or TBI. Measurements of the biomarkers are accomplished by obtaining at least one biological sample at a first time point from a subject presenting with clinical symptoms of a post-traumatic stress disorder (PTSD) or traumatic brain injury (TBI). In at least one embodiment measurement of the biomarkers is accomplished by using an agent which specifically hybridizes to each micro RNA on a quantitative PCR using amplification, hybridization, and/or sequencing methods.

The inventive process utilizes biological samples obtained at least thirteen days after a subject has been exposed to traumatic event likely to cause PTSD or TBI. In at least one embodiment a biological sample is obtained within one week after the subject presents with clinical symptoms of PTSD or TBI. In another embodiment, the sample is obtained after one week that the subject exhibits clinical symptoms of PTSD and/or TBI. In at least one embodiment, the biological samples are obtained within 24 hours after the subject presents with clinical symptoms of PTSD or TBI. In another embodiment, the biological samples are obtained within 24 hours after the subject experiences a traumatic episode. In at least one embodiment at least two biological samples are taken from a subject after presenting with clinical symptoms of PTSD or TBI. In some embodiments the at least two samples are obtained within 24 hours of the subjects clinical presence, while in other embodiments the at least two samples are obtained within two weeks of the subjects clinical presence. Exemplar biological samples for practicing the inventive process include whole blood, plasma, serum, CSF, urine, saliva, sweat, prefrontal cortex tissue, hippocampus tissue, or ipsilateral cortex tissue. It is appreciated that several methods exist for obtaining the aforementioned biological samples and are well known in the art and incorporated herein.

In some embodiments of the inventive process, a therapeutic agent is administered to a subject and an additional biological sample is obtained some time after the therapeutic has been administered and the biological sample is measured for the micro RNA biomarkers. In some embodiments, the therapeutic may be administered if the quantities of the measured biomarkers are modulated with respect to an amount present in a normal control, or modulated with respect to the amounts measured in a historical biological sample of the subject, where the historical biological sample was taken from the subject at some time prior to the subject experiencing the traumatic event or receiving the traumatic brain injury.

In at least one embodiment the therapeutic is an antidepressant, an antipsychotic, or combinations thereof. Exemplar antidepressant and antipsychotic therapeutics include fluoxitine (Prozac) and paroxatine (Paxil), venlafaxine (Effexor), sertraline (Zoloft), mirtazapine (Remeron), olanzapine (Zyprexa) and quetiapione (Seroquel), propranolol, or an al-selective adrenoceptor antagonist (Prazosin), or combinations thereof. In other embodiments, the administered therapeutic may be a therapeutically effective amount of a pharmaceutical composition including a pharmaceutically acceptable salt or ester for the treatment of PTSD or TBI, which may further include an anti-depressant or an antipsychotic.

Other embodiments include a process of determining the presence of a post-traumatic stress disorder (PTSD) or traumatic brain injury (TBI) in a subject. These embodiments include collecting a biological sample at a first time point from an affected subject suspected of having PTSD or TBI or presenting with clinical symptoms of PTSD or TBI, measuring the biological sample for an amount of at least one biomarker selected from miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674*, or combinations thereof, and comparing the amount of the biomarker with a normal amount of the biomarker measured in a normal subject not having PTSD or TBI. In at least one embodiment the measured levels of biomarkers in the affected subject are compared with a historical amount of the biomarkers measured in a historical biological sample from the subject prior to the subject being affected with the PTSD or TBI. Differential between the measured amount of biomarkers from the affected subject when compared to the normal levels or the historical levels is indicative of PTSD or TBI in the affected subject. In at least one embodiment, a plurality of markers are measured selected from the group consisting of miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674*. In at least one embodiment, the biomarkers miR-19b-3p, miR-223-3p and miR-221-3p are measured from the same sample and compared with normal amounts and/or historical amounts.

In additional embodiments, biological samples are collected and analyzed at least at a second time point to monitor the progression of PTSD and TBI in the subject over time.

In other embodiments, a therapeutic agent is administered to treat the PTSD or TBI and biological samples are collected and analyzed at least at a second time point to monitor the effectiveness of the therapeutic agent in treating PTSD and TBI in the subject over time. In at least one embodiment the µRNAs are detected by Northern blot.

In Vitro Diagnostic Device

FIG. **8** schematically illustrates the inventive in vitro diagnostic device. An inventive in vitro diagnostic device includes at least a sample collection chamber **803** and an assay module **802** used to detect biomarkers of psychiatric disorders. The in vitro diagnostic device may be a handheld device, a bench top device, or a point of care device.

The sample chamber **803** can be of any sample collection apparatus known in the art for holding a biological fluid. In one embodiment, the sample collection chamber can accommodate any one of the biological fluids herein contemplated, such as whole blood, plasma, serum, CSF, urine, saliva, sweat, buccal sample, prefrontal cortex tissue, hippocampus tissue, or ipsilateral cortex tissue.

The assay module **802** is preferably comprised of an assay which may be used for detecting RNA in a biological sample, for instance, through the use of probes and hybridization buffers in an immunoassay. In some embodiments the probes are labeled, such as radiolabeled. The assay module **802** may include of any assay currently known in the art; however the assay should be optimized for the detection of the micro RNA biomarkers used for detecting neural injuries, neuronal disorders or psychiatric disorders in a subject. The assay module **802** is in fluid communication with the sample collection chamber **803**. In one embodiment, the assay module **802** is comprised of an immunoassay where the immunoassay may be any one of a radioimmunoassay, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassay, immunoprecipitation assay, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assay, fluorescent immunoassay, chemiluminescent immunoassay, phosphorescent immunoassay, or an anodic stripping voltammetry immunoassay. In one embodiment a colorimetric assay may be used which may comprise only of a sample collection chamber **803** and an assay module **802** of the assay. Although not specifically shown these components are preferably housed in one assembly **807**. In one embodiment the assay module **802** contains agents specific for measuring miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* or any combination, fragment or breakdown product thereof. In another embodiment the assay module **802** contains reagents specific for measuring miR-19b-3p, miR-223-3p and miR-221-3p or any combination, fragment or breakdown product thereof. Still, in other embodiments the assay module **802** contains agents specific for measuring at least one micro RNA of miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* or any combination, fragment or breakdown product thereof. The assay module **802** may contain additional agents to detect additional biomarkers, as is described herein. Due to the co-morbidity of the PTSD with TBI, the inventive IVD may also measure the same biomarkers to correlate the presence or amount of the biomarkers with the presence and severity of TBI.

In another preferred embodiment, the inventive in vitro diagnostic device contains a power supply **801**, an assay module **802**, a sample chamber **803**, and a data processing module **805**. The power supply **801** is electrically connected to the assay module and the data processing module. The assay module **802** and the data processing module **805** are in electrical communication with each other. As described above, the assay module **802** may be comprised of any assay currently known in the art; however the assay should be optimized for the detection of neural biomarkers used for detecting neural injury, neuronal disorder or psychiatric disorders in a subject. The assay module **802** is in fluid communication with the sample collection chamber **803**. The assay module **802** is comprised of an immunoassay where the immunoassay may be any one of a radioimmunoassay, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassay, immunoprecipitation assay, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assay, fluorescent immunoassay, chemiluminescent immunoassay, phosphorescent immunoassay, or an anodic stripping voltammetry immunoassay. A biological sample is placed in the sample chamber **803** and assayed by the assay module **802** detecting for a biomarker of psychiatric disorder. The measured amount of the biomarker by the assay module **802** is then electrically communicated to the data processing module **804**. The data processing **804** module may comprise of any known data processing element known in the art, and may comprise of a chip, a central processing unit (CPU), or a software package which processes the information supplied from the assay module **802**.

In one embodiment, the data processing module **804** is in electrical communication with a display **805**, a memory device **806**, or an external device **808** or software package (such as laboratory and information management software (LIMS)). In one embodiment, the data processing module **804** is used to process the data into a user defined usable format. This format comprises of the measured amount of neural biomarkers detected in the sample, indication that a neural injury, neuronal disorder, or psychiatric disorder is present, or indication of the severity of the neural injury, neuronal disorder or psychiatric disorder. The information from the data processing module **804** may be illustrated on the display **805**, saved in machine readable format to a memory device, or electrically communicated to an external device **808** for additional processing or display. Although not specifically shown these components are preferably housed in one assembly **807**. In one embodiment, the data processing module **804** may be programmed to compare the detected amount of the biomarker transmitted from the assay module **802**, to a comparator algorithm. The comparator algorithm may compare the measure amount to the user defined threshold which may be any limit useful by the user. In one embodiment, the user defined threshold is set to the amount of the biomarker measured in control subject, or a statistically significant average of a control population.

The methods and in vitro diagnostic tests described herein may indicate diagnostic information to be included in the current diagnostic evaluation in patients suspected of having neural injury, neuronal disorder or psychiatric disorder. In another embodiment, the methods and in vitro diagnostic tests described herein may be used for screening for risk of progressing from at-risk, non-specific symptoms possibly associated with psychiatric disorders, and/or fully-diagnosed psychiatric disorders. In certain embodiments, the methods and in vitro diagnostic tests described herein can be used to rule out screening of diseases and disorders that share symptoms with psychiatric disorder.

In one embodiment, an in vitro diagnostic test may comprise one or more devices, tools, and equipment configured to hold or collect a biological sample from an individual. In one embodiment of an in vitro diagnostic test, tools to collect a biological sample may include one or more of a swab, a scalpel, a syringe, a scraper, a container, and other devices and reagents designed to facilitate the collection, storage, and transport of a biological sample. In one embodiment, an in vitro diagnostic test may include reagents or solutions for collecting, stabilizing, storing, and processing a biological sample. Such reagents and solutions for nucleotide collecting, stabilizing, storing, and processing are well known by those of skill in the art and may be indicated by specific methods used by an in vitro diagnostic test as described herein. In another embodiment, an in vitro diagnostic test as disclosed herein, may comprise a micro array apparatus and reagents, a flow cell apparatus and reagents, a multiplex nucleotide sequencer and reagents, and additional hardware and software necessary to assay a genetic sample for certain genetic markers and to detect and visualize certain biological markers.

Biomarkers

The present invention provides a process to detect micro RNAs, for the detection of psychiatric disorders, for example PTSD. These same micro RNAs may also be used to detect neural injuries and neuronal disorders, such as TBI, which is often comorbid with many psychiatric disorders. In one embodiment, at least one, more than one, or all micro RNAs specific to PTSD are detected and is selected from: miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* or any combination, fragment or breakdown product thereof. In at least one embodiment that real-time polymerase chain reaction (PCR) measures the micro RNA level of the biomarker in a biological sample taken from a patient presenting with symptoms of PTSD or TBI and compared with levels of the micro RNA in samples from normal patients or historical levels of the patient. Without being bound to any particular theory, and without limiting the invention to these particular μRNAs, Table 1 provides at least one embodiment of exemplary micro RNA biomarkers as a result of their modulation after a PTSD inducing event is experienced by a subject, and detected fourteen (14) days after a trauma inducing event.

TABLE 1

					Serum Day 14		Amygdala Day 14	
S#	TLDA ID	MicroRNA Symbol	MirBase ID	Mature Sequence	Fold change	P value	Fold change	P value
1	mmu-miR-142-5p-002248	rno-miR-142-5p	MIMAT0000847	cauaaaguagaaagcacuacu	2.95	0.029	2.1	0.001
2	mmu-miR-19b-000396	rno-miR-19b-3p	MIMAT0000788	ugugcaaauccaugcaaaacuga	3.13	0.018	2.37	0
3	mmu-miR-1928-121164_mat	rno-miR-221-3p	MIMAT0000890	agcuacauugucugcugguuuc	11.23	0.001	7.85	0
4	mmu-miR-223-002295	rno-miR-223-3p	MIMAT0000892	ugucaguuugucaaauacccc	4.25	0.001	2.16	0.033
5	mmu-miR-322#-002506	rno-miR-322-3p	MIMAT0000547	aaacaugaagcgcugcaaca	2.25	0.048	2	0.013
6	mmu-miR-324-3p-002509	rno-miR-324-3p	MIMAT0000554	ccacugccccaggugcugcugg	2.06	0.015	2.42	0.007

TABLE 1-continued

					Serum Day 14		Amygdala Day 14	
S#	TLDA ID	MicroRNA Symbol	MirBase ID	Mature Sequence	Fold change	P value	Fold change	P value
7	hsa-miR-421-002700	rno-miR-421-3p	MIMAT0017175	aucaacagacauuaauuggg	3.96	0.001	2.1	0.009
8	mmu-miR-463#-002582	rno-miR-463-5p	MIMAT0017309	uaccuaauuuguuguccauca	9.97	0.006	3.16	0.01
9	mmu-miR-674#-001956	rno-miR-674-3p	MIMAT0005330	cacagcucccaucucagaacaa	2.3	0.037	2.22	0.016

Other micro RNAs may alternatively be used. Table 2 provides PTSD potential biomarker micro RNA candidates experimentally validated targets from miRWalk database suitable for the diagnostics of PTSD or TBI.

TABLE 2

S#	MicroRNA Name	StemLoop Name	miR_Chr	Gene Name	EntrezID	Pubmed ID
1	rno-miR-322*	rno-mir-322	X	Egfr	24329	17889671
2	rno-miR-322*	rno-mir-322	X	MBP_RAT	24547	20215419
3	rno-miR-223	rno-mir-223	X	Stx1a	116470	18258830
4	rno-miR-223	rno-mir-223	X	Akt1	24185	19074548
5	rno-miR-223	rno-mir-223	X	Igf1r	25718	22425712
6	rno-miR-223	rno-mir-223	X	Blr1	29363	22984081
7	rno-miR-223	rno-mir-223	X	Fgf16	60464	18258830
8	rno-miR-223	rno-mir-223	X	Mmp9	81687	18258830
9	rno-miR-223	rno-mir-223	X	NOTC1_RAT	25496	20826802
10	rno-miR-223	rno-mir-223	X	Adora1	29290	18258830
11	rno-miR-223	rno-mir-223	X	Scn3a	497770	18258830
12	rno-miR-223	rno-mir-223	X	Itch	311567	19074548
13	rno-miR-223	rno-mir-223	X	Frap1	56718	22425712
14	rno-miR-223	rno-mir-223	X	Cd4	24932	23153510
15	rno-miR-223	rno-mir-223	X	Capn8	170808	18258830
16	rno-miR-223	rno-mir-223	X	Kcnj16	29719	18258830
17	rno-miR-223	rno-mir-223	X	Kitl	60427	20826802
18	rno-miR-223	rno-mir-223	X	CPG2	499010	18258830
19	rno-miR-223	rno-mir-223	X	Gad1	24379	18258830
20	rno-miR-223	rno-mir-223	X	Zap70	301348	19144983
21	rno-miR-223	rno-mir-223	X	Cd4	24932	22527633
22	rno-miR-223	rno-mir-223	X	Bcl2	24224	23208072
23	rno-miR-223	rno-mir-223	X	Dhcr24	298298	18258830
24	rno-miR-223	rno-mir-223	X	Runx1	50662	18416028
25	rno-miR-223	rno-mir-223	X	Frap1	56718	20826802
26	rno-miR-223	rno-mir-223	X	Ptges	59103	18258830
27	rno-miR-223	rno-mir-223	X	Fgfr1	79114	18258830
28	rno-miR-223	rno-mir-223	X	Lmo2	362176	19278969
29	rno-miR-223	rno-mir-223	X	Tnf	24835	22562984
30	rno-miR-223	rno-mir-223	X	Tra1_predicted	362862	23208072
31	rno-miR-223	rno-mir-223	X	Madd	94193	18258830
32	rno-miR-223	rno-mir-223	X	Stmn1	29332	18555017
33	rno-miR-223	rno-mir-223	X	Zap70	301348	20862275
34	rno-miR-223	rno-mir-223	X	Vsnl1	24877	18258830
35	rno-miR-223	rno-mir-223	X	Il6	24498	22959936
36	rno-miR-223	rno-mir-223	X	Dclk1	83825	18258830
37	rno-miR-223	rno-mir-223	X	Cd4	24932	19297609
38	rno-miR-223	rno-mir-223	X	Akt1	24185	23208072
39	rno-miR-223	rno-mir-223	X	Klf15	85497	18258830
40	rno-miR-223	rno-mir-223	X	Ifng	25712	18791161
41	rno-miR-223	rno-mir-223	X	NP_001102651.1	499593	21109969
42	rno-miR-223	rno-mir-223	X	Golph3	78961	18258830
43	rno-miR-223	rno-mir-223	X	Casp4	114555	22959936
44	rno-miR-223	rno-mir-223	X	Neurod1	29458	18258830
45	rno-miR-223	rno-mir-223	X	Frap1	56718	23208072
46	rno-miR-223	rno-mir-223	X	Slc17a7	116638	18258830
47	rno-miR-223	rno-mir-223	X	Rhob	64373	19850724
48	rno-miR-223	rno-mir-223	X	Bcl2	24224	17260024
49	rno-miR-223	rno-mir-223	X	Nos2	24599	18791161
50	rno-miR-223	rno-mir-223	X	NP_001099865.1	294515	21926415
51	rno-miR-223	rno-mir-223	X	Nol3	85383	18258830
52	rno-miR-223	rno-mir-223	X	LOC685953	29184	22959936
53	rno-miR-223	rno-mir-223	X	Itgb1	24511	18258830

TABLE 2-continued

S#	MicroRNA Name	StemLoop Name	miR_Chr	Gene Name	EntrezID	Pubmed ID
54	rno-miR-223	rno-mir-223	X	Mgst1	171341	18258830
55	rno-miR-223	rno-mir-223	X	Sars1	266975	19915717
56	rno-miR-223	rno-mir-223	X	Runx1	50662	17996649
57	rno-miR-223	rno-mir-223	X	Hyou1	192235	18258830
58	rno-miR-223	rno-mir-223	X	Cd4	24932	19014482
59	rno-miR-223	rno-mir-223	X	Smad7	81516	21940491
60	rno-miR-223	rno-mir-223	X	Il10	25325	22959936
61	rno-miR-223	rno-mir-223	X	Vim	81818	18258830
62	rno-miR-223	rno-mir-223	X	Gpd1	60666	18258830
63	rno-miR-223	rno-mir-223	X	Cd4	24932	19931339
64	rno-miR-223	rno-mir-223	X	Aqp4	25293	18258830
65	rno-miR-223	rno-mir-223	X	Akap6	64553	18258830
66	rno-miR-223	rno-mir-223	X	Lmo2	362176	19017354
67	rno-miR-223	rno-mir-223	X	Clec4d	362432	22145958
68	rno-miR-223	rno-mir-223	X	Tnf	24835	22959936
69	rno-miR-223	rno-mir-223	X	Ogt	26295	18258830
70	rno-miR-223	rno-mir-223	X	Gnb1	24400	18258830
71	rno-miR-223	rno-mir-223	X	Slc2a4	25139	20080987
72	rno-miR-223	rno-mir-223	X	Syt4	64440	18258830
73	rno-miR-223	rno-mir-223	X	Tagln	25123	18258830
74	rno-miR-223	rno-mir-223	X	Lmo2	362176	19047678
75	rno-miR-223	rno-mir-223	X	NOTC1__RAT	25496	22424712
76	rno-miR-223	rno-mir-223	X	Fos	314322	22959936
77	rno-miR-223	rno-mir-223	X	Tpm1__v7	24851	18258830
78	rno-miR-223	rno-mir-223	X	Mapre1	114764	18258830
79	rno-miR-223	rno-mir-223	X	Cd4	24932	20448109
80	rno-miR-223	rno-mir-223	X	Hmox1	24451	18258830
81	rno-miR-223	rno-mir-223	X	Acvr1	79558	18258830
82	rno-miR-223	rno-mir-223	X	Itgam	25021	19059913
83	rno-miR-223	rno-mir-223	X	Igf1r	25718	22424712
84	rno-miR-223	rno-mir-223	X	Scd2	83792	22959936
85	rno-miR-223	rno-mir-223	X	Mapk1	116590	18258830
86	rno-miR-223	rno-mir-223	X	Nr4a1	79240	18258830
87	rno-miR-223	rno-mir-223	X	NP__001102651.1	499593	20676373
88	rno-miR-223	rno-mir-223	X	Gmfb	81661	18258830
89	rno-miR-221	rno-mir-221	X	Zbtb16	353227	18417445
90	rno-miR-221	rno-mir-221	X	Zfhx1b	311071	20516212
91	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19767219
92	rno-miR-221	rno-mir-221	X	Met	24553	21537871
93	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19150885
94	rno-miR-221	rno-mir-221	X	NP__001028929.1	246060	20975375
95	rno-miR-221	rno-mir-221	X	Pten	50557	20021821
96	rno-miR-221	rno-mir-221	X	Icam1	25464	22535415
97	rno-miR-221	rno-mir-221	X	BIM__RAT	64547	19438724
98	rno-miR-221	rno-mir-221	X	Agt	24179	21310411
99	rno-miR-221	rno-mir-221	X	Mycn	298894	17943719
100	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20428775
101	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	18417445
102	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20547861
103	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19859555
104	rno-miR-221	rno-mir-221	X	Cd4	24932	21788445
105	rno-miR-221	rno-mir-221	X	NP__001028929.1	246060	19150885
106	rno-miR-221	rno-mir-221	X	NP__001102171.1	362686	21076613
107	rno-miR-221	rno-mir-221	X	Kras	24525	20093556
108	rno-miR-221	rno-mir-221	X	Tnf	24835	22562984
109	rno-miR-221	rno-mir-221	X	Mapk3	50689	19438724
110	rno-miR-221	rno-mir-221	X	Vcam1	25361	21310411
111	rno-miR-221	rno-mir-221	X	Myc	24577	17943719
112	rno-miR-221	rno-mir-221	X	NP__001028929.1	246060	20428775
113	rno-miR-221	rno-mir-221	X	Stmn1	29332	18555017
114	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20618998
115	rno-miR-221	rno-mir-221	X	Dbi	25045	19953484
116	rno-miR-221	rno-mir-221	X	Adam17	57027	22009755
117	rno-miR-221	rno-mir-221	X	Cdkn1a	114851	19153141
118	rno-miR-221	rno-mir-221	X	Runx1	50662	21076613
119	rno-miR-221	rno-mir-221	X	Map2k1	170851	20299489
120	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	22992757
121	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19615744
122	rno-miR-221	rno-mir-221	X	Socs1	252971	21355095
123	rno-miR-221	rno-mir-221	X	Dnd1	307492	18155131
124	rno-miR-221	rno-mir-221	X	Ttpa	25571	20435889
125	rno-miR-221	rno-mir-221	X	Cxcr4	60628	18647411
126	rno-miR-221	rno-mir-221	X	NP__001028929.1	246060	20618998
127	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19953484
128	rno-miR-221	rno-mir-221	X	Akt1	24185	22009755
129	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19153141
130	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	21109963

TABLE 2-continued

S#	MicroRNA Name	StemLoop Name	miR_Chr	Gene Name	EntrezID	Pubmed ID
131	rno-miR-221	rno-mir-221	X	Fos	314322	20299489
132	rno-miR-221	rno-mir-221	X	Met	24553	23380809
133	rno-miR-221	rno-mir-221	X	Bmf	246142	19671867
134	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	21355095
135	rno-miR-221	rno-mir-221	X	Tnf	24835	18246122
136	rno-miR-221	rno-mir-221	X	Hnrpd	79256	20435889
137	rno-miR-221	rno-mir-221	X	Ephb1	24338	18704095
138	rno-miR-221	rno-mir-221	X	Pten	50557	20618998
139	rno-miR-221	rno-mir-221	X	Amacr	25284	20014922
140	rno-miR-221	rno-mir-221	X	Nos3	24600	22037549
141	rno-miR-221	rno-mir-221	X	Kras	24525	19153141
142	rno-miR-221	rno-mir-221	X	Adm	25026	21122348
143	rno-miR-221	rno-mir-221	X	Ephb1	24338	20299489
144	rno-miR-221	rno-mir-221	X	Axin2	29134	23380809
145	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19671867
146	rno-miR-221	rno-mir-221	X	Bcl2	24224	21400558
147	rno-miR-221	rno-mir-221	X	Tnfsf10	246775	18246122
148	rno-miR-221	rno-mir-221	X	Tnf	24835	20435889
149	rno-miR-221	rno-mir-221	X	Pten	50557	18704095
150	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20818387
151	rno-miR-221	rno-mir-221	X	Ddit4	140942	20018759
152	rno-miR-221	rno-mir-221	X	Cdkn2a_v1	25163	22037549
153	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	19153141
154	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	21226887
155	rno-miR-221	rno-mir-221	X	Tpm1_v7	24851	20417062
156	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	19671867
157	rno-miR-221	rno-mir-221	X	Tp53	24842	21400558
158	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	18246122
159	rno-miR-221	rno-mir-221	X	Bcl2	24224	20460378
160	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	18708351
161	rno-miR-221	rno-mir-221	X	Pdc	25343	20822813
162	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20018759
163	rno-miR-221	rno-mir-221	X	Mmp14	81707	22213426
164	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19264608
165	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	21226887
166	rno-miR-221	rno-mir-221	X	LOC685953	29184	17379065
167	rno-miR-221	rno-mir-221	X	Rtn4	83765	20417062
168	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19730150
169	rno-miR-221	rno-mir-221	X	Fas	246097	21400558
170	rno-miR-221	rno-mir-221	X	Bcl2	24224	18382364
171	rno-miR-221	rno-mir-221	X	Stat5a	24918	20489169
172	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19088079
173	rno-miR-221	rno-mir-221	X	Cd4	24932	20822813
174	rno-miR-221	rno-mir-221	X	Frap1	56718	20018759
175	rno-miR-221	rno-mir-221	X	Cdkn2a_v1	25163	22213426
176	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19351832
177	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	21273047
178	rno-miR-221	rno-mir-221	X	NP_001099207.1	89804	17379831
179	rno-miR-221	rno-mir-221	X	Inppl1	65038	20417062
180	rno-miR-221	rno-mir-221	X	NP_001099886.1	294790	20492666
181	rno-miR-221	rno-mir-221	X	Pten	50557	19730150
182	rno-miR-221	rno-mir-221	X	Akt1	24185	21481725
183	rno-miR-221	rno-mir-221	X	Tp53	24842	18382364
184	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19107213
185	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20822813
186	rno-miR-221	rno-mir-221	X	Bcl2	24224	20021821
187	rno-miR-221	rno-mir-221	X	Egfr	24329	22213426
188	rno-miR-221	rno-mir-221	X	Npepps	50558	19351832
189	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	21278784
190	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	17569667
191	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	20417062
192	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20492666
193	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19749093
194	rno-miR-221	rno-mir-221	X	Pten	50557	21481725
195	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	18413744
196	rno-miR-221	rno-mir-221	X	Fabp4	79451	19126397
197	rno-miR-221	rno-mir-221	X	Cxcl12	24772	20975375
198	rno-miR-221	rno-mir-221	X	Tp53	24842	20021821
199	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	22473819
200	rno-miR-221	rno-mir-221	X	Akt1	24185	19401561
201	rno-miR-221	rno-mir-221	X	Kcnh8	246325	21310411
202	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	17627278
203	rno-miR-221	rno-mir-221	X	Pdcd4	64031	20417062
204	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	18413744
205	rno-miR-221	rno-mir-221	X	Pi3	408230	20505758
206	rno-miR-221	rno-mir-221	X	Tnfsf10	246775	19767219
207	rno-miR-221	rno-mir-221	X	Eno2	24334	21487968

TABLE 2-continued

S#	MicroRNA Name	StemLoop Name	miR_Chr	Gene Name	EntrezID	Pubmed ID
208	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	19126397
209	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20975375
210	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	20021821
211	rno-miR-221	rno-mir-221	X	NP_001028929.1	246060	22473819
212	rno-miR-221	rno-mir-221	X	Ephb1	24338	19438724
213	rno-miR-221	rno-mir-221	X	Agtr1a	24180	21310411
214	rno-miR-221	rno-mir-221	X	Cdkn1b	83571	17721077
215	rno-miR-221	rno-mir-221	X	NP_001102171.1	362686	20425795
216	rno-miR-19b	rno-mir-19b-1	15	Scpep1	114861	21527938
217	rno-miR-19b	rno-mir-19b-2	X	Hoxa7	500126	22362744
218	rno-miR-19b	rno-mir-19b-2	X	Bace1	29392	18434550
219	rno-miR-19b	rno-mir-19b-2	X	Pten	50557	20851997
220	rno-miR-19b	rno-mir-19b-1	15	Nr3c2	25672	19944075
221	rno-miR-19b	rno-mir-19b-1	15	Myc	24577	21664042
222	rno-miR-19b	rno-mir-19b-2	X	BIM_RAT	64547	22362744
223	rno-miR-19b	rno-mir-19b-2	X	Socs1	252971	18728182
224	rno-miR-19b	rno-mir-19b-2	X	Ctgf	64032	21501375
225	rno-miR-19b	rno-mir-19b-1	15	Myc	24577	20008931
226	rno-miR-19b	rno-mir-19b-1	15	BIM_RAT	64547	21664042
227	rno-miR-19b	rno-mir-19b-2	X	Tp53	24842	18728182
228	rno-miR-19b	rno-mir-19b-2	X	Rhob	64373	21527938
229	rno-miR-19b	rno-mir-19b-1	15	Cdkn1a	114851	20089119
230	rno-miR-19b	rno-mir-19b-1	15	Aps	114203	21794077
231	rno-miR-19b	rno-mir-19b-2	X	Stat3	25125	19713220
232	rno-miR-19b	rno-mir-19b-2	X	Scpep1	114861	21527938
233	rno-miR-19b	rno-mir-19b-1	15	Kras	24525	20089119
234	rno-miR-19b	rno-mir-19b-1	15	Bcl2	24224	21883694
235	rno-miR-19b	rno-mir-19b-2	X	Nr3c2	25672	19944075
236	rno-miR-19b	rno-mir-19b-2	X	Myc	24577	21664042
237	rno-miR-19b	rno-mir-19b-1	15	Fmr1	24948	20435064
238	rno-miR-19b	rno-mir-19b-1	15	BIM_RAT	64547	21883694
239	rno-miR-19b	rno-mir-19b-2	X	Myc	24577	20008931
240	rno-miR-19b	rno-mir-19b-1	15	NP_00100814.1	306825	17575136
241	rno-miR-19b	rno-mir-19b-2	X	BIM_RAT	64547	21664042
242	rno-miR-19b	rno-mir-19b-1	15	Myc	24577	20851997
243	rno-miR-19b	rno-mir-19b-1	15	Runx1	50662	22362744
244	rno-miR-19b	rno-mir-19b-2	X	Cdkn1a	114851	20089119
245	rno-miR-19b	rno-mir-19b-1	15	Hipk3	83617	17575136
246	rno-miR-19b	rno-mir-19b-2	X	Aps	114203	21794077
247	rno-miR-19b	rno-mir-19b-1	15	Kras	24525	20851997
248	rno-miR-19b	rno-mir-19b-1	15	Hoxa7	500126	22362744
249	rno-miR-19b	rno-mir-19b-2	X	Kras	24525	20089119
250	rno-miR-19b	rno-mir-19b-1	15	Bace1	29392	18434550
251	rno-miR-19b	rno-mir-19b-2	X	Bcl2	24224	21883694
252	rno-miR-19b	rno-mir-19b-1	15	Pten	50557	20851997
253	rno-miR-19b	rno-mir-19b-1	15	BIM_RAT	64547	22362744
254	rno-miR-19b	rno-mir-19b-2	X	Fmr1	24948	20435064
255	rno-miR-19b	rno-mir-19b-1	15	Socs1	252971	18728182
256	rno-miR-19b	rno-mir-19b-2	X	BIM_RAT	64547	21883694
257	rno-miR-19b	rno-mir-19b-1	15	Ctgf	64032	21501375
258	rno-miR-19b	rno-mir-19b-2	X	NP_001100814.1	306825	17575136
259	rno-miR-19b	rno-mir-19b-2	X	Myc	24577	20851997
260	rno-miR-19b	rno-mir-19b-1	15	Tp53	24842	18728182
261	rno-miR-19b	rno-mir-19b-2	X	Runx1	50662	22362744
262	rno-miR-19b	rno-mir-19b-1	15	Rhob	64373	21527938
263	rno-miR-19b	rno-mir-19b-2	X	Hipk3	83617	17575136
264	rno-miR-19b	rno-mir-19b-2	X	Kras	24525	20851997
265	rno-miR-19b	rno-mir-19b-1	15	Stat3	25125	19713220
266	rno-miR-142-5p	rno-mir-142	10	Ifng	25712	21085987
267	rno-miR-142-5p	rno-mir-142	10	Nos2	24599	21085987
268	rno-miR-142-5p	rno-mir-142	10	Adarb1	25367	16369484
269	rno-miR-142-5p	rno-mir-142	10	Phb2	114766	21569818
270	rno-miR-142-5p	rno-mir-142	10	Scpep1	114861	16369484
271	rno-miR-142-5p	rno-mir-142	10	Ifit3	309526	22367717
272	rno-miR-142-5p	rno-mir-142	10	Apcs	29339	19794140
273	rno-miR-142-5p	rno-mir-142	10	Apcs	29339	22549634
274	rno-miR-142-5p	rno-mir-142	10	Cxcl9	246759	20178649
275	rno-miR-142-5p	rno-mir-142	10	Cd4	24932	22549634
276	rno-miR-142-5p	rno-mir-142	10	Twist2	59327	20178649
277	rno-miR-142-5p	rno-mir-142	10	Elovl6	171402	20178649
278	rno-miR-142-5p	rno-mir-142	10	Ddit4l	140582	20178649
279	rno-miR-142-5p	rno-mir-142	10	Fmr1	24948	20435064
280	rno-miR-142-5p	rno-mir-142	10	Tnf	24835	21085987
281	rno-miR-421	rno-mir-421	X	Pten	50557	19175831
282	rno-miR-421	rno-mir-421	X	Mycn	298894	20080624
283	rno-miR-421	rno-mir-421	X	Smad4	50554	21352803
284	rno-miR-421	rno-mir-421	X	Nr1h4	60351	22146319

TABLE 2-continued

S#	MicroRNA Name	StemLoop Name	miR_Chr	Gene Name	EntrezID	Pubmed ID
285	mmu-miR-674	mmu-mir-674	2	Mbp	17196	20215419
286	mmu-miR-674	mmu-mir-674	2	Lin28	83557	20413612
287	mmu-miR-463	mmu-mir-463	X	Tnp2	21959	15901636
288	mmu-miR-463	mmu-mir-463	X	Mat1a	11720	19507003
289	mmu-miR-463	mmu-mir-463	X	Mbp	17196	20215419
290	mmu-miR-463	mmu-mir-463	X	Lin28	83557	20413612
291	mmu-miR-324-3p	mmu-mir-324	11	Ctdspl	69274	17369397
292	mmu-miR-324-3p	mmu-mir-324	11	Hprt1	15452	17369397
293	mmu-miR-324-3p	mmu-mir-324	11	Oog4	242737	17369397
294	mmu-miR-324-3p	mmu-mir-324	11	Dnmt3b	13436	17369397
295	mmu-miR-324-3p	mmu-mir-324	11	H2afx	15270	17369397
296	mmu-miR-324-3p	mmu-mir-324	11	Fgf21	56636	17369397
297	mmu-miR-324-3p	mmu-mir-324	11	Mos	17451	17369397
298	mmu-miR-324-3p	mmu-mir-324	11	Mtpn	14489	15538371
299	mmu-miR-324-3p	mmu-mir-324	11	Mt1	17748	17369397
300	mmu-miR-324-3p	mmu-mir-324	11	Cdh1	12550	19559694
301	mmu-miR-324-3p	mmu-mir-324	11	Ccne1	12447	17369397
302	mmu-miR-324-3p	mmu-mir-324	11	Ccnb2	12442	17369397
303	mmu-miR-324-3p	mmu-mir-324	11	Stat3	20848	19559694
304	mmu-miR-324-3p	mmu-mir-324	11	Zp3	22788	17369397
305	mmu-miR-324-3p	mmu-mir-324	11	Rfpl4	192658	17369397
306	mmu-miR-324-3p	mmu-mir-324	11	Fgf10	14165	19559694
307	mmu-miR-324-3p	mmu-mir-324	11	Sycp3	20962	17369397
308	mmu-miR-324-3p	mmu-mir-324	11	H2afz	51788	17369397
309	mmu-miR-324-3p	mmu-mir-324	11	Bmp4	12159	19559694
310	mmu-miR-324-3p	mmu-mir-324	11	Camk2g	12325	17369397
311	mmu-miR-324-3p	mmu-mir-324	11	Dicer1	192119	17369397
312	mmu-miR-324-3p	mmu-mir-324	11	Mapk14	26416	19559694
313	mmu-miR-324-3p	mmu-mir-324	11	Pou5f1	18999	17369397
314	mmu-miR-324-3p	mmu-mir-324	11	H1foo	171506	17369397
315	mmu-miR-324-3p	mmu-mir-324	11	Mbp	17196	20215419
316	mmu-miR-324-3p	mmu-mir-324	11	Ifitm3	66141	17369397
317	mmu-miR-324-3p	mmu-mir-324	11	Dppa3	73708	17369397
318	mmu-miR-324-3p	mmu-mir-324	11	Lin28	83557	20413612
319	mmu-miR-324-3p	mmu-mir-324	11	Cpeb1	12877	17369397

In some embodiments of the inventive methods quantization of micro RNA is performed by Real-time RT-PCR. In at least one embodiment, about 1.5 μg total micro RNA is isolated from the biological samples of patients presenting with clinical symptoms of TBI and PTSD and reverse transcribed in a reaction volume of 20 μl using Taqman RT kit and micro RNA-specific primers. The product is diluted to a volume of 150 μl and 6 μl aliquots are used as templates for amplification using conventional PCR reagent kit components and gene-specific primers. In some embodiments, micro RNA can correlated with normal controls or historical controls with that of the corresponding micro RNA to detect modulation of the micro RNA in the injured patient, and based on the modulation of one or all of the micro RNAs a clinician can determine whether the patient is suffering from PTSD or TBI.

Kits

The process of measuring for micro RNA biomarkers or diagnosing PTSD or TBI may also be included as part of a kit for use in an ELISA, Northern Blot, Northern Analysis, hybridized buffers, probes, labeled probes or Western Blot, a bench top platform, a point of care device, or handheld device for diagnosing PTSD or other psychiatric disorders or TBI and other neural injuries. The PTSD and TBI biomarkers can also be used to screen for therapeutic targets for treating PTSD or TBI and to monitor a patient's progression or recovery from PTSD or TBI.

In certain embodiments, the diagnostic process and kits includes one or more agents for detecting one or more micro RNA biomarkers. The diagnostic process and kits also comprise two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more agents or antibodies that bind to a protein identified as specific to a PTSD or TBI cluster to diagnose PTSD or TBI in a patient. The kits can support for the simultaneous measurement of a panel of micro RNA biomarkers, 3 micro RNA biomarkers, or at least one micro RNA biomarker.

An inventive kit is also provided for aiding a diagnosis of a PTSD or TBI wherein the kits can be used to detect any number of the diagnostic proteins of the present invention. For example, the kits can be used to detect whether the diagnostic protein markers are present in samples of a patient and normal subjects. An inventive kit is used to identify compounds that modulate expression of one or more of the markers using in vitro or in vivo animal models to determine the effects of treatment. An inventive kit includes (a) a composition or panel of biomarkers; (b) a substrate; and (c) a detection agent. Such kits are prepared from the materials described above, and the previous discussion regarding the materials (e.g., antibodies, detection reagents, immobilized supports, etc.) is fully applicable to this section and will not be repeated. Optionally, the kit includes pre-fractionation spin columns. In some embodiments, the kit optionally further includes instructions for reacting the agent with the biological sample, or other operation parameter to afford a diagnosis of the condition. The instructions, in the form of a label or a separate insert.

A kit is also provided that includes (a) a substrate with an adsorbent thereon, wherein the adsorbent is suitable for binding a marker, (b) any biomarker of the present invention to be tested, and (c) instructions to detect the marker or markers by contacting a sample with the adsorbent and detecting the marker or markers retained by the adsorbent.

In some embodiments, the kit includes an eluent (as an alternative or in combination with instructions) or instructions for making an eluent, wherein the combination of the adsorbent and the eluant allows detection of the markers using gas phase ion spectrometry. Such kits are prepared from the materials described above, and the previous discussion of these materials (e.g., probe substrates, adsorbents, washing solutions, etc.) is fully applicable to this section and is not repeated.

A kit is also provided that includes a first substrate with an adsorbent thereon such as a particle functionalized with an adsorbent and a second substrate onto which the first substrate is positioned to form a probe which is removable and insertable into a gas phase ion spectrometer. The kit optionally includes single substrate which is in the form of a removable and insertable probe with adsorbents on the substrate. The kit also optionally includes a prefractionation spin column (e.g., Cibacron blue agarose column, anti-HSA agarose column, size exclusion column, Q-anion exchange spin column, single stranded DNA column, lectin column, etc.).

Optionally, the kit also optionally includes instructions for suitable operational parameters in the form of a label or a separate insert. For example, the kit may have standard instructions informing a consumer how to wash the probe after a sample is contacted on the probe. In another example, the kit may have instructions for pre-fractionating a sample to reduce complexity of proteins in the sample. In another example, the kit may have instructions for automating the fractionation or other processes.

It should be appreciated that although serum and amygdala are illustrated in the following Examples the inventive biomarkers for PTSD and TBI may be detected identically using the same procedures identified, the only difference being how the biological sample is drawn, as the varying biological samples have different methods for collection as one having skill in the art should readily know.

EXAMPLES

Reference will now be made in detail to the exemplary embodiments of the invention. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention and it is to be understood that other embodiments may be utilized and that changes may be made without departing from the scope of the invention. The following description is, therefore, merely exemplary.

Example 1—Animals and Stress Protocol

Male albino Sprague Dawley rats (Taconic Farms, Germantown, N.Y., USA) weighing 76 to 100 g and aged between 4-6 weeks old were used. These animals were kept for acclimation for a week and then the rats were grouped into two groups of six animals each for stress and control. Young animals were used for this study to give sufficient time for simulating PTSD progression as seen in the battlefield scenario. Development of PTSD like symptoms take at least two weeks after the cessation of stressors in the animal model and hormonal changes occur immediately after stress exposure as compared to the molecular level changes (Servatius et al. 1995). Hence, young animals were used to give sufficient time for studying the molecular level changes like protein or gene expression during PTSD development. Housing conditions, acclimation of rats and the stress protocol were followed as previously described (Jia et al., 2012). The stress protocol consisted of a 2 h per day session of immobilization along with tail shocks for three consecutive days. These animals were restrained and exposed to 40 electric shocks (2 mA, 3 s duration) at varying intervals of 150-210 s. Control groups were handled similar to stress group such as acclimation and housing except for the stress protocol. The Institutional Animal Care and Use Committee of the USUHS approved all the experimental procedures. Not being bound by any particular theory it is understood that similar models can be used for TBI, and presentation of symptoms of TBI appear within 24 hours of the initial stress tests.

Example 2—RNA Isolation, Quantity and Quality Check

Total RNA including micro RNA was isolated from the serum samples using the miRNeasy Serum/Plasma Kit (Qiagen, Valencia, USA) according to the manufacturer's protocol. QIAzol lysis reagent (1 ml) was added to the serum sample (200 μL) and vortexed. After incubating at room temperature for 5 min, 200 μL of chloroform was added and the samples were incubated at room temperature for 2-3 min and centrifuged for 15 min at 12,000×g at 4° C. The aqueous phase obtained after centrifugation was mixed with 1.5 volume of 100% ethanol and loaded into an RNeasy MiniElute spin column in a 2 ml collection tube. The flow through after centrifugation was discarded and the column was washed with 700 μL of Buffer RWT, 500 μL of Buffer RPE, 500 μL of 80% ethanol and then finally eluted with 14 μL of RNase-free water.

Total RNA was isolated from the amygdala tissue by combining a protocol of TRIzol reagent (Ambion/Life Technologies, Carlsbad, Calif., USA) and the mirVana μRNA isolation kit (Ambion/Life Technologies, Carlsbad, Calif., USA) according to the manufacturer's protocol. Briefly, two volumes of Trizol were added to the samples along with 1 volume of chloroform. After centrifugation, the aqueous layer was collected and mixed with 1.25 volume of absolute ethanol and passed through the RNAqueous micro kit cartridge and RNA eluted in TE buffer. Quality and quantity of small RNA for both serum and amygdala samples were analyzed using Agilent Small RNA kit (Agilent Technologies, Santa Clara, Calif., USA) in Agilent 2100 Bioanalyzer. Bioanalyzer data indicated the presence of good quality micro RNA in total serum RNA extractions. However, the micro RNA quantity in serum was an average of 15 ng/μl (FIG. **5**). This was expected since micro RNAs have been reported to be present in serum at low concentration and most of them are secreted out of the cells (Sayed et al., 2013). micro RNA concentrations of 30 ng of serum and 5 ng of amygdala μRNAs were used for the PCR reactions.

Example 3—Reverse Transcription, Pre-Amplification and Real Time Quantitative PCR

Reverse transcription (RT) was performed with TaqMan micro RNA RT Kit (Life Technologies, Carlsbad, Calif., USA) as described with slight modifications (Balakathiresan et al., 2012). micro RNA quantity was measured from the total RNA of bioanalyzer data and was used as template RNA (5 ng-brain μRNA; 30 ng-serum μRNA) for RT reactions (FIG. **5**). Briefly, the RT reaction mixture contained 0.8 μl Megaplex RT primers Rodent Pool A/B (v3.0), 0.2 μl 100 mM dNTPs (with dTTP), 1.5 μl Multiscribe reverse transcriptase (50 U/μl), 0.8 μl 10×RT Buffer, 0.9 μl $MgCl_2$ (25 mM), 0.1 μl RNAse inhibitor (20 U/μl), RNA template and nuclease free water to a final volume of 7.5 μl.

RT reaction was carried out on Veriti 96-Well Thermal Cycler (Life Technologies, Carlsbad, Calif., USA) according to manufacturer's recommended thermal cycling conditions. Pre-amplification of RT products, cycles and conditions were followed according to the manufacturer's protocol (Life Technologies, Carlsbad, Calif., USA). The undiluted pre-amplification products were used for the micro RNA profiling using TaqMan Low Density Rodent microRNAs Array (TLDA) Set v3.0 (Applied Biosystems, Inc) containing 692 rodent micro RNAs. The quantitative PCR (qPCR) reaction was carried out at default thermal-cycling conditions in ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Life Technologies, Foster City, Calif.).

Example 4—TaqMan Micro RNA Assay

TaqMan micro RNA assays (Applied Biosystems, Life Technologies, Foster City, Calif.) were carried out to validate the changes in the expression of selected micro RNAs in serum and amygdala. RT was performed as per manufacturer's protocol using micro RNA specific RT primers and mammalian U6 small nuclear RNA (U6 snRNA) was used as an endogenous control for the validation of all selected micro RNAs. RT and RT-qPCR reactions were carried out as described in Balakathiresan et al (2012). TaqMan micro RNA assays were carried out in triplicate. For relative quantification, each micro RNA was calibrated to the expression of U6 snRNA, which then gave a delta CT (ΔCt) value for each μRNA (μRNA Ct value-U6 Ct value). The fold changes were calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$).

Micro RNA expression profiles for Ct values were analyzed using real-time StatMiner software (Integromics Inc) to identify significantly modulated stress-responsive micro RNAs. For relative quantification of μRNAs between control and traumatic stress exposed animals, the following steps were performed in the StatMiner software suite: quality control of biological replicates, selection of U6 snRNA as an optimal endogenous control, filtering of micro RNAs expression having Ct values below 35 cycles and the detection of expression in all biological replicates of calibrator and target. Statistically significant micro RNAs were selected based on p-value lower than 0.05.

Predicted targets of differentially expressed serum and amygdala micro RNAs downloaded from miRWalk, a target prediction algorithm, were analyzed. MiRWalk is a combinatorial μRNA-target prediction tool and is able to identify both predicted and validated targets (Dweep et al., 2011). Both functional and network analysis of altered micro RNA and their gene targets associated with fear responses were performed using Ingenuity Pathway Analysis (IPA) program (Ingenuity Systems Inc, Redwood City, Calif.).

Example 5—Analysis of RNA Signatures in Serum and Correlation with Amygdala

The micro RNA expression profiling identified 82 micro RNAs, which were differentially expressed at day 14 after traumatic stress, whereas only 18 micro RNAs were modulated in serum at day 0 after the cessation of stress. Thus micro RNA candidates in serum to diagnose PTSD are hereby presented. micro RNA expression in amygdala due to its critical role in fear conditioning (Morey et al., 2012) was also established. A comparison of micro RNAs expression profile in amygdala at day 0 and day 14 with serum μRNAs indicated a similar μRNA modulation pattern (Table 3).

TABLE 3

	Amygdala Day 0			Amygdala Day 14		
S#	Detector	RQ_Stress-Control	P. Value Stress-Control	Detector	RQ_Stress-Control	P. Value Stress-Control
1	mmu-miR-429	2.02	0.01	rno-miR-632	742.43	0.01
2	mmu-miR-29b	2.58	0.05	hsa-miR-190b	14.49	0
3	mmu-miR-205	2.31	0.01	mmu-miR-1928	7.85	0
4	mmu-miR-130b*	2.25	0.04	hsa-miR-124*	4.93	0
5	mmu-miR-690	2.16	0.05	mmu-miR-141	4.47	0
6	mmu-miR-186	−3.02	0	mmu-miR-706	3.73	0
7	mmu-miR-449a	−2.84	0.01	mmu-miR-291a-3p	3.67	0
8	mmu-miR-331-5p	−2.44	0.01	mmu-miR-1982.2	3.49	0
9	rno-miR-632	−25.01	0.03	rno-miR-673	3.43	0.01
10	mmu-miR-342-3p	−2.24	0.02	mmu-miR-1896	3.32	0
11	mmu-miR-376a*	−2.16	0.02	hsa-miR-653	3.29	0.03
12	mmu-miR-467b	−2.07	0.01	mmu-miR-362-5p	3.28	0.01
13	mmu-miR-16	−2	0.02	mmu-miR-463*	3.16	0.01
14	hsa-miR-27b*	−2	0.01	rno-miR-547	3.05	0.01
15				rno-miR-219-1-3p	3.01	0.02
16				mmu-miR-146b	2.93	0
17				mmu-miR-204	2.85	0.03
18				mmu-miR-300*	2.84	0
19				mmu-miR-1188	2.83	0.01
20				mmu-miR-433-5p	2.8	0
21				mmu-miR-200c	2.79	0
22				mmu-miR-487b	2.77	0
23				rno-miR-345-3p	2.59	0
24				mmu-miR-130b*	2.58	0
25				mmu-miR-363	2.51	0
26				rno-miR-409-3P	2.49	0
27				mmu-miR-10a	2.45	0
28				mmu-miR-342-3p	2.42	0.01
29				mmu-miR-199b	2.41	0
30				mmu-miR-28*	2.38	0.01
31				mmu-miR-19b	2.37	0
32				hsa-miR-28-3p	2.36	0.02

TABLE 3-continued

	Amygdala Day 0			Amygdala Day 14		
S#	Detector	RQ_Stress-Control	P. Value Stress-Control	Detector	RQ_Stress-Control	P. Value Stress-Control
33				hsa-miR-136*	2.35	0.05
34				mmu-miR-124	2.35	0
35				mmu-miR-125b*	2.32	0.03
36				mmu-miR-217	2.25	0.02
37				hsa-miR-412	2.23	0.01
38				hsa-miR-875-5p	2.23	0.01
39				mmu-miR-674*	2.22	0.02
40				mmu-miR-103	2.21	0
41				mmu-miR-671-3p	2.19	0
42				hsa-miR-30e-3p	2.18	0
43				mmu-miR-134	2.17	0.02
44				mmu-miR-223	2.16	0.03
45				rno-miR-146B	2.14	0.01
46				mmu-miR-467b	2.12	0
47				hsa-miR-421	2.1	0.01
48				mmu-miR-142-5p	2.1	0
49				hsa-miR-151-5P	2.09	0.02
50				hsa-miR-455	2.07	0
51				mmu-miR-9	2.06	0
52				mmu-miR-216b	2.05	0
53				mmu-miR-99a	2.05	0.03
54				rno-miR-344-3p	2.04	0
55				hsa-miR-340	2.04	0
56				mmu-miR-383	2.03	0.01
57				mmu-miR-140	2.02	0
58				mmu-miR-188-5p	2.01	0.02
59				hsa-miR-189	2	0.02
60				mmu-miR-322*	2	0.01

Fourteen micro RNAs were modulated at day 0 whereas 60 micro RNAs were modulated at day 14 after the cessation of stress. It was also observed that most of the modulated micro RNAs at day 0 were significantly downregulated in both serum (27 out of 31) and amygdala (8 out of 14). However, this trend of micro RNA downregulation at day 0 was reversed at day 14 post stress where 78 out of 82 micro RNAs were upregulated in serum and all 60 significantly modulated micro RNAs were upregulated in amygdala. No common micro RNAs were found between all four groups. However, comparison of serum and amygdala profiles showed 9 common micro RNAs at Day 14. No similar micro RNAs between serum and amygdala were observed at day 0. Comparison of micro RNAs in serum samples at day 0 and 14 showed 18 common micro RNAs whereas only 4 micro RNAs were common in amygdala profiling data at day 0 and day 14 (FIG. **1**). The symptoms and pathophysiology of PTSD in this model has been previously reported to develop at day 14 after stress exposure, which also correlates with the changes in the RNA expression profile. Moreover, PTSD in humans is shown to develop over a period of time after the traumatic stress (Jia et al., 2012). Therefore, micro RNA profiles of day 14 serum and amygdala were compared to diagnose PTSD in the stress animal model and 9 upregulated micro RNAs were identified as common viz., miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* (Table 1). This panel of micro RNAs represented a small subset of micro RNAs, but it is nonetheless possible that the other serum micro RNAs could serve as biomarkers of traumatic stress, such as those presented in Table 2.

Example 6—Validation of Differential Expression in Taqman µRNA Assay

Global micro RNA screening platforms can introduce bias in the micro RNA profiling which can occur because of the reproducibility of the platform used, pre amplification step due to low serum concentration and stable endogenous controls. All these factors may contribute and lead to an identification of false positive (Balakathiresan et al., 2012). Therefore, validation of the micro RNA profiling data was obtained from low-density array platform by performing individual micro RNA assay. MiR-223 was selected as a representative for a validation study since it is reported that miR-223 is enriched in hippocampus, midbrain, and cortex (Harraz et al., 2012). MiR-223 is also implicated in studies related to brain injury and stroke, thus it is appreciated that the discovered micro RNA also detect TBI or Stroke, for which PTSD is usually co-morbid. MiR-223 is reported to be prevalent in the relatively large vessel-like structures scattered throughout the brain after TBI (Redell et al., 2009). In stroke animal model, miR-223 overexpression in hippocampus shows the neuroprotective effect by regulating the expression of glutamate receptor subunits, GluR2 and NR2B (Harraz et al., 2012). In this validation assay with miR-223, U6 small nucleolar RNA were chosen as an endogenous control. The singleplex PCR assay for miR-223 confirms and validates the expression for the same set of animals from the multiplex platform (FIG. **2**). Validation of miR-128 expression in serum and amygdala samples of day 14 is also confirmed (FIG. **6**).

Example 7—Prediction of Traumatic Stress Altered µRNA Targets and their Pathway Analysis

To understand the role of the nine micro RNAs which are common to both serum and amygdala in PTSD pathophysiology, a bioinformatics analysis was performed to identify gene targets. Analysis in MiRWalk database showed 331 experimentally validated gene targets (Table 2). Among these genes, it can be found that genes involved in anxiety regulation or developments are among the targets of the modulated micro RNAs. Two genes stathmin 1 (STMN1) and aquaporin 4 (AQP4) were identified and the role of these two genes have been well-studied in anxiety disorder. Moreover, they have been identified as direct target of miR-223. Pathway analysis of validated gene targets by IPA program suggested cell death and survival as one of the top most biofunctions in the molecular and cellular functional category (FIG. **3**A). In canonical pathways, glucocorticoid receptor signaling pathway was among the top five pathways which is regulated by micro RNAs (FIG. **3**B). Molecular functional network was constructed using fear related genes and molecules suggested that miR-223, miR-1928 (miR-221) may have direct role in STMN1 regulation (FIG. **4**). Taken together these data suggest that the selected nine micro RNAs have a role in PTSD development as their modulation was observed in both serum and amygdala and thus can serve as biomarkers.

The micro RNA expression at day 0 immediately after the cessation of stress showed that most of the micro RNAs were found to be downregulated in amygdala. Without being bound by any particular theory, this downregulation may be due to the "de novo protein synthesis" that supports long-lasting functional and structural plasticity which is a molecular requirement for new memory formation. (Griggs et al., 2013). The downregulated micro RNAs were also shown to regulate memory formation in amygdala by repressing actin-regulating proteins that are involved in plasticity and memory (Griggs et al., 2013). Furthermore, the global reduction of several micro RNAs expression in rodents forebrain such as amygdala, hippocampus and cortex have been shown to regulate learning and memory (Gao et al., 2010; Konopka et al., 2010; Lin et al., 2011; Griggs et al., 2013).

Much evidence indicates that the newly formed fear memories are being consolidated into stable long-term memories in the amygdala which are believed to be the site of fear memory storage (Fanselow et al., 1999; Nader et al., 2000). To identify the micro RNAs that are involved in consolidation and long-term stability of fear memories, micro RNA profiling was performed in amygdala at day 14 after the cessation of traumatic stress. Analysis of day 14 micro RNAs in amygdala revealed a substantial alteration of the posttranscriptional machinery characterized by a global increase in micro RNA expression. This change indicated the development and ongoing pathophysiology of the PTSD, as each microRNA was able to regulate the expression of several target genes (Beveridge et al., 2010). For example, it was observed two fold upregulation of miR-124, which has been shown to directly target mineralocorticoid receptor (MR) which regulates CORT secretion (Mannironi et al., 2013). Interestingly, Jia et al (2012) demonstrated the down-regulation of MR in amygdala enhanced the secretion of CORT for several days and the development of anxiety. Due to the alteration of large number of micro RNAs (60 μRNAs; >2 fold) in day 14 amygdala, only those micro RNAs were selected that were common (9 micro RNAs) between serum and amygdala of day 14 for further analysis such as correlation with fear related genes. Network analysis of these 9 micro RNAs with their fear-related gene targets that are available in IPA showed only 5 of them were correlated with fear related genes (FIG. **4**). For instance, cAMP responsive element binding protein 1 (Creb1) was identified as a direct target of miR-142-3p. Creb1 was recently reported to be down regulated in rat brain exposed to repeated inescapable shock (Smalheiser et al., 2011), suggesting that miR-142-3p may regulate the expression of Creb1 and may play an important role in stress related response (FIG. **4**). Further, miR-221 and miR-223 were also found to regulate the expression of STMN1, an important amygdala molecule involved in fear conditioning (Shumyatsky et al., 2005).

IPA analysis suggested involvement of five micro RNAs viz., miR-142-5p, miR-19b, miR-1928, miR-223 and miR-421-3p in the regulation of genes associated with delayed and exaggerated fear. These five micro RNAs were explored for their brain specificity and/or their functions related to any neurological conditions. MiR-142-5p was found to be enriched in microglia and was shown to be upregulated after brain injury (Lei et al., 2009; Wu et al., 2011; Lau et al 2013). Further, auditory fear training in rats down regulated the expression of miR-142-5p in lateral amygdala of naïve animals, suggesting its involvement in memory formation dysfunction (Griggs et al., 2013). MiR-19b-3p that copurifies with polyribosomes in mammalian neurons show significantly higher expression in 6-hydroxydopamine-injured MN9D cells, indicating its role in neurodegenerative diseases by contributing to dopaminergic neuronal apoptosis (Li et al 2013). MiR-221-3p expression was also upregulated in distal axons of superior cervical ganglia (SCG) after spinal cord injury (Liu et al., 2009, Wu et al., 2011). MiR-223 and miR-19 were also enriched in glial cells and were shown to inhibit aberrant glial expression of neuronal proteins and phenotypes (Jovicid et al., 2013). The miR-421 was first identified in neocortex and hippocampus from developing rat brain and also plays a role in neurodegenerative disorders (Miska et al., 2004; Taguchi 2013). Recent studies also suggested participation of miR-421 in the regulation of plasminogen activator Inhibitor-1 (PAI-1) which is known to induce neuronal apoptosis, disrupt the blood-brain barrier (BBB) and contribute to neurotoxicity in ischemic brain damage after stroke (Abu Fanne et al., 2010; Marchand et al 2012).

For biomarker identification, only day 14 serum micro RNA profiles were selected for the analysis, since the day 14 animals showed delayed and exaggerated startle response, enhanced plasma CORT and retarded body weight gain after several days (10-21 days) of posttraumatic stress in rats (Jia et al., 2012). Modulation of micro RNAs in serum can occur either because of the change in the micro RNAs expression in the regions of the brain which controls the stress response. These micro RNAs can leach out in the serum by different ways as previously described (Andrews and Neises 2012). However, there is a possibility that serum micro RNA modulation may occur due to a bystander effect of the stress on other organs which can potentially alter the serum micro RNA expression profile. Such micro RNAs can be a marker for organ stress but cannot be used as marker for psychological stress. To identify the true candidates biomarkers, micro RNA profiling was performed for amygdala which is believed to play a critical role in regulation of fear conditioning in this animal model (Andero et al., 2013). Nine micro RNAs that were upregulated in both amygdala and in serum were selected and analysed for their correlation with PTSD pathophysiology by computational analysis to validate their potential as diagnostic biomarkers of PTSD. Since micro RNA regulates the cell physiology by targeting the mRNA and altering the protein expression, the validated gene targets of the 9 candidate micro RNAs were identified using miRWalk program. These gene targets were used to identify the pathways involved using IPA. Interestingly, stress-related glucocorticoid receptor signalling pathway appeared as one of the major canonical pathway which was regulated by the 9 micro RNAs. These computational analyses suggest that the candidate biomarkers of PTSD have an important role in stress response and hence are good candidates for further biomarker validation studies.

Thus it is shown that traumatic stress associated with a global decrease in day 0 and global increase in day 14 in micro RNA expression in amygdala has profound psychopathological implications in the context of PTSD development by influencing genes involved in fear memory formation and consolidation. A panel of dysregulated micro RNAs present in both serum and amygdala after exposure to traumatic stress and their correlation with PTSD pathophysiology suggests them as promising candidates for biomarkers.

Example 8—Analysis of μRNAs in Serum for Biomarkers of PTSD

Altered expressions of serum and amygdala micro RNAs in an animal model of PTSD were examined. Differentially expressed and statistically significant micro RNAs in serum were validated for their presence in amygdala of corresponding animals. A panel of nine stress-responsive micro RNAs viz., miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* were identified in serum at 14 days post exposure to traumatic stress. The animal model used induces enhanced fear response in the animals at day 14 which is evident from the increased startle response. Fear and stress both are the key features in PTSD diagnosis. Therefore, to identify putative serum biomarkers to diagnose PTSD, the role of these micro RNAs in both psychological stress and fear response were analyzed. The data was analyzed with ingenuity pathway analysis (IPA) which identifies the relationship of micro RNAs towards a specific pathway by predicting the binding affinity of a micro RNA with the proteins of the pathway. In addition, the current literature was also used to identify role of μRNAs in a specific pathway.

This analysis showed that among the nine micro RNAs 3 micro RNAs had a direct interaction with genes regulating the stress and fear response. These micro RNAs were miR-19b-3p, miR-223-3p and miR-221-3p. MiR-19b and miR-223 are found to regulate the proteins which are involved in regulation of both fear and stress response. Both of these molecules are found to regulate many proteins involved in stress and fear regulation. Among these, one protein which is common to these micro RNAs is adregenic receptor beta-1 (adrb-1). Volk et. al. (November 2014) reports, increased expression of miR-19b in amygdala which regulates the levels of adrb-1 regulate fear response. Direct correlation with the increased miR-19b expression in serum and amygdala is thus shown. In addition, the role of these micro RNAs is found in regulation of stathmin 1 which has been reported to play a crucial role in stress and fear response. Interaction of miR-221 with cnr-1, a molecule of stress responsive pathway, was found. Based on these analysis and other reports, it is clear that miR-19b-3p, miR-221-3p and miR-223-3p are involved in regulation of stress and fear responsive pathways and their appearance in serum post-traumatic stress is a direct results of the traumatic stress. Based on these results miR-19b-3p, miR-223-3p and miR221-3p are biomarkers of PTSD.

The trends in micro RNA levels detailed herein are found to correlate with other samples collected from human subjects, the samples including whole blood, cerebral spinal fluid (CSF), plasma, serum, urine, and saliva. miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463* and miR-674* levels were also confirmed to trend as detailed above. Thus animal data for PTSD, TBI and control groups performed in the Examples correlated with that of human subject who have been diagnosed with PTSD or a TBI, thus confirming the protocol as an animal model for human PTSD and TBI.

While at least one exemplary embodiment has been presented in the foregoing detailed description, it should be appreciated that a vast number of variations exist. It should also be appreciated that the exemplary embodiment or exemplary embodiments are only examples, and are not intended to limit the scope, applicability, or configuration of the described embodiments in any way. Rather, the foregoing detailed description will provide those skilled in the art with a convenient road map for implementing the exemplary embodiment or exemplary embodiments. It should be understood that various changes can be made in the function and arrangement of elements without departing from the scope as set forth in the appended claims and the legal equivalents thereof.

Patent documents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. These documents and publications are incorporated herein by reference to the same extent as if each individual document or publication is specifically and individually incorporated herein by reference.

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 9

<210> SEQ ID NO 1
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 1

cauaaaguag aaagcacuac u                                          21


<210> SEQ ID NO 2
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)
```

```
<400> SEQUENCE: 2

ugugcaaauc caugcaaaac uga                                              23


<210> SEQ ID NO 3
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 3

agcuacauug ucugcugggu uuc                                              23


<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 4

ugucaguuug ucaaauaccc c                                                21


<210> SEQ ID NO 5
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 5

aaacaugaag cgcugcaaca                                                  20


<210> SEQ ID NO 6
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 6

ccacugcccc aggugcugcu gg                                               22


<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 7

aucaacagac auuaauuggg                                                  20


<210> SEQ ID NO 8
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 8

uaccuaauuu guuguccauc a                                                21


<210> SEQ ID NO 9
```

-continued

```
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic (micro RNA biomarker)

<400> SEQUENCE: 9

cacagcuccc aucucagaac aa                                          22
```

The invention claimed is:

1. A process for administering a therapeutic agent for treating post-traumatic stress disorder (PTSD), the process comprising:

measuring levels of miR-19b-3p, miR-223-3p and miR-221-3p micro RNA in a biological sample obtained at a first time point from a subject suspected of suffering from PTSD, comprising hybridizing agents that specifically hybridize to each of the micro RNA, amplifying each of the micro RNA and sequencing the micro RNA to confirm the identity of each of the micro RNA and quantifying the identified RNA, and

administering a therapeutic for the treatment of PTSD based on the measurements of the micro RNA.

2. The process of claim **1** wherein said biological sample is obtained at least thirteen days after said subject has been exposed to traumatic event likely to cause PTSD.

3. The process of claim **1** wherein said biological sample is obtained within one week after said subject presents with clinical symptoms of PTSD.

4. The process of claim **1**, wherein said biological sample is obtained within 24 hours after said subject presents with clinical symptoms of PTSD.

5. The process of claim **1** wherein said therapeutic agent is an antidepressant, an antipsychotic, or combinations thereof.

6. The process of claim **5**, wherein said therapeutic is fluoxitine and paroxatine, venlafaxine, sertraline, mirtazapine, olanzapine and quetiapione, propranolol, or an α_1-selective adrenoceptor antagonist, or combinations thereof.

7. The process of claim **1** wherein said biological sample is whole blood, plasma, serum, CSF, urine, saliva, sweat, prefrontal cortex tissue, hippocampus tissue, or ipsilateral cortex tissue.

* * * * *