

What is claimed is:

1. A nucleic acid extraction from one or more microvesicles isolated from a eukaryotic biological sample, wherein 18S rRNA and 28S rRNA are detectable in the extraction.
2. The nucleic acid extraction of claim 1, wherein the biological sample is a bodily fluid.
3. The nucleic acid extraction of claim 2, wherein the bodily fluid is urine.
4. The nucleic acid extraction of claim 2, wherein the bodily fluid is serum or plasma.
5. The nucleic acid extraction of any of claims 1-4, wherein the biological sample is from a mammal.
6. The nucleic acid extraction of claim 5, wherein the biological sample is from a human.
7. The nucleic acid extraction of any of claims 1-6, wherein the quantitative ratio of 18S rRNA to 28S rRNA detectable in the extraction is within the range of approximately 1:1 to approximately 1:2; and is preferably approximately 1:2.
8. The nucleic acid extraction of claim 7, wherein the biological sample is a bodily fluid with a protein concentration of less than 10 mg/ml, such as urine, and the extraction has an RNA Integrity Number of greater than or equal to 5.
9. The nucleic acid extraction of claim 7, wherein the biological sample is a bodily fluid with a protein concentration of greater than 10 mg/ml, such as serum or plasma, and the extraction has an RNA Integrity Number of greater than or equal to 3.
10. The nucleic acid extraction of claim 8, wherein the nucleic acid yield from 20 ml of biological sample is greater than or equal to 50 pg/ml.
11. The nucleic acid extraction of claim 9, wherein the nucleic acid yield from 1 ml of biological sample is greater than or equal to 50 pg/ml.
12. A profile of nucleic acid from one or more microvesicles isolated from a eukaryotic biological sample, wherein 18S rRNA and 28S rRNA are detectable in the profile.
13. The profile of claim 12, wherein the biological sample is a bodily fluid.

14. The profile of claim 13, wherein the bodily fluid is urine.
15. The profile of claim 13, wherein the bodily fluid is serum or plasma.
16. The profile of any of claims 12-15, wherein the biological sample is from a mammal.
17. The profile of claim 16, wherein the biological sample is from a human.
18. The profile of any of claims 12-18, wherein the quantitative ratio of 18S rRNA to 28S rRNA is within the range of approximately 1:1 to approximately 1:2; and is preferably approximately 1:2.
19. The profile of claim 18, wherein the biological sample is a bodily fluid with a protein concentration of less than 10 mg/ml, such as urine, and the nucleic acid has an RNA Integrity Number of greater than or equal to 5.
20. The profile of claim 18, wherein the biological sample is a bodily fluid with a protein concentration of greater than 10 mg/ml, such as serum or plasma, and the nucleic acid has extraction has an RNA Integrity Number of greater than or equal to 3.
21. The profile of claim 19, wherein the nucleic acid yield from 20 ml of biological sample is greater than or equal to 50 pg/ml.
22. The profile of claim 20, wherein the nucleic acid yield from 1 ml of biological sample is greater than or equal to 50 pg/ml.
23. A method of evaluating the quality of a nucleic acid extraction from microvesicles isolated from a eukaryotic biological sample, comprising the steps of:
 - (a) extracting RNA from microvesicles; and
 - (b) measuring the quality of the RNA by determining the quantity of 18S and 28S rRNA in the extraction.
24. The method of claim 23, wherein the biological sample is a bodily fluid.
25. The method of claim 24, wherein the bodily fluid is urine.
26. The method of claim 24, wherein the bodily fluid is serum or plasma.
27. The method of any of claims 23-26, wherein the biological sample is from a mammal.

28. The method of claim 27, wherein the biological sample is from a human.
29. The method of any of claims 23-28, wherein the quantitative ratio of 18S rRNA to 28S rRNA is within the range of approximately 1:1 to approximately 1:2; and is preferably approximately 1:2.
30. The profile of claim 29, wherein the biological sample is a bodily fluid with a protein concentration of less than 10 mg/ml, such as urine, and the nucleic acid has an RNA Integrity Number of greater than or equal to 5.
31. The profile of claim 29, wherein the biological sample is a bodily fluid with a protein concentration of greater than 10 mg/ml, such as serum or plasma, and the nucleic acid has extraction has an RNA Integrity Number of greater than or equal to 3.
32. The profile of claim 30, wherein the nucleic acid yield from 20 ml of biological sample is greater than or equal to 50 pg/ml.
33. The profile of claim 31, wherein the nucleic acid yield from 1 ml of biological sample is greater than or equal to 50 pg/ml.
34. A method of obtaining nucleic acid from a biological sample, comprising the steps of:
- (a) obtaining a biological sample;
 - (b) performing an extraction enhancement operation on the biological sample; and
 - (c) extracting nucleic acid from the biological sample.
35. The method of claim 34, wherein the extraction enhancement operation is comprised of:
- (a) the addition of one or more of the following agents to the biological sample:
 - (i) RNase inhibitor;
 - (ii) protease;
 - (iii) reducing agent;
 - (iv) decoy substrate, such as synthetic RNA;
 - (v) soluble receptor;
 - (vi) small interfering RNA,
 - (vii) RNA binding molecule, such as anti-RNA antibody, chaperone protein, or an RNase inhibitory protein;

- (ix) RNase denaturing substance, such as high osmolarity solution, detergent; or

(b) the performance of one or more of the following steps prior to nucleic acid extraction:

- (x) washing;
- (xi) size-separating RNase from the sample;
- (xii) effecting RNase denaturation through a physical change, such as by decreasing temperature, freeze/thaw cycle; or

(c) any combination of the foregoing agents or steps.

36. The method of claim 34 or claim 35, wherein the biological sample is a bodily fluid.

37. The method of claim 36, wherein the bodily fluid is urine.

38. The method of claim 36, wherein the bodily fluid is serum or plasma.

39. The method of any of claims 34-38, wherein a derivative is obtained from the biological sample and subjected to the extraction enhancement operation before extracting nucleic acid.

40. The method of claim 39, wherein the derivative is a microvesicle fraction from the biological sample.

41. The method of claim 40, wherein the microvesicle fraction is obtained by a technique selected from the group consisting of differential centrifugation, affinity purification, filtration concentration, buoyant density gradient, microfluidic separation, chromatography, affinity chromatography, ion exchange, and any combination of any of the foregoing.

42. The method of claim 41, wherein the microvesicle fraction is obtained by a filtration concentration technique.

43. The method of claim 42, wherein the biological sample is passed through a filter having a pore size of less than or equal to 0.8 μm .

44. The method of any of claims 34-43, wherein the biological sample or the derivative is treated with a ribonuclease, deoxyribonuclease, or a combination thereof, prior to performance of the enhancement extraction operation.
45. The method of any of claims 32-44, wherein the extraction enhancement operation comprises the addition of an RNase inhibitor to the biological sample, or to the derivative, prior to extracting nucleic acid.
46. The method of claim 45, wherein the RNase inhibitor has a concentration of greater than [1X] concentration; alternatively, greater than or equal to [5X] concentration; alternatively, greater than or equal to [10X] concentration; alternatively, greater than or equal to [25X] concentration; and alternatively, greater than or equal to [50X] concentration.
47. The method of claim 45 or claim 46, wherein the RNase inhibitor is a protease.
48. A kit for obtaining nucleic acids from microvesicles, comprising in one or more containers:
- (a) a nucleic acid extraction enhancement agent;
 - (b) DNase, RNase, or both; and
 - (c) a lysis buffer.
49. The kit of claim 48, further comprising instructions for using the kit.
50. The kit of claim 48 or claim 49, wherein the nucleic acid extraction enhancing agent is selected from the group consisting of:
- (a) RNase inhibitor;
 - (b) protease;
 - (c) reducing agent;
 - (d) decoy substrate;
 - (e) soluble receptor;
 - (f) small interfering RNA;
 - (g) RNA binding molecule;
 - (h) RNase denaturing substance; or
 - (i) any combination of any of the foregoing.
51. A method of analyzing RNA from microvesicles, comprising the steps of:

- (a) obtaining a sample of microvesicles;
 - (b) treating the sample with DNase to eliminate all or substantially all of any DNA located outside of or on the surface of the microvesicles in the sample;
 - (c) extracting RNA from the sample; and
 - (d) analyzing the extracted RNA.
52. The method of claim 51, wherein the microvesicles are isolated from a biological sample.
53. The method of claim 52, wherein the biological sample is a bodily fluid.
54. The method of claim 53, wherein the bodily fluid is urine.
55. The method of claim 53, wherein the bodily fluid is serum or plasma.
56. The method of any of claims 51-55, wherein the biological sample is from a mammal.
57. The method of claim 56, wherein the biological sample is from a human.
58. A method for diagnosing, monitoring, or treating a subject, comprising the steps of:
- (a) isolating a microvesicle fraction from a urine sample from a subject;
 - (b) detecting the presence or absence of a biomarker within the microvesicle fraction;
- wherein the biomarker is selected from the group consisting of (i) a species of nucleic acid, (ii) the level of expression of a nucleic acid, (iii) a nucleic acid variant, and (iv) any combination of any of the foregoing; and
- wherein the biomarker is associated with the presence or absence of a disease or other medical condition, or the viability of a treatment option.
59. The method of claim 58, wherein the biomarker is an mRNA transcript.
60. The method of claim 59, wherein the mRNA transcript is selected from the group consisting of: NPHS2 (podocin), LGALS1 (Galectin-1), HSPG2 (heparin sulfate proteoglycan); CUBN (cubilin), LRP2 (megalin), AQP1 (aquaporin 1), CA4 (carbonic anhydrase 4), CLCN5 (chloride channel protein 5), BDKRB1 (bradykinin B1 receptor), CALCR (calcitonin receptor), SCNN1D (amiloride-sensitive sodium channel subunit delta), SLC12A3 (thiazide-sensitive sodium-chloride cotransporter), AQP2

(aquaporin 2), ATP6V1B1 (V-ATPase B1 subunit), SLC12A1 (kidney-specific Na-K-Cl symporter via RT-PCR of RiboAmped mRNA).

61. The method of claim 60, wherein the mRNA transcript is AQP2 (aquaporin 2) or ATP6V1B1 (V-ATPase B1 subunit).
62. The method of claim 58, wherein the biomarker and disease or other medical condition are selected from the group consisting of:
 - (a) NPHS2 (podocin) and glomerular disease, such as steroid-resistant nephritic syndrome;
 - (b) CUBN (cubilin) and proteinuria, such as in Imerslund-Gräsbeck syndrome;
 - (c) AQP2 (aquaporin 2) and diabetes insipidus.
63. An isolated polynucleotide molecule comprising a first nucleotide sequence that is at least 90% identical to a second nucleotide sequence selected from the group consisting of SEQ ID NOS: 1-29.
64. The isolated polynucleotide molecule in claim 63, wherein the polynucleotide molecule is an deoxyribonucleotide.
65. The isolated polynucleotide molecule in claim 63, wherein the polynucleotide molecule is an ribonucleotide.
66. A vector comprising the isolated nucleic acid molecule in claim 63.
67. A host cell comprising the isolated nucleic acid molecule in claim 63.
68. An isolated polynucleotide comprising a segment of a nucleotide sequence selected from SEQ ID NOS: 1-29.
69. The isolated polynucleotide molecule in claim 68, wherein the polynucleotide molecule is an deoxyribonucleotide.
70. The isolated polynucleotide molecule in claim 68, wherein the polynucleotide molecule is an ribonucleotide.
71. A vector comprising the isolated nucleic acid molecule in claim 68.
72. A host cell comprising the isolated nucleic acid molecule in claim 68.

73. An isolated polynucleotide comprising a sequence of at least 13 nucleotides that are the same as any 13-nucleotide sequence in any one of SEQ ID NOS: 1-29.
74. The isolated polynucleotide molecule in claim 73, wherein the polynucleotide molecule is an deoxyribonucleotide.
75. The isolated polynucleotide molecule in claim 79, wherein the polynucleotide molecule is an ribonucleotide.
76. A vector comprising the isolated nucleic acid molecule in claim 73.
77. A host cell comprising the isolated nucleic acid molecule in claim 73.
78. A method of assessing the quality of a nucleic acid extraction from a biological sample, comprising:
 - a. providing a biological sample;
 - b. obtaining an extraction of nucleic acids from the biological sample;
 - c. measuring the amount of a polynucleotide molecule comprising a segment having a nucleotide sequence selected from SEQ NOS: 1-29 in the extraction; and
 - d. comparing the amount of the polynucleotide molecule against a standard to assess the quality of the nucleic acid extraction.
79. The method in claim 78, wherein the biological sample is a bodily fluid.
80. The method of claim 78, wherein the bodily fluid is urine.
81. The method of claim 78, wherein the bodily fluid is serum or plasma.
82. The method of any of claims 78-81, wherein the biological sample is from a mammal.
83. The method of claim 82, wherein the biological sample is from a human.
84. The method in claim 78, wherein the extraction is an extraction in claim 1.
85. The method in claim 78, wherein the extraction is obtained with a method in claim 34.
86. The method in claim 78, wherein the standard is derived by measuring the amount of a polynucleotide molecule comprising a segment having the nucleotide sequence

selected from SEQ NOS: 1-29 in nucleic acid extractions from more than 5 biological samples.

CLAIMS

1. A method of obtaining and analyzing RNA from a urine sample, comprising the steps of:
 - (a) obtaining a urine sample and pre-processing the urine sample by filtration through a 0.8 μ m filter;
 - (b) obtaining a microvesicle fraction from the pre-processed urine sample by ultracentrifugation or filtration concentration;
 - (c) washing the microvesicle fraction;
 - (d) extracting RNA from the microvesicle fraction;
 - (e) measuring the quality of the RNA from the microvesicle fraction by determining the quantity of 18S and 28S rRNA in the extraction for the purpose of evaluating the quality of the RNA extraction, where detection of 18S and 28S rRNA in the RNA extraction indicates that the RNA extraction is high yield and/or high integrity; and
 - (f) analyzing one or more RNAs from the RNA extraction of step e) which has been evaluated as high yield and/or high integrity.
2. A method according to Claim 1, in which the microvesicle fraction is obtained by filtration concentration.
3. A method according to Claim 2, in which the filtration concentration comprises passing the urine sample through a filter having a pore size of less than or equal to 0.8 μ m.
4. A method according to any of Claims 1, 2 or 3, in which the urine sample or microvesicle fraction is treated with a ribonuclease, deoxyribonuclease, or a combination thereof, prior to performance of the washing in step (c).
5. A method according to any preceding claim, in which step (c) comprises the addition of an RNase inhibitor, prior to extracting RNA.
6. A method according to Claim 5, in which the RNase inhibitor has a concentration of greater than [1X] concentration; alternatively, greater than or equal to [5X] concentration; alternatively, greater than or equal to [10X] concentration; alternatively, greater than or equal to [25X] concentration; and alternatively, greater than or equal to [50X] concentration.

7. A method according to Claim 5 or 6, in which the RNase inhibitor is a protease.
8. A method according to any preceding claim, further comprising determining a quantitative ratio of 18S rRNA to 28S rRNA in the extraction.
9. A method according to Claim 8, in which the quantitative ratio of 18S rRNA to 28S
5 rRNA is within the range of approximately 1:1 to approximately 1:2.
10. A method according to Claim 8 or 9, in which the quantitative ratio of 18S rRNA to 28S rRNA is approximately 1:2.
11. A method according to Claim 4 comprising:
treating the microvesicle fraction with DNase to eliminate all or substantially all of any
10 DNA located outside of or on the surface of the microvesicles in the fraction; extracting RNA from the sample; and analyzing the extracted RNA.