

CLAIMS

1. A method for RNA profiling and analysis of small volume samples from a patient or individual comprising:
 - (a) obtaining one or more small volume sample self-collected by the patient or individual or by a non-medical person, wherein the sample is collected in or otherwise combined with an RNA stabilization solution whereby cells in the sample are lysed and RNA is stabilized; and
 - (b) isolating RNA using a process adapted for small volume samples wherein the amount of any and all solutions or buffers utilized are reduced and adjusted for small volume samples;wherein the RNA is of sufficient quality and quantity for whole transcriptome analysis and transcriptomic profiling through RNA sequencing (RNAseq).
2. The method of claim 1 wherein the RNA is isolated using a process comprising:
 - (a) contacting the sample with a protease to form a protease treated small volume sample;
 - (b) contacting the protease treated sample with an ethanol or salt solution forming a precipitate containing the RNA, wherein the precipitate containing the RNA is then resuspended in a buffer or solution, or contacting the protease treated sample with an organic extraction solution, forming a solution having an aqueous phase containing the RNA and an organic phase;
 - (c) contacting the resuspended precipitate containing the RNA or the aqueous phase containing the RNA with DNase to form a DNase-treated resuspended precipitate or DNase-treated aqueous phase;
 - (d) binding the RNA to a silica based solid phase or column by contacting the resuspended precipitate or aqueous phase with said silica based solid phase; and
 - (e) eluting the RNA from the silica based solid phase comprising contacting the silica based solid phase with a solution or buffer to provide isolated RNA;wherein all buffer and solution volumes are reduced and adjusted for small volume samples.
3. The method of claim 2 wherein between steps (b) and (c), the resuspended precipitate containing the RNA or the aqueous phase containing the RNA is contacted with a solution or column to remove residual sample cell debris and/or to homogenize the sample cell lysate.
4. The method of claim 1, wherein the RNA is isolated using a process comprising:

- (a) contacting the sample with an RNA stabilization solution, wherein the solution has capability to lyse cells and inactivate adventitious agents;
 - (b) optionally further contacting the sample with a salt, a reducing agent, and/or a detergent;
 - (c) contacting the solution contacted sample of (a) or (b) with silica, silica based solid phase or carboxylated magnetic beads which bind RNA and seves to purify the RNA from other components in the sample; and
 - (d) eluting the RNA from the silica or silica based solid phase or the magnetic beads comprising contacting the silica, silica based solid phase or magnetic beads with a solution or buffer to provide isolated RNA;
- wherein all buffer and solution volumes are reduced and adjusted for small volume samples.

5. The method of any of claims 1-4, wherein the small volume sample is a small volume blood sample collected via fingerstick.

~~6. The method of any of claims 1-5, wherein the small volume blood sample is collected via fingerstick.~~

~~7.6.~~ The method of any of claims ~~1-6~~ 1-5, wherein the sample volume is less than 500µl, less than 300µl, less than 250µl, about 200-300µl, less than 200µl, about 100-300µl, about 150-300µl, about 100-250µl, about 50-300µl, less than 100µl, less than 50µl, less than 25µl, 10µl or less.

~~8.7.~~ The method of any of claims ~~1-7~~ 1-6, wherein buffer and solution volumes are reduced to 20-40% or 20-30% or about 25% of those utilized for isolation of RNA from a standard venipuncture blood sample.

~~9.8.~~ The method of any of claims ~~1-8~~ 1-7, wherein said protease is proteinase K.

~~10.9.~~ The method of any of claims ~~1-9~~ 1-8, further comprising sequencing the RNA.

~~11.10.~~ The method of claim ~~10~~ 9, wherein abundant RNA species or RNA species not of interest are removed prior to sequencing.

~~12.11.~~ The method of claim ~~11~~ 10, wherein globin mRNA, ribosomal RNA(s) or species specific RNAs are removed prior to sequencing.

~~13.12.~~ The method of any of claims ~~1-12~~ 1-11, wherein the patient or individual has a disease or infection or is at risk of or suspected of disease or infection.

~~14~~.13._____The method of any of claims ~~1-13~~ 1-12 for longitudinal screening by RNA profiling and analysis of small volume samples from one or more patient or individual, wherein the patient or individual has a disease or infection or is at risk of or suspected of disease or infection.

~~15~~.14._____The method of claim ~~14~~ 13, wherein small volume samples are collected via fingerstick in series or in regular or designated increments of hours, days, weeks or months.

~~16~~.15._____The method of claim ~~15~~ 14, wherein small volume blood samples are collected via fingerstick in series or in regular or designated increments of hours, days, weeks or months.

~~17~~.16._____ A system or kit for RNA profiling and analysis of small volume samples from a patient or individual comprising:

- (a) a means for self-collection of a small volume sample by the patient or individual or by a non-medical person, comprising a lancet, swab or receptacle for a wash, spit or aspirate;
- (b) a tube or receptacle for receiving the small volume sample on collection and containing a volume of RNA stabilization solution whereby cells in the sample are lysed and RNA is stabilized; and
- (c) one or more appropriate label(s) for designating the name or identity of the patient or individual, date of sample collection and time of sample collection.

~~18~~.17._____The system or kit of claim ~~17~~ 16, further comprising an envelope or mailing container for shipment of the sample to a laboratory or facility for RNA isolation and analysis.

~~19~~.18._____The system or kit of claim ~~17~~ 16, for longitudinal RNA profiling and analysis of multiple small volume samples collected in series from a patient or individual over days, weeks or months comprising:

- (a) a set of numerous means for self-collection of individual small volume samples by the patient or individual or by a non-medical person, each comprising a lancet, swab or receptacle for a wash, spit or aspirate;
- (b) a set of numerous tubes or receptacles each individually for receiving a small volume sample on collection and containing a volume of RNA stabilization solution whereby cells in the sample are lysed and RNA is stabilized;
- (c) numerous appropriate label(s) for designating the name or identity of the patient or individual, date of sample collection and time of sample collection; and
- (d) numerous envelopes or mailing containers for shipment of each sample or several samples to a laboratory or facility for RNA isolation and analysis.

~~20~~.19. The system or kit of any of claims ~~17-19~~ 16-18, wherein the volume of RNA stabilization solution is less than 1ml, about 500µl or less, about 300µl or less, about 200-300µl, about 250µl, less than 200µl, less than 100µl, less than 50µl, less than 25µl, or 10µl or less.

~~21~~.20. The system or kit of any of claims ~~17-20~~ 16-19, wherein the tube or receptacle for receiving the small volume sample and containing RNA stabilization solution has a total volume capacity of 1.5 ml or less, 1.2 ml or less, or 1ml or less.