

WHAT IS CLAIMED IS: CLAIMS

1. A method for preparing a library of nucleic acids from a plurality of polynucleotides, comprising:

(a) hybridizing ~~a~~ the plurality of polynucleotides with a plurality of primers comprising random hexamer sequences and tags, wherein the plurality of polynucleotides comprises RNA and DNA;

(b) extending the hybridized primers with a reverse transcriptase to obtain extended primers from the RNA polynucleotides; and

(c) generating a library of nucleic acids ~~from comprising~~ the extended primers and the DNA ~~polynucleotides~~; and

~~The method of claim 1, further comprising~~

2. (d) sequencing the library of nucleic acids.

~~3.2.~~ 3. The method of claim ~~2~~ 1, further comprising (e) identifying polynucleotide sequences comprising the tags, thereby identifying sequences derived from the RNA polynucleotides of the plurality of polynucleotides.

~~4.3.~~ 4. The method of claim ~~3~~ 2, further comprising identifying polynucleotide sequences lacking the tags, thereby identifying sequences derived from the DNA polynucleotides of the plurality of polynucleotides.

~~5.4.~~ 5. The method of any one of claims 1-~~4~~ 3, wherein at least one of

(i) the plurality of primers comprises different sequences~~;~~

~~The method of any one of claims 1-5, wherein~~

~~6.~~ 6. (ii) each primer comprises a different sequence~~;~~

~~The method of any one of claims 1-6, wherein~~

~~7.~~ 7. (iii) the plurality of primers comprises greater than 10,000 different sequences~~;~~
and/or

~~The method of any one of claims 1-7, wherein~~

~~8.~~ 8. (iv) the plurality of primers comprises greater than 100,000 different sequences.

~~9.~~ 9. ~~The method of any one of claims 1-8, wherein the plurality of primers comprises random hexamer sequences.~~

~~10.5.~~ The method of any one of claims 1-~~94~~, wherein the plurality of primers comprises the same tag.

~~11.6.~~ The method of any one of claims 1-~~105~~, wherein the reverse transcriptase lacks a DNA-dependent polymerase activity.

~~12.7.~~ The method of any one of claims 1-~~116~~, wherein the reverse transcriptase is selected from the group consisting of avian myeloblastosis virus (AMV) reverse transcriptase, moloney murine leukemia virus (MMLV) reverse transcriptase, human immunovirus (HIV) reverse transcriptase, equine infectious anemia virus (EIAV) reverse transcriptase, Rous-associated virus-2 (RAV2) reverse transcriptase, *C. hydrogeniformans* DNA polymerase, *T. thermus* DNA polymerase, *T. flavus* DNA polymerase, and functional variants thereof.

~~13.8.~~ The method of any one of claims 1-~~127~~, wherein step (b) is performed in the presence of the DNA polynucleotides.

~~14.9.~~ The method of any one of claims 1-~~138~~, wherein step (b) comprises generating double-stranded cDNA from the extended primers.

~~15.10.~~ The method of any one of claims 1-~~149~~, wherein step (c) comprises contacting the extended primers and DNA polynucleotides with a reagent selected from the group consisting of a kinase, a ligase, a transposon, a polymerase, and a sequencing adaptor.

~~16.11.~~ The method of any one of claims 1-~~1510~~, wherein the plurality of polynucleotides is cell-free.

~~17.12.~~ The method of claim ~~16~~11, wherein the plurality of polynucleotides is obtained from a sample selected from the group consisting of serum, interstitial fluid, lymph, cerebrospinal fluid, sputum, urine, milk, sweat, and tears.

~~18.~~ A method for preparing a library of nucleic acids comprising:

- ~~(a) hybridizing a plurality of polynucleotides with a plurality of primers, wherein the plurality of polynucleotides comprises RNA and DNA;~~
- ~~(b) extending the hybridized primers with a reverse transcriptase; and~~
- ~~(c) generating a library of nucleic acids from the extended primers and the DNA.~~

~~19.~~ The method of claim 18, wherein the plurality of polynucleotides is cell free.

~~20.— The method of claim 18 or 19, wherein the plurality of polynucleotides is obtained from a sample selected from the group consisting of serum, interstitial fluid, lymph, cerebrospinal fluid, sputum, urine, milk, sweat, and tears.~~

~~21.— The method of any one of claims 18-20, wherein the plurality of primers comprises different sequences.~~

~~22.— The method of any one of claims 18-21, wherein each primer comprises a different sequence.~~

~~23.— The method of any one of claims 18-22, wherein the plurality of primers comprises greater than 10,000 different sequences.~~

~~24.— The method of any one of claims 18-23, wherein the plurality of primers comprises greater than 100,000 different sequences.~~

~~25.— The method of any one of claims 18-24, wherein the plurality of primers comprises random hexamer sequences.~~

~~26.— The method of any one of claims 18-25, wherein the reverse transcriptase lacks a DNA-dependent polymerase activity.~~

~~27.— The method of any one of claims 18-26, wherein the reverse transcriptase is selected from the group consisting of avian myeloblastosis virus (AMV) reverse transcriptase, moloney murine leukemia virus (MMLV) reverse transcriptase, human immunovirus (HIV) reverse transcriptase, equine infectious anemia virus (EIAV) reverse transcriptase, Rous-associated virus 2 (RAV2) reverse transcriptase, *C. hydrogenoformans* DNA polymerase, *T. thermus* DNA polymerase, *T. flavus* DNA polymerase, and functional variants thereof.~~

~~28.— The method of any one of claims 18-27, wherein (b) is performed in the presence of the DNA polynucleotides.~~

~~29.— The method of any one of claims 18-28, wherein (b) comprises generating double-stranded cDNA from the extended primers.~~

~~30.— The method of any one of claims 18-29, wherein (c) comprises contacting the extended primers and DNA polynucleotides with a reagent selected from the group consisting of a kinase, a ligase, a transposon, a polymerase, and a sequencing adaptor.~~

~~31.13. A method of identifying a nucleic acid in a sample of nucleic acids any one of claims 1-12, further comprising:~~

(i) obtaining sequence data from ~~a the~~ library of nucleic acids ~~prepared from a sample of nucleic acids by the method of any one of claims 1-30~~; and

(ii) identifying a polynucleotide sequence comprising a tag, thereby identifying a sequence derived from a RNA polynucleotide of the plurality of polynucleotides.

~~32.14.~~ The method of claim ~~3413~~, further comprising (iii) identifying a variant in the polynucleotide sequence comprising a tag.

~~33.15.~~ The method of claim ~~3214~~, wherein the variant is selected from the group consisting of a single nucleotide polymorphism (SNP), a deletion, an insertion, a substitution, a duplication, a translocation, and a gene fusion.

~~34.16.~~ The method of any one of claims ~~3413-3315~~, further comprising identifying a reverse transcription error in the polynucleotide sequence comprising a tag.

~~35.17.~~ The method of any one of claims ~~3413-3416~~, further comprising comparing the polynucleotide sequence comprising a tag with a reference sequence.

~~36.18.~~ The method of claim ~~3517~~, wherein the reference sequence is derived from a DNA polynucleotide of the library of nucleic acids.

~~37.~~ ~~The method of any one of claims 31-36, wherein the sample comprises cell free nucleic acids.~~

~~38.19.~~ The method of any one of claims ~~3413-3718~~, wherein the RNA polynucleotide is an RNA selected from the group consisting of mRNA, tRNA, ribosomal RNA, non-coding RNA, piRNA, siRNA, lncRNA, shRNA, snRNA, miRNA, snoRNA, viral RNA, bacterial RNA, and a ribozyme.

20. The method of any one of claims 13-19, wherein the RNA polynucleotide is a long non-coding RNA (lncRNA).

~~39.~~ ~~A kit for preparing a library of nucleic acids comprising:~~

~~a reverse transcriptase; and~~

~~a plurality of primers comprising tags, wherein each primer is different.~~

~~40.~~ ~~The kit of claim 39, wherein the plurality of primers comprises the same tag.~~

~~41.~~ ~~The kit of claim 39 or 40, further comprising a component selected from the group consisting of a kinase, an RNase, a ligase, a transposon, a polymerase, and a sequencing adaptor.~~

42. — ~~The kit of any one of claims 39-41, wherein the reverse transcriptase lacks DNA-dependent polymerase activity.~~

43. — ~~The kit of any one of claims 39-42, wherein the reverse transcriptase is selected from the group consisting of avian myeloblastosis virus (AMV) reverse transcriptase, moloney murine leukemia virus (MMLV) reverse transcriptase, human immunovirus (HIV) reverse transcriptase, equine infectious anemia virus (EIAV) reverse transcriptase, Rous associated virus 2 (RAV2) reverse transcriptase, *C. hydrogenoformans* DNA polymerase, *T. thermus* DNA polymerase, *T. flavus* DNA polymerase, and functional variants thereof.~~