CLAIMS

1. A method of extracting nucleic acids from a wax-embedded biological sample, comprising:

(a) combining the biological sample with a wax-solubilizing organic solvent comprising a hydrocarbon of at least 10 carbon atoms to form a mixture; and

(b) adding an alcohol to the mixture;

whereby a precipitate is formed from the mixture that contains the nucleic acids after step (a) and before or after step (b); then

(c) separating the organic solvent and the alcohol from the precipitate;

(d) combining the precipitate with a lysis buffer so as to dissolve at least some of the precipitated nucleic acids from the sample that are in the precipitate to form a lysis solution;

(e) combining the lysis solution with a first aliquot of beads adapted to bind nucleic acids;

(f) recovering nucleic acids derived from the biological sample from the first aliquot of beads;

(g) treating the recovered nucleic acids in a buffer that contains DNase to remove deoxyribonucleic acids; and thereafter

(h) isolating RNA from the buffer containing DNase using a second aliquot of beads adapted to bind nucleic acids;

wherein the lysis buffer contains a protease and one or more detergents that include nonyl-phenoxypolyethoxylethanol (NP40), sodium dodecyl sulfate (SDS), or a combination of both NP40 and SDS.

2. The method of claim 1, wherein the one or more detergents include NP40.

3. The method of claim 1, wherein the one or more detergents include SDS at a concentration between 0.25% and 1% (w/v), wherein the concentration of the SDS is effective as an RNase inhibitor.

4. The method of claim 1, wherein the one or more detergents include both NP40 and SDS.

5. The method of claim 1, wherein the biological sample is a human tissue sample embedded in paraffin.

6. The method of claim 1, wherein the organic solvent comprises hexadecane.

7. The method of claim 1, wherein the alcohol is ethanol.

8. The method of claim 1, wherein the lysis buffer comprises proteinase K.

9. The method of claim 1, wherein the organic solvent is removed from the precipitate before the alcohol is added.

10. The method of claim 1, wherein the precipitate is dried before the lysis buffer is added.

11. The method of claim 1, further comprising determining nucleic acid sequences for nucleic acids or analyzing gene expression from nucleic acids recovered from the second aliquot of beads.

12. The method of claim 1 wherein the beads are magnetic particles coated with carboxyl groups in the form of succinic acid.

13. A method for improving the recovery of nucleic acids in a protocol for isolating nucleic acids from wax-embedded biological samples,

wherein the protocol comprises dissolving the wax-embedded sample in an organic solvent, extracting nucleic acids into an aqueous buffer, and isolating the nucleic acids from the aqueous buffer;

wherein the improvement comprises the following:

(1) using a saturated hydrocarbon comprising at least 10 carbon atoms as the organic solvent;

(2) causing the nucleic acids in the organic solvent to form a precipitate, which is then washed to remove the organic solvent and alcohol;

(3) including the non-ionic detergent nonyl-phenoxypolyethoxylethanol (NP40) and an RNA inhibitor in the aqueous buffer;

(4) treating the nucleic acids with DNase; and

(5) isolating the nucleic acids both before and after the DNase treatment using beads.

14. The method of claim 13, whereby the protocol is improved by one or both of the following criteria:

(1) a median percentage of fragments over 200 base pairs in length (DV200) of at least 40%; and

(2) a median number of unique mRNA transcripts detected at Cq < 22 of at least 100.

15. The method of claim 13, wherein the RNase inhibitor is sodium dodecyl sulfate (SDS) and the saturated hydrocarbon is hexadecane.

16. A method of extracting ribonucleic acids (RNA) from a wax-embedded biological sample, comprising:

(a) combining the biological sample with a wax-solubilizing organic solvent to form a mixture;

(b) recovering from the mixture a precipitate that contains nucleic acids from the biological sample;

(c) washing the precipitate with a solution comprising an alcohol

(d) combining the washed precipitate with a lysis buffer that contains nonyl phenoxypolyethoxylethanol (NP40), sodium dodecyl sulfate (SDS), and proteinase K so as to dissolve at least some of the nucleic acids from the sample that are in the precipitate to form a lysis solution;

(e) combining the lysis solution with a first aliquot of magnetic beads adapted to reversibly bind nucleic acids;

(f) recovering nucleic acids derived from the biological sample from the first aliquot of beads;

(g) treating the recovered nucleic acids in a buffer that contains DNase to remove deoxyribonucleic acids;

(h) combining the lysis solution containing the DNase treated nucleic acids with a second aliquot of magnetic beads adapted to reversibly bind nucleic acids; and

(i) recovering nucleic acids derived from the biological sample from the second aliquot of beads, thereby obtaining the ribonucleic acids (RNA) from the wax-embedded biological sample.

17. The method of claim 16, wherein the organic solvent comprises hexadecane and the alcohol is ethanol.