

Claims

1. A method of preparing a sample for library amplification and subsequent amplification comprising the following steps:
 - (a) providing a nucleic acid-containing cellular sample;
 - (b) lysing cells of the sample with a lysis reagent to liberate nucleic acid from within the cells of the cellular sample, thereby forming a lysate; and
 - (c) amplifying the nucleic acid from the lysed samples to form amplified nucleic acids;wherein there is no purification of the nucleic acid from the lysate prior to beginning the amplification step (c); and
wherein amplifying the nucleic acid in step (c) comprises tagmentation.
2. The method of claim 1, wherein the nucleic acid is DNA.
3. The method of claims 1 or 2, wherein the sample is a blood sample, a whole blood sample, or a dried blood sample.
4. The method of claims 1 or 2, wherein the sample is a non-blood sample, a tissue sample, a tumor sample, a biopsy sample, an aspirate, or an FFPE sample.
5. The method of any preceding claim, wherein the lysis reagent is water or purified/distilled water.
6. The method of any of claims 1-4, wherein the lysis reagent is a detergent, a base, an acid, and/or an enzyme.
7. The method of any preceding claim, wherein step (b) comprises treating the cells of the sample or the lysate with an enzyme to disrupt the DNA structure.
8. The method of claim 7, wherein the enzyme that disrupts the DNA structure is proteinase K.

9. The method of any preceding claim, further comprising neutralizing the lysis reagent and/or the enzyme that disrupts DNA structure prior to the amplification step (c) to inactivate the lysis reagent and/or the enzyme that disrupts the DNA.
10. The method of claim 9, wherein the neutralizing step is carried out via a neutralising agent or via heat.
11. The method of any preceding claim, wherein there is an incubation period after combining the sample and the lysis reagent and before the amplification step.
12. The method of any preceding claim, wherein the steps of lysing the sample and amplifying the nucleic acid contained in the lysate are conducted in a single pot reaction.
13. The method of any preceding claim, wherein the time taken from combining the sample and lysis reagent to beginning the amplification process is less than about 20 minutes, preferably less than about 15 minutes, preferably less than about 10 minutes, preferably less than about 5 minutes, preferably less than about 4 minutes, preferably less than about 3 minutes, preferably less than about 2 minutes, preferably between about 1-5 minutes, preferably between about 2-4 minutes.
14. The method of any preceding claim, wherein the amplified nucleic acids are sequenced to ascertain their sequences.
15. The method of claim 14, wherein the sequencing protocol is high throughput sequencing or a sequence-by-synthesis protocol.
16. The method of any preceding claim, wherein the tagmentation comprises fragmenting the nucleic acid in the lysate.

17. The method of any preceding claim, further comprising:

- (d) exposing the amplified nucleic acids to a solid surface with immobilized amplification primers, thereby immobilizing the amplified nucleic acids on the solid surface; and
- (e) clonally amplifying the immobilized, amplified nucleic acids on the solid surface in a clustering reaction to generate clusters.

18. The method of claim 17, wherein the clustering reaction is performed in the presence of one or more of proteinase K, formalin, paraffin, cellular components, protein, extracellular matrix components, collagen, and tissue debris.

19. The method of claim 18, wherein the sample is an FFPE sample and the clustering reaction is performed in the presence of at least 0.001 pg paraffin.

20. The method of claim 19, wherein the clustering reaction is performed in the presence of at least 0.01 pg, 0.1pg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, 1 µg, 10 µg, 100 µg, or at least 1 mg paraffin.

21. The method of claim 18, wherein the clustering reaction is performed in the presence of at least 0.001 pg proteinase K.

22. The method of claim 21, wherein the clustering reaction is performed in the presence of at least 0.01 pg, 0.1pg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, 1 µg, 10 µg, 100 µg, or at least 1 mg proteinase K.

23. The method of any one of claims 17-22, wherein the immobilized, amplified nucleic acids are sequenced to ascertain their sequences.