PalArch's Journal of Archaeology of Egypt / Egyptology

SANGER SEQUENCING AND ITS RECENT ADVANCES - A REVIEW

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Aleena Alex, Dr. M.P.Brundha, Dr. Lavanya Prathap. SANGER SEQUENCING AND ITS RECENT ADVANCES - A REVIEW-- Palarch's Journal Of Archaeology Of Egypt/Egyptology 17(7), 698-705. ISSN 1567-214x

Keywords: DNA sequencing; RNA sequence; metagenomics ; amplification ;Genome; transcription

ABSTRACT

The main aim of the study is to analyse the role of Sanger sequencing and its recent advances .DNA sequencing has revolutionized biomedicine, and progress in the field has been unrelenting since it was invented over 30years ago. As a complete DNA sequence of the human genome is obtained from decades of work by a large number of scientists. Less than a decade later the next-generation' instruments can make it possible for a single lab to produce the same amount of data in a week. The instruments are now increasingly automated, upstream sample processing remains a challenge. An extensive review of literature of Sanger sequencing and its advances by collection of data Pubmed, core, google scholar, Cochran and semantic scholar base medicine Gor-Mesk from the articles dates from 2000 - 2020.From which data is analysed to interpret the results.Inclusion criteriaArticles related to Sanger sequencing , human genome amplification, DNA profiling methods for forensic identification and Advanced DNA sequencing techniques.Exclusion criteria : articles related to other categories.The recent articles discussed in this review helps in attaining knowledge and awareness about Sanger sequencing and its recent advanced techniques. Advanced DNA sequencing techniques such as metagenomics, transcriptome sequencing, genome sequencing, re-sequencing, chromatin immunoprecipitation and diagnosis of genetic diseases in human genetic history.This review gives a clear view about knowledge and awareness

about Sanger sequencing and its advances , advantages and uses in medical field,genetics and forensic medicine.

INTRODUCTION

The vast majority of microbial species remain uncultivated until recently about half of all known bacterial phyla was identified only from the 16s ribosomal RNA Gene sequence. With the advent of single cell sequencing the genomes of uncultivated species have rapidly filled in the unsequenced branches of the tree of microbial phylogenetic. The wealth of new insight from these previously inaccessible groups is providing a deeper understanding of their basic biology, taxonomy and evolution as well as their diverse roles in environmental ecosystems and human health(1)

Successful mapping of the draft human genome in the year of 2001 and also more recent mapping of the human microbiome genome in the year 2012 has relied heavily on the parallel processing of the second generation or Next Generation Sequencing (NGS) DNA machines at a cost of several millions dollars and long computer processing times. These have been mainly biochemical approaches(2) Here a system analysis approach is used to review these techniques by identifying the requirements, specifications, test methods, error estimates, repeatability, reliability and trends in the cost reduction.

ROLE OF SANGER SEQUENCING

DNA sequencing is a method to obtain the exact order of occurrence of nucleotides in a DNA. With the help of DNA sequence the researchers can illuminate the genetic information from a biological system and can decipher the DNA sequences necessary for almost all branches of life sciences and its understanding has grown exponentially in the past many decades(3)The first-generation sequencing method known as Sanger sequencing was developed by Edward Sanger in the year of 1975. Sanger sequencing is considered as the gold standard for DNA sequencing(4).The first major breakthrough of the first-generation sequencing was the Human Genome Project (HGP). But due to inherent limitations in throughput, speed, scalability and resolution of the first-generation Sanger sequencing approach, second-generation sequencing method or next-generation sequencing (NGS) has been developed to cater out a high demand for cheaper as well as faster sequencing technology(5)

The NGS is fundamentally a different approach for sequencing that leads to several ground-breaking discoveries and brought a new revolution in genomic research by revealing limitless insight related to genome, transcriptome and epigenome of any species. Hence, technology has brought a new revolution in the welfare of human society(6).

Principally, this next-generation sequencing is similar to capillary electrophoresis (CE)-based Sanger sequencing but the NGS extends the idea to perform massive parallel sequencing where a millions of fragments of DNA from a single sample can be accurately sequenced. The NGS can enable sequencing of large stretches of DNA base pairs and producing hundreds of gigabases of data in a single sequential run. The NGS is also known as massive parallel sequencing(7) which can allow the complete genome of a human to be sequenced in less than a day.

ADVANCEMENT IN NEXT-GENERATION SEQUENCING TECHNIQUES

- A high-throughput sequencing (HTS) of the human genome lets us discover the genes and regulatory pathways associated with the disease(8).
- The targeted sequencing of specific genes or genomic regions helps in the identifying of the disease-causing mutations which can help in the faster diagnosis and outcome of disease-targeted sequencing that may help in better therapeutic decision-making for several genetic diseases which includes many cancers(9).
- The RNA-Seq (NGS of RNA) can provide an entire transcriptomic information of a sample without any need of previous knowledge related to the genetic sequence of an organism. The RNA-Seq provides a strong alternative approach to Microarrays for gene expression studies and lets the researchers visualise RNA expression in the form of sequence(10) .

This variant study is quite common in medical genetics where the DNA sequence and data are compared with a reference sequence to catalogue the differences in between. These differences may range from single nucleotide polymorphisms (SNPs) to complex chromosomal rearrangement. (11)
DNA sequencing is a truly powerful approach for decoding a number of human diseases that is even cancer. After the advent of next-generation sequencing (NGS) technologies it has reduced the sequencing cost by orders of magnitude and significantly increased it throughput by making the whole-genome sequencing a possible way for obtaining the global genomic information about the patients on whom the clinical actions can be taken(12,13). The benefits offered by the NGS technologies has a number of challenges that must be addressed adequately before it can be transformed from research tools to routine clinical practices(14).

ADVANCES IN DNA SEQUENCING

DNA sequencing is the procedure of verifying nucleotides the precise order inside a DNA molecule. It incorporates any technique or technology that is used to figure out the order of the four bases-adenine, guanine, cytosine, and thymine-in a strand of DNA. The advance of rapid DNA sequencing methods has significantly quickened the biological ,medical(15) and educational research(16,17).

It has become fundamental for basic biological research, and in various connected fields, for example analytic, biotechnology, forensic biology, and biological systematics. A quick speed of sequencing is achieved with advanced DNA sequencing technology which has been instrumental in the sequencing of complete DNA sequences or genomes of various types and species of life in which it incorporates the human genome and other complete DNA sequences of numerous animal, plant, and microbial species.
The main DNA sequences were acquired in the year of 1970s by scholarly researchers by using laborious methods which depend upon two-dimensional chromatography. Accompanying the advancement of fluorescence-based sequencing strategies with automated analysis, the DNA sequencing has become less demanding and orders of magnitude in faster rate.

The two basic methods of

- DNA sequencing are: Maxam-Gilbert sequencing and Chain-termination methods (Sanger Method)
- Advanced Methods, includes Shotgun Sequencing(18) and Bridge PCR.
- Next Generation Sequencing methods are, Massively Parallel Signature Sequencing (MPSS),Polony sequencing, 454 pyrosequencing,Illumina (Solexa)(19) sequencing Solid sequencing,Ion Torrent semiconductor sequencing,DNA nanoball sequencing, Heliscope single molecule sequencing and Single molecule real time (SMRT) sequencing.

USE OF SANGER **SEQUENCING IN GENOMICS OF INFECTIOUS DISEASES**

Sanger sequencing uses the SBS approach in which a DNA polymerase generates DNA reads from a template in which the DNA molecule can be analyzed. The nature of the nucleotide in a given position can be determined by using specific dyes(20,21)

Although Sanger sequencing is too laborious and expensive for WGS it still remains routinely used when sequencing of specific genes or fragment of genes is needed such as for viral or bacterial genotyping ,for resistance testing when SNPs are associated with specific genome regions. For the bacterial WGS the biological amplification by culture and single colony picking is needed whereas the PCR amplification of specific genes is done for both viruses and bacteria before amplicons are to be sequenced. The most used are ABI sequencers instruments, a brand that now proposes a series of capillary electrophoresis sequencers ranging from 1 to 96 capillaries and covering the needs of different laboratories.

SANGER SEQUENCING USED FOR DETECTION OF SINGLE NUCLEOTIDE MUTATIONS

Sanger sequencing is a method based on selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. The modern Sanger sequencing usually uses the fluorescently labeled dideoxynucleotides that are detected by a laser after capillary electrophoresis(22) to generate a sequence chromatogram with fluorescent peaks corresponding to incorporation of the four different fluorescent dyes which are coupled to ddATP, ddCTP, ddGTP, and ddTTP. The Sanger sequencing has proven useful for assessing the presence or absence of the recurrent single nucleotide mutations or small insertions/deletions in oncogenes and tumor suppressor genes in the surgically resected pathology specimens(23). The genomic DNA is extracted from snap-frozen or formalin-fixed, paraffin-embedded tumor tissue. The polymerase chain reaction (PCR) is performed by using oligonucleotide primers and genomic DNA which is isolated from the tumor tissue as a template to amplify the genetic region of interest . Sanger sequencing reactions are usually performed on the PCR amplicons to determine their composition of nucleotides. Such as examples of recurrent single nucleotide mutations with diagnostic, prognostic, or

therapeutic relevance that are now routinely assessed by Sanger sequencing in surgical specimens includes:

- IDH1 exon 4 contains the p.R132 hotspot and IDH2 exon 4 contains the p.R172 hotspot which are frequently mutated in WHO grade II and III oligodendrogliomas, in grade II and III diffuse/anaplastic astrocytomas and also in IDH-mutant glioblastomas .
- H3F3A and HIST1H3B containing the p.K27 hotspot frequently mutated in diffuse midline gliomas and rarely other midline tumor entities, including ganglioglioma and pilocytic astrocytoma
- BRAF exon 15 contains the p.V600 hotspot which frequently mutates in pleomorphic xanthoastrocytoma, ganglioglioma(24), extra cerebellar pilocytic astrocytoma and epithelioid glioblastoma. It also diffuses gliomas in children and other tumor entities.
- TERT promoter region contains the c.-124C and c.-146C hotspots upstream of the ATG translational start site which are frequently mutated in IDH-wildtype glioblastoma in adults, IDH-mutant and 1p/19-codeleted oligodendroglioma, anaplastic meningioma and also other tumor entities.

While mutant-specific antibodies have been generated to detect some of the recurrent mutations found in oncogenes in brain tumors and genetic analysis through Sanger sequencing or next-generation sequencing is required for their assessment. (25)(26)

BENEFITS

Sanger sequencing is a hearty testing system ready to decide if a point change or little cancellation or duplication is available. It has been broadly utilized for quite a few years in numerous settings, including characterizing the mutational range of a tumor just as distinguishing a sacred variation in demonstrative testing. It is routinely utilized in clinical consideration for the recognition of DNA arrangement variations, single nucleotide changes, or little additions or cancellations, when the range of DNA variety is obscure. As substantial DNA arrangement variety is frequently the reason for strange cell development as well as guideline and eventually tumorigenesis. Distinguishing proof of these oncogenic DNA grouping variations effectively prompts the advancement of disease including dental(27) treatments. Groundworks can be made to cover a few districts (amplicons) to cover any measure locale of intrigue. Additionally utilized in variety examines, genomics,forensic and demonstrative and applied therapeutics.

LIMITATIONS

Albeit one could utilize singular Sanger sequencing responses to cover any ideal locale, this testing approach can be expensive when contrasted and other multiplex testing frameworks. Thus, most of the present accessible Sanger sequencing tests are quality explicit or examine a little subset of qualities. Sanger sequencing can distinguish mosaic transformations including as low as 20% of the cells but however Sanger sequencing isn't decisively quantifiable. For instance as one can't finish up if a change is available in 25% versus 40% of cells dependent on top sizes so therefore extra testing techniques must be utilized for evaluation.

FUTURE ADVANCES FOR NGS/ SANGER SEQUENCING

With steady advances in NGS and associated technologies the costs of NGS are expected to decrease and speed and processing accuracy are expected to increase.

NGS technology will become increasingly accessible to researchers and clinicians and will continue to transform cancer genomics, leading to identification of all the major alterations in the cancer genomes.The incorporation of NGS in patient management holds the promise of advancing personalised cancer treatment with the goal of maximizing efficacy and minimizing toxicity.

CONCLUSION

The Sanger process has made it possible for researchers to sequence stretches of DNA at speeds never before possible. It has increased the sequencing rates ,drastically cut the cost of sequencing and may eventually allow every person the possibility of personalized genomic information. It also offers the promise of advanced medical treatments,which require a considerable amount of work to generate, understand, organize, and apply this massive amount of data to human disease.

REFERENCES

1. Men AE, Wilson P, Siemering K, Forrest S. Sanger DNA Sequencing [Internet]. Next Generation Genome Sequencing. p. 1–11. Available from: http://dx.doi.org/10.1002/9783527625130.ch1
2. Gupta SK, Viswanatha DS, Patel KP. Evaluation of Somatic Hypermutation Status in Chronic Lymphocytic Leukemia (CLL) in the Era of Next Generation Sequencing. Front Cell Dev Biol. 2020 May 19;8:357.
3. Thaventhiran JED, Lango Allen H, Burren OS, Rae W, Greene D, Staples E, et al. Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature [Internet]. 2020 May 6; Available from: http://dx.doi.org/10.1038/s41586-020-2265-1
4. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine – A Literature Review. Research Journal of Pharmacy and Technology. 2019;12(8):4052–6.
5. Head SR, Ordoukhanian P, Salomon DR. Next Generation Sequencing: Methods and Protocols. Humana Press; 2017. 266 p.
6. Preethikaa S, Brundha MP. Awareness of diabetes mellitus among general population. J Pharm Res [Internet]. 2018; Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=11&issue=5&article=024
7. Ballard D, Winkler-Galicki J, Wesoły J. Massive parallel sequencing in forensics: advantages, issues, technicalities, and prospects. Int J Legal Med [Internet]. 2020 May 25; Available from: http://dx.doi.org/10.1007/s00414-020-02294-0
8. Shenoy PB, Brundha MP. Awareness of polycystic ovarian disease among females of age group 18-30 years. J Pharm Sci [Internet]. 2016; Available from:

http://search.proquest.com/openview/a8a09e7b2e9d2f967bf3fee479c7018a/1?pq-origsite=gscholar&cbl=54977

9. Balaji S, Brundha. MP, Path DNB. Awareness of About Breast Cancer among Dental Surgeons.Journal of Pharmaceutical Sciences and Research, 8 (8), 797–800.
10. Hannah R, Ramani P, Brundha MP, Herald. J. Sherlin, Ranjith G, Ramasubramanian A, et al. Liquid Paraffin as a Rehydrant for Air Dried Buccal Smear [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 1197. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00199.9
11. Lee D, Kim J, Kim H, Heo H, Park K, Lee Y. High-performance transparent pressure sensors based on sea-urchin shaped metal nanoparticles and polyurethane microdome arrays for real-time monitoring. Nanoscale. 2018 Oct 21;10(39):18812–20.
12. Ravichandran H, Brundha MP. Awareness about Personal Protective Equipments in Hospital Workers (Sweepers and Cleaners) – Research.International Journal of Pharmaceutical Sciences Review and Research, 40(1),28–29.
13. Bridge PD, Pearce DA, Rivera A, Rutherford MA. VNTR derived oligonucleotides as PCR primers for population studies in filamentous fungi [Internet]. Vol. 24, Letters in Applied Microbiology. 1997. p. 426–30. Available from: http://dx.doi.org/10.1046/j.1472-765x.1997.00060.x
14. Kumar MDA, Ashok Kumar MD, Brundha MP. Awareness about nocturia-A questionnaire survey [Internet]. Vol. 9, Research Journal of Pharmacy and Technology. 2016. p. 1707. Available from: http://dx.doi.org/10.5958/0974-360x.2016.00344.9
15. Swetha S, Brundha MP. Analysis of knowledge about the hospital warning symbols among the postgraduate dental students-A comparative study [Internet]. Vol. 10, Research Journal of Pharmacy and Technology. 2017. p. 975. Available from: http://dx.doi.org/10.5958/0974-360x.2017.00177.9
16. Brundha MP, Nallaswamy D. Hide and seek in pathology-A research on game-based histopathology learning. International Journal of Research Pharmaceutical Sciences. 2019;10 (2):1410–4.
17. Prashaanthi N, Brundha MP. A Comparative Study between Popplet Notes and Conventional Notes for Learning Pathology [Internet]. Vol. 11, Research Journal of Pharmacy and Technology. 2018. p. 175. Available from: http://dx.doi.org/10.5958/0974-360x.2018.00032.x
18. Feldmeyer B, Hoffmeier K, Pfenninger M. The complete mitochondrial genome of Radix balthica (Pulmonata, Basommatophora), obtained by low coverage shot gun next generation sequencing [Internet]. Vol. 57, Molecular Phylogenetics and Evolution. 2010. p. 1329–33. Available from: http://dx.doi.org/10.1016/j.ympev.2010.09.012
19. He P, Wei P, Chen X, Lin Y, Peng J. Identification and characterization of microRNAs in the gonad of Trachinotus ovatus using Solexa sequencing. Comp Biochem Physiol Part D Genomics Proteomics. 2019 Jun;30:312–20.
20. Shreya S, Brundha MP. Alteration of Haemoglobin Value in Relation to Age, Sex and Dental Diseases-A Retrospective Correlation Study

[Internet]. Vol. 10, Research Journal of Pharmacy and Technology. 2017. p. 1363. Available from: http://dx.doi.org/10.5958/0974-360x.2017.00241.4

21. Brundha MP, Pathmashri VP, Sundari S. Quantitative Changes of Red Blood cells in Cancer Patients under Palliative Radiotherapy-A Retrospective Study [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 687. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00122.7
22. Brundha MP, Saivignesh S. Myeloid sarcoma [Internet]. Vol. 3, International Journal of Clinicopathological Correlation. 2019. p. 41. Available from: http://dx.doi.org/10.4103/ijcpc.ijcpc_11_19
23. Kalaiselvi R, Brundha MP. Prevalence of hysterectomy in South Indian population [Internet]. Vol. 9, Research Journal of Pharmacy and Technology. 2016. p. 1941. Available from: http://dx.doi.org/10.5958/0974-360x.2016.00398.x
24. Kendall BE, Hunter JV. Diagnostic Imaging of Brain Tumours [Internet]. Malignant Brain Tumours. 1995. p. 221–64. Available from: http://dx.doi.org/10.1007/978-1-4471-1877-0_10
25. Ferdioz, J., & Brundha, M. P. (2016). Awareness of stye. International Journal of Pharmaceutical Sciences Review and Research, 40(1), 30–32.[Internet]. [cited 2020 Jun 9]. Available from: https://www.scopus.com/record/display. uri?origin=recordpage&eid=2-s2.0- 84989287584
26. Brundha, M. P. (2015). A comparative study- the role of skin and nerve biopsy in hansen's disease. Journal of Pharmaceutical Sciences and Research, 7 (10), 837–844.[Internet]. [cited 2020 Jun 9]. Available from: https://www.scopus.com/record/display. uri?origin=recordpage&eid=2-s2.0- 84944874047
27. Harsha L, Brundha MP. Prevalence of Dental Developmental Anomalies among Men and Women and its Psychological Effect in a Given Population.Journal of Pharmaceutical Sciences and Research, 9(6), 869–873.