DNA Sequencing Using Ion Torrent Technology

Alexandra Burnett
Outline

- Introduction
- Background
- Case Study-Fungi
- Case Study-Forensics
- Conclusion
Introduction: History

- DNA Sequencers
- Sanger method, 1977
- 25 years later, NGS
- Optics sequencers
- 2010, Ion Torrent

Introduction: Development

- Still a new technique
- Not yet widespread
- Increasing attention
- Used in more and more applications

http://massgenomics.org/2010/03/agbt-ion-torrent-semiconductor-sequencin.html
Introduction: Uses

- Genomics
- Medicine
- Forensics
- Anthropology
- Environmental DNA

http://blog.dnagenotek.com/topic/buccal-swab
Background: Sample Prep

- DNA/RNA is purified from the sample of interest
- Sample is fragmented
- Convert to DNA if sample is RNA
- Oligonucleotide adapters attached

Background: Analysis

- Fragments multiplied on beads
- Each bead goes in a well in the chip
- Wells flooded with nucleotide
- $H^+$ released only when there is a match
- Amount of $H^+$ determines how many matches in a row
- The fragments are added up to provide the complete sequence

http://www.wright.edu/~oleg.paliy/NGS.html
Background: Other Techniques

**Ion Torrent**
- Cheapest
- ~2 hours
- 1.71% error
- ~200 bases
- 100-1000ng DNA Required

**Pac Bio**
- Most Expensive
- ~2 hours
- 12.86% error
- Reads~1500 bases
- ~1µg DNA Required

**Illumina**
- Moderately Expensive
- ~27 hours-11 days
- >1% error
- Up to 150 bases
- 500-1000ng Required
RESEARCH ARTICLE

Deep Ion Torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystem

Shawn P. Brown¹, Mac A. Callaham Jr², Alena K. Oliver¹ & Ari Jumpponen¹

¹Division of Biology, Kansas State University, Manhattan, KS, USA; and ²Center for Forest Disturbance Science, USDA Forest Service, Southern Research Station, Athens, GA, USA
Case Study: Soil Fungi

- Studied fungi in soil samples
- Forest fires
- Fungi DNA used to compare samples
- Fungi specific primers
- Used Ion Torrent sequencing

https://en.wikipedia.org/wiki/Ectomycorrhiza
Case Study: Soil Fungi

Results:
- Found diverse fungi samples
- 6 years may not be a sufficient interval

Ion Torrent:
- Low cost
- Small fragments

Fig. 1. Venn diagram displaying shared and unique OTU counts among the three long-term prescribed fire treatments.
Case Study: Soil Fungi
Strategies for complete mitochondrial genome sequencing on Ion Torrent PGM™ platform in forensic sciences

Yishu Zhou¹, Fei Guo², Jiao Yu², Feng Liu², Jinling Zhao², Hongying Shen², Bin Zhao², Fei Jia², Zhu Sun², He Song¹, Xianhua Jiang¹,²,⁎

¹ China Medical University School of Forensic Medicine, No. 77, Puhe Road, Shenyang North New Area, Shenyang, Liaoning 110122, PR China
² Criminal Science and Technology Institute of Liaoning Province, No. 2, Qishan Middle Road, Huanggu District, Shenyang, Liaoning 110032, PR China
³ Department of Forensic Medicine, National Police University of China, No. 83, Taiwan Street, Huanggu District, Shenyang, Liaoning 110854, PR China
Case Study: Forensics

- Application of Ion Torrent
- Used mitochondrial DNA
  - Inherited from the mother
- CR region used
- Compared DNA from 3 generations

https://en.wikipedia.org/wiki/Mitochondrial_DNA
Case Study: Forensics

Averaged coverage (n = 107)

Strand balance (n = 107)
Case Study: Forensics

Distribution of homopolymers (rCRS)

Distribution of variants (n = 107)
Case Study: Forensics

Conclusion:
- Ion torrent has potential
- Sample prep
- Error
- Data analytical programs

https://en.wikipedia.org/wiki/Mitochondrial_DNA
Summary

**Advantages**
- Low cost
- Fast
- Low amount of DNA needed

**Disadvantages**
- Lower accuracy
- Long sample prep time
- Small fragments required
Conclusion

Ion Torrent sequencing has the advantage with low cost and fast analysis, but the small fragments required and higher error rate limits its uses.
References

Thanks!

Any questions?