DNA sequencing
Third-generation sequencing

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Outline

- Introduction to DNA sequencing methods (1).
- Mechanism of Single Molecule Real-Time sequencing (1)(2)(3)(4).
- Mechanism of Nanopore Sequencing (1)(2)(3).
- Applications (1)(2).
- Conclusion.
- References.

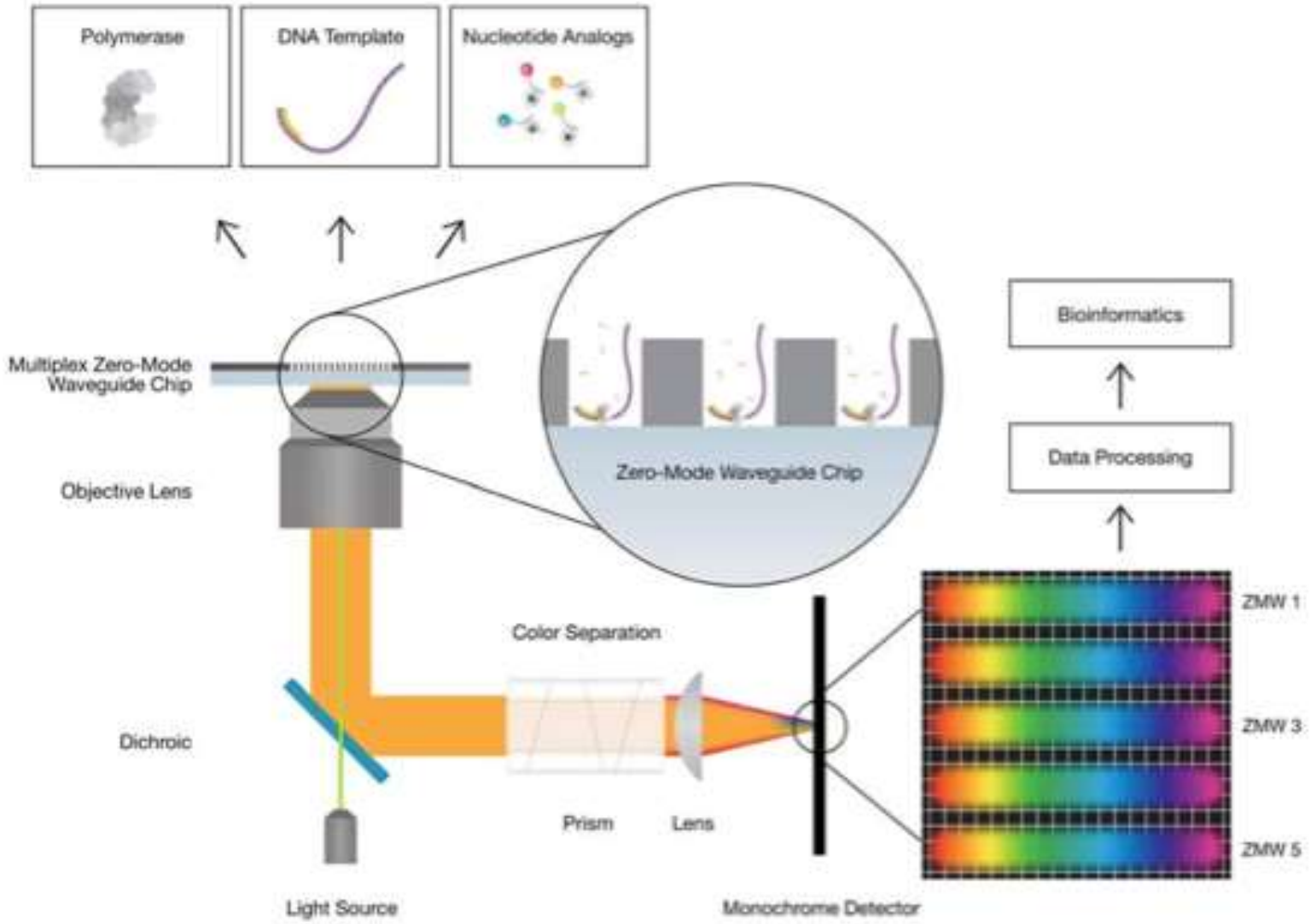
DNA sequencing methods

- Sanger's chain termination method
- Next generation sequencing method
  - 454 pyrosequencing
  - Illumina sequencers
  - Ion Torrent
- Third-generation sequencing method
  - Single Molecule Real-Time (SMRT) sequencing (Pacific Biosystems)
  - Nanopore sequencing (Oxford Nanopore Technologies)

Table 1. Comparison of the output of selected sequencing platforms. Numbers are according to companies or recent publications.

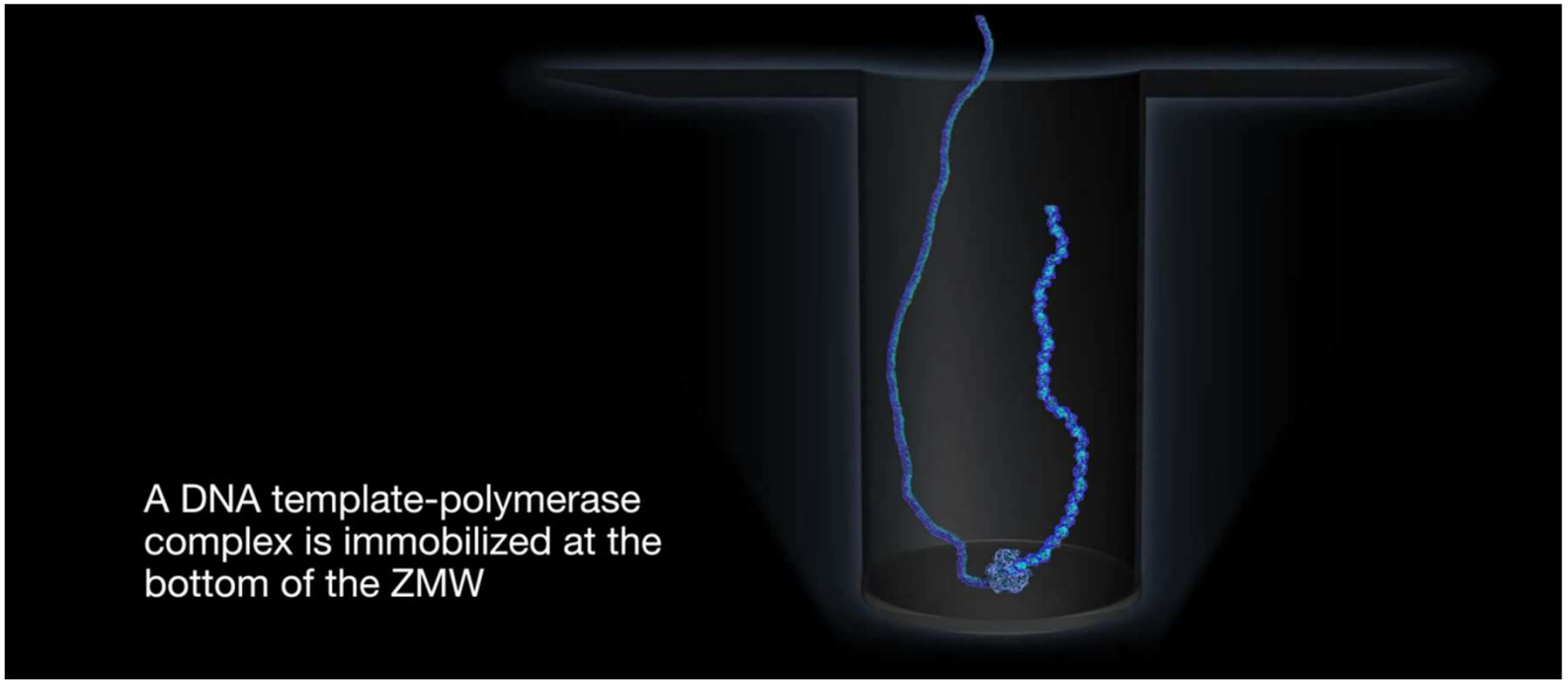
Platform	Sequencer	Costs sequencing platform	Reads per run/lane	Output per run/lane	Maximal read lengths[1]	Average run duration
Sanger	ABI 3730xl	$100,000	96	100 kbp	1000 bp	2–3 hours
454	GS FLX	$450,000	1,000,000	700 mpb	1000 bp	24 hours
Illumina	HiSeq 3000	$750,000	300,000,000[2]	150 gbp[3]	250 bp	4 days
Illumina	NextSeq500	$250,000	400,000,000	120 gbp[3]	150 bp	30 hours
Illumina	MiSeq	$100,000	25,000,000	15 gbp[3]	300 bp	24 hours
Ion Torrent	Proton II	$224,000	330,000,000	66 gbp	200 bp	4 hours
Ion Torrent	PGM 318	$50,000	5,000,000	2 gbp	400 bp	7 hours
PacBio	RS II	$700,000	50,000	400 mbp	54 kbp	3 hours
Nanopore	MinION	$1,000	80,000[4]	490 mbp[4]	150 kbp	n.a.[4]

SMRT sequencing



https://petridishtalk.com/2011/10/06/zero-mode-waveguide-single-molecule-real-time-sequencing/ 2019/6/19

SMRT sequencing



https://www.pacb.com/smrt-science/smrt-sequencing/ 2019/6/24

Nanopore sequencing

- The device：the size of a small cell phone and can be plugged into the USB of a laptop.

https://www.genengnews.com/magazine/310/sequencing-goes-long/ 2019/6/19

Nanopore sequencing

https://www.youtube.com/watch?v=GUb1TZvMWsw 2019/6/24

Application

- The potential of SMRT long reads has recently been demonstrated as they are powerful in resolving long repeat regions and might soon become the gold standard in prokaryote genome sequencing (Chin et al.,2013).
- SMRT long reads have been used to resolve complex genomic regions of chimp and human (Chaisson et al.,2015; Huddleston et al.,2014).
- Initial results from nanopore sequencing also show that it is possible to reconstruct a bacterial genome sequence solely (Loman, Quick, & Simpson,2015).

Conclusion

- Third-generation sequencing aims to improve on the next-generation methods by carrying out sequencing in real time.
- Real-time sequencing also enables read lengths to be longer, as the regular pauses that occur during the next-generation methods reduces the processivity of the polymerase.
- Despite having many advantages, third-generation sequencing methods still have problem regarding of high error rate. However, it has been demonstrated that methodological improvements strongly improved the quality and length of sequence reads.

References

- (1) : Brown T.A. (2016). *Gene cloning & DNA analysis an introduction 7th edition*. Manchester : Wiley.
- (2) : Christoph Bleidorn. "Systematics and Biodiversity". *Perspective Third generation sequencing : technology and its potential impact on evolutionary biodiversity research* 21 Aug.2015 : 8. Print.
- (3) : Videos : Pacific Biosystems (PacBio) and National DNA Database channels.
- (4) : Figures : https://petridishtalk.com/2011/10/06/zero-mode-waveguide-single-molecule-real-time-sequencing/

(accessed date : 18 Jan.2019)

Thank you for listening!