

Sequencing technologies – the next generation

Ann-Kathrin Wagner

1 July 2015



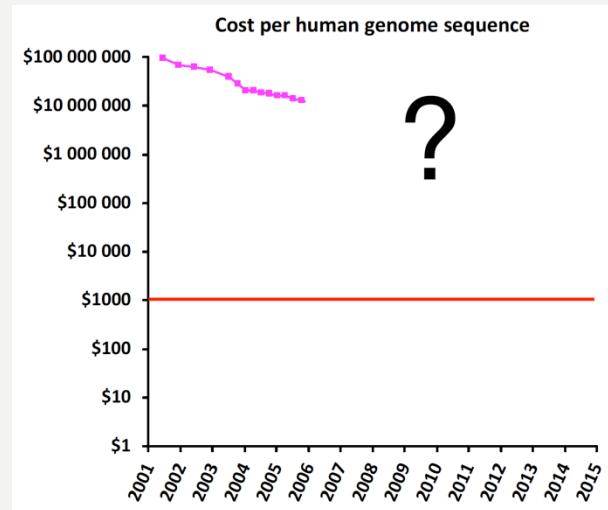


1. Introduction and repetition
2. Next generation sequencing based on fluorescence dyes
3. Comparison of methods used by different instruments

Introduction



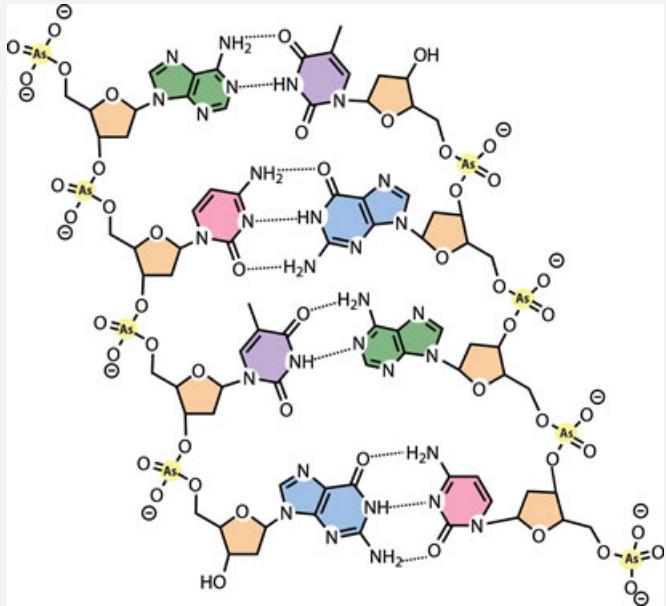
- US\$ 1000 Human Genome Project by 2014



- Personalized genomics for medical purposes



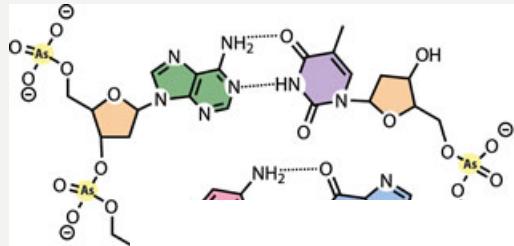
Sequence: order of nucleotides within DNA



http://www.rsc.org/images/Arsenic-DNA-chemical-structure_410_tcm18-214805.jpg



Sequence: order of nucleotides within DNA



ORIGINAL SEQUENCE

- UGUAC AUG UAU ACG UCU CAA UGA UCCA
Met Tyr Ser Thr Gln STOP

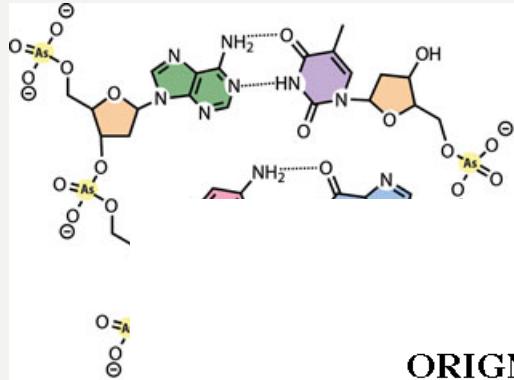
POINT MUTATIONS

- UGUAC AUG UAU ACG UCU CAG UGA UCCA
Met Tyr Ser Thr Gln STOP
- UGUAC AUG UAU ACG CCU CAA UGA UCCA
Met Tyr Ser Pro Gln STOP
- UGUAC AUG UAA ACG UCU CAA UGA UCCA
Met **STOP**

<http://www.ucl.ac.uk/~sjjgsca/PointMutations.gif>



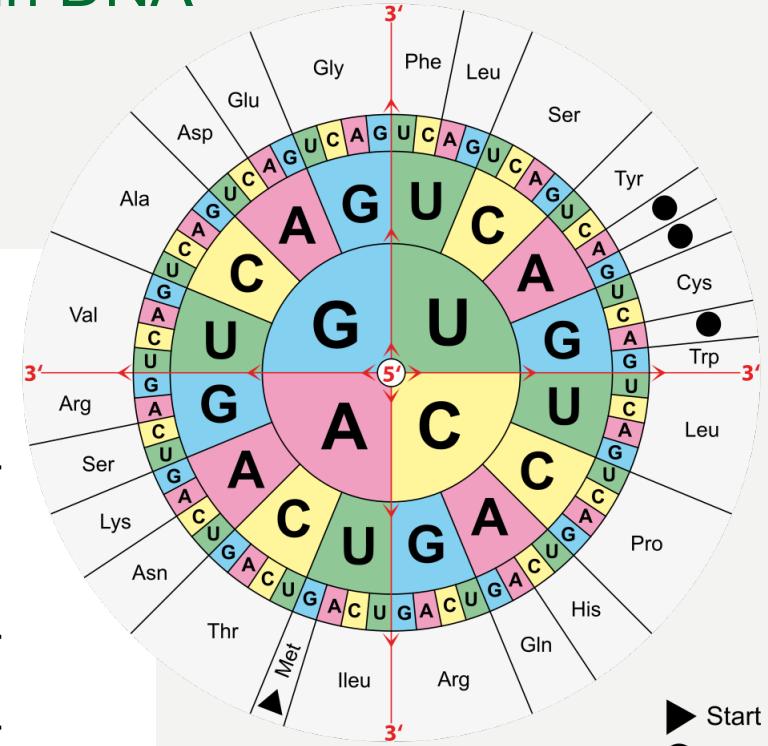
Sequence: order of nucleotides within DNA



- UGUAC AUG UAU ACG UCU CAA UGA UCCA
Met Tyr Ser Thr Gln STOP

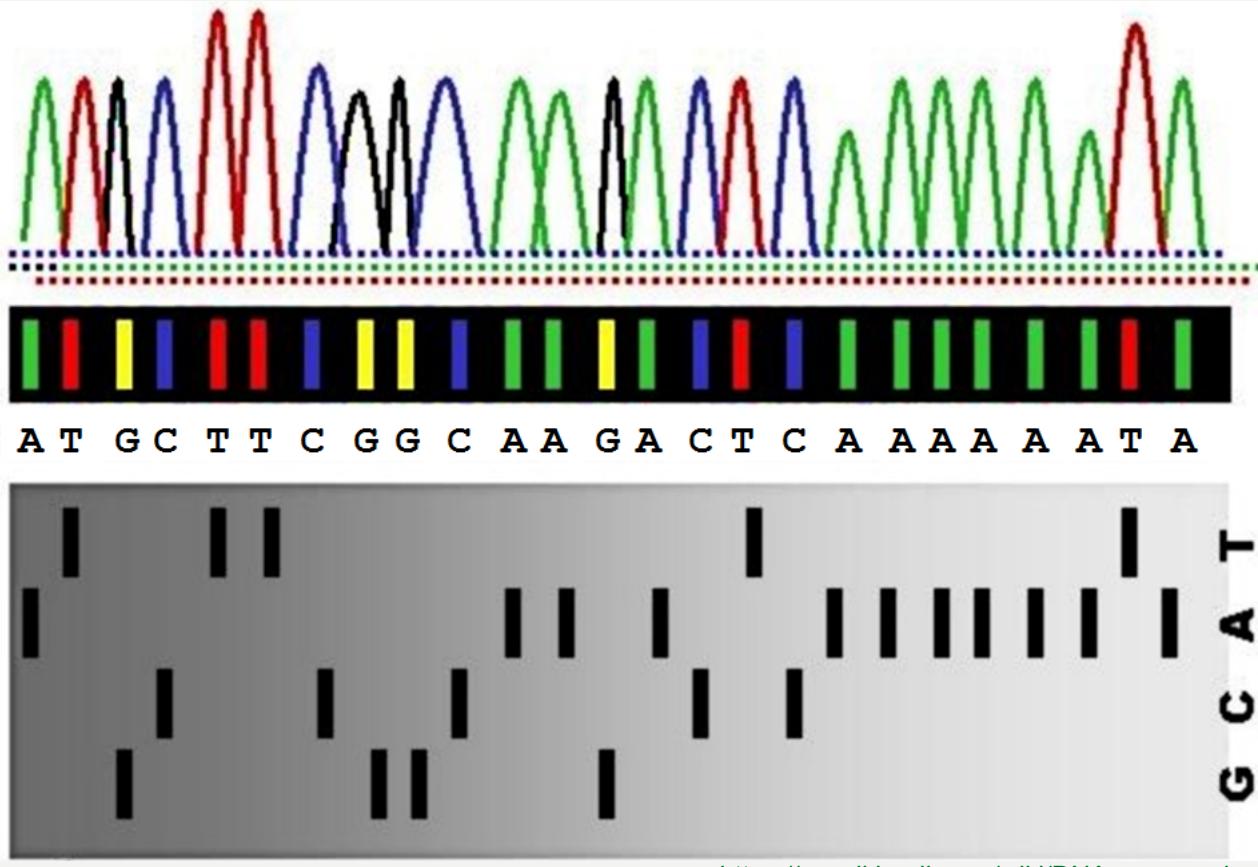
POINT MUTATIONS

- UGUAC AUG UAU ACG UCU **CAG** UGA UCCA
Met Tyr Ser Thr Gln STOP
- UGUAC AUG UAU ACG **CCU** CAA UGA UCCA
Met Tyr Ser **Pro** Gln STOP
- UGUAC AUG **UAA** ACG UCU CAA UGA UCCA
Met **STOP**



http://biobook.nerinxs.org/bb/genetics/dna/1000px-Aminoacids_table.png

<http://www.ucl.ac.uk/~sjjgsca/PointMutations.gif>



https://en.wikipedia.org/wiki/DNA_sequencing

→ use of fluorophores instead of radiophosphates

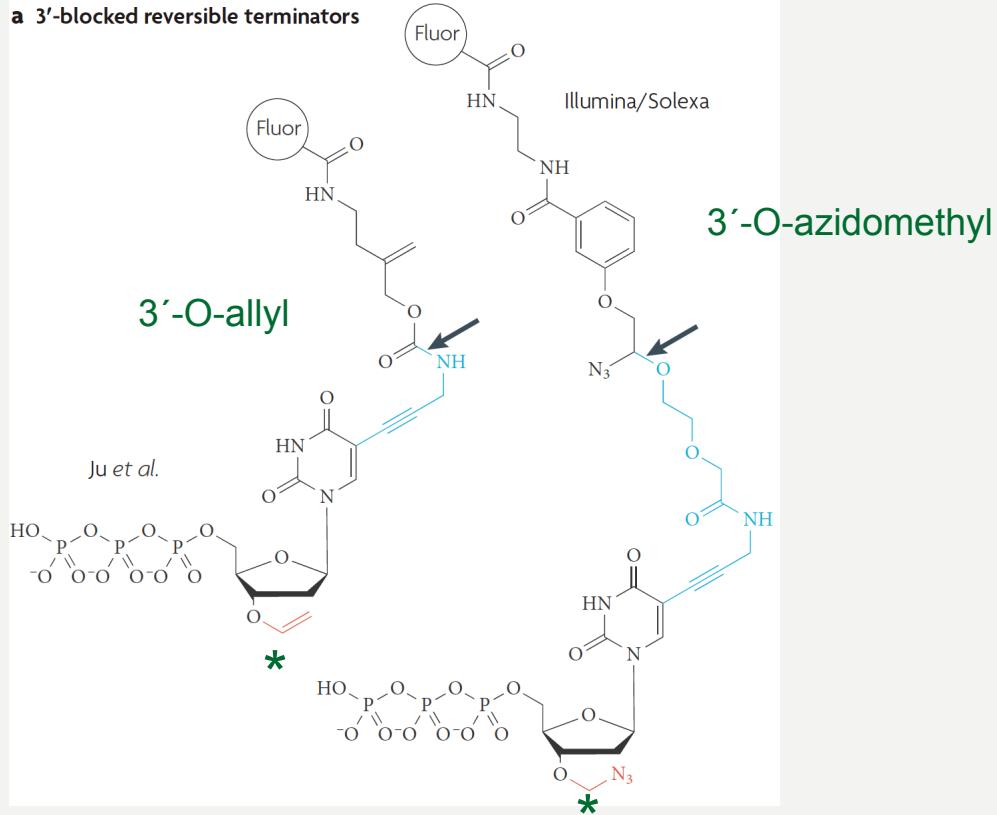


1. Fluorophore attachment
2. Template prep
3. Sequencing
4. Data analysis

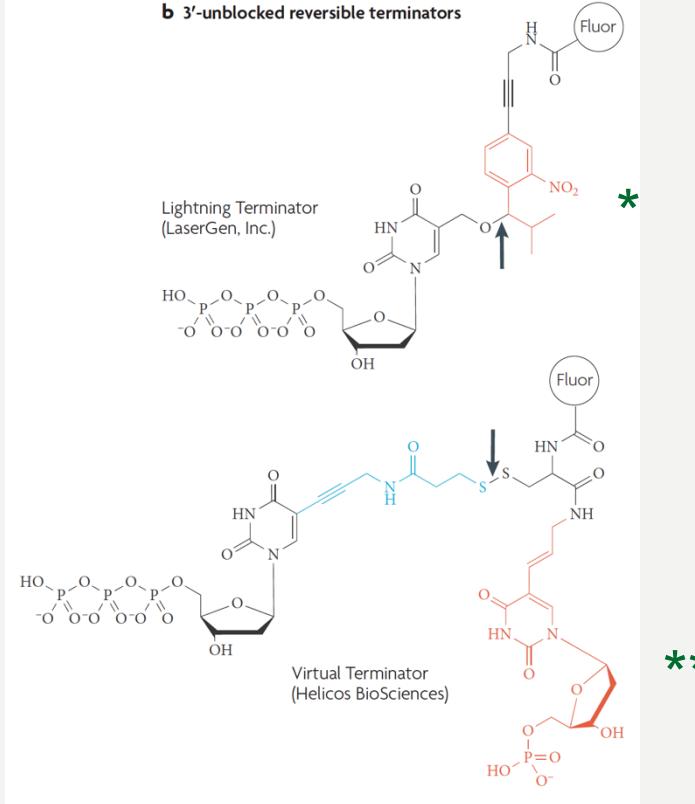
Fluorescence signal – base linked fluorescent nucleotides



a 3'-blocked reversible terminators



b 3'-unblocked reversible terminators



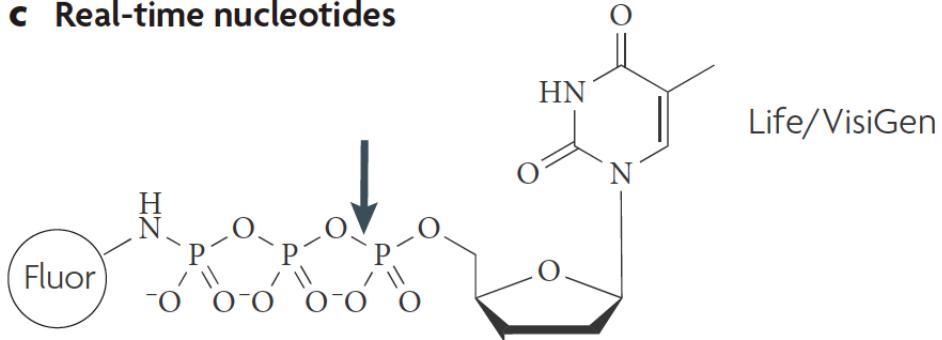
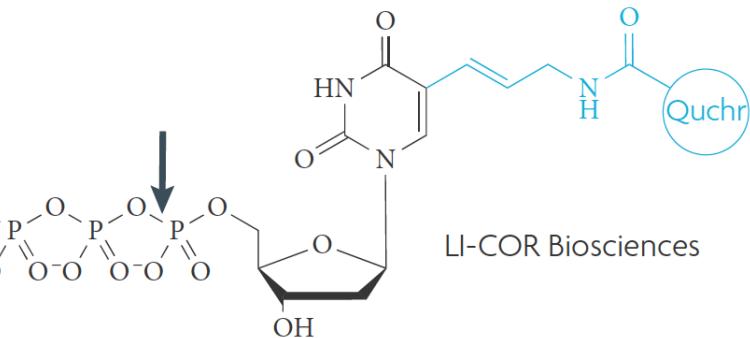
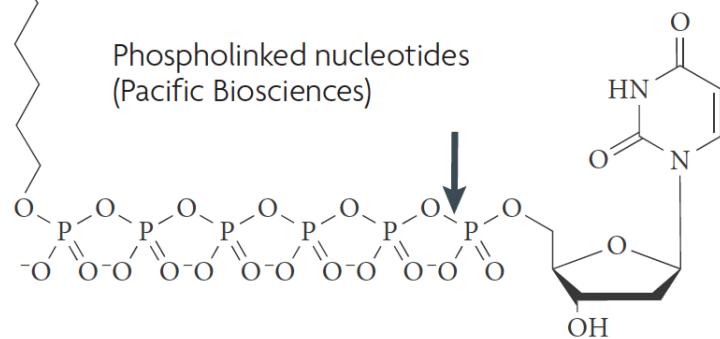
* Terminating functional group

** inhibitory function

Metzker, M.; Nature Reviews (2010)



c Real-time nucleotides

Phospholinked nucleotides
(Pacific Biosciences)

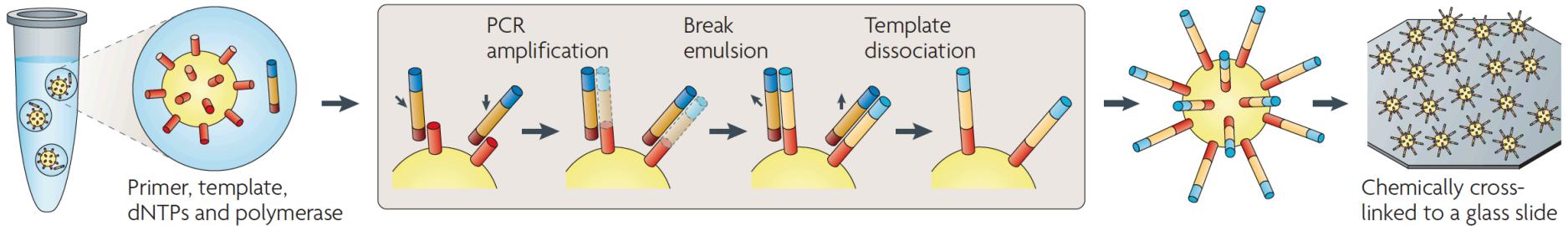
→ no terminating group

Metzker, M.; Nature Reviews (2010)

Template prep – clonally amplified

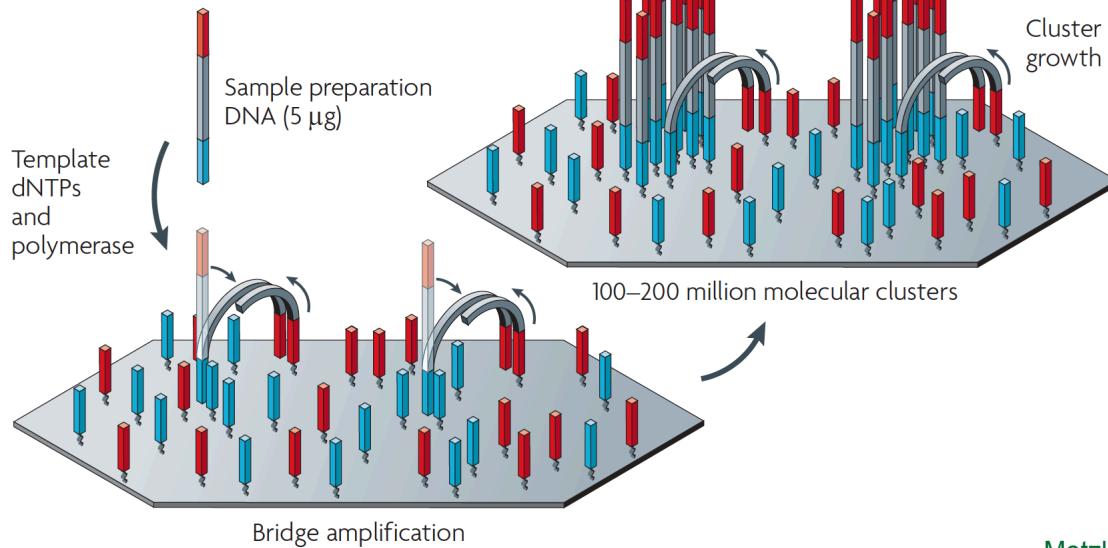
a Roche/454, Life/APG, Polonator Emulsion PCR

One DNA molecule per bead. Clonal amplification to thousands of copies occurs in microreactors in an emulsion



b Illumina/Solexa Solid-phase amplification

One DNA molecule per cluster

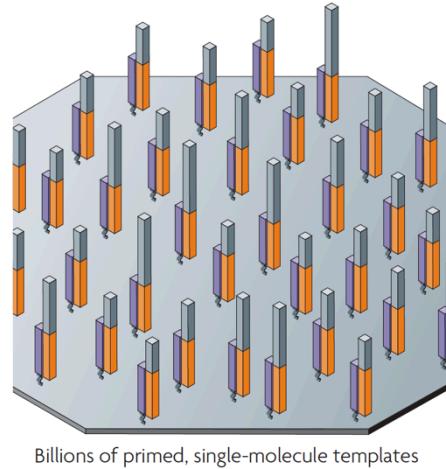


Metzker, M.; Nature Reviews (2010)

Template prep – single molecule

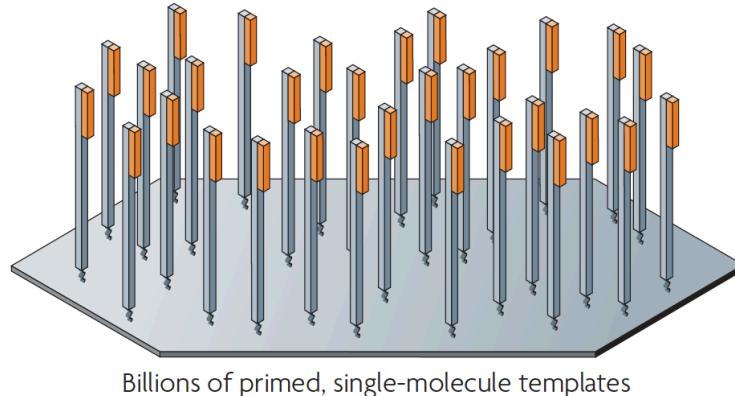


c Helicos BioSciences: one-pass sequencing
Single molecule: primer immobilized

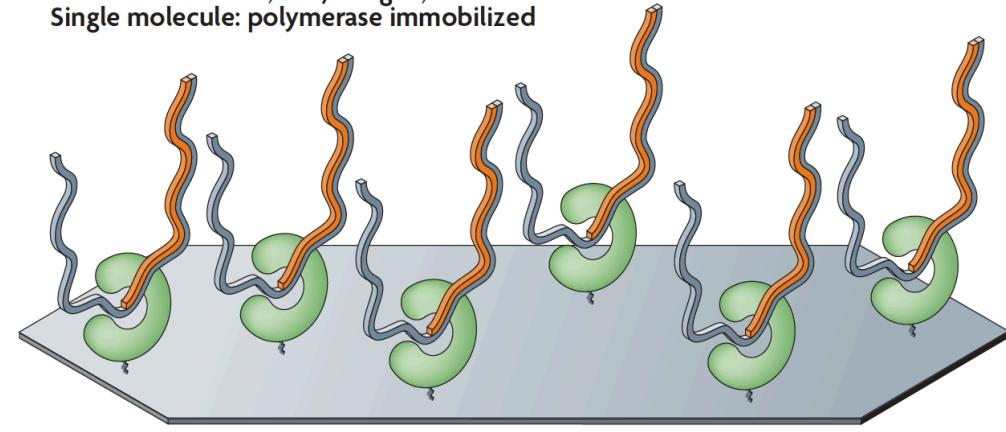


- C: hybridization to primer
- D: attachment to solid support
- E: polymerases attachment

d Helicos BioSciences: two-pass sequencing
Single molecule: template immobilized



e Pacific Biosciences, Life/Visigen, LI-COR Biosciences
Single molecule: polymerase immobilized



Thousands of primed, single-molecule templates

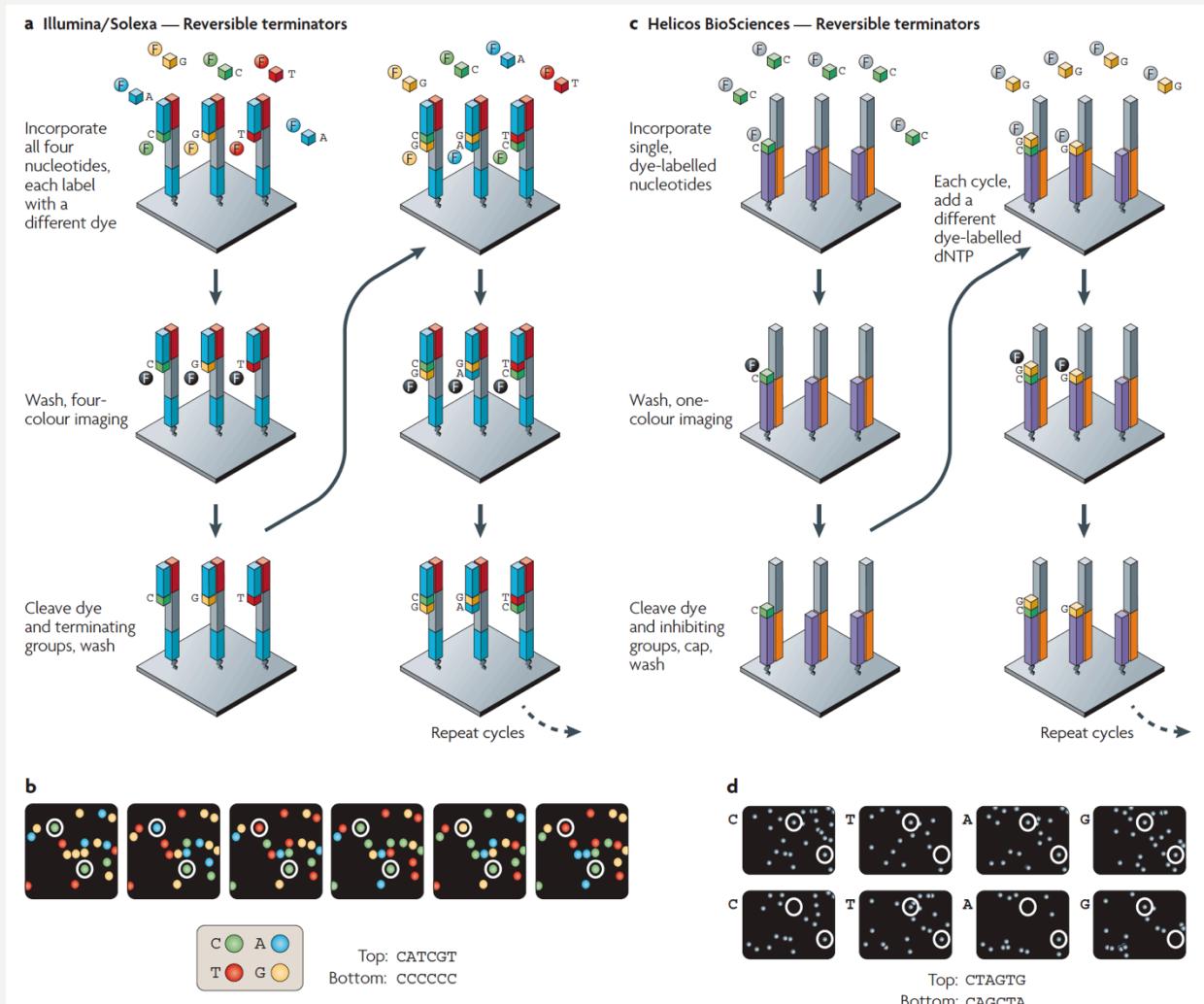
Metzker, M.; Nature Reviews (2010)

Sequencing – cyclic reversible termination



4-color
cyclic
reversible
termination

1-color at
a time

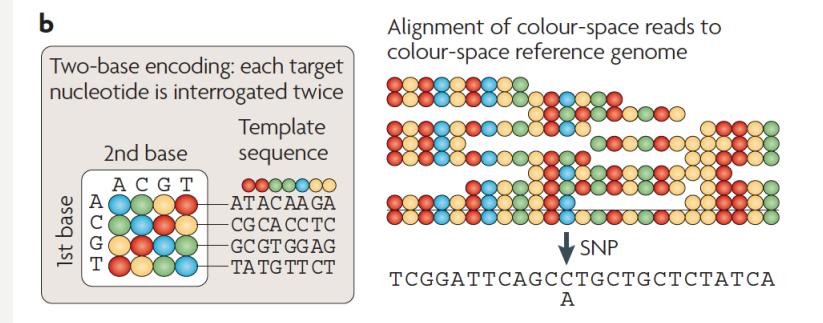
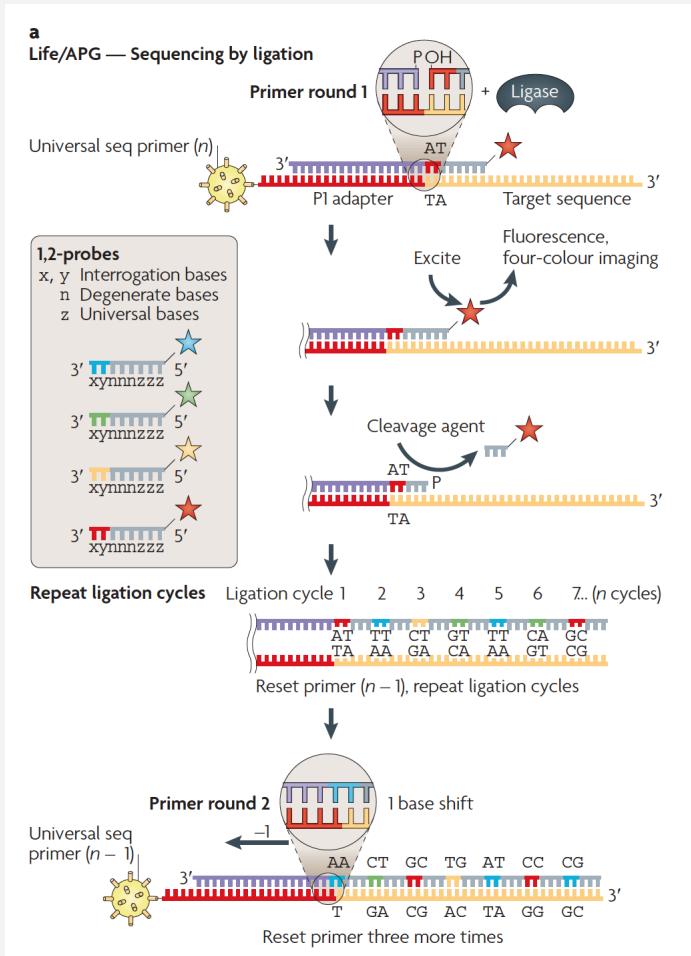


Metzker, M.; Nature Reviews (2010)

Sequencing – sequencing by ligation



SoLiD:

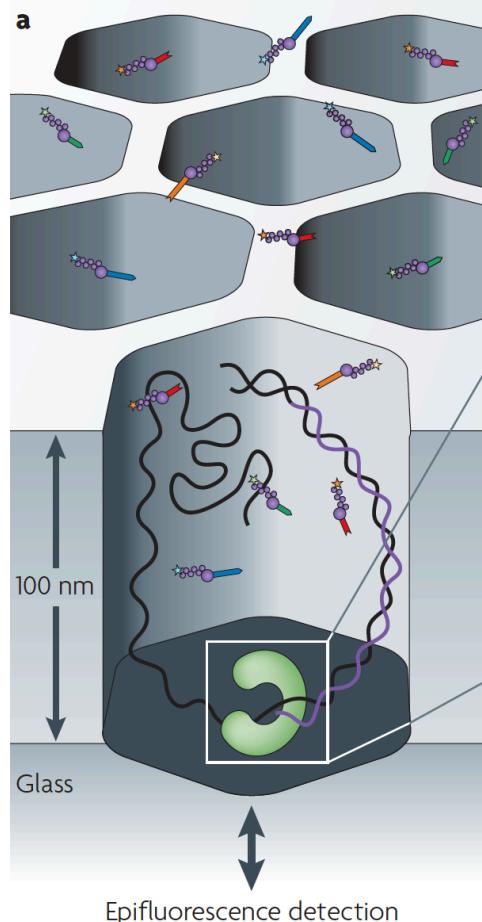


→decode DNA sequence

Metzker, M.; Nature Reviews (2010)

Sequencing - SMRT

Pacific Biosciences — Real-time sequencing

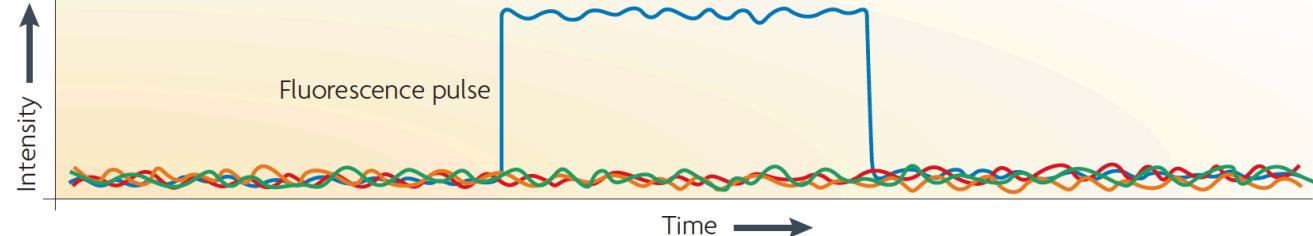


Phospholinked hexaphosphate nucleotides



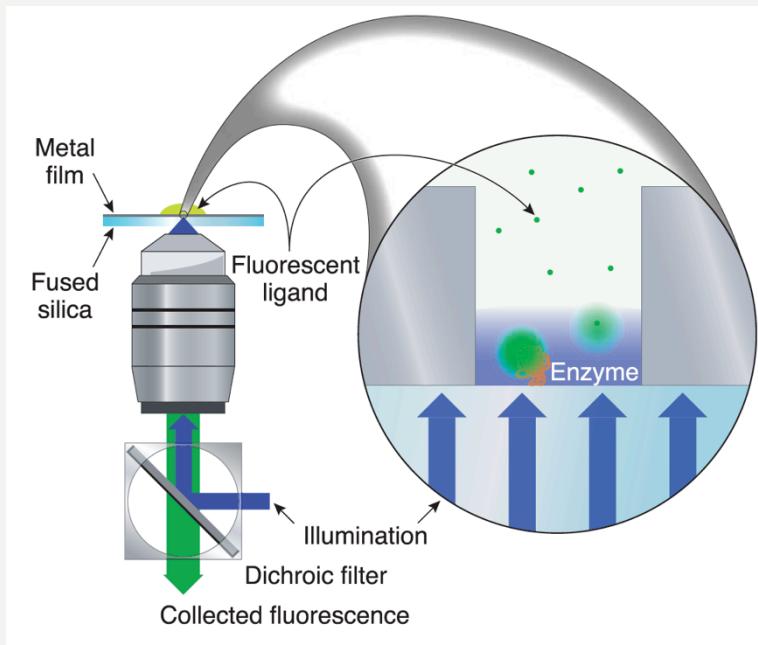
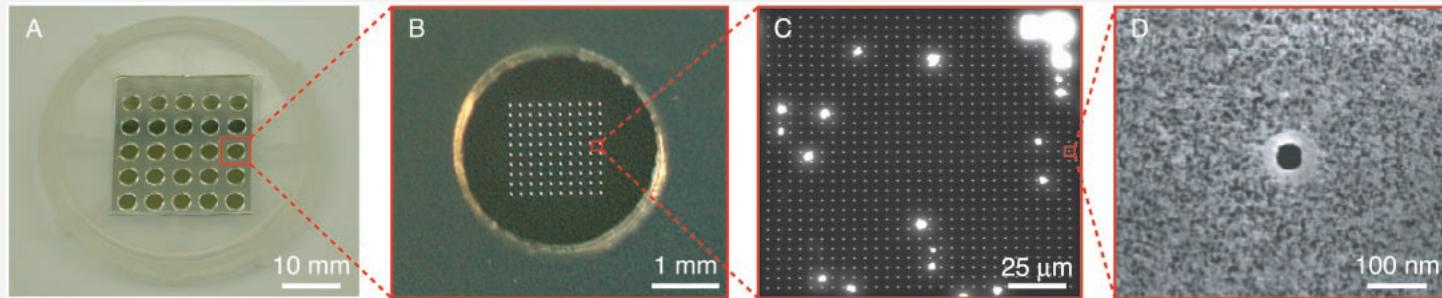
b

Limit of detection zone

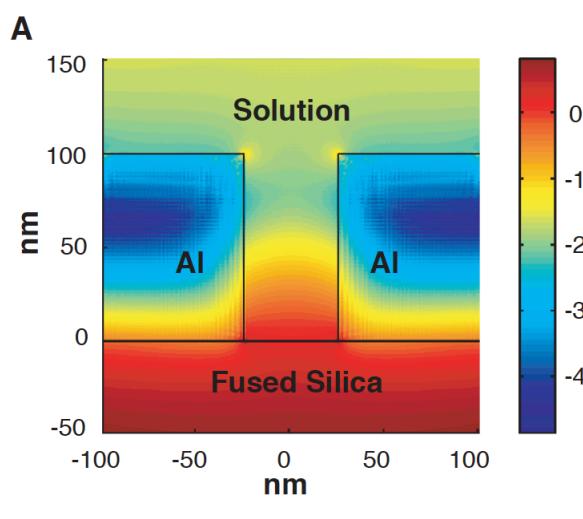


Metzker, M.; Nature Reviews (2010)

Zero Mode Waveguides (ZMW)



- subwavelength holes in metal film
- studies at μM concentration & μs temporal resolution



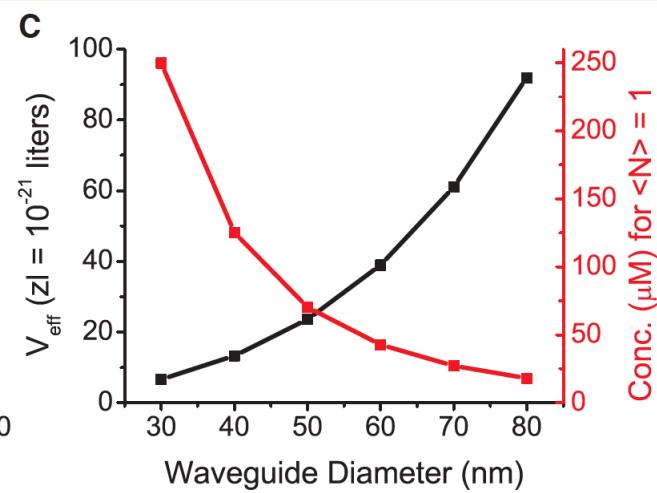
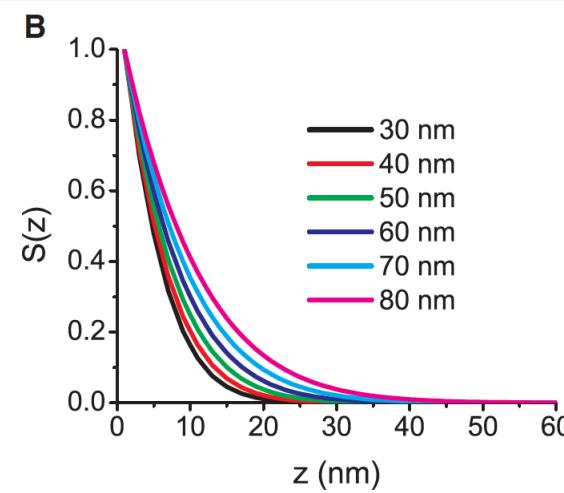
→ circular polarized light

$$S(z) = I(z)p(z) \frac{p(z)}{p(z)+C}$$

eff. observation profile

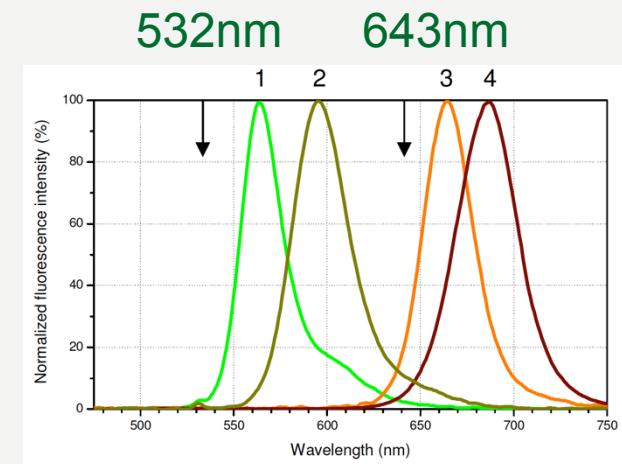
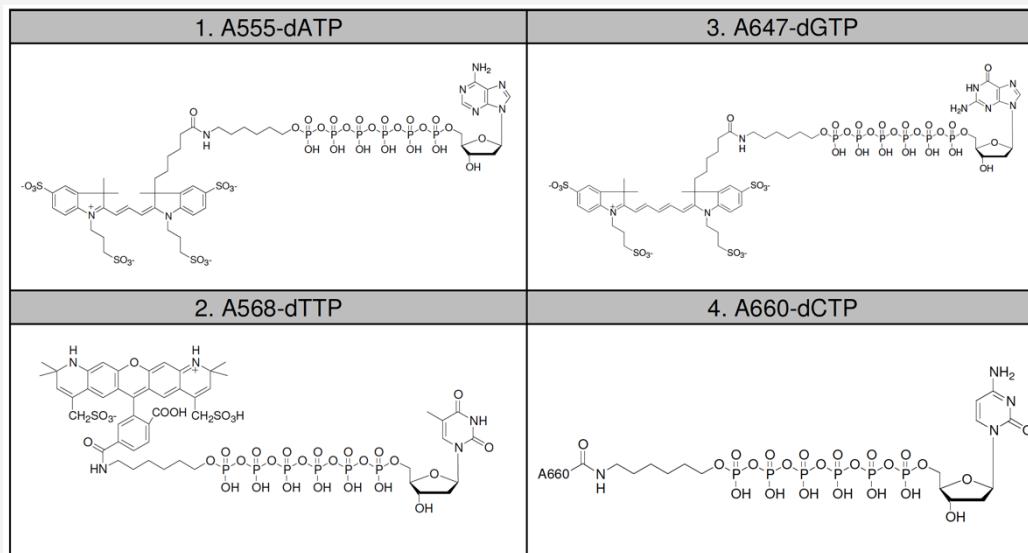
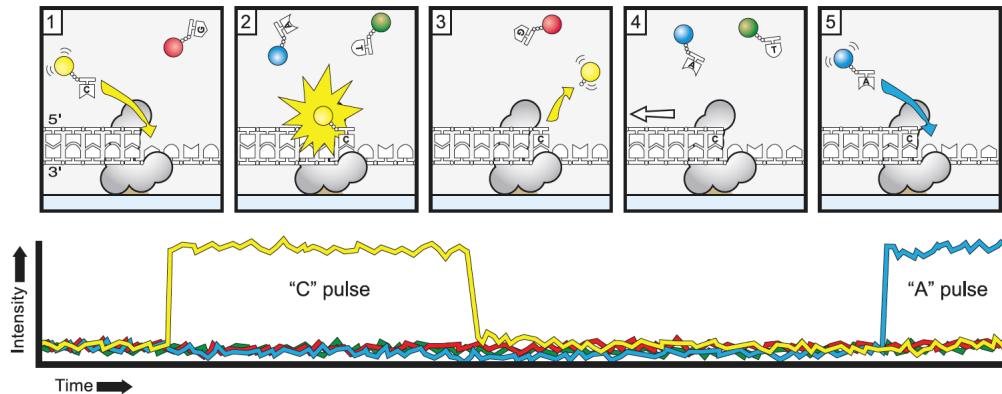
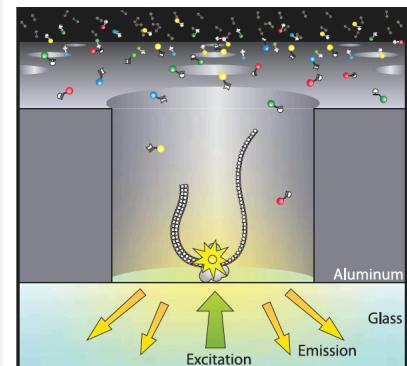
$$V_{\text{eff}} = \frac{\pi d^2}{4} \left(\int S(z) dz \right)^2$$

eff. observation volume



→ limits V_{eff} to first
10-20 nm

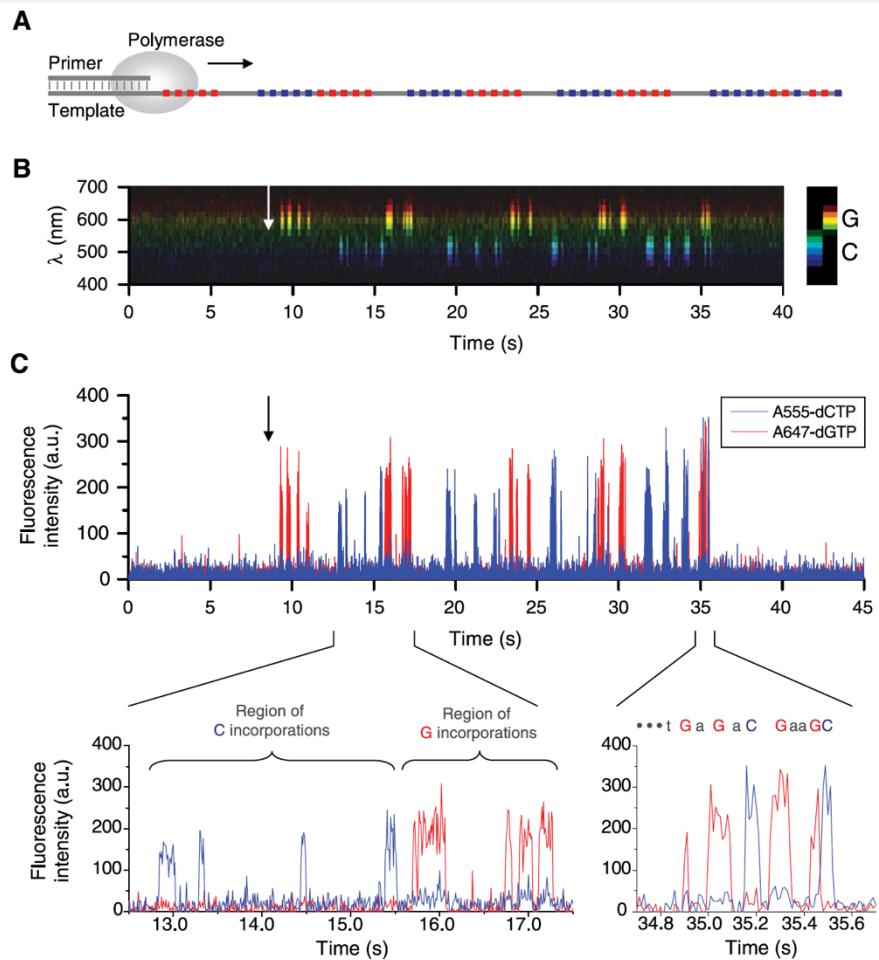
Signal detection & data analysis



Eid, J.; Science (2009)



ssDNA 2-base artificial sequence pattern (CG)



| | A | |
|--------|------------------|-------------------------|
| | Single molecule | Bulk |
| | Pulse width (ms) | Incorporation time (ms) |
| pH 6.5 | A555-dCTP | 115 ± 6 |
| | A647-dGTP | 168 ± 5 |
| pH 7.1 | A555-dCTP | 94 ± 14 |
| | A647-dGTP | 126 ± 10 |
| | | 250 ± 21 |
| | | 211 ± 13 |
| | | 72 ± 5 |
| | | 55 ± 4 |

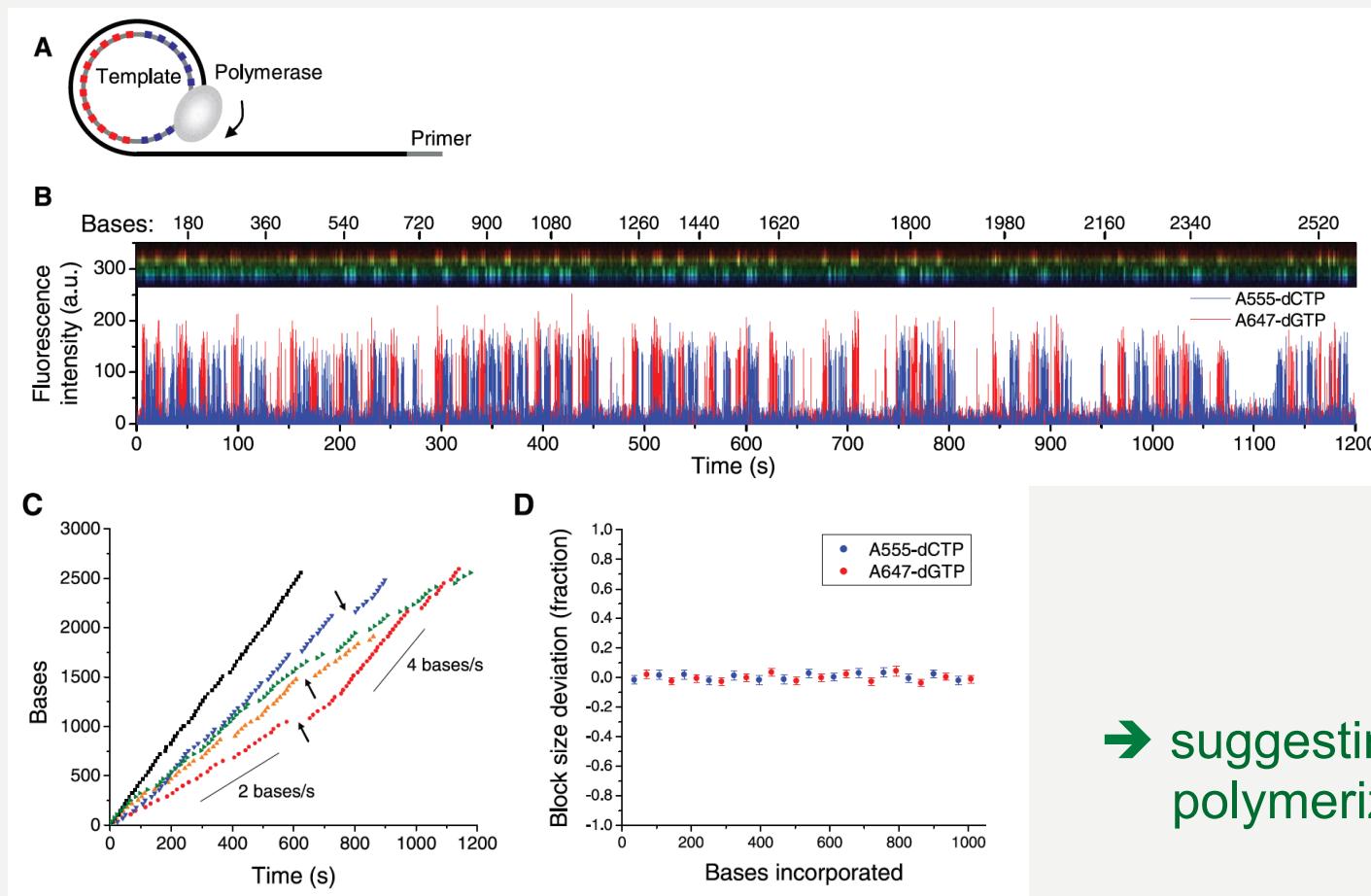
| | A | |
|--------|------------------------------|-----------------|
| | Single molecule | Bulk |
| | DNA synthesis rate (bases/s) | |
| 100 nM | 1.40 ± 0.03 | 1.10 ± 0.04 |
| 250 nM | 3.75 ± 0.10 | 2.20 ± 0.10 |

→ sensitive to pH and concentration change

Eid, J.; Science (2009)



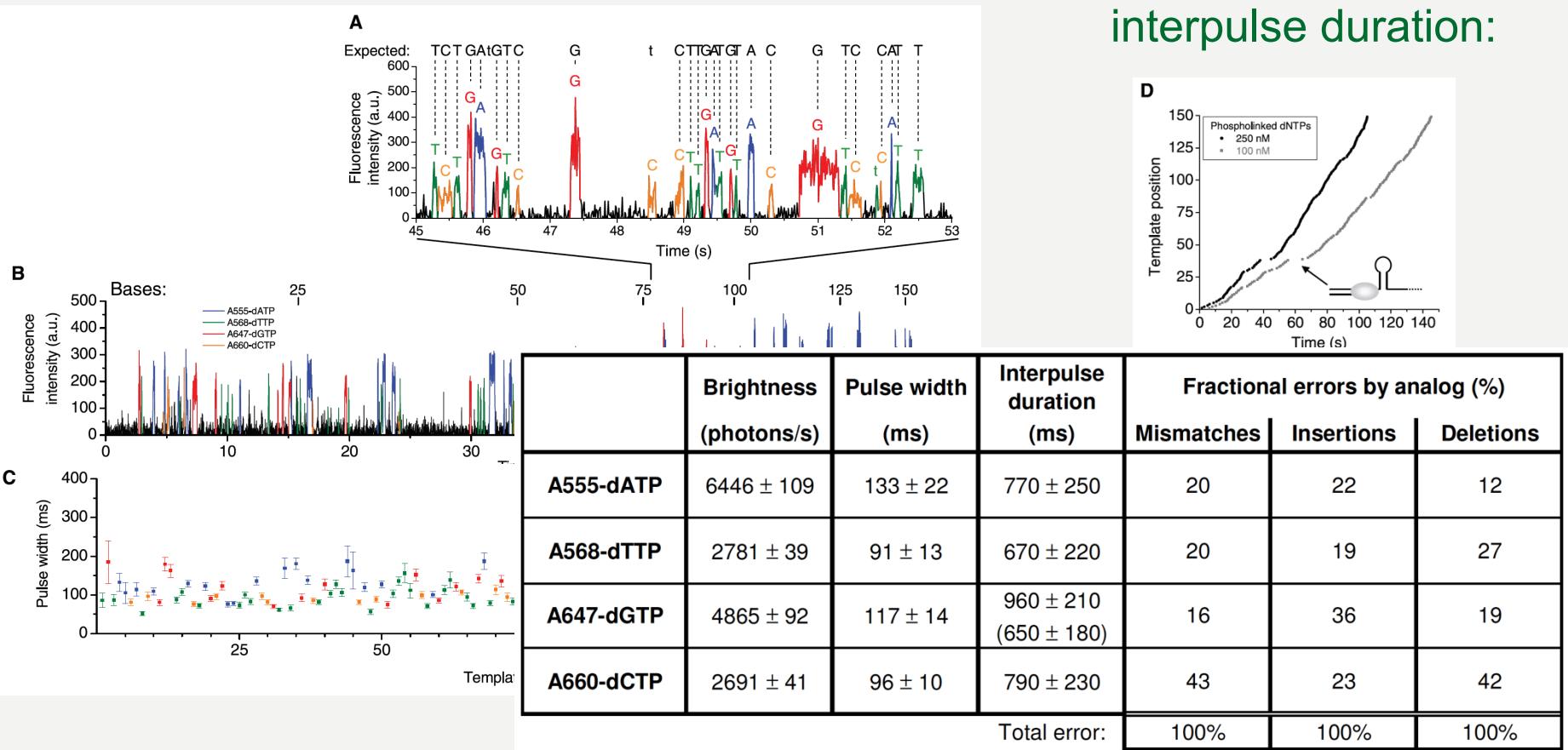
Long-read DNA sequencing for 2 bases



Eid, J.; Science (2009)



Four-color sequencing

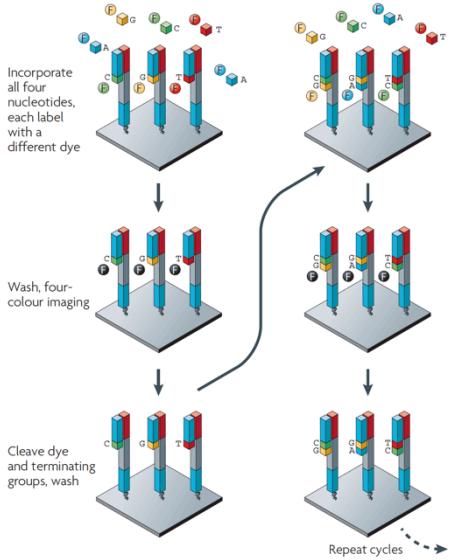


Eid, J.; Science (2009)

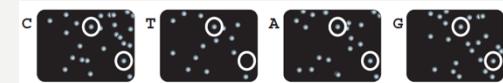
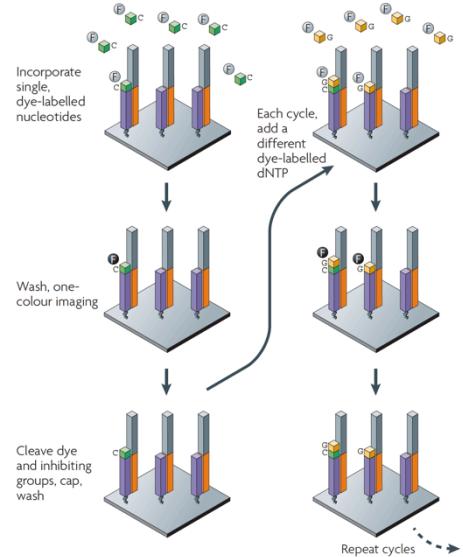
Summary of commercially available techniques



a Illumina/Solexa — Reversible terminators

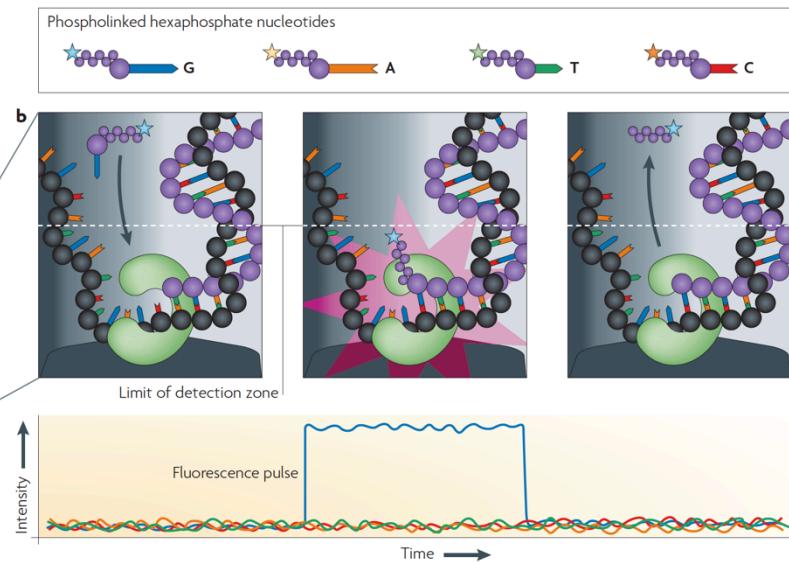
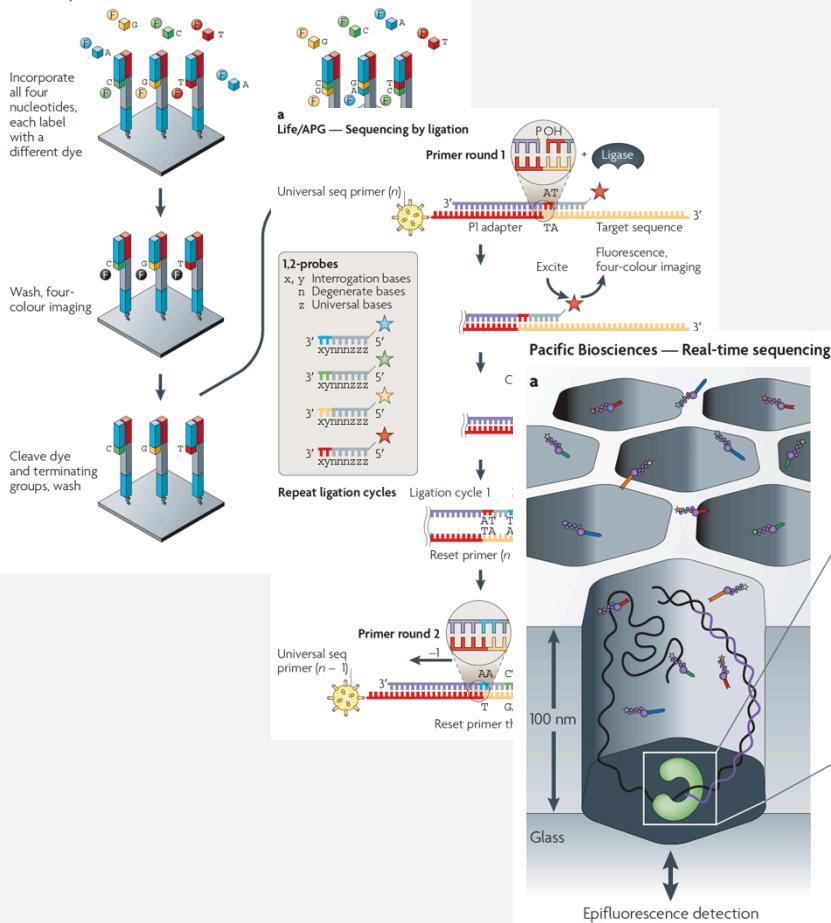


c Helicos BioSciences — Reversible terminators



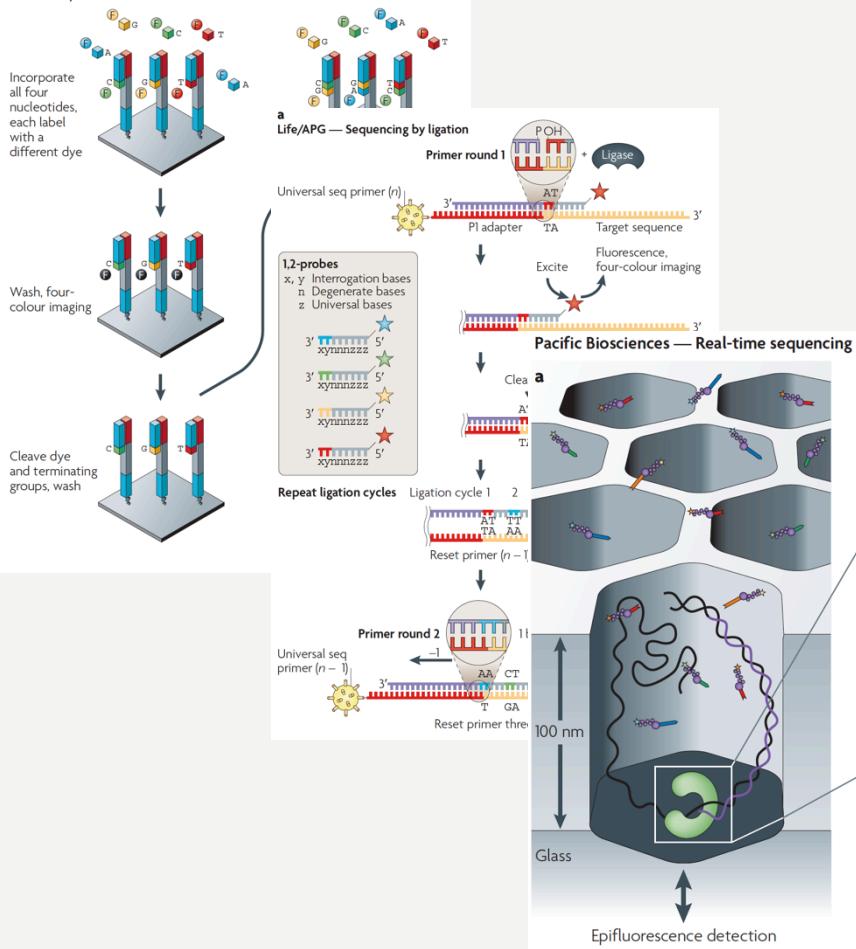
Metzker, M.; Nature Reviews (2010)

Summary of commercially available techniques


a Illumina/Solexa — Reversible terminators


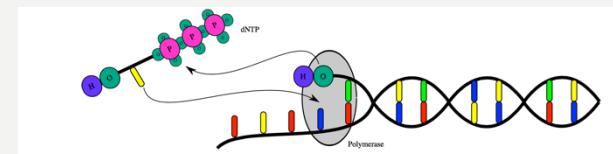
Metzker, M.; Nature Reviews (2010)

Summary of commercially available techniques

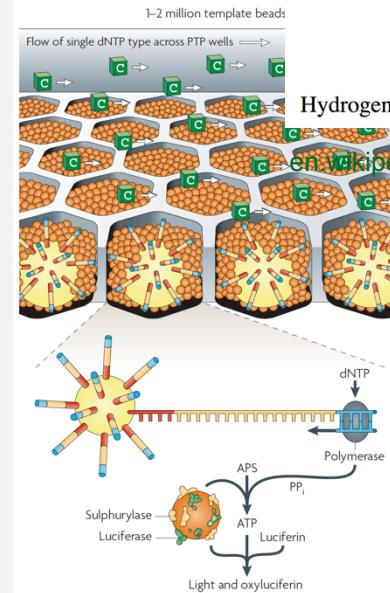

a Illumina/Solexa — Reversible terminators


Summary of commercially available techniques

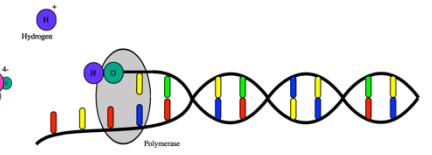
ion torrent



en.wikipedia.org/wiki/Ion_semiconductor_sequencing

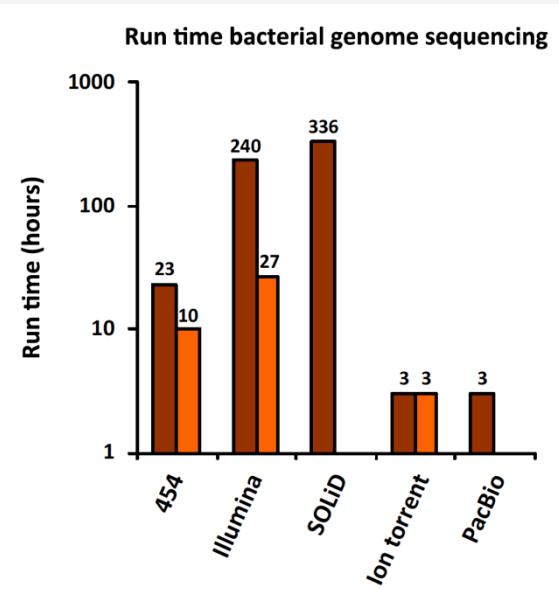
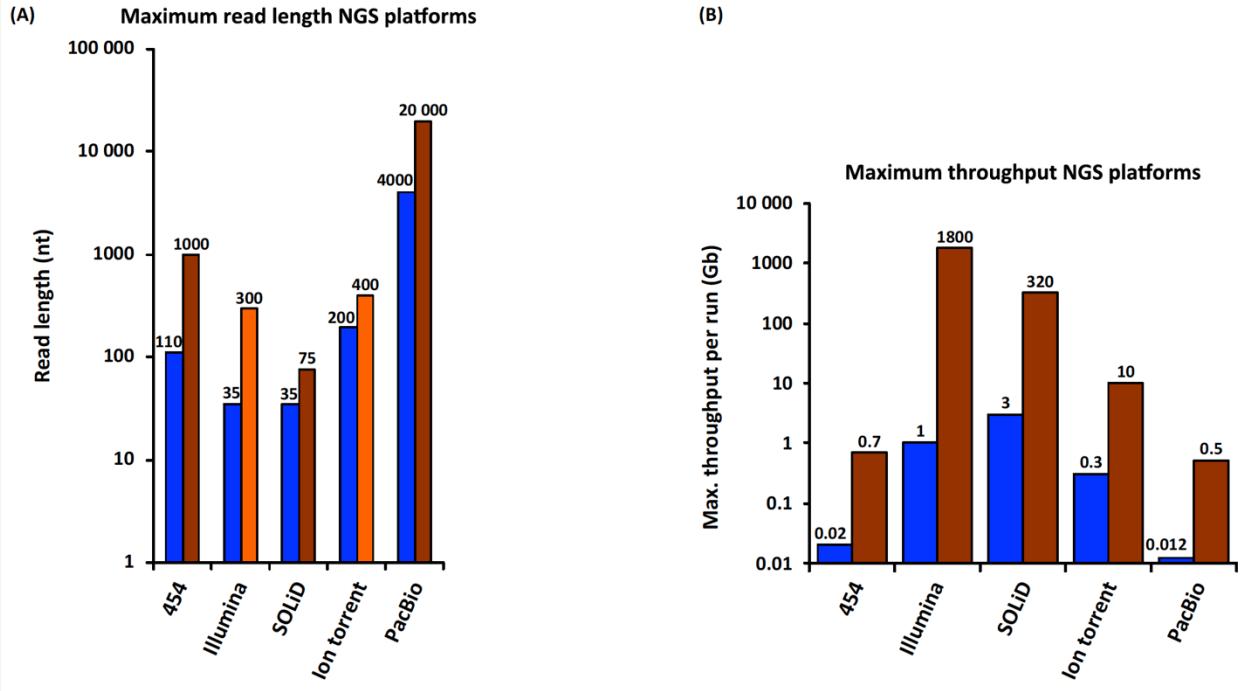
c Roche/454 — Pyrosequencing


Hydrogen and pyrophosphate are released.



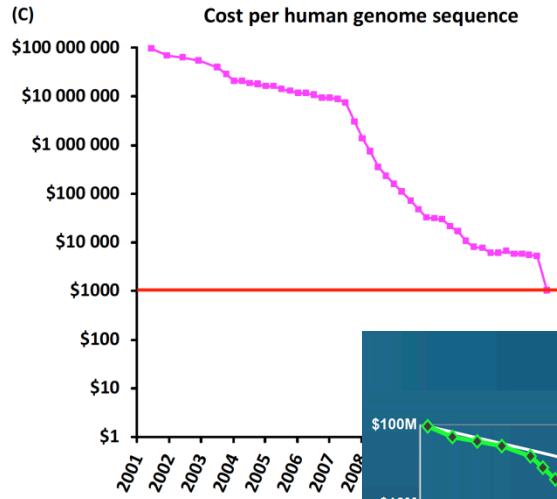
Metzker, M.; Nature Reviews (2010)

Comparison

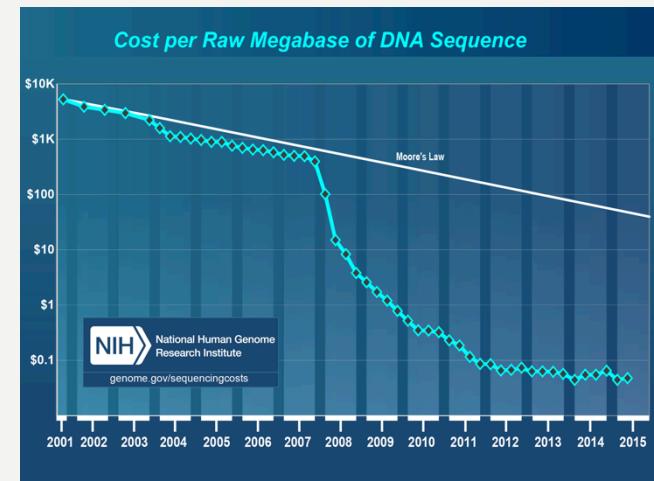
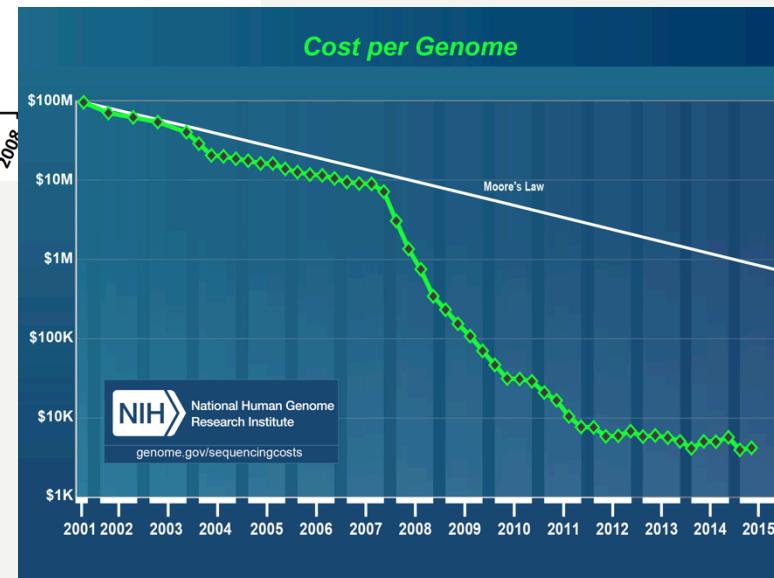


→ PacBio max. read length
Illumina max. throughput
Ion torrent fastest sequencing

van Dijk, E.; Elsevier (2014)



→ Illumina's HiSeq X Ten system



National Human Genome Research Institute

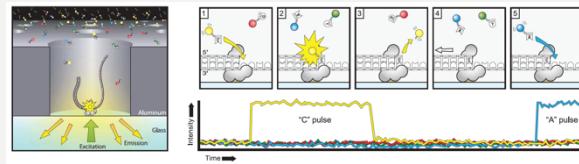


- 3 major improvements of NGS methods:

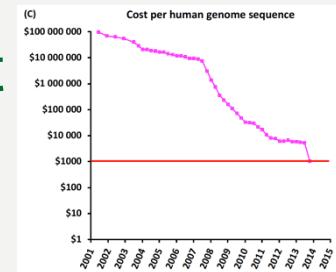
1. Prep in cell free environment → no cloning
2. Lots of parallel measurements
3. Direct detection → no electrophoresis

- 2 types of fluorophore attachment

- ZMW

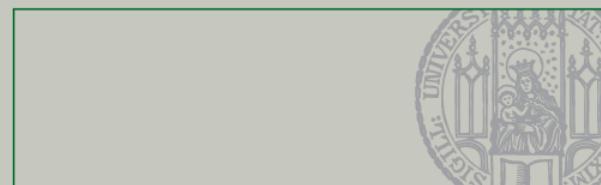
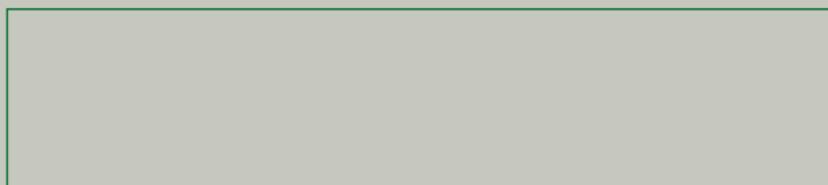


→ Reduce of cost and time & increase of throughput





- Metzker, M.; Sequencing technologies – the next generation; Nature Reviews (2010)
- Levene, M.; Zero-mode waveguides for single-molecule analysis at high concentrations; Science (2003)
- Eid, J.; Real-time DNA sequencing from single polymerase molecules; Science (2009)
- van Dijk, E.; Ten years of next-generation sequencing technology; Elsevier (2014)
- Pushkarev, D.; Single-molecule sequencing of an individual human genome; Nature Biotechnology (2009)
- Metzker, M.; Emerging technologies in DNA sequencing; Cold Spring Harbor Laboratory Press (2005)
- wikipedia/DNA-sequencing
- datasheet-hiseq-x-ten from Illumina
- PacBio_RS_II_Brochure from Pacific Biosciences
- Seo, T.; Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides; PNAS (2005)
- http://biobook.nerinxhs.org/bb/genetics/dna/1000px-Aminoacids_table.png
- <http://www.ucl.ac.uk/~sjjgsca/PointMutations.gif>
- http://www.rsc.org/images/Arsenic-DNA-chemical-structure_410_tcm18-214805.jpg



Thank you
for
your attention!