

Gel electrophoresis
Polymerase chain reaction
DNA cloning
DNA sequencing

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Polymerase Chain Reaction (PCR)

- Template DNA
- DNA polymerase
- Primers (forward and reverse)
- Deoxynucleoside triphosphates (dNTPs: dATP, dCTP, dGTP, and dTTP)
- Required cofactor for activity of DNA polymerases: Mg^{2+}
- Buffer: suitable chemical environment for activity of DNA polymerase



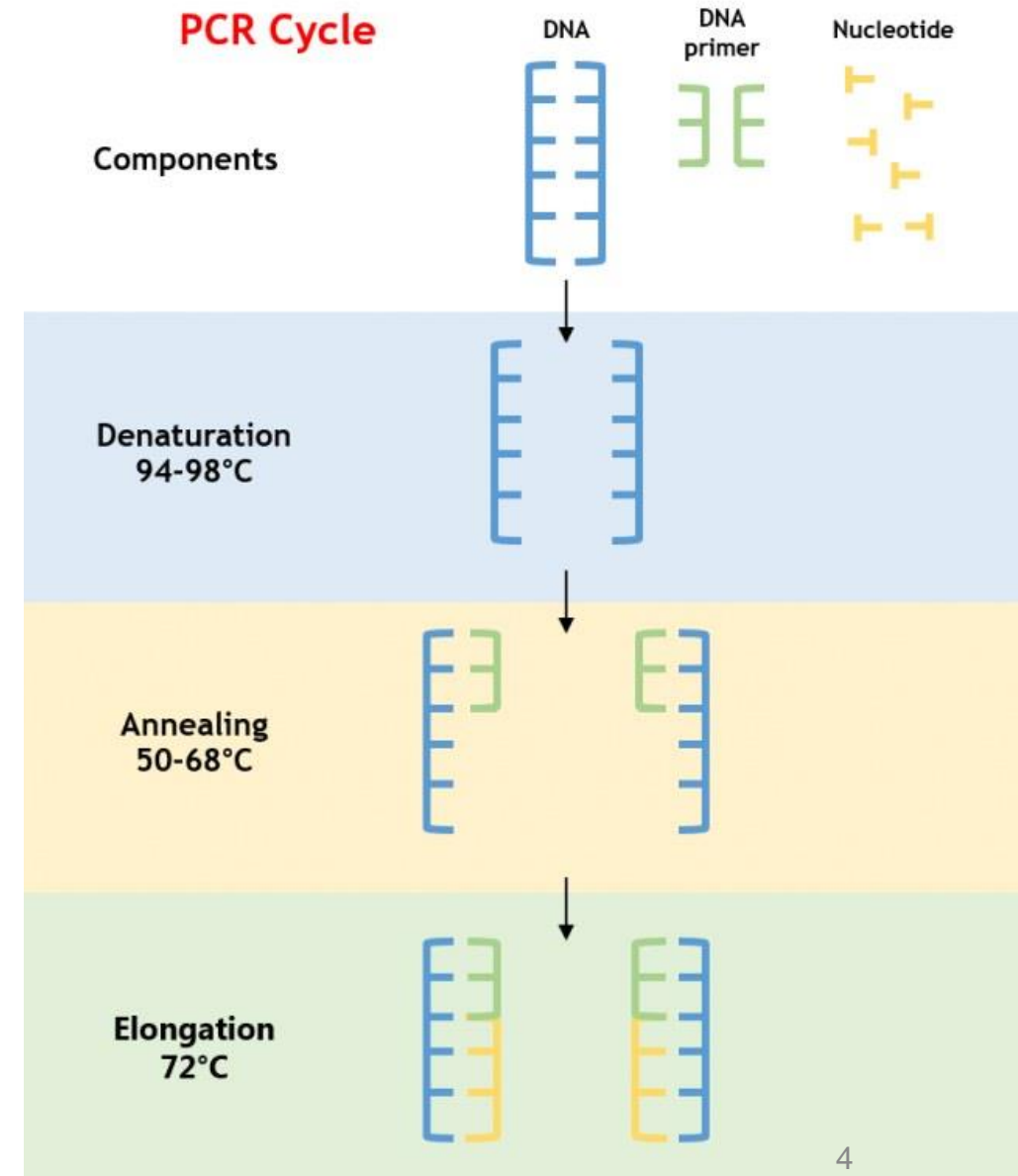
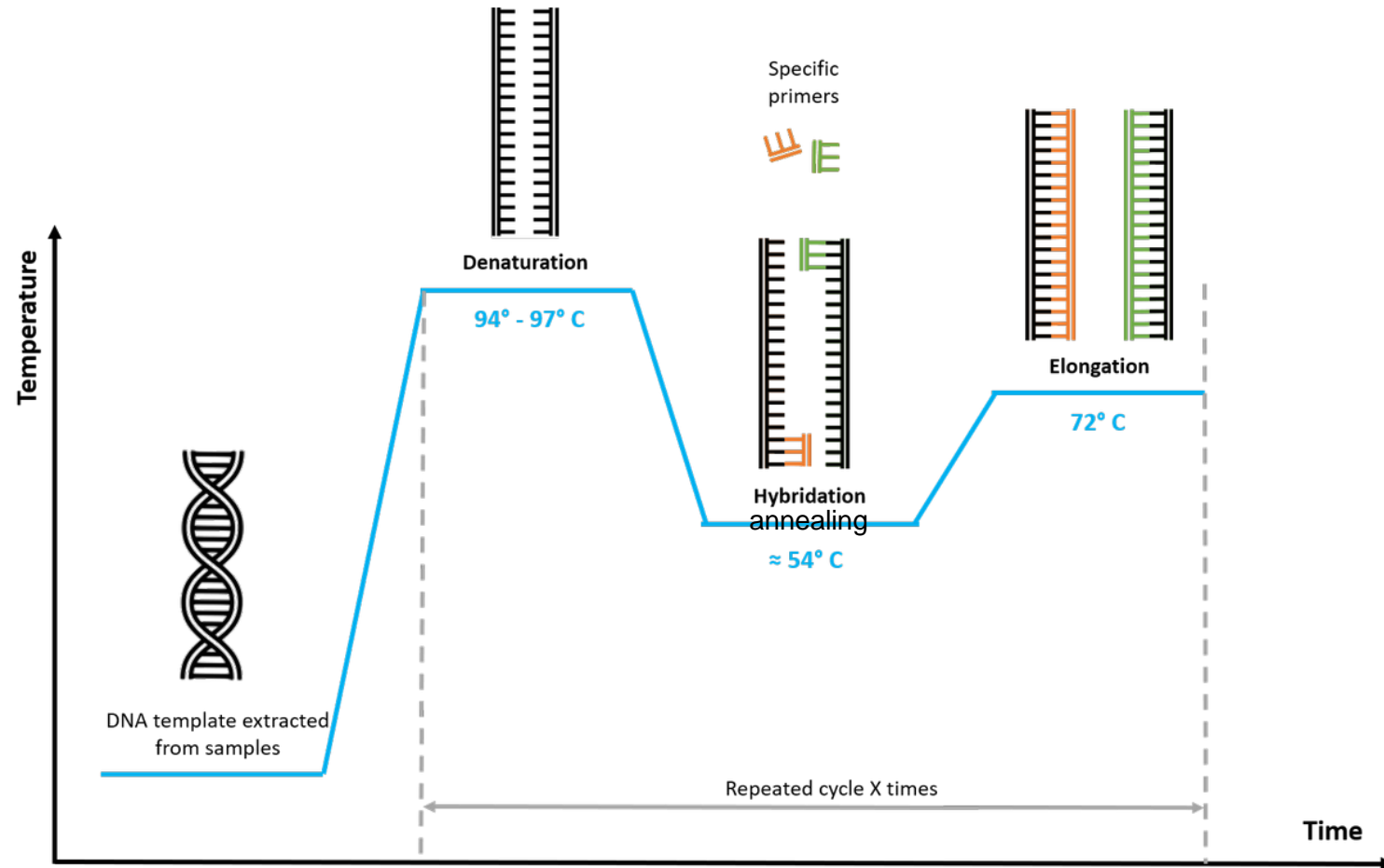
Polymerase Chain Reaction (PCR)

PCR thermocycler

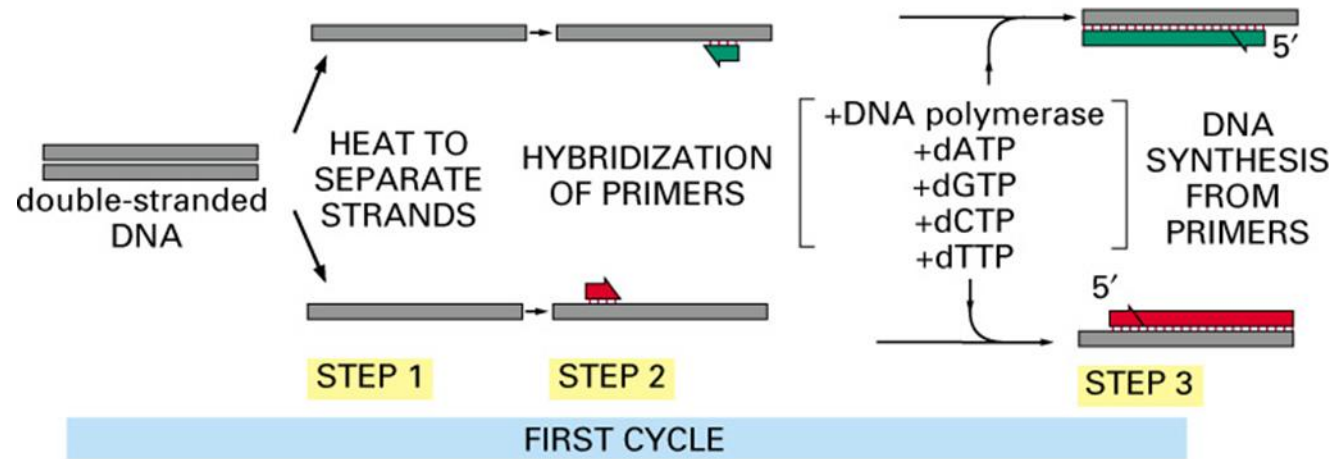


Polymerase Chain Reaction (PCR)

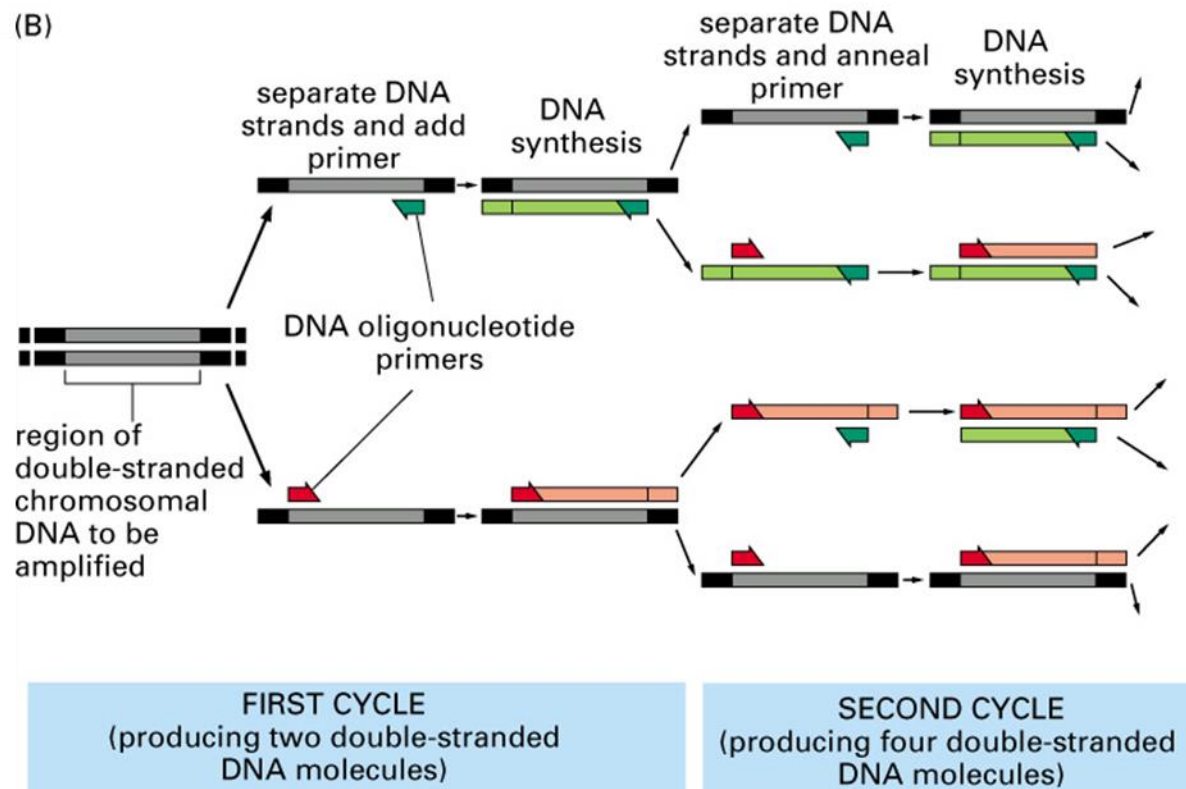
Steps



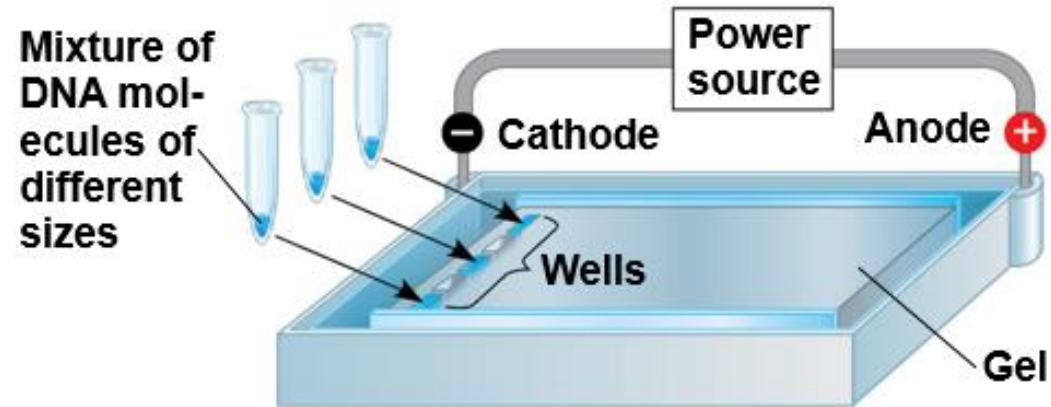
(A)



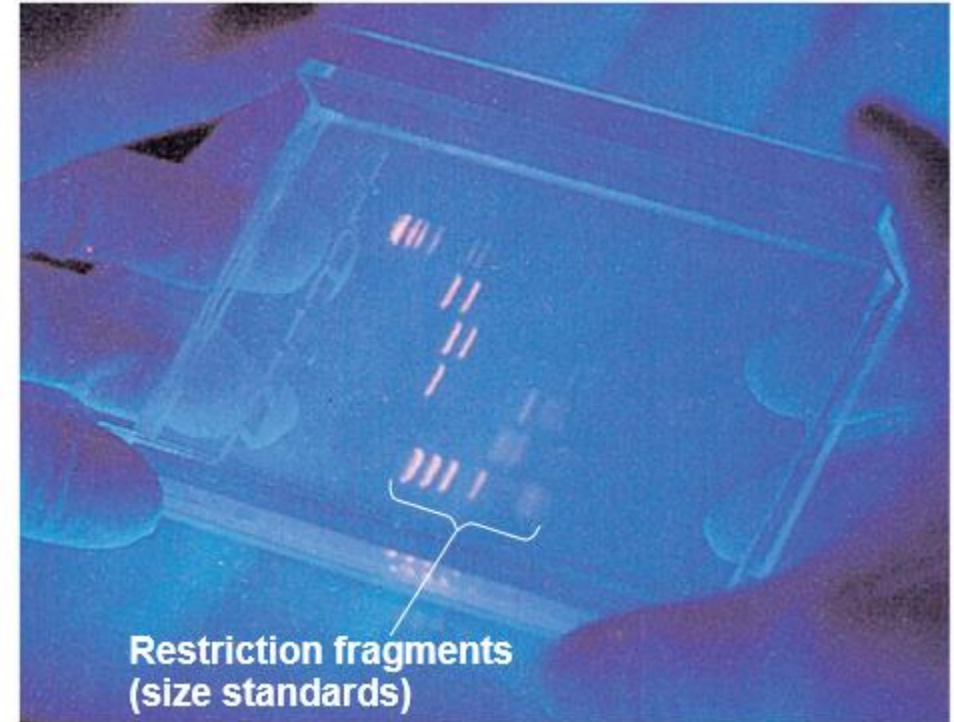
(B)



Gel Electrophoresis



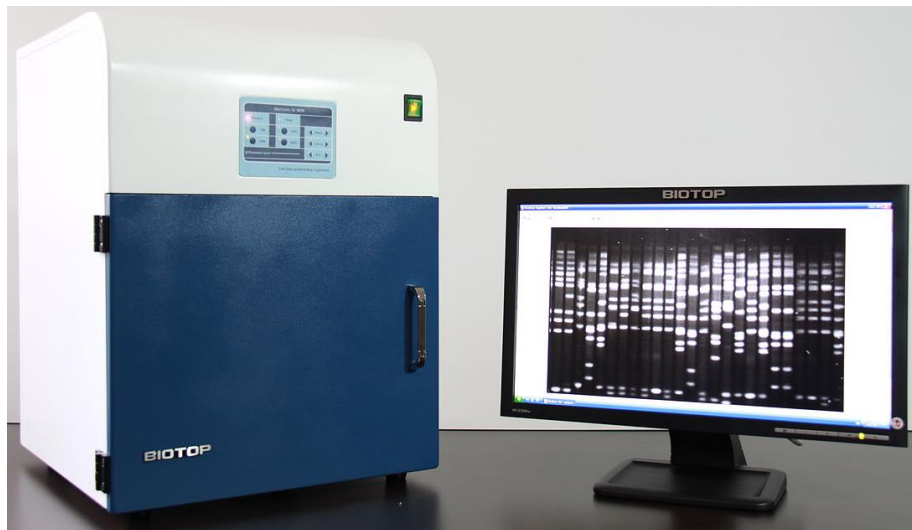
a) Negatively charged DNA molecules will move toward the positive electrode.

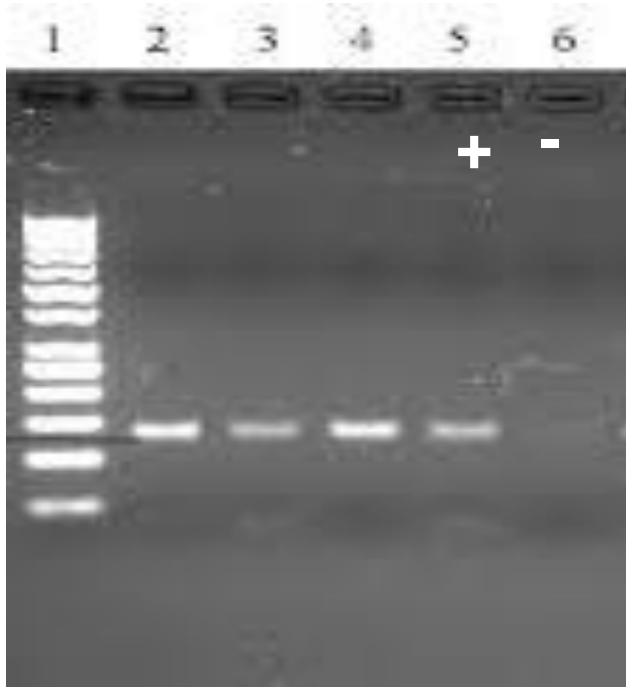


b) Shorter molecules are slowed down less than longer ones, so they move faster through the gel.

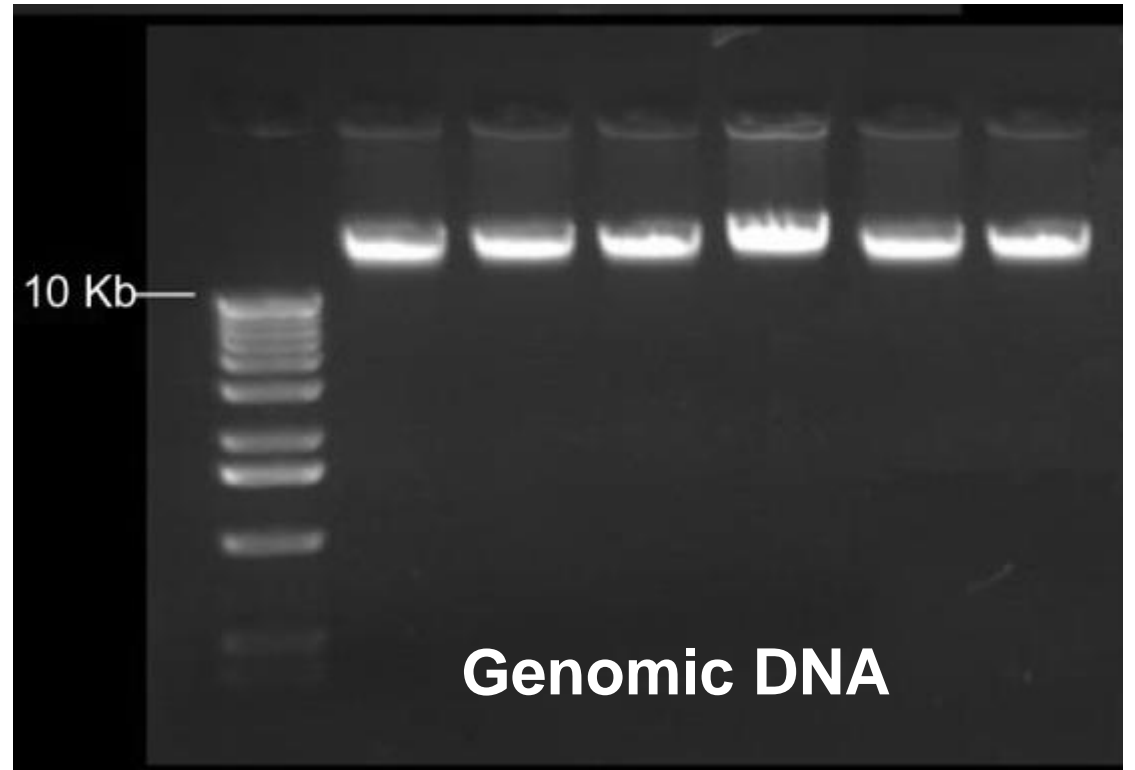


Gel documentation system



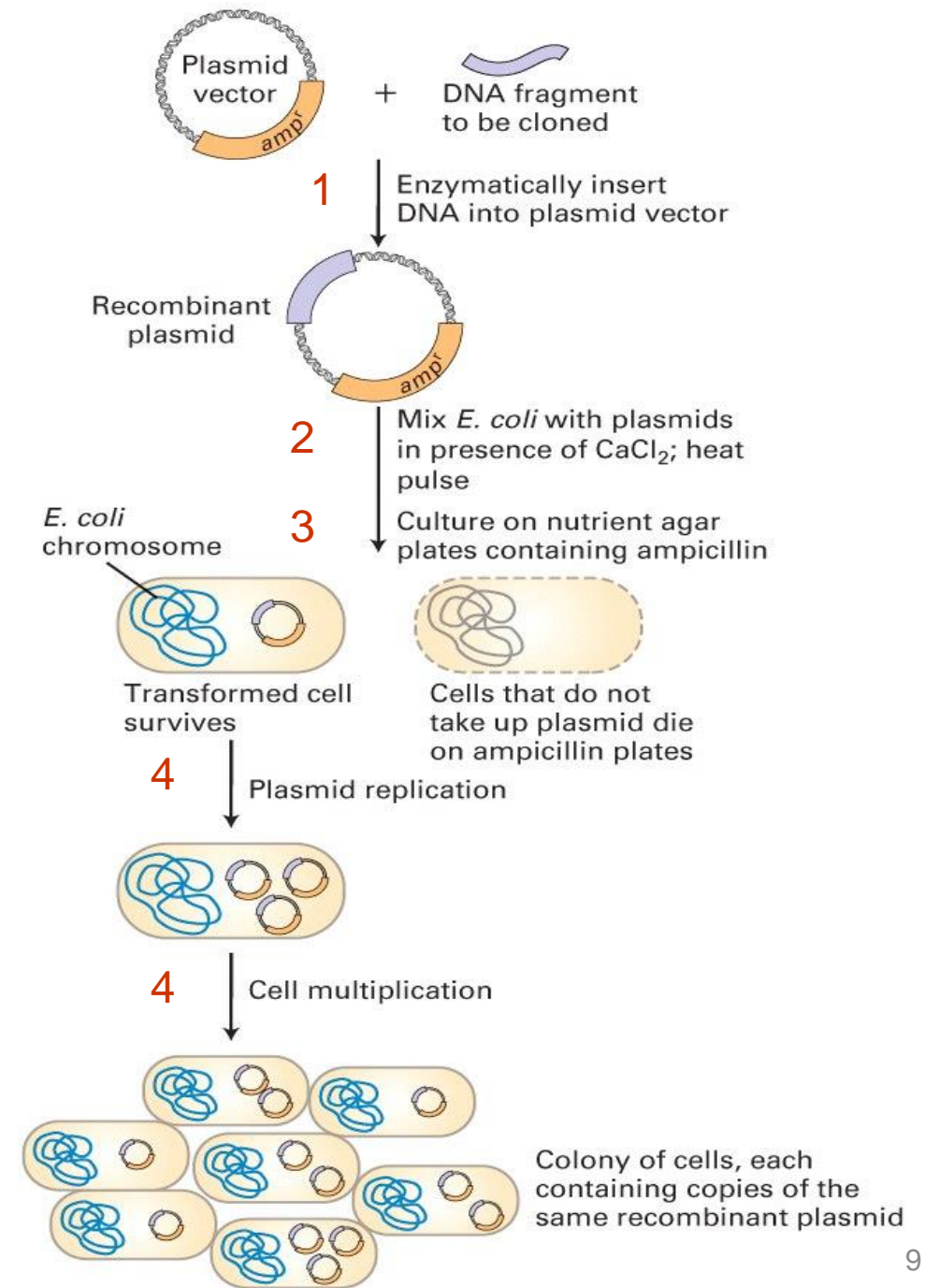
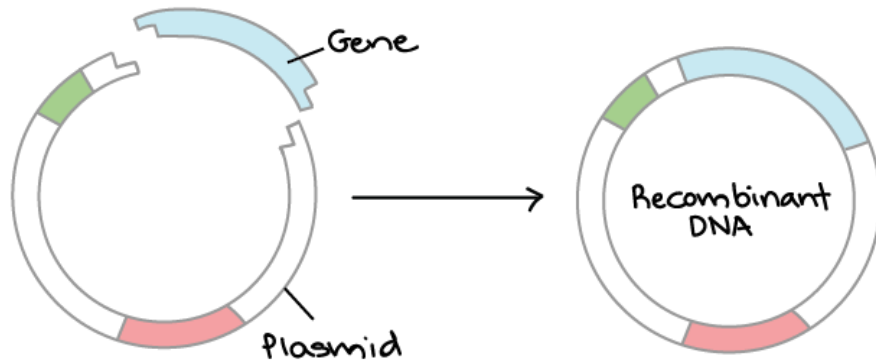


**PCR product:
Amplicon**

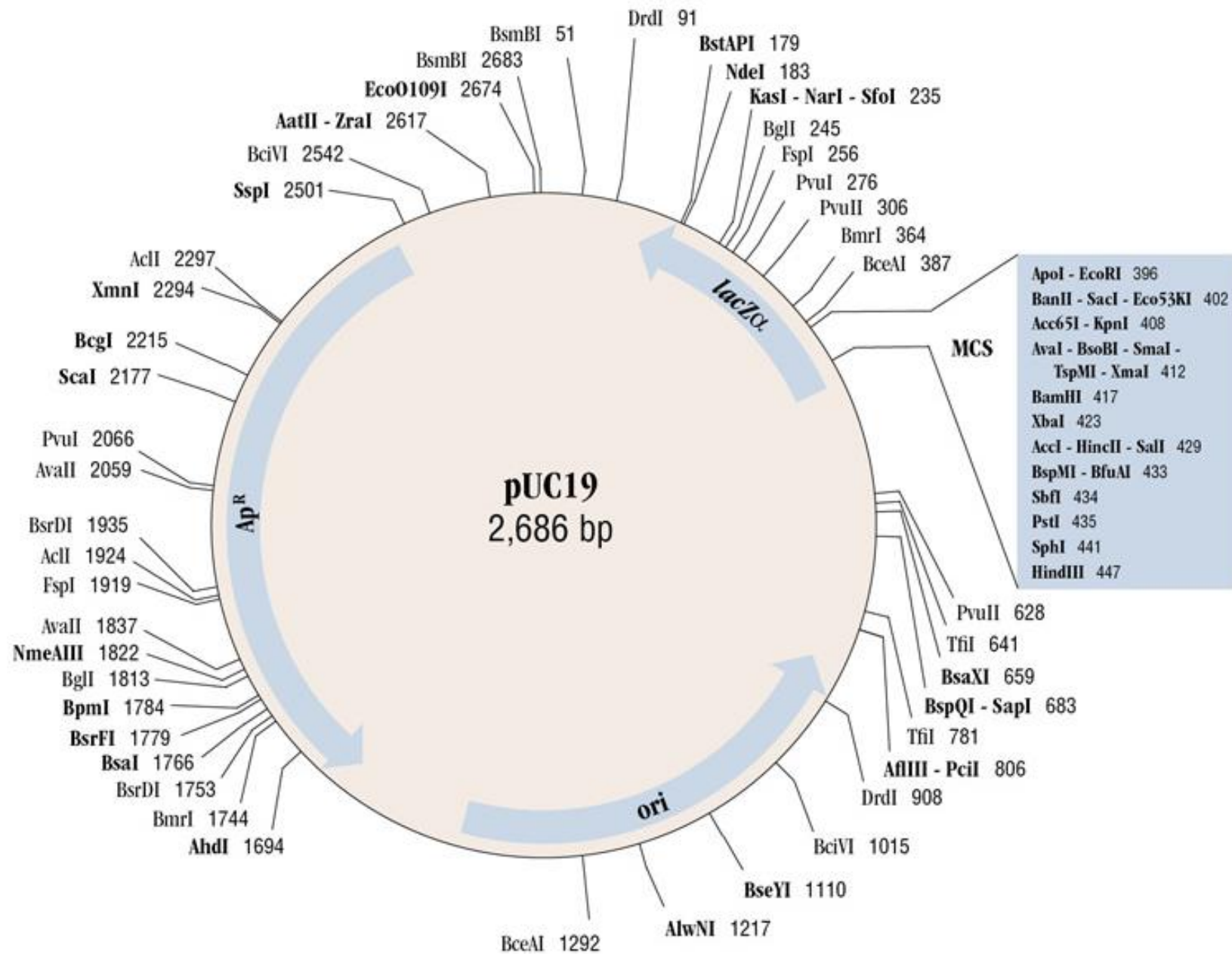


Genomic DNA

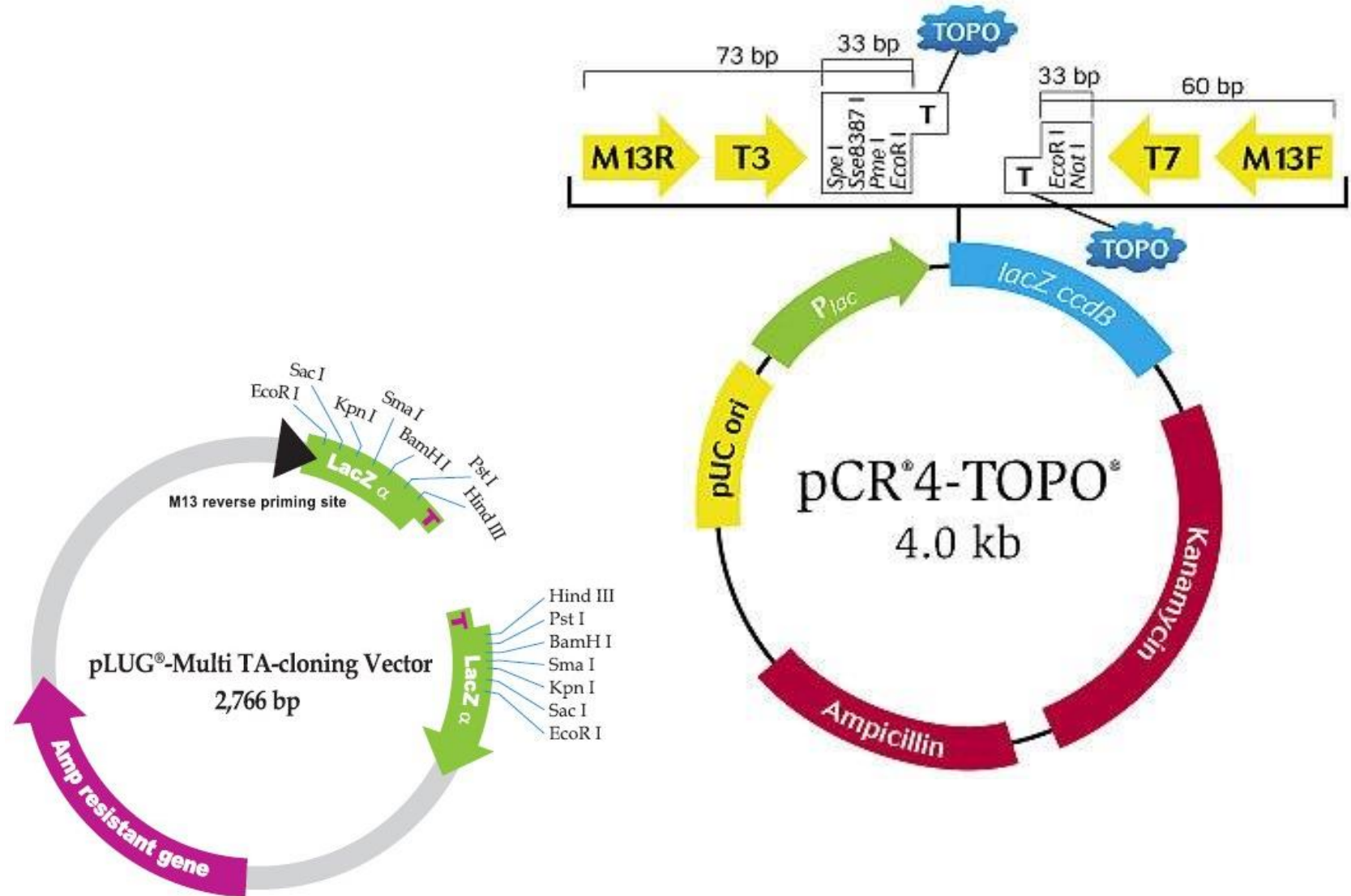
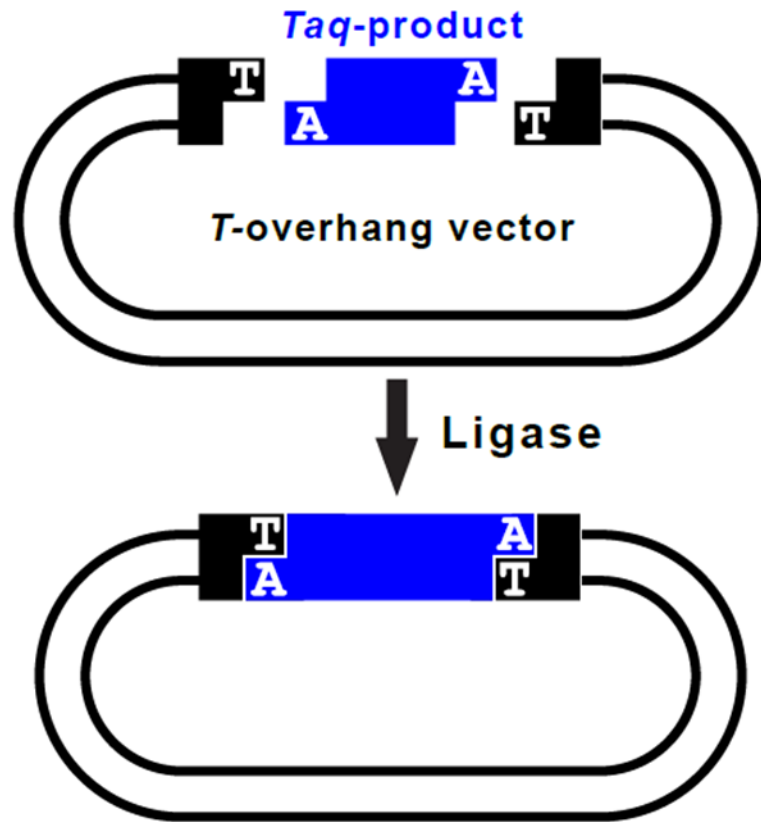
DNA cloning







DNA cloning

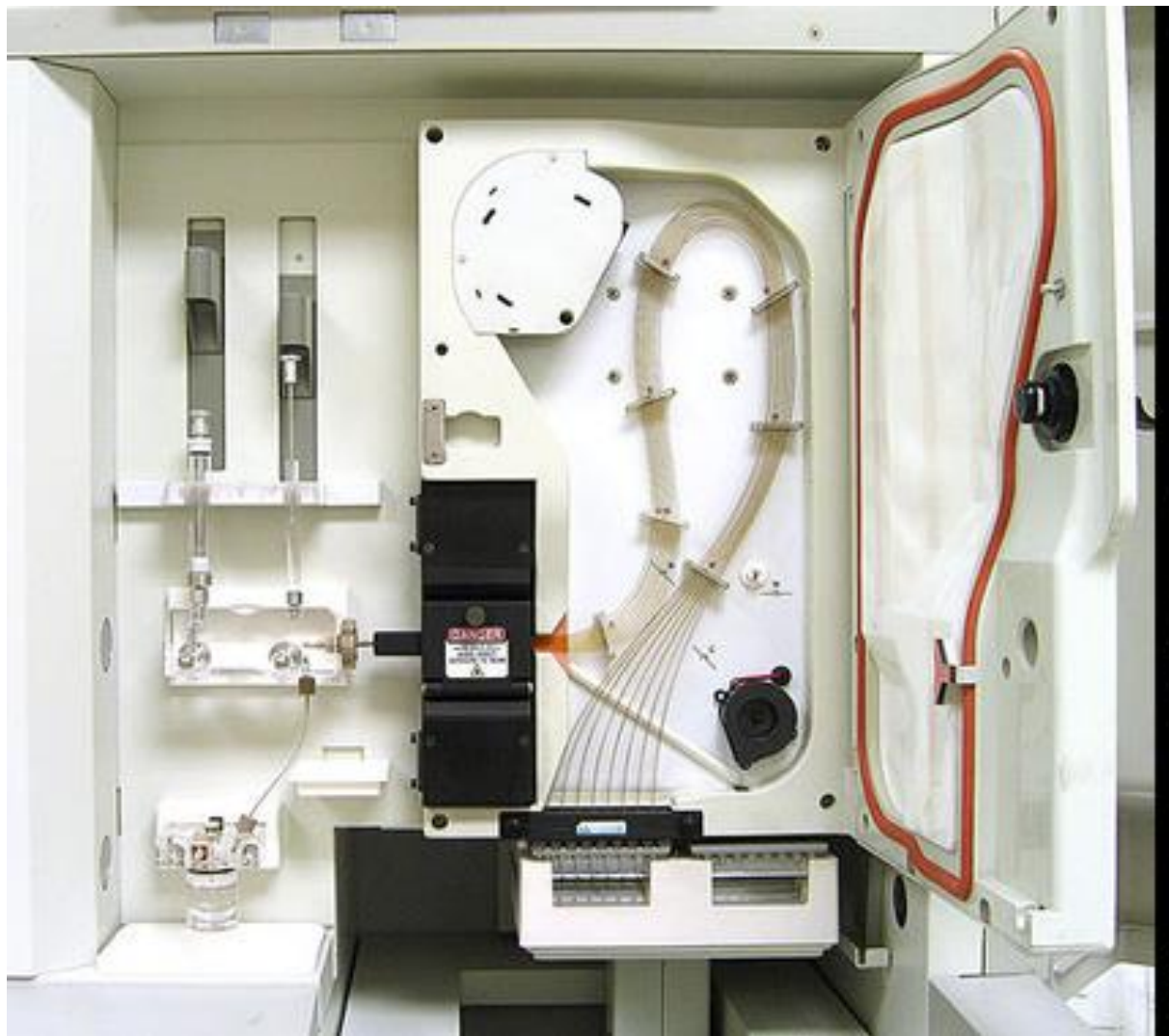


How do you sequence DNA?

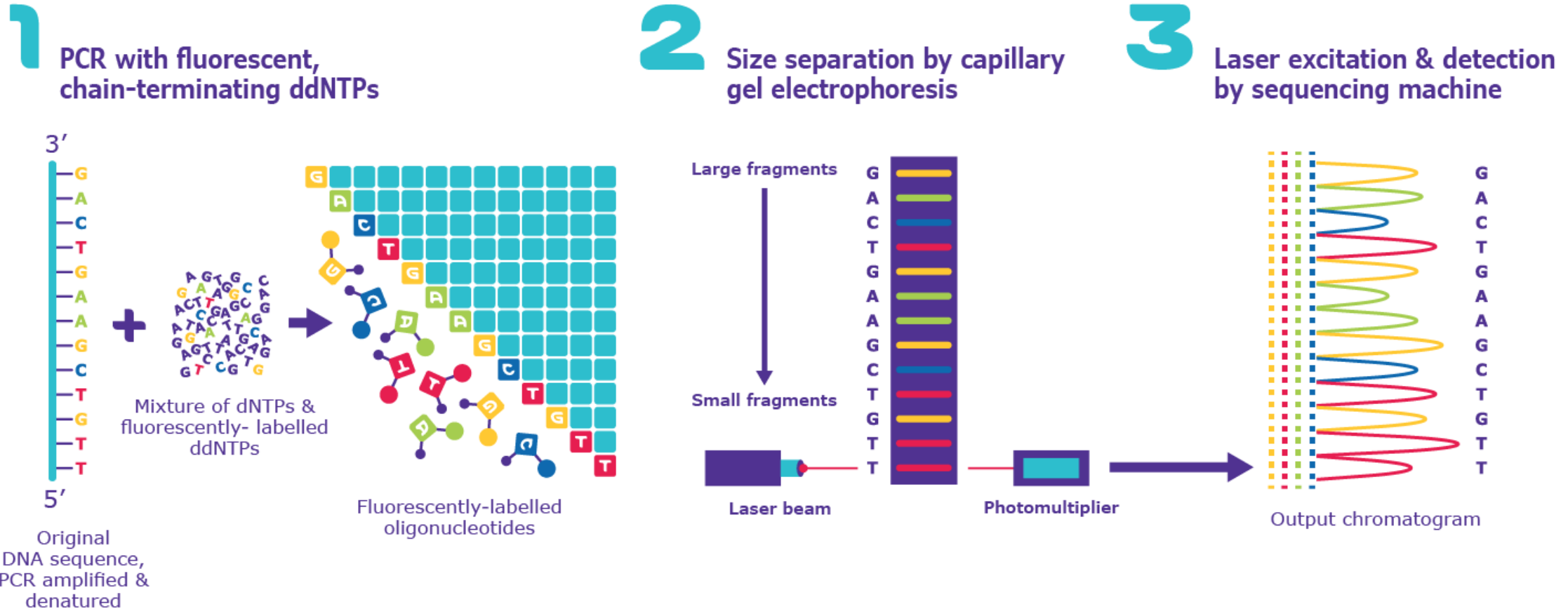
- Sanger (**dideoxy**, enzymatic) – developed by Frederick Sanger and is still used today with little change to the basic method.

High-throughput DNA sequencing

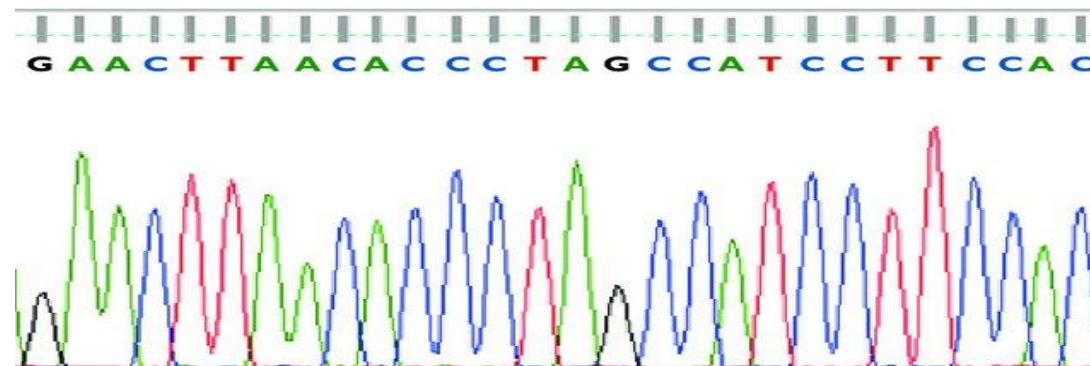
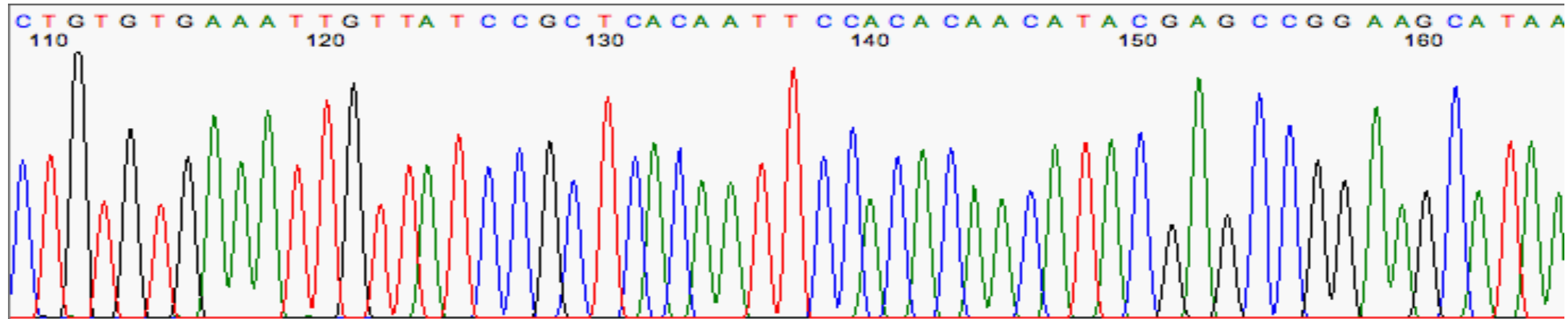
- **Next-generation sequencing**



1. All 4 fluorescently-labeled ddNTPs are used in 1 reaction, each a different “color”
2. Fragments are separated in matrix-filled capillary tubes, 1 capillary per reaction
3. Laser detects fluorescence automatically as each fragment exits capillary
4. Computer software “calls bases” and processes sequence files



Sequencing chromatogram



A quick history of sequencing

- 1869 – Discovery of DNA
- 1909 – Chemical characterization
- 1953 – Structure of DNA solved
- 1977 – Sanger sequencing invented
 - First genome sequenced – bacteriophage Phi X 174 (5 kb)
- 1986 – First automated sequencing machine
- 1990 – Human Genome Project started
- 1992 – First “sequencing factory” at TIGR



A quick history of sequencing

- 1995 – First bacterial genome – *H. influenzae* (1.8 Mb)
- 1998 – First animal genome – *C. elegans* (97 Mb)
- 2003 – Completion of Human Genome Project (3 Gb). 13 years, \$2.7 bn
- 2005 – First “next-generation” sequencing instrument
- 2021 – >60,000 genome sequences in NCBI database



Published: 15 February 2001

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium

Nature 409, 860–921(2001) | [Cite this article](#)

121k Accesses | 14976 Citations | 1081 Altmetric | [Metrics](#)

Genomics Revolution? *Completion* of the Human Genome





Published: 05 November 2008

My genome. So what?

[Open Access](#) | Published: 06 November 2008

The diploid genome sequence of an Asian individual

Jun Wang , Wei Wang, [...] Jian Wang 

Nature **456**, 60–65(2008) | [Cite this article](#)

3520 Accesses | **673** Citations | **75** Altmetric | [Metrics](#)

Han Chinese

[Open Access](#) | Published: 06 November 2008

Accurate whole human genome sequencing using reversible terminator chemistry

David R. Bentley , Shankar Balasubramanian, [...] Anthony J. Smith

Nature **456**, 53–59(2008) | [Cite this article](#)

20k Accesses | **2171** Citations | **80** Altmetric | [Metrics](#)

Illumina sequencing (Solexa)

Published: 01 September 2005

Initial sequence of the chimpanzee genome and comparison with the human genome

The Chimpanzee Sequencing and Analysis Consortium

Nature **437**, 69–87(2005) | [Cite this article](#)

29k Accesses | **1454** Citations | **384** Altmetric | [Metrics](#)

Some examples of sequenced genomes:

Species	Genome size (C)
<i>Mycoplasma genitalium</i> (bacteria)	580 Kb
<i>Haemophilis influenzae</i> (bacteria)	1.8 Mb
<i>Escherichia coli</i> (bacteria)	4.7 Mb
<i>Saccharomyces cerevisea</i> (yeast)	12.5 Mb
<i>Caenorhabditis elegans</i> (worm)	97 Mb
<i>Arabidopsis thaliana</i> (mustard weed)	125 Mb
<i>Drosophila melanogaster</i> (fruit fly)	180 Mb
<i>Fugu rubripes</i> (puffer fish)	400 Mb
<i>Oryza sativa</i> (rice)	400 Mb
<i>Homo sapiens</i> (human)	3.2 Gb

NGS technologies

illumina

- **Illumina:** works by simultaneously identifying DNA bases, as each base emits a unique fluorescent signal, and adding them to a nucleic acid chain.
- **Roche 454:** based on pyrosequencing, a technique which detects pyrophosphate release, using fluorescence, after nucleotides are incorporated by polymerase to a new strand of DNA.
- **Ion Torrent:** measures the direct release of H⁺ (protons) from the incorporation of individual bases by DNA polymerase. Differs from the previous two methods as it does not measure light.

Illumina sequencing

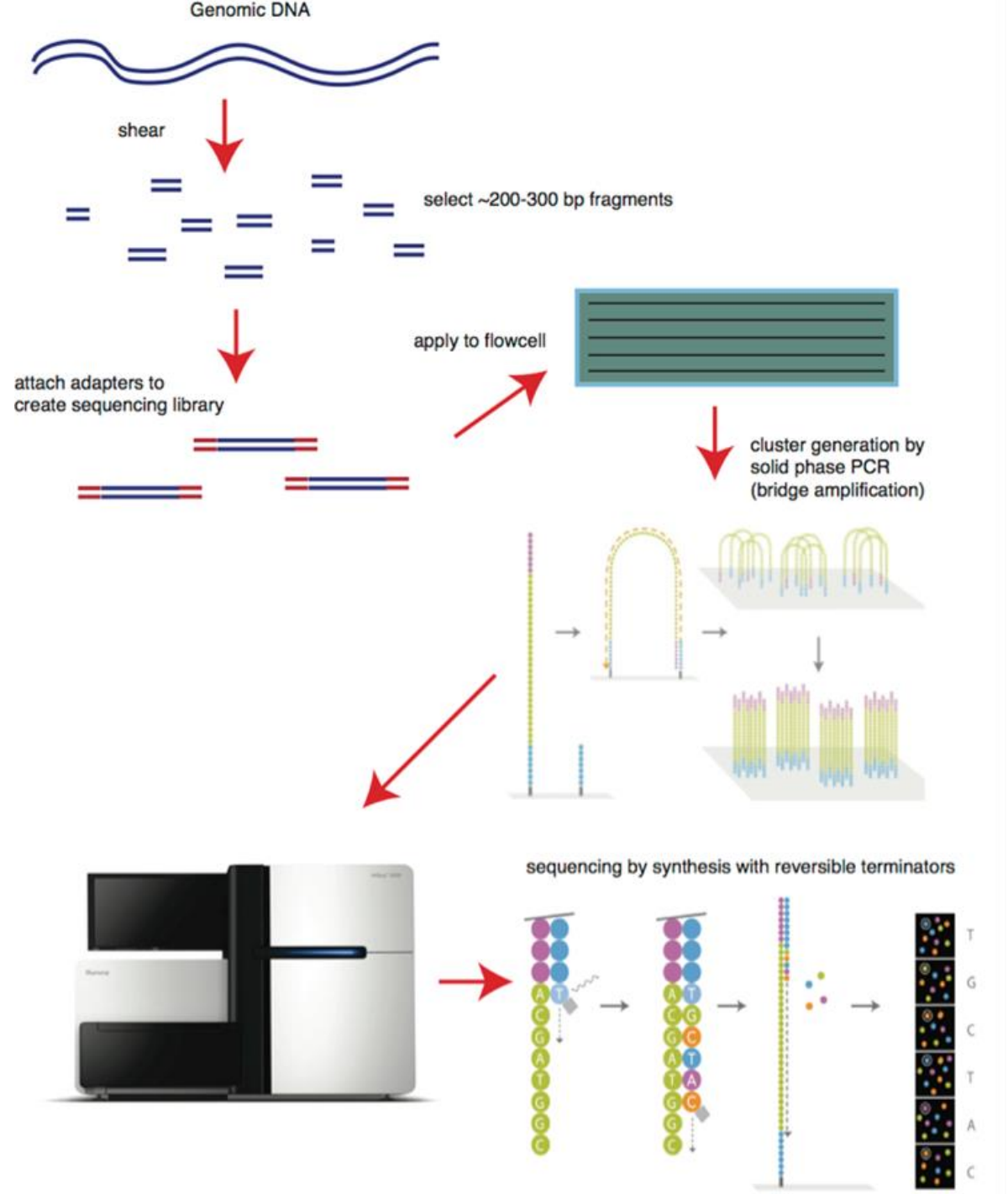
Sequencing-by-synthesis

Vast numbers of short reads are sequenced in a single stroke.

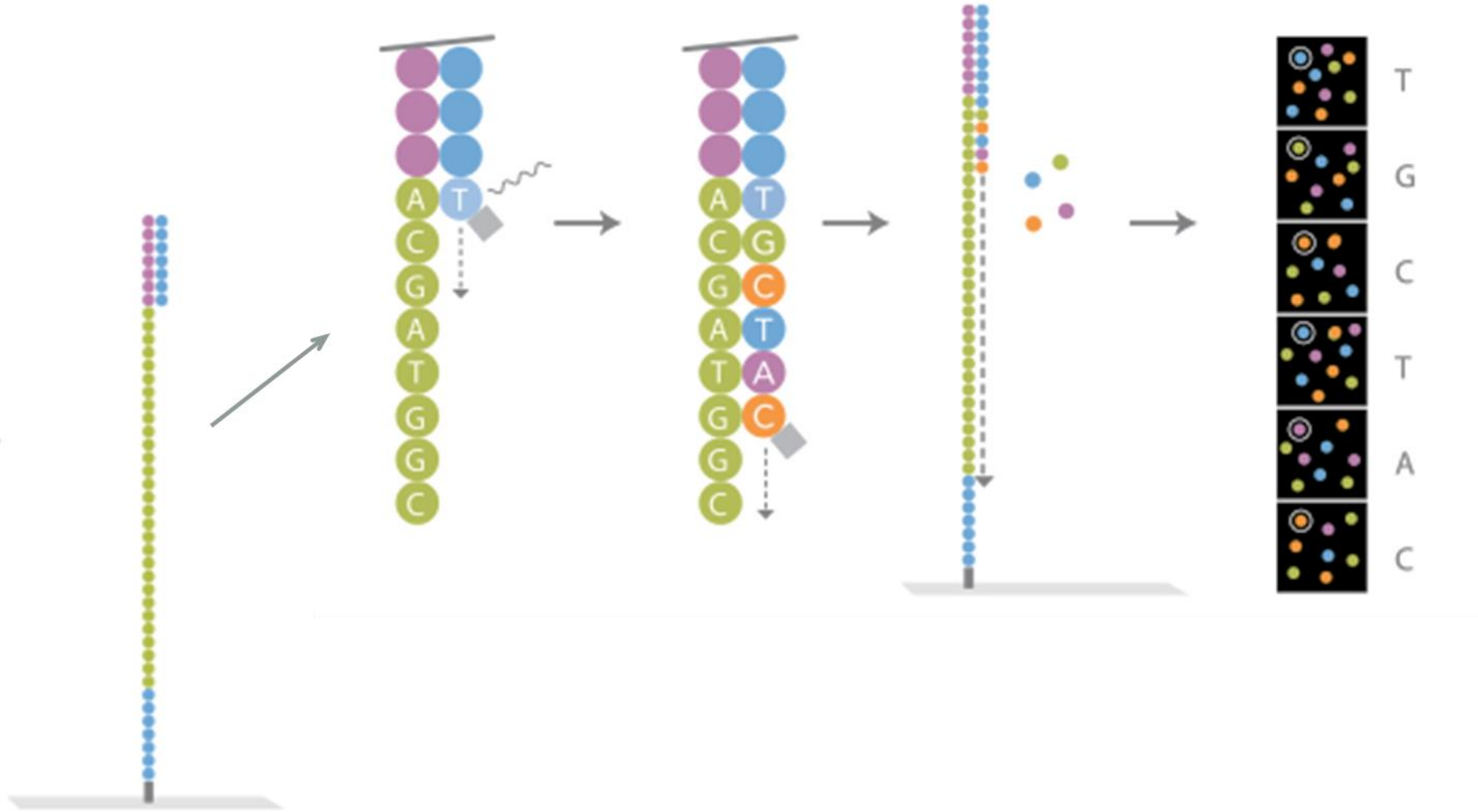
The input sample must be cleaved into short sections (100-300bp).

Illumina sequencing

- Library construction
Fragment, attach adapter DNA
- Cluster generation
Add to flow cell
Bridge amplification
- Sequencing
Single base at a time, imaging
- Data analysis
Images transformed into base calls and 'reads'



Illumina sequencing



Long reads sequencing



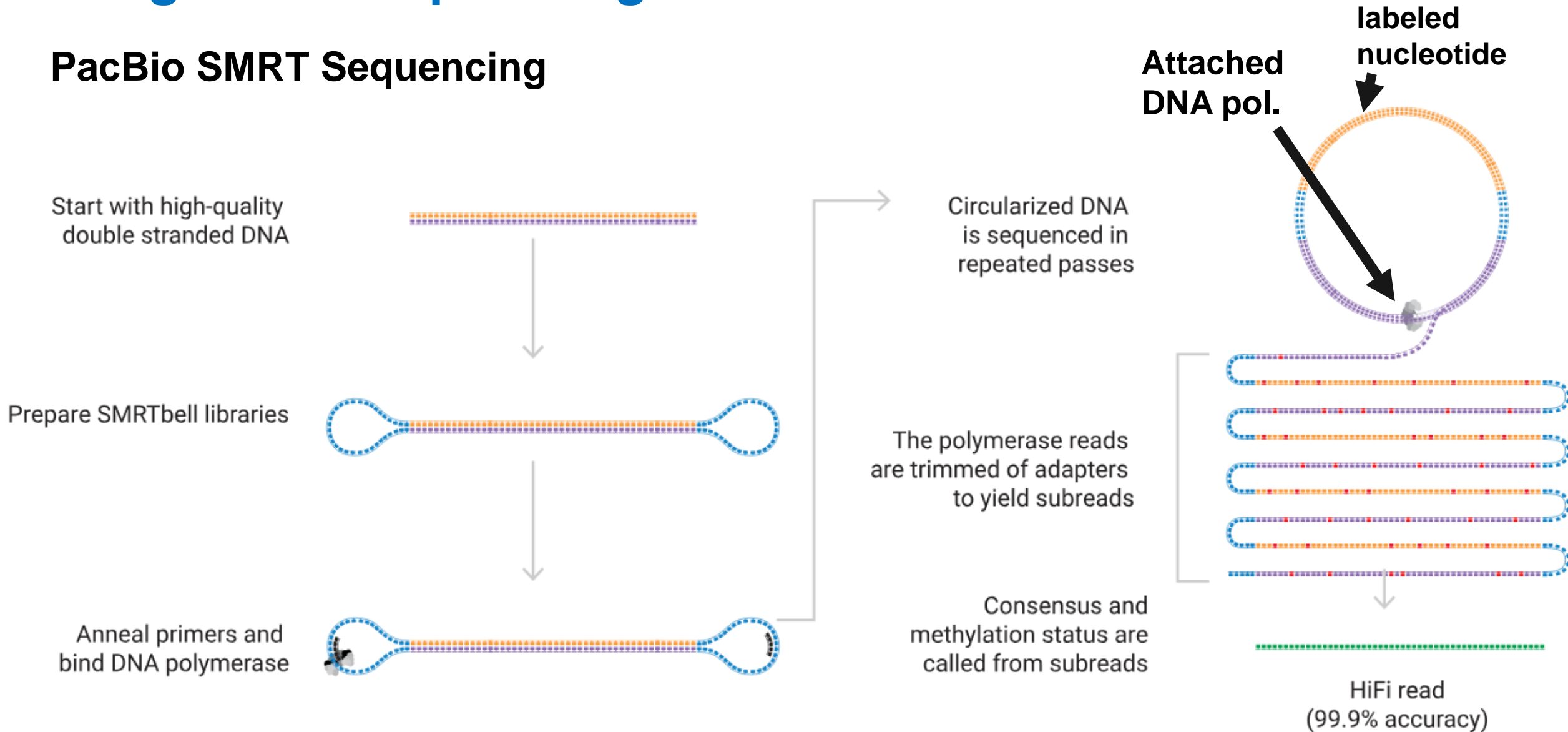
- Pacific Biosciences (**PacBio**)
'single-molecule real-time' (SMRT) sequencing



- Oxford **Nanopore** Technologies (ONT)
Nanopore sequencing

Long reads sequencing

PacBio SMRT Sequencing





Assembly of the durian chloroplast genome using long PacBio reads

Jeremy R. Shearman¹, Chutima Sonthirod¹, Chaiwat Naktang¹, Duangjai Sangsrakru¹, Thippawan Yoocha¹, Ratchanee Chatbanyong², Siriporn Vorakuldumrongchai², Orwintinee Chusri², Sithichoke Tangphatsornruang¹ & Wirulda Pootakham¹✉

<https://doi.org/10.1038/s41598-020-73549-4>

scientific reports

<https://doi.org/10.1038/s41598-022-24823-0>

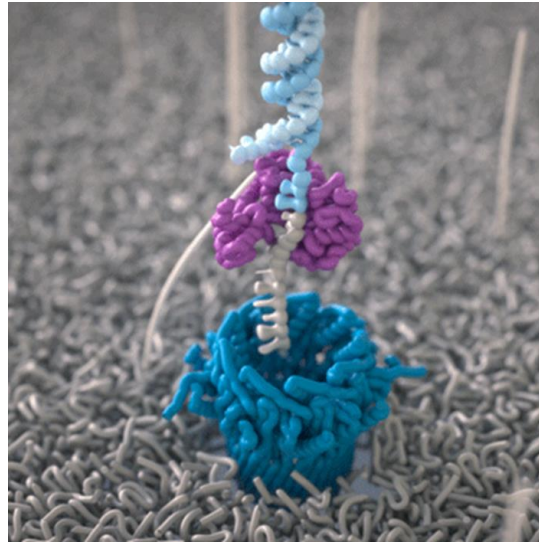


OPEN A draft chromosome-scale genome assembly of a commercial sugarcane

Jeremy R. Shearman¹✉, Wirulda Pootakham¹, Chutima Sonthirod¹, Chaiwat Naktang¹, Thippawan Yoocha¹, Duangjai Sangsrakru¹, Nukoon Jomchai¹, Sissades Tongsim², Jittima Piriyaongsa², Chumpol Ngamphiw², Nanchaya Wanasen³, Kittipat Ukoskit⁴, Prapat Punpee^{3,5}, Peeraya Klomsa-ard⁵, Klanarong Sriroth⁵, Jisen Zhang⁶, Xingtian Zhang⁶, Ray Ming⁶, Somvong Tragoonrungs³ & Sithichoke Tangphatsornruang¹✉

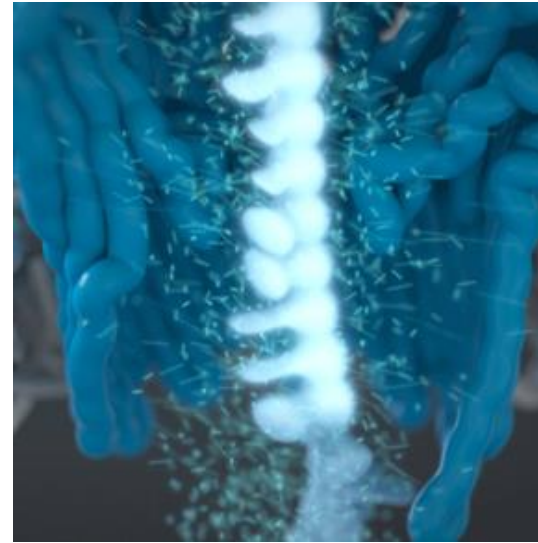
Long reads sequencing

Nanopore Sequencing: ONT



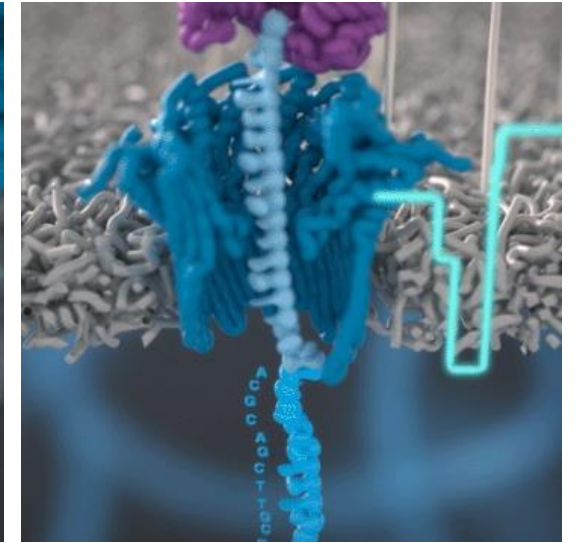
1.

A motor feeds DNA through a nanopore



2.

The DNA blocks the flow of current through the pore



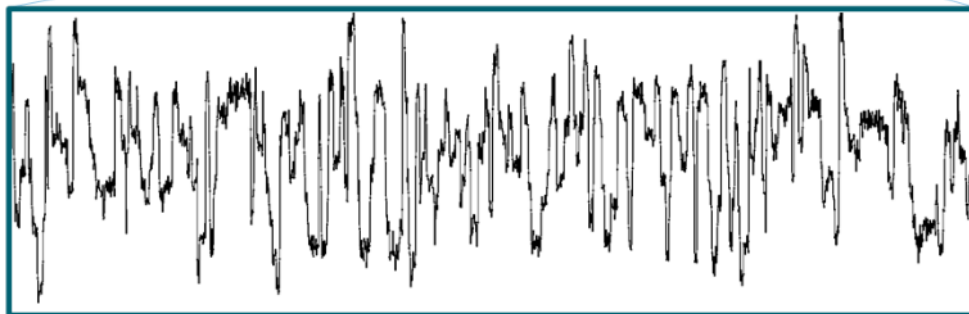
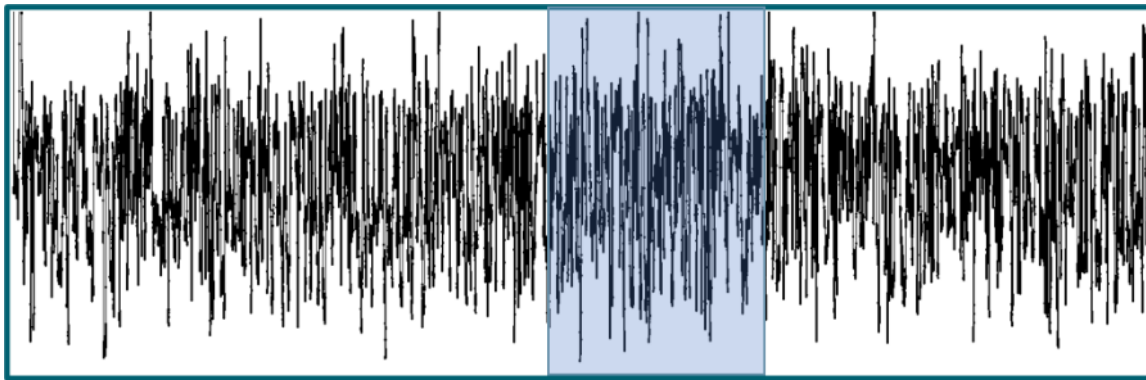
3.

The changes in current are decoded into the DNA sequence – this is called basecalling

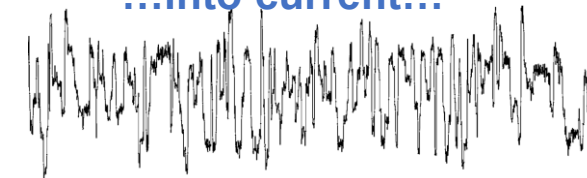
Long reads sequencing

Nanopore Sequencing: ONT

Nanopore signal data



...into current...



...and the current
into bases

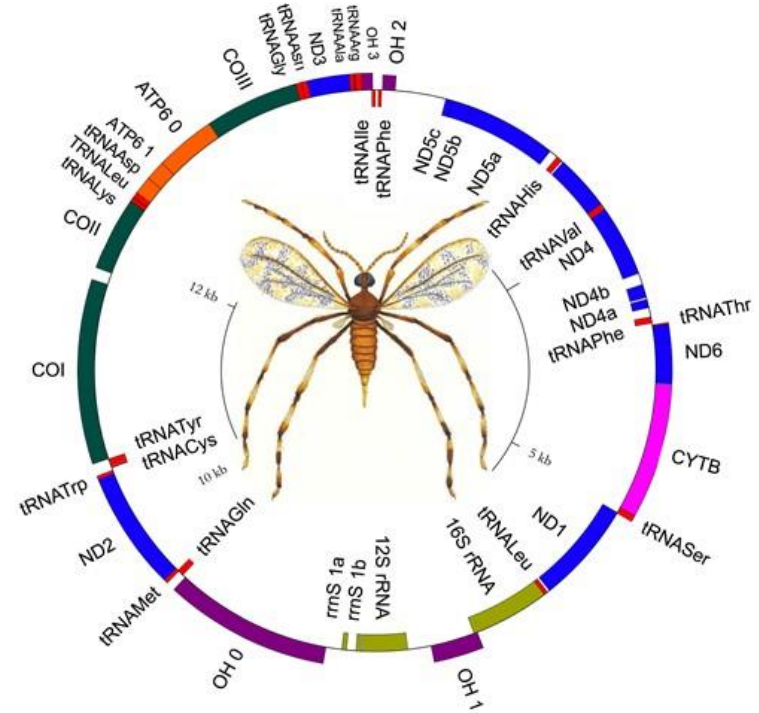
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TCATTATTCTATCACGCTTTCGAGTGACTGGTGACGGATATCCCCACGAATGAAATCTTTTTC
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CGGAGTTTCGGCGCTGTGCTGCCGAGAGCCGGCCATTGTCTATTGGGGCCTCACTTGAGGATACCC
GACCTATTTGTGCGGACCACTCGGGGTAGTCGTTGGGCTTATGCACCGTAAAGTCCTCCGCCGGC
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GATTCGTGCT
```

Students in a Course-Based Undergraduate Research Experience Course Discovered Dramatic Changes in the Bacterial Community Composition Between Summer and Winter Lake Samples

Stokes S. Baker^{1*}, Mohamed S. Alhassan¹, Kristian Z. Asenov¹, Joyce J. Choi^{1,2}, Griffin E. Craig¹, Zayn A. Dastidar^{1,3}, Saleh J. Karim¹, Erin E. Sheardy¹, Salameh Z. Sloulin¹, Nitish Aggarwal¹, Zahraa M. Al-Habib¹, Valentina Camaj¹, Dennis D. Cleminte¹, Mira H. Hamady¹, Mike Jaafar¹, Marcel L. Jones¹, Zayan M. Khan¹, Evileen S. Khoshaba¹, Rita Khoshaba¹, Sarah S. Ko¹, Abdulmalik T. Mashrah¹, Pujan A. Patel¹, Rabeeh Rajab¹ and Sahil Tandon¹



<https://doi.org/10.3389/fmicb.2021.579325>



G3, 2023, 13(4), jkad046

<https://doi.org/10.1093/g3journal/jkad046>

Advance Access Publication Date: 2 March 2023

Genome Report

The genome of the soybean gall midge (*Resseliella maxima*)

Gloria Melotto,¹ Megan W. Jones,¹ Kathryn Bosley,² Nicole Flack,³ Lexi E. Frank,³ Emily Jacobson,¹ Evan J. Kipp,³ Sally Nelson,¹ Mauricio Ramirez,¹ Carrie Walls,² Robert L. Koch ,¹ Amelia R.I. Lindsey ,¹ Christopher Faulk ^{2,*}

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<https://doi.org/10.1093/g3journal/jkad046>