Started on	Thursday, 4 April 2024, 3:06 PM
State	Finished
Completed on	Thursday, 4 April 2024, 3:14 PM
Time taken	8 mins 9 secs
Marks	16.00/16.00
Grade	**10.00** out of 10.00 (**100**%)

Question 1

Correct

Mark 4.00 out of 4.00

1. Sanger sequencing

Answer the following questions regarding to Sanger sequencing

1.1. Regarding to template amplification in Sanger sequencing it is true:

- a- It can be done by cloning ✔
- b- It is carried out by emulsion PCR
- c- it is carried out by solid-phase bridge amplification
- d- None of the above

1.2 How does the Sanger sequencing reaction works?:

- a- Cyclic reversible termination
- b- Nucleotide addition ✔
- c- Real time long read sequencing
- d- None of the above

1.3 Most representative features of Sanger sequencing is/are:

- a- Produce high quality reads
- b- Low throughput
- c- Short reads
- d- A and B ✔
- e All are correct

2. Read the descriptions and figures below. What description and figure belong to Sanger sequencing reaction?:

- a- description E and figure 4
- b- description F and figure 7 ✔
- c- description A and figure 5
- d- description H and figure 1

Descriptions

A After bead-based template enrichment, the beads are arrayed onto a microtitre plate along with primers and different beads that contain an enzyme cocktail. During the first cycle, a single nucleotide species is added to the plate and each complementary base is incorporated into a newly synthesized strand by a DNA polymerase. The by-product of this reaction is a pyrophosphate molecule (PP_i). The PP_i molecule, along with ATP sulfurylase, transforms adenosine 5′ phosphosulfate (APS) into ATP. ATP, in turn, is a cofactor for the conversion of luciferin to oxyluciferin by luciferase, for which the by-product is light. Finally, apyrase is used to degrade any unincorporated bases and the next base is added to the wells. Each burst of light, detected by a charge-coupled device (CCD) camera, can be attributed to the incorporation of one or more bases at a particular bead.

B Fragmented DNA templates are ligated to adapter sequences and are captured in an aqueous droplet (micelle) along with a bead covered with complementary adapters, deoxynucleotides (dNTPs), primers and DNA polymerase. PCR is carried out within the micelle, covering each bead with thousands of copies of the same DNA sequence.

C Cutting (with restriction enzymes) or shearing (with mechanical forces) large DNA fragments into shorter DNA fragments. The fragmented DNA may then be cloned into a DNA vector and amplified in a bacterial host such as Escherichia coli.

D Fragmented DNA is ligated to adapter sequences and bound to a primer immobilized on a solid support, such as a patterned flow cell. The free end can interact with other nearby primers, forming a bridge structure. PCR is used to create a second strand from the immobilized primers, and unbound DNA is removed.

E DNA is initially fragmented to 8–10 kb. Two different adapters, a leader and a hairpin, are ligated to either end of the fragmented dsDNA. Currently, there is no method to direct the adapters to a particular end of the DNA molecule, so there are three possible library conformations: leader–leader, leader–hairpin and hairpin–hairpin. The leader adapter is a double-stranded adapter containing a sequence required to direct the DNA into the pore and a tether sequence to help direct the DNA to the membrane surface. Without this leader adapter, there is minimal interaction of the DNA with the pore, which prevents any hairpin–hairpin fragments from being sequenced. The ideal library conformation is the leader–hairpin. In this conformation the leader sequence directs the DNA fragment to the pore with current passing through. As the DNA translocates through the pore, a characteristic shift in voltage through the pore is observed. Various parameters, including the magnitude and duration of the shift, are recorded and can be interpreted as a particular k-mer sequence. As the next base passes into the pore, a new k-mer modulates the voltage and is identified. At the hairpin, the DNA continues to be translocated through the pore adapter and onto the complement strand. This allows the forward and reverse strands to be used to create a consensus sequence called a '2D' read.

F Sequencing uses ddNTPs (dideoxynucleotide triphosphates) which do not have a free 3' OH mixed in with dNTPs. Whenever the DNA polymerase incorporates a ddNTP it won't be able to add any other nucleotides. Then gel electrophoresis is used to separate the DNAvv.

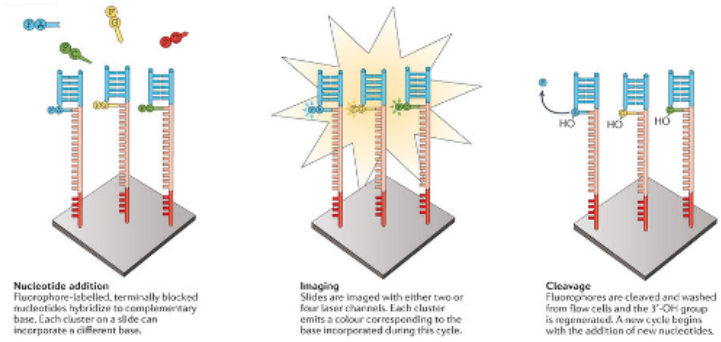
G Template fragments are processed and ligated to hairpin adapters at each end, resulting in a circular DNA molecule with constant single-stranded DNA (ssDNA) regions at each end with the double-stranded DNA (dsDNA) template in the middle. The resulting 'SMRTbell' template undergoes a size-selection protocol in which fragments that are too large or too small are removed to ensure efficient sequencing. Primers and an efficient φ29 DNA polymerase are attached to the ssDNA regions of the SMRTbell. The prepared library is then added to the zero-mode waveguide (ZMW) SMRT cell, where sequencing can take place. To visualize sequencing, a mixture of labelled nucleotides is added; as the polymerase-bound DNA library sits in one of the wells in the SMRT cell, the polymerase incorporates a fluorophore-labelled nucleotide into an elongating DNA strand. During incorporation, the nucleotide momentarily pauses through the activity of the polymerase at the bottom of the ZMW, which is being monitored by a camera.

H After solid-phase template enrichment, a mixture of primers, DNA polymerase and modified nucleotides are added to the flow cell. Each nucleotide is blocked by a 3′-*O*-azidomethyl group and is labelled with a base-specific, cleavable fluorophore (F). During each cycle, fragments in each cluster will incorporate just one nucleotide as the blocked 3′ group prevents additional incorporations. After base incorporation, unincorporated bases are washed away and the slide is imaged by total internal reflection fluorescence (TIRF) microscopy using either two or four laser channels; the colour (or the lack or mixing of colours in the two-channel system used by NextSeq) identifies which base was incorporated in each cluster. The dye is then cleaved and the 3′-OH is regenerated with the reducing agent tris(2-carboxyethyl)phosphine (TCEP). The cycle of nucleotide addition, elongation and cleavage can then begin again.

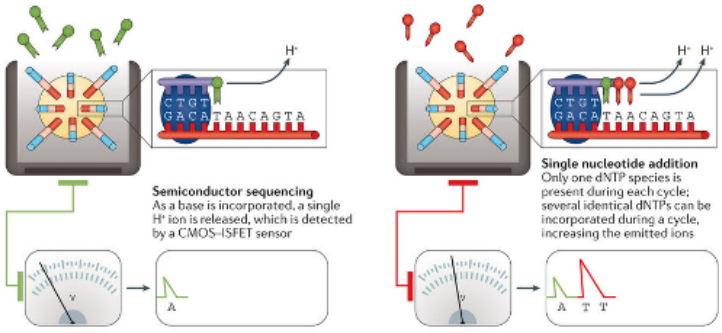
I After bead-based template enrichment, beads are carefully arrayed into a microtitre plate where one bead occupies a single reaction well. Nucleotide species are added to the wells one at a time and a standard elongation reaction is performed. As each base is incorporated, a single H^+ ion is generated as a by-product. The H^+ release results in a 0.02 unit change in pH, detected by an integrated complementary metal-oxide semiconductor (CMOS) and an ion-sensitive field-effect transistor (ISFET) device. After the introduction of a single nucleotide species, the unincorporated bases are washed away and the next is added.

Images.

1.



2.



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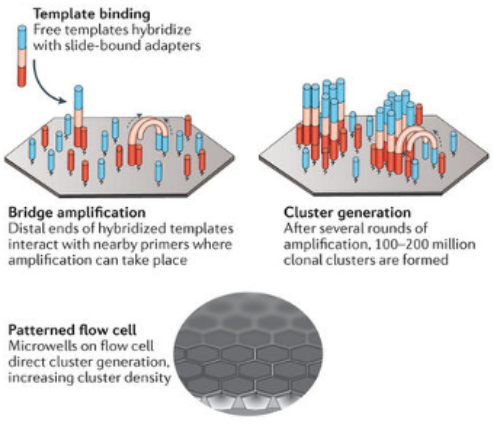

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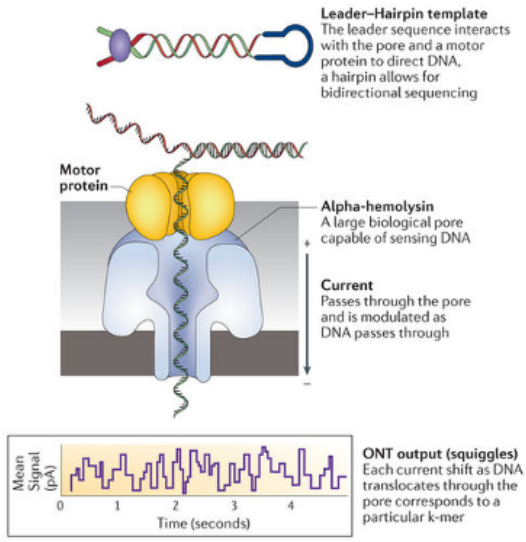
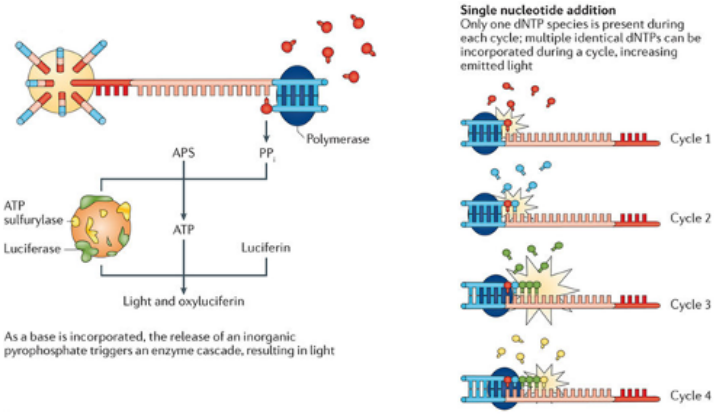
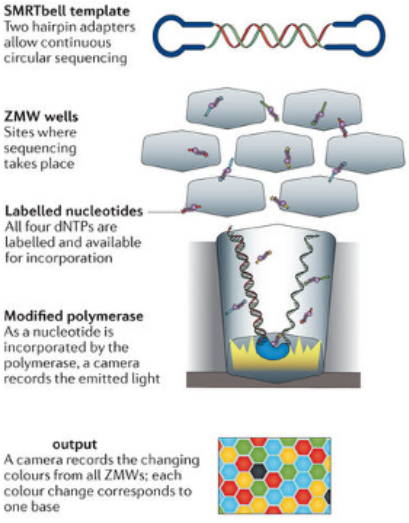

Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing
Motor protein
Alpha-hemolysin
A large biological pore capable of sensing DNA
Current
Passes through the pore and is modulated as DNA passes through
Mean Signal (pA)
0
1
2
3
4
Time (seconds)
ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

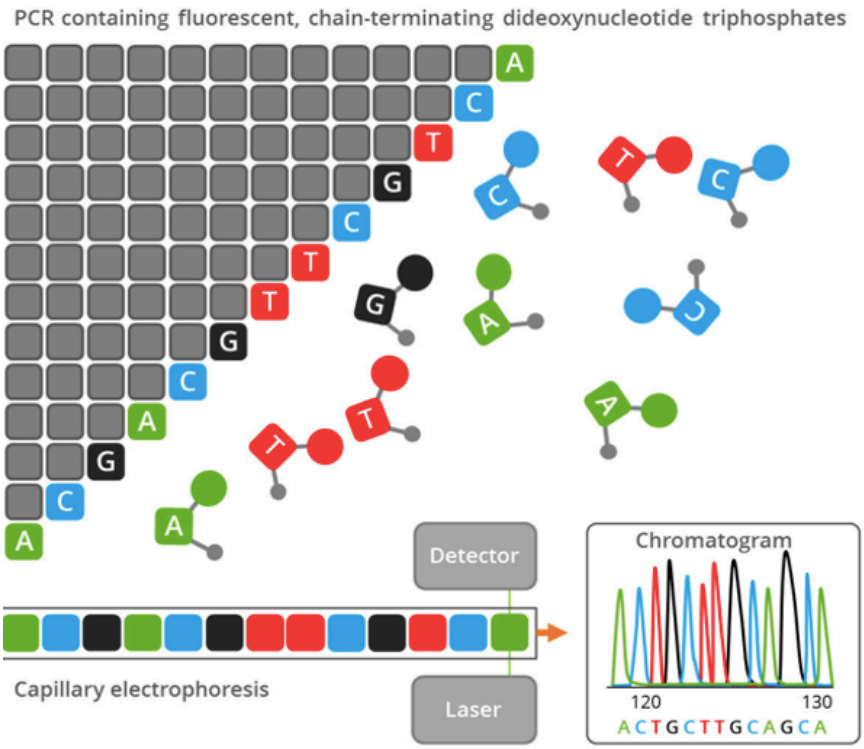
5.


Polymerase
APS
PPi
ATP sulfurylase
Luciferase
ATP
Luciferin
Light and oxyluciferin
As a base is incorporated, the release of an inorganic pyrophosphate triggers an enzyme cascade, resulting in light
Single nucleotide addition
Only one dNTP species is present during each cycle; multiple identical dNTPs can be incorporated during a cycle, increasing emitted light
Cycle 1
Cycle 2
Cycle 3
Cycle 4

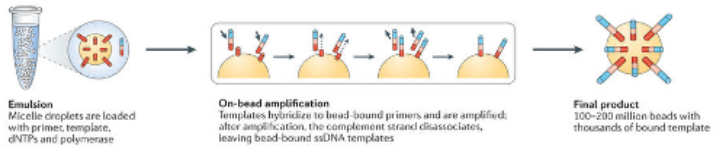
6.


SMRTbell template
Two hairpin adapters allow continuous circular sequencing
ZMW wells
Sites where sequencing takes place
Labelled nucleotides
All four dNTPs are labelled and available for incorporation
Modified polymerase
As a nucleotide is incorporated by the polymerase, a camera records the emitted light
output
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

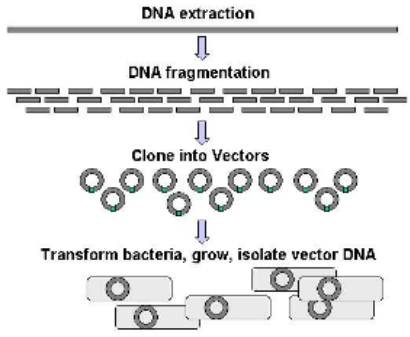
7.


PCR containing fluorescent, chain-terminating dideoxynucleotide triphosphates
Detector
Laser
Capillary electrophoresis
Chromatogram
120
130
ACTGCTTGCAGCA

8.


Emulsion
Micelle droplets are loaded with primer, template, dNTPs and polymerase
On-bead amplification
Templates hybridize to bead-bound primers and are amplified; after amplification, the complement strand disassociates, leaving bead-bound ssDNA templates
Final product
100–200 million beads with thousands of bound template

9.


DNA extraction
DNA fragmentation
Clone into Vectors
Transform bacteria, grow, isolate vector DNA

Question 2

Correct

Mark 4.00 out of 4.00

2. Illumina sequencing

Answer the following questions regarding to Illumina sequencing

2.1. Regarding to template amplification in Illumina sequencing it is true:

- a- It can be done by cloning
- b- It is carried out by emulsion PCR
- c- it is carried out by solid-phase bridge amplification✔
- d- None of the above

2.2 How does the Illumina sequencing reaction works?:

- a- Cyclic reversible termination✔
- b- Nucleotide addition
- c- Real time long read sequencing
- d- None of the above

2.3 The most representative features of Illumina sequencing is/are:

- a- High quality reads
- b- High throughput
- c- Short reads
- d- A and C
- e- All are correct✔

2.4. Read the descriptions and figures below. What description and figure belong to Illumina sequencing reaction?:

- a- description E and figure 4
- b- description F and figure 7
- c- description A and figure 5
- d- description H and figure 1✔
- e- description G and figure 6

Descriptions

A After bead-based template enrichment, the beads are arrayed onto a microtitre plate along with primers and different beads that contain an enzyme cocktail. During the first cycle, a single nucleotide species is added to the plate and each complementary base is incorporated into a newly synthesized strand by a DNA polymerase. The by-product of this reaction is a pyrophosphate molecule (PP_i). The PP_i molecule, along with ATP sulfurylase, transforms adenosine 5′ phosphosulfate (APS) into ATP. ATP, in turn, is a cofactor for the conversion of luciferin to oxyluciferin by luciferase, for which the by-product is light. Finally, apyrase is used to degrade any unincorporated bases and the next base is added to the wells. Each burst of light, detected by a charge-coupled device (CCD) camera, can be attributed to the incorporation of one or more bases at a particular bead.

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C Cutting (with restriction enzymes) or shearing (with mechanical forces) large DNA fragments into shorter DNA fragments. The fragmented DNA may then be cloned into a DNA vector and amplified in a bacterial host such as Escherichia coli.

D Fragmented DNA is ligated to adapter sequences and bound to a primer immobilized on a solid support, such as a patterned flow cell. The free end can interact with other nearby primers, forming a bridge structure. PCR is used to create a second strand from the immobilized primers, and unbound DNA is removed.

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G Template fragments are processed and ligated to hairpin adapters at each end, resulting in a circular DNA molecule with constant single-stranded DNA (ssDNA) regions at each end with the double-stranded DNA (dsDNA) template in the middle. The resulting 'SMRTbell' template undergoes a size-selection protocol in which fragments that are too large or too small are removed to ensure efficient sequencing. Primers and an efficient φ29 DNA polymerase are attached to the ssDNA regions of the SMRTbell. The prepared library is then added to the zero-mode waveguide (ZMW) SMRT cell, where sequencing can take place. To visualize sequencing, a mixture of labelled nucleotides is added; as the polymerase-bound DNA library sits in one of the wells in the SMRT cell, the polymerase incorporates a fluorophore-labelled nucleotide into an elongating DNA strand. During incorporation, the nucleotide momentarily pauses through the activity of the polymerase at the bottom of the ZMW, which is being monitored by a camera.

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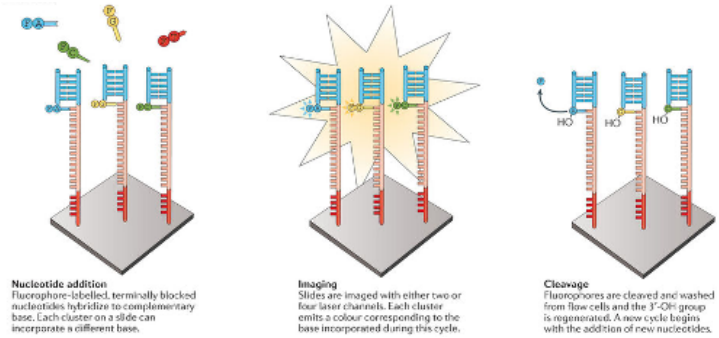
Images.

1.



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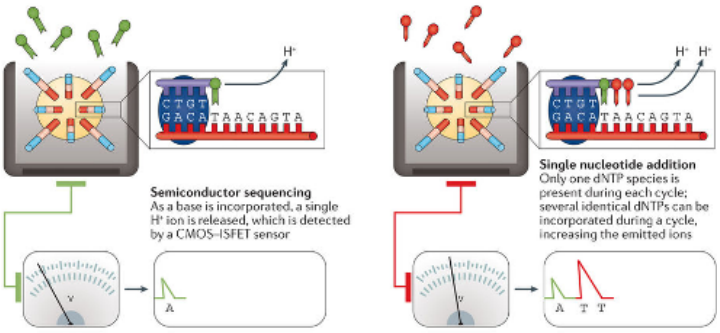

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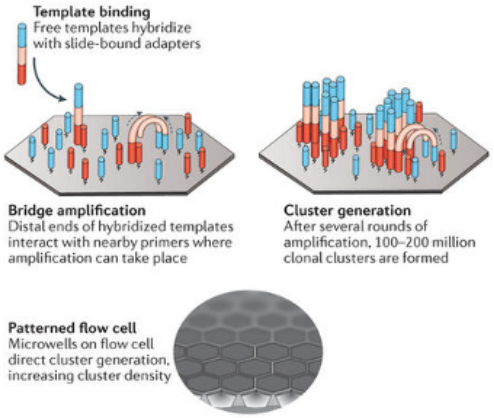
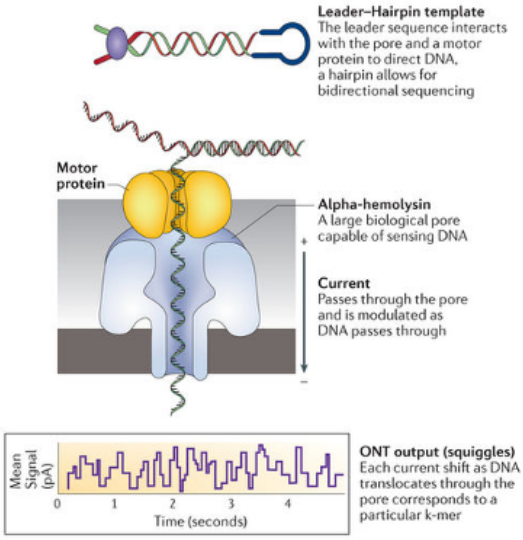
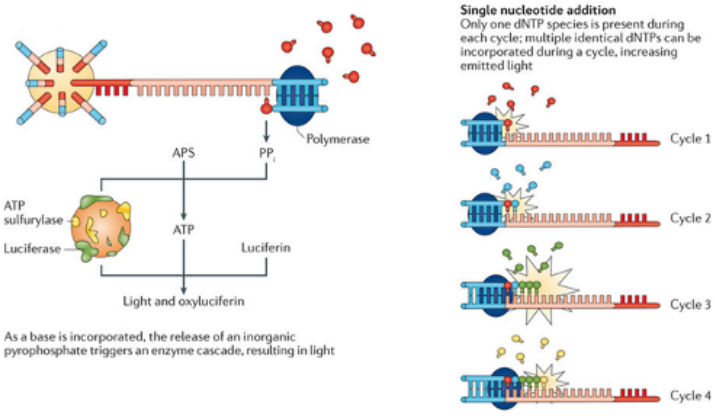

Template binding
Free templates hybridize with slide-bound adapters
Bridge amplification
Distal ends of hybridized templates interact with nearby primers where amplification can take place
Cluster generation
After several rounds of amplification, 100–200 million clonal clusters are formed
Patterned flow cell
Microwells on flow cell direct cluster generation, increasing cluster density

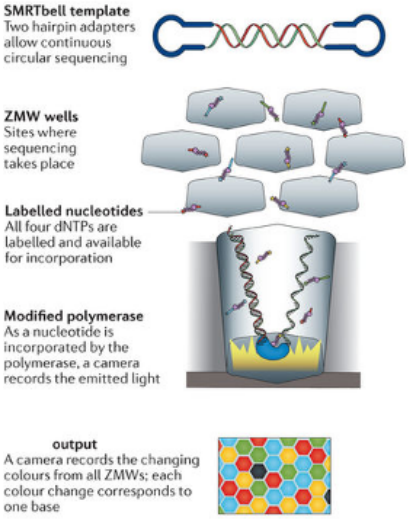
4.


Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing
Motor protein
Alpha-hemolysin
A large biological pore capable of sensing DNA
Current
Passes through the pore and is modulated as DNA passes through
Mean Signal (pA)
0
1
2
3
4
Time (seconds)
ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

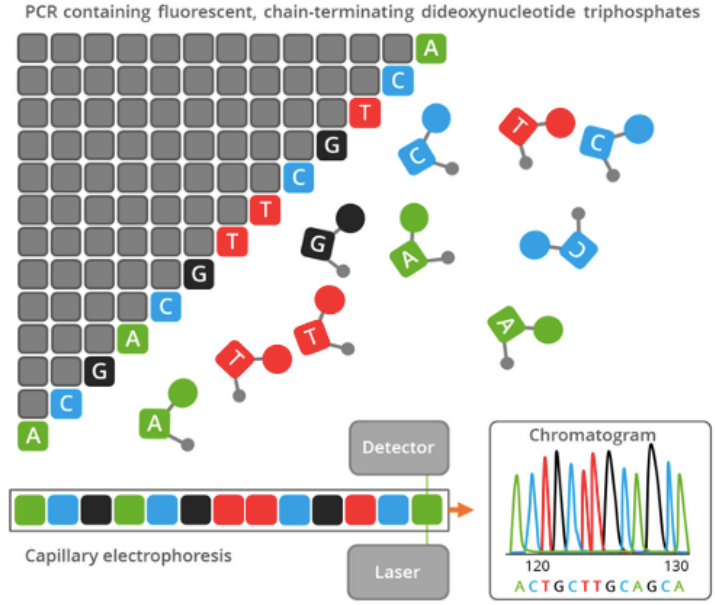
5.


Polymerase
APS
PPi
ATP sulfurylase
Luciferase
ATP
Luciferin
Light and oxyluciferin
As a base is incorporated, the release of an inorganic pyrophosphate triggers an enzyme cascade, resulting in light
Single nucleotide addition
Only one dNTP species is present during each cycle; multiple identical dNTPs can be incorporated during a cycle, increasing emitted light
Cycle 1
Cycle 2
Cycle 3
Cycle 4

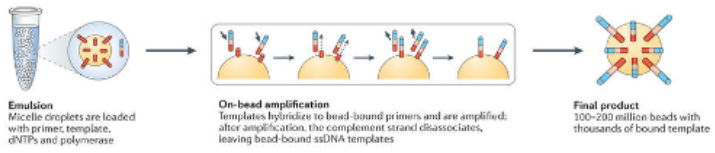
6.


SMRTbell template
Two hairpin adapters allow continuous circular sequencing
ZMW wells
Sites where sequencing takes place
Labelled nucleotides
All four dNTPs are labelled and available for incorporation
Modified polymerase
As a nucleotide is incorporated by the polymerase, a camera records the emitted light
output
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

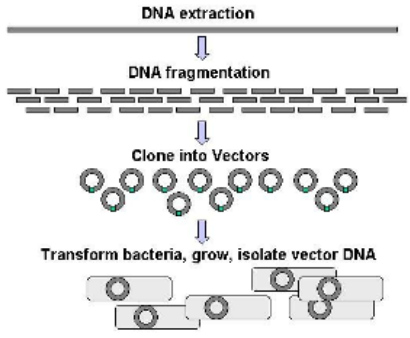
7.


PCR containing fluorescent, chain-terminating dideoxynucleotide triphosphates
Detector
Laser
Capillary electrophoresis
Chromatogram
120
130
ACTGCTTGCAGCA

8.


Emulsion
Micelle droplets are loaded with primer, template, dNTPs and polymerase
On-bead amplification
Templates hybridize to bead-bound primers and are amplified; after amplification, the complement strand disassociates, leaving bead-bound ssDNA templates
Final product
100–200 million beads with thousands of bound template

9.


DNA extraction
DNA fragmentation
Clone into Vectors
Transform bacteria, grow, isolate vector DNA

Question 3

Correct

Mark 4.00 out of 4.00

3. Nanopore sequencing

Answer the following questions regarding to Nanopore sequencing

3.1. Regarding to template amplification in Nanopore sequencing it is true:

- a- It can be done by cloning
- b- It is carried out by emulsion PCR
- c- it is carried out by solid-phase bridge amplification
- d- None of the above ✔

3.2 How does the Nanopore sequencing reaction works?:

- a- Cyclic reversible termination
- b- Nucleotide addition
- c- Real time long read sequencing ✔
- d- None of the above

3.3 Most representative features of Nanopore sequencing are:

- a Produce high quality reads
- b- High throughput
- c- Real time and portable long read sequencing ✔
- d- A and C
- e- All are correct

3.4. Read the descriptions and figures below. What description and figure belong to Nanopore sequencing reaction?

- a- description E and figure 4 ✔
- b- description F and figure 7
- c- description A and figure 5
- d- description H and figure 1
- e- description G and figure 6

Descriptions

A After bead-based template enrichment, the beads are arrayed onto a microtitre plate along with primers and different beads that contain an enzyme cocktail. During the first cycle, a single nucleotide species is added to the plate and each complementary base is incorporated into a newly synthesized strand by a DNA polymerase. The by-product of this reaction is a pyrophosphate molecule (PP_i). The PP_i molecule, along with ATP sulfurylase, transforms adenosine 5′ phosphosulfate (APS) into ATP. ATP, in turn, is a cofactor for the conversion of luciferin to oxyluciferin by luciferase, for which the by-product is light. Finally, apyrase is used to degrade any unincorporated bases and the next base is added to the wells. Each burst of light, detected by a charge-coupled device (CCD) camera, can be attributed to the incorporation of one or more bases at a particular bead.

B Fragmented DNA templates are ligated to adapter sequences and are captured in an aqueous droplet (micelle) along with a bead covered with complementary adapters, deoxynucleotides (dNTPs), primers and DNA polymerase. PCR is carried out within the micelle, covering each bead with thousands of copies of the same DNA sequence.

C Cutting (with restriction enzymes) or shearing (with mechanical forces) large DNA fragments into shorter DNA fragments. The fragmented DNA may then be cloned into a DNA vector and amplified in a bacterial host such as Escherichia coli.

D Fragmented DNA is ligated to adapter sequences and bound to a primer immobilized on a solid support, such as a patterned flow cell. The free end can interact with other nearby primers, forming a bridge structure. PCR is used to create a second strand from the immobilized primers, and unbound DNA is removed.

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Images.

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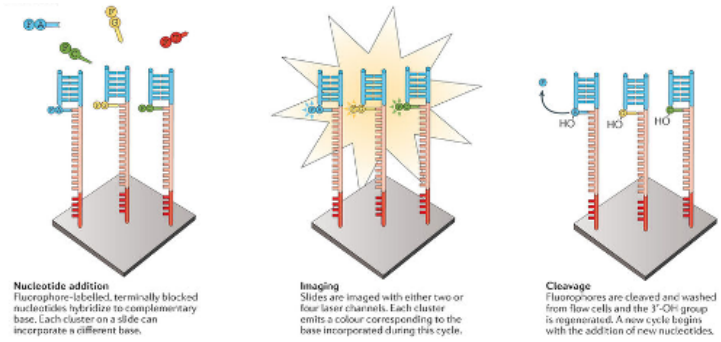

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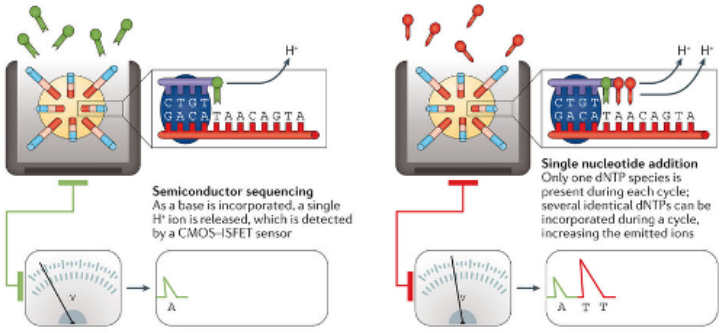

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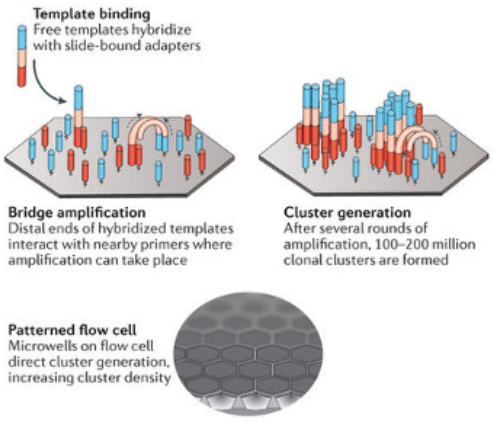

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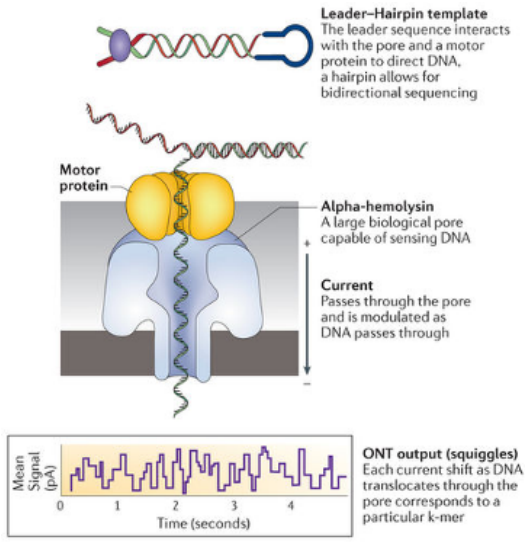
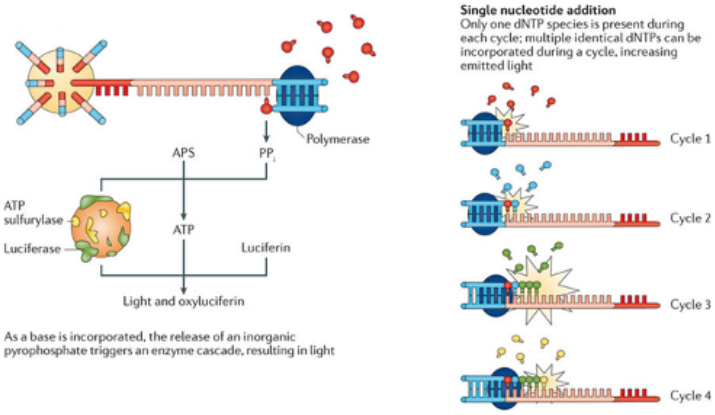

Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing
Motor protein
Alpha-hemolysin
A large biological pore capable of sensing DNA
Current
Passes through the pore and is modulated as DNA passes through
Mean Signal (pA)
0
1
2
3
4
Time (seconds)
ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

5.


Polymerase
APS
PPi
ATP sulfurylase
Luciferase
ATP
Luciferin
Light and oxyluciferin
As a base is incorporated, the release of an inorganic pyrophosphate triggers an enzyme cascade, resulting in light
Single nucleotide addition
Only one dNTP species is present during each cycle; multiple identical dNTPs can be incorporated during a cycle, increasing emitted light
Cycle 1
Cycle 2
Cycle 3
Cycle 4

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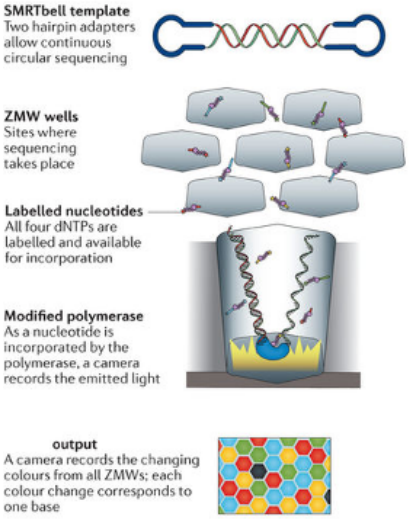
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As a nucleotide is incorporated by the polymerase, a camera records the emitted light
output
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

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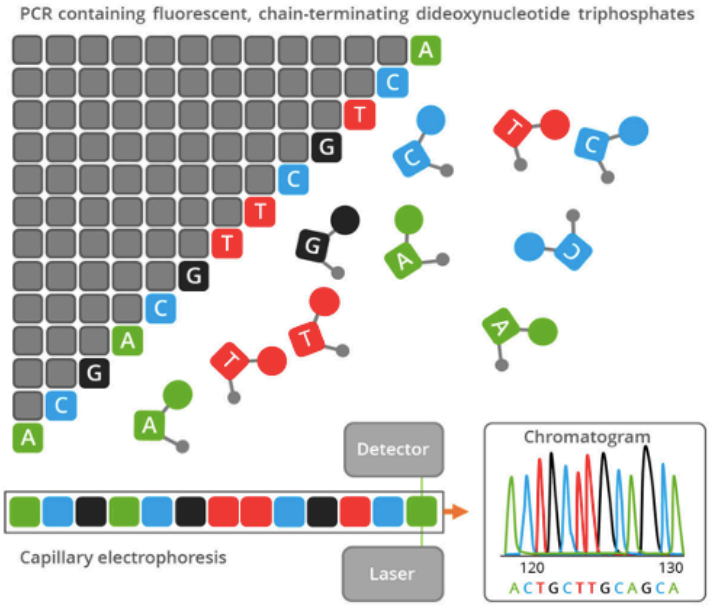
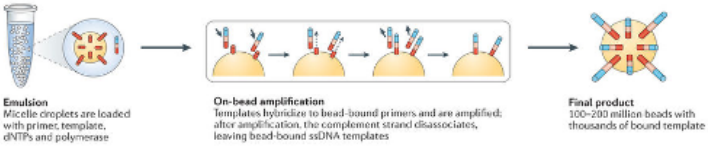

PCR containing fluorescent, chain-terminating dideoxynucleotide triphosphates
Detector
Chromatogram
Capillary electrophoresis
Laser
120
130
ACTGCTTGCAGCA

8.


Emulsion
Micelle droplets are loaded with primer, template, dNTPs and polymerase
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100–200 million beads with thousands of bound template

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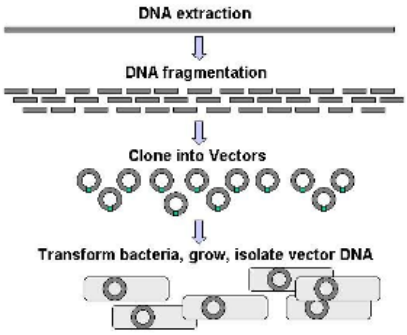
DNA extraction
DNA fragmentation
Clone into Vectors
Transform bacteria, grow, isolate vector DNA

Question 4

Correct

Mark 4.00 out of 4.00

1. PacBio sequencing

Answer the following questions regarding to PacBio sequencing

1.1. Regarding to template amplification in PacBio sequencing it is true:

- a- It can be done by cloning
- b- It is carried out by emulsion PCR
- c- it is carried out by solid-phase bridge amplification
- d- None of the above ✔

1.2 How does the PacBio sequencing reaction works?:

- a- Cyclic reversible termination
- b- Nucleotide addition
- c- Real time long read sequencing ✔
- d- None of the above

1.3 Most representative features of PacBio sequencing is/are:

- a- Real time sequencing
- b- Low throughput
- c- Long reads
- d- A and C ✔
- e All are correct

2. Read the descriptions and figures below. What description and figure belong to PacBio sequencing reaction?:

- a- description G and figure 6 ✔
- b- description F and figure 7
- c- description A and figure 5
- d- description H and figure 1

Descriptions

A After bead-based template enrichment, the beads are arrayed onto a microtitre plate along with primers and different beads that contain an enzyme cocktail. During the first cycle, a single nucleotide species is added to the plate and each complementary base is incorporated into a newly synthesized strand by a DNA polymerase. The by-product of this reaction is a pyrophosphate molecule (PP_i). The PP_i molecule, along with ATP sulfurylase, transforms adenosine 5′ phosphosulfate (APS) into ATP. ATP, in turn, is a cofactor for the conversion of luciferin to oxyluciferin by luciferase, for which the by-product is light. Finally, apyrase is used to degrade any unincorporated bases and the next base is added to the wells. Each burst of light, detected by a charge-coupled device (CCD) camera, can be attributed to the incorporation of one or more bases at a particular bead.

B Fragmented DNA templates are ligated to adapter sequences and are captured in an aqueous droplet (micelle) along with a bead covered with complementary adapters, deoxynucleotides (dNTPs), primers and DNA polymerase. PCR is carried out within the micelle, covering each bead with thousands of copies of the same DNA sequence.

C Cutting (with restriction enzymes) or shearing (with mechanical forces) large DNA fragments into shorter DNA fragments. The fragmented DNA may then be cloned into a DNA vector and amplified in a bacterial host such as Escherichia coli.

D Fragmented DNA is ligated to adapter sequences and bound to a primer immobilized on a solid support, such as a patterned flow cell. The free end can interact with other nearby primers, forming a bridge structure. PCR is used to create a second strand from the immobilized primers, and unbound DNA is removed.

E DNA is initially fragmented to 8–10 kb. Two different adapters, a leader and a hairpin, are ligated to either end of the fragmented dsDNA. Currently, there is no method to direct the adapters to a particular end of the DNA molecule, so there are three possible library conformations: leader–leader, leader–hairpin and hairpin–hairpin. The leader adapter is a double-stranded adapter containing a sequence required to direct the DNA into the pore and a tether sequence to help direct the DNA to the membrane surface. Without this leader adapter, there is minimal interaction of the DNA with the pore, which prevents any hairpin–hairpin fragments from being sequenced. The ideal library conformation is the leader–hairpin. In this conformation the leader sequence directs the DNA fragment to the pore with current passing through. As the DNA translocates through the pore, a characteristic shift in voltage through the pore is observed. Various parameters, including the magnitude and duration of the shift, are recorded and can be interpreted as a particular k-mer sequence. As the next base passes into the pore, a new k-mer modulates the voltage and is identified. At the hairpin, the DNA continues to be translocated through the pore adapter and onto the complement strand. This allows the forward and reverse strands to be used to create a consensus sequence called a '2D' read.

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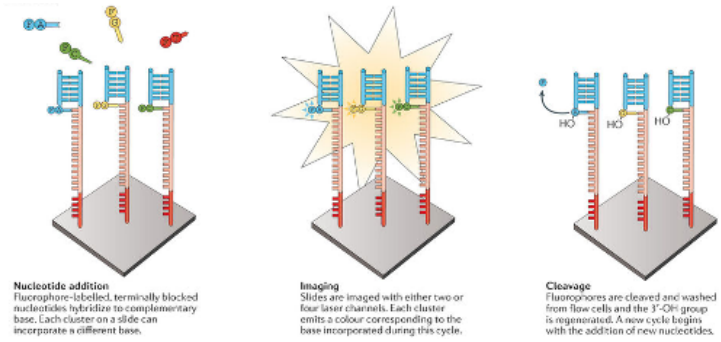

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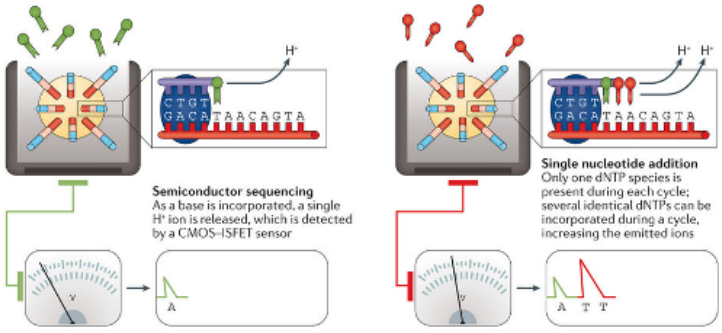

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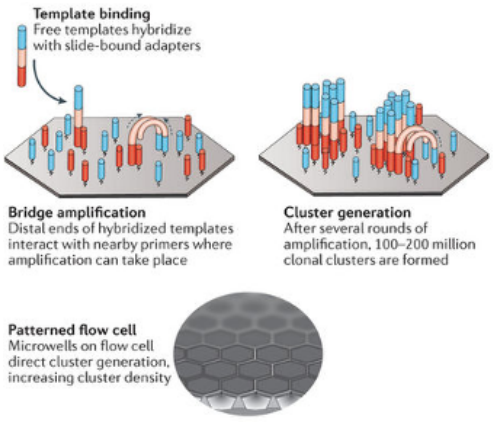

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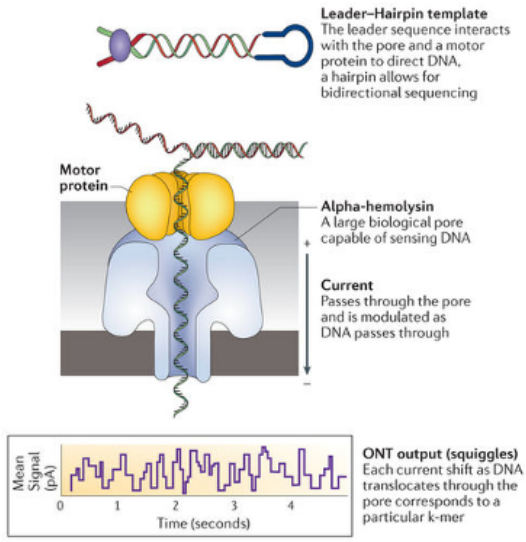
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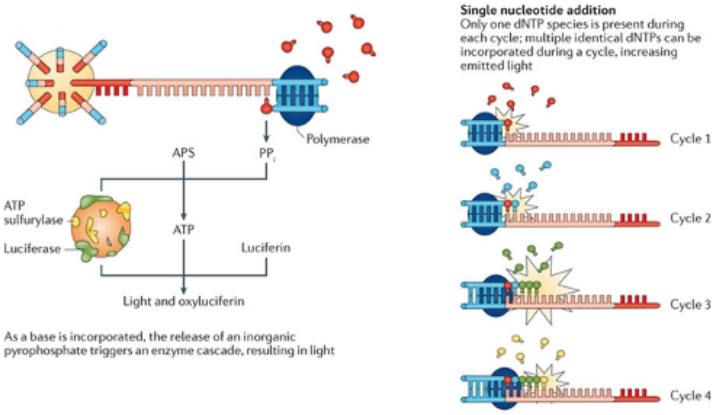
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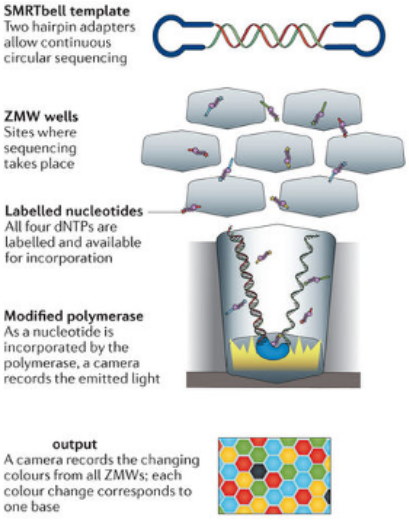
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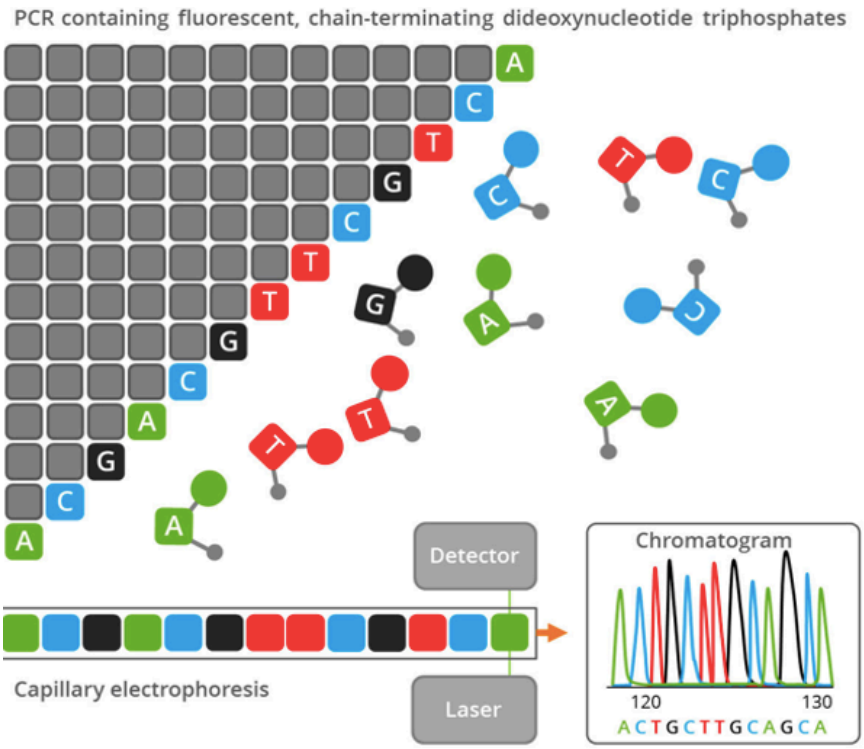
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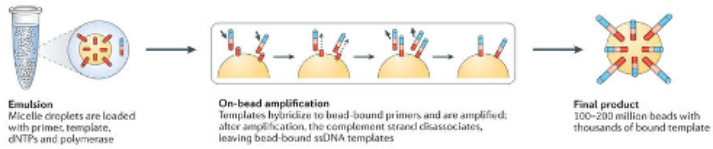
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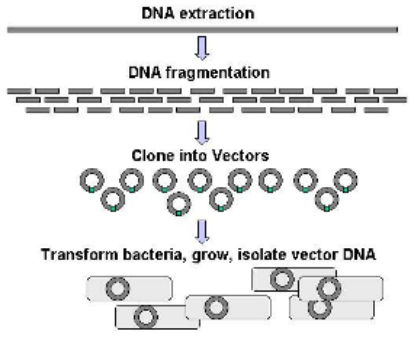
DNA extraction
DNA fragmentation
Clone into Vectors
Transform bacteria, grow, isolate vector DNA