Genetic Analysis

Phenotype analysis: biological-biochemical analysis

Behaviour under specific environmental conditions

Behaviour of specific genetic configurations

Behaviour of progeny in crosses -

Genotype analysis: molecular and physical analysis

DNA Fragments/Molecules
Sequence homology
Sequence

Genetic Analysis

Phenotype analysis: biological-biochemical analysis

Behaviour under specific environmental conditions

Some examples:

Utilization of specific substrates
Loss fo function (e.g. Auxotrophy)
Înduction behaviour

Behaviour of specific genetic configurations

Some examples:

Complementation analysis: e.g. function (auxotrophy) complementation
Cis-trans test → see regulation: *lac* operon

Phenotype analysis: biological-biochemical analysis

Behaviour of progeny in crosses – see also introduction → Mendel

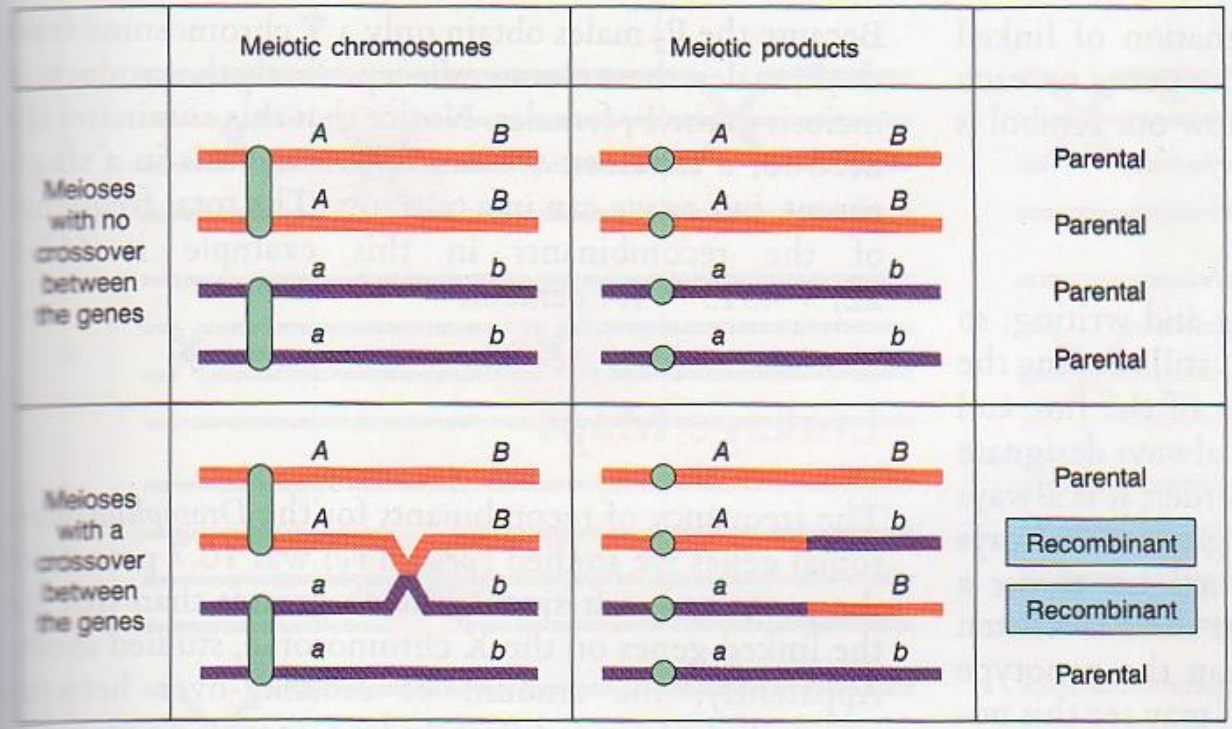
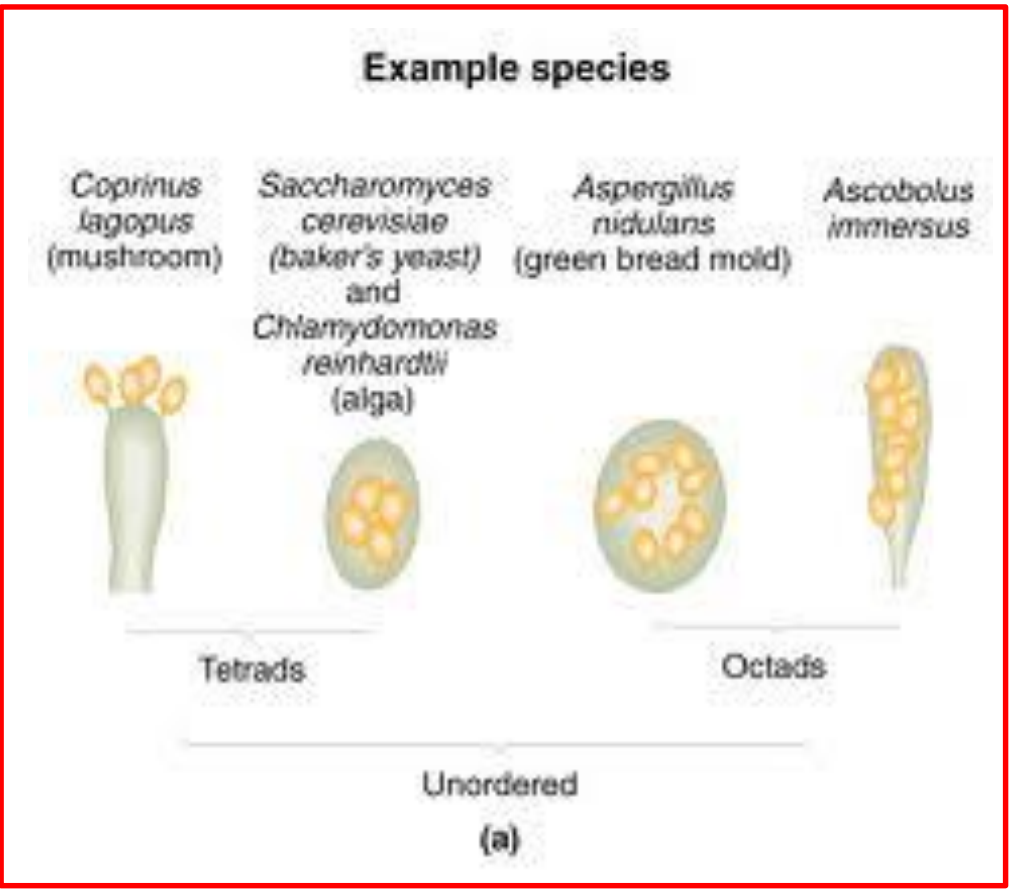
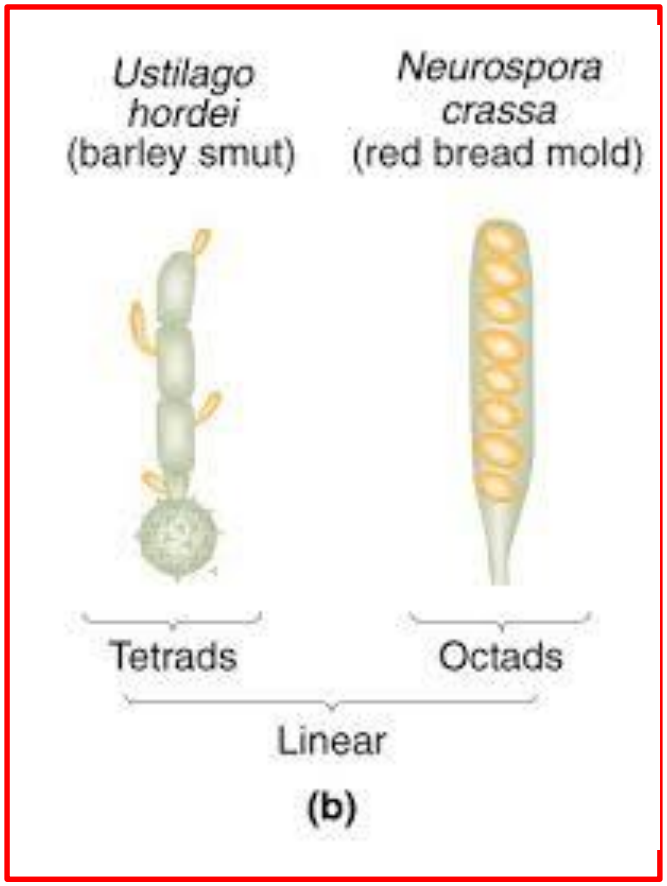


Figure 5-7 Intrachromosomal recombinants arise from meioses in which nonsister chromatids cross over between the genes under study.

Product from sexual reproduction

Specific systems with fungi





Unordered
No connection to position in meiosis

Ordered
Position in meiosis reflected

First division segregation pattern

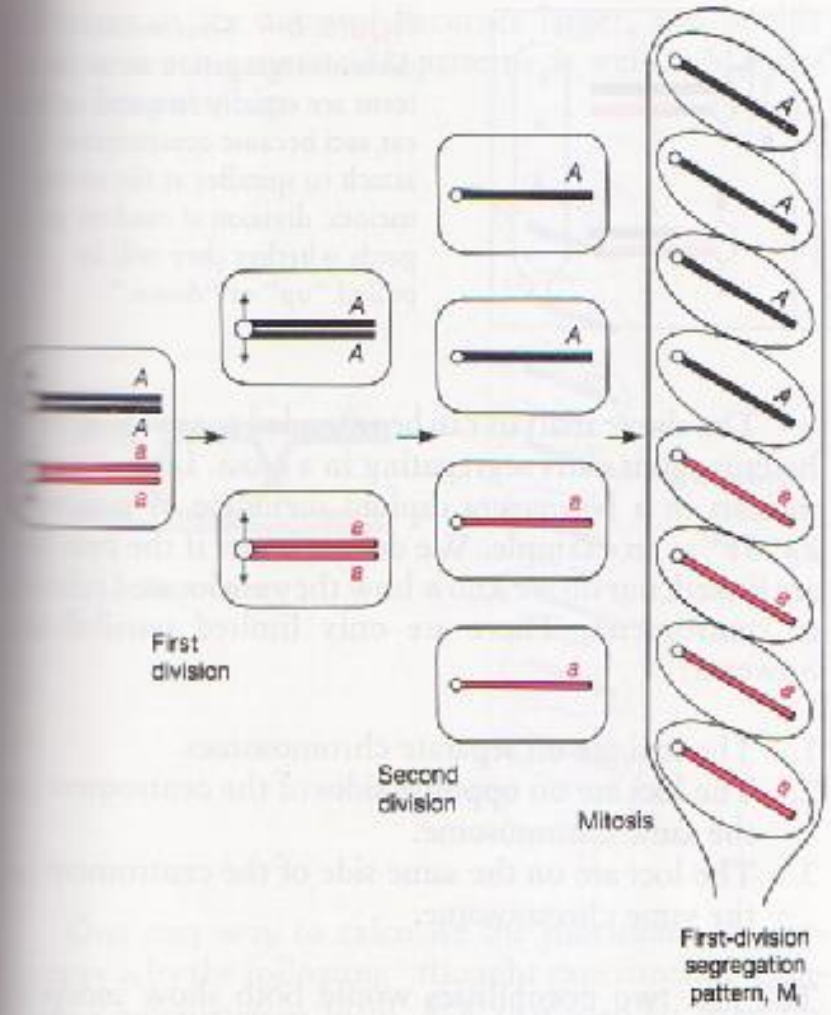


Figure 6-7 *A* and *a* segregate into separate nuclei at the first meiotic division when there is no crossover between the centromere and the locus. The resultant allele pattern in the octad is called a first-division segregation pattern.

Second division segregation pattern

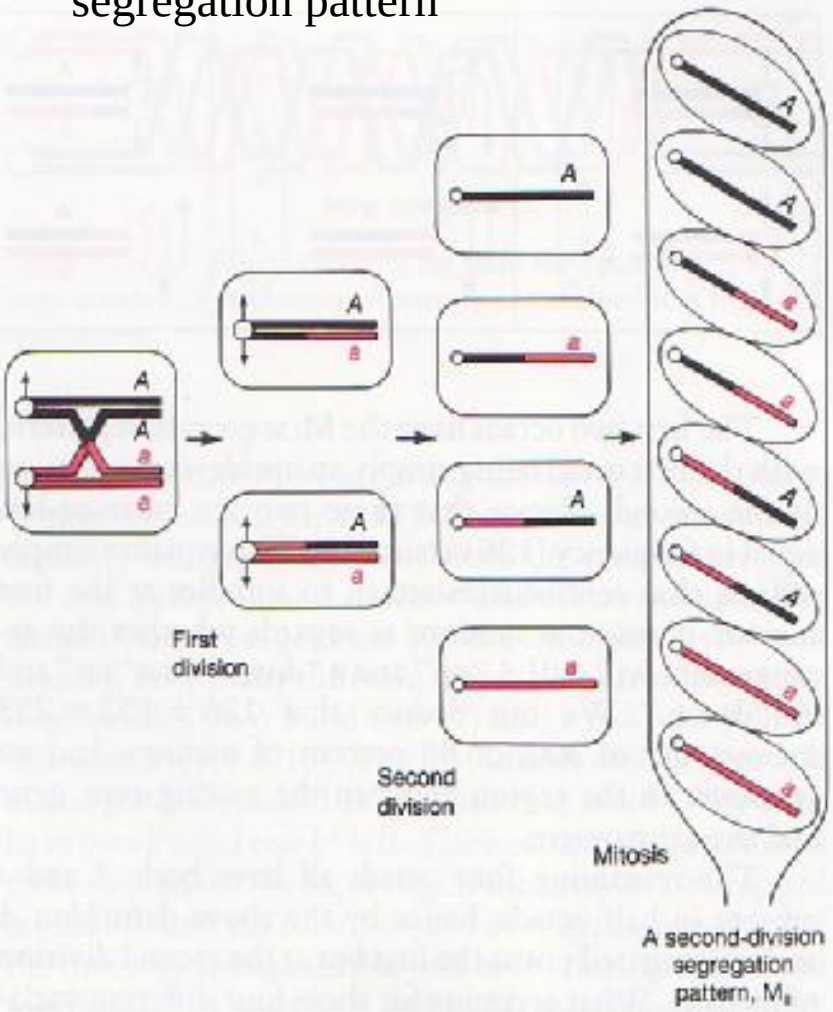
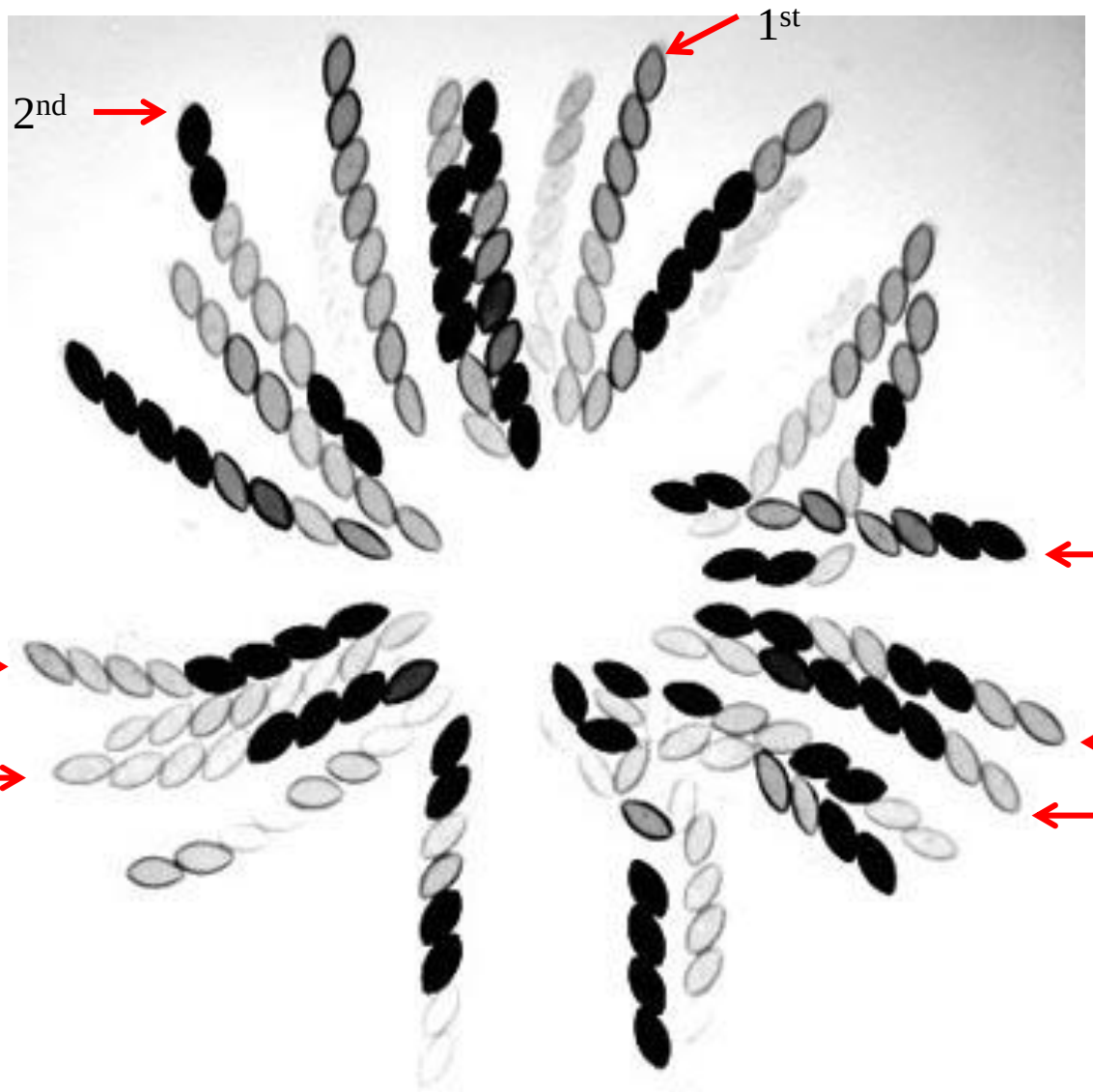


Figure 6-8 *A* and *a* segregate into separate nuclei at the second meiotic division when there is a crossover between the centromere and the locus.

Ordered linear octads in Neurospora crassa



Examples for first and second division segregation patterns

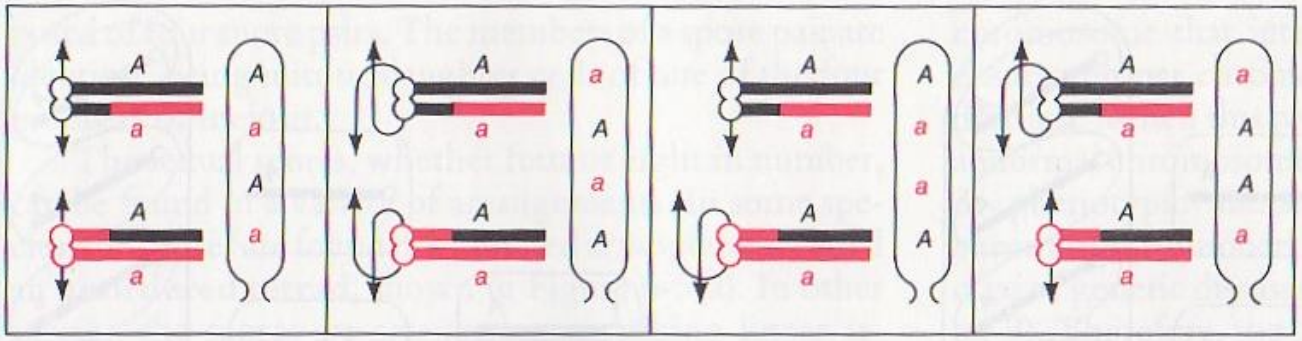


Figure 6-9 Four second-division segregation ascus patterns are equally frequent in l ear asci because centromeres attach to spindles at the secon meiotic division at random as gards whether they will be pulled "up" or "down."

Octads					
A	*a*	*A*	*a*	*A*	*a*
A	*a*	*A*	*a*	*A*	*a*
A	*a*	*a*	*A*	*a*	*A*
A	*a*	*a*	*A*	*a*	*A*
a	*A*	*A*	*a*	*a*	*A*
a	*A*	*A*	*a*	*a*	*A*
a	*A*	*a*	*A*	*A*	*a*
a	*A*	*a*	*A*	*A*	*a*
126	132	9	11	10	12

Total = 300

map unit (m.u.) = RF (Recombination Frequency, as %)
For example: 1 map unit: RF = 0,01 = 1%

1 m.u. = 1 centi Morgan (cM)

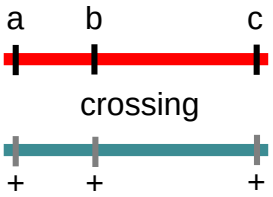
Ordered Tetrade:
Distance Locus – Centromer:

$$0{,}5 \times \frac{\text{(second division pattern tetrads)}}{\text{Total tetrads}} \times 100$$

Unordered tetrads

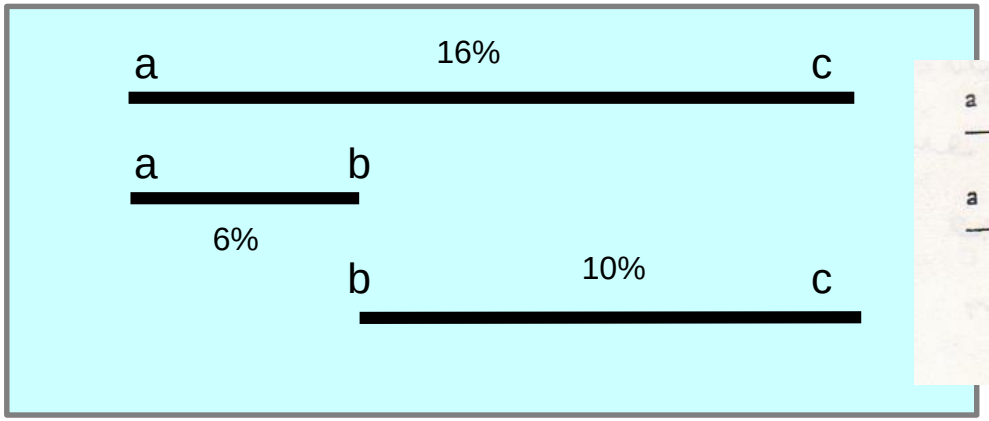
2 - factor cross analysis

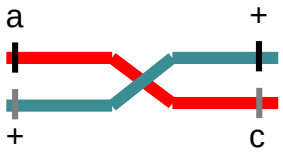
loci

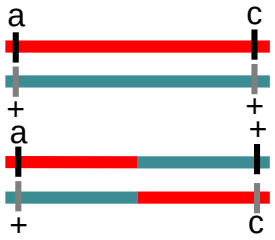


Mutant alleles

wild type alleles



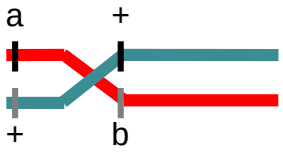


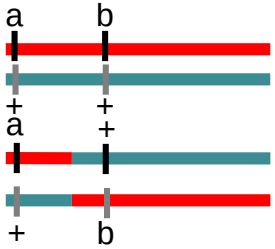


parentals 42% 42% } 84%

recombinants 8% 8% } 16%

Crossover between **a** and **c**

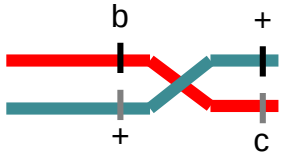


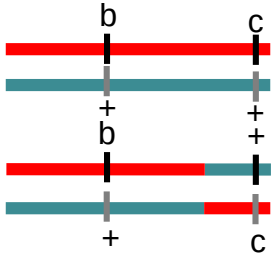


parentals 47% 47% } 94%

recombinants 3% 3% } 6%

Crossover between **a** and **b**





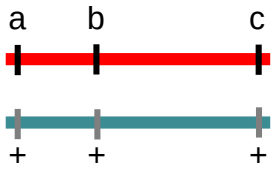
parentals 45% 45% } 90%

recombinants 5% 5% } 10%

Crossover between **b** and **c**

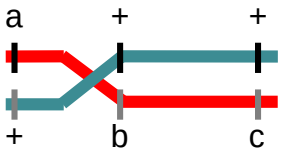
Unordered tetrads

3- factor cross analysis



parentals

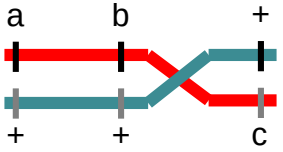
40,2%
40,2%
} 80,4%



recombinants

2,9%
2,9%
} 5,8%

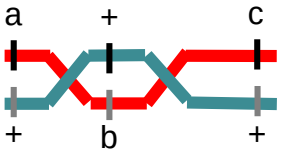
exchange of locus **a**



recombinants

4,9%
4,9%
} 9,8%

exchange of locus **c**



recombinants

0,2%
0,2%
} 0,4%

exchange of locus **b**

Exchange of middle locus needs 2 cross-overs: → lowest probability

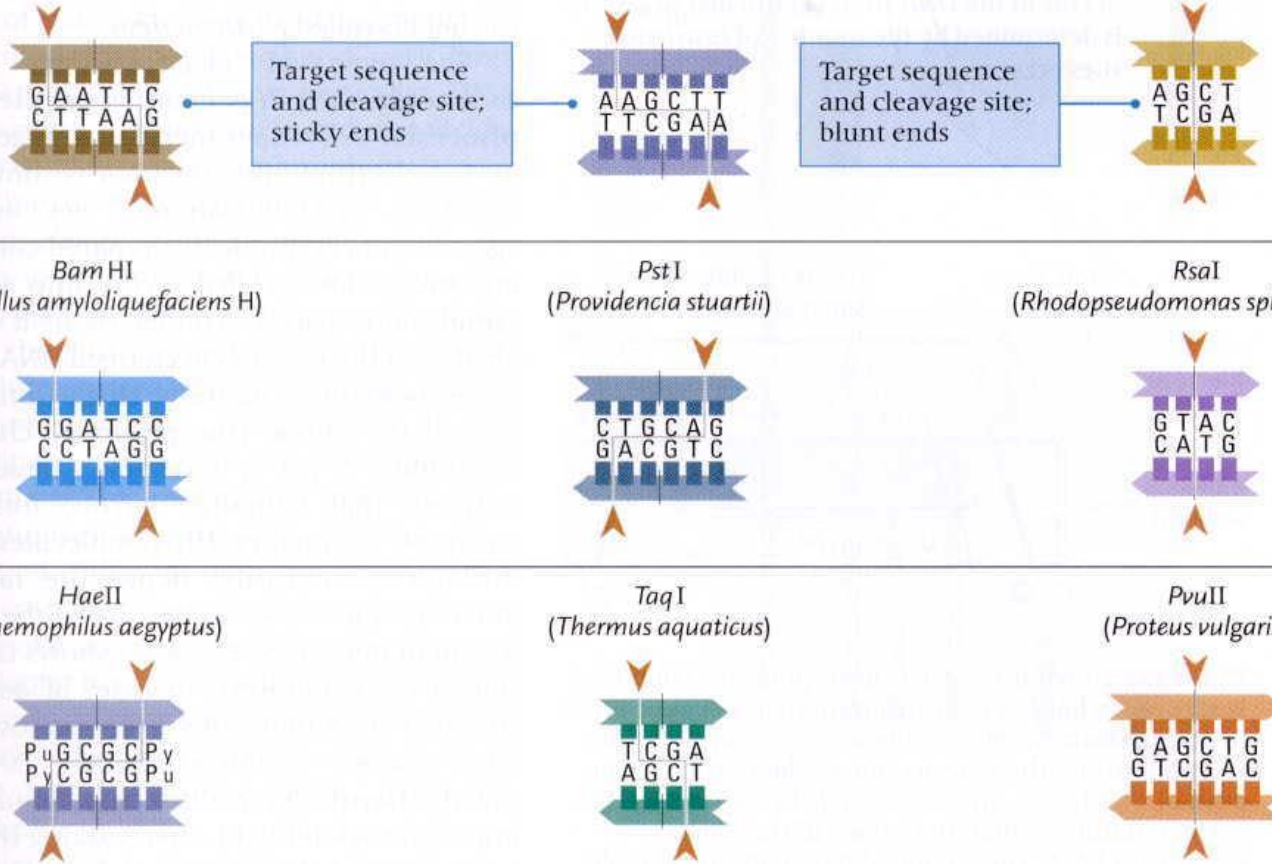
Genetic Analysis

Genotype analysis: molecular and physical analysis

DNA Fragments/Molecules
Sequence homology
Sequence

Table 2.3 Some restriction endonucleases, their sources, and their cleavage sites

Enzyme (Microorganism)	Enzyme (Microorganism)	Enzyme (Microorganism)
*Eco*RI (*Escherichia coli*)	*Hind* III (*Haemophilus influenzae*)	*Alu*I (*Arthrobacter luteus*)
Bam HI (*Bacillus amyloliquefaciens* H)	*Pst* I (*Providencia stuartii*)	*Rsa*I (*Rhodopseudomonas sphaeroides*)
*Hae*II (*Haemophilus aegyptus*)	*Taq* I (*Thermus aquaticus*)	*Pvu*II (*Proteus vulgaris*)



Note: The vertical dashed line indicates the axis of symmetry in each sequence. Red arrows indicate the sites of cutting. The enzyme *Taq*I yields cohesive ends consisting of two nucleotides, whereas the cohesive ends produced by the other enzymes contain four nucleotides. Pu and Py refer to any purine and pyrimidine, respectively.

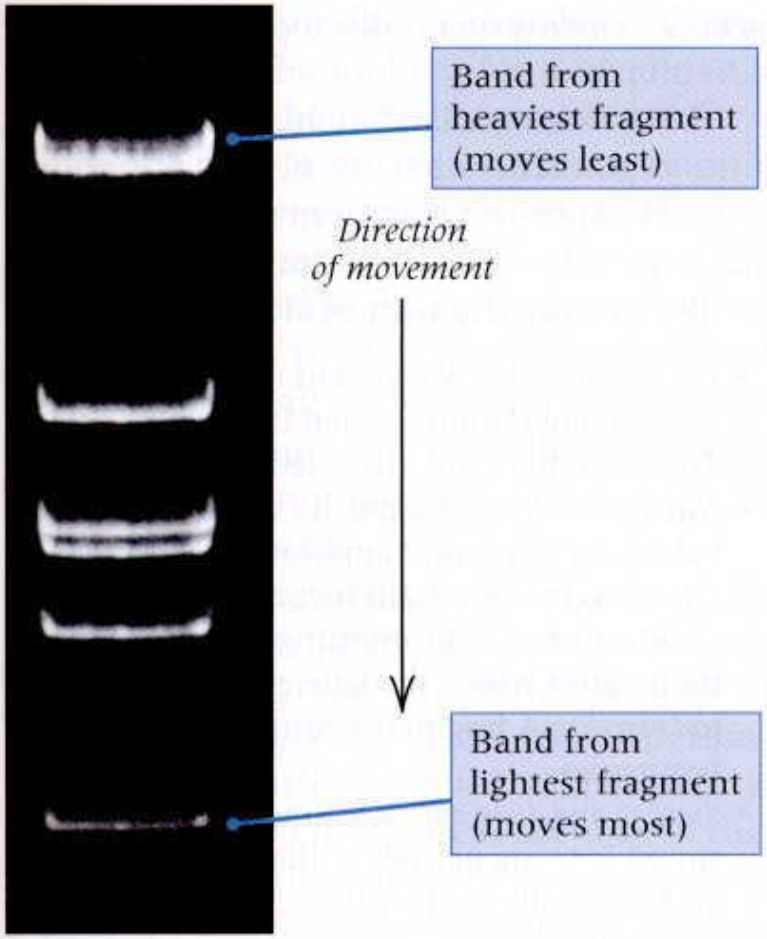


Figure 2.12 Gel electrophoresis of DNA. Fragments of different sizes were mixed and placed in a well. Electrophoresis was in the downward direction. The DNA has been made visible by the addition of a dye (ethidium bromide) that binds only to DNA and that fluoresces when the gel is illuminated with short-wavelength ultraviolet light.

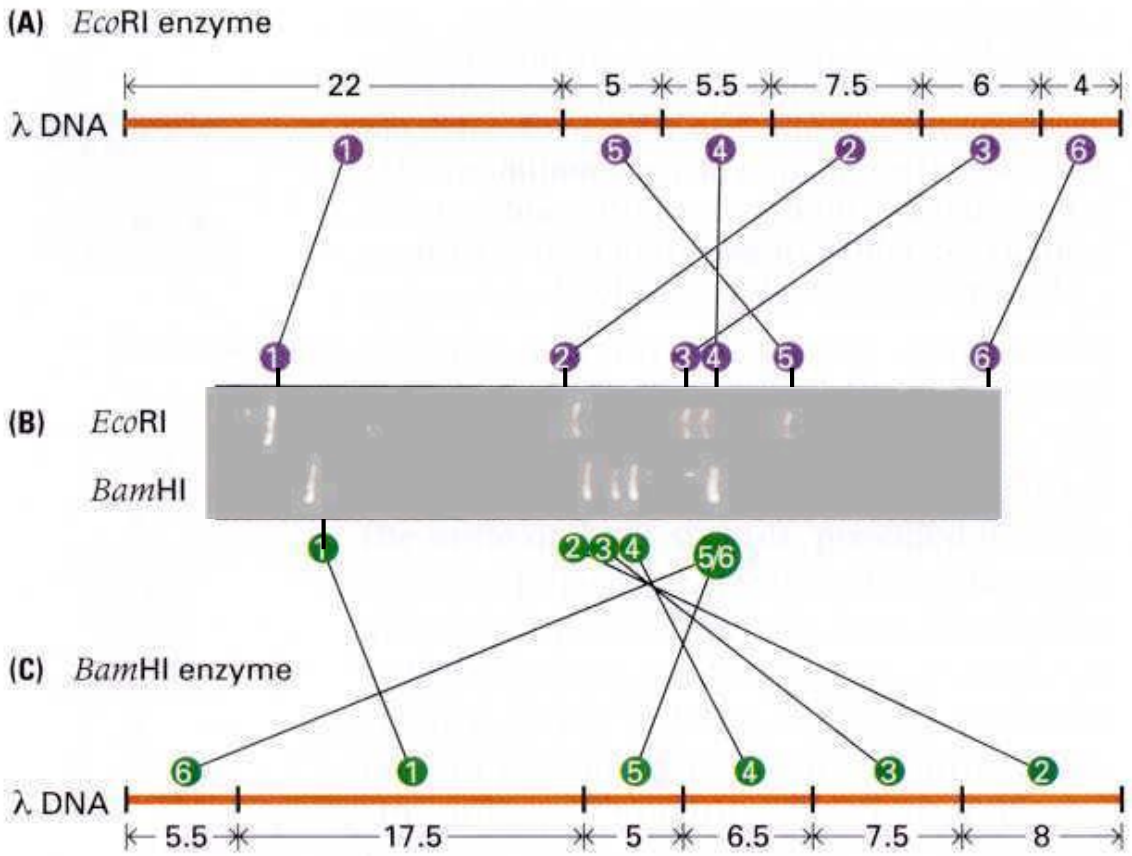


Figure 2.14 Restriction maps of λ DNA for the restriction enzymes (A) *Eco*RI and (C) *Bam*HI. The vertical bars indicate the sites of cutting. The numbers within the arrows are the approximate lengths of the fragments in kilobase pairs (kb). (B) An electrophoresis gel of *Bam*HI and *Eco*RI enzyme digests of λ DNA. Numbers indicate fragments in order from largest (1) to smallest (6); the circled numbers on the maps correspond to the numbers beside the gel. The DNA has not undergone electrophoresis long enough to separate bands 5 and 6 of the *Bam*HI digest.

Note: In Problem 2 at the end of this chapter (Guide to Problem Solving), we show how to use the results of a double digest to determine the particular order of fragments for a pair of restriction enzymes.

Pulsed Field Gel Electrophoresis

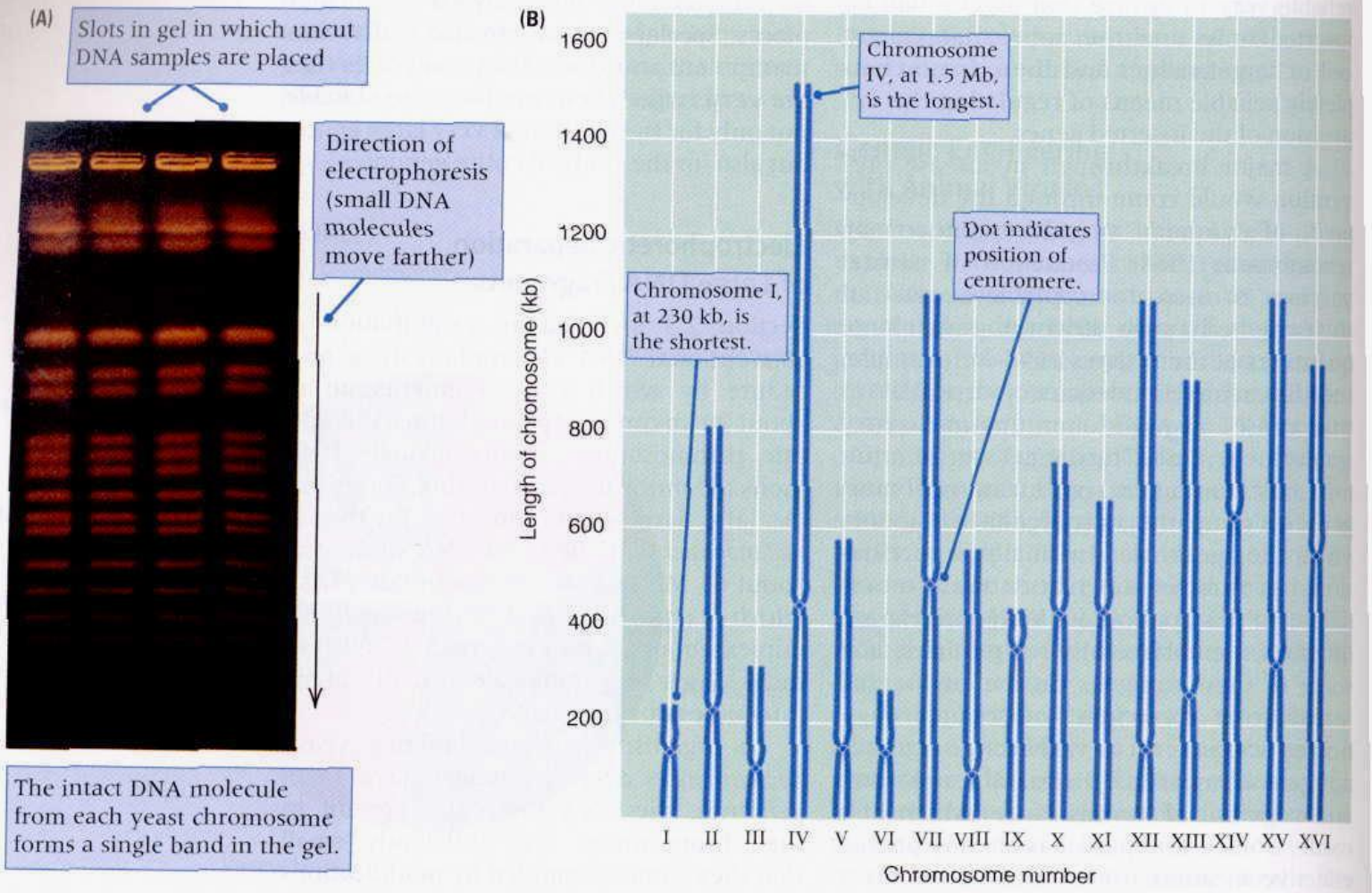


Figure 13.23 (A) Separation of chromosomes of the yeast *Saccharomyces cerevisiae* by pulsed-field gel electrophoresis, in which there is regular change in the orientation of the electric field. (B) Histogram of sizes of the 16 yeast chromosomes. [©1988 BioRad Laboratories, Inc.; permission to use this image has been granted by Bio-Rad Laboratories Inc.]

Nucleic Acid Hybridization

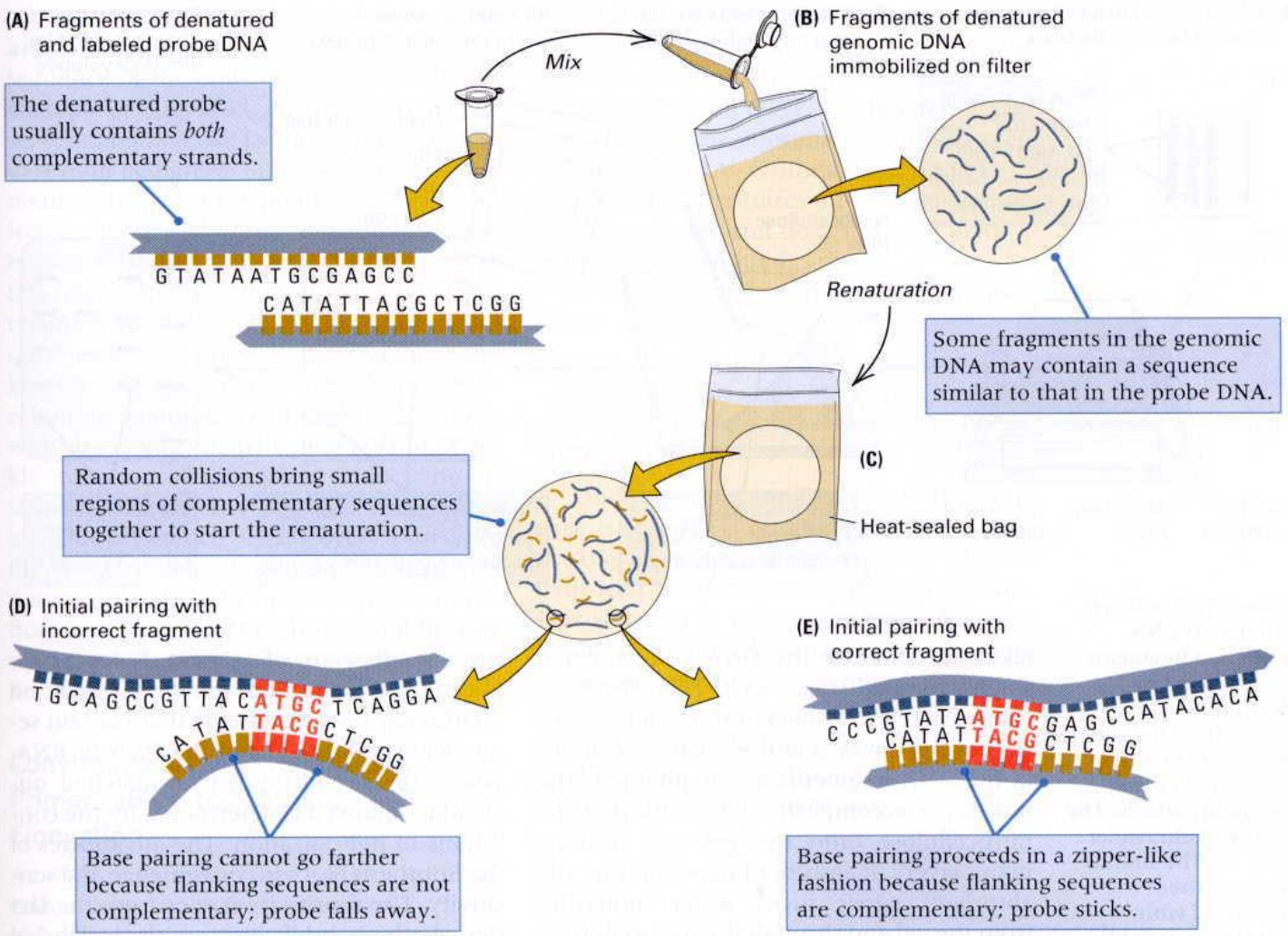
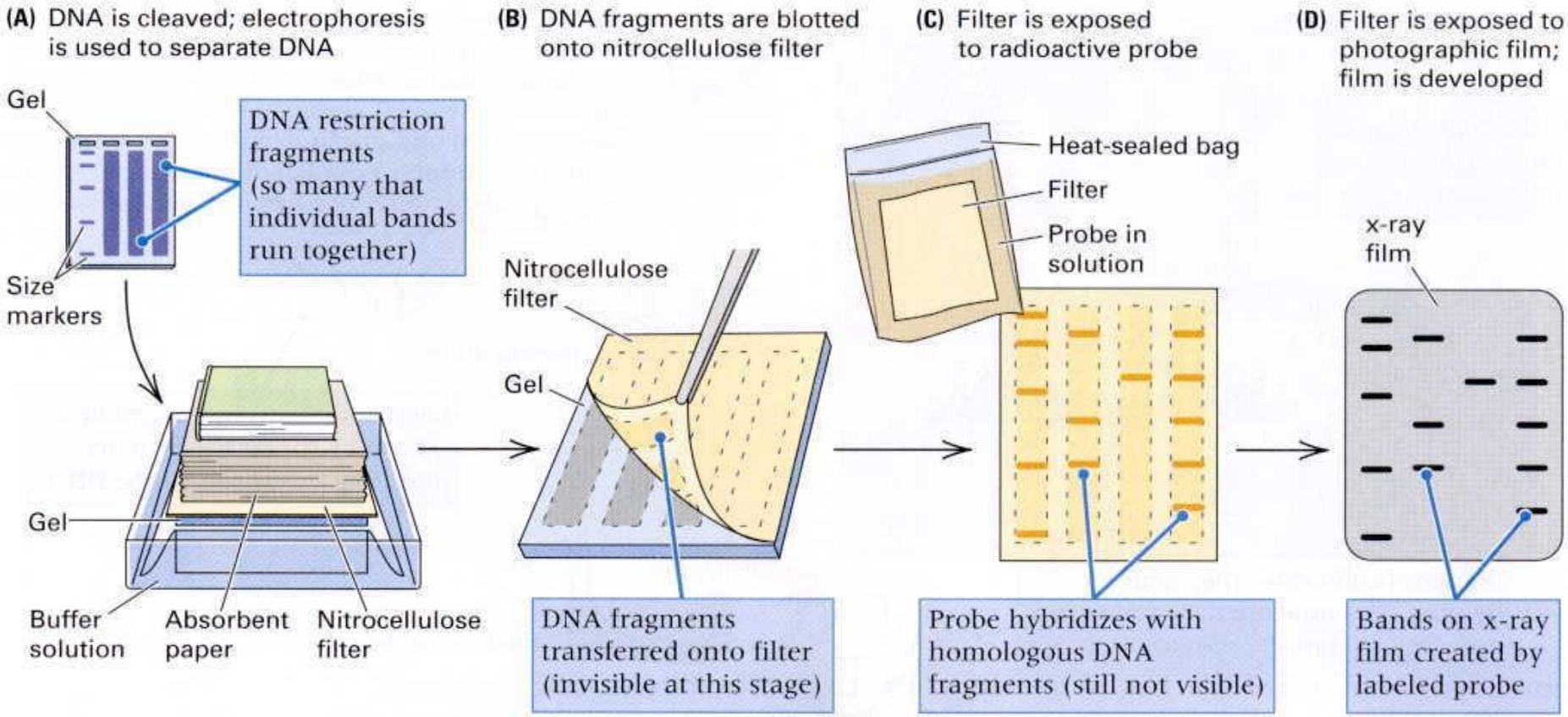


Figure 2.16 Nucleic acid hybridization. (A) Duplex molecules of probe DNA (obtained from a clone) are denatured and (B) placed in contact with a filter to which is attached denatured strands of genomic DNA. (C) Under the proper conditions of salt concentration and temperature, short complementary stretches come together by random collision. (D) If the sequences flanking the paired region are not complementary, then the pairing is unstable and the strands come apart again. (E) If the sequences flanking the paired region are complementary, then further base pairing stabilizes the renatured duplex.

Nucleic Acid Hybridization



Southern Blot → analysis of (genomic) DNA

Northern Blot → analysis of mRNA populations separated on denaturing gels

Restriction site Polymorphism

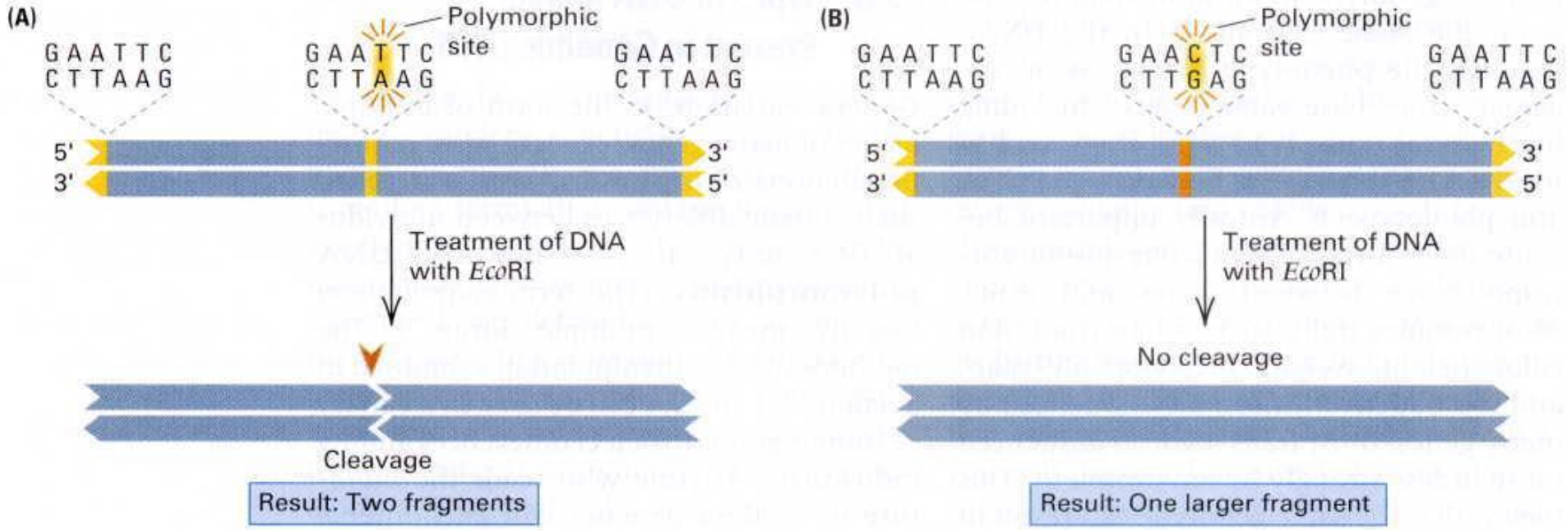
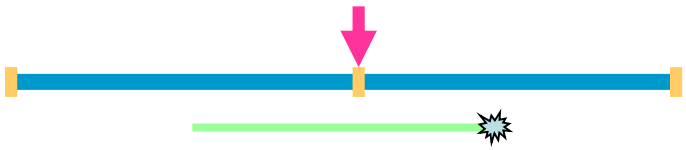
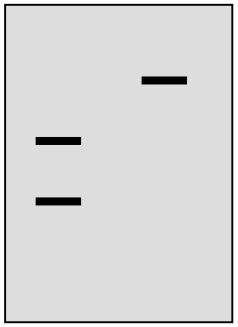


Figure 2.23 A minor difference in the DNA sequence of two molecules can be detected if the difference eliminates a restriction site. (A) This molecule contains three restriction sites for *Eco*RI, including one at each end. It is cleaved into two fragments by the enzyme. (B) This molecule has an altered *Eco*RI site in the middle, in which 5'-GAATTC-3' becomes 5'-GAACTC-3'. The altered site cannot be cleaved by *Eco*RI, so treatment of this molecule with *Eco*RI results in one larger fragment.

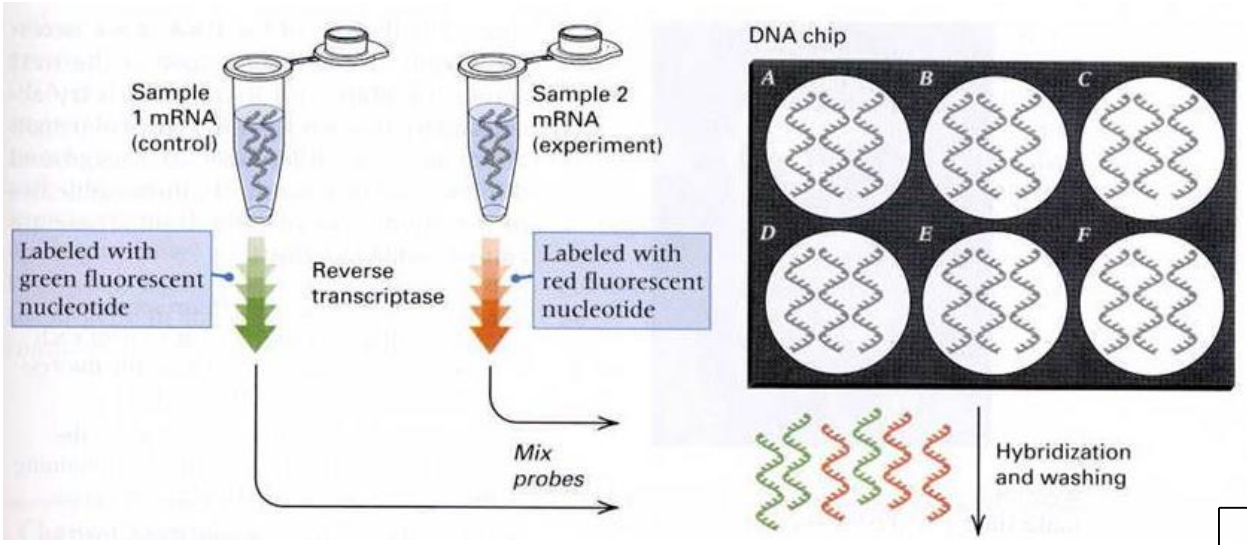


labelled probe



DNA Microarrays

(DNA Chips)



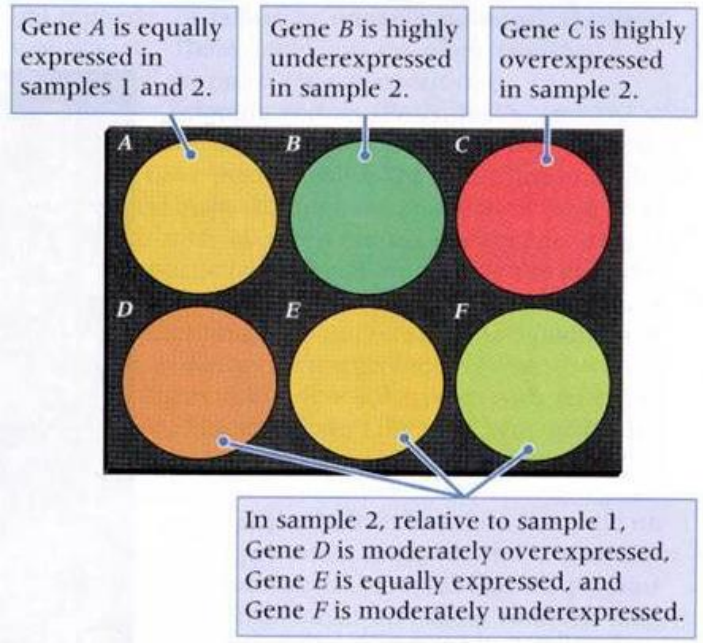
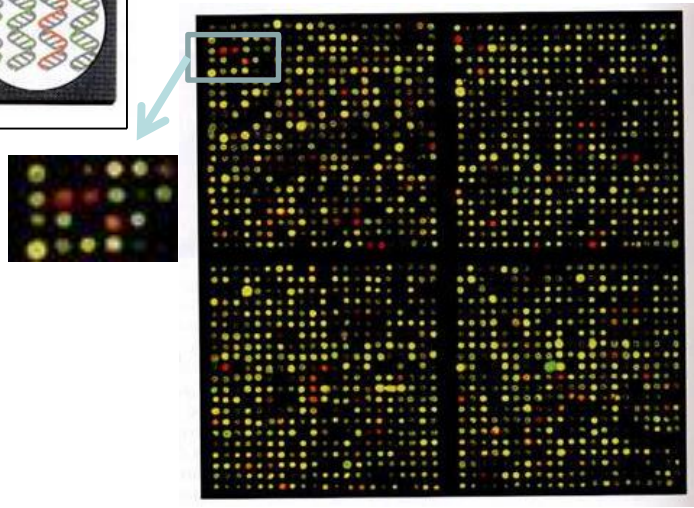


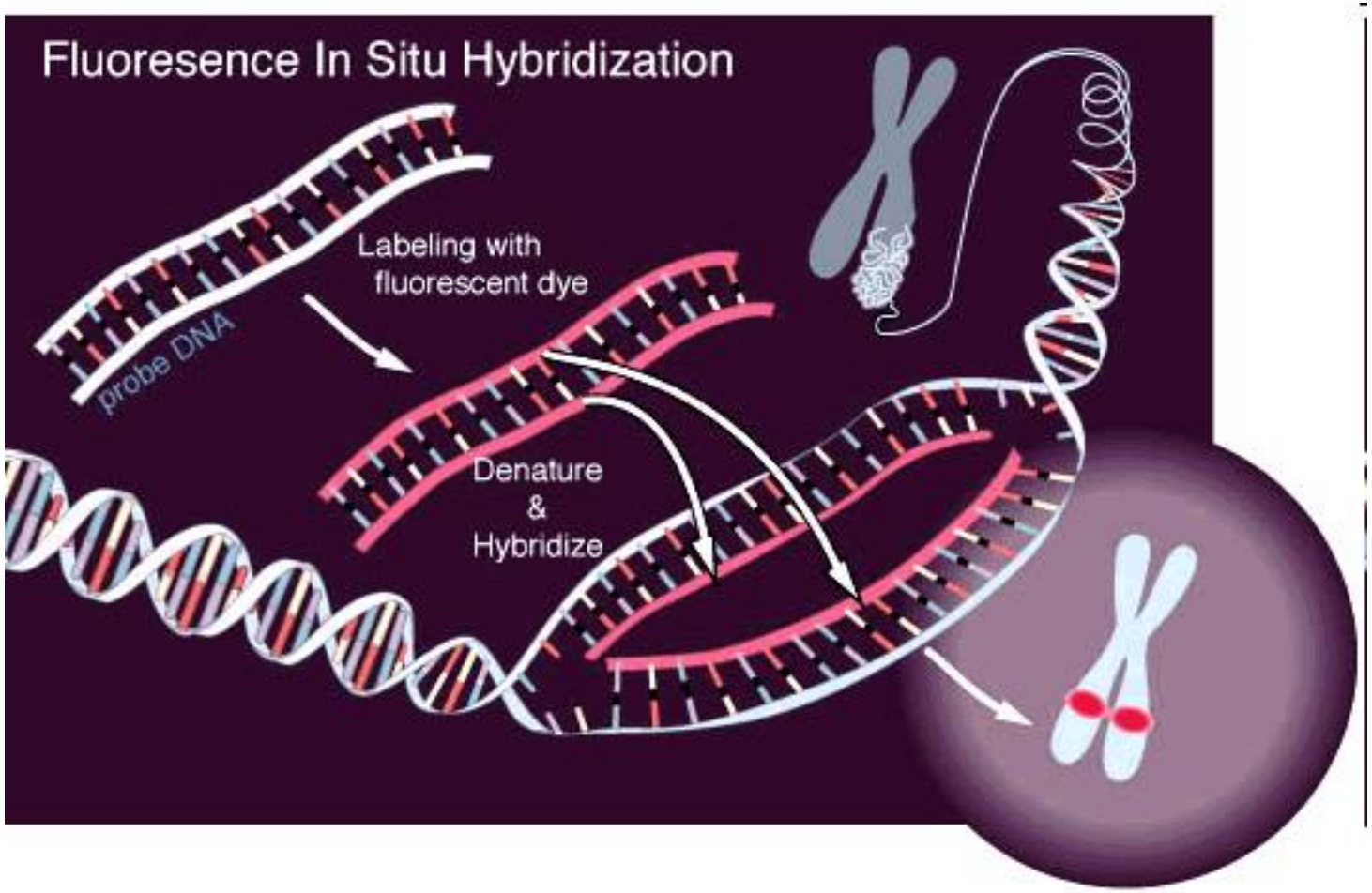
Figure 13.30 Principle of operation of one type of DNA chip. At the top are dried microdrops, each of which contains immobilized DNA strands from a different gene (*A*–*F*). These are hybridized with a mixture of fluorescence-labeled DNA samples obtained by reverse transcription of cellular mRNA. Competitive hybridization of red (experimental) and green (control) label is proportional to the relative abundance of each mRNA species in the samples. The relative levels of red and green fluorescence of each spot are assayed by microscopic scanning and displayed as a single color. Red or orange indicates overexpression in the experimental sample, green or yellow-green underexpression in the experimental sample, and yellow equal expression.

Figure 13.31 Small part of a yeast DNA chip showing 1764 spots, each specific for hybridization with a different mRNA sequence. The color of each spot indicates the relative level of gene expression in experimental and control samples. The complete chip for all yeast open reading frames includes over 6200 spots. [Courtesy of Jeffrey P. Townsend, Duccio Cavalieri, and the Harvard Center for Genomics Research.]



In situ Hybridization

FISH - (Fluoresence In Situ Hybridization)



www.accessexcellence.org/RC/VL/GG/fish.php

PCR
Polymerase Chain Reaction

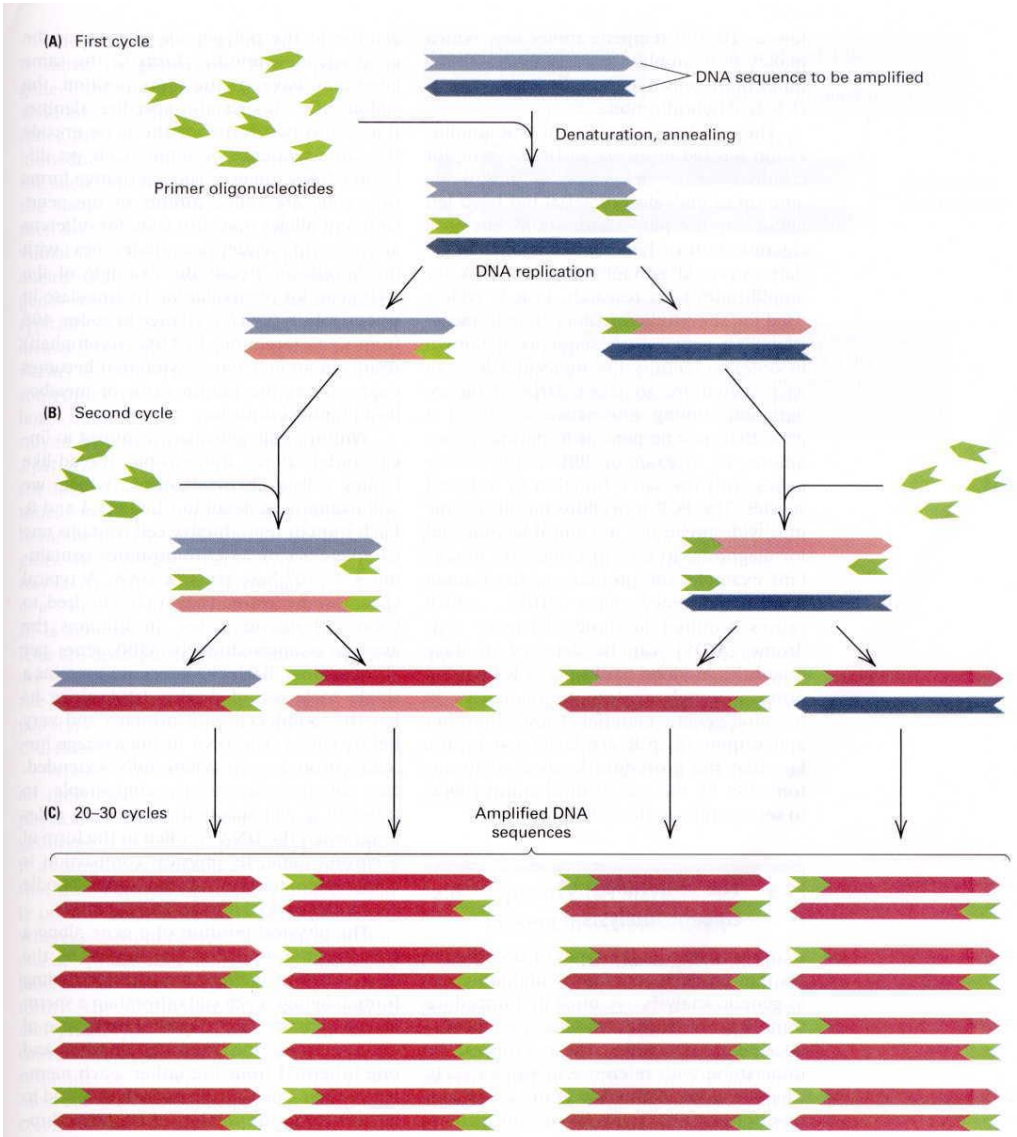
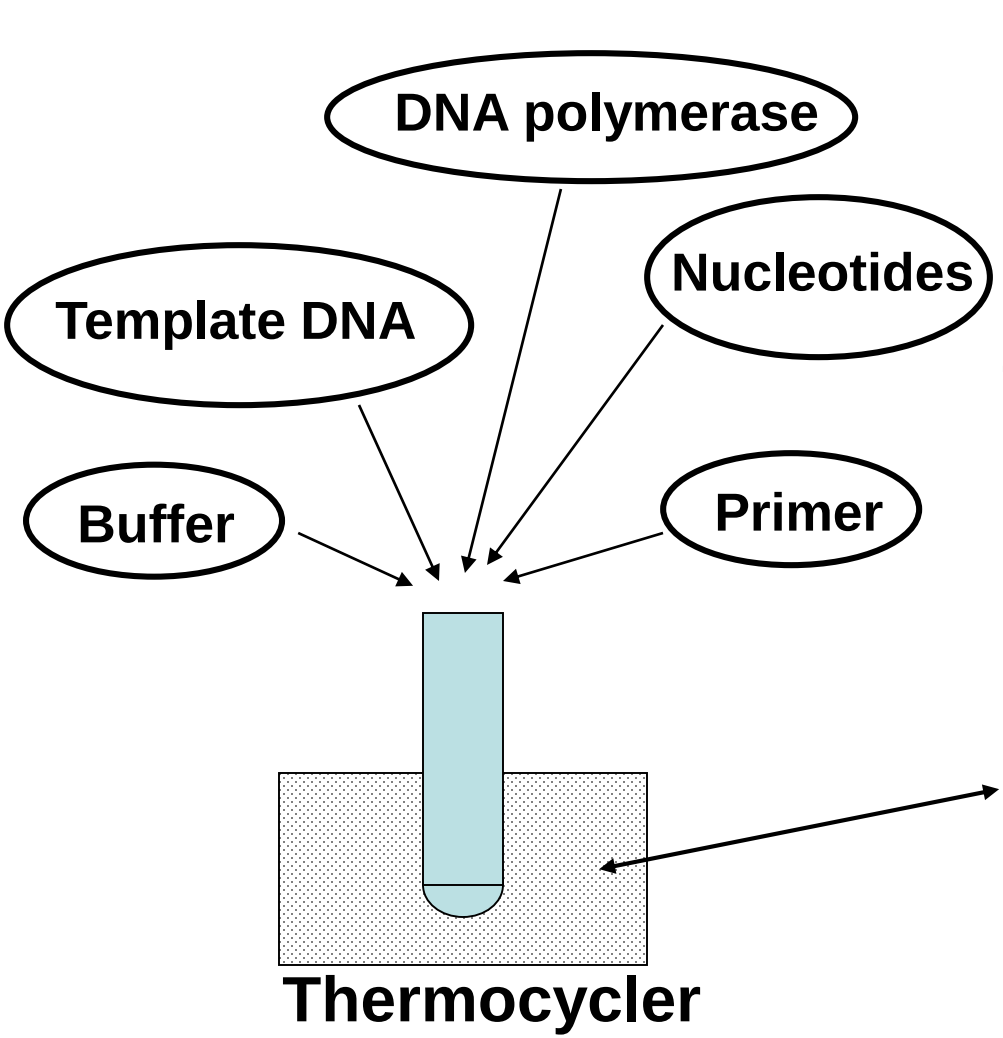
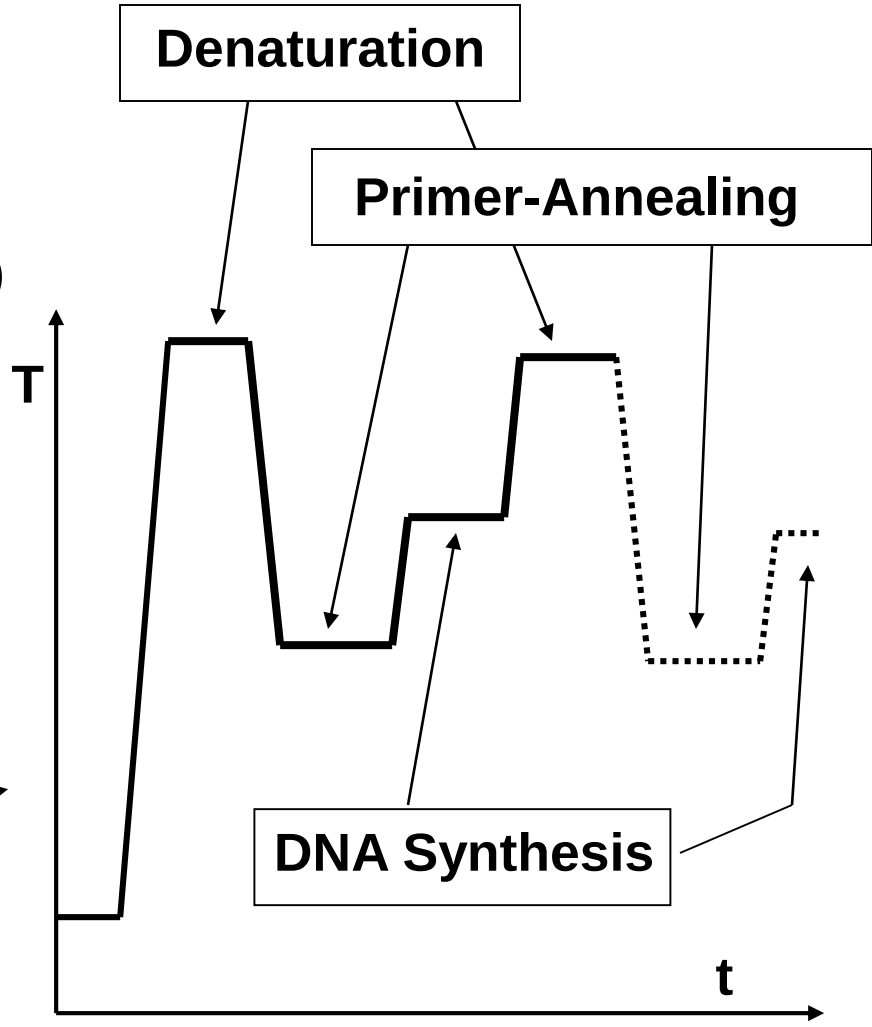


Figure 2.21 Polymerase chain reaction (PCR) for amplification of particular DNA sequences. Only the region to be amplified is shown. Oligonucleotide primers (green) that are complementary to the ends of the target sequence (blue) are used in repeated rounds of denaturation, annealing, and DNA replication. Newly replicated DNA is shown in pink. The number of copies of the target sequence doubles in each round of replication, eventually overwhelming any other sequences that may be present.

Amplification of DNA between two primers

PCR


DNA polymerase
Template DNA
Nucleotides
Buffer
Primer
Thermocycler


Denaturation
Primer-Annealing
T
DNA Synthesis
t

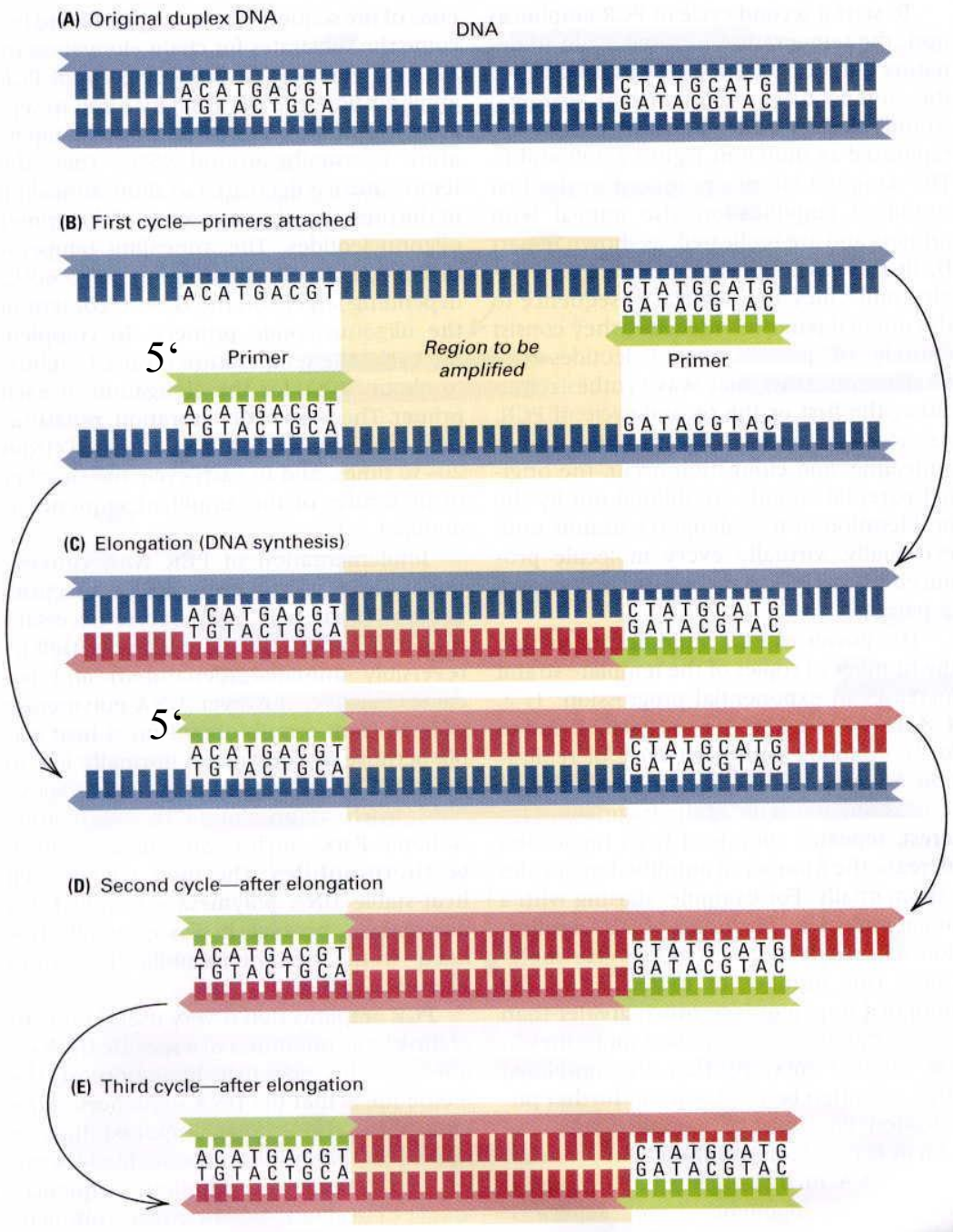


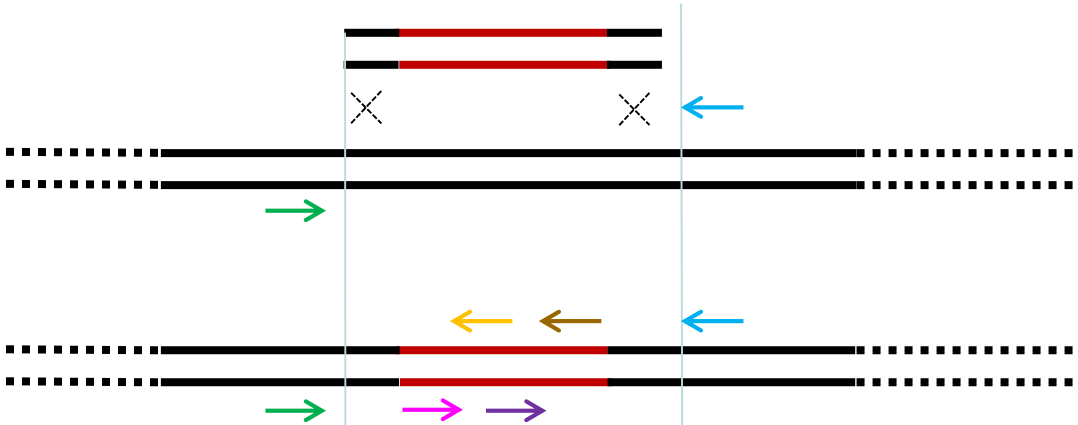
Figure 2.20 Role of primer sequences in PCR amplification. (A) Target DNA duplex (blue), showing sequences chosen as the primer-binding sites flanking the region to be amplified. (B) Primer (green) bound to denatured strands of target DNA. (C) First round of amplification. Newly synthesized DNA is shown in pink. Note that each primer is extended *beyond* the other primer site. (D) Second round of amplification (only one strand shown); in this round, the newly synthesized strand terminates at the opposite primer site. (E) Third round of amplification (only one strand shown); in this round, both strands are truncated at the primer sites. Primer sequences are normally about twice as long as shown here.

PCR
Polymerase Chain Reaction

Amplification of DNA between two primers

Analysis of genomic constellations by PCR

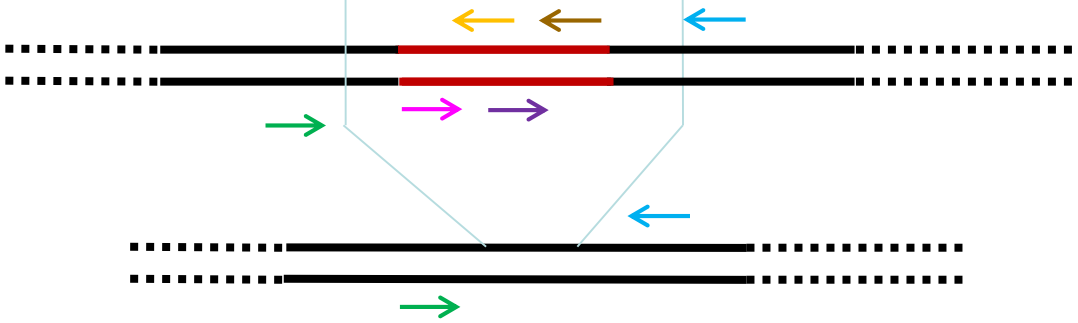
Example: Integration of a DNA fragment



PCR Product formation

→ ←	→ ←	→ ←	→ ←
+	-	-	-
+	+	+	+

Example: Deletion of a DNA fragment



→ ←	→ ←	→ ←	→ ←
+ large	+	+	+
+ small	-	-	-

Real Time PCR -- qPCR

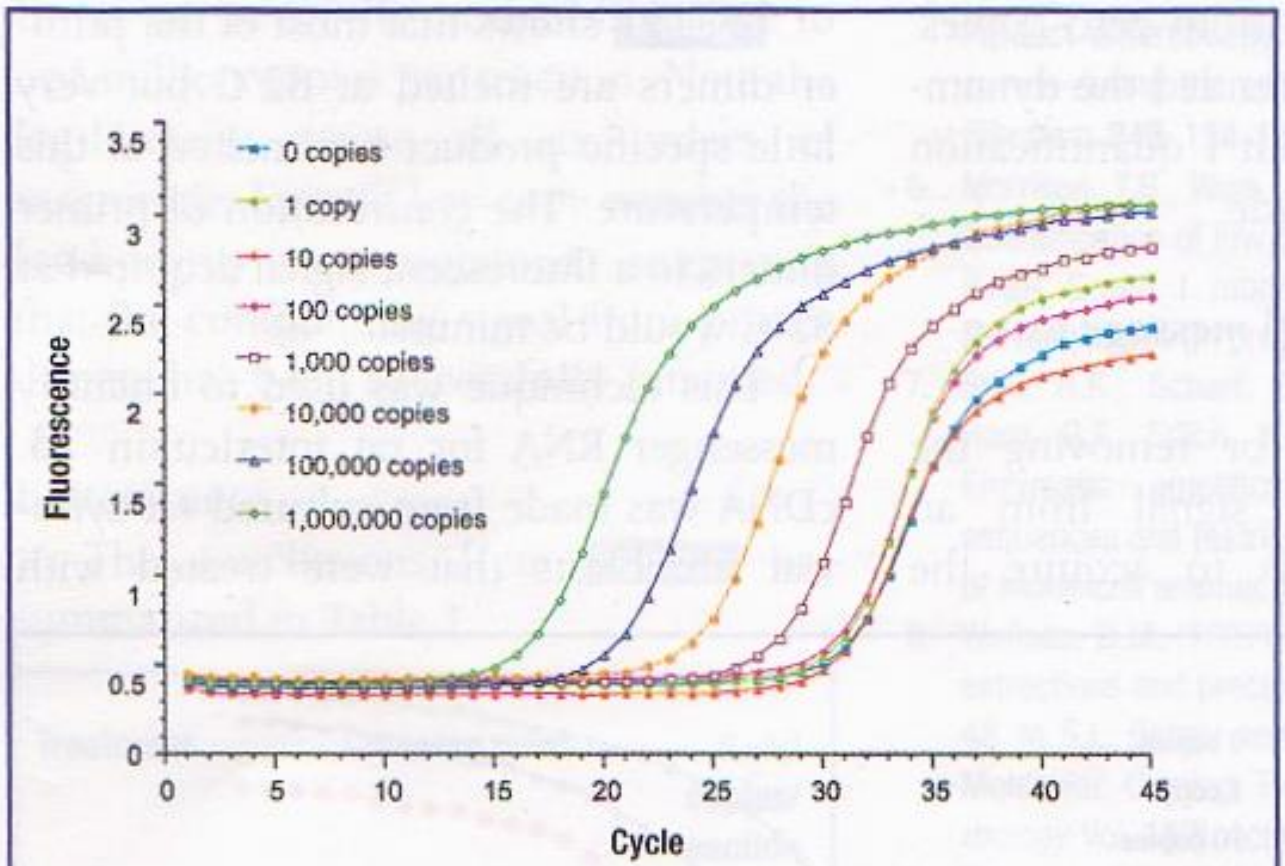


Figure 1 **Amplification from zero to one million copies of a fragment of the human β-globin gene.** All samples contained SYBR Green I. The reactions from one million to one thousand copies are easily distinguished. Below 1000 copies the reactions begin to overlap due to the predominance of primer dimers and non-specific PCR products at these lower copy numbers.

SYBR Green Incorporation into dsDNA

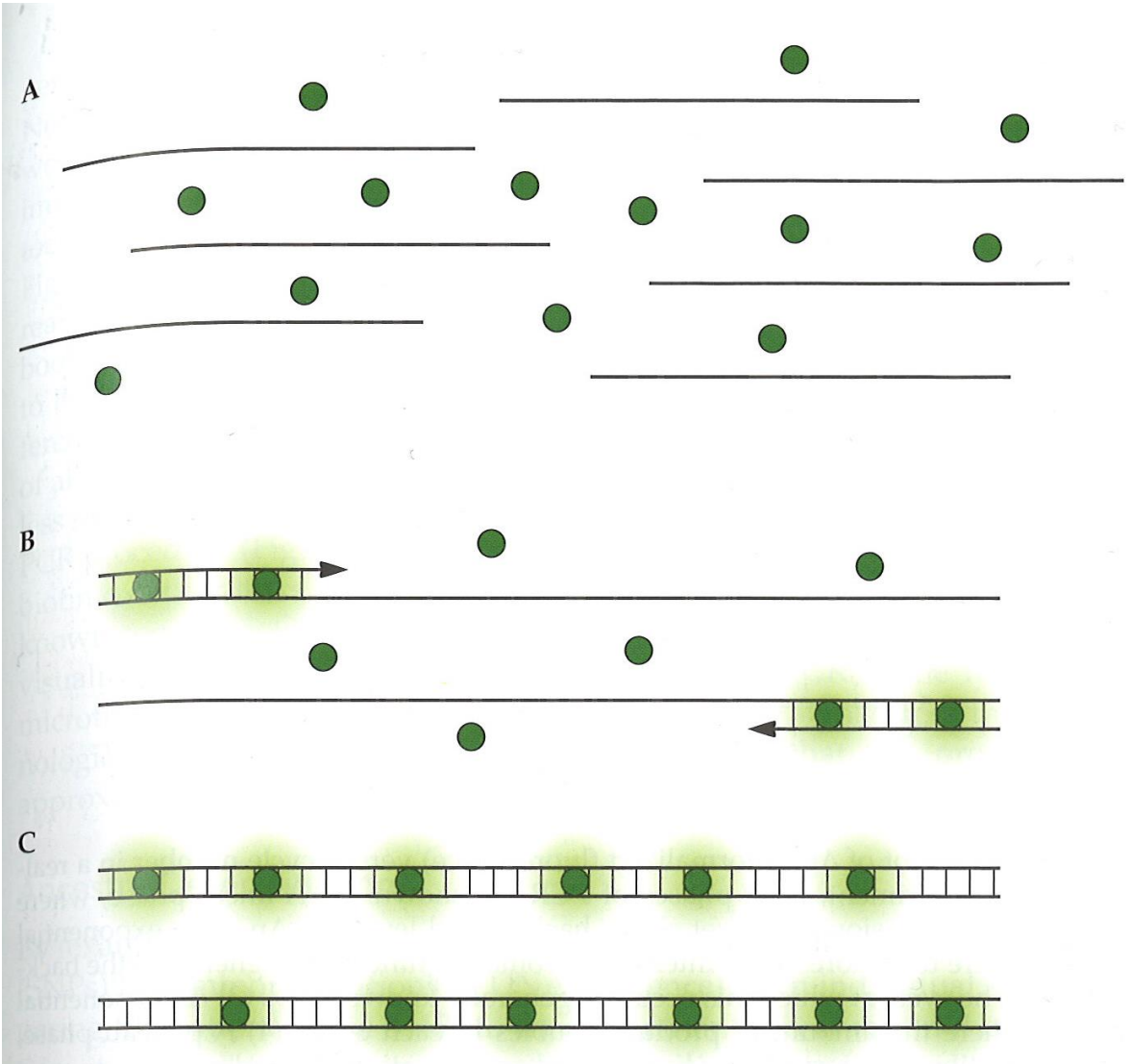


FIGURE 9.20 The fluorescent dye SYBR green does not bind to single-stranded DNA **(A)**, binds to double-stranded DNA as it is synthesized **(B)**, and is bound to the double-stranded amplified DNA **(C)**. Only the bound DNA fluoresces.

Random **A**mplified **P**olymorphic **D**NA

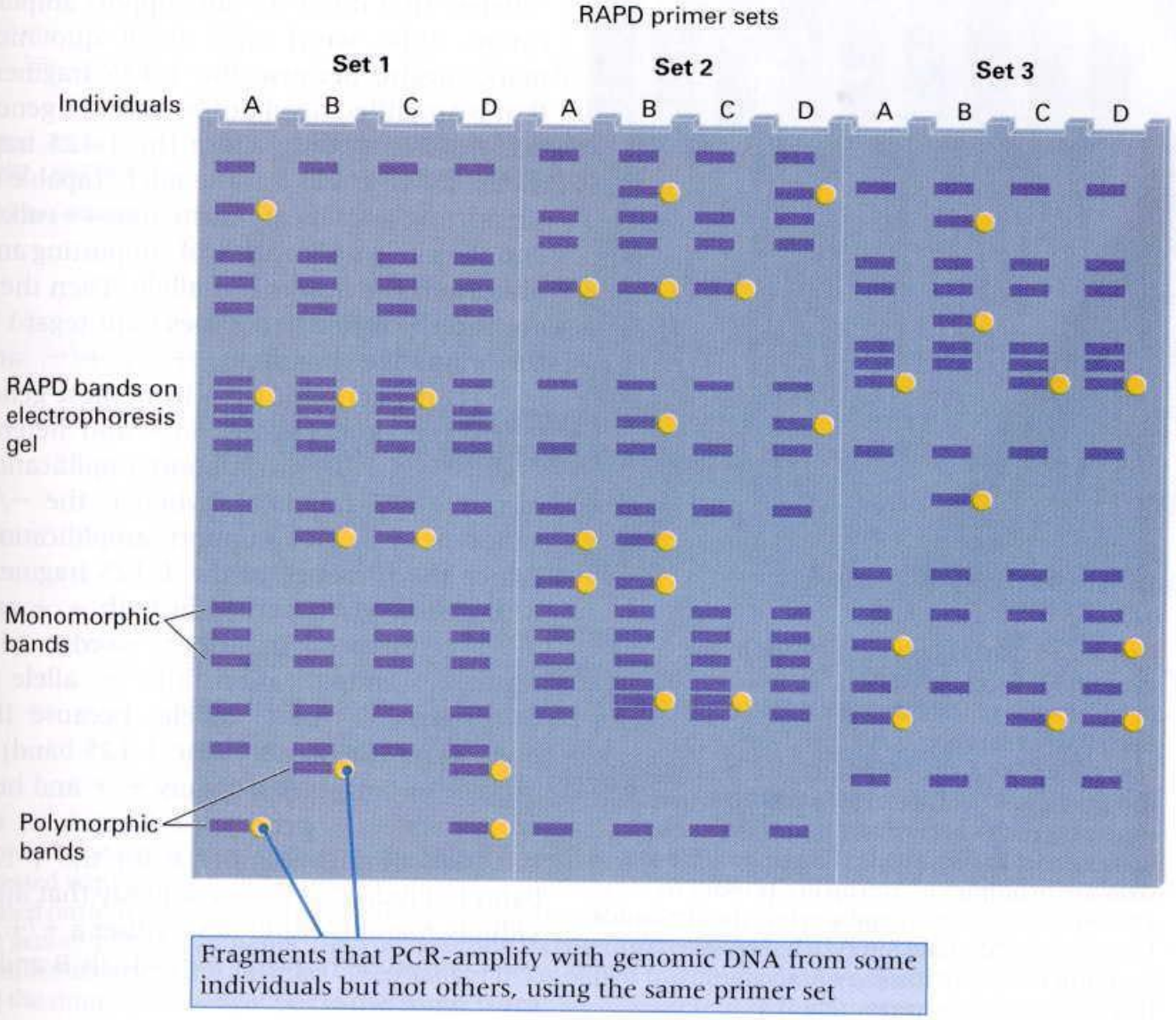
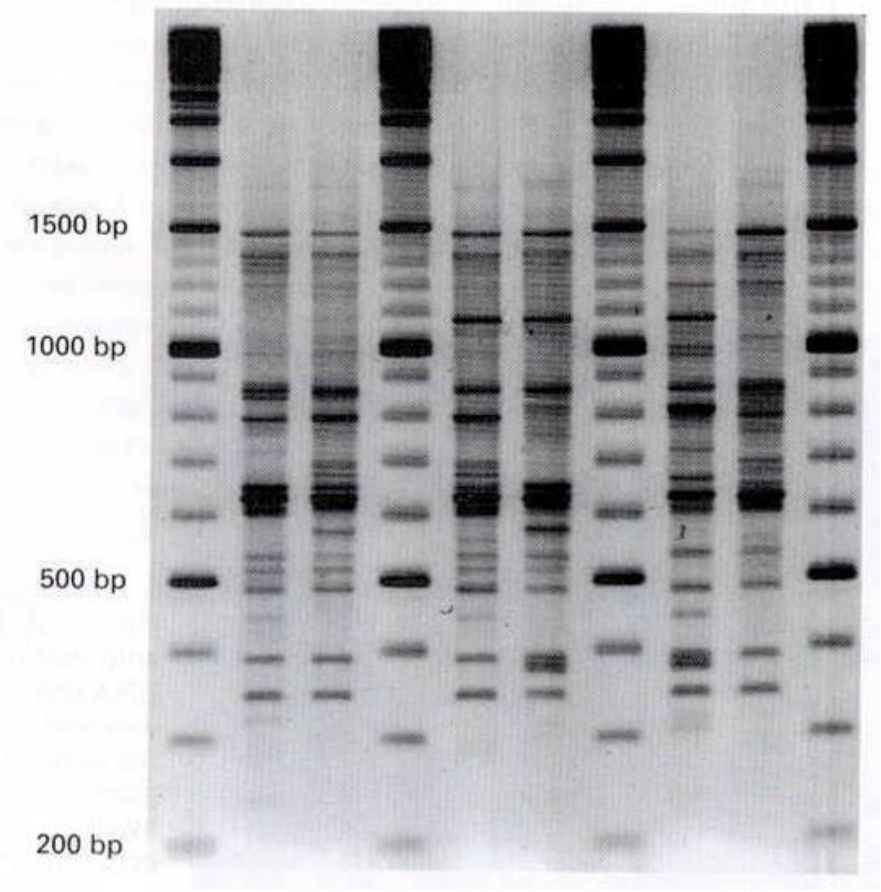


Figure 2.25 Random amplified polymorphic DNA (RAPD) is detected through the use of relatively short primer sequences that, by chance, match genomic DNA at multiple sites that are close enough together to support PCR amplification. Genomic DNA from a single individual typically yields many bands, only some of which are polymorphic in the population. Different sets of primers amplify different fragments of genomic DNA.

Random **A**mplified **P**olymorphic **D**NA



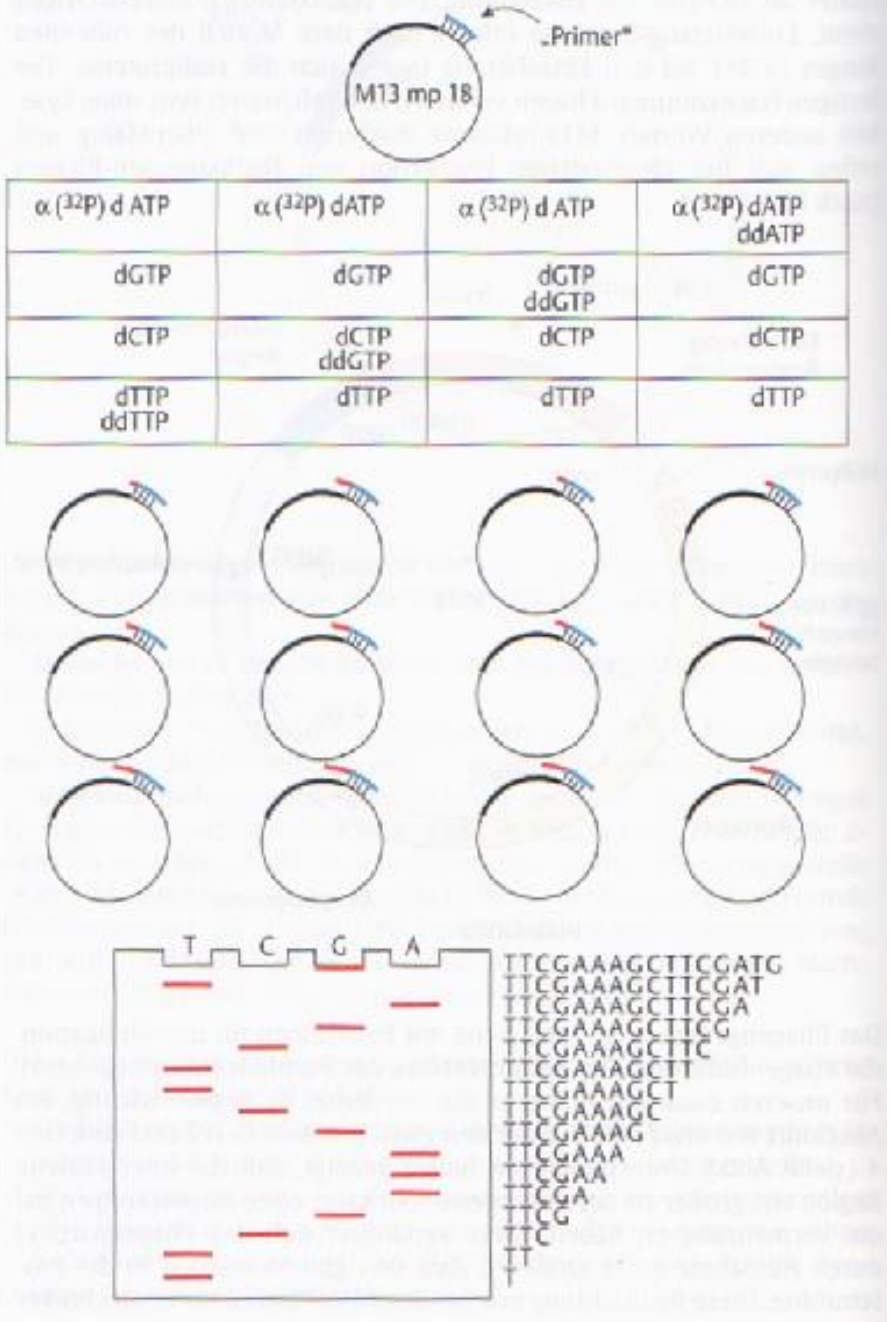
→ Short Primers
→ Bind to many sequences within a genome
→Set of amplified fragments depending on sequence # defined priming sites

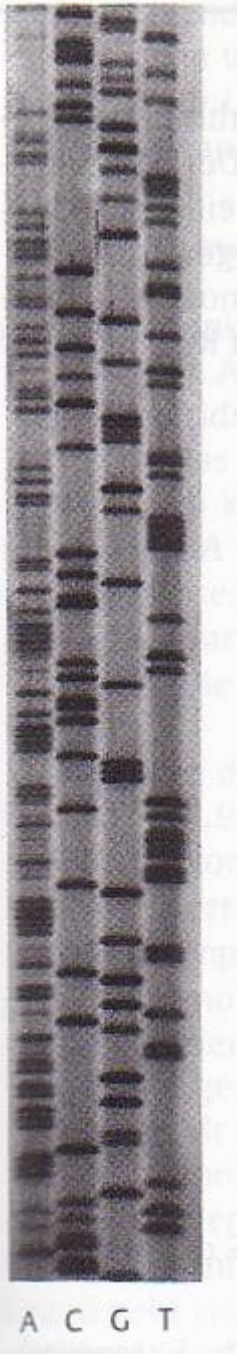
Figure 2.26 RAPD polymorphisms in the stoneroller fish (*Campostoma anomalum*) trapped in tributaries of the Great Miami River in Ohio. Each pair of samples is flanked by a lane containing DNA size standards; in these lanes, the smallest DNA fragment is 100 base pairs (bp), and each successively larger fragment increases in size by 100 bp. Fragments whose sizes are multiples of 500 bp are present in greater concentration and so yield darker bands. [Courtesy of Michael Simonich, Manju Garg, and Ana Braam (Pathology Associates International, Cincinnati, Ohio).]

DNA Sequencing

Abb. 9.15 Prinzip der Sequenz-Reaktion nach der Didesoxy- oder Kettenabbruch-Methode.

- Vier Ansätze werden parallel vorbereitet. Jeder Ansatz enthält ein radioaktiv oder anders markiertes Nucleotid (hier α-[^{32}P]-dATP) und die drei anderen nichtmarkierten Desoxynucleosid-Triphosphate. Jeder Ansatz enthält zudem ein Didesoxynucleotid, entweder ddTTP, ddCTP, ddGTP oder ddATP. Je nach experimenteller Situation wird ein Verhältnis von 1/50, 1/100 oder 1/200 von ddNTP zu dNTP gewählt.
- Nach Zusatz der DNA-Polymerase beginnt die Komplementärstrang-Synthese. Sie kommt zum Halt, wenn zufällig ein Didesoxynucleotid statt des normalen Desoxynucleotids in das aktive Zentrum der DNA-Polymerase gelangt. Im ersten Ansatz wird das der Fall sein, wenn die Sequenz des Matrizenstranges den Einbau eines Thymin-Nucleotids verlangt. Mit anderen Worten, im ersten Ansatz erhält man eine Kollektion von DNA-Fragmenten, deren Längen die Positionen von Adenin-Resten im Matrizen-Strang wiedergeben. Entsprechendes gilt für die Längen der Syntheseprodukte in den anderen Ansätzen.
- Der kritische methodische Vorgang ist die genaue Auftrennung der Syntheseprodukte. Dazu werden Matrize und synthetisierte Komplementärstränge durch Denaturierung voneinander gelöst und auf dünnen, besonders zubereiteten Polyacrylamid-Gelen analysiert. Die Markierung der Syntheseprodukte ermöglicht ihre Darstellung durch Autoradiographie oder geeignete Färbemethoden. Die Auswertung der Gel-Elektrophorese ist hier gezeigt. Durch das kleinste, am weitesten gewanderte Fragment wird das erste Nucleotid in der Sequenz angezeigt, durch das nächst größte Fragment das zweite usw. [nach 13].





Next Generation Sequencing – Deep Sequencing

Sequencing platform	ABI3730xl Genome Analyzer	Roche (454) FLX	Illumina Genome Analyzer	ABI SOLiD
Sequencing chemistry	Automated Sanger sequencing	Pyrosequencing on solid support	Sequencing-by-synthesis with reversible terminators	Sequencing by ligation
Template amplification method	In vivo amplification via cloning	Emulsion PCR	Bridge PCR	Emulsion PCR
Read length	700–900 bp	200–300 bp	32–40 bp	35 bp
Sequencing throughput	0.03–0.07 Mb/h	13 Mb/h	25 Mb/h	21–28 Mb/h

Applications:
Genome sequencing, Metagenome Sequencing,
Transcriptome Sequencing (incl. Non-coding RNA)
Gene Expression profiling

www.seas.gwu.edu/~rhyspj/fall09cs144/presentations/sam.ppt

Ilumina

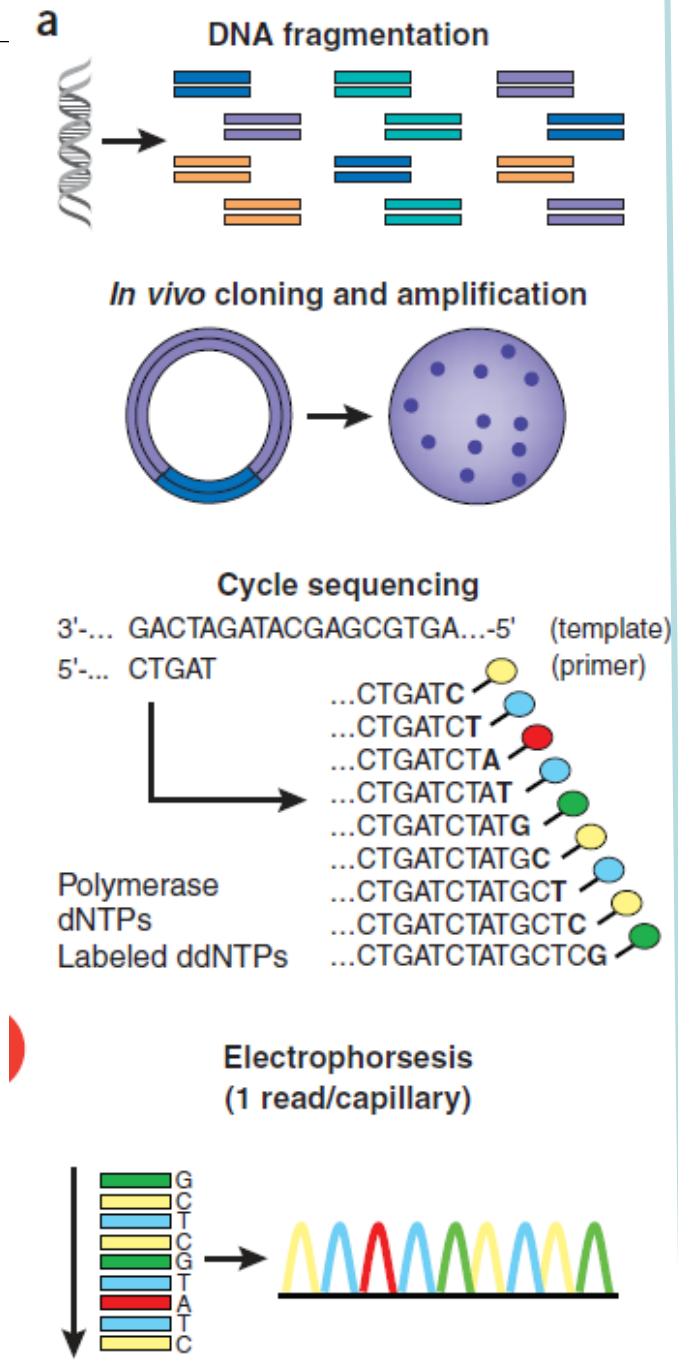
Roche 454 FLX

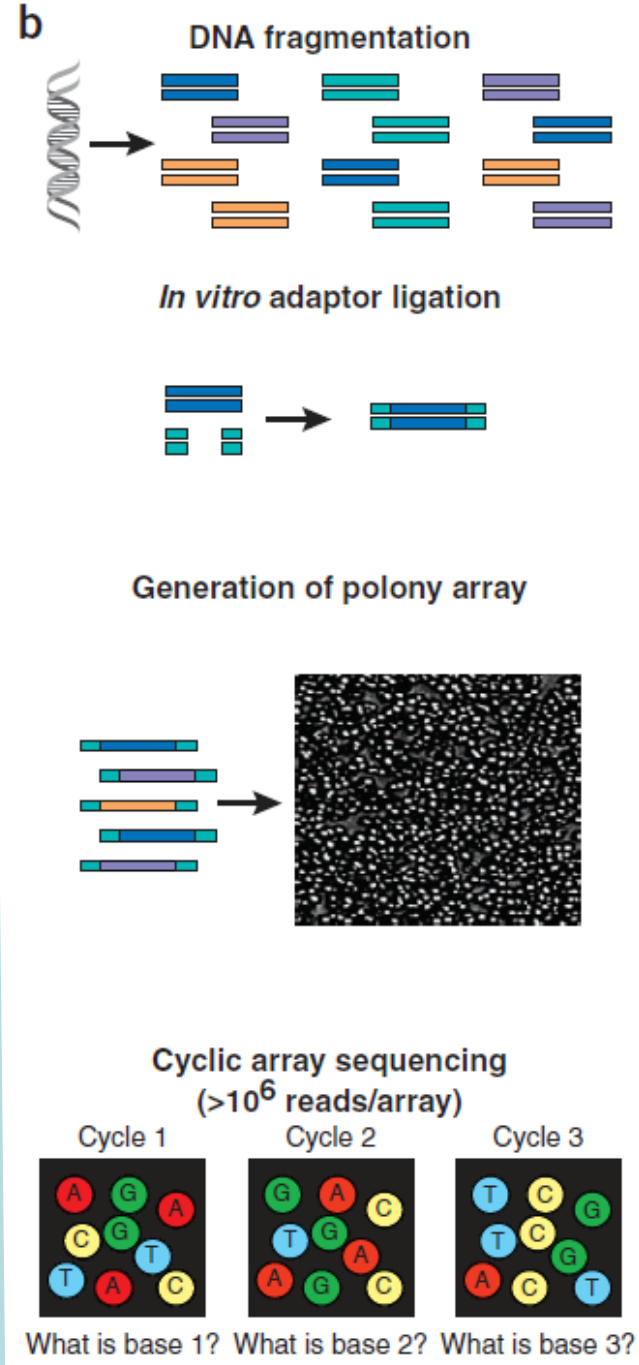
	APPLICATION	HISEQ 2500	MISEQ	PACBIO RS II	GS FLX++	GS JUNIOR+	ION PROTON
GENOME SEQ	De novo sequencing of bacterial & fungal genomes	✓✓	✓✓✓	✓✓	✓✓✓		
	De novo sequencing of higher eukaryotic genomes	✓✓✓		✓	✓		
	Resequencing of genomes	✓✓✓	✓✓✓				
TRANSCRIP-TOME SEQ	De novo transcriptome sequencing	✓✓	✓✓✓		✓✓✓		✓
	Expression profiling	✓✓✓	✓				
EXOME & AMPLICONS	Ultra deep amplicon sequencing	✓	✓✓✓		✓✓✓	✓✓✓	✓✓
	Exome sequencing	✓✓✓	✓✓				✓✓

Our ratings are based on factors such as data output, read length, turnaround time, data quality and cost efficiency.

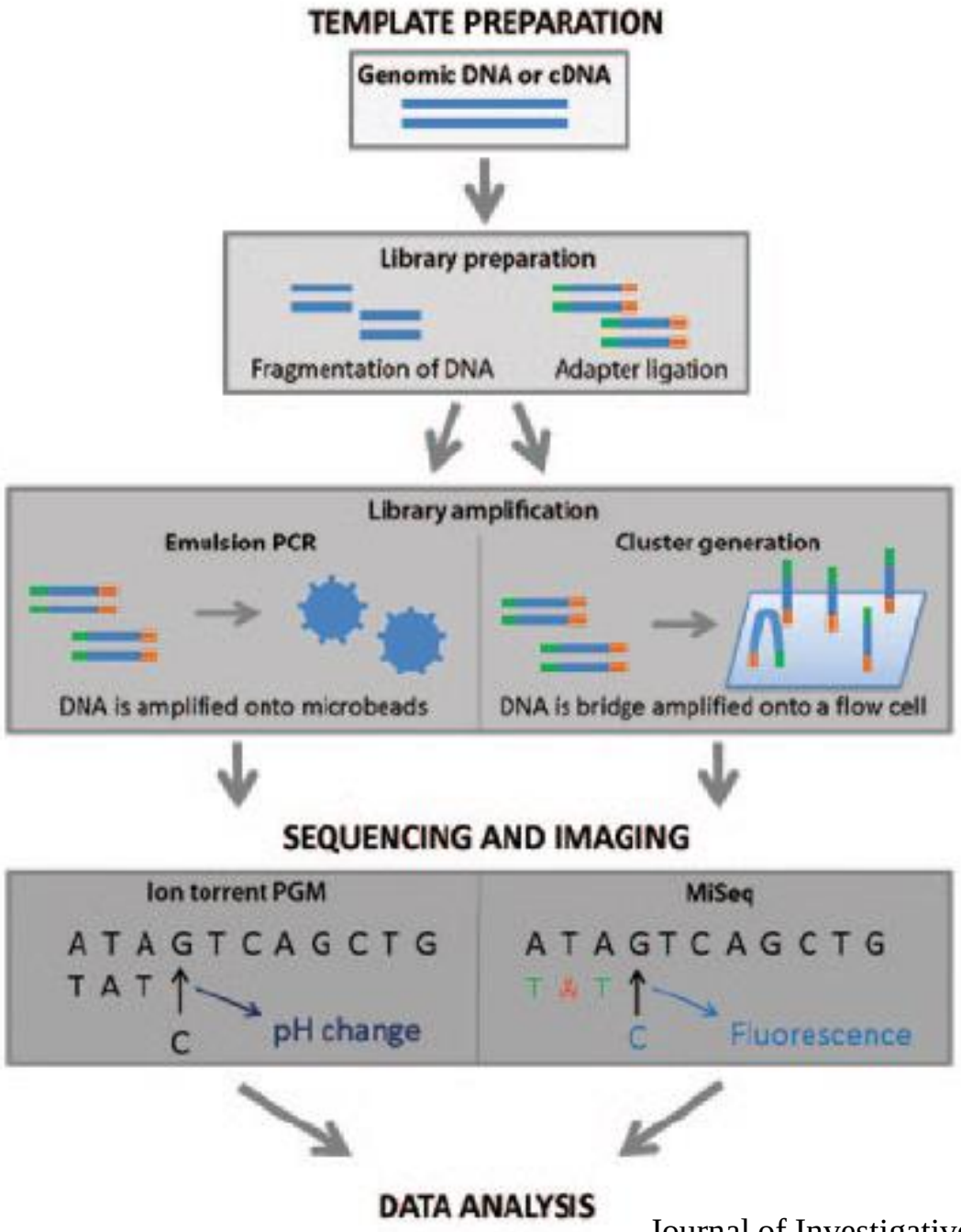
Single-molecule real-time sequencing

Sanger sequencing





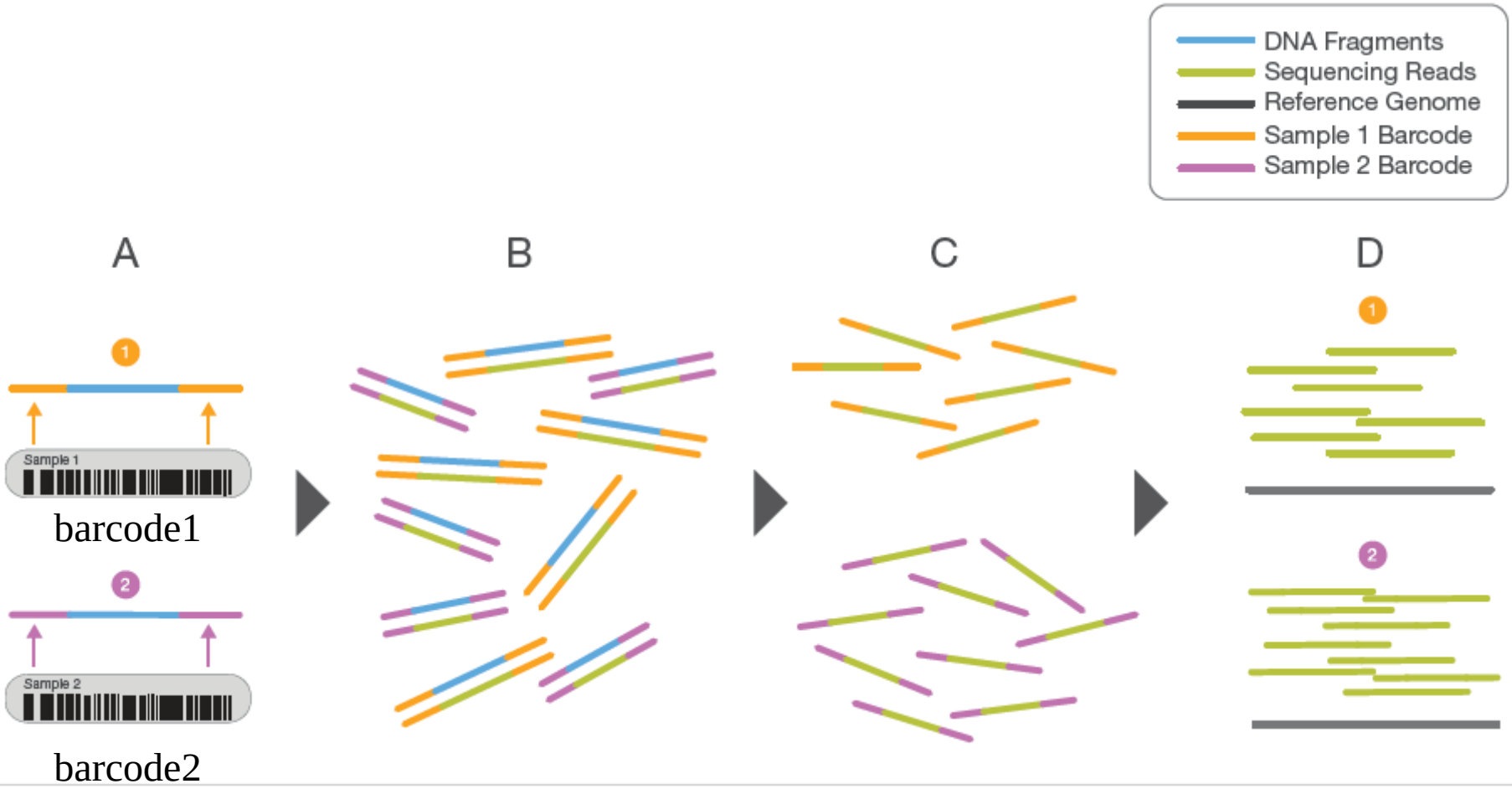
Next generation sequencing



Next generation sequencing

Journal of Investigative Dermatology (2013), Volume 133

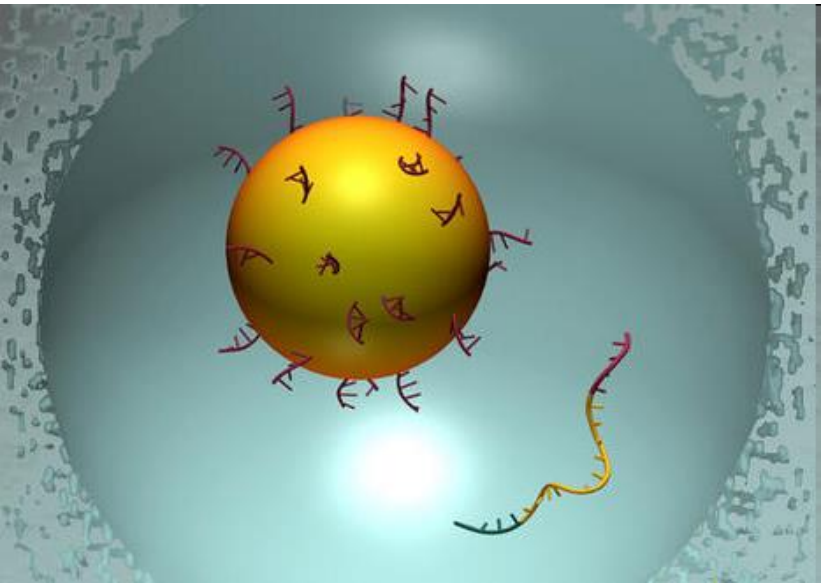
Sample multiplexing



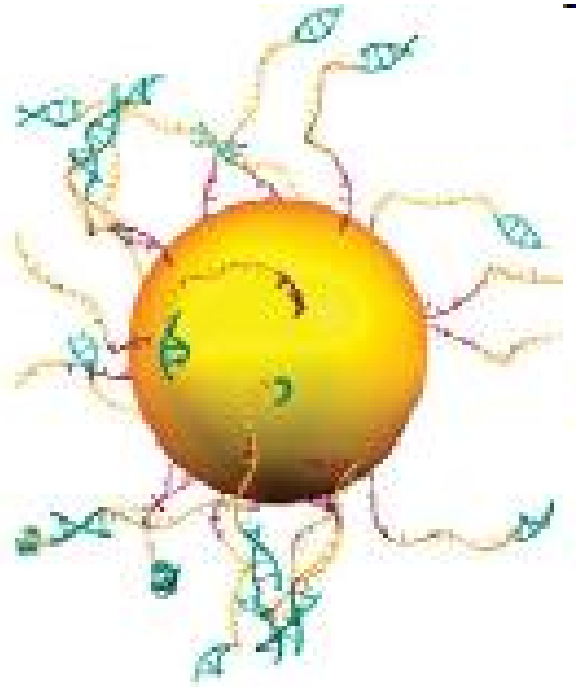
Codes defined by sequence of adaptor

http://www.illumina.com

The Roche 454/GS FLX Sequencing Technology

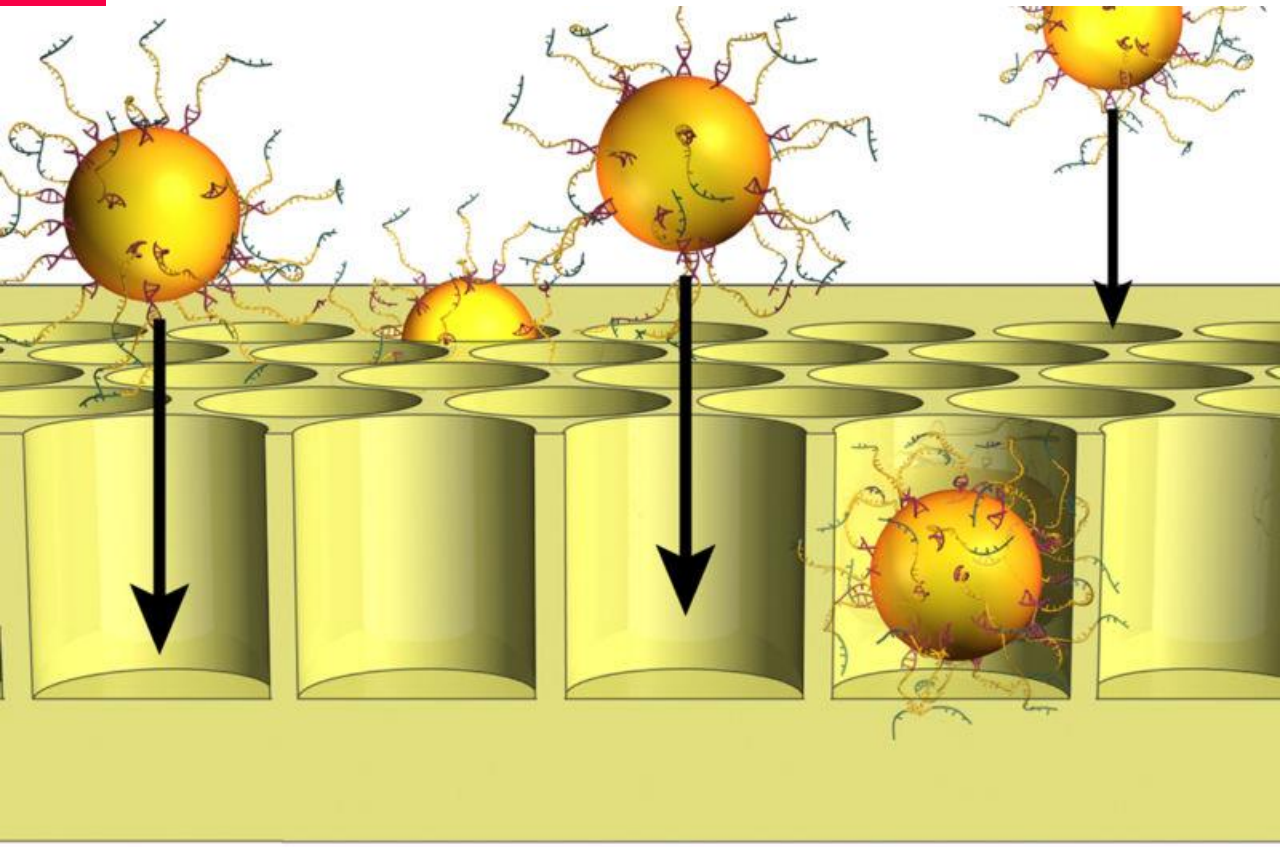


Aided by the adaptors individual fragments are captured on their own unique beads. A bead and the bound fragment together with a water-in-oil emulsion form a microreactor so that each fragment can be amplified without contamination via the so called emulsion PCR (emPCR). The entire fragment collection is amplified in parallel.



The emPCR amplifies each fragment several million times. After amplification the emulsion shell is broken and the clonally amplified beads are ready for loading onto the fibre-optic PicoTiterDevice for sequencing

Pyro-Sequencing – 454 Technology



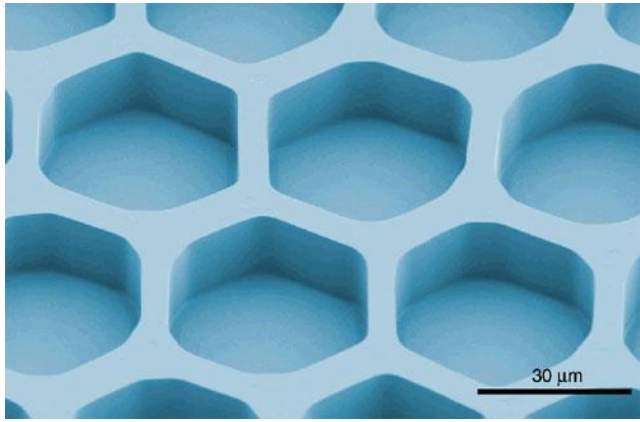
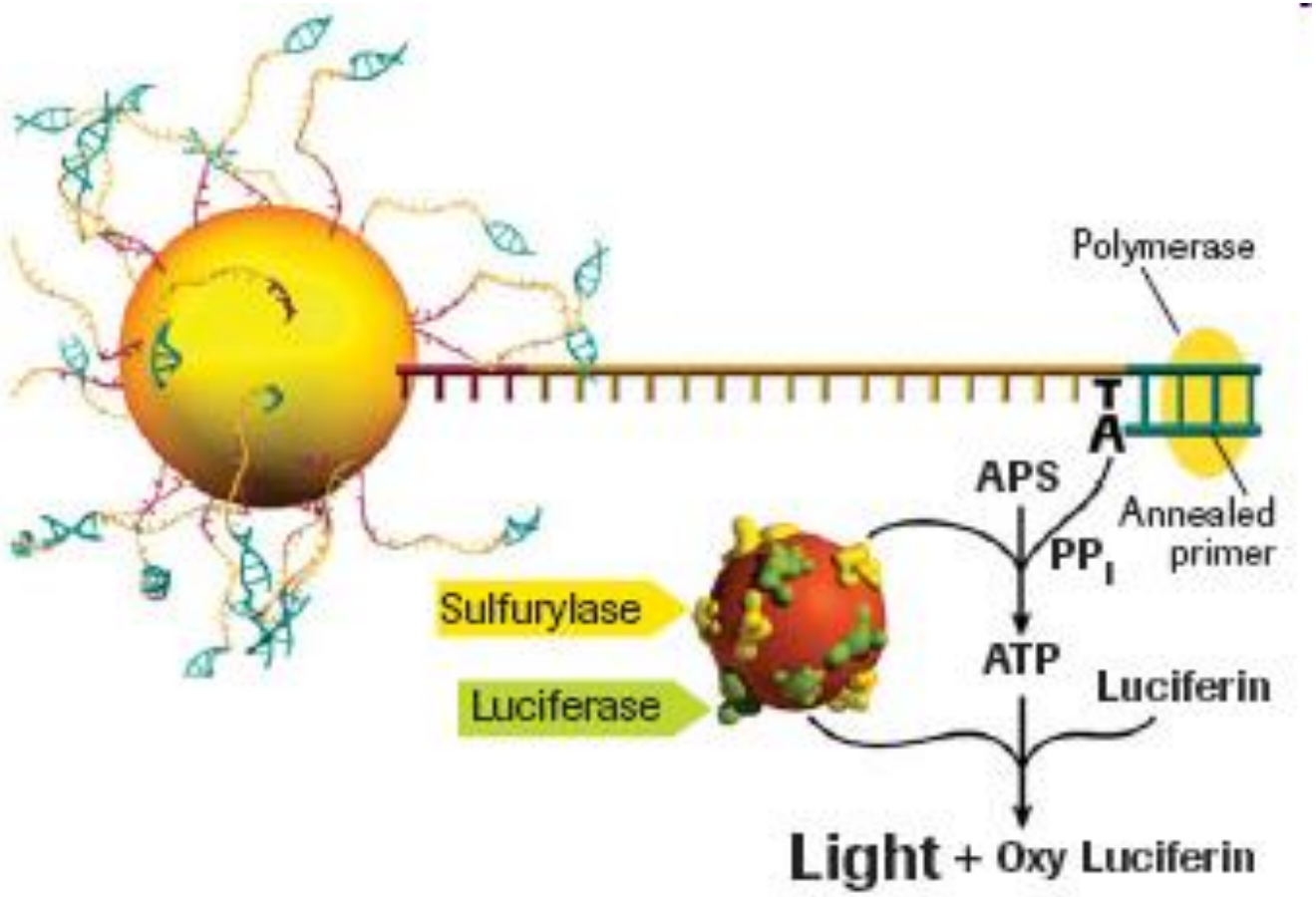


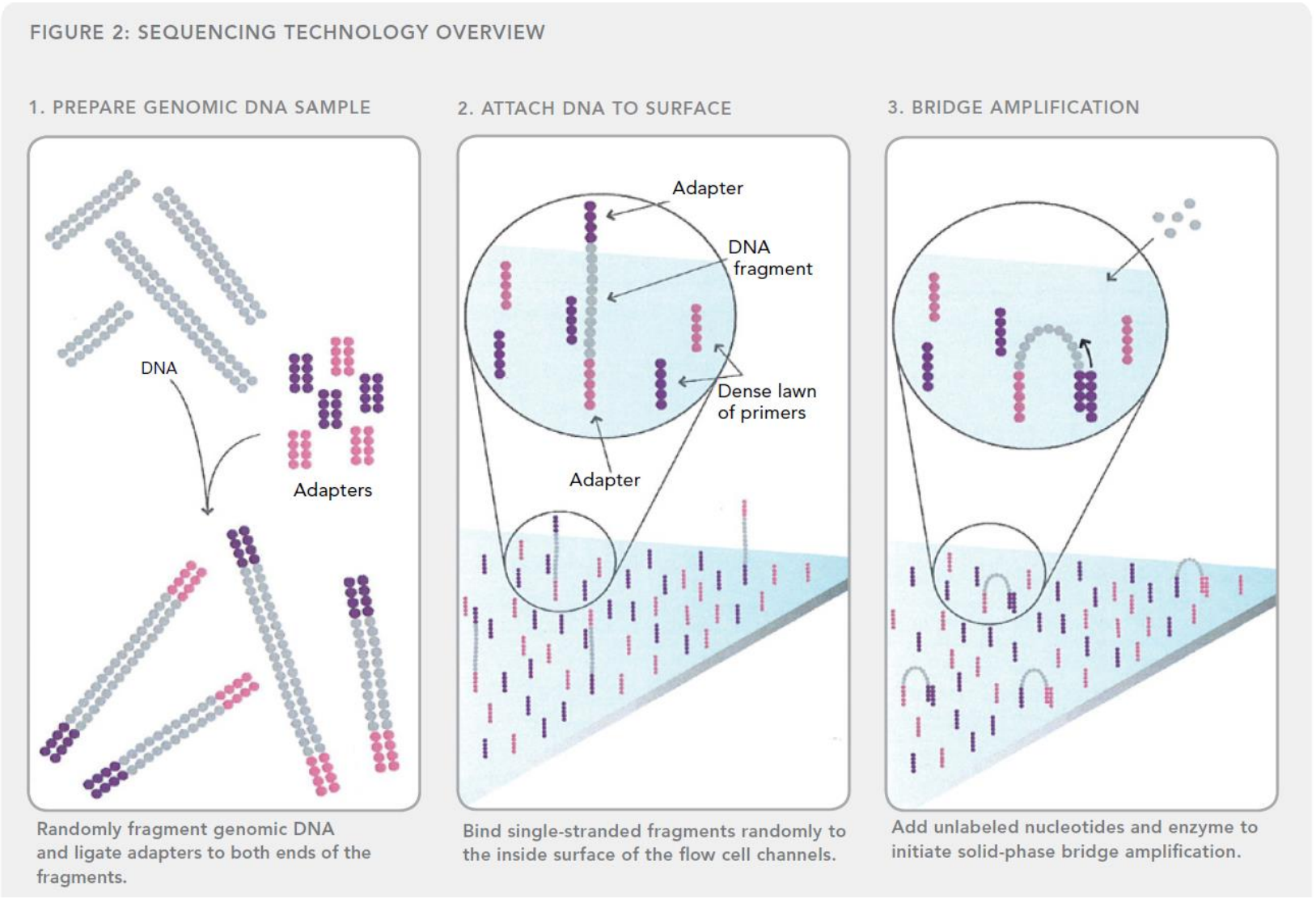
Figure 1. Scanning electron micrograph of etched well in 454 PicoTiter sequencing plate.
454's technology is based on performing hundreds of thousands of simultaneous sequencing reactions in 75 picoliter (44 µm) wells. All molecular biology reactions—DNA amplification, sequencing by synthesis, and signal light generation—occur in a single well.

The Roche 454/GS FLX Sequencing Technology



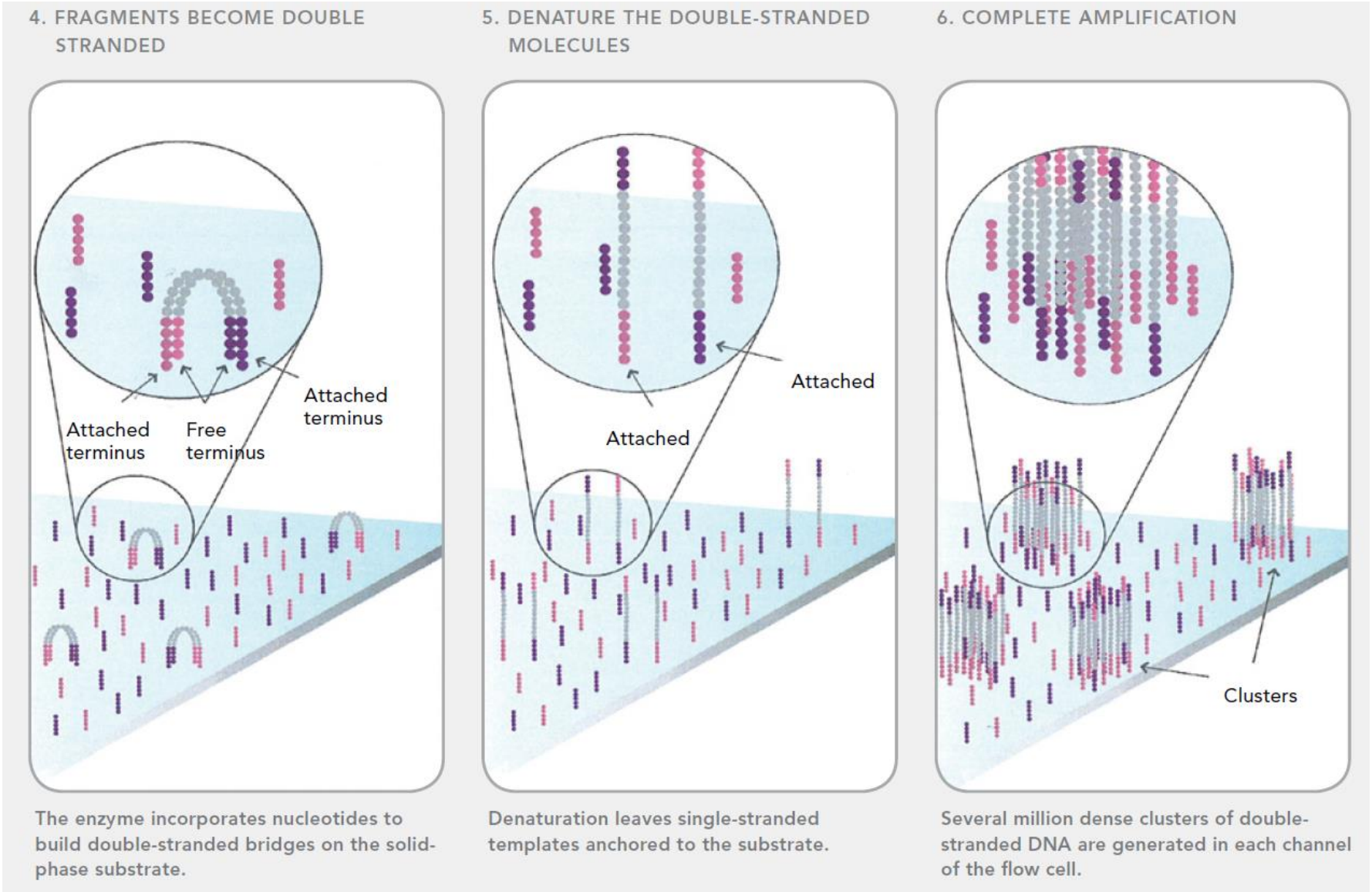
Sequencing is accomplished by synthesizing the complementary strands of the bead attached templates. In a number of cycles the four bases (ATGC) are sequentially washed over the PicoTiterPlate. The incorporation of a new base is associated with the release of inorganic pyrophosphate starting a chemical cascade. This results in the generation of a light signal which is captured by a CCD camera.

DNA Sequencing with Illumina (Solexa®) Technology



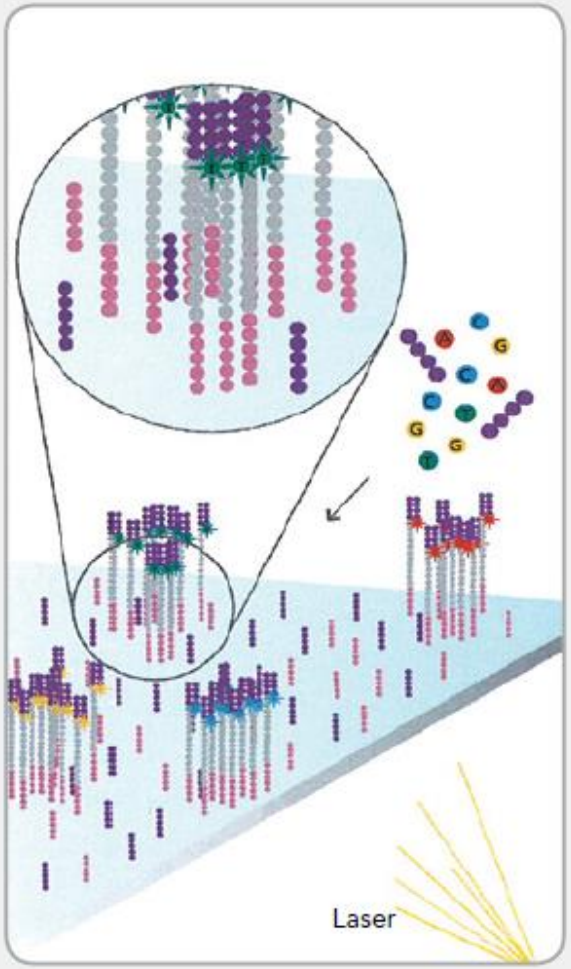
*www.**illumina**.com/documents/products/.../techspotlight_**sequencing**.pdf*

DNA Sequencing with Illumina (Solexa®) Technology



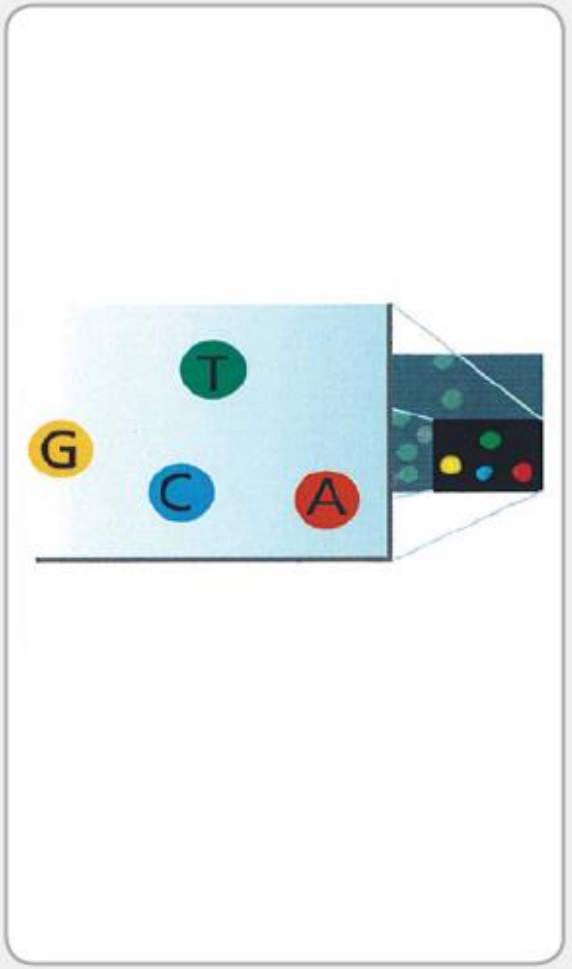
DNA Sequencing with Illumina (Solexa®) Technology

7. DETERMINE FIRST BASE



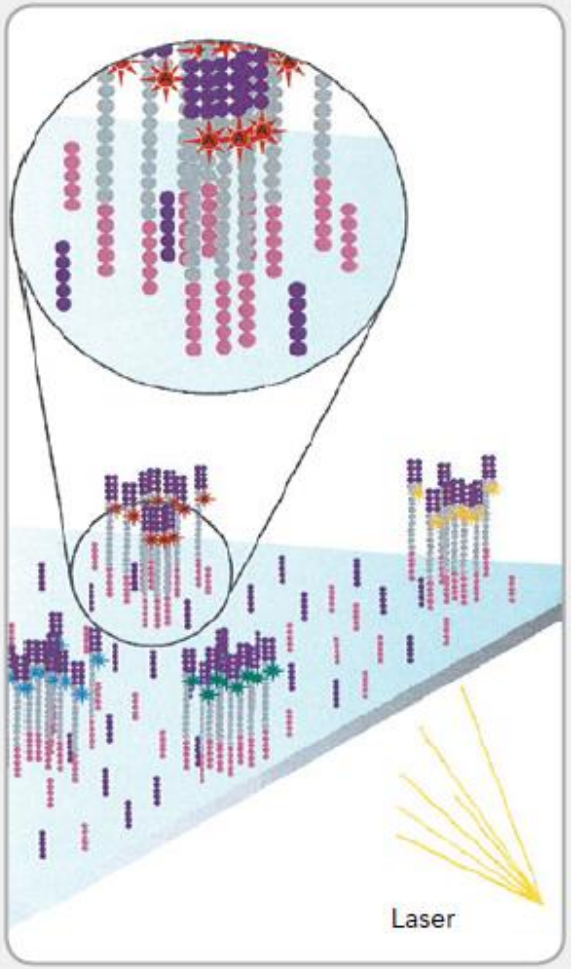
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE



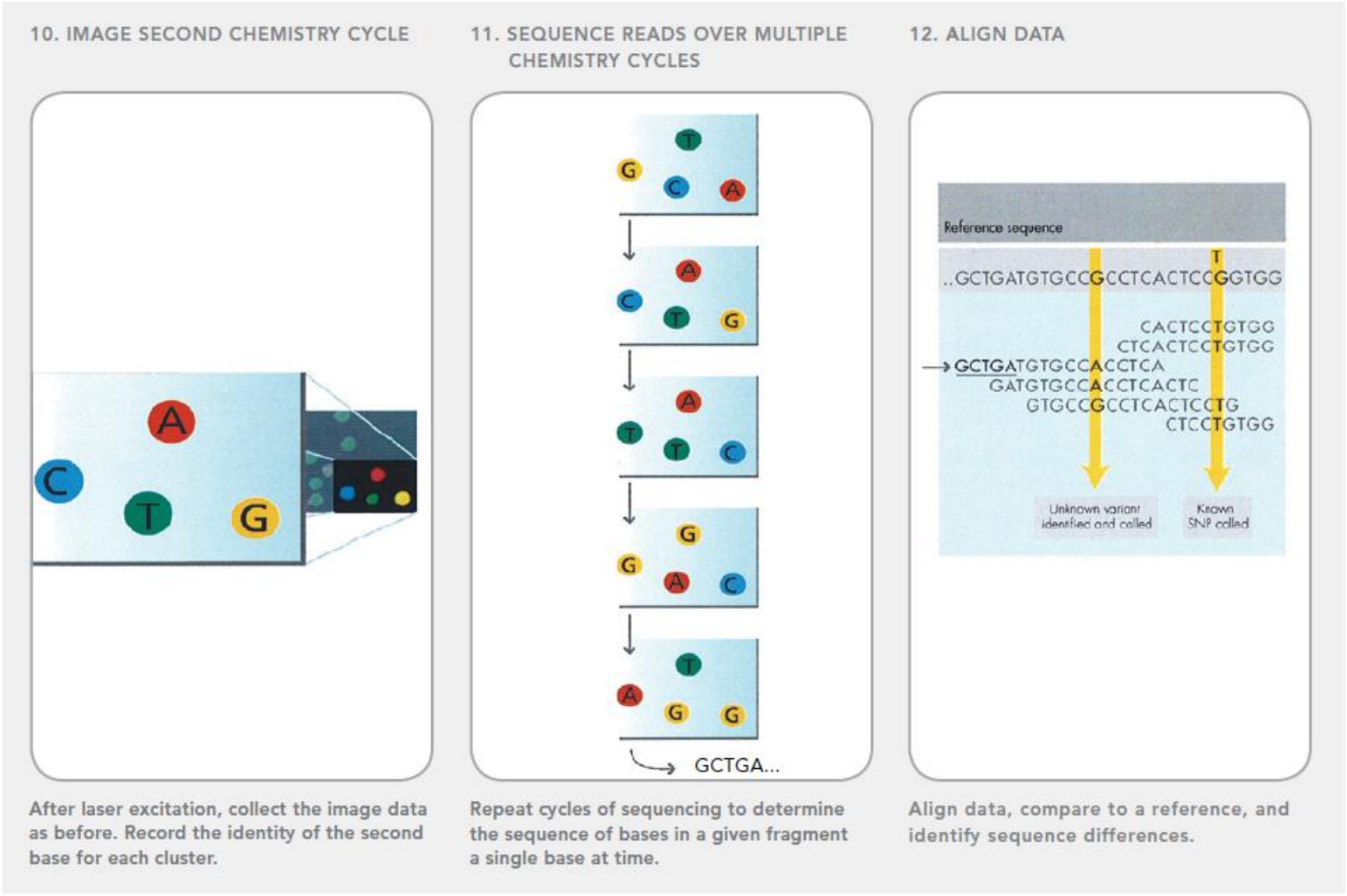
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

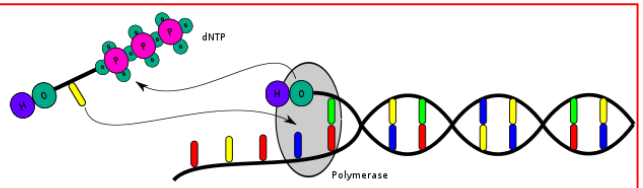
9. DETERMINE SECOND BASE



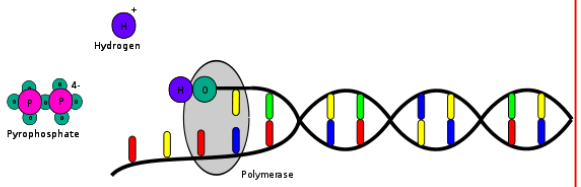
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

DNA Sequencing with Illumina (Solexa®) Technology



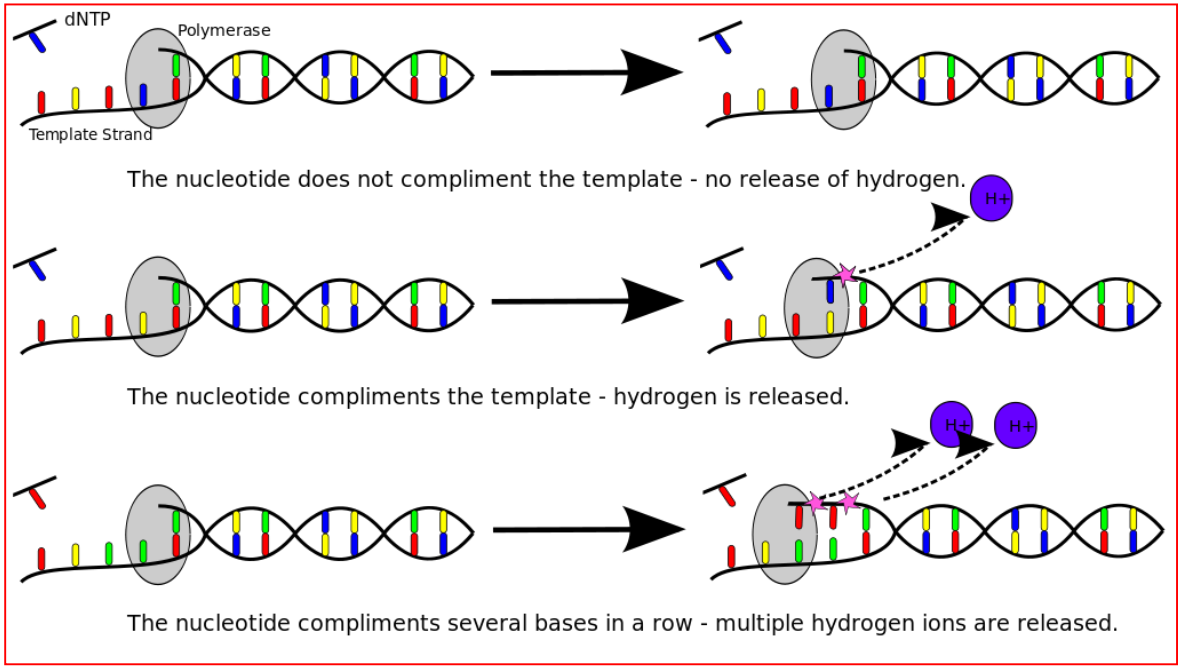


Polymerase integrates a nucleotide.



Hydrogen and pyrophosphate are released.

Ion Torrent Sequencing



http://en.wikipedia.org/wiki/Ion_semiconductor_sequencing