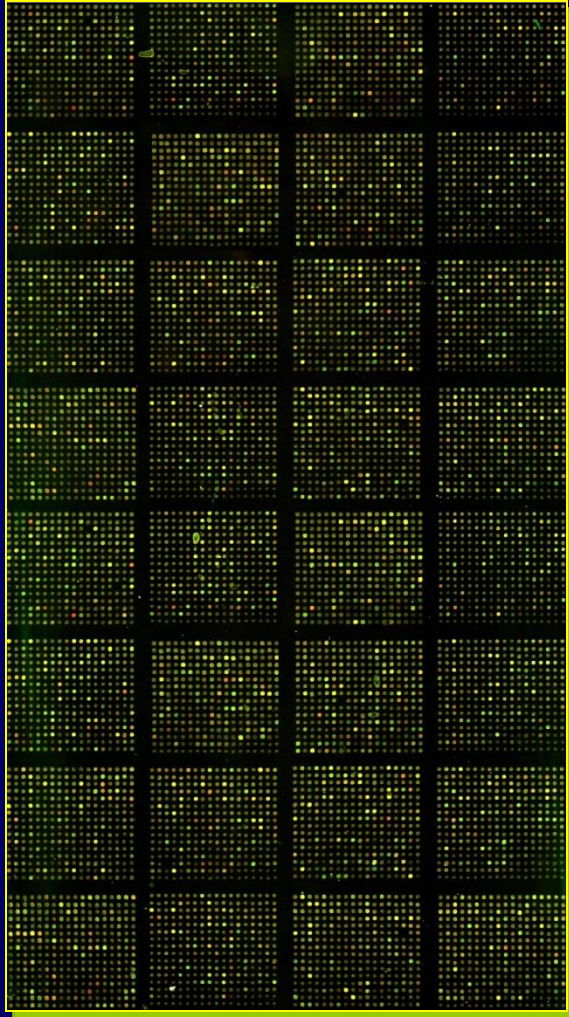


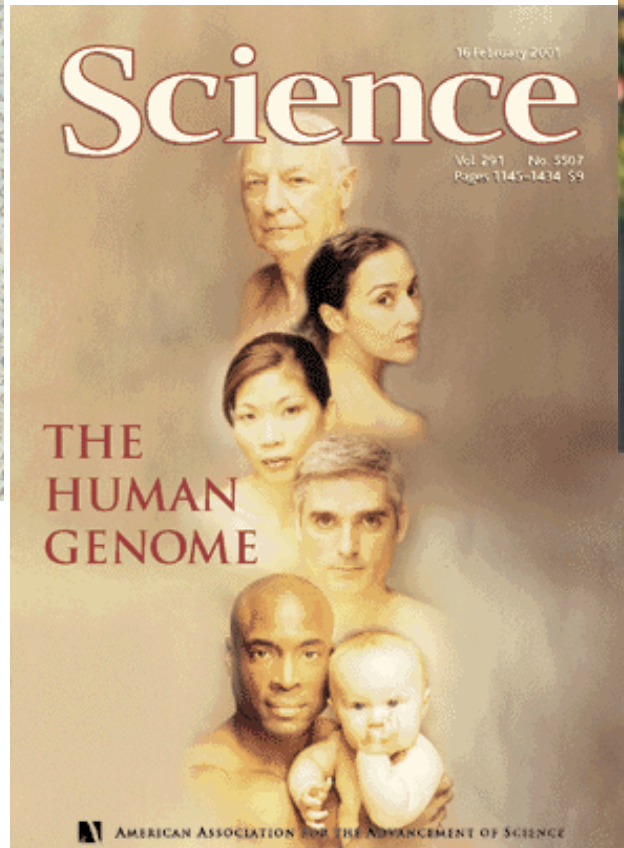
Introduction to Genomics



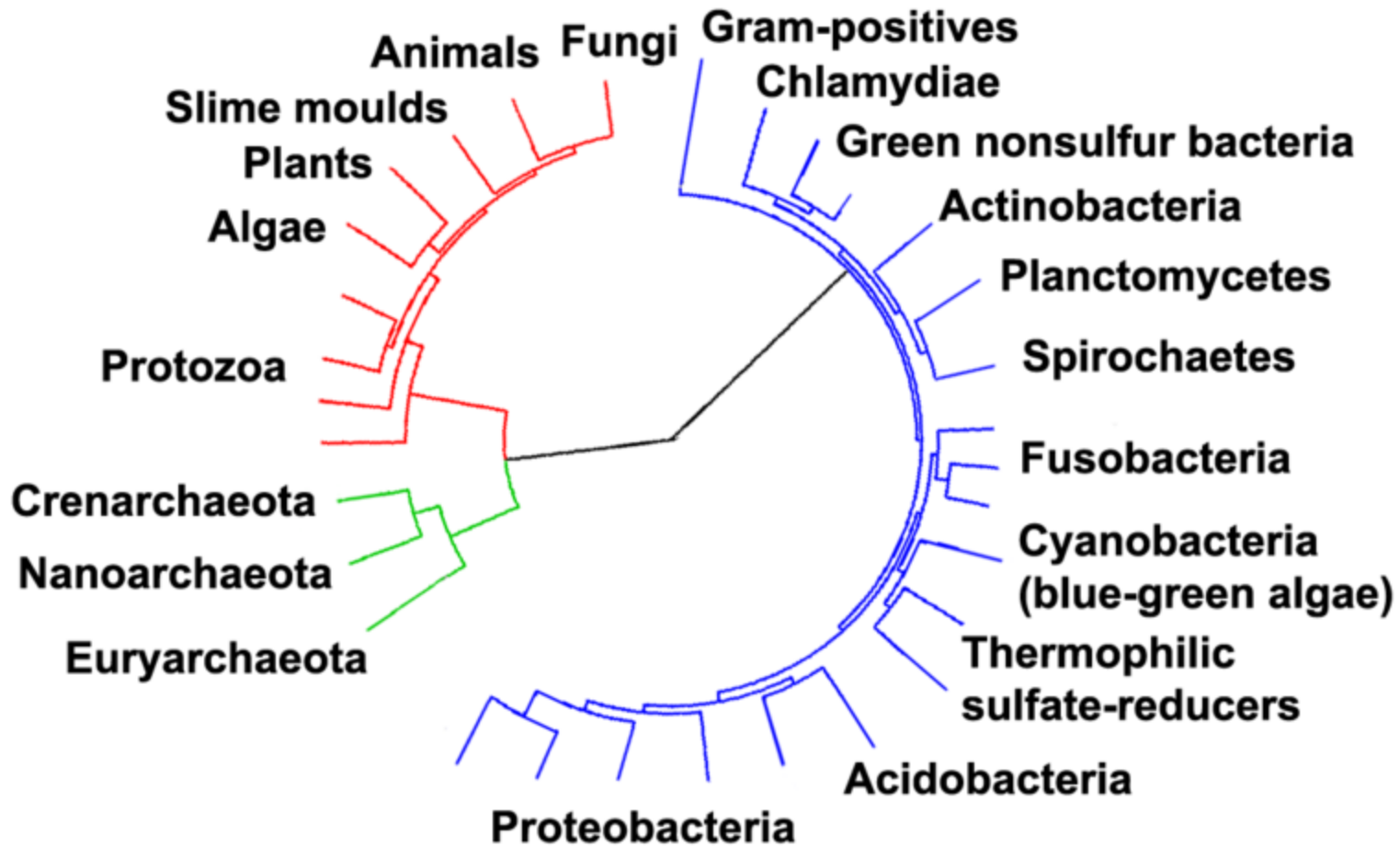
Genomics: is the study of the genome. The full DNA sequence of an organism



GENOMES



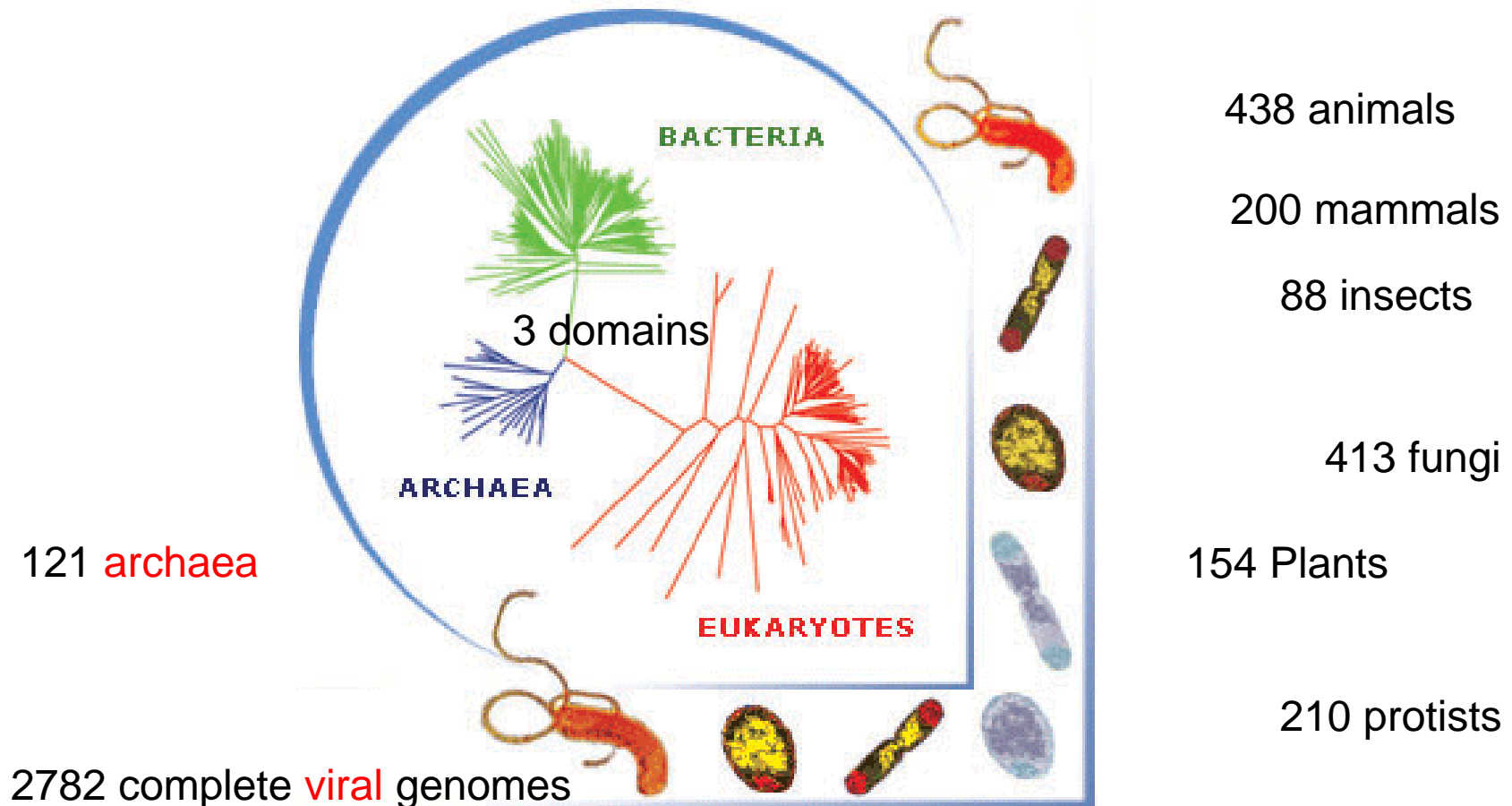
3 domains of life: **Archaea**, **Bacteria** and **Eukaryote**



Thousands of completed genomes

1742 **bacteria**

1569 **Eukaryote**



Genome sequencing: ways to go

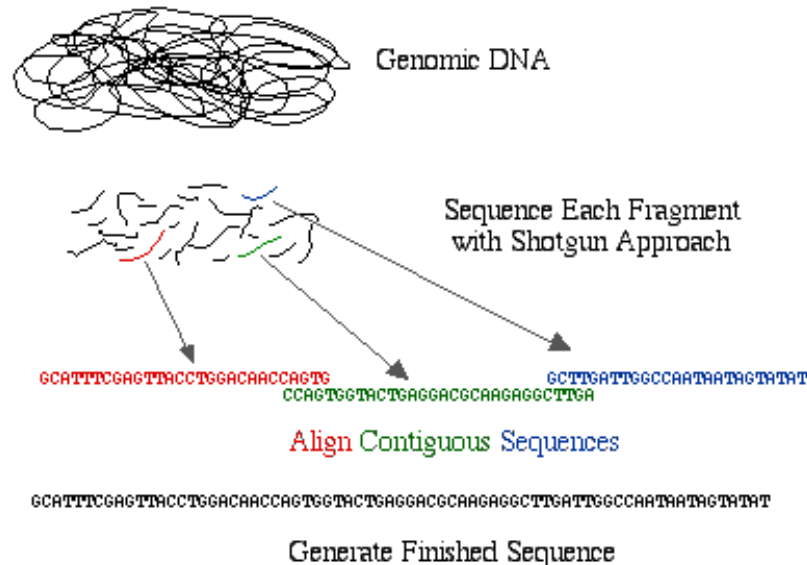
a) Clone-by-clone (e.g. *C. elegans*)

very expensive, slowest, most informative

b) Shotgun

cheapest, fastest, less informative

Whole Genome Shotgun Sequencing Method

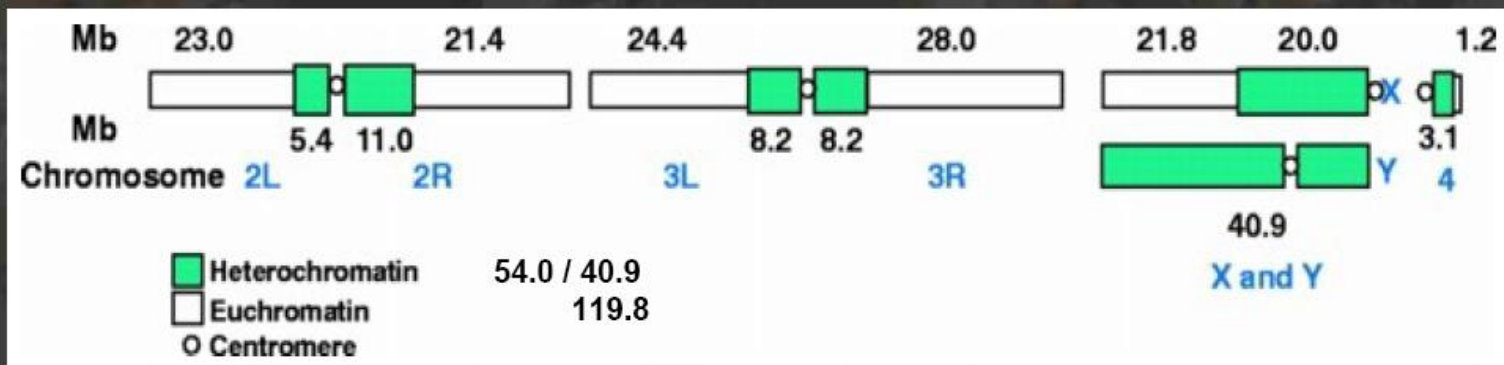
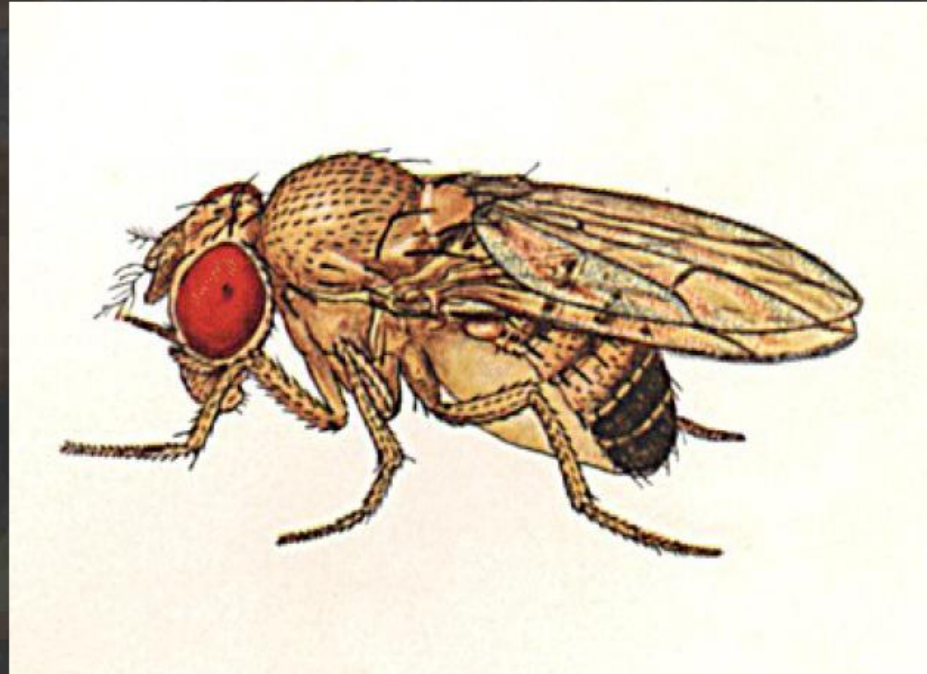


What does one want to know from GENOMES?

- ✓ *Genes*: Function, genomic location, fine structure, expression, alleles, comparative data, etc.
- ✓ *Gene products*: All available data on conceptually translated proteins and more...
- ✓ *Genetic markers*: Location of "old ones" (RAPDs, microsatellites, allozymes, mtDNA, etc.), and "new ones" (e.g. SNPs).

Drosophila Genomics

4 chromosomes



Genome sequencing: Comparison of *Drosophila* and *Anopheles*

- Fruit fly
- 4. Initiated in 1986 as a physical mapping project in Europe (EDGP).
 - ✓ Sequence components (STSs) added in 1990.
 - ✓ Actual genome sequencing (clone-by-clone) started in 1996.
 - ✓ Celera (shotgun sequencing) joined in 1998.
 - ✓ "Finished" in 2000.
 - ✓ Still not finished!

Eukaryote genome annotation

Find locus



Genome



Transcription

Primary Transcript



RNA processing

Find exons
using transcripts

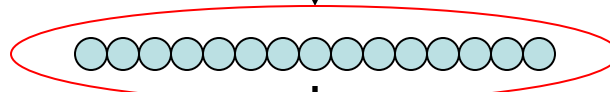
Processed mRNA



Translation

Polypeptide

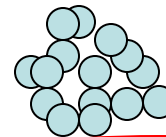
Find exons
using peptides



Protein folding

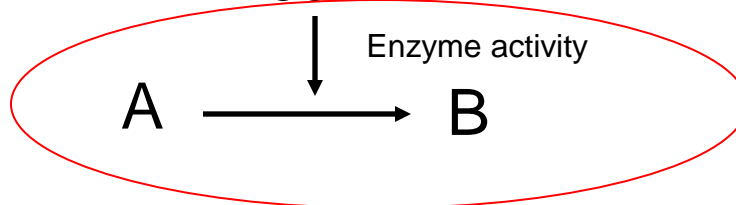
Folded protein

Find function



Enzyme activity

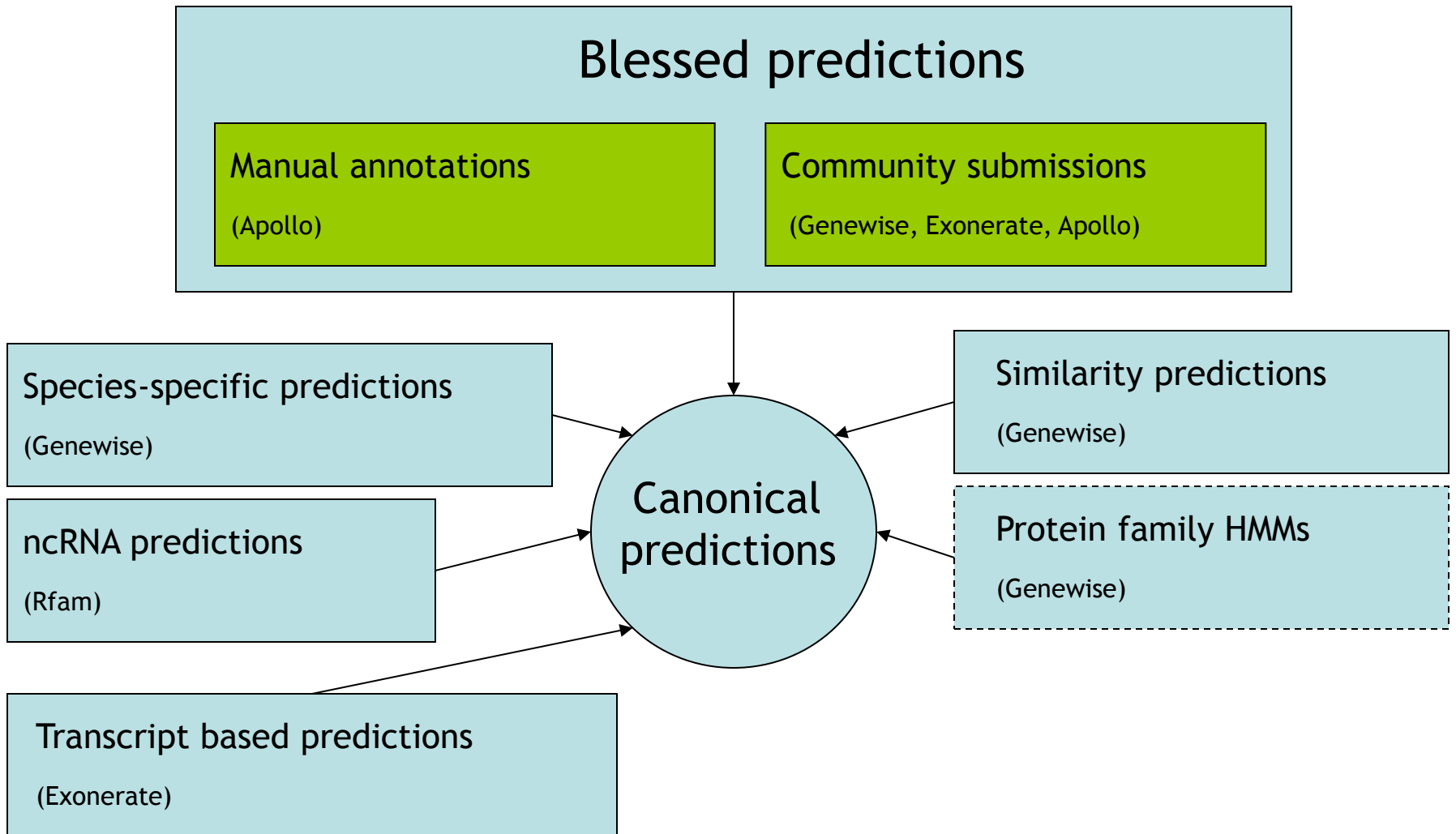
Functional activity



Annotation aims

- Produce a canonical gene set for each genome
 - Protein-coding genes (CDS)
 - ncRNA genes (rRNA, tRNA, miRNA etc)
- Functional annotation consisting of:
 - Brief description
 - Protein domains (InterPro)
 - Gene Ontology (GO) terms
 - Enzyme Classification (EC) numbers
- Release of these data on website
- Submission of these data to public repositories (GenBank/EMBL/DDBJ)
- (Re)annotation of the genome in a timely manner depending on available data and resources

Gene prediction pipeline



Genome browser



VectorBase Gene Expression

The VectorBase consortium is working to provide a repository for microarray data produced by the insect vector community. The repository will have three main roles:

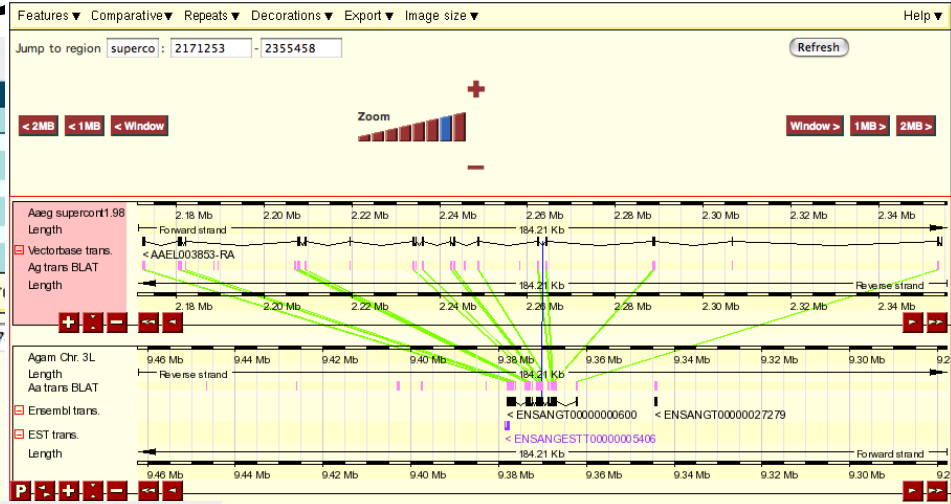
1. To serve public data via genome browsers, search, file export and clustering tools.
2. To facilitate collaboration between groups on pre-publication data.
3. To be the first step in **ArrayExpress** submissions for the insect vector community.

Browse gene expression data:

- **By VectorBase gene id:** (e.g. ENSANGG0000007634, AAEL006624)
 - in species:
- **By reporter/probe/spot id:** (e.g. 4A3B-AAJ-B-05)
 -
- **In the VectorBase genome browser:** For example, **this region on 3R**. Click on the green microarray features for further information and expression data links.
- **Using the BASE web interface:** log in using the panel on the left hand side (username=vbguest, password=readonly) - full **BASE** documentation is available [here](#) (you will need it!). Contact the administrator (details below) to apply for an account so that you can use the analysis tools.

Submit expression data:

- All two-colour data is welcome, please see the [submission guidelines](#).
- Affymetrix data will be handled as soon as possible (we hope in early 2007).



VectorBase: *A. gambiae*

All Organisms: *A. gambiae* | *A. aegypti* | *I. scapularis* | *C. pipiens* | *P. humanus*

Sequence Data | Expression Data | Images | Documents | More... | Tools | Get Data | Search | User | **Get VectorBase Help**

Details: ENSANGG0000002044

- Gene information
- Genomic sequence
- Genomic sequence alignment
- Gene splice site image
- Gene tree info.
- Gene variation info.
- ID history
- Transcript information
- Exon information
- Protein information
- Export gene data

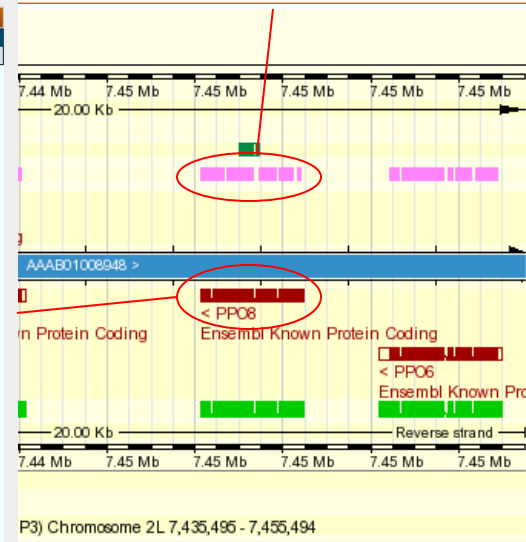
Chromosome 2L
7,441,521 - 7,444,137

- View of Chromosome 2L
- Graphical view
- Graphical overview
- Export information about region
- Export sequence as FASTA
- Export EMBL file
- Export Gene info in region
- Export SNP info in region



Ensembl Gene Report for ENSANGG0000002044

Gene	PP03 (Anopheles symbol) To view all Ensembl genes linked to the name click here .
Ensembl Gene ID	ENSANGG0000002044
Genomic Location	This gene can be found on Chromosome 2L at location 7,441,521-7,444,137. The start of this gene is located in Chunk AAAB01008948_47 .
Description	Pro-phenol oxidase subunit 2. <i>Source</i> : Uniprot/SPTREMBL O44251
Prediction Method	Genes were annotated by the Ensembl automatic analysis pipeline using either a GeneWise/Exonerate model from a database protein or a set of aligned cDNAs/ESTs followed by an ORF prediction. GeneWise/Exonerate models are further combined with available aligned cDNAs/ESTs to annotate UTRs (For more information see V.Curwen et al., Genome Res. 2004 14:942-50)
Transcripts	<p>ENSANGT0000002437 ENSANGP0000002437 PP03 [Transcript info] [Exon info] [Peptide info]</p> <p>Features</p> <p>Chr. 2L 7.43 Mb 7.44 Mb 7.44 Mb 7.44 Mb 7.45 Mb 7.45 Mb Length: 22.62 Kb Forward strand</p> <p>Ensembl trans. GPROR31 > Ensembl Known Protein Coding</p> <p>DNA(contigs) AAAB01008948_47 ></p> <p>Ensembl trans. < PP03 Ensembl Known Protein Coding</p> <p>Ensembl trans. < PP06 Ensembl Known Protein Coding</p> <p>Length: 22.62 Kb Reverse strand</p>

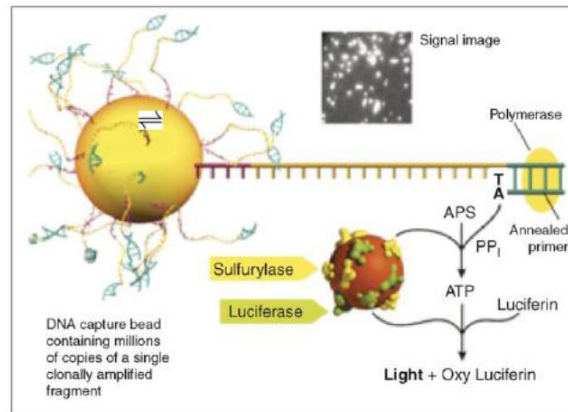


Functional Genomics: is the area of genomics that study the biological activity of the genes and their products

DNA Micro-arrays

RNA interference

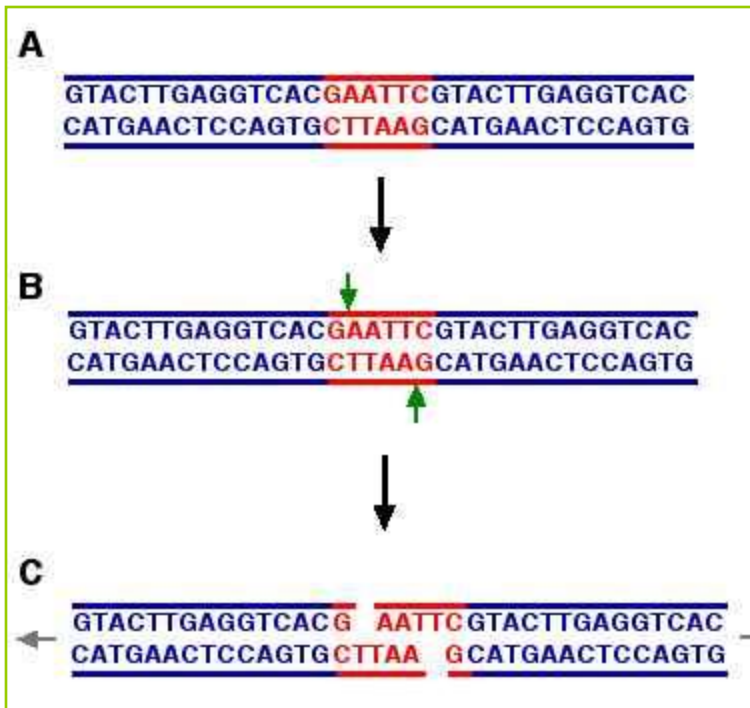
New generation sequencing



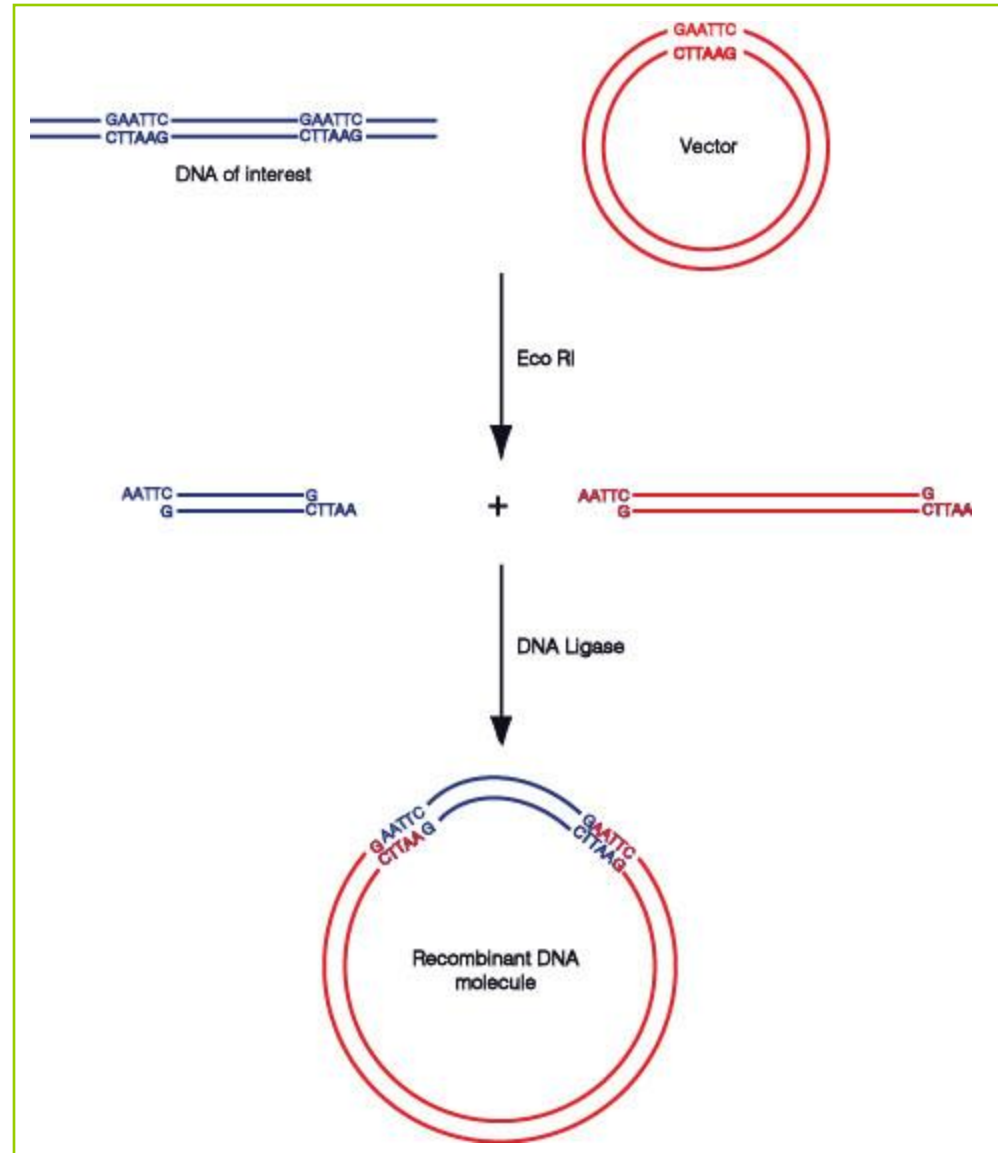
Recombinant DNA technology

Artificial rearrangement of DNA; segments of DNA from one organism can be incorporated into the genetic makeup of another organism. Using these techniques, researchers can study the characteristics and actions of specific genes.

Restriction endonucleases: are enzymes that recognize and cleave specific palindromic sequences in DNA.



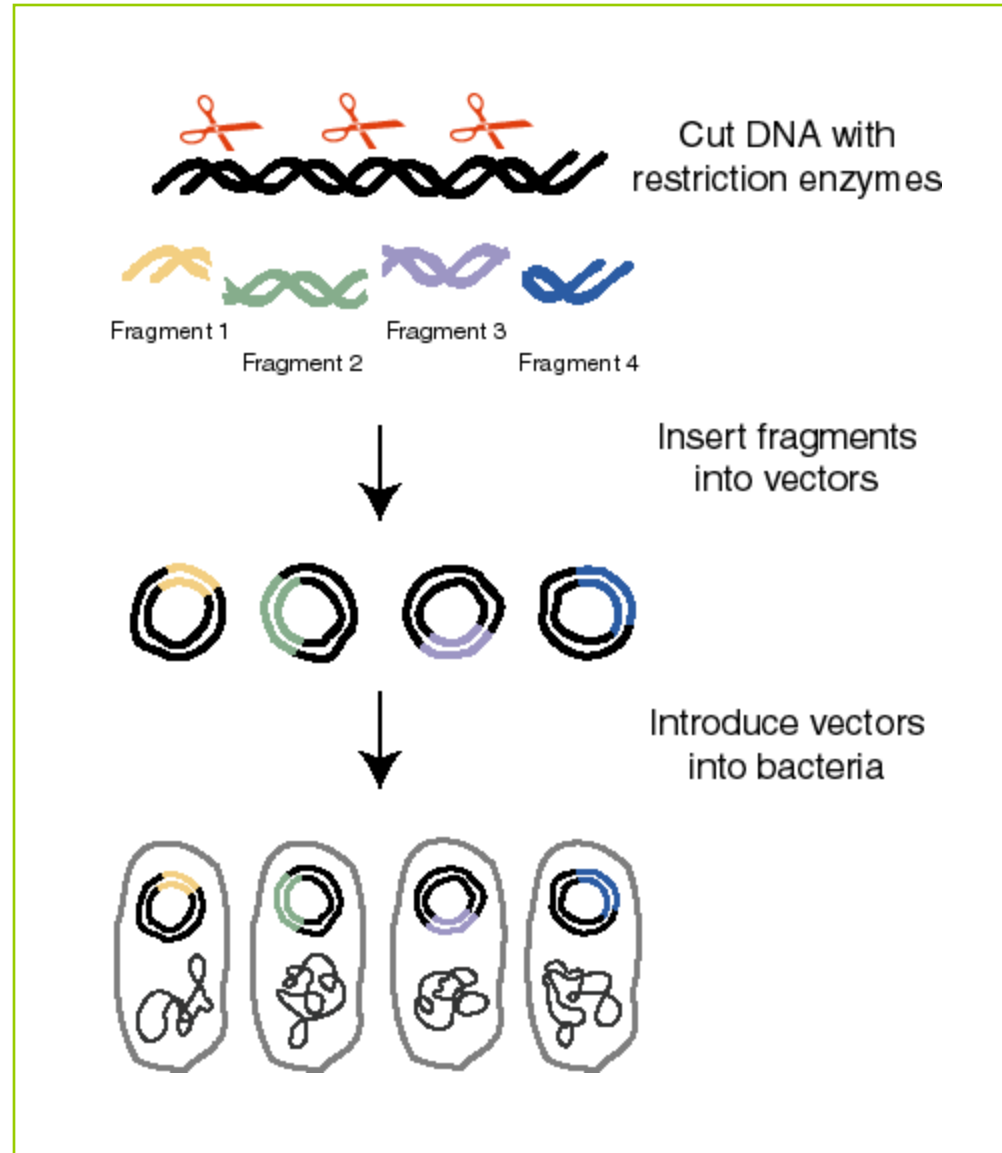
DNA segments of interest may be isolated by "**cloning**" - cutting the DNA with a restriction enzyme, and ligating it into a vector, such as a plasmid, which can then be reproduced in bacteria.



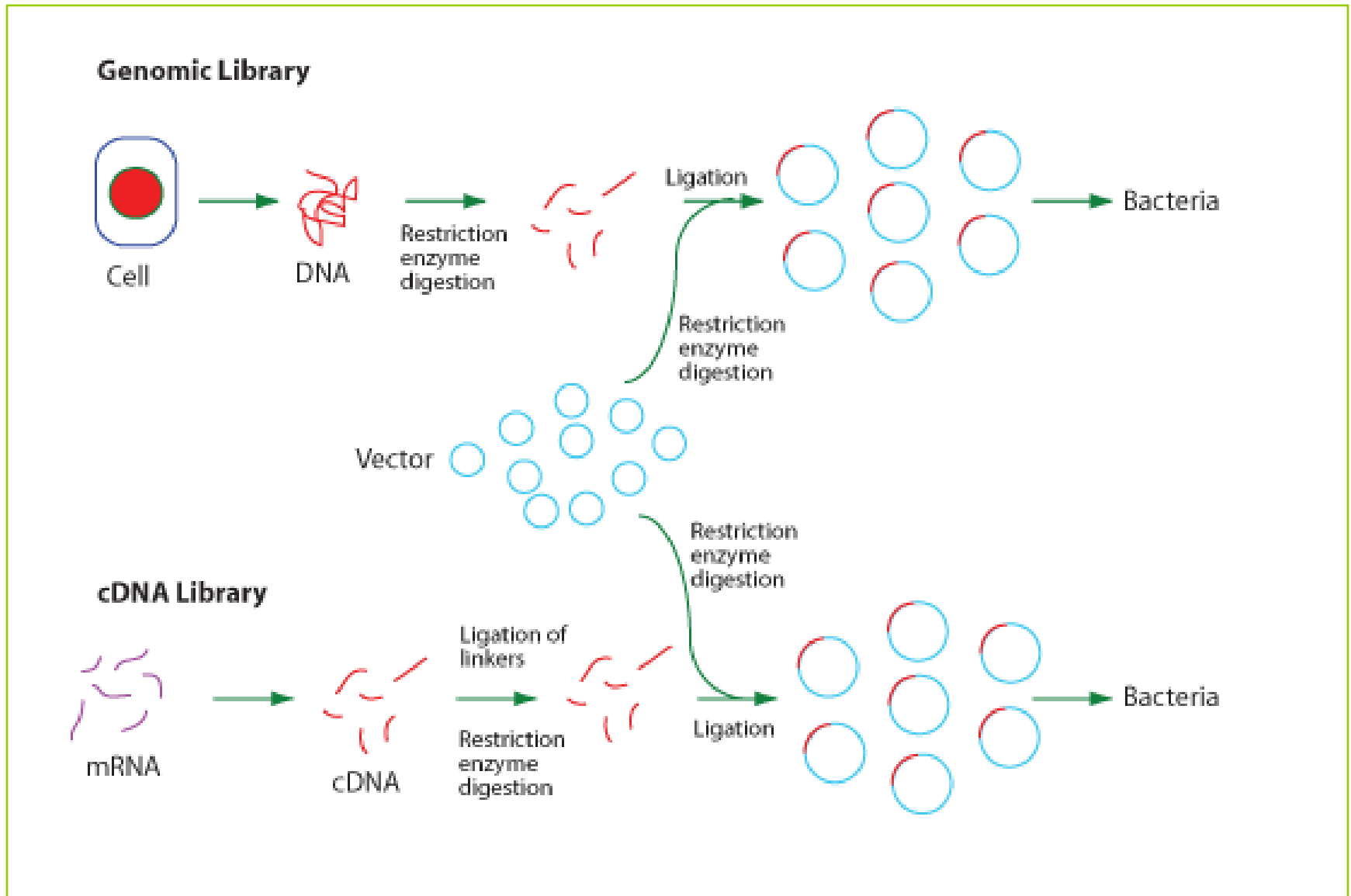
Recombinant DNA technology

DNA libraries are bacterial or viral cultures that contain mixtures of DNA molecules inserted into vectors.

Once cloned, DNA fragments can be **characterized** by DNA sequencing.



Recombinant DNA technology



- **QUANTITATION OF mRNA**

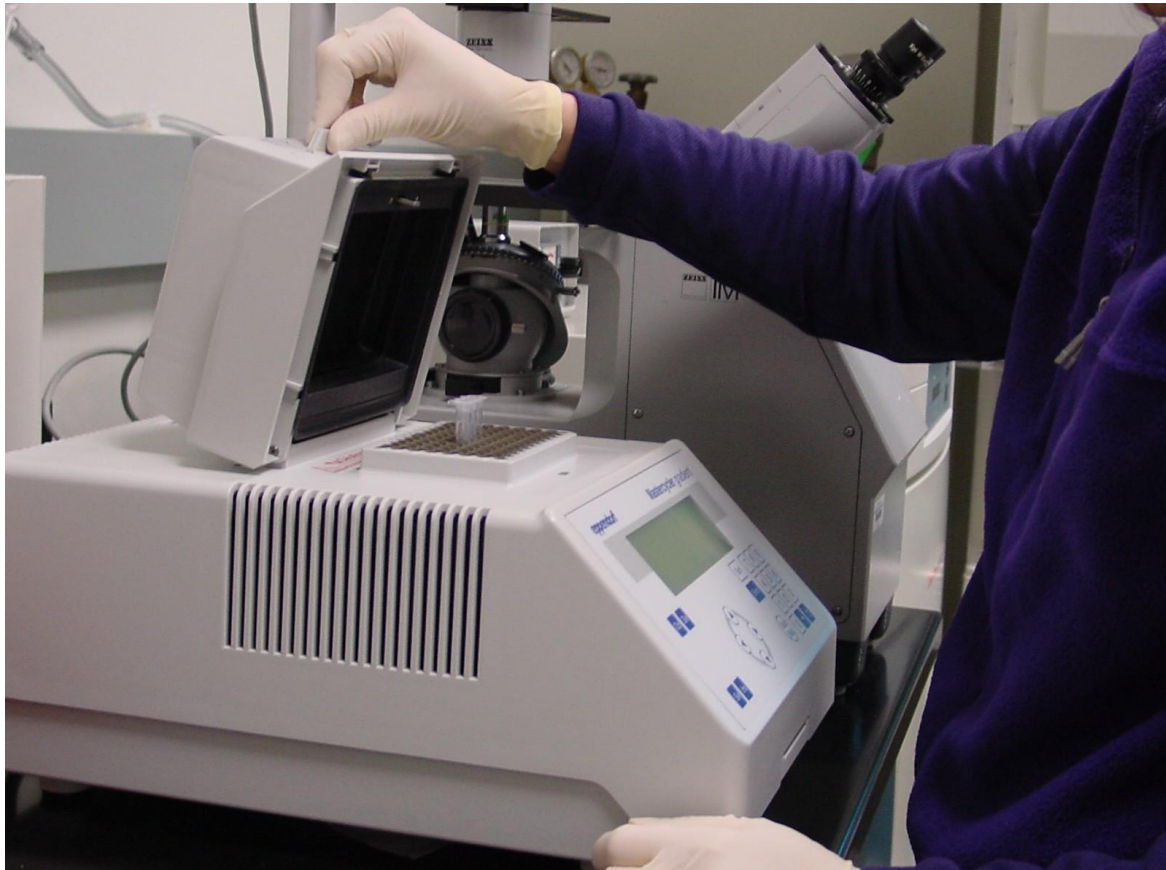
- PCR

- Northern blotting

- In situ hybridization

PCR

Polymerase Chain Reaction (PCR) is a technique for the amplification of DNA. This technique requires knowledge of the sequence of the DNA to be amplified.



PCR

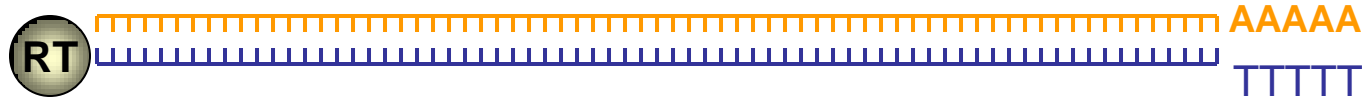
The Nobel Prize in Chemistry 1993



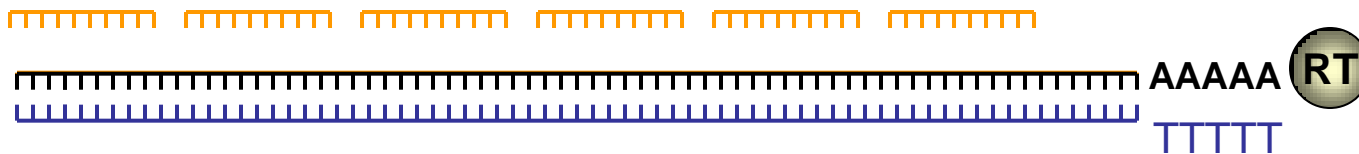
Kary B. Mullis



Oligo dT primer is bound to mRNA



Reverse transcriptase (RT) copies first cDNA strand

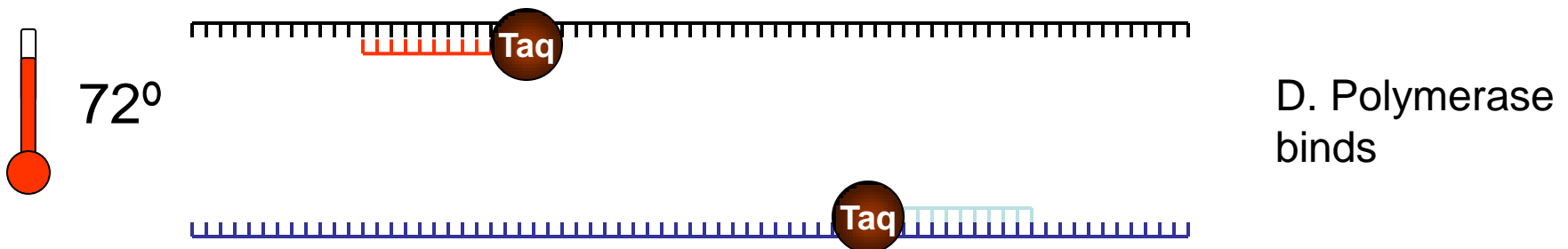
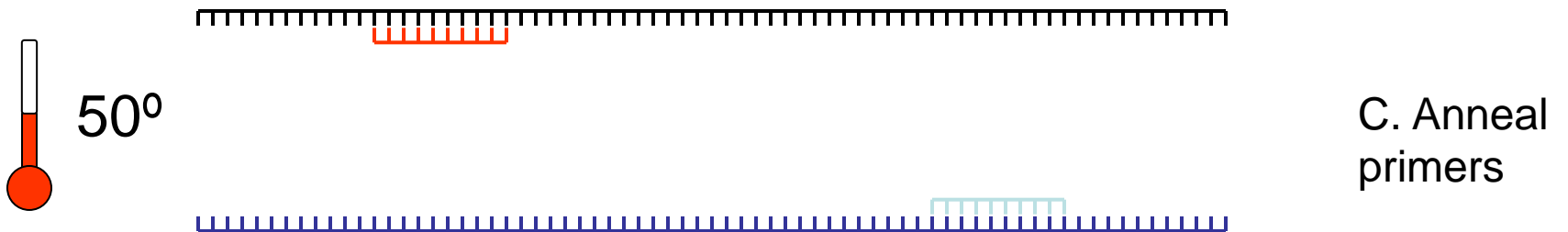
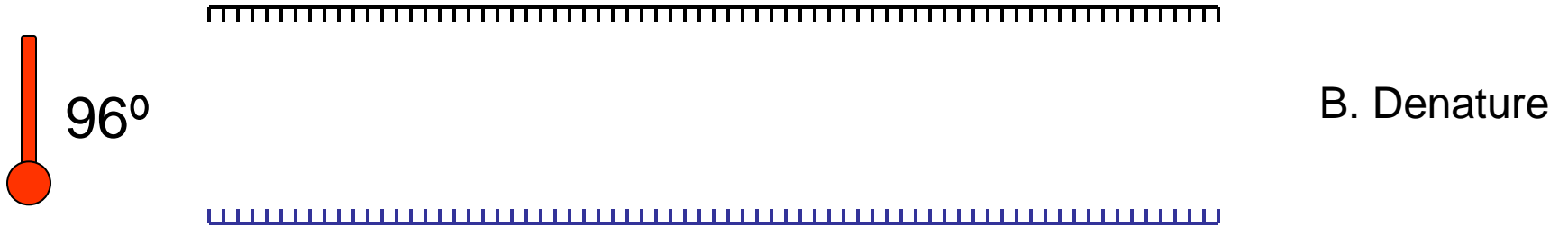
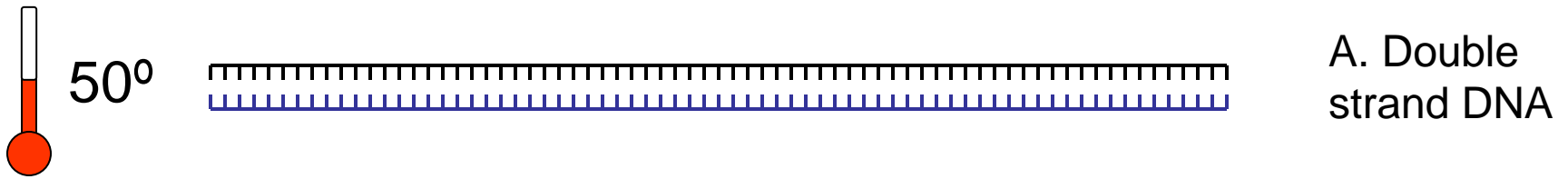


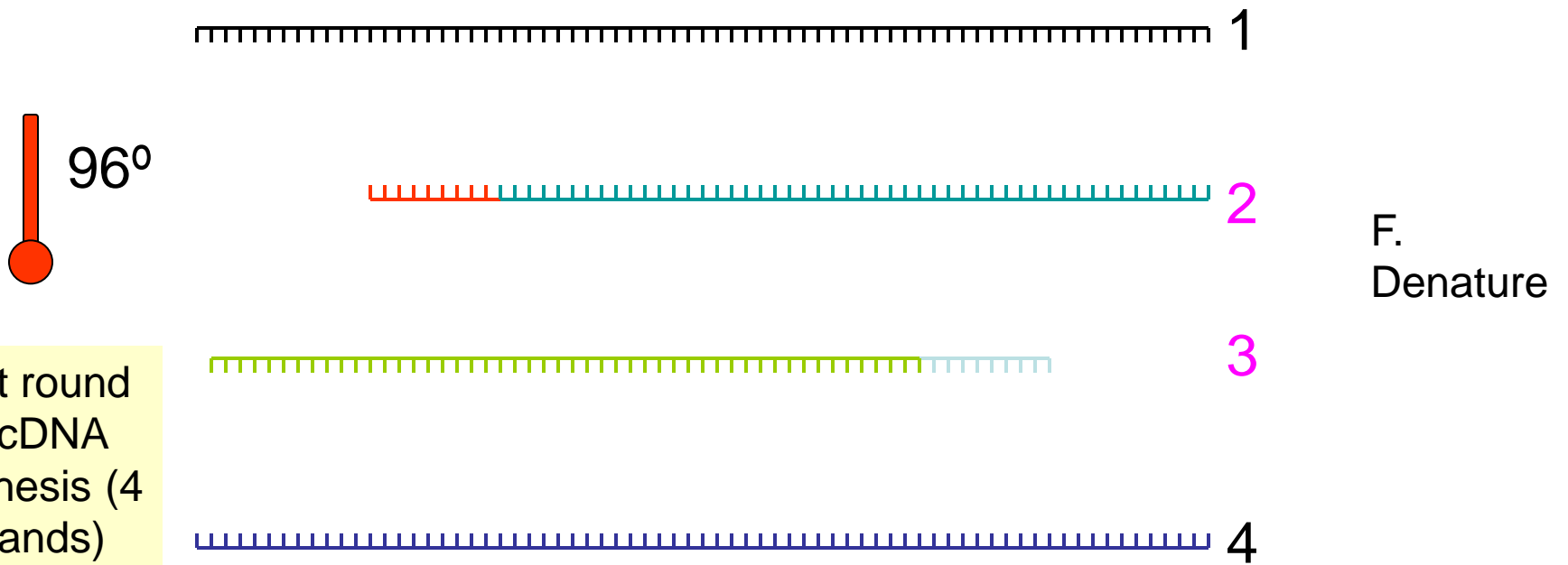
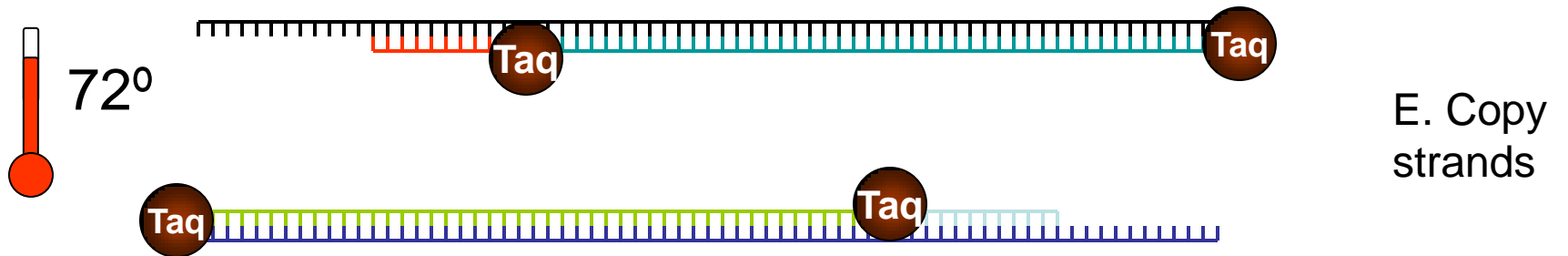
Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA



Double strand cDNA

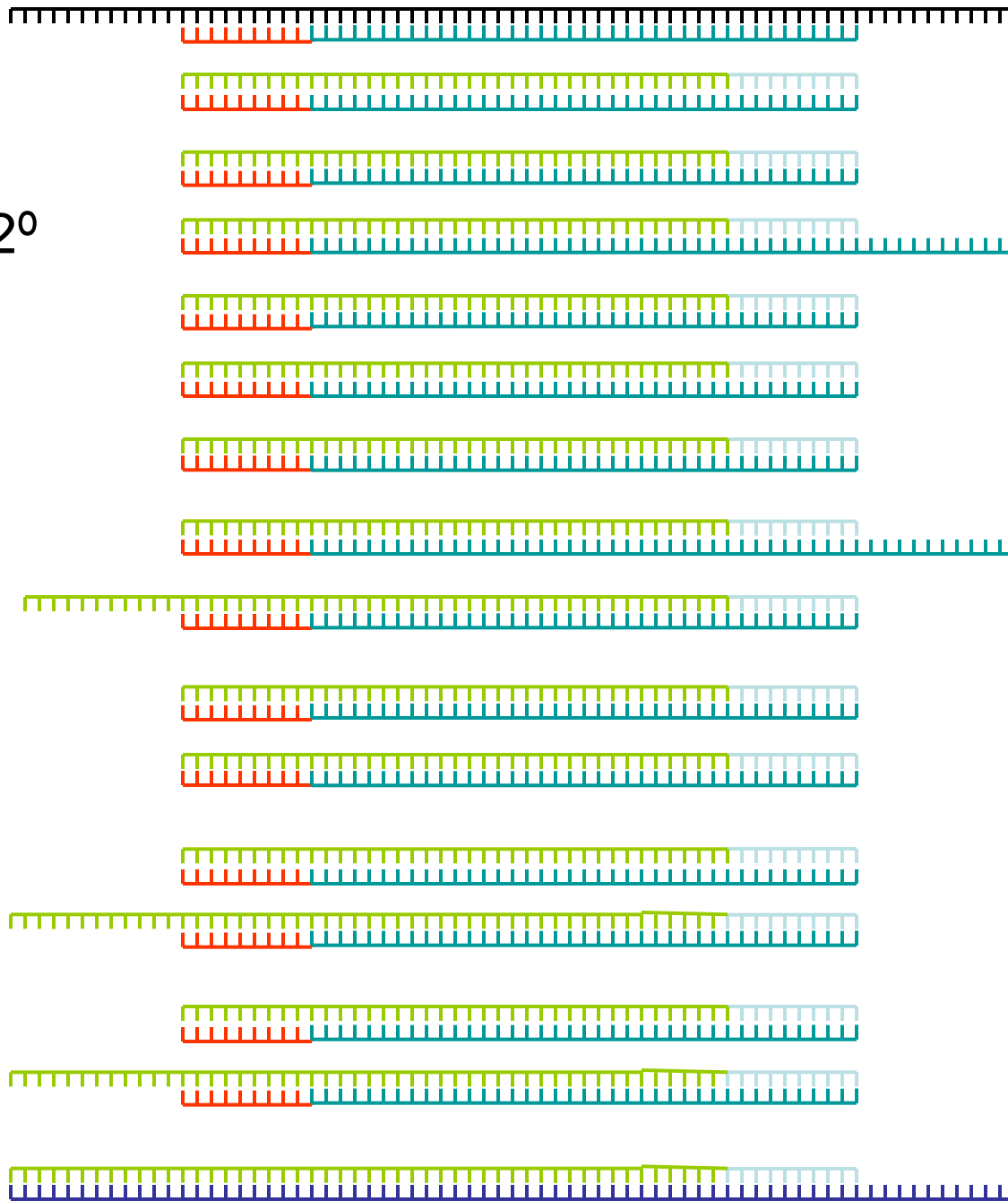
Conversion of mRNA to cDNA by Reverse Transcription







72°



1

M.
Copy strands at
72°

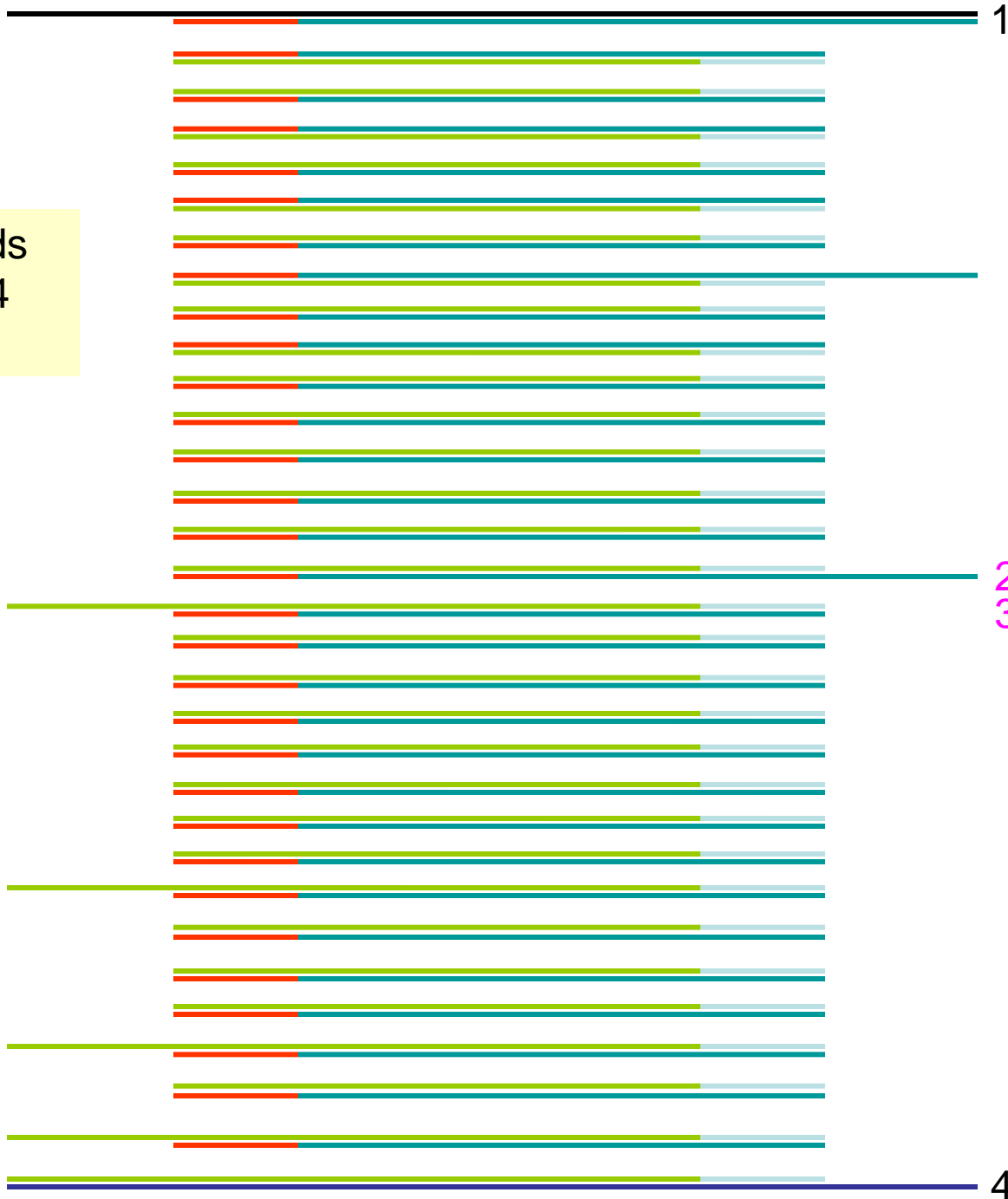
2

3

4

Fourth
round of
cDNA
synthesis
(32
strands)

After 5 rounds
there are 64
strands



PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :

Step 1 : denaturation

1 minut 94 °C

Step 2 : annealing

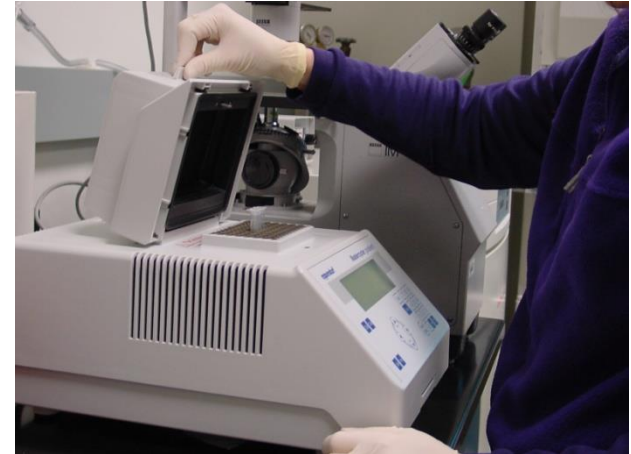
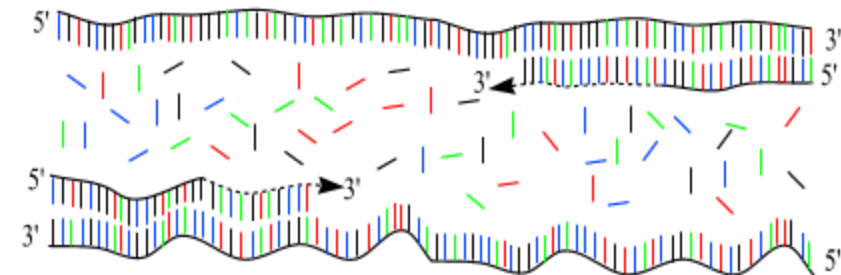
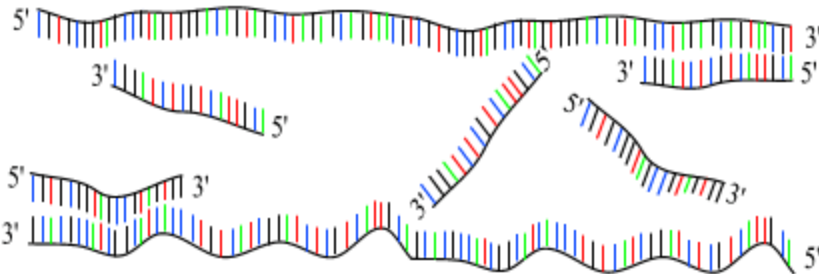
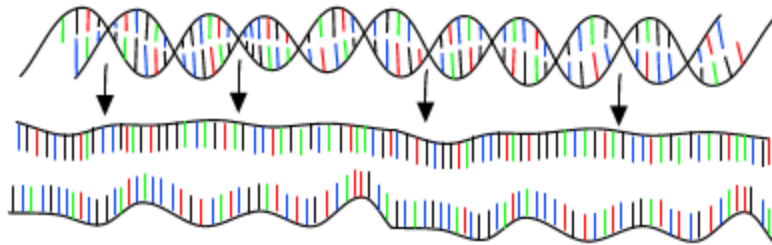
45 seconds 54 °C

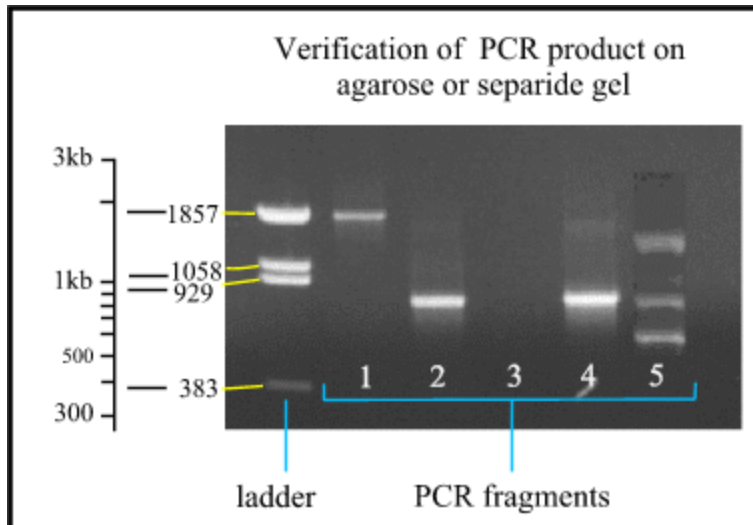
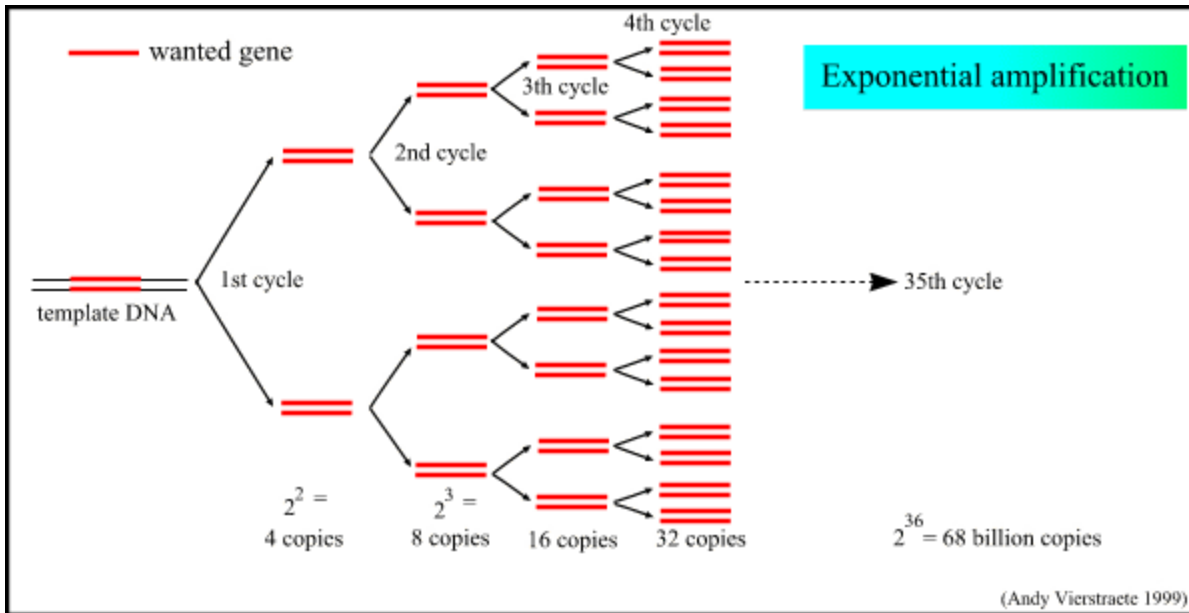
forward and reverse primers !!!

Step 3 : extension

2 minutes 72 °C

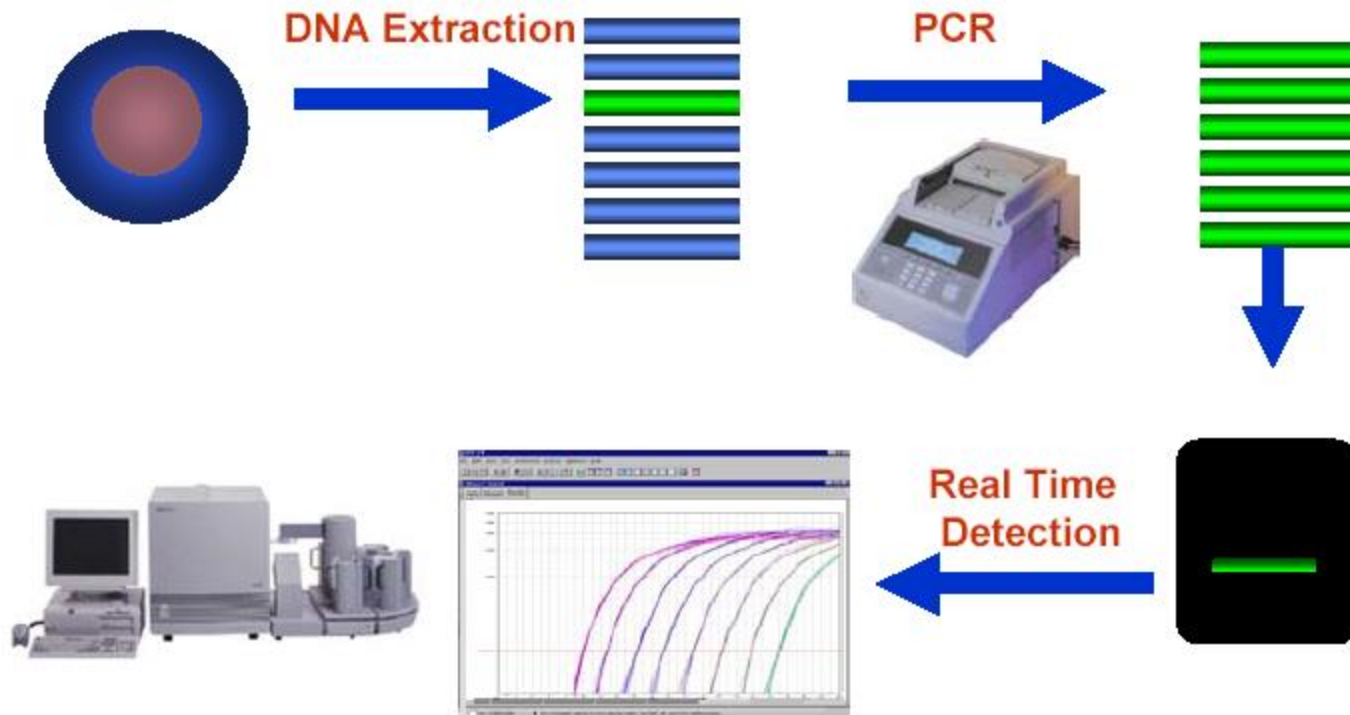
only dNTP's





- Sensitive
- Can discriminate closely related mRNAs
- Technically simple

Real time PCR





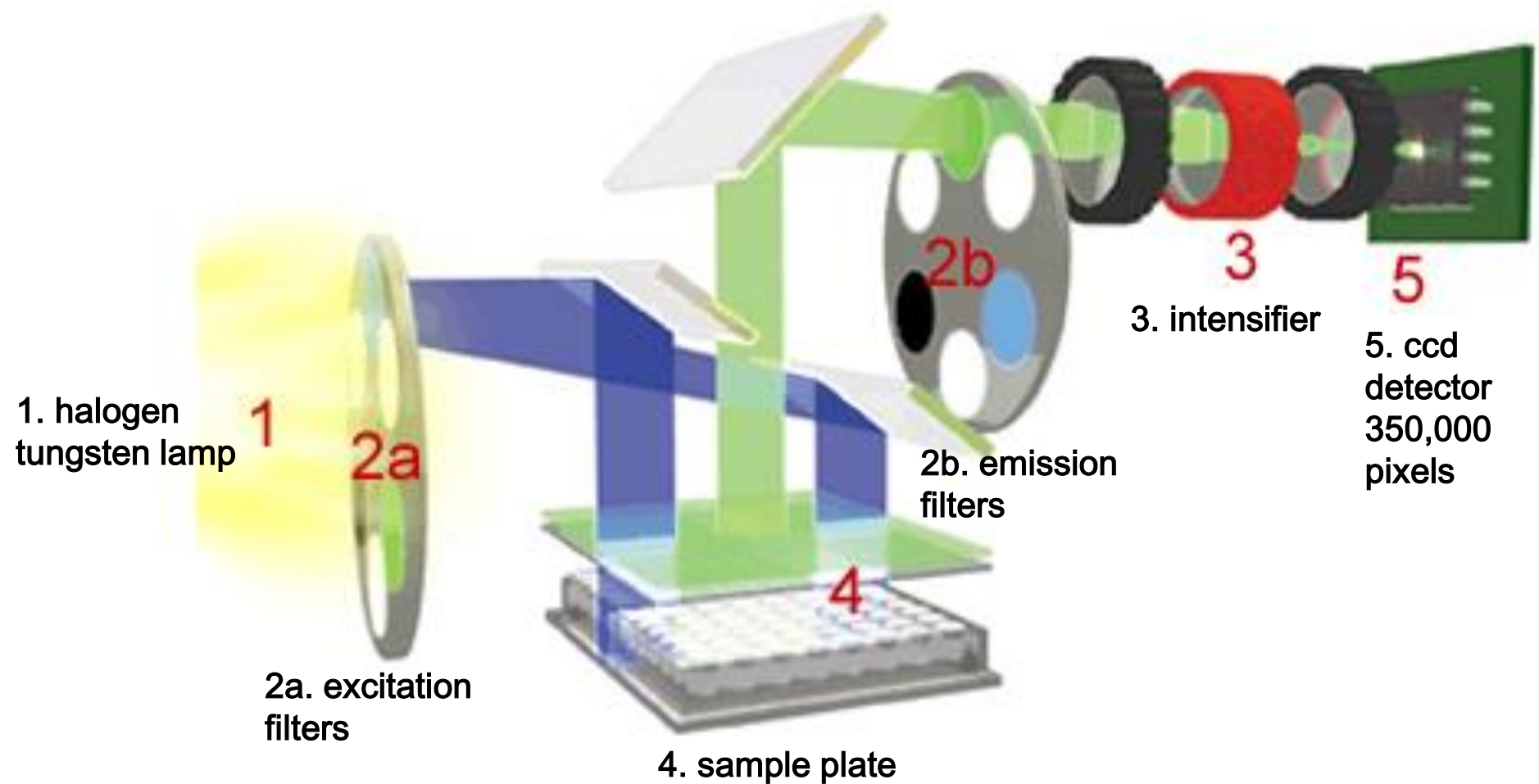


Fig. 1.2. Representation of Optical Detection System layout.

Unbound SYBR[®] Green



Fluorescent Bound SYBR[®] Green

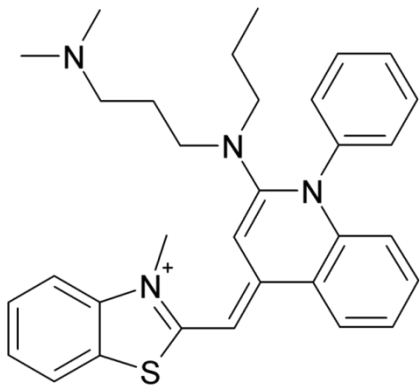
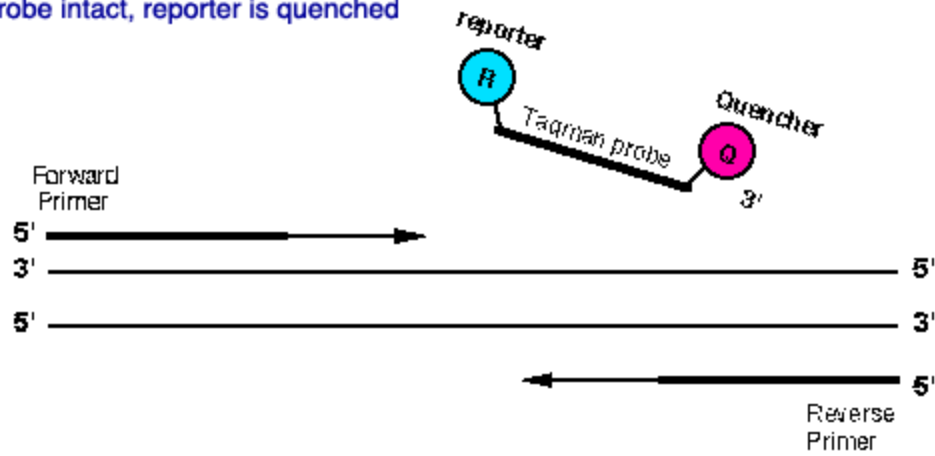


Figure 4

SYBR[®] Green I detection mechanism; double-stranded DNA in the reaction is bound by the dye. In the bound state, SYBR Green I is 1000 fold more fluorescent than in the unbound state. As PCR amplification increases the amount of dsDNA present, the fluorescence signal increases proportionately.

Probe intact, reporter is quenched



TaqMan

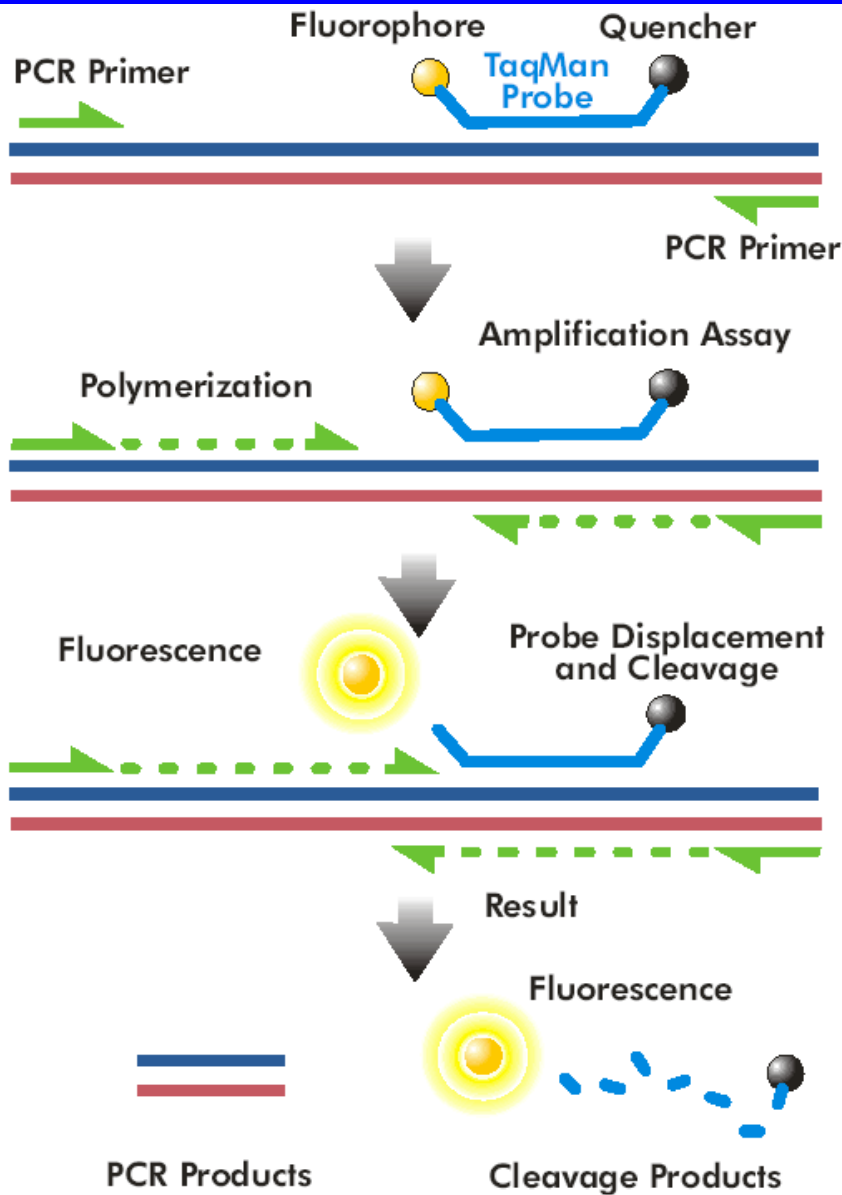


Figure 5
TaqMan[®] probe chemistry mechanism. These probes rely on the 5'-3' nuclease activity of *Taq* DNA polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence.

Figure 12: The 5' Nuclease Assay

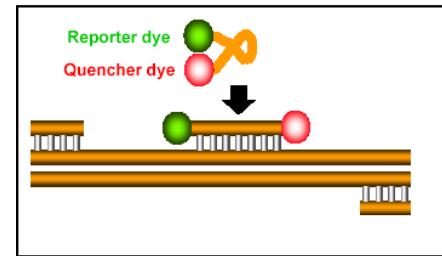
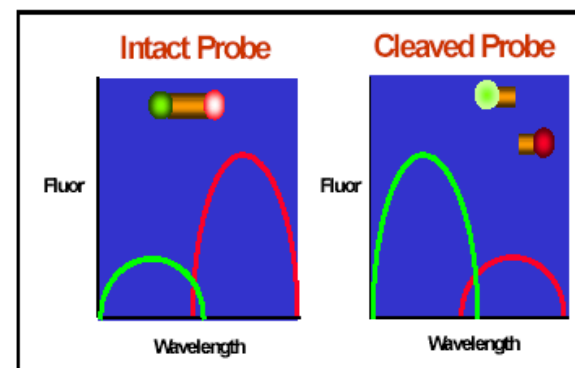
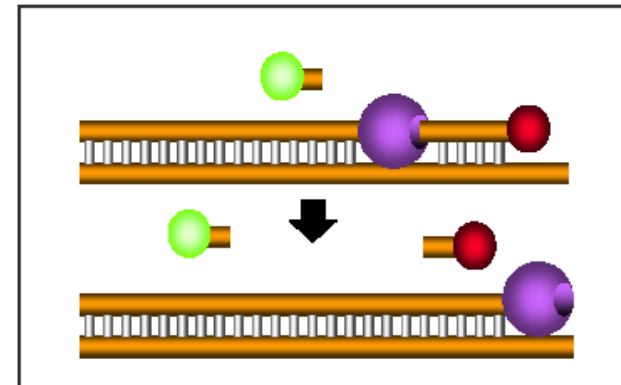
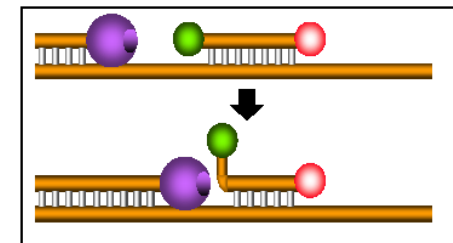


Figure 13: Polymerase collides with TaqMan[®] Probe



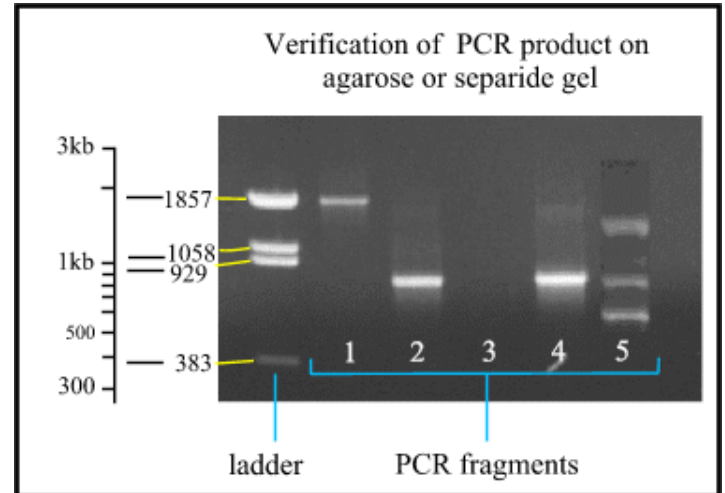


Figure 4: PCR Phases in Log view

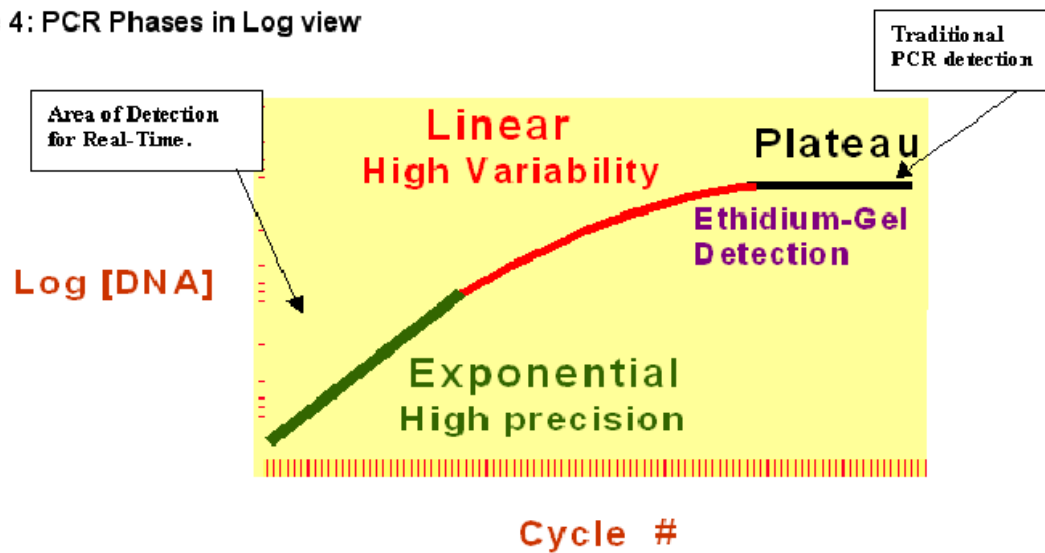


Figure 8: Log view 5-fold dilution series

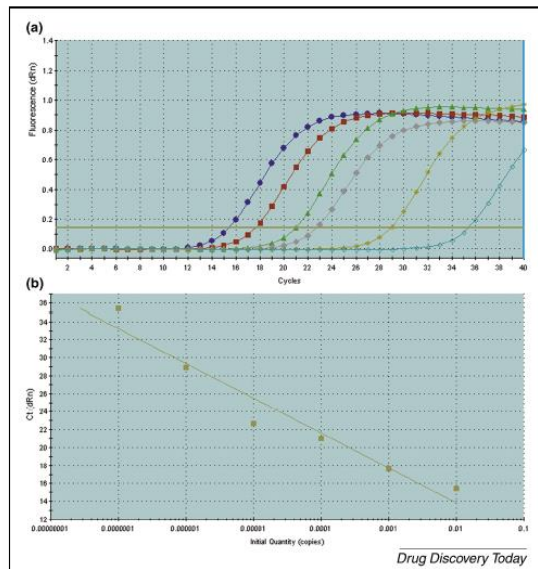
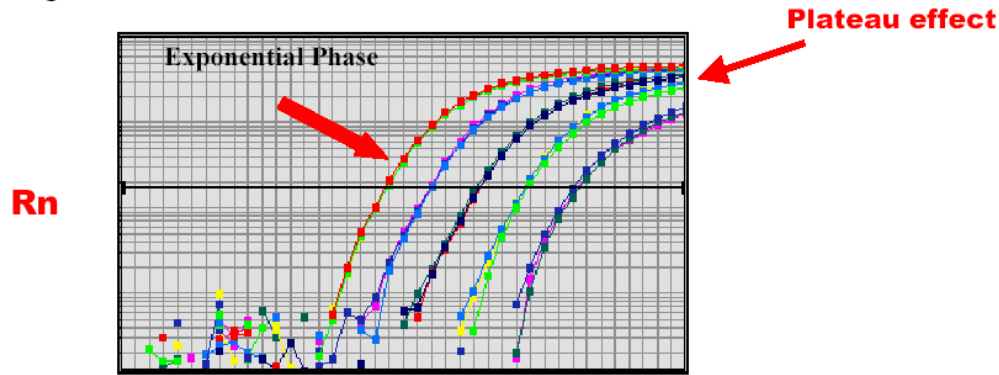


Figure 3. Real-time PCR amplification plot for a series of standards for human cytochrome P450 1A2. Each curve shows increasing fluorescence with each amplification cycle for a dilution series ranging from 10^{-2} to 10^{-7} . The resultant standard curve, which is linear over six orders of magnitude ($r^2=0.975$), is based on the critical threshold (C_t) values. C_t is determined for each curve as the fractional amplification cycle at which the baseline-corrected normalized fluorescence is determined to be statistically significant above background signal.

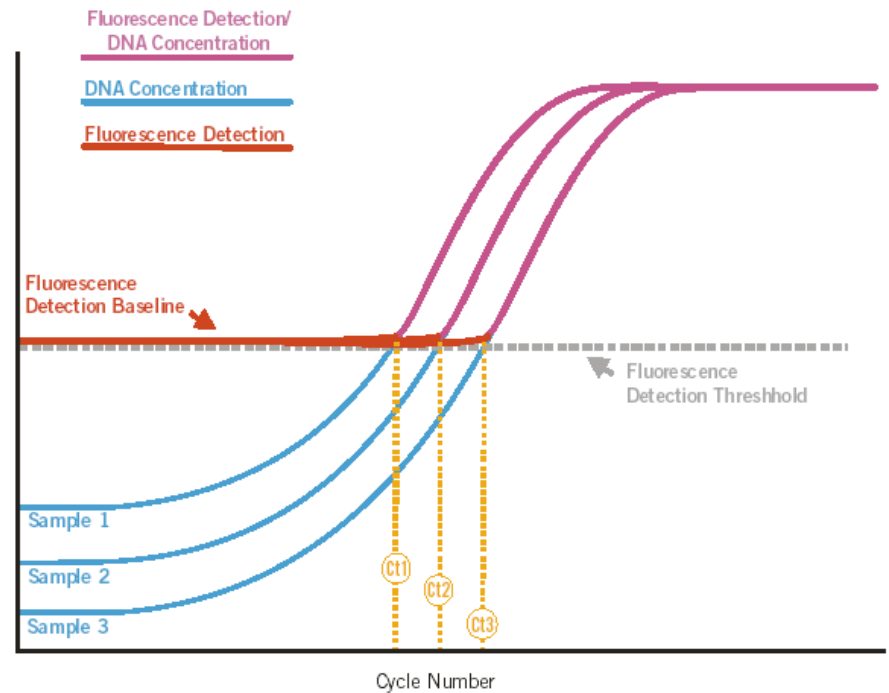
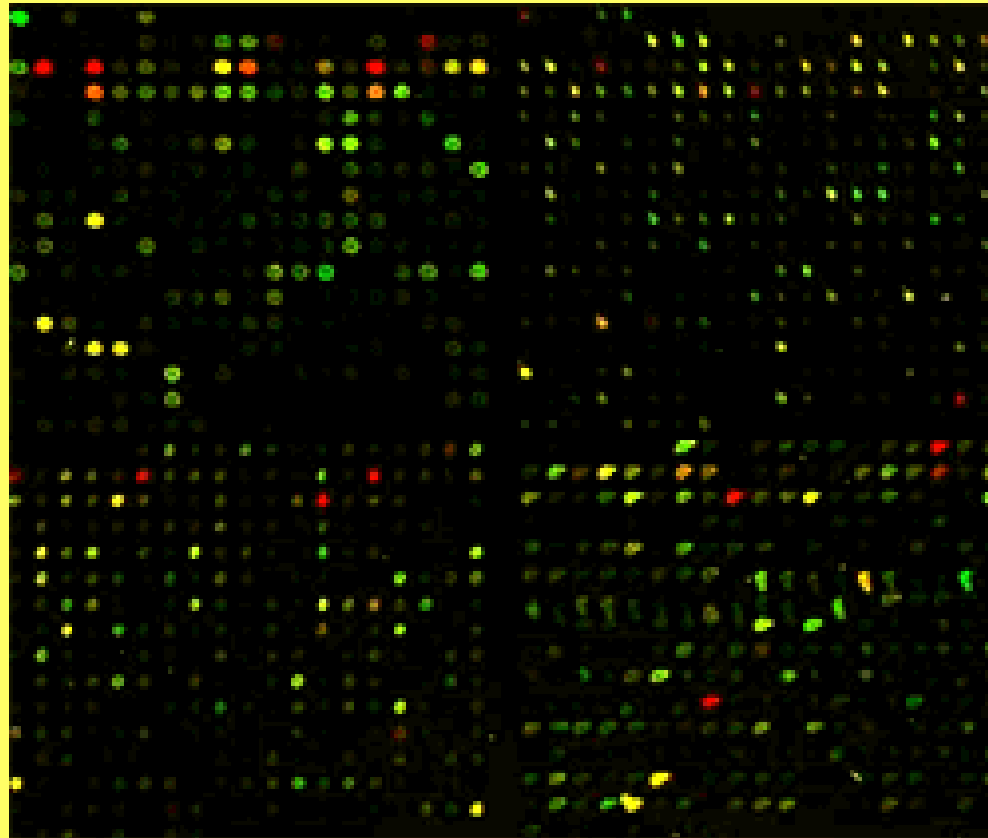


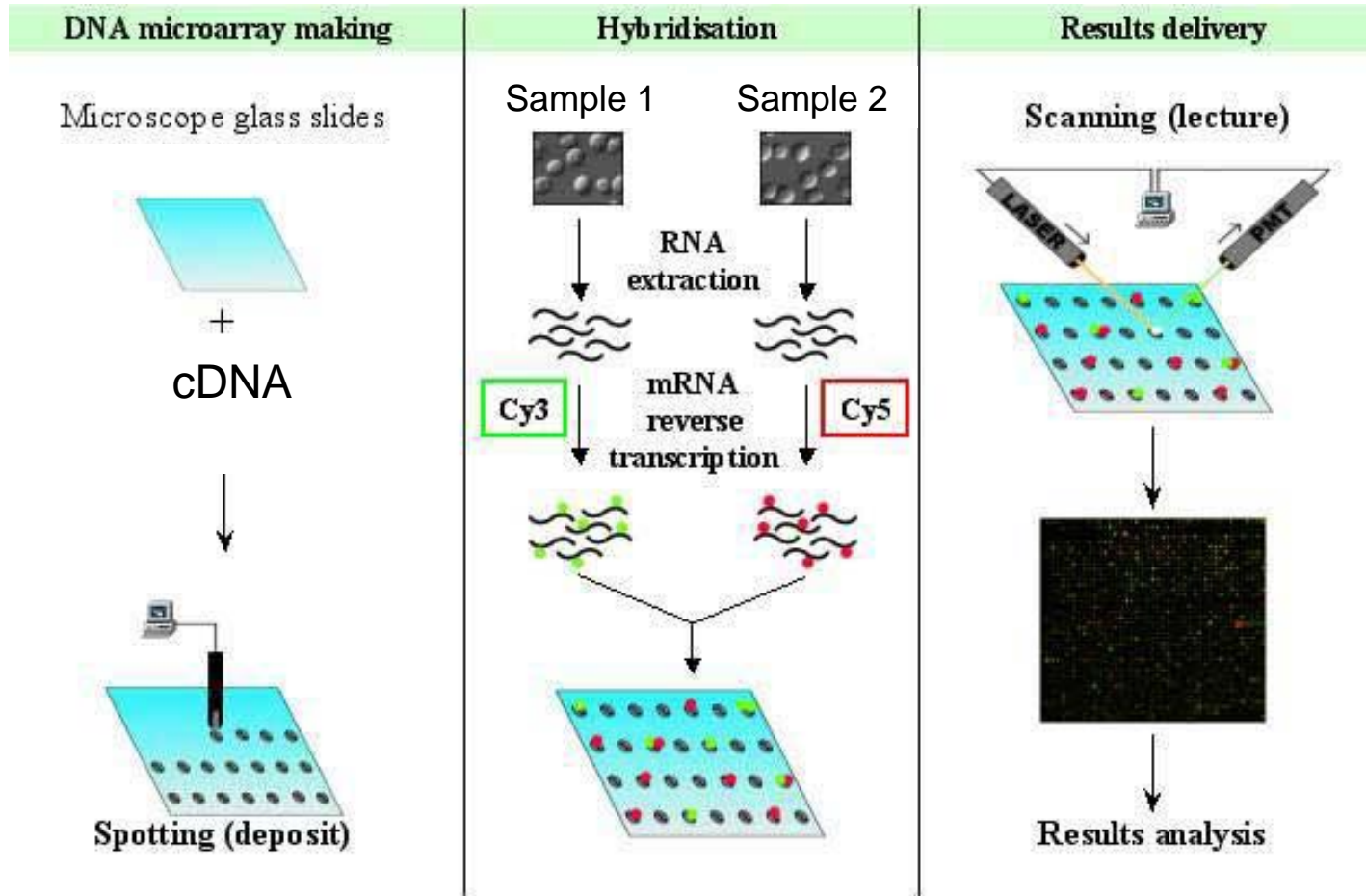
Figure 2

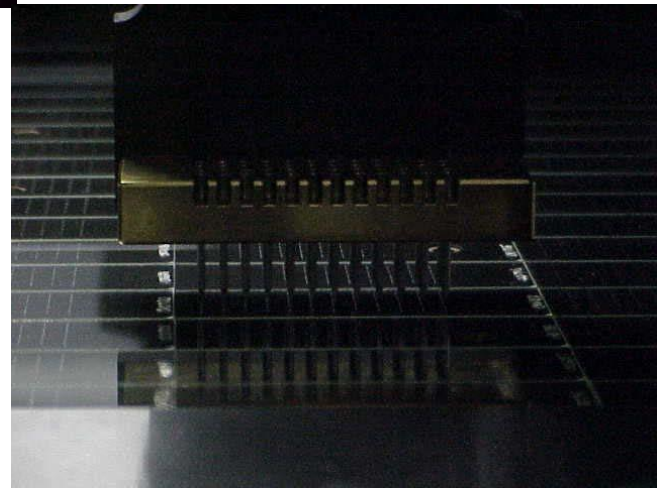
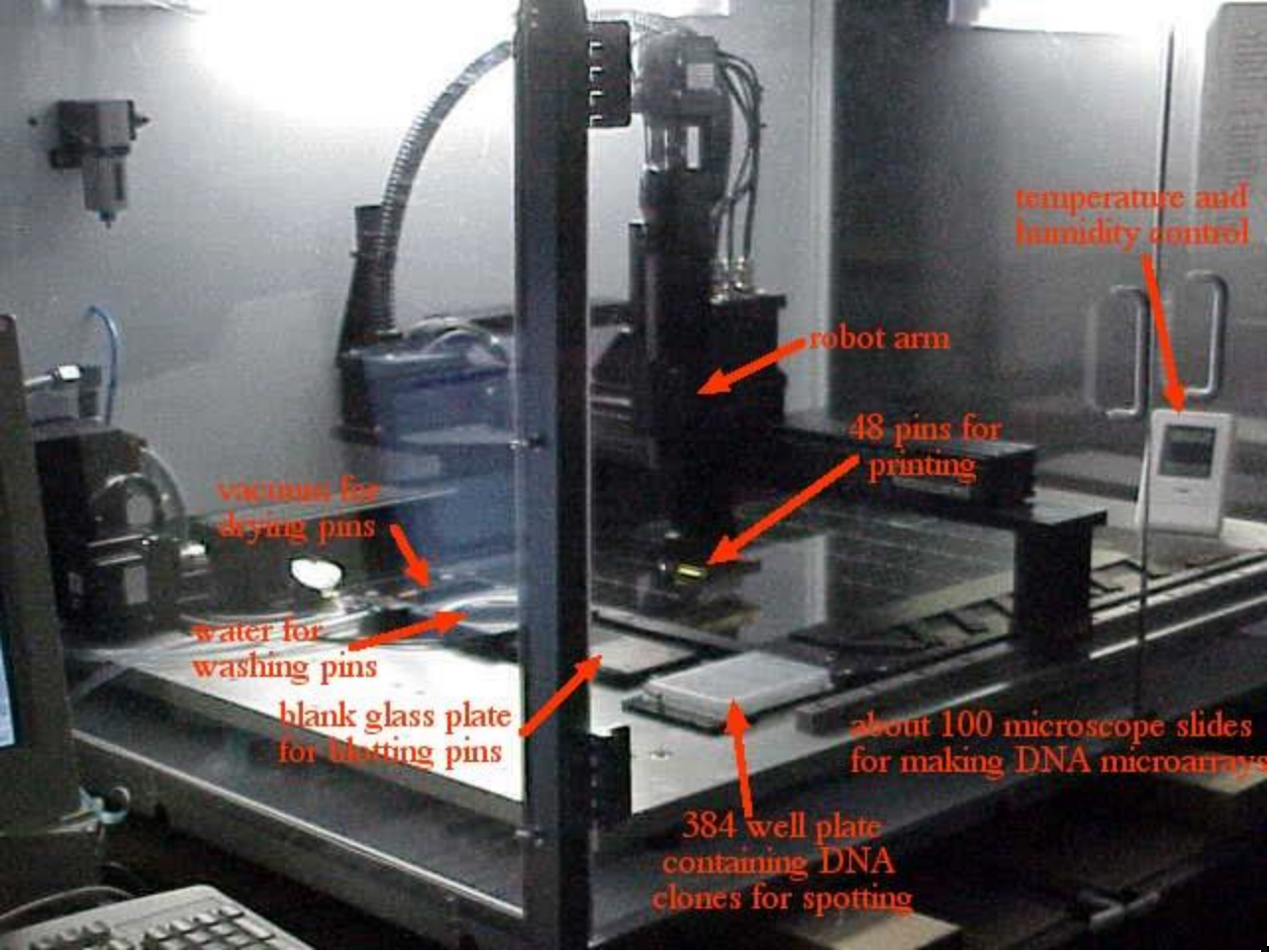
Principles of real-time fluorescence detection and QPCR target concentration measurements using threshold cycle (C_t). The C_t is inversely proportional to the initial copy number. Only when the DNA concentration has reached the fluorescence detection threshold can the concentration be reliably inferred from the fluorescence intensity. A higher initial copy number will correlate to a threshold cycle.

DNA microarray

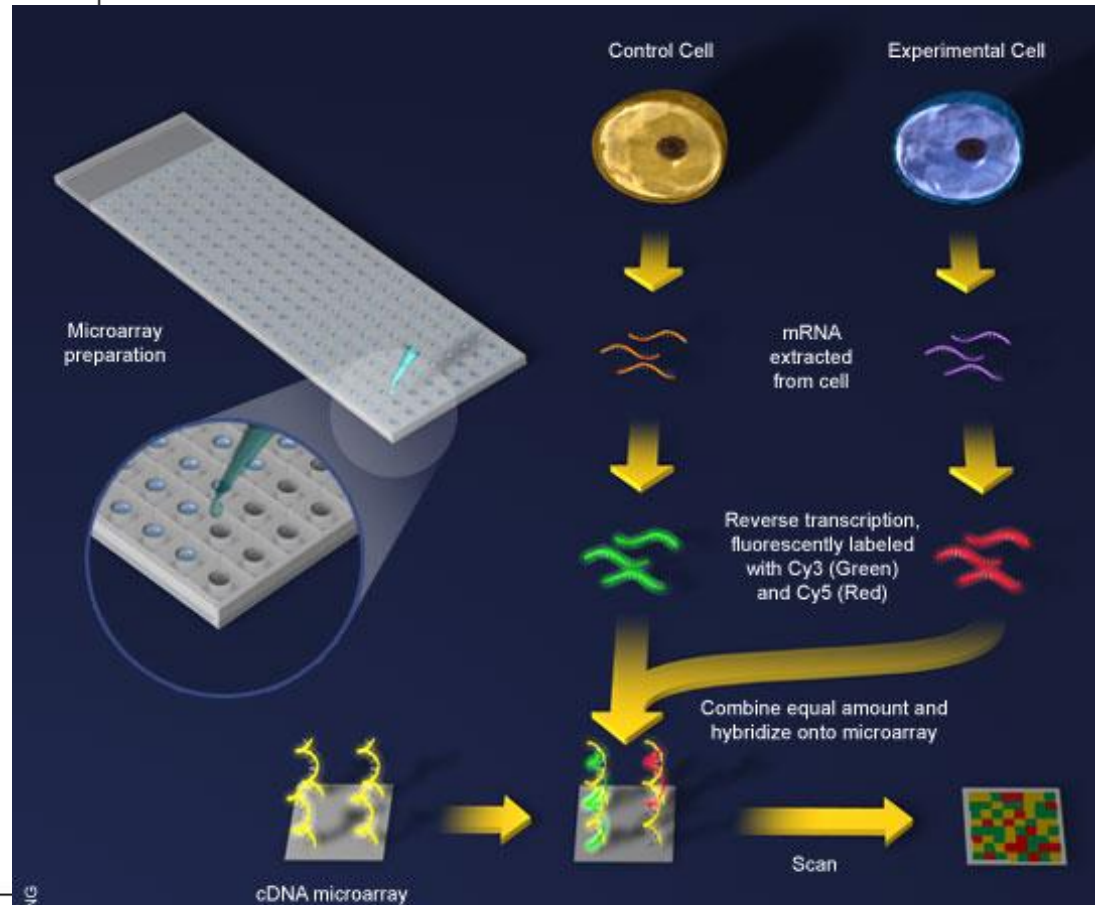
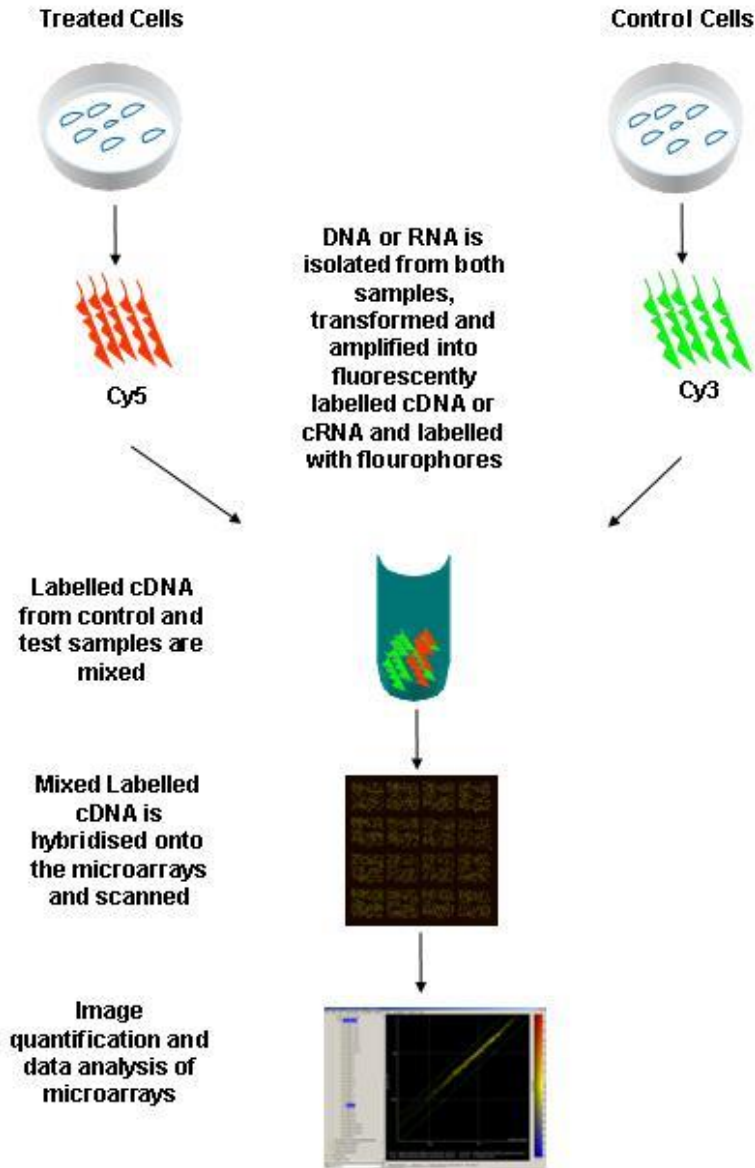


Expression profiling array platform



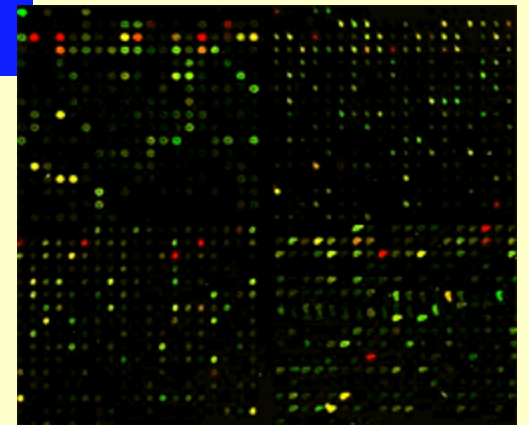
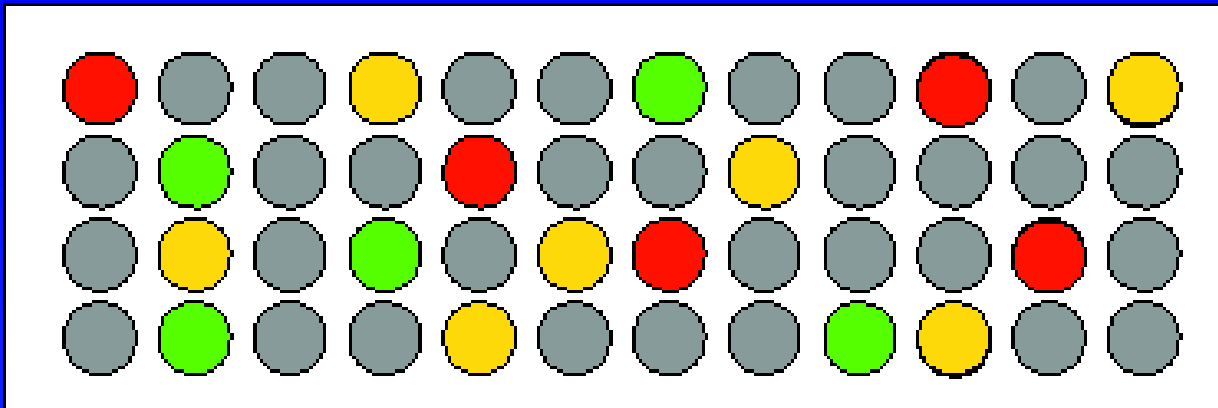
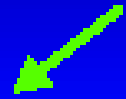
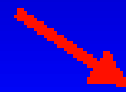
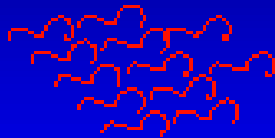
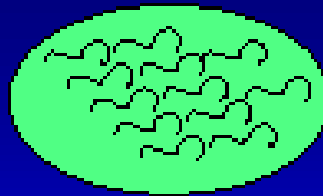
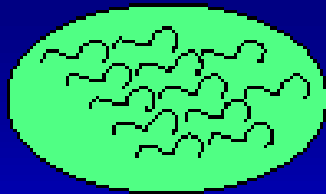


Target preparation and array hybridization

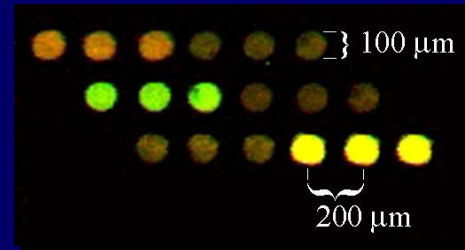
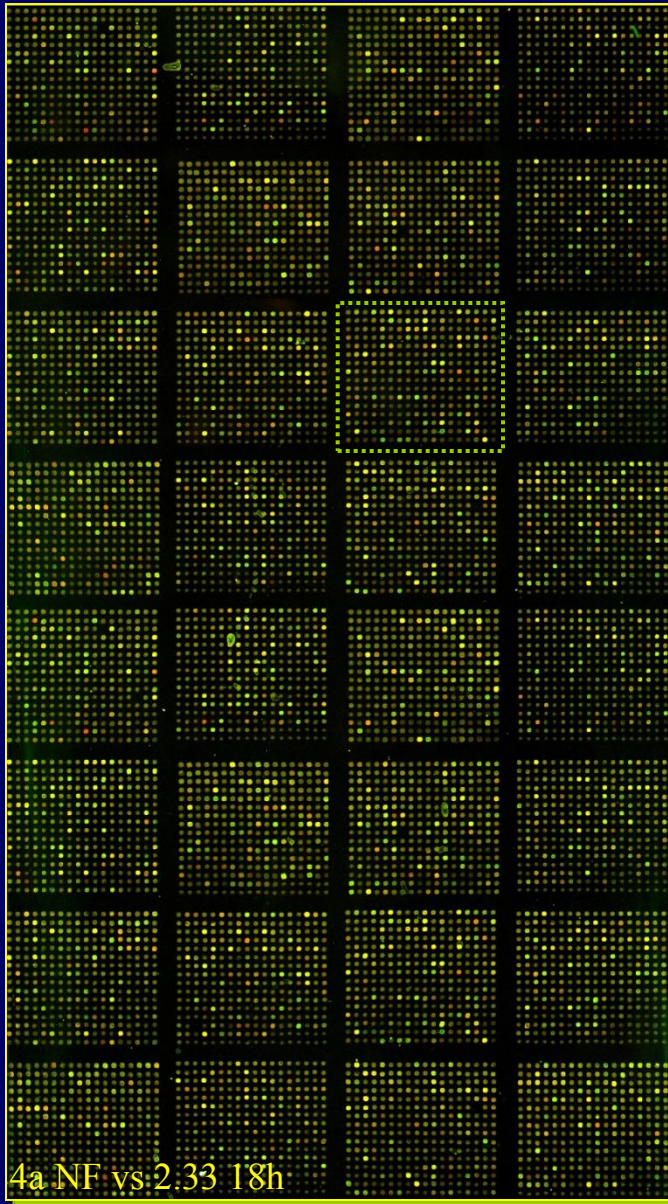
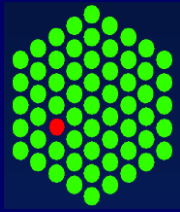


Condition A

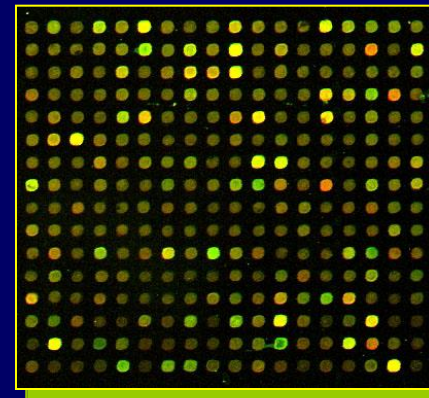
Condition B



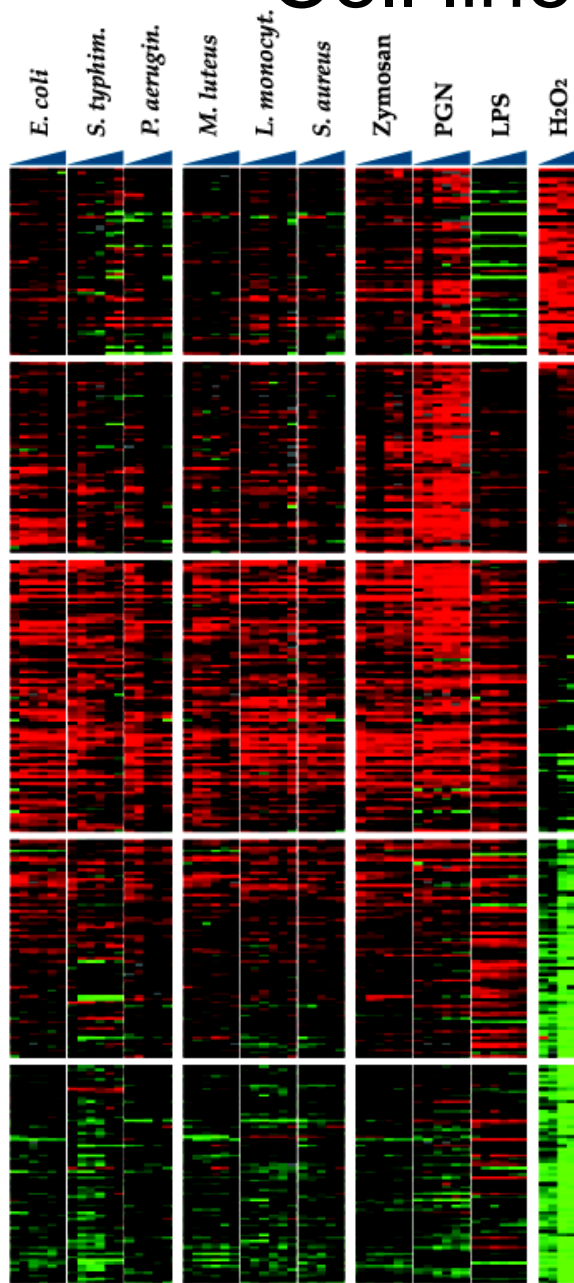
Anopheles gambiae cell line EST 4000 array



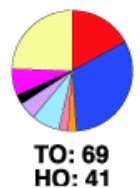
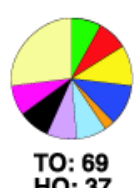
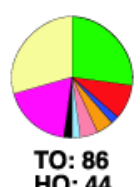
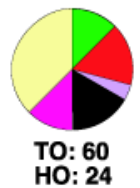
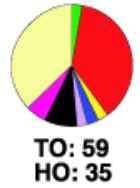
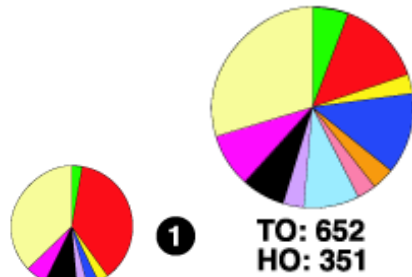
Cy-3 Cy-5



Cell line immune responses



REF: naive cell line



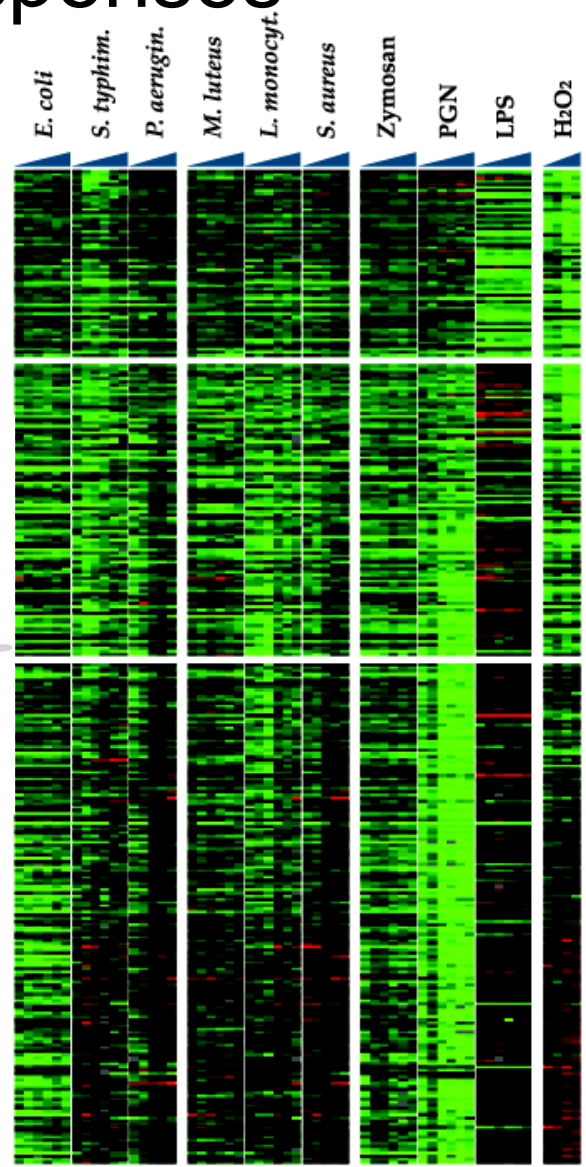
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2

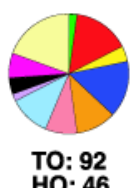
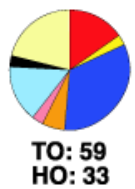
3

4

5



REF: naive cell line



6

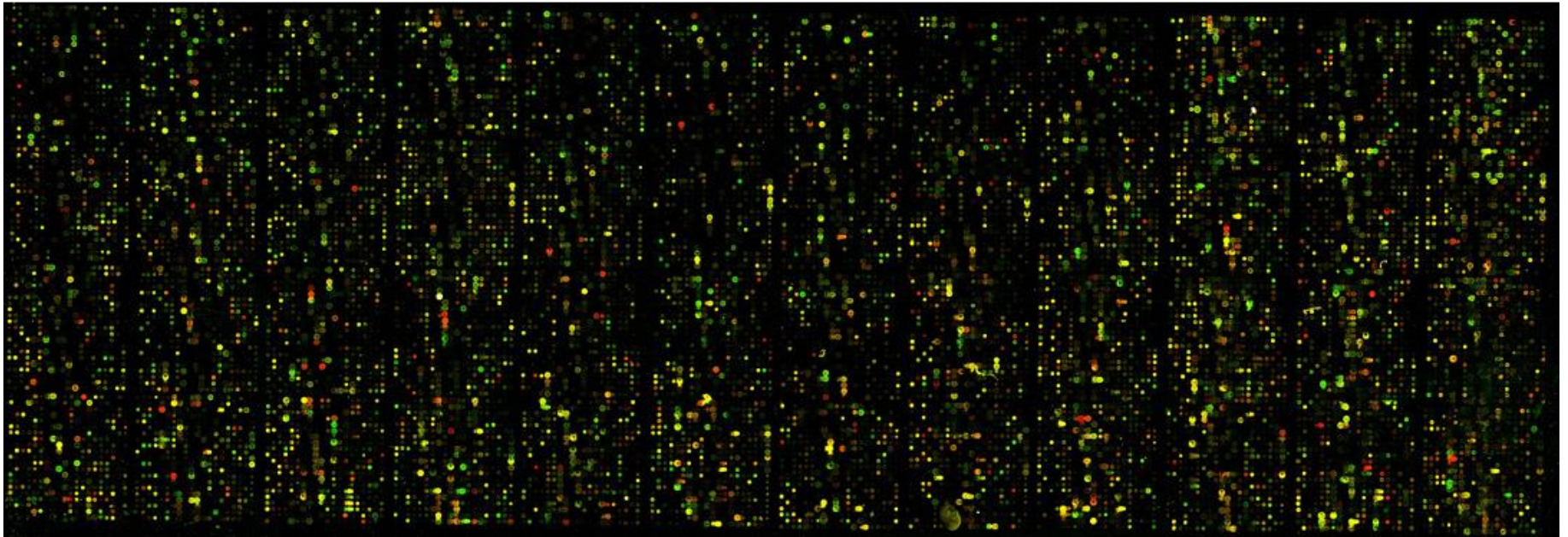
7

8

High density array platform

20K array platform

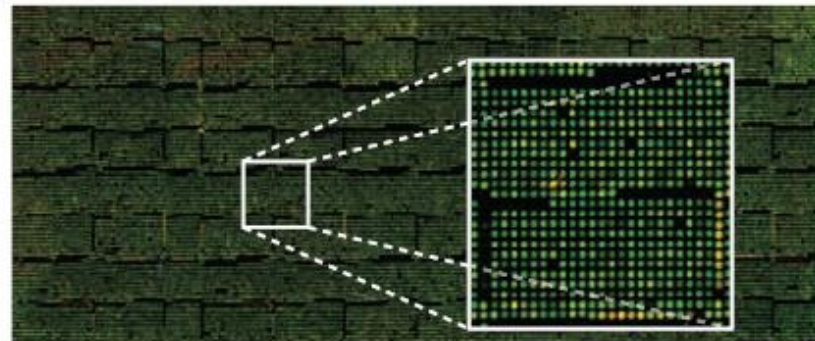
- *15,360 EST clones from a pooled mosquito stages library*
- *480 EST clones from an adult head library*
- *20K EST array construction (16K+4K)*
- *EST clustering & assembling database -AnoEST*



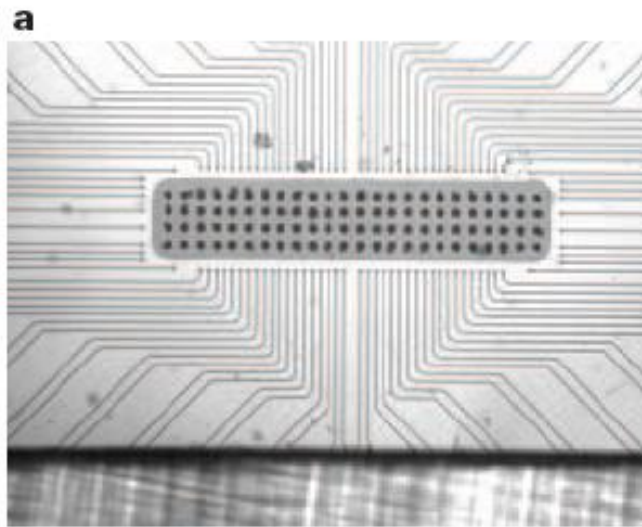
0.25–1nl of DNA solution is printed on a slide, creating spots that range from 100 to 150 μm in diameter



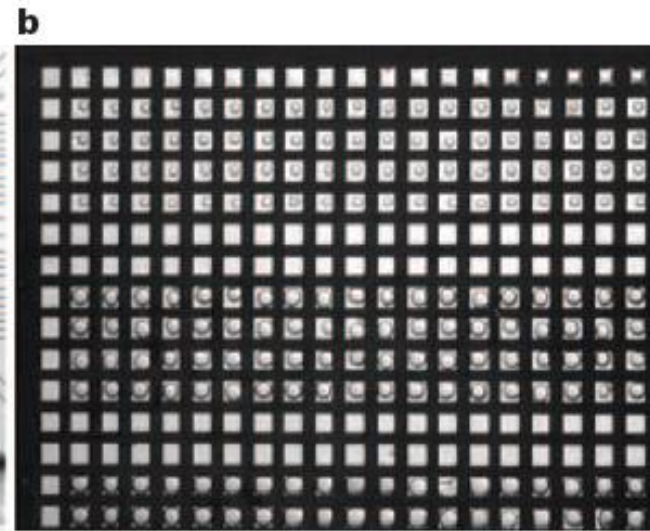
A typical fluorescent read-out of highly parallel hybridization on a cDNA microarray



- 0.25 nl of DNA, spots range of 100 μm in diameter, 50,000 DNA/array
- 20 μm^2 in diameter (5 μm^2), 400,000 DNAs/array



a) Nanodispenser



b) High-density nanowell plate

- Nanowell plates: volumes of 1 nl
- miniaturized protein assays.
- Cell-free transcriptions and translations assays transfer into wells

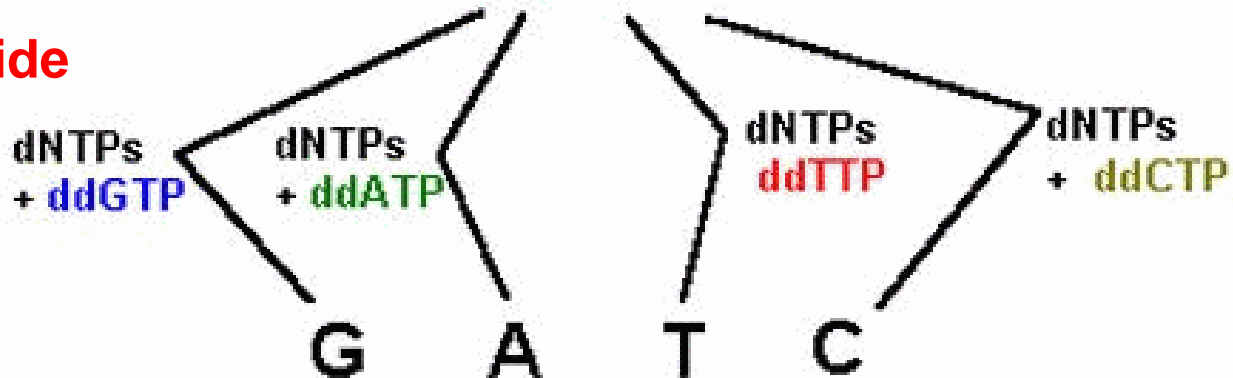
Sequencing Approaches



Sanger ddNTP Chain Termination Sequencing

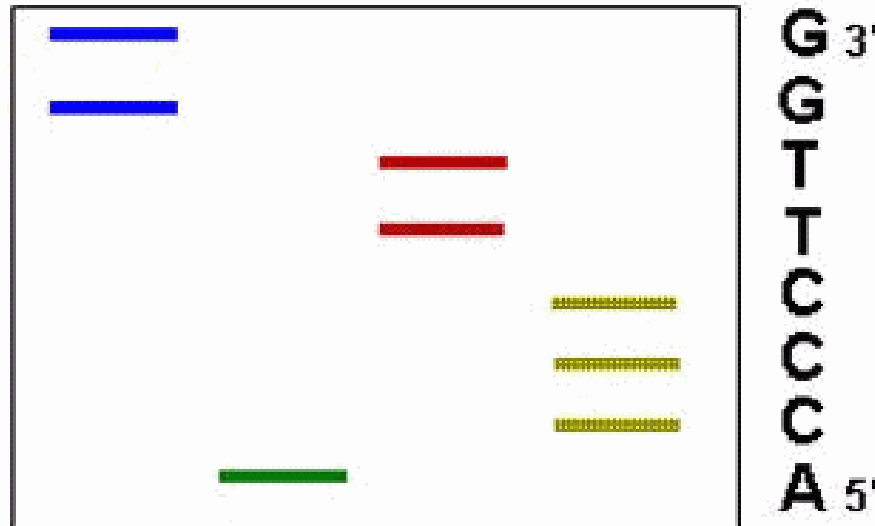
Template: 3' -----GCATTGGGAACC----- 5'
 Primer: 5' -----CGTA 3'

dideoxynucleotide



Analogues of the dNTPs which **lack the hydroxyl group at the 3' position.**

Incorporation of a ddNTP into a growing DNA strand gives rise to **termination** of strand synthesis.



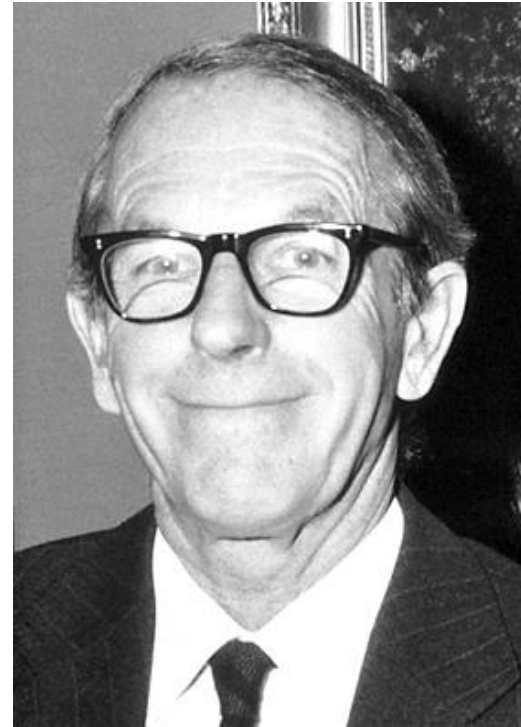
Frederick Sanger

The Nobel Prize in Chemistry 1958



Prize motivation: "for his work on the structure of proteins, especially that of **insulin**".

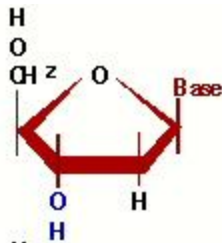
The Nobel Prize in Chemistry 1980



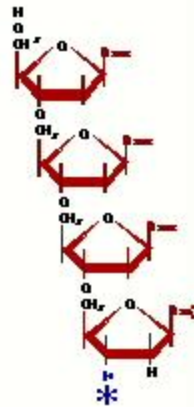
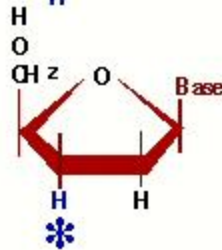
Prize motivation: "for their contributions concerning the determination of **base sequences in nucleic acids**"

Sequencing

Normal nucleotides:



Dideoxy Chain Terminators:



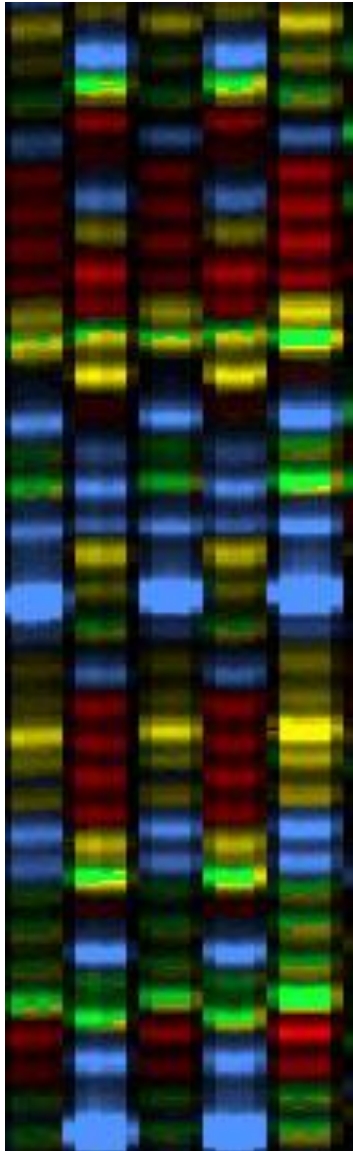
DNA Polymerase reads the template strand and synthesizes a new second strand to match:

5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCTACCGAT
 3' - ATGCGCCATTGCCATACAGCTGGCAATTCGATGGCTAGAGATCAA - 5'



IF 5% of the T nucleotides are actually dideoxy T, then each strand will terminate when it gets a ddT on its growing end:

5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCTACCGAT•
 5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCT•
 5' - TACGCGGTACGGTATGTTTCGACCGTTT•
 5' - TACGCGGTACGGTATGTTTCGACCGTT•
 5' - TACGCGGTACGGTATGTTTCGACCGT•
 5' - TACGCGGTACGGTATGTTTCGACCGT•
 5' - TACGCGGTACGGTATGTT•
 5' - TACGCGGTACGGTATGT•
 5' - TACGCGGTACGGTAT•
 5' - TACGCGGTACGGT•
 5' - TACGCGGT•



Mezcla dNTP

Tinte terminador ddUTP



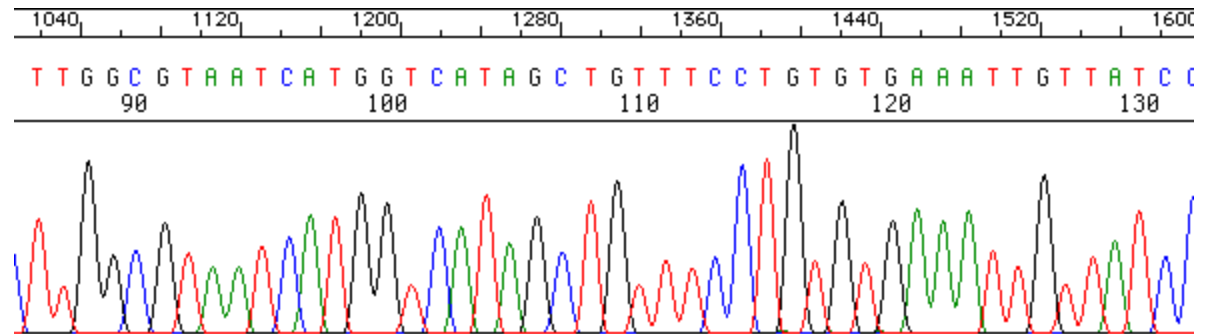
Tinte terminador ddGTP



Tinte terminador ddCTP



Tinte terminador ddATP

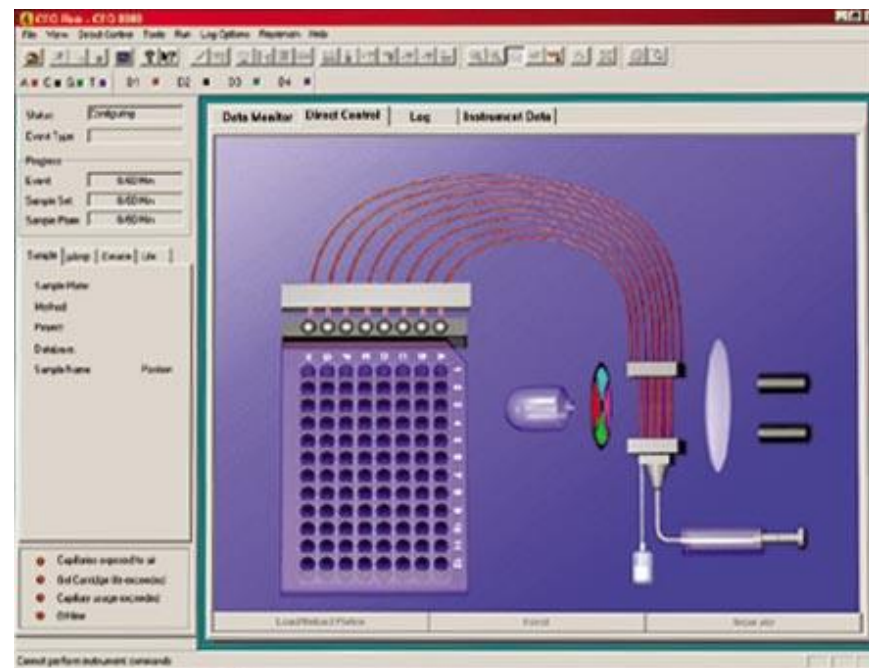


Sequencing

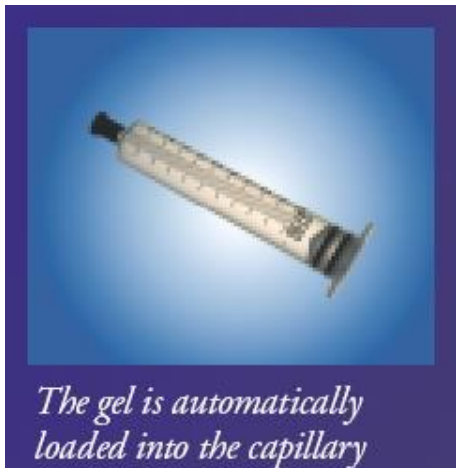
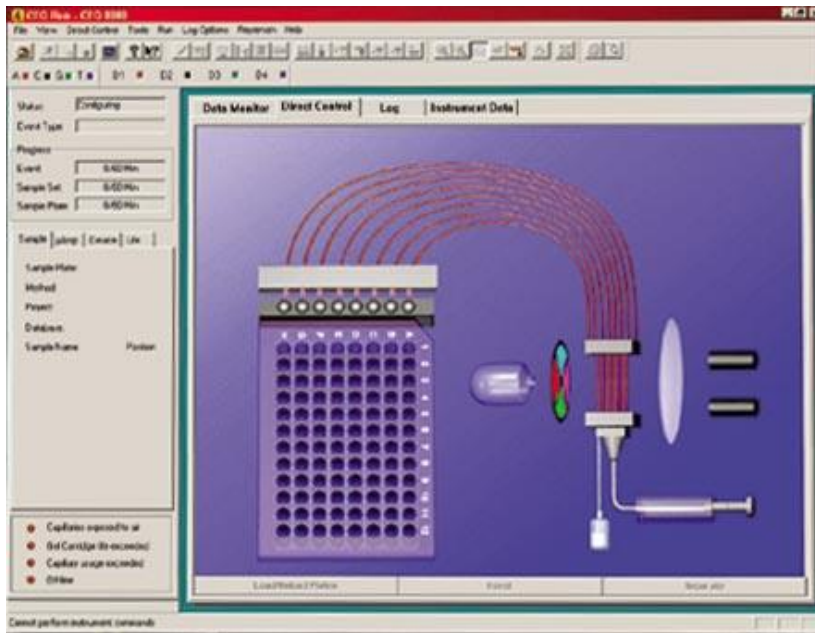
A fully automated sequencing system. This system automatically fills the capillary array with a gel, denatures and loads the sample, applies the voltage program, and analyzes the data.



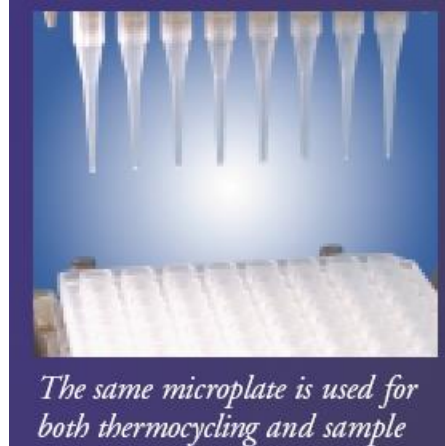
Sequencing



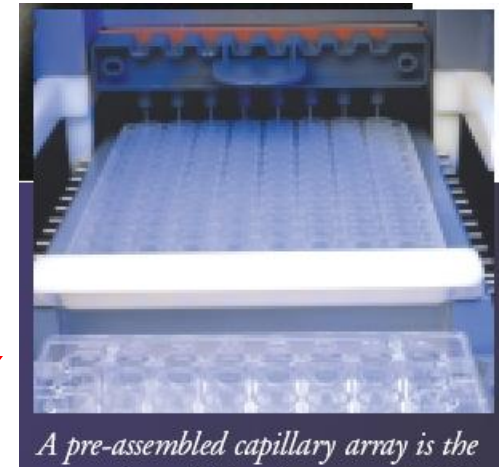
Sequencing



The gel is automatically loaded into the capillary array,

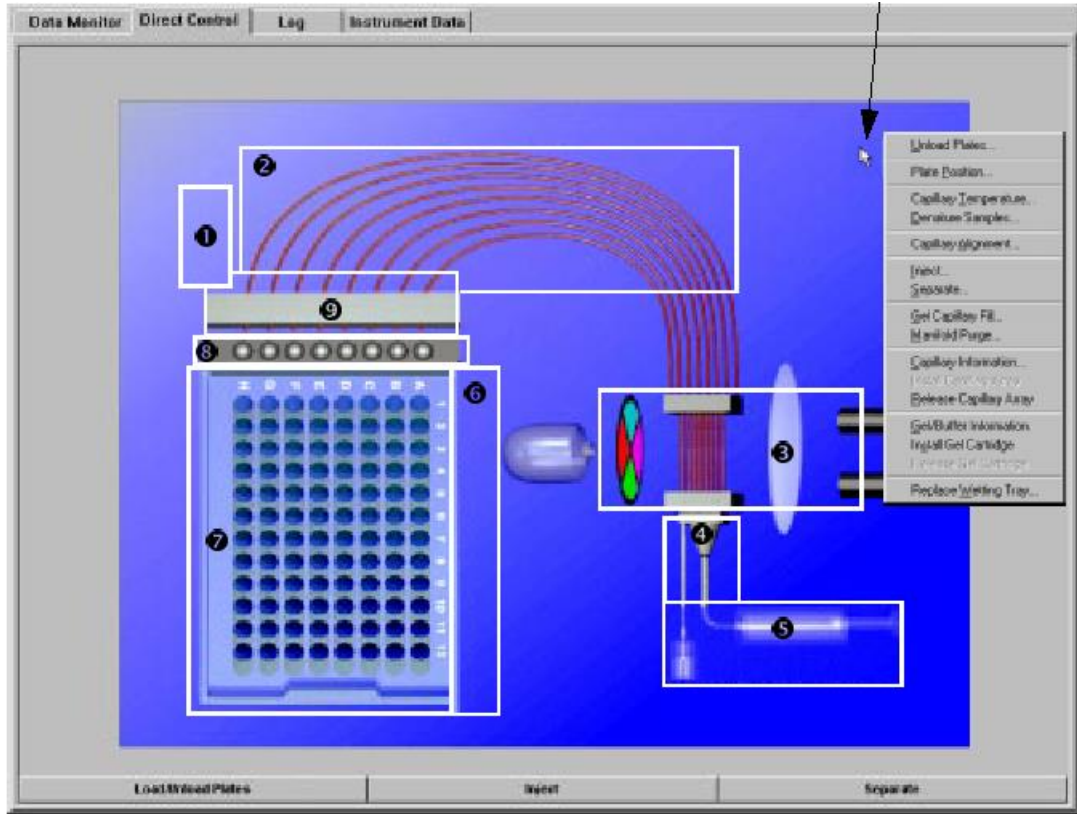
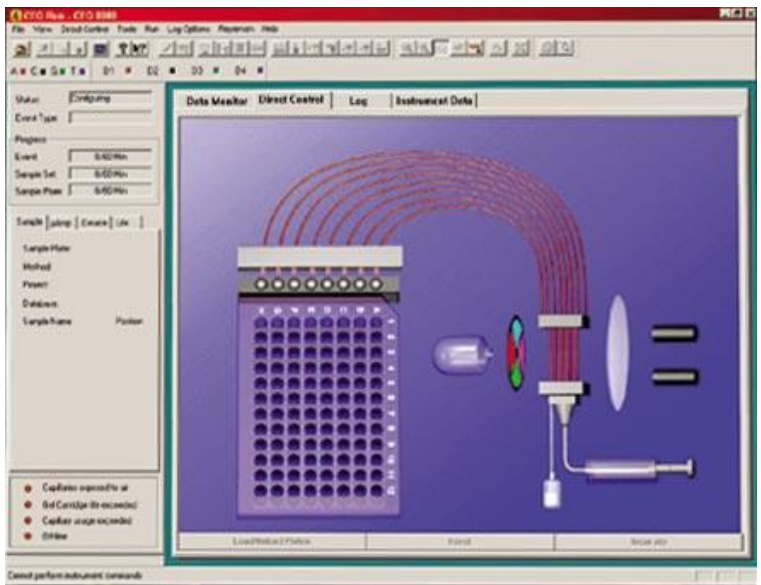


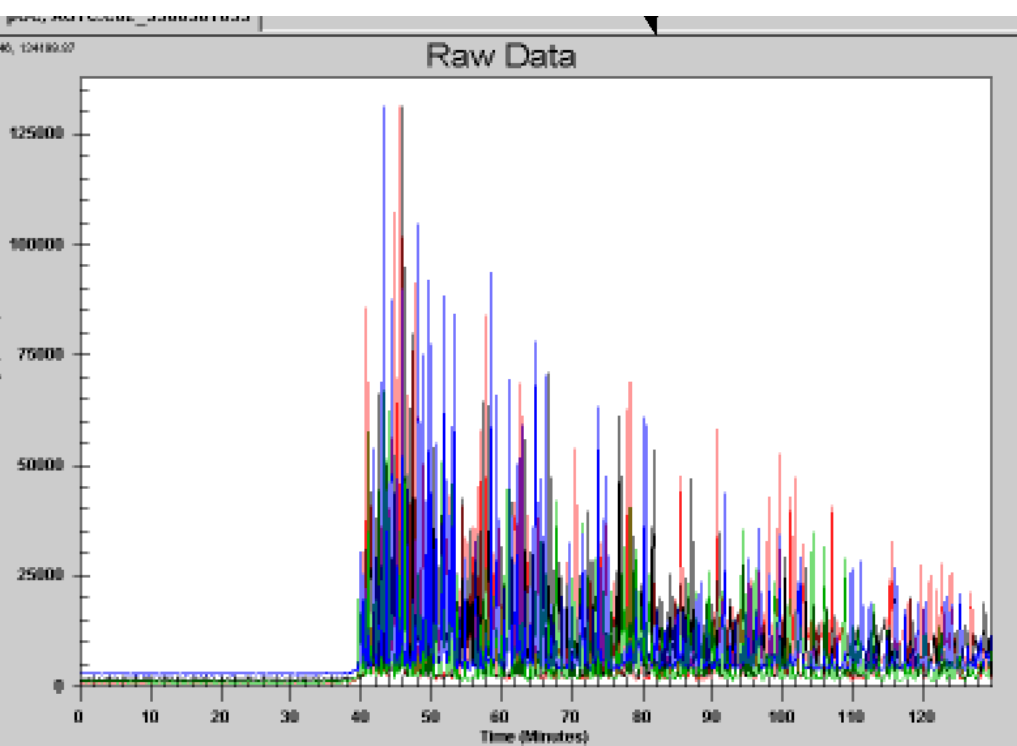
The same microplate is used for both thermocycling and sample introduction,



A pre-assembled capillary array is the separation vehicle.

Sequencing

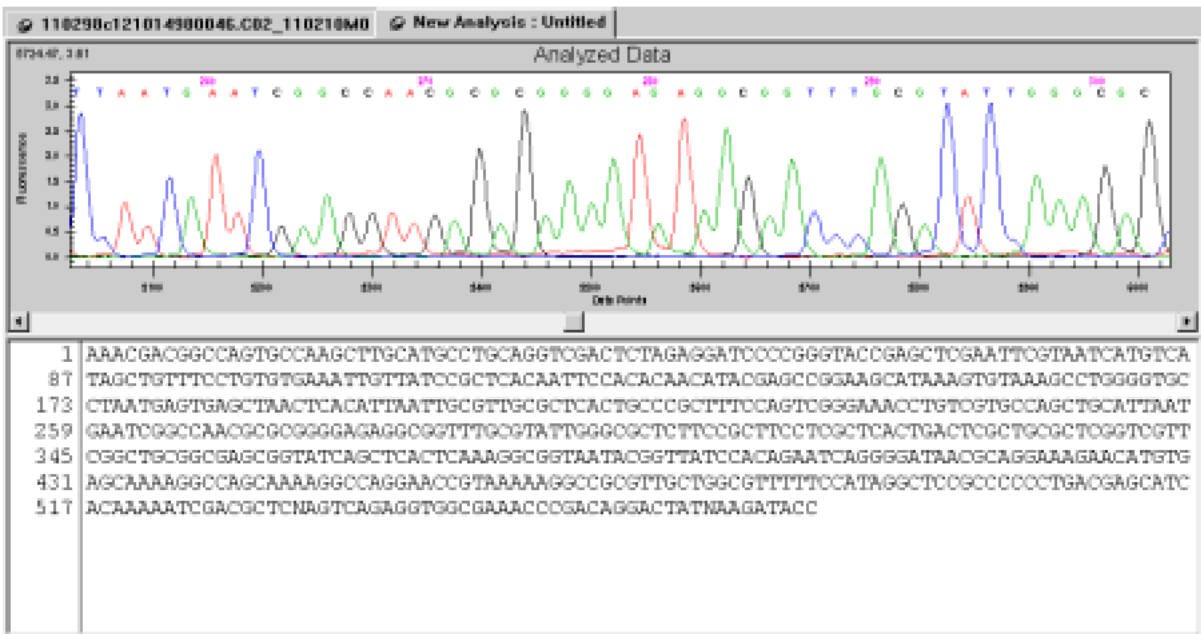
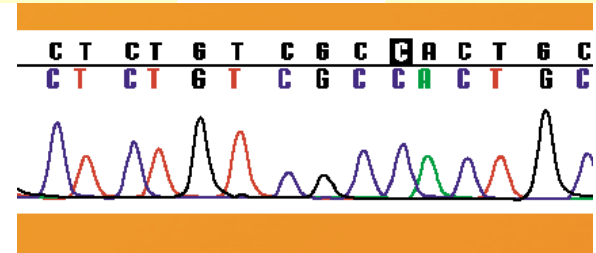




Sequencing

Legend for sequencing dyes:

- ddUTP (Red dot)
- ddGTP (Black dot)
- ddCTP (Blue dot)
- ddATP (Green dot)



```

filename="AECAM_B12_P2.seq"
GGCCATTACGGCCGGGGACAGTATTTCTTGTCATTCCACAAGGATAGAT
TGCTTTGGCCCTGTACCTTACCG
TCCTGGTAAATCTTCTGTCACAAACCAGTGGCAATCTTCAAAGATCAAC
AACGACAACAACAGAGAAGACGC
CTACGGCGGATTCAACTAAGATCGGTGCTTGTACTTCGGCACTCTCGT
GTACGTGAATGCCACAAAGGATC
AGGAACTCATCAATGGCGTCTTCGACGCCACTTTGAATCGCTTGACCAA
GCTGAATGTGGAAGATGTCCACG
AGAAGAACGTCGGTCGCTACGATATGACCACTCTGCTCTGCTGGGCGG
TCAATAATTACTTGCTTTATGATA
AATATAACGAAAAGCGGATATGGTGTATAAGCTTGCGAAGAAGTTCTA
TCCCGAACGAAACGATAAAGCCA
AAACGGAACGGGAAATACTGGCTCGGCAACCCTGTAAGGCAAATCTGC
CGGTAACGGTCATCACGGGATTCG
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TACTTGGACGATGAATAAAAAATC
TAATACTATGTCCAAA

```

14

```

;
filename="AECAM_B2_P2.seq"
TCGGCCATTACGGCCGGGGAGTGTATAAATCCGTATCAGTCAATTAGAA
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AGAAGATGATTTTTCTCGTTT
GCATCACCATGCTTACAGCCGCCAACAAATTTCAAGAAACGATATTTGT
ACATAACAACATACACGTTACG
TTCTTGAAGCATGGAGACAGTGTCAAGCCATTGGTCTTCGCTTAGCTA
CCATTACATCTGAGGAGGATAGT
AAACTCATGCAGGAAACCATTGCAAATTTCAACTAACACGAAAGGTCCTT
GGTATATCGGTGGCACCGACTTA
GGGAATGAAGGACACTTTGTTTGGATATCTACCAATAAGCCTGTCCGGAC
ACATGACAGGATACTTCAACTAT
TCACCCGGACAACCAGACAATGCTGGAGGAAGCGAAAACGTCTCGAG
ATCGGTGCGTGGGGTGGTGTAGTC
TGGAATGATGTGCCATGCGATGCAAAGCTACCCTACATTTGTAATCTG
TCNCTCCAAACTGGTCGTAATAAAA
ACAATGAACATAAATAAATCATNTGTTNGTTTATNANTNGAACCCCTTTC

```



```

>AECAL_A1_P1.seq L776 N%1 CAP11 SZ757 AT49 NF%1
gi|21288014|gb|EAA00335.1| (AAAB01008986) agCP9558 [Anopheles ga... 226 2e-058
gi|21291336|gb|EAA03481.1| (AAAB01008704) ebiP2250 [Anopheles ga... 194 8e-049
gi|21358457|ref|NP_651703.1| (NM_143446) CG1907 gene product [Dr... 153 2e-036
gi|7292529|gb|AAF47931.1| (AE003482) CG7514-PA [Drosophila melan... 130 1e-029
gi|21355497|ref|NP_647924.1| (NM_139667) CG18418 gene product [D... 129 5e-029
---->Results for cdd:
gn|Pfam|pfam00153 mito_carr, Mitochondrial carrier protein 105 8e-024

```

```

>AECAL_A11_P1.seq L857 N%9 SN631 SZ631 AT49 NF%2
gi|22987835|gb|ZP_00032908.1| (NZ_AAAC01000288) hypothetical pro... 33 3.5
gi|7662182|ref|NP_055649.1| (NM_014834) KIAA0563 gene product [H... 32 6.0
gi|20344445|ref|XP_109199.1| (XM_109199) nuclear receptor co-rep... 32 7.8
gi|20881184|ref|XP_126352.1| (XM_126352) nuclear receptor co-rep... 32 7.8
gi|6754802|ref|NP_035438.1| (NM_011308) nuclear receptor co-repr... 32 7.8
---->Results for cdd:
No matches to database

```

```

>AECAL_A12_P1.seq L685 N%3 CAP20 SZ654 AT49 NF%3
gi|16799875|ref|NP_470143.1| (NC_003212) similar to two-componen... 32 6.5
---->Results for cdd:
No matches to database

```

```

>AECAL_A2_P1.seq L499 N%5 SN153 SZ153 AT52 NF%5
No matches to database
---->Results for cdd:
No matches to database

```

```

>AECAL_A4_P1.seq L134 N%2 CAP5 SZ119 PA96 AT55 NF%0
No matches to database
---->Results for cdd:
No matches to database

```

```

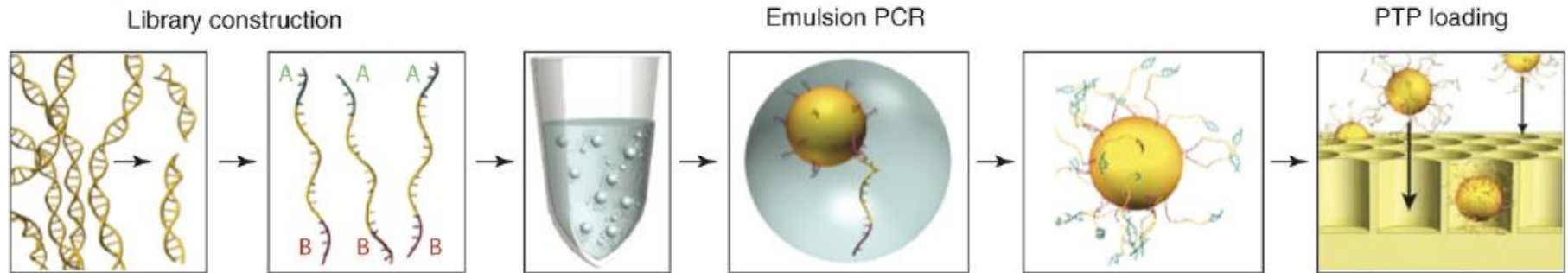
>AECAL_A5_P1.seq L788 N%2 CAP14 SZ766 AT54 NF%2
gi|18568320|gb|AAL76030.1|AF466607_1 (AF466607) putative 30.5 kD... 356 2e-097
gi|11497204|ref|NP_051320.1| (NC_000951) conserved hypothetical ... 35 1.3
gi|23025069|gb|ZP_00064247.1| (NZ_AAAO01000060) hypothetical pro... 34 1.7
gi|20808196|ref|NP_623367.1| (NC_003869) Methyl-accepting chemot... 34 1.7
gi|13358128|ref|NP_078402.1| (NC_002162) unique hypothetical mem... 34 2.3
---->Results for cdd:
No matches to database

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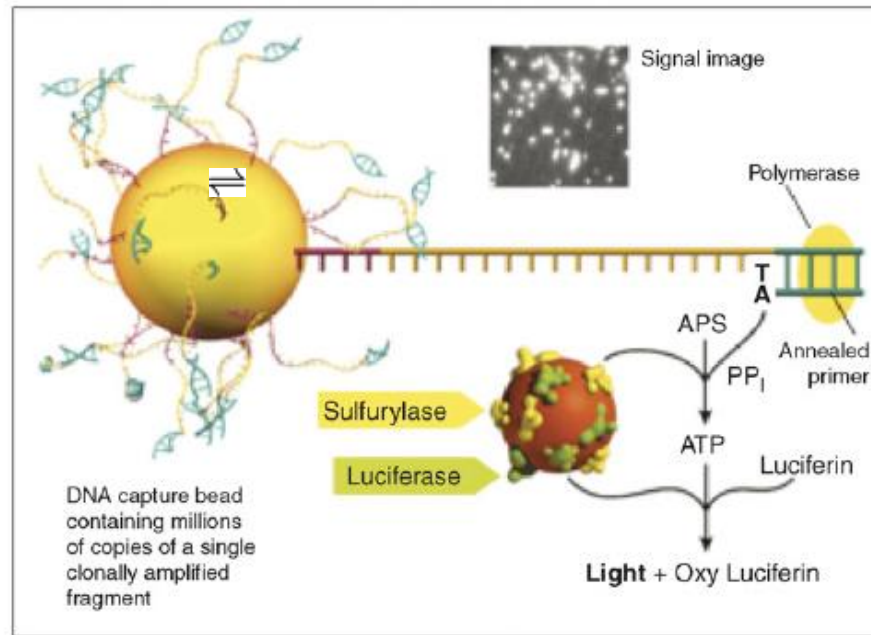
Sequencing

NEXT generation: 454 sequencing

Roche (454) GSFLX Workflow:



250 bp/sample
100 Mb/7 h run
\$2000/100 Mb



adenosine 5' phosphosulfate

Pyrosequencing reaction

TRENDS in Genetics

Figure 1. 454 Workflow: library construction ligates 454-specific adapters to DNA fragments and couples amplification beads with DNA in an emulsion PCR to amplify fragments before sequencing. The beads are loaded into the picotiter plate (PTP). The bottom panel illustrates the pyrosequencing reaction that occurs on nucleotide incorporation to report sequencing by synthesis.

I. Template preparation

- 1. DNA fragmentation**
- 2. DNA immobilization**
- 3. DNA amplification**

II. Sequencing

III. Analysis

I. Template preparation

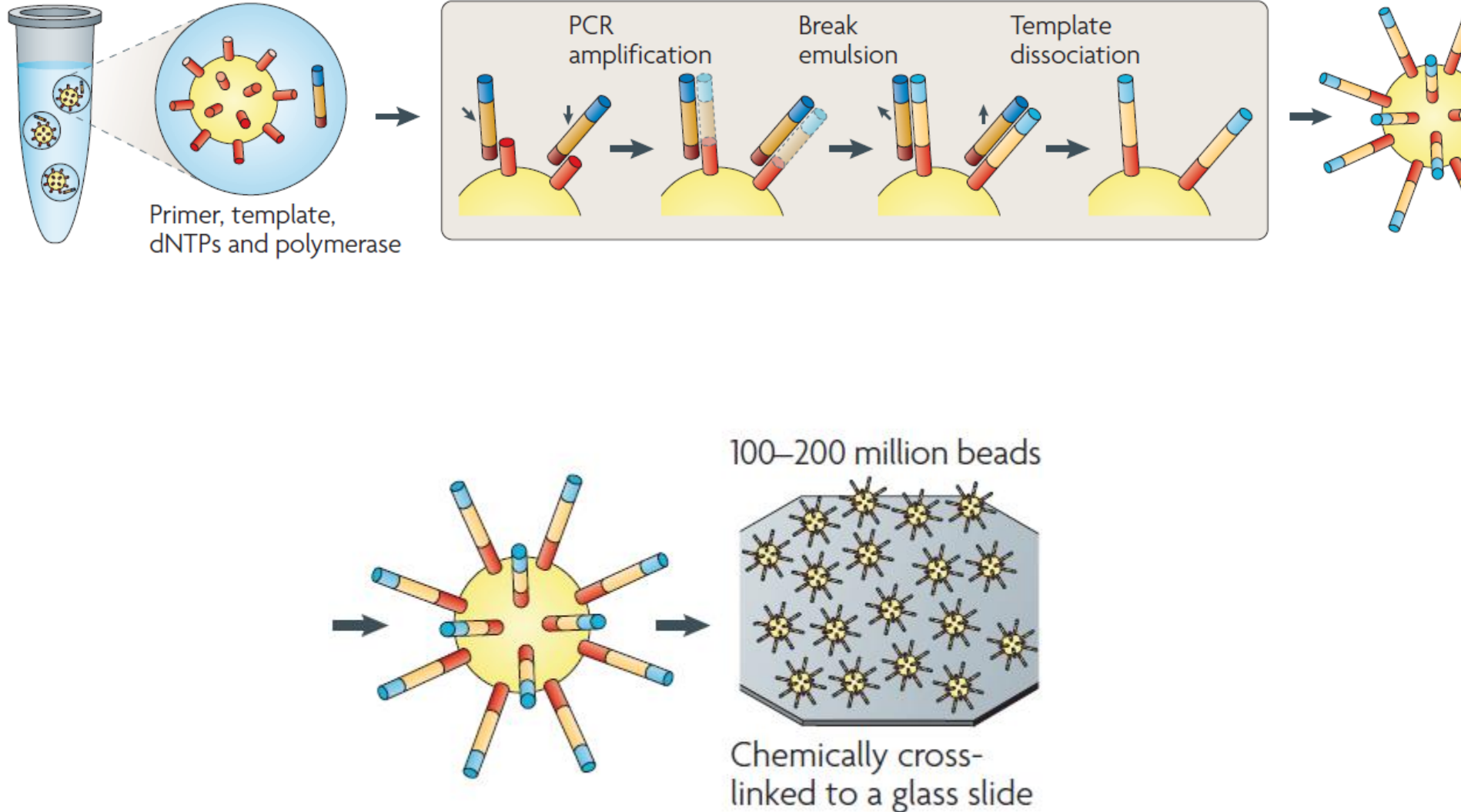
1. Emulsion PCR

2. Solid-Phase amplification

Emulsion PCR

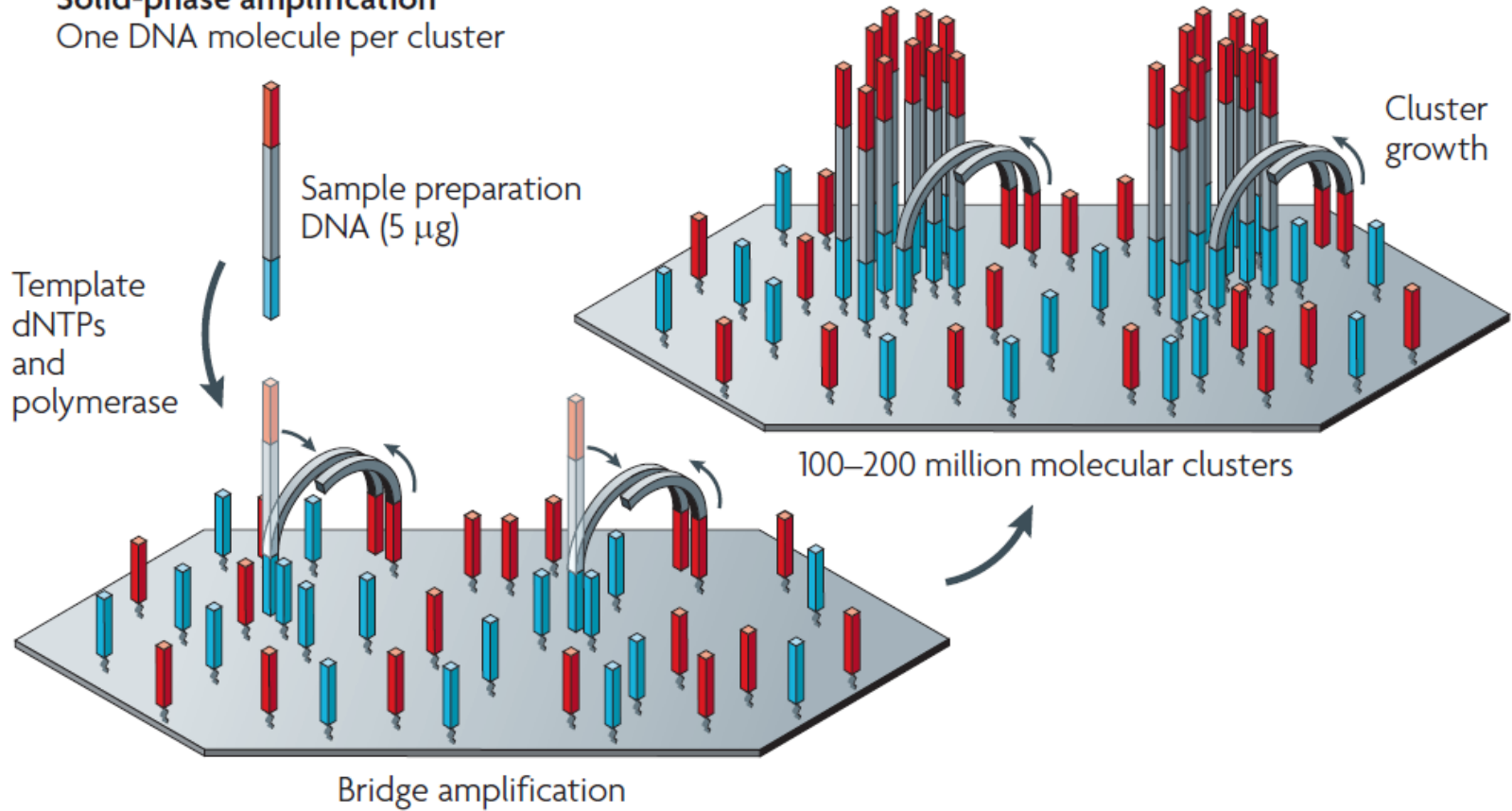
a Roche/454, Life/APG, Polonator Emulsion PCR

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



Solid-Phase amplification

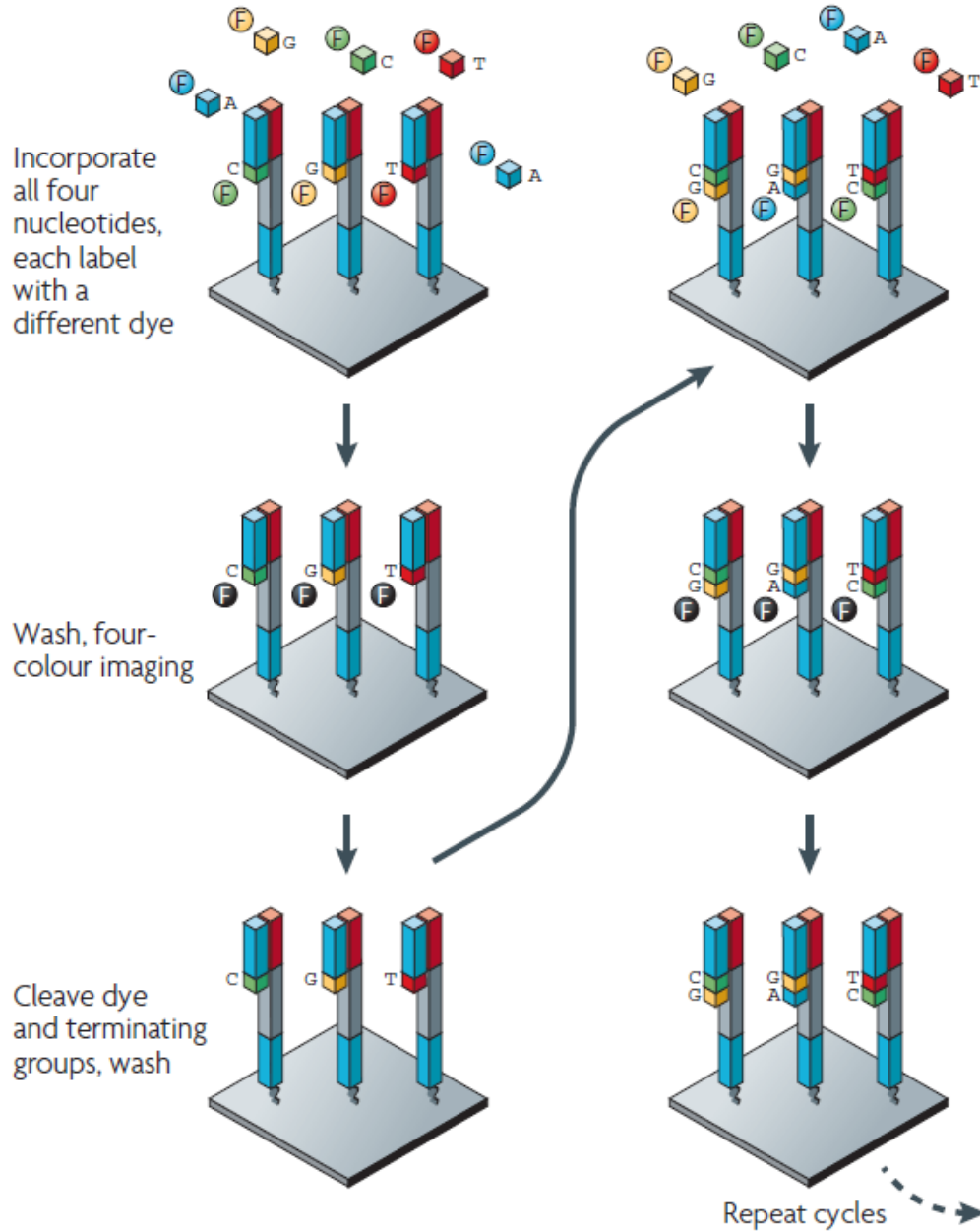
b Illumina/Solexa
Solid-phase amplification
One DNA molecule per cluster



II. Sequencing

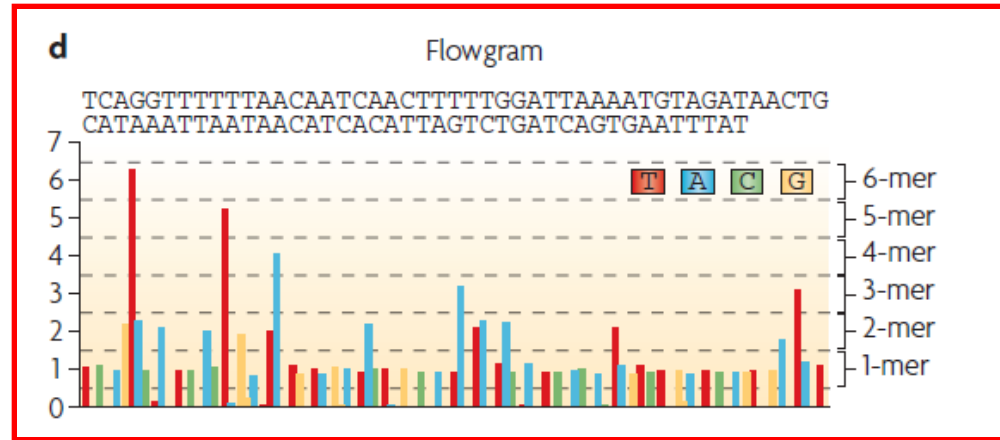
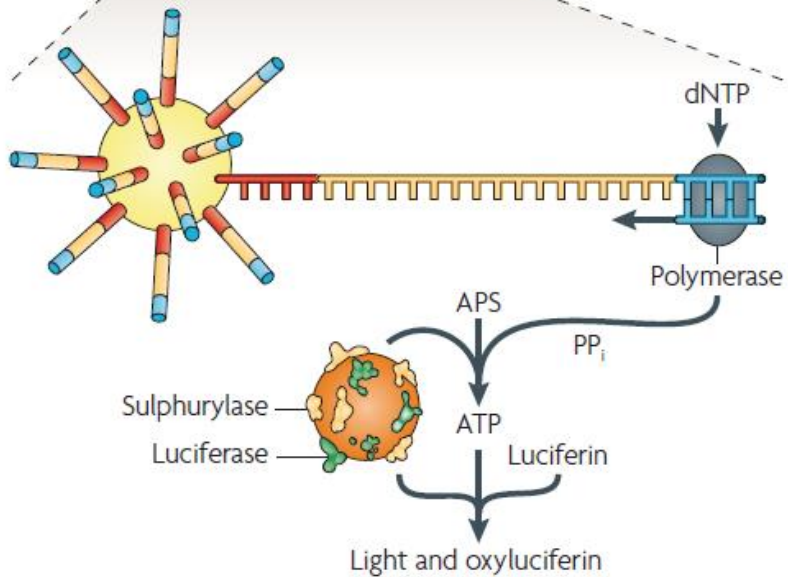
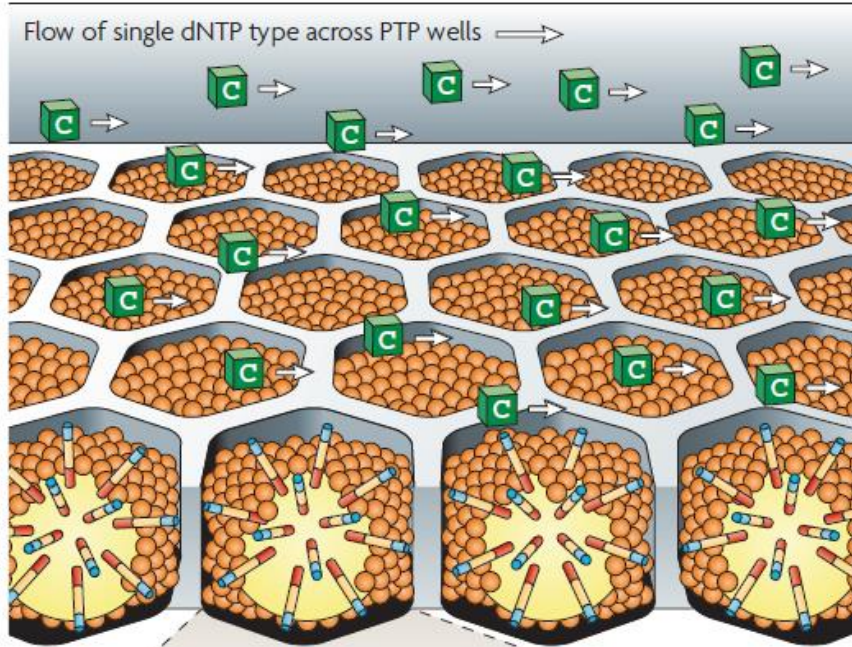
- 1. Cyclic reversible termination**
- 2. Single nucleotide addition (Pirosequencing)**

a Illumina/Solexa — Reversible terminators



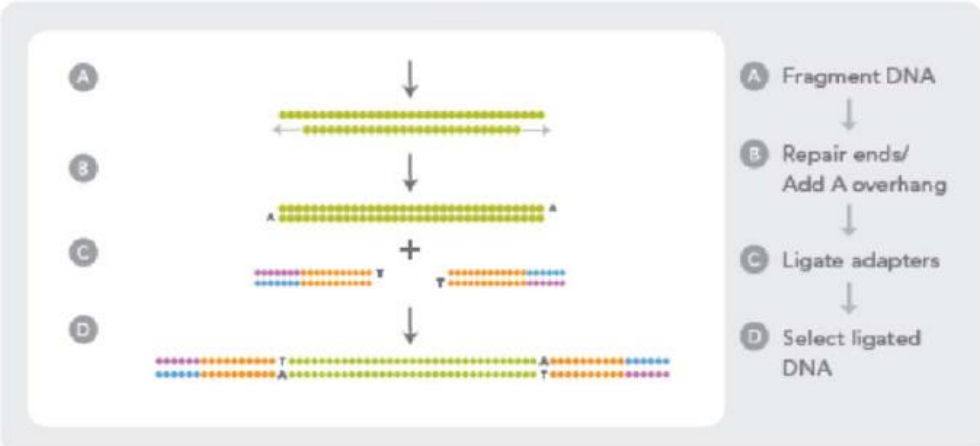
c
Roche/454 — Pyrosequencing

1–2 million template beads loaded into PTP wells



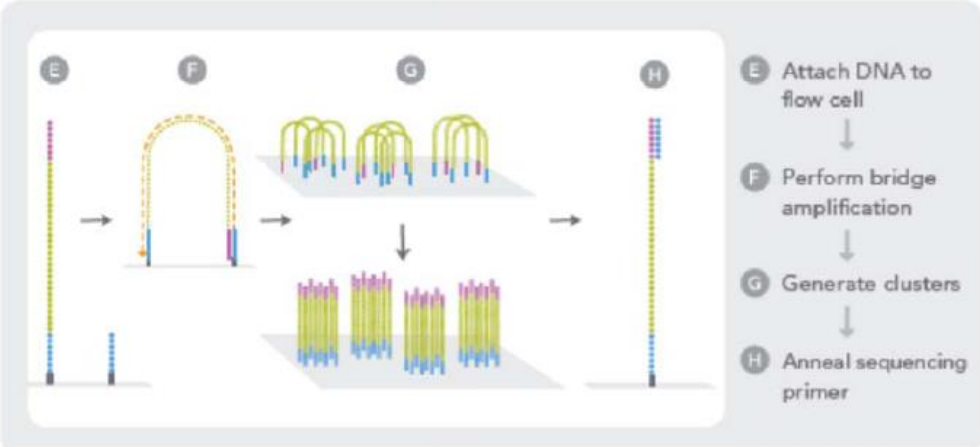
illumina system

I.



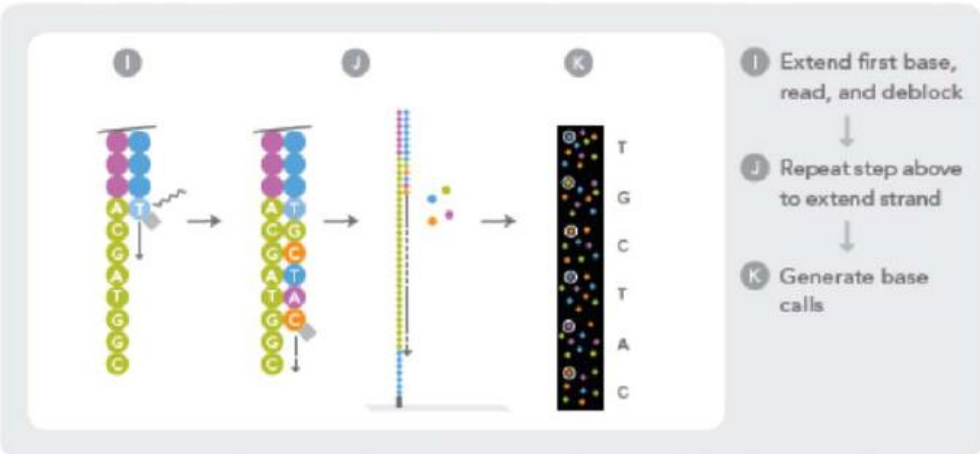
Solid-Phase amplification

II.



Cyclic reversible termination

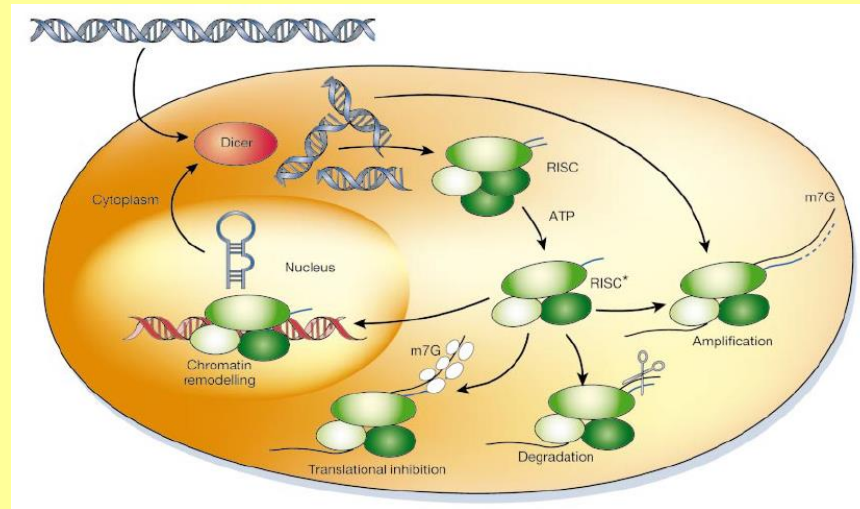
III.



Show all platforms
 454
 GA2
 SOLiD
 Heliscope
 Polonator
 Affymetrix arrays
 Illumina arrays
 Agilent arrays
 HiSeq



Reverse genetic: test the effect of inhibiting the expression of a gene on a phenotype of interest.



RNA interference

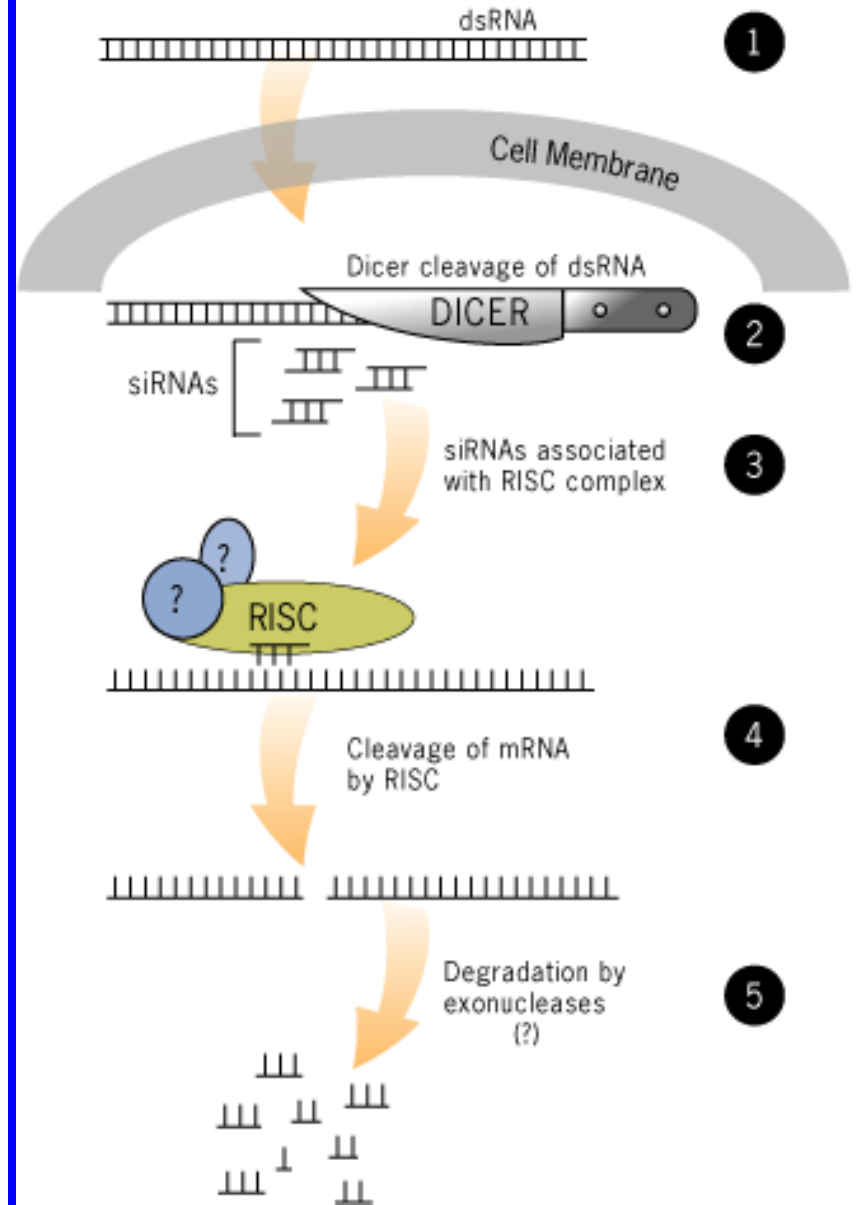
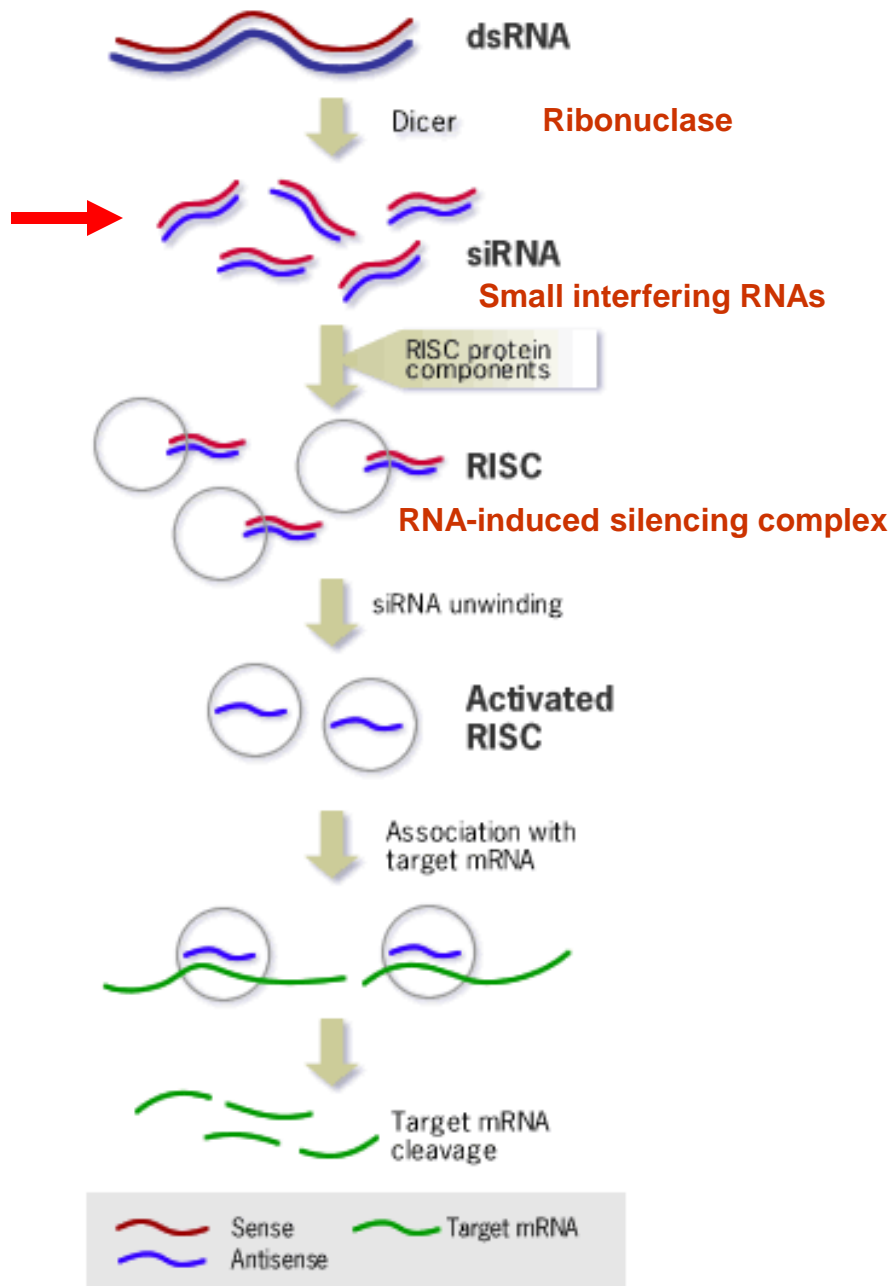
RNA interference (RNAi):

A method for evaluating gene function

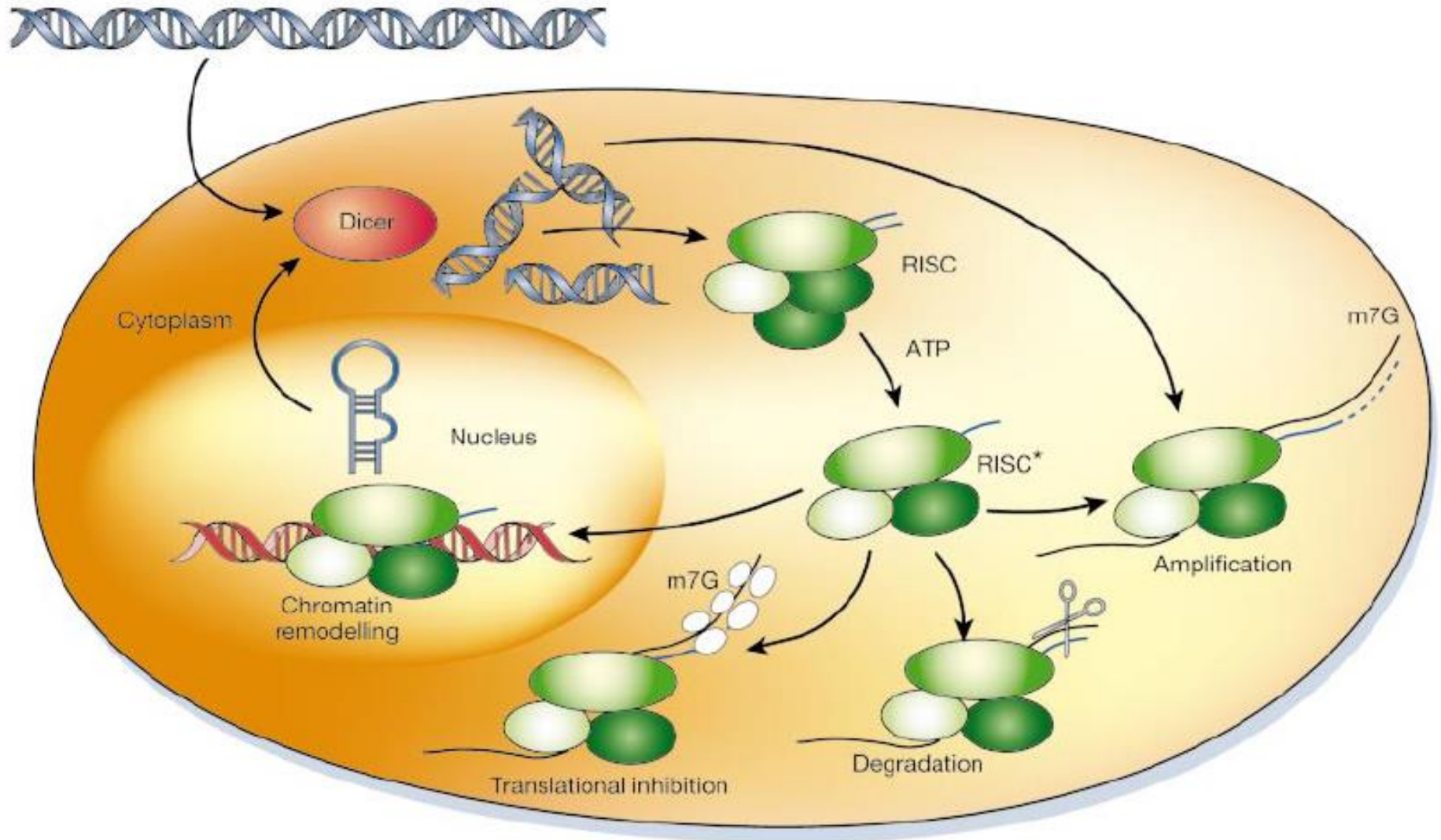
RNAi is the **sequence-specific**, **post-transcriptional** gene silencing mediated by double-stranded RNA (dsRNA).

```
AGGTGAAACAGTTTAGGATCAACTTATAAGACTCTAAACCCCTCTGACA  
AACGTCACGCATACAGTTGGAGATCTGACTTCAAACCTATAGAAATCAAG  
CAAACGAAATTTCAAGGTCGAAACATAAATATGTTTCGTTAATCTATTTTA  
CAACGTGCAGATTATTTTCAGACACATGGCTCGAAATATATCATAAATTTT  
GTTTCTCATAC
```

How does RNAi work ?



How does RNAi work ?



Hannon (2002). RNA interference. Nature 418, 244-251

RNA interference (RNAi):

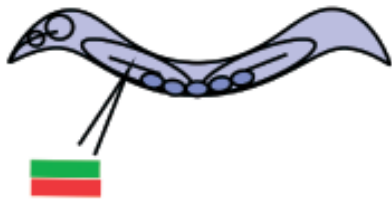
Introduced by:

- injection of dsRNA
- feeding of dsRNA
- bathing the cells with dsRNA
- feeding of bacteria expressing dsRNA
- Transgenic approaches



Effect can spread throughout treated animal (**systemic**)

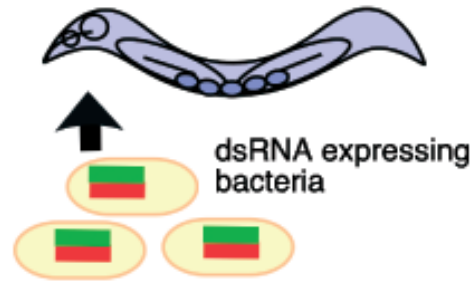
Effect can be transmitted to F1 generation



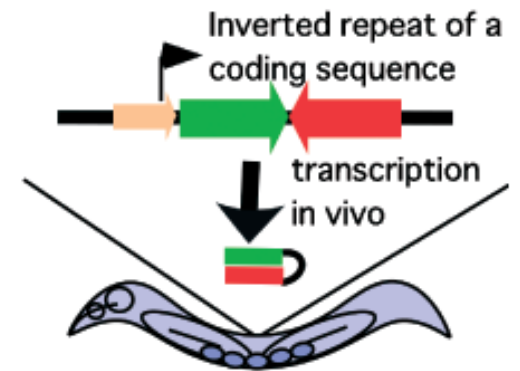
A Microinjection



B Soaking

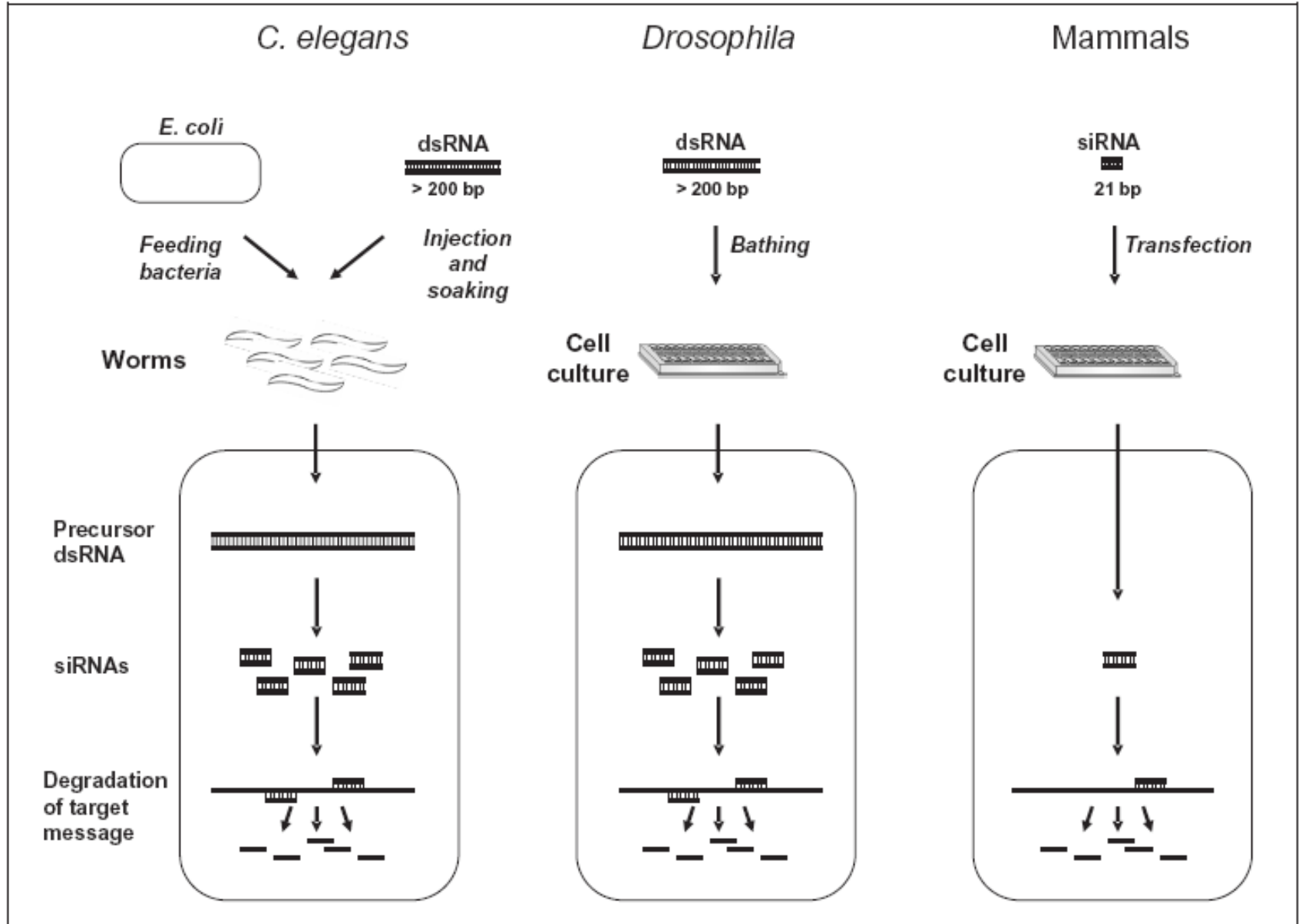


C Feeding



D Transgene

RNA interference (RNAi)



Why RNA interference (RNAi)?

- Mechanism of defense to silence expression of exogenous genes ?
- Mechanism for regulation of gene expression?

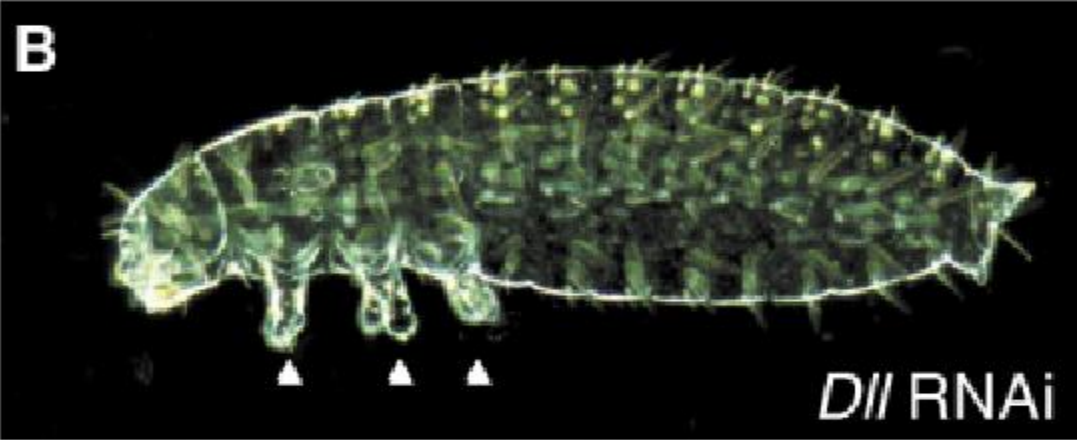
RNAi analysis of *proboscipedia*
in the milkweed bug
Oncopeltus fasciatus



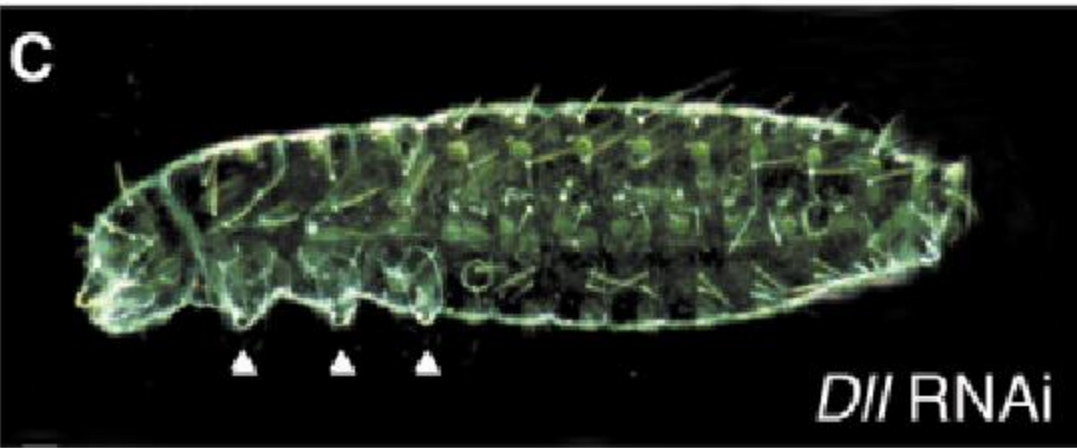
proboscipedia-RNAi
causes transformation
of labium towards legs



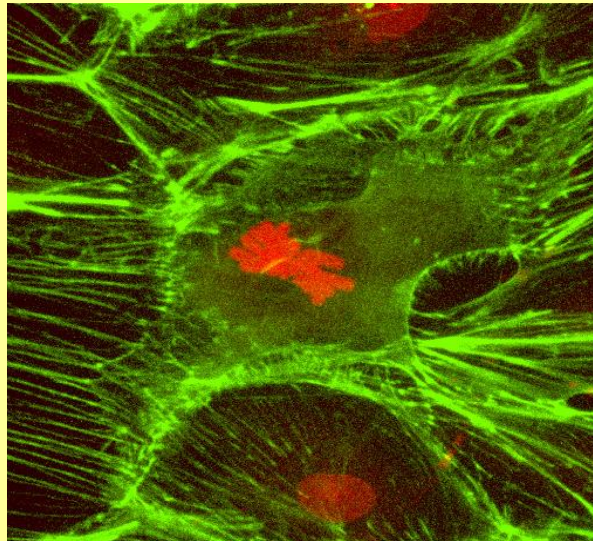
Parental RNAi in *Tribolium* (Coleoptera)



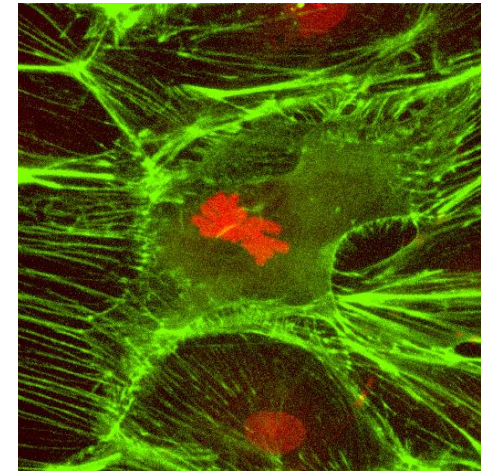
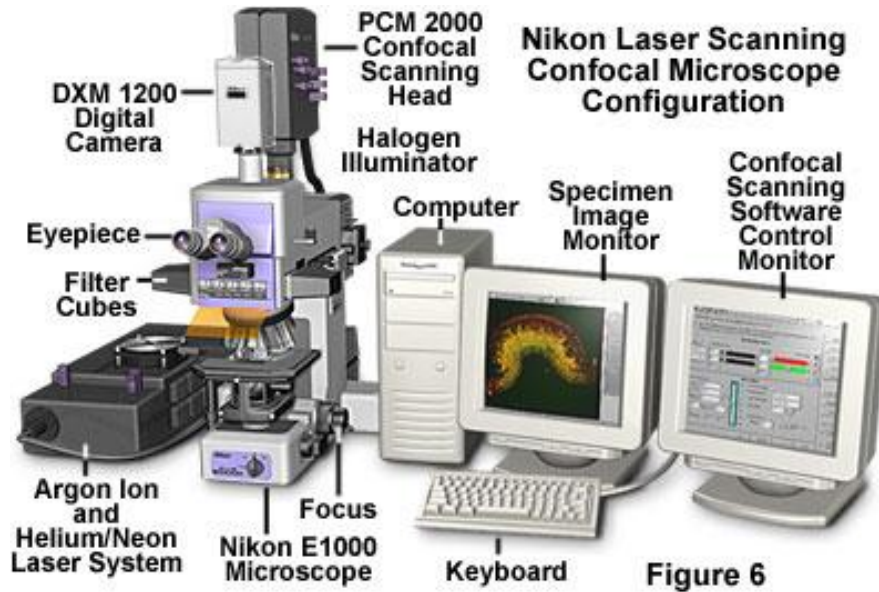
Injection of dsRNA
into haemolymph
of female pupae
results in lack-of-function
Phenotype in embryos
derived from such females



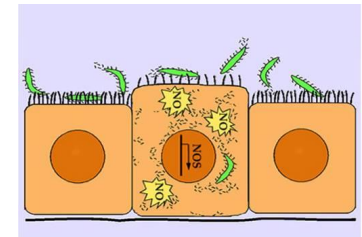
Confocal Microscopy



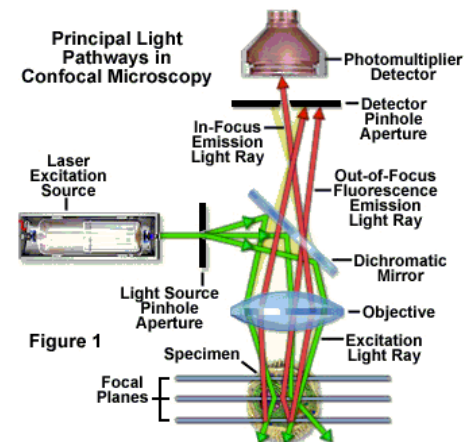
Confocal Microscopy



The illumination is achieved by scanning one or more focused beams of light, usually from a laser, across the specimen. The images produced by scanning the specimen in this way are called **optical sections**. This terminology refers to the noninvasive method by which the instrument collects images, using focused light rather than physical means to section the specimen.



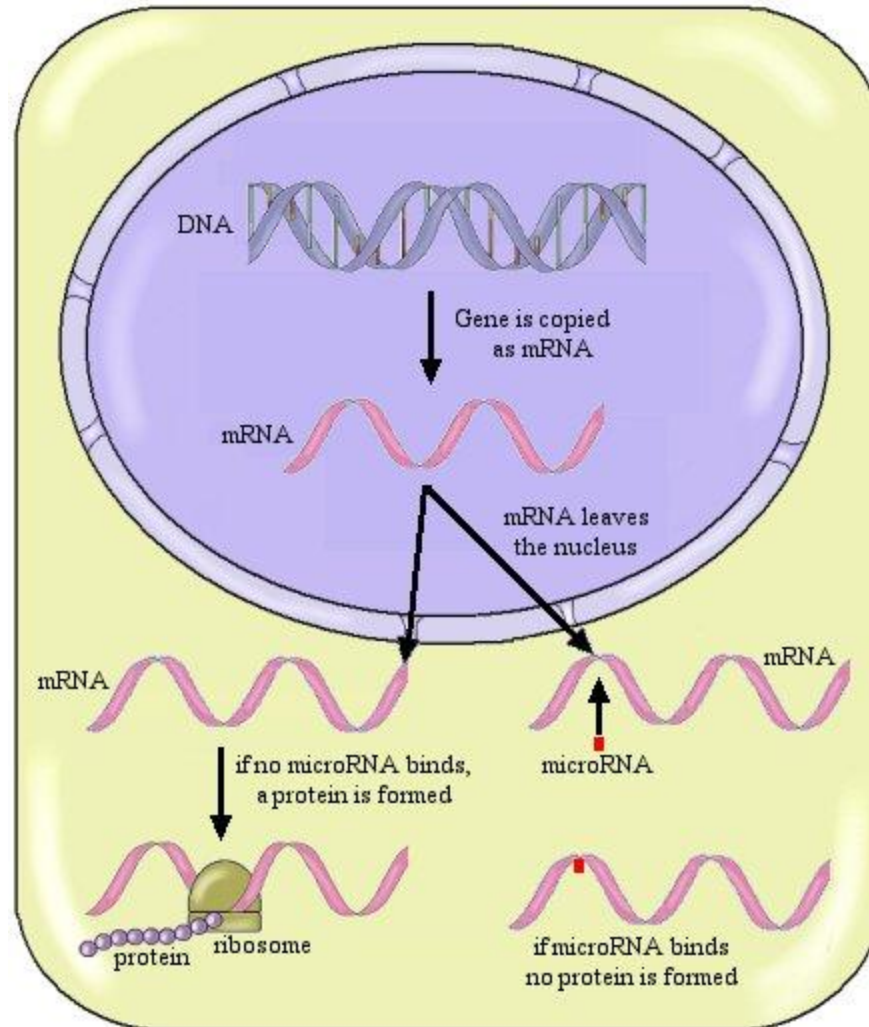
Widefield Versus Point Scanning of Specimens



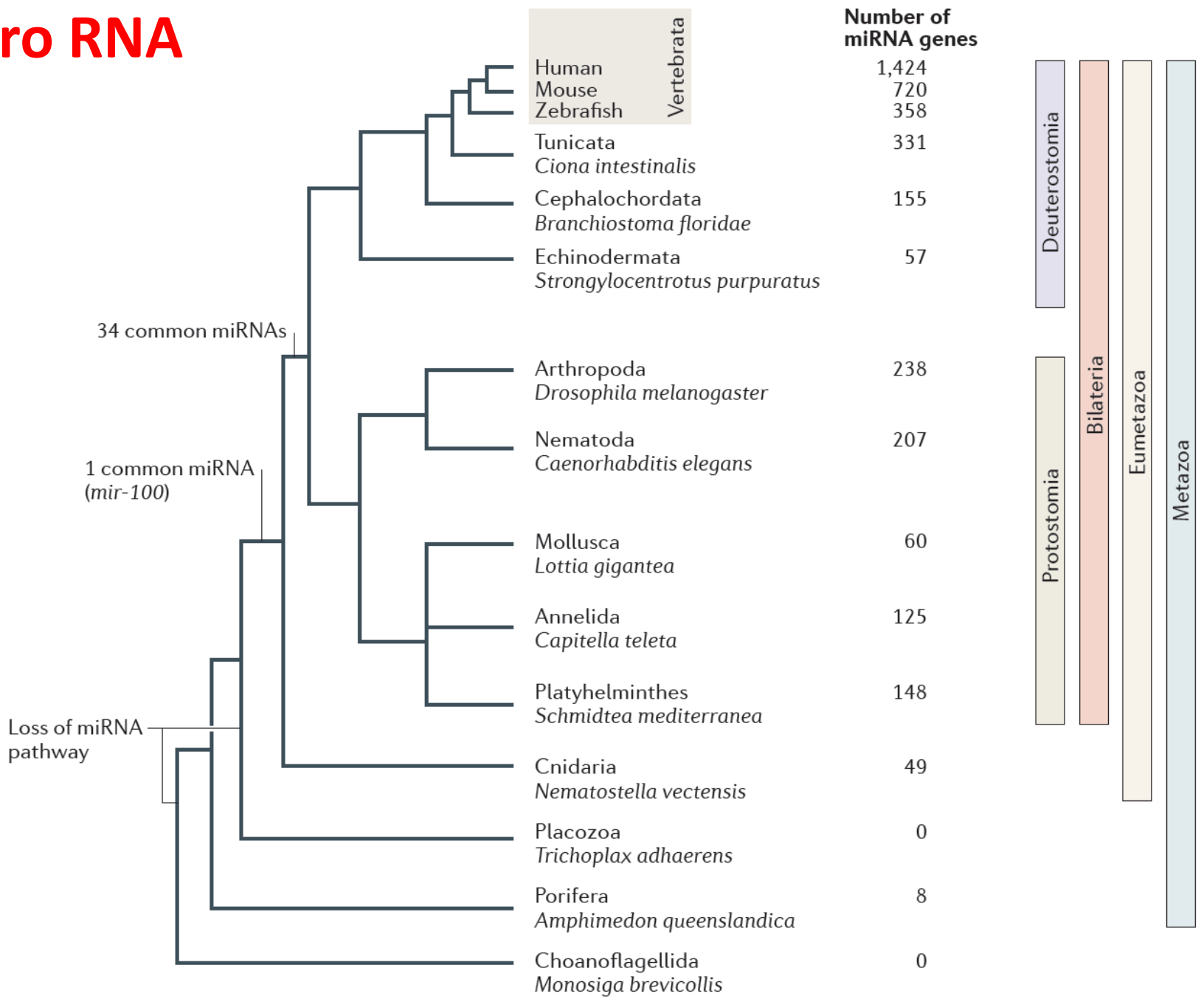
A **microRNA** is a short RNA (an average of 22).

miRNAs are post-transcriptional regulators that bind to complementary sequences on target mRNA resulting in translational repression or target degradation and gene silencing

Micro RNA



Micro RNA



Epigenetics

Epigenetics is the study of heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence (nucleotide sequence).

Examples: DNA methylation and histone deacetylation (suppress gene expression)

