INTRODUCTION TO SOME BASIC METHODS IN MOLECULAR BIOLOGY

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PROBLEM :

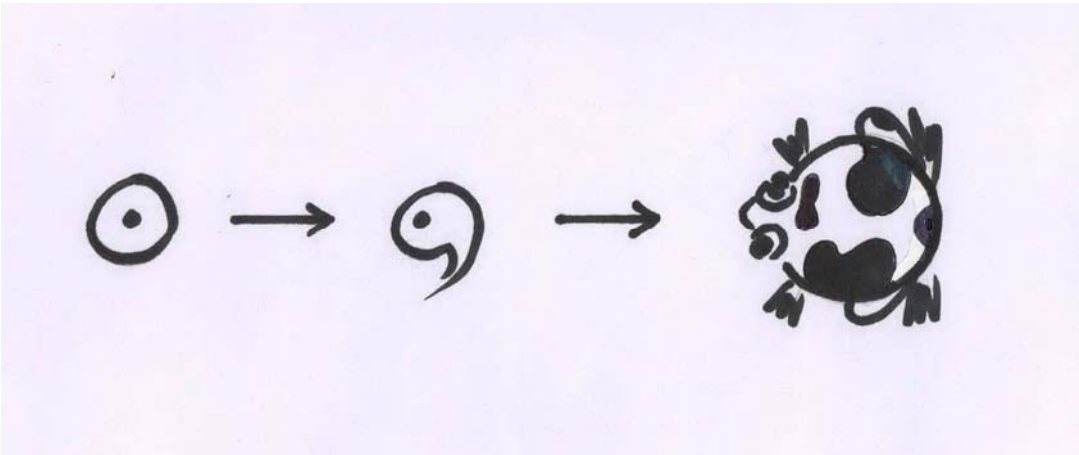
Metamorphosis in amplibian is a process involving many radical molecular, morphological and biochemical changes transforming an aquatic larvae to a terrestrial animal. This phenomenon is triggered by thyroid hormones. Thyroid hormones act through thyroid hormone receptors (THR) which are encoded by α and β THR genes. Determining the role of THR genes can aid to better understanding the phenomenon. Let's begin with THR α gene of *Xenopus laevis*, an African frog.

Some questions have to be answered :

➔ **What is the structure of THR α gene ?**

➔ **How is it expressed (at which time, in which tissues, at what amount) ?**

➔ **How does it interact with other genes to control metamorphosis ?**



SOME METHODS ARE INTRODUCED BELOW AS SUGGESTED SOLUTIONS. OTHER (BETTER) STRATEGIES AND METHODS COULD BE IMAGINED

➔ **What is the structure of *Xenopus laevis* THR α gene?**

- **Firstly, the genetic material of *X. laevis* is extracted from frog tissue ⇨ DNA/RNA extraction methods**
- **Purified DNA/RNA are qualitatively and quantitatively analyzed before further investigation ⇨ Electrophoresis and Spectrophotometer-based methods**
- **THR α gene is isolated and amplified from genomic DNA/mRNA ⇨ Cloning, PCR (Polymerase Chain Reaction), RT-PCR methods**
- **The gene is detected by Southern blotting**
- **Finally, the nucleotide sequence of THR α gene is determined by Sequencing methods**

➔ **How is it expressed (at which time, in which tissues, at what amount) ?**

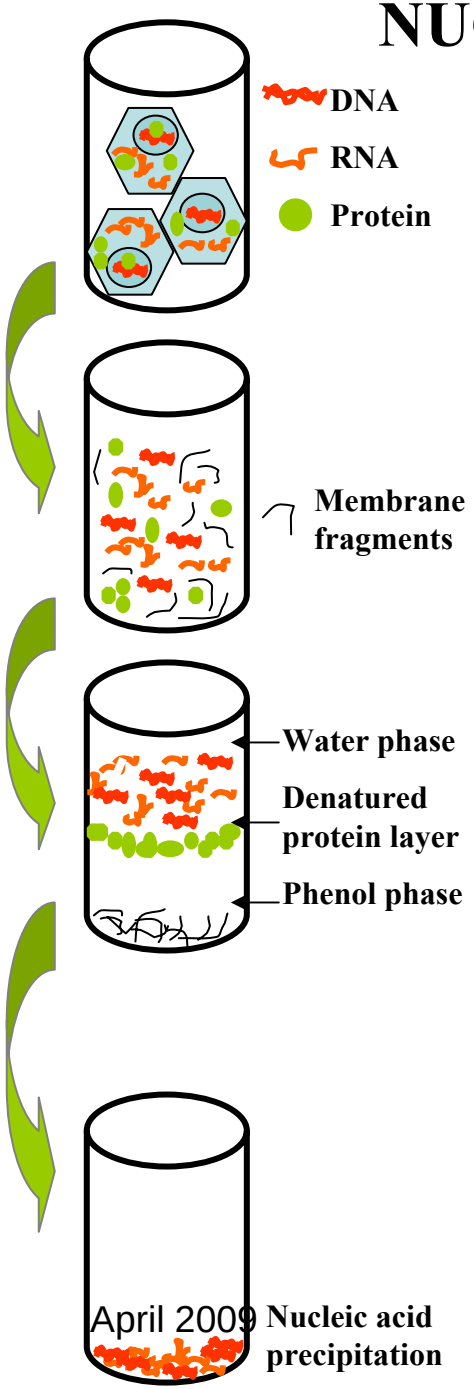
- **Differential expression of THR α gene is evaluated by Northern blotting,**

  or Real-time RT-PCR

➔ **How does it interact with other genes to control metamorphosis ?**

- **Interaction between two genes at RNA level is assessed by In situ Hybridization**
- **Interaction between two gene products (proteins) is determined by Two-hybrid System.**

NUCLEIC ACID EXTRACTION METHODS



According to the nature of sample (animal, plant tissues or microbial organisms, …), and the kind of nucleic acids to be extracted, different extraction agents are used. Nevertheless, DNA/RNA extraction methods are usually composed of three steps which could be separated or combined.

Step 1 : Cell membrane lysis

Tissues are mechanically (ground) and/or enzymatically broken. Cell membrane are lyzed by detergents (SDS, ..) or chaotropic agents, such as Guanidinium thiocyanate which is commonly used for RNA extraction.

Step 2 : Protein elimination

A Phenol:Chloroform:Isoamyl alcohol solution is usually used to denature proteins which are consecutively eliminated by centrifugation. Some resins could also be used to eliminate proteins.

This step could be combined with step 1 to simultaneously lyzing membranes and eliminating proteins. Acidic Phenol is used for RNA extraction instead of basic Phenol used in DNA extraction.

Step 3 : Obtention of purified nucleic acids. Many approaches can be used:

- **DNA/RNA precipitation by Ethanol/Isopropanol combined with salt. DNA/RNA are recovered by centrifugation and redissolved.**
- **Adsorption of DNA/RNA on a matrix (silica,…) followed by washing and DNA/RNA elution.**

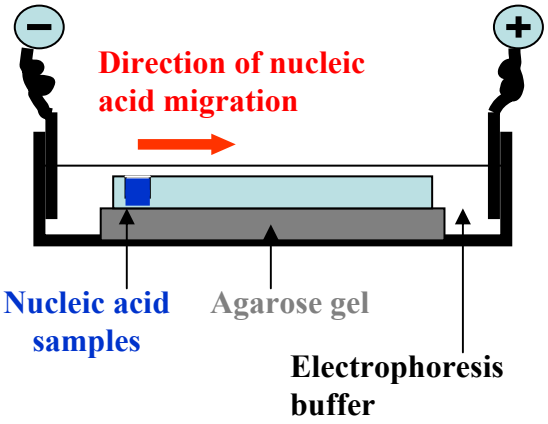
….

The purified nucleic acids must then be analyzed to assess their amount as well as their quality (integrity, purity).

The two common methods used include Electrophoresis and Spectrophotometric measure of Optical Density (OD).

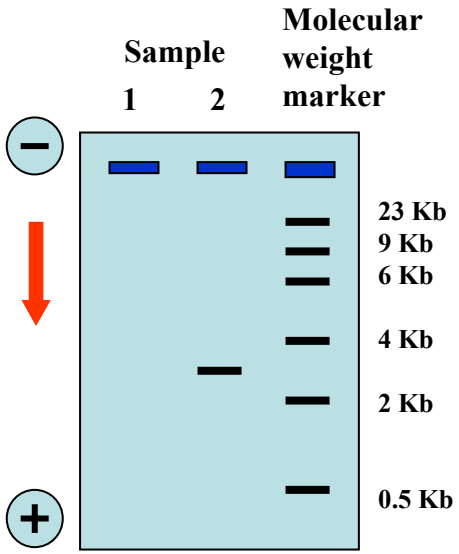
Electrophoresis methods are usually considered as qualitative analysis whereas Spectrophotometric measures are mostly used as quantitative analysis. Nevertheless, combined with other methods they can be used indifferently for qualitative as well as for quantitative analysis.

ELECTROPHORESIS



Nucleic acids which are negatively charged in solution will migrate in an electric field toward the positive pole. The electric field is usually established in a semi-solid matrix (gel) made of high-molecular weight polysaccharides such as agarose or acrylamide. Electrophoresis are used to separate different nucleic acid fragments in a mixture.

During electrophoresis, nucleic acids have to "creep" through the network established by agarose or acrylamide molecules. The migration rate of nucleic acids depends on many factors : their size, the gel concentration which determines "loose" or "tighten" meshes, the voltage,…



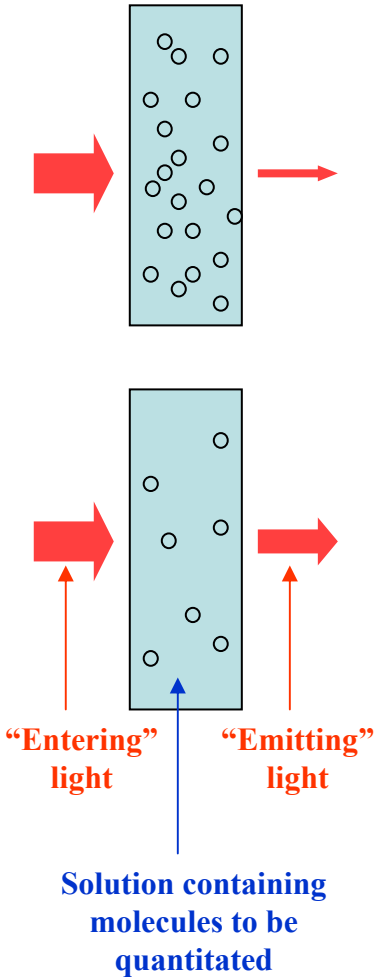
Agarose gels are commonly used for separation and detection of nucleic acid fragments. Acrylamide (usually in the form of polyacrylamide) gels which have high resolution capacity are used for specific purposes, e.g. Sequencing gels where a one-nucleotide difference in size can be recognized.

Nucleic acids are detected after electrophoresis by staining with some fluorescent agents (Ethidium bromide, SYBrgreen, ..). These agents are incorporated in nucleic acids and fluoresce under UV light.

Molecular weight markers are used in electrophoresis for detecting the size of the desired fragment.

Electrophoretic analysis can answer the question about the presence and the integrity (molecular size) of nucleic acid fragments. It also contributes to identify a particular DNA/RNA fragment when combined with other methods such as Molecular hybridization.

SPECTROPHOTOMETRIC ANALYSIS



Light particles entering a solution will be absorbed by molecules present in the solution. Light particles absorption increases with increasing numbers of molecules present in the solution.

Optical density (OD) measure is performed by a spectrophometer, based on the difference of light intensity before and after entering a solution containing the molecules to be quantitated. The intensity decrease after light entering a solution is due to absorption by these molecules.

In nucleic acids it is purines and pyrimidines which absorb light ; absorption is maximal at a wavelength of 260 nm. Nucleic acids concentration can be calculated based on their OD values as follows :

$$1\ OD_{260\ nm} \approx 50\ \mu g/ml \text{ double-stranded nucleic acids}$$
$$\approx 40\ \mu g/ml \text{ single-stranded nucleic acids}$$
$$\approx 20\ \mu g/ml \text{ oligonucleotides}$$

Furthermore, the purity (protein contamination-free) of nucleic acid solutions can be assessed by the ratio of $OD_{260\ nm}$/ $OD_{280\ nm}$ values. A ratio of 1.8 – 2 shows good DNA purity whereas an inferior value means protein contamination.

OD measurement of nucleic acid solutions are used to calculate their concentration as well as their purity before further studies.

The purified nucleic acids are now ready for further use.

They can be cut and ligated, lyzed or replicated.

**All these activities are performed by enzymes –
nucleases, ligases, polymerases.**

UTILIZATION OF SOME ENZYMES IN MOLECULAR TECHNIQUES

Nucleases include two groups :

- Exonucleases : Nucleases which digest DNA fragments from the 5'/3' ends, e.g Exonuclease III, used to create overhang DNA fragments.
- Endonucleases : Nucleases which cut inside a DNA fragment ; e.g DNase I creates "nicks" in double-stranded DNA, RNase A/H eliminate RNA, …

Ligases :

- Joint two RNA sequences, e.g T4 RNA ligase

or

- Two DNA sequences, e.g T4 DNA ligase

Polymerases :

- Enzymes synthesizing DNA (DNA polymerases, e.g E.coli DNA pol I, Taq polymerase,)
- RNA sequences (RNA polymerases, e.g T3/T7/SP6 RNA polymerases)

Other enzymes:

- Kinases add phosphate group to DNA sequences
- Phosphatases remove phosphate groups from DNA sequences,
- Methylases

A group of particular endonucleases consists of restriction enzymes type II. These enzymes are named according to their origine, e.g : *EcoRI*

Escherichia coli Strain Order of discovery

Restriction enzymes cut at specific sequences called restriction sites. Restriction site are usually palindromes of 4-10 bp which are composed of the same nucleotide sequence in the two complementary strand. Examples of restriction sites recognized by some enzymes :

EcoRI : 5'…G A A T T C…3'
3'…C T T A A G…5'

HpaI : 5'…G T T A A C…3'
3'…C A A T T G…3'

Restriction enzymes can create blunt-end (*HpaI*) or cohesive ends (*EcoRI*)

Restriction enzymes have numerous applications and are indispensable tools for molecular cloning

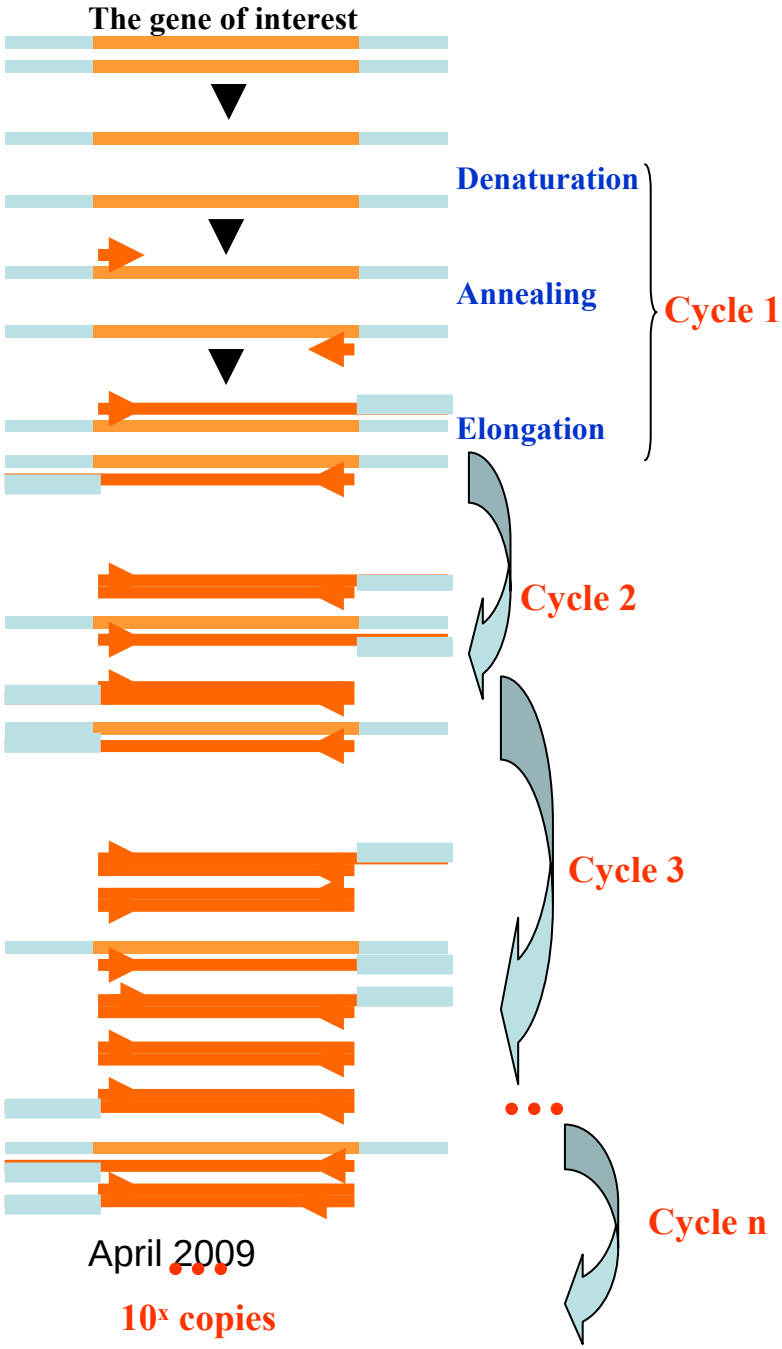
To isolate a particular gene, two methods are commonly used : PCR (Polymerase Chain reaction) and Molecular Cloning.

Both methods are based on the amplification of gene copies.

In Molecular cloning, also called *in vivo* amplification, gene copies are replicated by living cells. Molecular cloning must be followed by other methods (Molecular hybridization or PCR) if a specific gene has to be isolated.

PCR, an *in vitro* amplification of gene copy number, is performed in lab tubes. The amplification is gene-specific thus PCR products consist of large amount of the gene of interest.

PCR (POLYMERASE CHAIN REACTION)



PCR consists of repeated rounds of DNA replication resulting in important amplification of a specific DNA fragment.

PCR is composed of many cycles, each includes three steps : (1) Denaturation, the two DNA strand are separate by heating, (2) Annealing, target-specific primers (forward and reverse) hybridize to complementary sequence in the DNA target, (3) Elongation, thermophilic DNA polymerases synthesize complementary strands from annealed primers. According to theoretical calculations, amplification can result in 10^6 copies from the initial one after 30 cycles of PCR.

PCR can be used to obtained a large amount of a particular DNA fragment for further manipulations (cloning, sequencing), or to detect a specific target DNA at very low concentration in samples.

Many PCR-based methods were developed : RT-PCR combining reverse transcription with PCR to amplifying RNA, quantitative PCR (qPCR or real-time PCR) allowing the quantitation of initial DNA target number, in situ PCR amplifying target DNA in tissues without prior extraction, RAPD using random primers to amplifying large regions of genomic DNA, …

qPCR (REAL-TIME PCR)

In this technique, fluorescent particles are integrated into the newly synthesized double-stranded DNA during the amplification process. The increasing amount of fluorescent signals reflect the increasing amount of replicated DNA and is recorded at each moment of the amplification reaction.

Real-time PCR is used to quantitate DNA content in a sample or to compare DNA amount in different samples. RNA can be quantitated by real-time RT-PCR combining a reverse transcription with the real-time PCR.

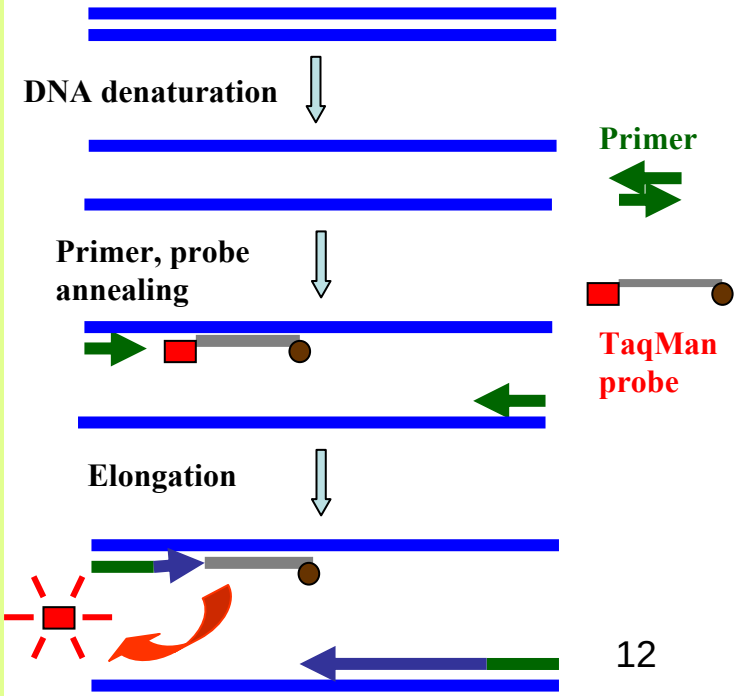
Fluorescent agents are of two types : (1) DNA binding dyes, e.g SYBrGreen, which fluoresce when intercalating into doubled-strand DNAs, (2) Fluorescent agents used to label target-sepecific probes, e.g Hydrolysis probes, Hybridization probes, Molecular beacon, …

Example of target-specific probes, Hydrolysis or Taqman probe

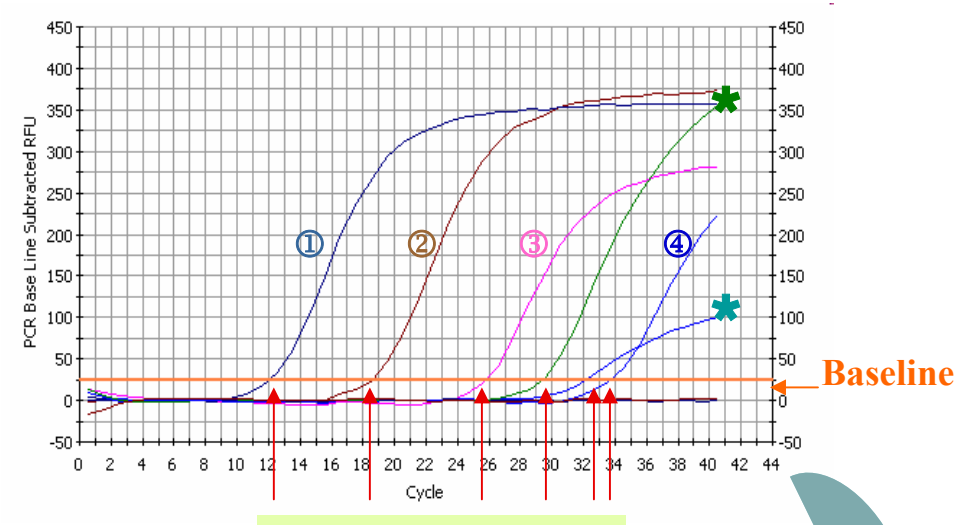
Taqman probe is labelled at the 5'end by a reporter fluorophore and at the 3'end by a quencher fluorophore which "quenches" the reporter.

During elongation step,Taq polymerase displaces the annealed probe, releasing the reporter fluorophore which is no longer quenched by the quencher. Each signal emitted by the released reporter group is equivalent to a newly synthesized strand and can be recorded precisely at any moment of the amplification process, whre the term "real-time".

How can the initial amount of DNA in the sample be calculated ?



qPCR (REAL-TIME PCR) (continued)



Ct

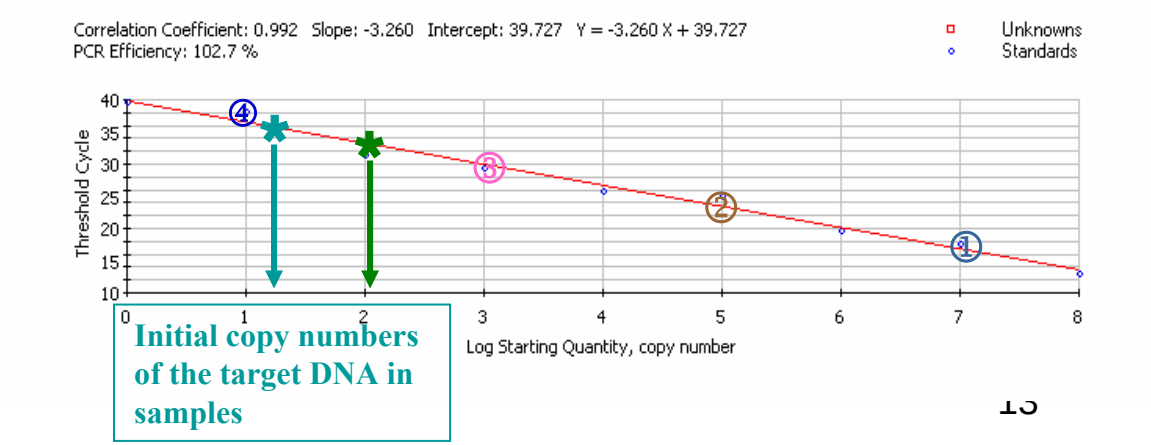
An important notion of real-time PCR is the Ct (Cycle threshold). Ct is the amplification cycle at which the signal is recognized as specific signal, arising from the non specific background context.

To calculate the initial amount of DNA in the sample, a standard curve is established with predetermined concentration of a standard DNA.

Cts from different concentrations of standard DNA : Ct①, ②, ③, ④

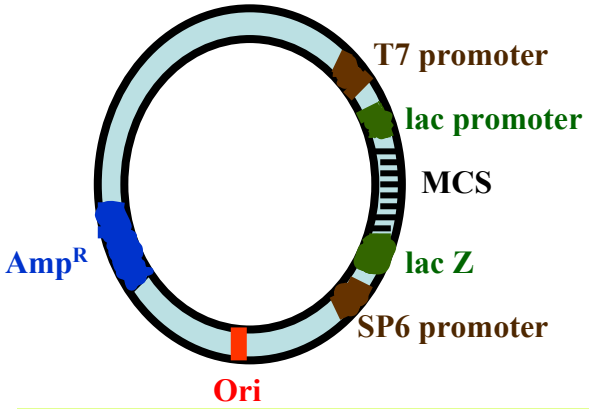
Cts from samples : Ct✱, ✱

Standard Curve Graph for FAM-490



The initial concentration of target DNAs in samples can be determined from the established standard curve

MOLECULAR CLONING



Example of a vector :

- ***Ori : origin of replication***
- ***Amp^R : Ampicillin-resistant gene***
- ***MCS : Multicloning site (PCS -polycloning site, polylinker), sequence containing many restriction sites***
- ***Lac promoter/lac Z : region flanking the MCS, allowing the detection of cells containing the recombinant vector***
- ***T7/SP6 promoter : allowing the expression of cloned gene into RNAs***

Molecular cloning refer to the amplification of DNA fragments by cellular DNA replication system. The approach consists of introducing the DNA fragment into a host cell and inducing its replication. To create a self-replicating DNA molecule, the target DNA (insert) is incorporated into a vector ; the vector become a recombinant vector.

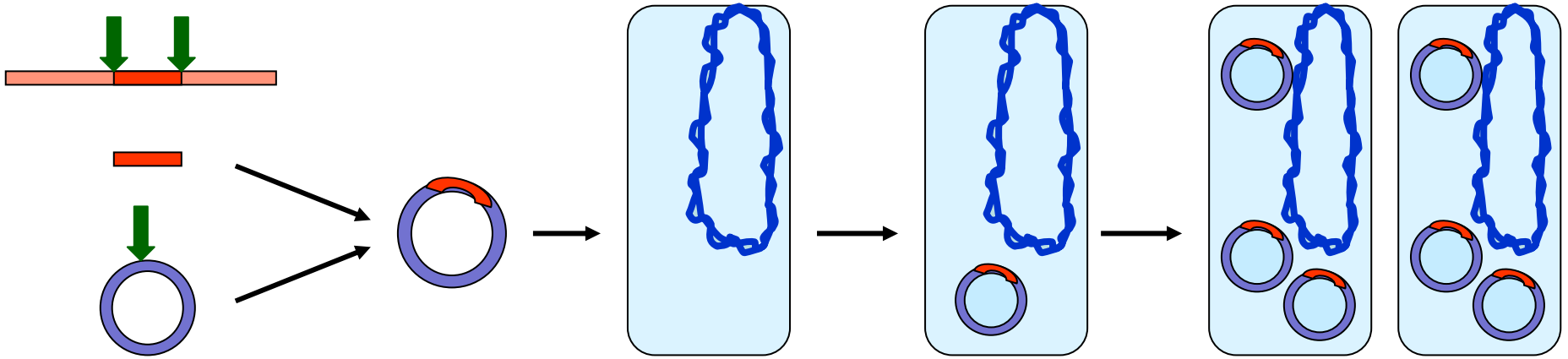
Vectors are DNA molecules having some characteristics :

- **They contain an origine of replication which allows them to replicate independently in host cell**
- **They contain many restriction sites which are substrate for restriction enzymes.**
- **They contain markers allowing identification of cells being transformed with these vectors, such as antibiotic resistance genes which permit transformed cells to grow in media containing antibiotics.**
- **Vectors can bear other sequences with specific utility ; e.g vectors containing promoters are used to express cloned genes into RNAs.**

Depending on research purposes, vectors of different kinds can be used : plasmid, cosmid, phage, animal virus, plant virus, BAC (Bacterial Artificial Chromosome), YAC (Yeast Artificial Chromosome), MAC (Mammalian Artificial Chromosome), …

MOLECULAR CLONING (continued)

Cloning process includes many steps :



The insert can be obtained by digestion of genomic DNA with restriction enzymes, or reverse transcribed from RNA, or artificially synthesized

The vector is opened with restriction enzymes having recognition sites within the MCS

"Opened" vector and insert are joined by ligase to form recombinant vector

The recombinant vector is introduced into host cell rendered competent by transformation or transfection

Transformed cells are selected on selective media (containing antibiotics) and form colonies, also called clones.

Each clone is composed of cells containing the same recombinant vector at numerous copies.

Clones can be cultivated in liquid media and recombinant vectors collected by DNA extraction methods

Selection of a specific clone is made by a molecular hybridization process performed in colonies.

MOLECULAR HYBRIDIZATION

The pase-pairing of two single strand DNAs from different source but having complementary sequence is called Molecular Hybridization.

In Hybridization, a probe is used to detect a specific nucleic acid sequence. Probes have some charateristics : (1) Having sequence complementarity with the target sequence, (2) Being chemically or radioisotopically labelled. Probes could be single-stranded DNA, RNA or oligonucleotide.

Based on the location of target sequences, hybridization can be classified as :

- **Hybridization in liquid phase between targets and probes present in solution**
- **Hybridization on solid substrate : the target sequences are fixed on a subtrate such as membrane, plate, slides, … , e.g Southern/Northern/Dot blot hybridization, technique of microarray, …**
- ***In situ* hybridization : taking place directly on tissues without prior extraction of the target sequences.**

Hybridization in colonies can be considered as an *in situ* hybridization process. Bacterial cell membrane are lyzed and the recombinant vectors present inside the cells are hybridized with a probe.

Southern and Northern blot hybridization are introduced as follows

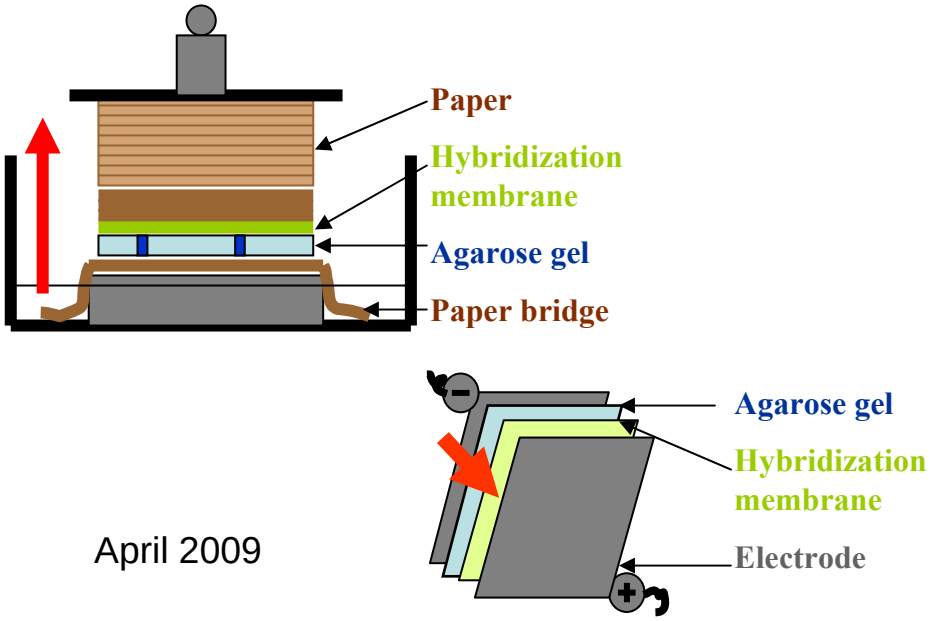
SOUTHERN/NORTHERN BLOTTING

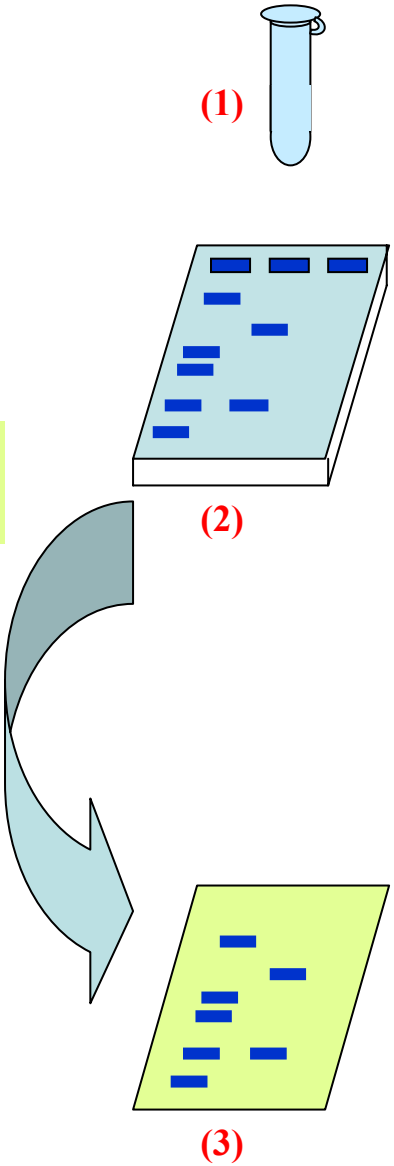
Southern blot is named according to Edward Southern (1975) who invented the technique.

The technique consisted of transferring DNA fragments from an agarose gel to a hybridization membrane made of nitrocellulose or nylon.

Other related techniques were developed : Northern blotting for RNA transfer, Western blotting for protein transfer.

Blotting can be done by passive transfer through capillarity (left) or by electroblotting (right)





Actually, Southern blotting concerns the whole molecular hybridization process, including :

(1) DNA extraction from samples

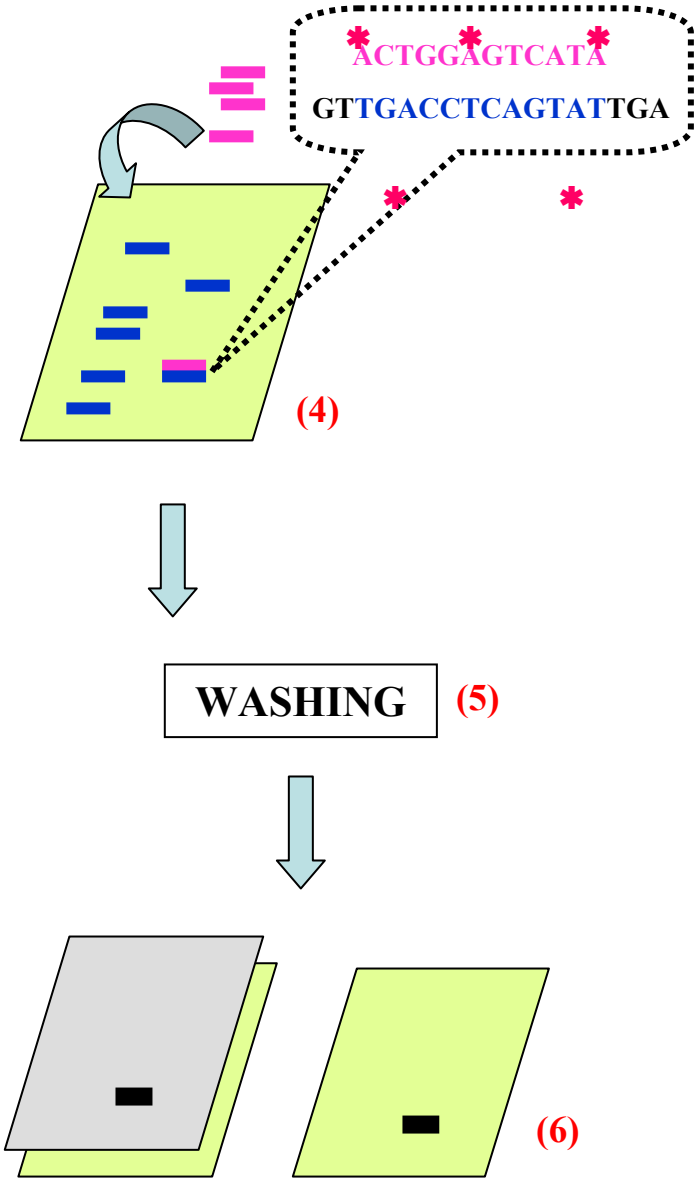
(2) DNA fragments separation by agarose gel electrophoresis.

The agarose gel is then soaked in denaturation buffer (NaOH) to denature the double-stranded DNA.

(3) DNA blotting

After blotting, the membrane is fixed by baking or UV crosslinking. The fixation step irreversibly immobilized the denatured DNA on the membrane.

SOUTHERN/NORTHERN BLOTTING (continued)



(4) Prehybridization is the preparation step before hybridization : the membrane is incubated in hybridization membrane

Probes are then added for hybridization. During hybridization, base-pairing between probes and complementary DNA fixed on the membrane take place.

(5) Washing : The membrane is washed many times to eliminate excess probes

(6) Detection : "target:probe" hybrids are detected different ly according to the labelling method :

- **Radiolabelled probes are detected by autoradiography. Signals are expressed as dark bands on an X-ray film.**
- **Chemically labelled probes can be detected by colorimetric, fluorescent, chemiluminescent, .. methods. Colorimetric or fluorescent detection is performed directly on the membrane whereas chemiluminescent signals are obtained through light-sensitive films.**

DIDEOXY SEQUENCING (METHOD OF SANGER)

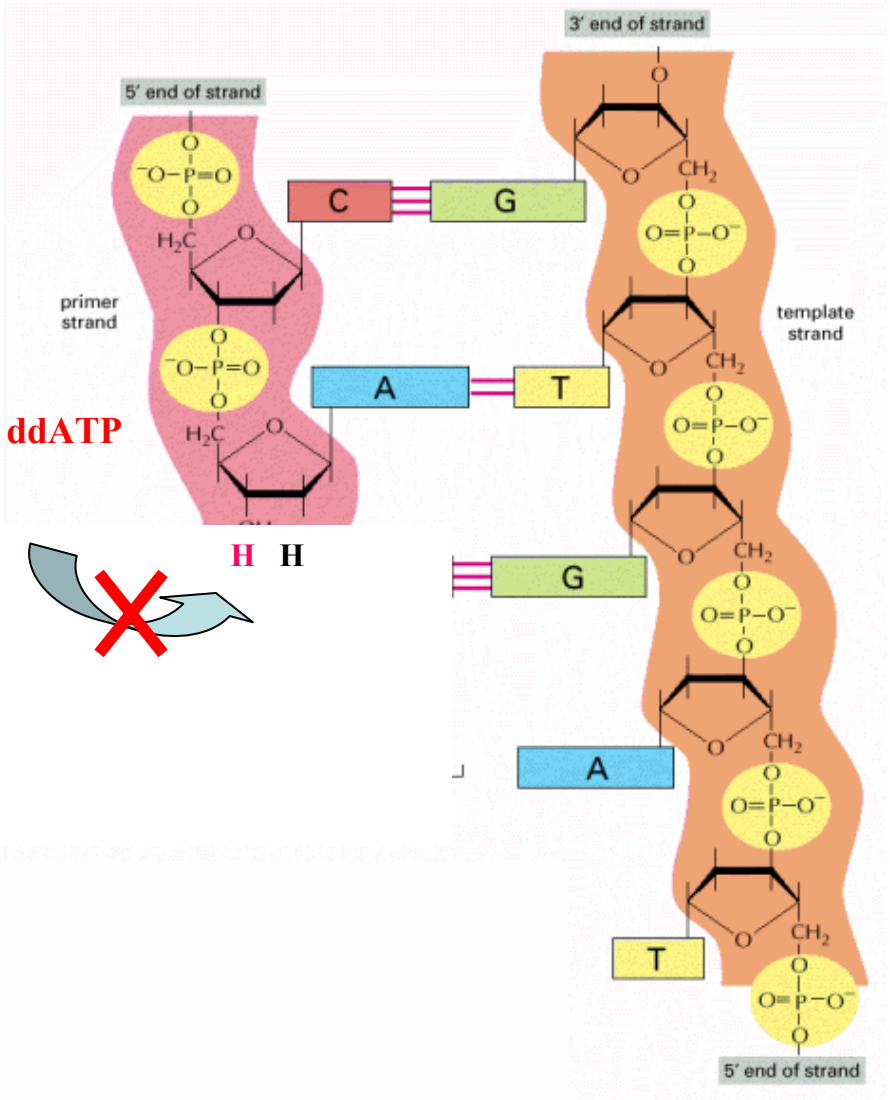
The principle of Dideoxynucleotide sequencing (or method of Sanger) is based on random incorporations of dideoxynucleotides into the ongoing replicated DNA strand.

Lacking the C3'-OH, a dideoxynucleotide (ddNTP), e.g dideoxyadenine – ddATP, or ddCTP, ddGTP, ddTTP, can not participate in the formation of phosphodiester bonds. Thus, at any time a ddNTP is randomly incorporated in the elongated strand, DNA synthesis is immediately interrupted.

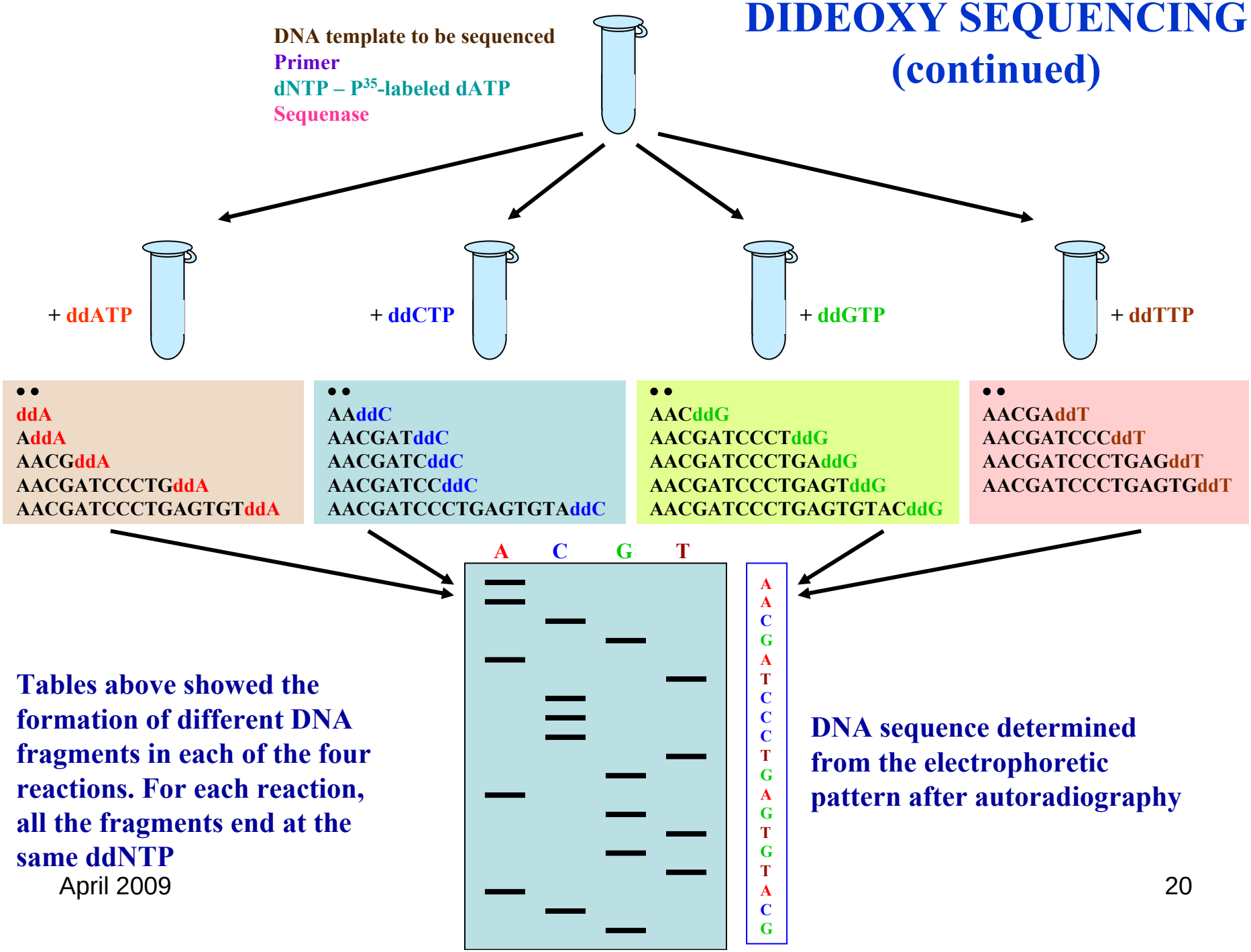
To determine the nucleotide sequence of a DNA fragment, 4 reactions are established, each including all the components necessary to DNA replication plus 1 of the 4 ddNTPs.

At the end of replication process, the four reactions are analyzed by polyacrylamide gel electrophoresis.

A portion of radiolabeled dATP is added to each replication reaction so that the DNA sequence can be read after an autoradiography of the polyacrylamide gel



DIDEOXY SEQUENCING (continued)



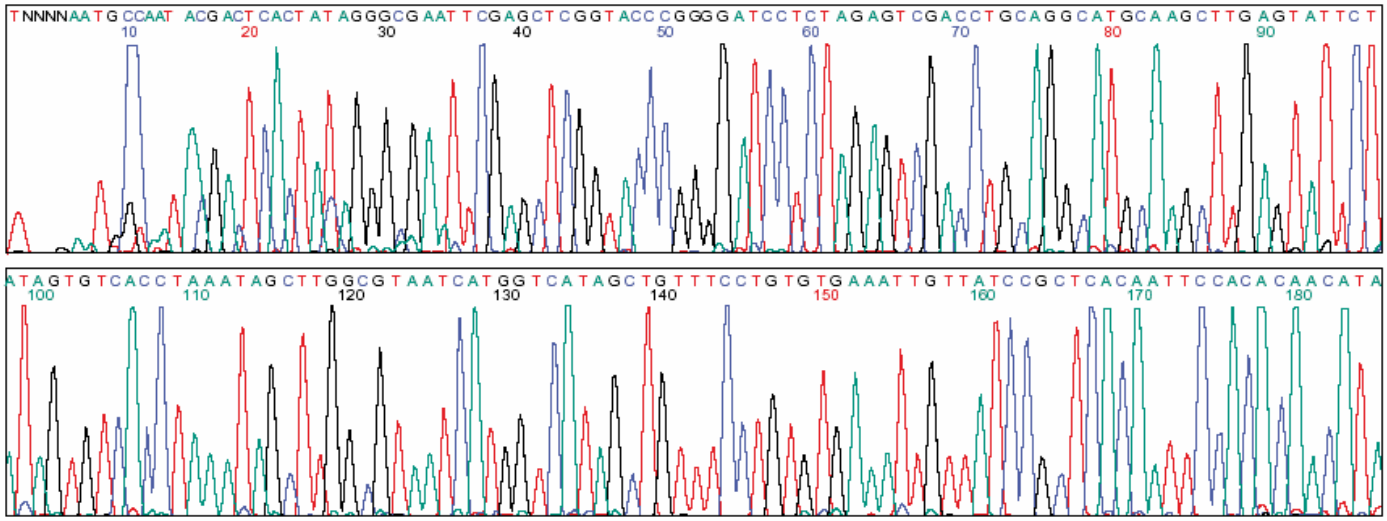
Tables above showed the formation of different DNA fragments in each of the four reactions. For each reaction, all the fragments end at the same ddNTP

DNA sequence determined from the electrophoretic pattern after autoradiography

DIDEOXY SEQUENCING (continued)

Automatic sequencing was developed based on the method of Sanger.

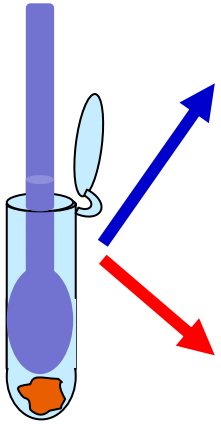
Automatic sequencing is performed in a sequencer and uses, instead of radiolabelling, different fluorescent dye-labelled ddNTPs. The terminating replication reaction are then analyzed by electrophoresis. During electrophoresis, migrating DNA fragments are induced and emit different fluorescent signals when crossing a detector. The signals are recorded and reflect the terminal ddNTP of each DNA fragments. The overall result is the nucleotide sequence to be determined (below).



Improvements of automatic sequencing involve more efficient sequencing enzymes, and particularly more efficient electrophoretic analysis, e.g capillary electrophoresis.

The great beneficiary of automatic sequencing are Genome projects, the most important of which is the Human Genome Project.

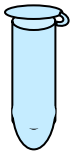
Let's come back to our *Xenopus laevis*. The structure and expression of THR α gene could be determined as follows :



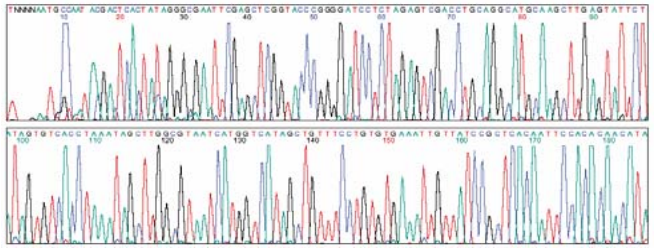
DNA extraction

RNA extraction

Amplification of THR α gene by PCR



Electrophoresis analysis of PCR products

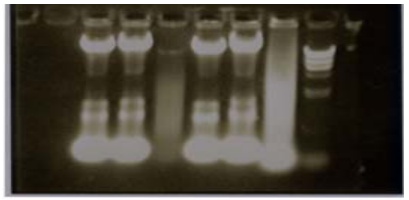


Sequencing of THR α gene

THR α GENE STRUCTURE

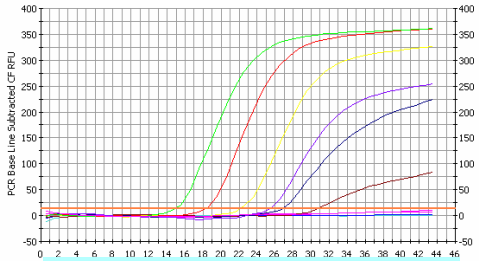
Agarose gel electrophoresis

1 2 3 4 RNA ladder



1 2 3 4 RNA ladder

Northern blot with THR α-specific probe. Sample 1, 2, 3, 4 correspond to different developmental stages



Real-time RT-PCR to quantitate THR α mRNAs at different developmental stages

THR α GENE EXPRESSION

Metamorphosis is a highly complicated process involving numerous genes interacting with each other. "What are the other genes, besides THR α gene, also included in the process of metamorphosis in *X. laevis* ?".

By means of the previously described methods, genes are analyzed individually, one after another. Analysises are slow and do not provide an overview of the whole biological network.

Similar questions concerning varied biological processes were at the origin of what is called Genomics. Genomics is the study of an organism entire genome, at one time.

Genomics was developed based on some methods : (1) Cloning methods to establish genomic libraries, (2) PCR to amplify specific genes, (3) Automatic sequencing for high-throughput determination of gene nucleotide sequences, (4) Computational science for data storage and analysis.

GENOMICS

Genomics includes many domains " : (1) Structural Genomics is based on the mapping and Sequencing of the entire genome, (2) Functional Genomics tends to analyze gene function and interaction by the utilisation of high-throughput techniques such as Microarray, (3) Bioinformatics concerns data storage and computational analysis of gene structure and function.

GENOME SEQUENCING – THE BASIS OF STRUCTURAL GENOMICS

The two strategies for genome sequencing :

Ordered clone approach (OCA)

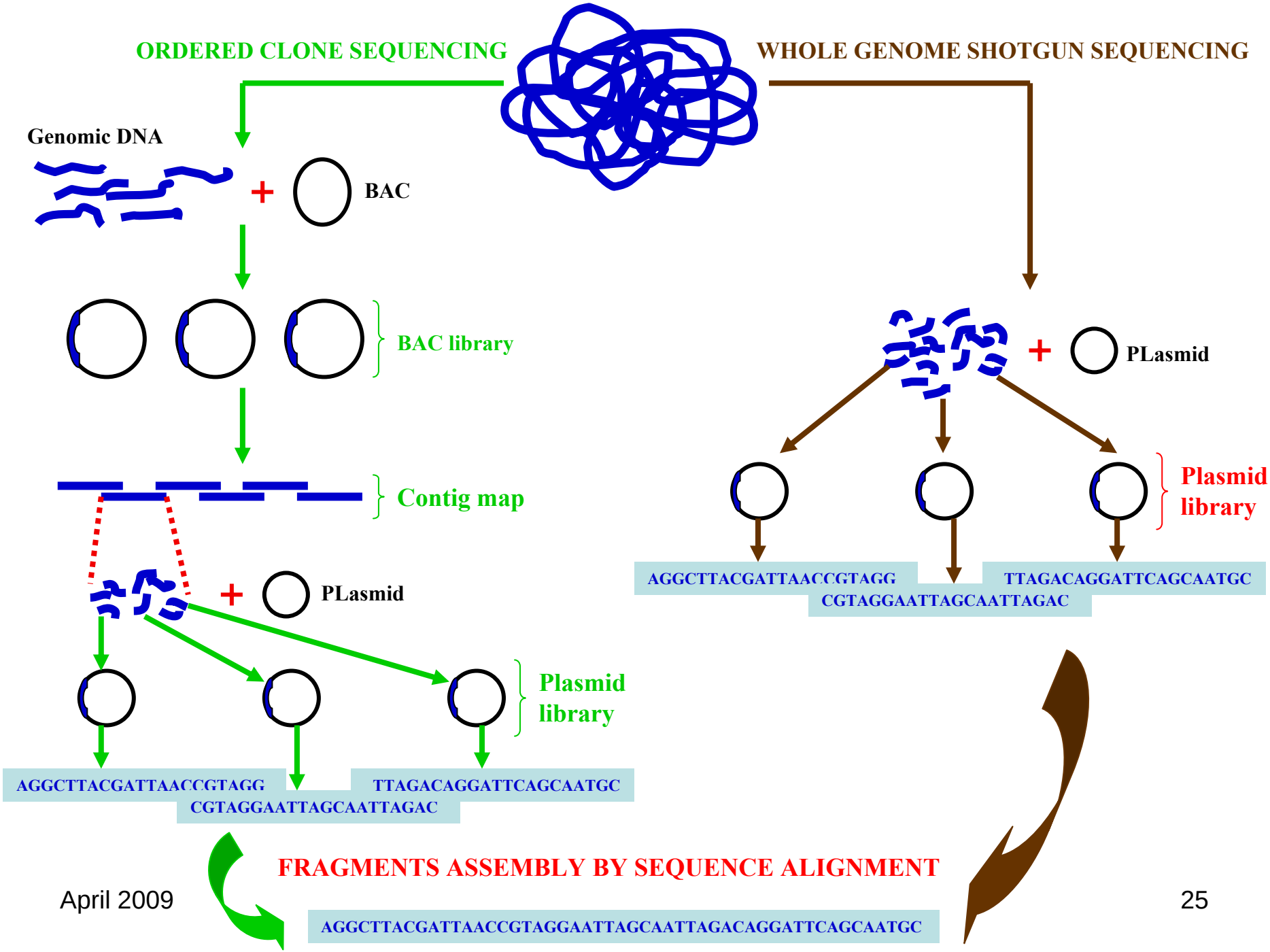
- **The genome is digested into large pieces which are cloned into vectors, usually BAC (Bacterial Artificial Chromosome). Recombinant BAC are then used to transform bacteria, forming genomic libraries**
- **A map including all BACs, called Contigs (Contiguous sequences), is established along the chromosomes**
- **Each BAC is cut into smaller pieces and sequenced.**
- **All the individual sequences are ordered into larger DNA fragments by alignment and finding of overlapping sequences. Final assembly of all Contigs.**

This approach largely avoids incorrect assemly of the predetermined Contigs but is time- and money-consuming

Whole genome shotgun approach

- **Genomic DNA is randomly sheared into small fragments. Each fragment is sequenced at the two ends**
- **Assembly of sequenced fragments by computational programs based on the finding of overlapping sequences**

This approach is more adapted to small-size prokaryotic genomes containing few repetitive sequences. It is faster and less expensive than the other approach but is more subject to errors in the assembly of sequenced fragments, in particular when repetitive sequences are present.


ORDERED CLONE SEQUENCING
WHOLE GENOME SHOTGUN SEQUENCING
Genomic DNA
BAC
BAC library
Contig map
PLasmid
Plasmid library
PLasmid
Plasmid library
AGGCTTACGATTAACCGTAGG
TTAGACAGGATTCAGCAATGC
CGTAGGAATTAGCAATTAGAC
AGGCTTACGATTAACCGTAGG
TTAGACAGGATTCAGCAATGC
CGTAGGAATTAGCAATTAGAC
FRAGMENTS ASSEMBLY BY SEQUENCE ALIGNMENT
AGGCTTACGATTAACCGTAGGAATTAGCAATTAGACAGGATTCAGCAATGC

Each genome project generates millions of DNA sequences. This huge and continuously growing information pool requires systematic storage and analysis methods to make them useful

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BIOINFORMATICS - THE STUDY OF INFORMATION ENCODED IN GENOME

Bioinformatics uses tools including applied mathematics, computer science, chemistry, biology, … to extract comprehensive information from the whole genome sequence.

- **Sequence alignment – a basic function of Bioinformatics – is firstly used to assemble fragments in genome shotgun sequencing. Analysis through sequence alignment is also used to compare sequences from different species or within species in order to deduce gene functions or to determine relations between species**

- **Seeking ORFs (Open Reading Frame which is considered as potential protein-coding sequence) through computational detection of special sequences corresponding to start and stop signals, intron-exon boundaries, …**

- **Prediction of docking sites (protein-nucleic acid binding sites) such as promoters, ribosome-binding site, regulators for replication, transcription and translation, …**

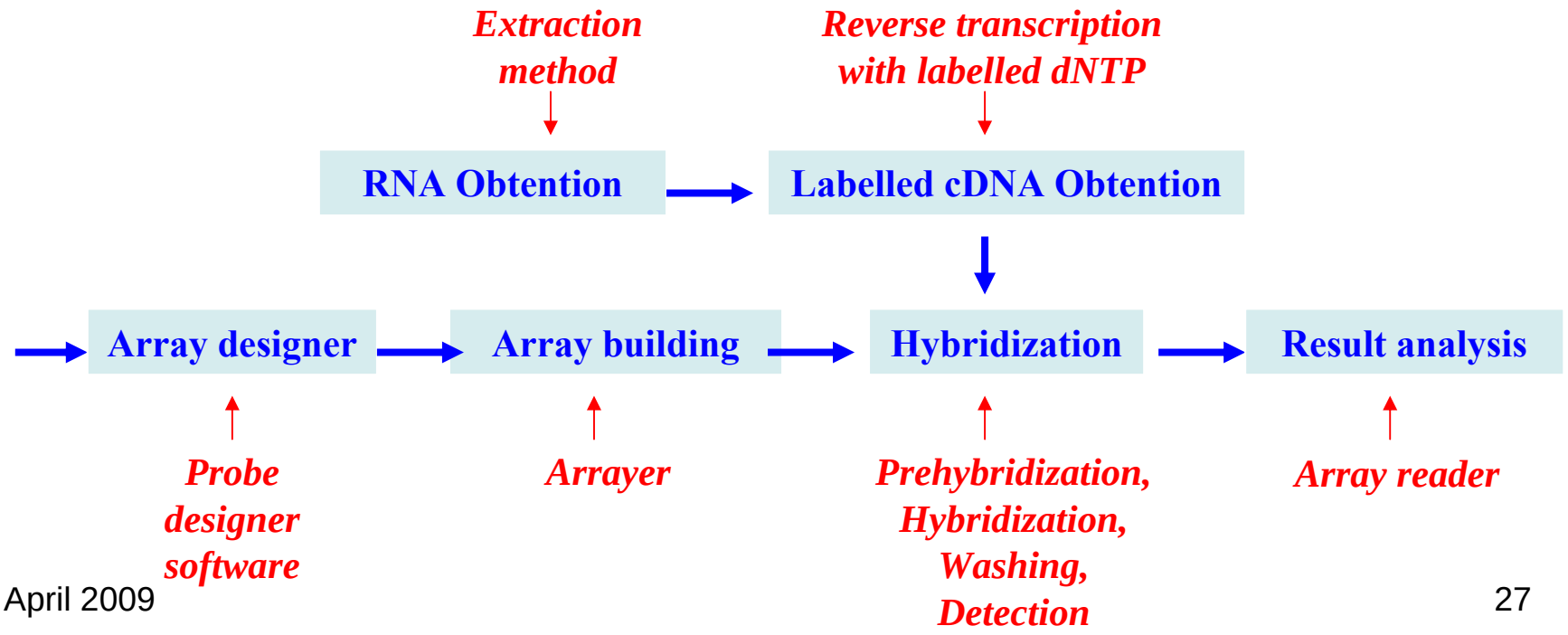
- **Prediction of gene function through sequence comparison between species,**

- **Study of the whole genome organization to understand the meaning of gene arrangements, e.g vertebrate genomes can be divided into regions having different G:C densities with G:C-dense regions containing more expressed genes.**

Whole genome sequence identification makes possible the study of functions and interactions between all genes involving in a biological process.

MICROARRAY – TOOL FOR FUNCTIONAL GENOMICS

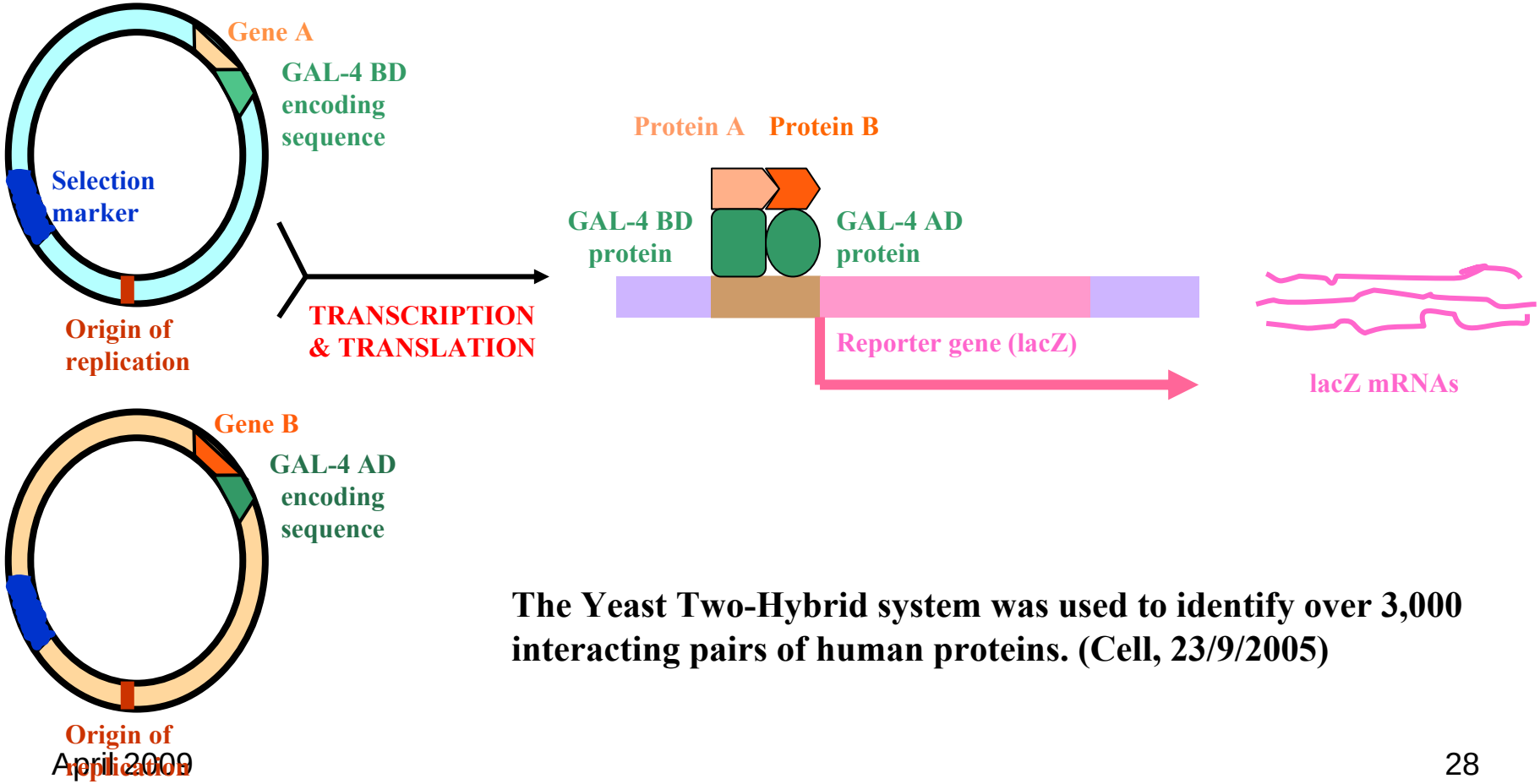
- **Microarry is based on the method of reverse dot blot. In this method, probes with known sequence, instead of targets, are fixed on a substrate. Labelled target sequences are then hybridized to fixed probes.**
- **A microarray can be made of glass, silicon or polypropylene and contain 3,000 to more than 40,000 sequences which are fixed or synthesized *in situ*. Target sequences are fluorescent-labelled.**
- **Microarray hybridization includes many steps :**



STUDY THE INTERACTOME

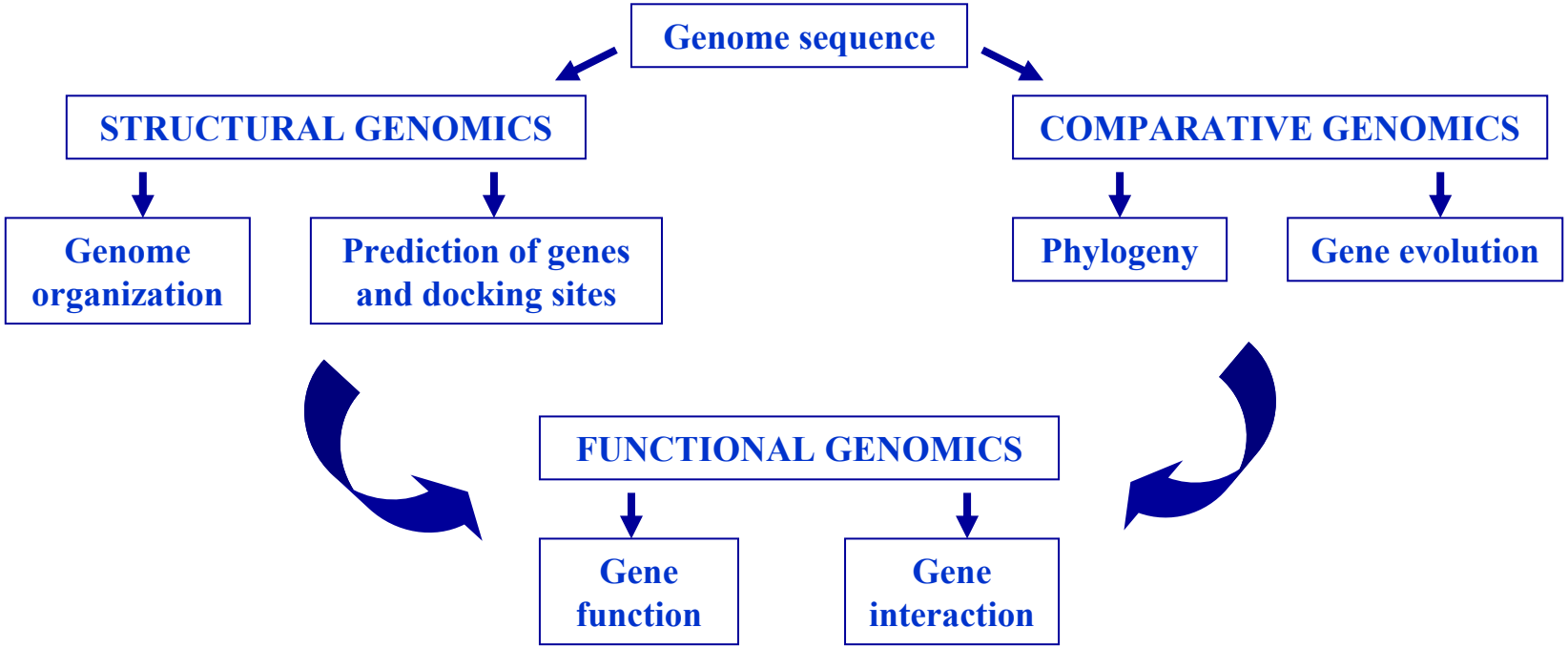
The Yeast Two-Hybrid System is used to study the interaction of two proteins, e.g protein A and B.

Gene A and gene B are respectively combined with sequences encoding a DNA Binding Domain (BD) and an Activation Domain (AD) of a yeast regulator gene – GAL 4 to form two hybrid proteins. Interaction between the A and B parts of the hybrid proteins restore the regulator gene structure and function leading to the induction of the reporter gene expression.



The Yeast Two-Hybrid system was used to identify over 3,000 interacting pairs of human proteins. (Cell, 23/9/2005)

Genomics and derivative domains can be summarized as follows :



Structural Genomics inspires other "omics" which are considered as post-genomics sciences

TRANSCRIPTOMICS studies the sequence and expression of all transcripts of a cell under defined conditions

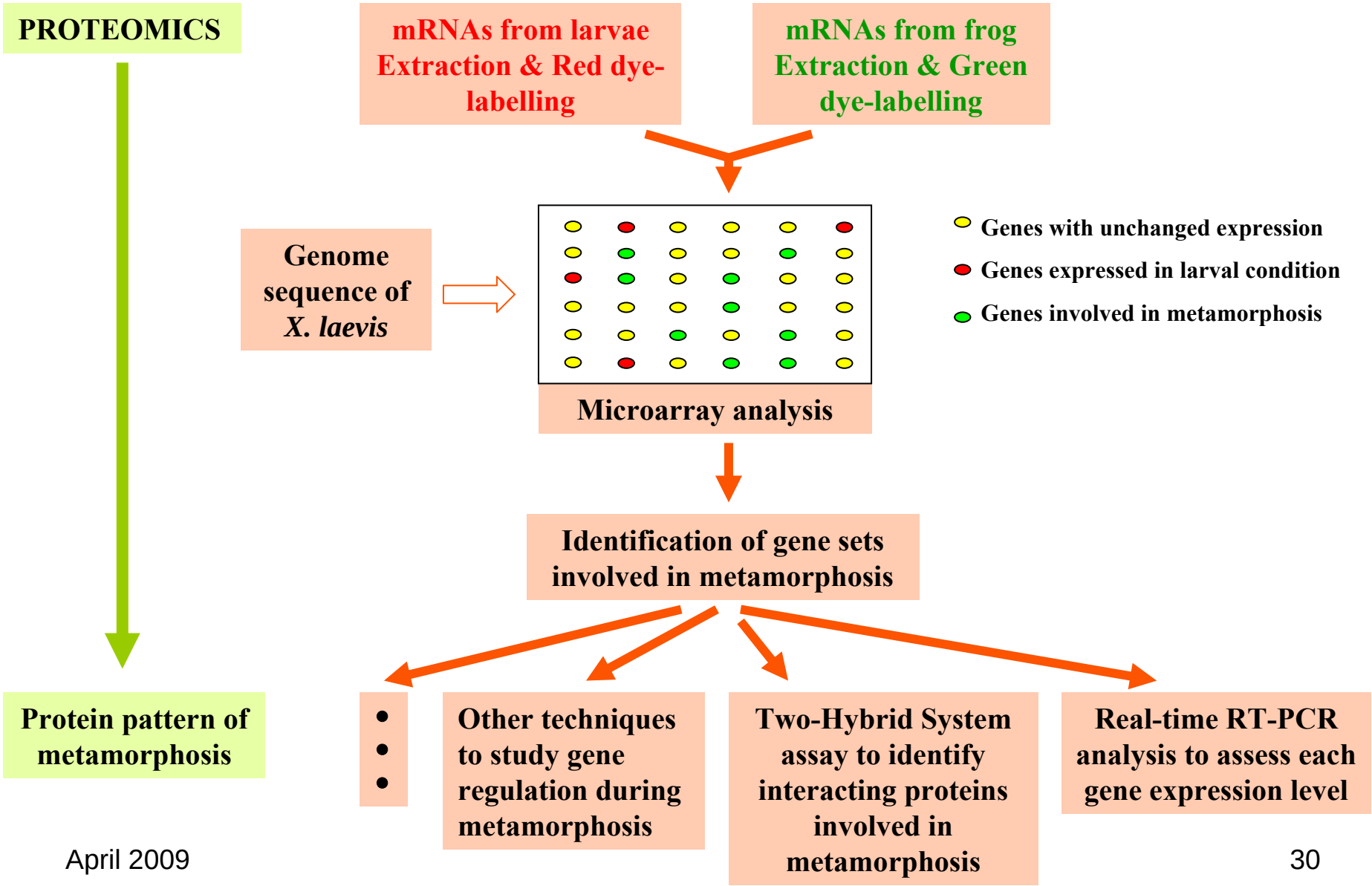
PROTEOMICS studies the structure and functions of all proteins of a cell under defined conditions

METABOLOMICS determines all metabolites of a cell under defined conditions

• • •

Unlike the genome, the transcriptome, proteome, interactome, metabolome, … are dynamic ensembles continuously changing, depending on internal and external stimuli. This considerably complicates their studies.

"What are the genes implied in *X. laevis* metamorphosis ? How are they expressed ? How do they interact to induce and control the process ?". A suggested study is presented below



SUMMARY

Individual gene structure and expression can be determined by sequential or simultaneous utilization of the following methods :

- **Nucleic acid extraction methods**
- **Qualitative and quantitative analysis based on Electrophoresis and Spectrophotometric measures**
- **Molecular Cloning**
- **PCR and other derivative techniques**
- **Molecular Hybridization – Southern/Northern Blot, Dot Blot, *in situ* Hybridization, …**
- **DNA Sequencing**
- **…**

Genomics - the study of the whole genome – comprises some domains :

- **Structural Genomics based on Genome sequencing**
- **Bioinformatics – the study of information from genome structure using computational methods**
- **Functional Genomics using methods such as microarray**

Other sciences – Transcriptomics, Proteomics, Metabolomics, … - respectively study the transcriptome, proteome, metabolome, interactome of a cell under defined conditions.