

*Lecture by:
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MICROARRAY

genetic expression analysis



INTRODUCTION

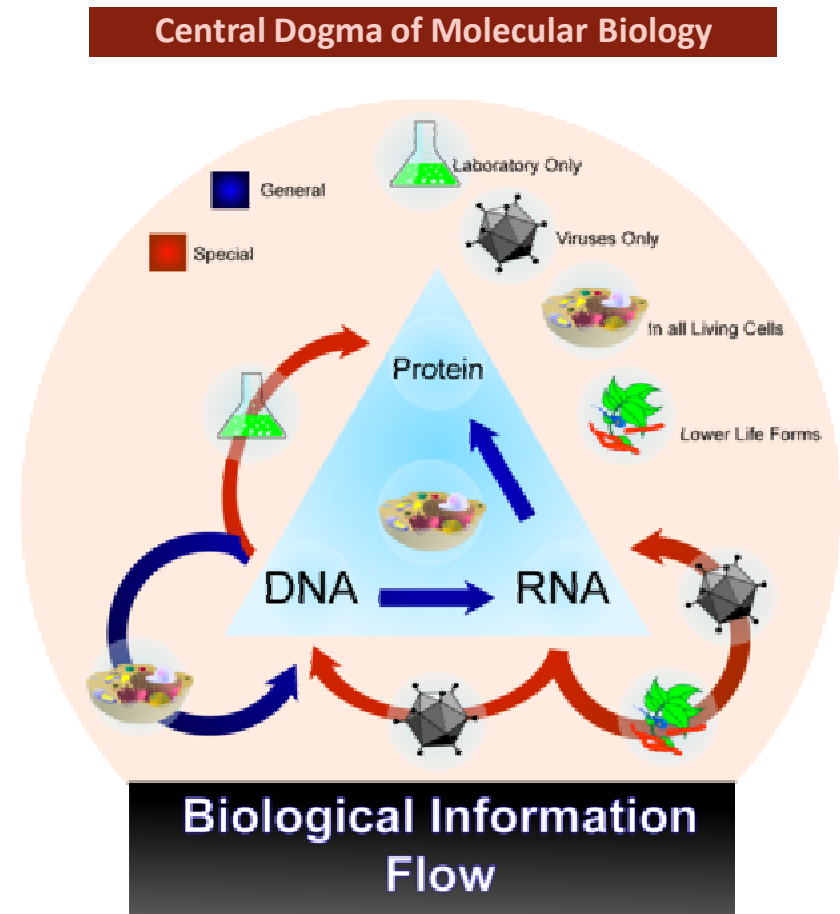
This section will discuss the foundations of
Microarray technology

Gene Expression

With only a few exceptions, **every cell** of the body contains a **full set of chromosomes** and **identical genes**.

Gene expression is a highly complex and tightly regulated process that allows a cell to **respond** dynamically both to environmental stimuli and to its own changing needs.

This mechanism acts as both an "**on/off**" **switch** to control which genes are expressed in a cell as well as a "**volume control**" that increases or decreases the level of expression of particular genes as necessary.



<http://en.wikipedia.org/wiki/Image:CDMB2.png>

Important Terms

Transcription

Messenger RNA

Polyadenylation

Reverse Transcription

Complementary DNA

Oligonucleotide synthesis

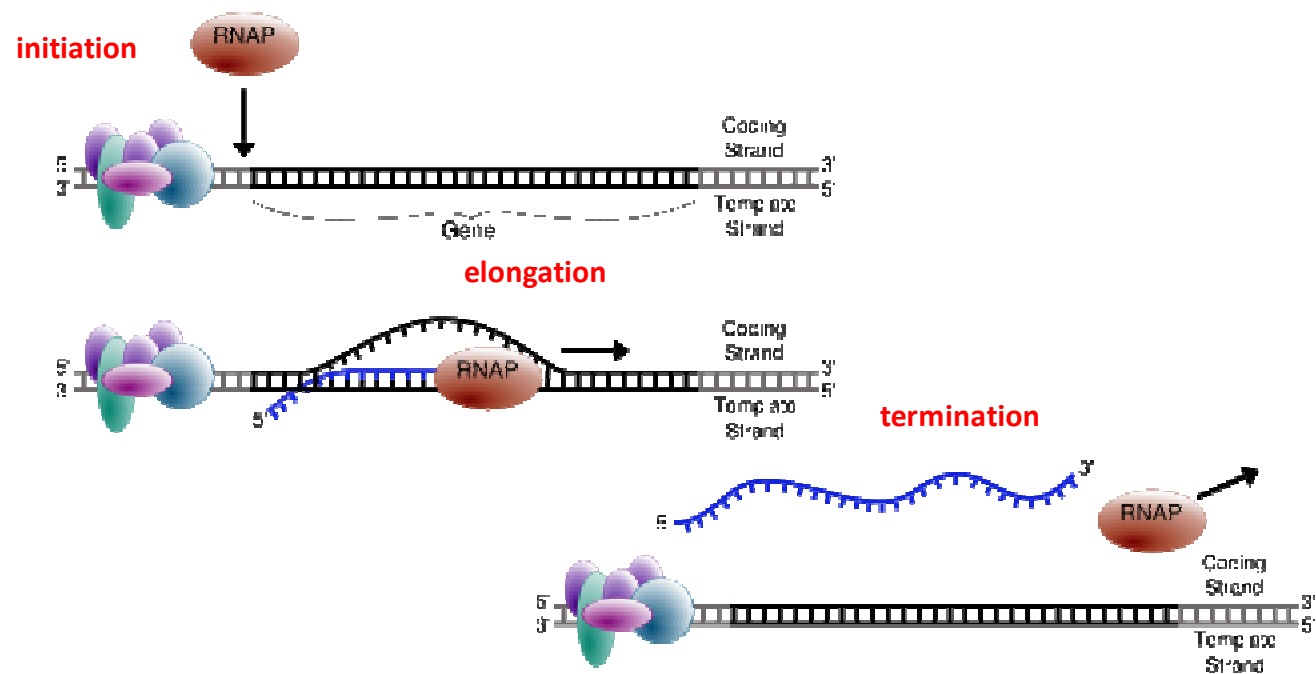
Nucleic acid hybridization

Fluorescence & Fluophore

DNA Labeling

Transcription

is the process by which the information contained in a section of DNA is **transferred** to a newly assembled piece of messenger RNA (mRNA).



http://en.wikipedia.org/wiki/Image:Simple_transcription_initiation1.svg

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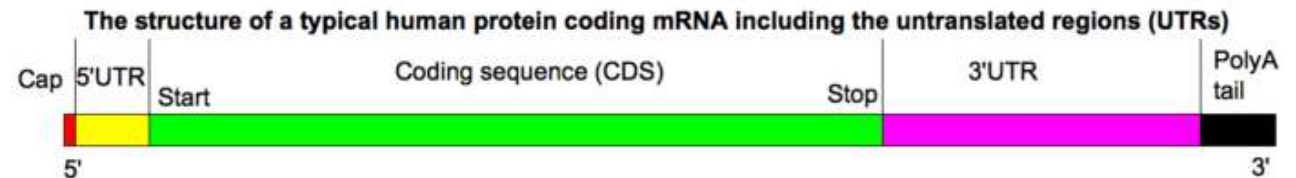
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Messenger ribonucleic acid (mRNA)

is a molecule of RNA encoding a chemical "**blueprint**" for a protein product. mRNA is **transcribed** from a DNA template, and **carries** coding information to the sites of protein synthesis: **the ribosomes**



http://en.wikipedia.org/wiki/Image:MRNA_structure.png

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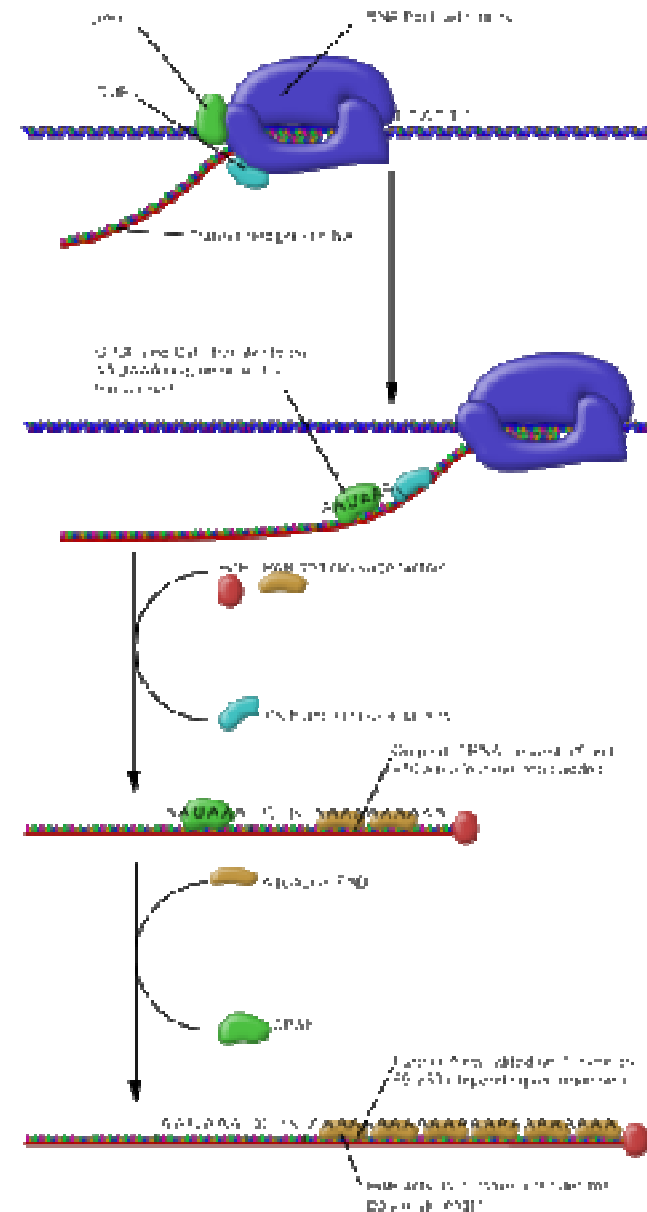
Polyadenylation

Polyadenylation is the covalent linkage of a polyadenylyl moiety to a messenger RNA molecule at 3' end (**3' poly(A) tail**).

protecting mRNA from degradation by exonucleases.

Important for transcription termination, export of the mRNA from the nucleus, and translation.

Occurs during and immediately after transcription of DNA into RNA.



<http://en.wikipedia.org/wiki/Image:Polyadenylation.png>

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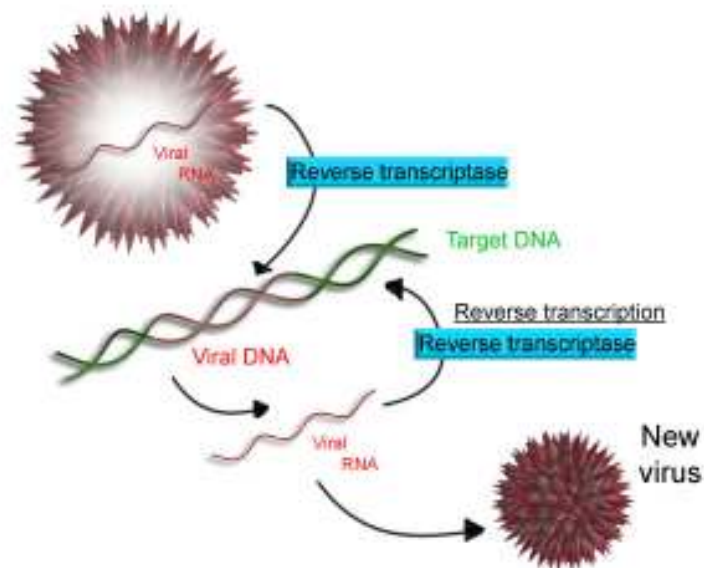
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Reverse Transcription

is the transfer of information from **RNA to DNA** (the reverse of normal transcription).

This is known to occur in the case of **retroviruses**, such as HIV, and, in higher eukaryotes, in the case of retrotransposons.

It is **not**, however, the **general case** in most living organisms.



<http://en.wikipedia.org/wiki/Image:RetroTranscription.jpg>

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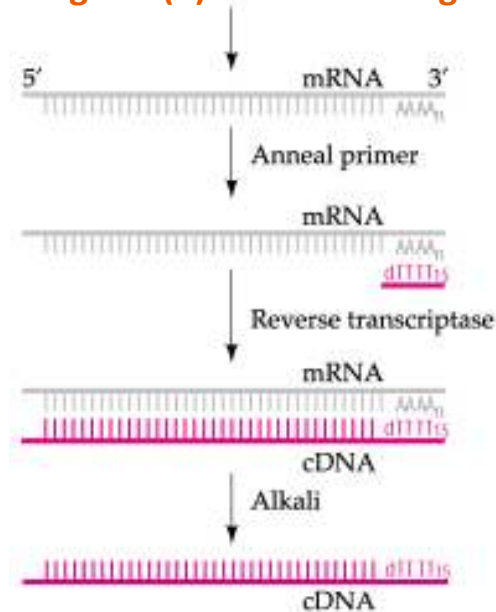
DNA Labeling

Complementary DNA (cDNA)

is DNA **synthesized from a mature mRNA** template in a reaction catalyzed by the enzyme **reverse transcriptase**.

This enzyme operates on a single strand of mRNA, generating its complementary DNA based on the pairing of RNA base pairs (A, U, G and C) to their DNA complements (T, A, C and G respectively).

We can use **oligo dT(n)** or **random oligo** as primer.



<http://8e.devbio.com/images/ch04/0405fig2.jpg>

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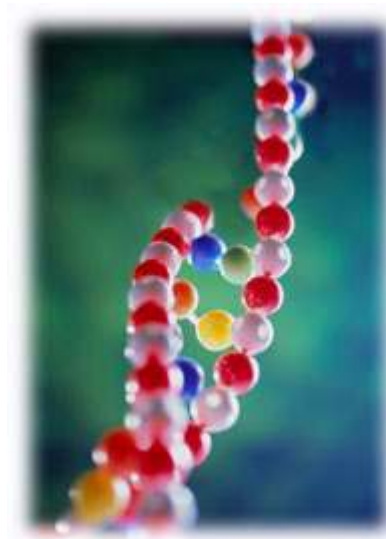
DNA Labeling

Oligonucleotide synthesis

is the non-biological, **chemical synthesis** of defined short sequences of nucleic acids

Automated synthesizers allow the synthesis of oligonucleotides (typically single stranded) up to **160 to 200 bases**

Commonly used as **primers, probes** and **restriction sites**



<http://www.ctaalliance.org/MCBI/images/oligo.jpg>

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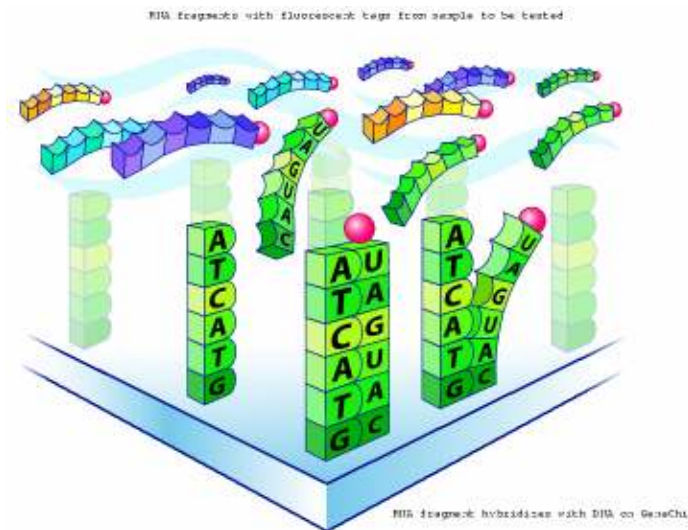
DNA Labeling

Nucleic acid hybridization

Hybridization is the process of combining complementary, single-stranded nucleic acids into a single molecule.

Nucleotides will bind to their complement under normal conditions, so two perfectly complementary strands will bind to each other readily.

Southern and Northern blotting are the exist two kind of nucleic acid hybridization



http://cswww.essex.ac.uk/staff/W.Langdon/genechip/hybridization_of_tagged_probes.gif

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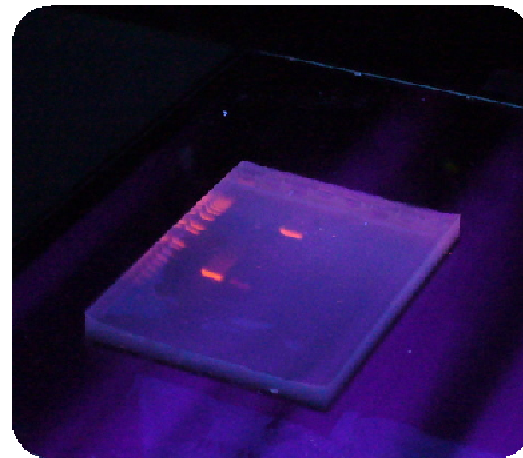
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Fluorescence

is a **luminescence** that is mostly found as an **optical phenomenon** in cold bodies, in which the molecular absorption of a photon triggers the emission of another photon with a longer wavelength.

Fluorophore

is a component of a molecule which causes a molecule to be **fluorescent**. It is a functional group in a molecule which will **absorb energy** of a specific wavelength and **re-emit** energy at a different (but equally specific) wavelength.



<http://en.wikipedia.org/wiki/Image:AgarosegelUV.jpg>

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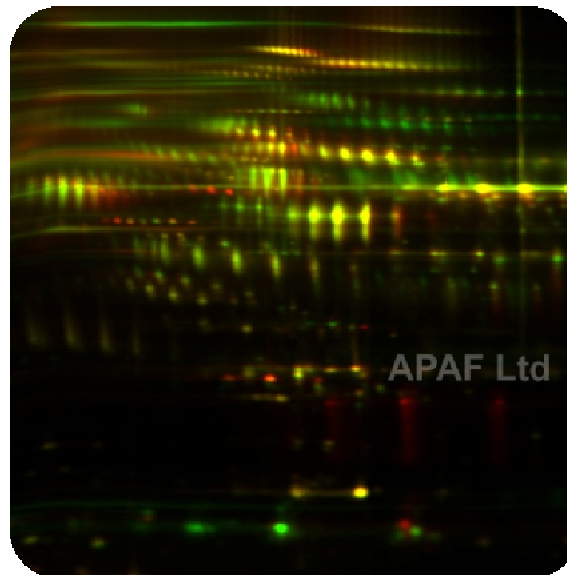
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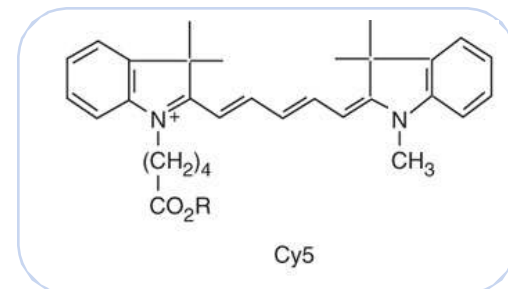
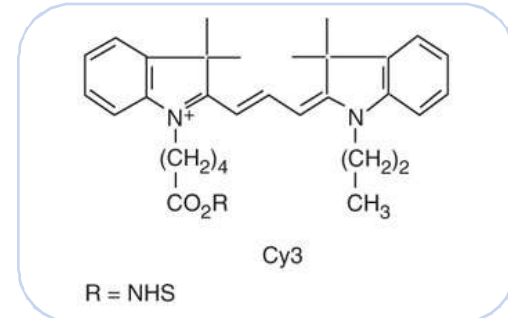
DNA Labeling

is an addition of a chemical into DNA strand to make them **visualizable**.
A label can be a radioactive compound or a **fluorophore**.

The most famous fluorophore family used in Microarray experiment is **Cyanine (Cy)**, there are two mostly used of Cy compounds, **Cy3** and **Cy5**.



<http://www.proteome.org.au/images/UserUploadedImages/saturation-dige.jpg>



<http://www.nature.com/nprot/journal/v1/n3/images/nprot.2006.234-F2.jpg>