hydrogen

G / C form 3 bridges of
hydrogen

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Double-stranded DNA (dsDNA) is a very regular structure whatever the sequence of bases. Each base pair consists of a purine and a pyrimidine, of so that the size of each pair is practically constant. Therefore, the distance between sugar residues it is also almost constant.

Chargaff Rules

- Valid for any dsDNA molecule
- A = T
- G = C
- A + G = C + T
- As a logical consequence of the above equations: A + C = G + T

TBP- (TATA binding protein)

It is not necessary to destroy the double helix conformation to have access to the base sequence this reading is carried out using proteins such as TATA.

The stability of the double helix

Hydrogen bridges are responsible for pairing, but are NOT the main responsible for the stability of the double helix. It is not because in one case there are 2 bridges hydrogen and another 3 that it takes “more or less” strength to break that bond. At responsible for the stability of the double helix are the stacking forces, depend on the adjacent bases of the same chain, and has a stronger performance in the G / C pair (hence the denaturation temperature is higher).

The fact that the interior of the DNA molecule is hydrophobic is essential because it stabilizes and protects hydrogen bonds from complementary bases. These connections would not form if the bases were surrounded by water molecules, as each of these bonds would be easily replaced by hydrogen bonds with water, ie the bases would form H bridges with the water. As I said earlier, the strongest forces important for the double-helix stability are the interactions of stacking between adjacent bases (interactions between electrons in upper and lower surfaces of the rings that form the bases)

Stacking interactions that involve G / C pairs are more stronger than those involving A / T pairs. This is the reason why Denaturation temperature depends on the sequence of bases.

The action of these forces causes a pile of pairs to form. bases, practically without any space between them which results also in the exclusion of water and keeps the two chains together.

How do you write the sequence of a DNA molecule?

We can get DNA from RNA through reverse transcriptase.

General properties of DNA polymerases

- Absolute need for mold chain, although they catalyze the phosphodiester bond
- Absolute need for a pre-existing polynucleotide (usually a primer or primer)
- Synthesis in the 5 '-> 3'- direction (establishes a phosphoester bond between the 3'-OH free of a pre-existing polynucleotide with the α – PO4 group of a 2′-deoxynucleoside 5′-triphosphate)

- Exonuclease 3 '□ 5' - DNA chain review properties, polymerase I of E.coli.

Replication of each of the chains

The replication of each of the chains of the pair propeller does not occur in the same way. The leading chain, also known as leading strand, is synthesized continuously. The other chain is synthesized discontinuous form by segments (Fragments of Okazaki) that have 1000-2000 nucleotides in bacteria and 150-200 nucleotides in eukaryotes. THE DNA ligase binds the fragments at the end.

Why the 2 chains do not separate completely for replication?

1) High temperatures were required
2) It would be difficult for maturation to occur so often without error

E.coli replication fidelity

1 error / 10^{9} - 10^{10} nucleotides ≈ 1 error / 10^{3} -10^{4} replications (E.coli genome size ≈ 4.6 x 10^{6} bp)

Contributions:

- Base pairing (H bridges)
- Geometric compatibility of base pairs. Mismatched bases are excluded from the active site of DNA polymerases
- Activity of intrinsic review of many DNA polymerases (proofreading)
- DNA polymerase I: in addition to synthesis activity, it has exonuclease activity 3'-5 ' (chain review). It can detect the error itself, rewind, cut the connection phosphodiester and replace the incorrect base. Endonucleases cut bases inside from jail.

DNA polymerase

The first DNA polymerase to be characterized was DNA polymerase

1) Responsible for replacing the RNA primer in an Okazaki fragment with DNA.

On the left, Escherichia coli DNA PolI, and on the right, Thermusaquaticus.

The image on the left represents a truncated version of the molecule, but perfectly functional (called Klenow fragment); the missing region is marked in green.

Green is the lost part with 5'-3 'exonuclease activity.

Klenow fragment - produced when E. coli poly I is enzymantically cleaved by a protease. Retains the 5´-3´ polymerase activity, but asks for the 5´-3´ exonuclease.

Okazaki fragment

Each Okazaki fragment must be initiated by an RNA primer (primer) that is synthesized by a primase DNA (which is a polymerase). Unlike DNA polymerases, this manages to initiate a polynucleotide linking two ribonucleosides triphosphate. In eukaryotic cells these initiators are approximately 10nt of size.

DNA exonuclease activity polymerase I

The 5 '=> 3' exonuclease activity of DNA polymerase I is responsible for replacement of RNA primers

through a process known as nick translation (cutting offset)

Nick translation: DNA ligase catalyzes bonding without add nucleotides.

DNA ligase

DNA ligase catalyzes the formation of phosphodiester bonds between terminal groups: 5 ' - phosphate and 3 'hydroxide juxtaposed, in double stranded DNA molecules.

ATP + (deoxyribonucleotide) $_n$ + (deoxyribonucleotide) $_m$ = AMP + 2Pi + (deoxyribonucleotide) $_{n+m}$

DNA ligase is the enzyme that unites Okasaki fragments, after the removal of primers (or primers) by DNA polymerase I. It is also used as a tool in Molecular Biology techniques to "stick" fragments of DNA of interest. In the figure, the yellow we have the phosphate balls that are released from ATP and that form the bonds phosphodiester among Okazaki fragments.

Replication source

Each origin of replication gives rise to an opening located on the double helix and two replication forks that progress in opposite directions. Each DNA strand has thus zones where it is the leading chain and the lagging strand. The chromosome bacterial ring typically has only one origin of replication that gives rise to two replication forks that progress in opposite directions.

Duplication of DNA in eukaryotic cells

DNA duplication in eukaryotic cells has to be regulated very it needs to ensure that the entire genome is duplicated once in each cell cycle. There are multiple sources of replication. The amount of DNA doubled by each source replication is relatively small. The existence of multiple sources of replication allowed the expansion of the genome during the evolution process.

Transcription and translation

Within the repeated sequences we have:

- Transposons- DA sequences capable of moving around a region to the other in a cell genome. The possibility of inserting themselves within the body's own genes it can cause several diseases. About 45%
  - the LINEs- Long Intersperced Nuclear Element, about 20%
  - SINEs- Short Interspersed Nuclear Elements, about 14%
- Retrovieal elements, about 3%
- Simple repeated sequences, about 2%
- Segmental duplications, about 6%

Within the unique sequences we have:

- Genes, about 20%
  - Introes, about 19%
  - o Protein coding regions (exons), about 2%

Não Non-repetitive DNA that is neither in the codons nor in the intros, about 28%

Promoters

RNA synthesis begins in special DNA sequences called promoters. An promoter sequence is an asymmetric sequence in the DNA that marks where the RNA polymerases should initiate synthesis and in which direction they should continue. In eukaryotes, the Poly RNA binds indirectly, and in prokaryotes it binds directly.

- mRNA
  - o Coding region with introns in the middle
  - the 5'-RTU- after the promoter region
  - o 3'- RTU- before terminating region
  - o The RNA sequence starts at +1 (after the promoter region) and ends before the terminating region. (includes 5'UTR and 3'-UTR)

Regions not encoder ss

RNA synthesis

RNA synthesis begins in special DNA sequences called promoters. Typically, in bacteria, promoters are formed by two elements positioned at -10 and -35 to the first mRNA nucleotide (+1).

of these.

Restriction enzymes

Restriction enzymes cleave DNA at specific locations:

1) Producing fragments of discrete size, which can be analyzed by electrophoresis (band pattern reflects enzyme cut sites)
2) Constituting a valuable helper in DNA cloning and allowing manipulation of relatively small DNA fragments (insertion into molecules)

Most restriction enzymes act as homodimers (proteins made up of two similar subunits) and leave small single chain ends, the cohesive ends.

EcoRI is a 31-kilodalton subinitial homodimer

Restriction endonuclease I + methylase

Bacteria are permanently under attack by bacteriophages, such as example phiX174. To protect themselves, they developed a method of attacking DNA strangers: restriction endonuclease - methylase, which is a system that protects DNA from host and digests the viral DNA.

1) Cut the DNA in the middle of the chain

At

Restriction enzymes (or restriction endonucleases) were discovered in 1970 during investigations on the phenomenon of modification and restriction of bacteriophages by host.

The restriction to infection results from the action of "restriction enzymes" and, in combination with DNA-methyltransferases (ECORI methylase - methyl active sites in the restriction enzyme DNA EcoRI). These methylate the bacterial DNA itself, methylate at the cutting sites of the

Transformation (of phenotype):

Incubation of a competent E. coli suspension (cells treated in such a way as to more easily incorporate DNA) with the ligation reaction product.

Exposure to CaCl induces the competence of DNA cells as it makes the membrane cell permeable to DNA. Competence is also due to thermal shock occurs at entry of the plasmid to bacteria, through pores in the wall. It is a rare event.

Basic characteristics of plasmids

Plasmids have:

- An origin of replication (ori) that allows autonomous replication of the plasmid *,
- A multiple cloning site (MCS) or polylinker **, which allows the insertion of the fragment to be cloned- large number of unique restriction sites
- A selection marker that allows you to select the bacteria that incorporate the plasmid (Ex: gene that confers resistance to antibiotics, such as ampicillin)

* if the plasmid does not have the zone recognizable by the replication machinery it is not divided.

** Specially designed to insert the DNA fragment; with restriction locations of all compressed enzymes, these sequences were not repeated in any other plasmid location allowing it to be cut only once by the restriction enzyme. Allows flexibility in choosing plasmid

Most used antibiotics in biology

The DNA topoisomerase I enzyme of the virus vaccine cleaves the phosphodiester bond of

Primers for cDNA synthesis	Considerations
Specific primers	Used to reverse specific RNA genetic sequences Primer only recommended for one RT-PCR step Can also be used in 2 steps of RT-PCR
Oligo (dt) 18	Used to reverse transcripts from eukaryotic mRNAs only and polyA tails retroviruses Not all genes have poly A chain. Do not reverse 18S rRNA transcripts They may have problems transcribing long transcripts or with pin structures May not efficiently reverse degraded RNA due to lack of the intact poly A
Random primers	Can simultaneously reverse transcripts of all mRNA and also 18S rRNA (ie endogenous controls and targets) Best to use with long transcripts, and pin transcripts and degraded RNA.

Poly-T primers are used to take advantage of the fact that eukaryotic RNA has the poly A tail and so we can copy all the mRNA (this only works for eukaryotes - only these have poly-A tail. Transcribes all the mRNA present in the cell (lets you know eg which genes are being transcribed in the cells. If we don't know the sequence then we use random primers with 6bp, these bind to sequences that are assigned to them complementary)

RT- PCR Only in one step

Reverse transcriptase is not thermostable - without interruption

RT PCR 2 steps - do the reaction transcriptase and PCR in times many different

Uses

- Expression studies in reference genes
- The AGP6 and AGP11 genes are expressed in unicamen in the pollen grain (GP) and tube pollen (TP). The amount of total cDNA used in each PCR reaction should be equivalent in all reactions of experience. The reference genes Ubc9 and Tub4 (housekeeping genes) are used to standardize the amount of cDNA in each reaction.

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qPCR - Real-time PCR

In contrast to conventional PCR, PCR in real-time (qPCR) allows the accumulation of amplified is estimated as the reaction progresses by increasing an alarm signal fluorescence. Special thermal cyclers measure the fluorescence signal.

The great advantage of qPCR is the determination of initial amount of mold molecules (as a rule, mRNA) in the sample, with high accuracy. qPCRé especially useful in gene expression studies, viral load measurement, pathogen detection

The threshold value must be the same for all samples analyzed in a given experience.

CT-Cycle in which the amount of accumulated amplifier produces a signal fluorescent above the threshold value.

Chemical systems used in qPCR-Fluorochromes specific for dsDNA: fluorescence increases intensely only when the fluorochromes and binds to dsDNA

Main disadvantage: they emit fluorescence in the presence of any dsDNA, including dimers of primers.

- Viral transfer of regulatory genes and oncogenes is dangerous and must be monitored
- The variation may not be adequate

• Many viruses are lytic

Transfection methods based on chemical reagents

Positively charged particles are mixed with DNA and added to the culture of cells:

• Liposomes
• Calcium phosphate

• DEAE dextran

1. **Lipofaction**

Lipid mediation also known as lipofation, or gene transfection based on liposome. Uses lipids to cause absorption of Exogenous DNA by the cell.
Transfer of genetic material to the cell occurs via liposomes, which are vesicles that can fuse with the cell membrane, given which are both made of a bilayer phospholipid

2. **Calcium phosphate**

This protocol involves mixing DNA with calcium chloride added in a controlled manner to a phosphate solution saline, allowing the mixture to incubate the temperature environment.
This step generates a precipitate that is dispersed in the cells of culture, it is absorbed by the cells via endocytosis or phagocytosis.

3. DEAE-Dextran

DEAE-dextram is a cationic polymer that is associated with nucleic acids with negative charge. The positively charged polymer-DNA complex is associated with

induce the transcription of the genes to come, and the consequent transfer of T-DNA and respective integration into the plant's genomic DNA.

5. Transcription and translation of T-DNA by the plant cell leads to the production of opines (nutrition) and tumors (logistics) for bacteria.

6. The Ti plasmid genes involved in the transport and catabolism of opines allow bacteria to use opines as a source of C, H, O, and N.

Transgenic plants

A disc is cut from a leaf and incubated on a plate cell culture Agrobacterium containing a recombinant plasmid, this in turn contains a checkmark and the desired transgene. the cells damaged are on the periphery and segregate substances that attract the cells of Agrobacterium and cause injection of DNA into these cells. Only the plants that absorb the DNA appropriate and express the checkmark will survive and form callus. THE manipulation of growth regulators and nutrients provided will promote the formation of small formations that later will originate an adult individual with the transgene.

Floral dip method

… Their work has made it possible to modify specific genes in the germlineof mammals and to raise offspring that carry and express the modified gene.

(www.nobelprize.org)

When an investigator intends to replace an allele without affecting any other in the genome, the method of choice is recombination homologous.

Conditions for the production of transgenic mice were gathered: the development of ES cell cultures, the demonstration that genetic changes in cells ES can be transmitted to the germline cells, and the observation that homologous recombination occurs with high frequency in mammalian genome

Homologous recombination associated with the LoxP / Cre system recombinase constitutes a powerful tool for studying gene activity in mice

Cre Recombinase

Cre recombinase is a type I topoisomerase of bacteriophage P1 that catalyzes the site-specific recombination of DNA between LoxP sites. 2 species of DNA containing

only one LoxP site will be fused, the DNA between the loxP sites will be excised in one circular shape, while the DNA between the local opposites of LoxP will be inverted

Applications of Cre Recombinase

- Excision of DNA between 2 LoxP sites
- Fusion of DNA molecules containing places of LoxP
- Inversion of DNA between sites from loxP

The Cre / lox system is one of more powerful tools and versatile for studies in mice. Provides sophisticated control over the location and timing of gene expression. Cre / loxé normally used to make alleles knockout, but can also be used to activate gene expression.

The insertion of lox-P to flank a Stop sequence (elements transcription terminal), between the promoter and the sequence transgenic will block gene expression.

The Cre / lox seismic can also be used to produce strains in which trangene is induced or expressed in certain fabrics. For example, mating the species of fig 1 with a species that

3. Adding restriction sites on the PCR product
   Uses oligonucleotide primers designed to include enzyme recognition sites from restriction near the 5 'terminal. After amplification, the resulting product is isolated by precipitation and cleaved by the restriction enzyme appropriate, resulting in a fragment of DNA with cohesive ends that will bind to sequences complementary elements in a prepared cloning vector.

Degenerate Primers Design

A Primer sequence is degenerated when some of its positions have several hypotheses. We use degenerate primers when we know the amino acid sequence of a protein. We can reverse the translation and determine the possible nucleotides of the sequence

W could use the blue string degenerate primer since has fewer chances and for each nucleotide there's only one bases different. Thus 32 oligonucleotides could be made from those combinations.

CDNA Libraries

- Cardosine A - aspartic proteinase
- Isolate protein - obtain gene - gene library

We can use cDNA, using the cell's mRNA (we must be careful, because despite all cells have the same DNA, mRNA can be different for each that is, we have to be careful and extract the mRNA from a place where it is expressed)

To transcribe to cDNA (5 'and 3' RACE) using reverse transcriptase we use the polyT primer that pairs with the poly A tail, which is characteristic of mRNA. The 2 chain of cDNA, (same as the original mRNA strand, except that it has R's instead of U's). Yes yes is equal to the initial mRNA strand, it is complementary to the strand copied by the transcriptase reverse that is formed by DNA polymerase as soon as the mRNA chain is destroyed. It is placed also an adapter at the end.

When inserting in a vector for expression, it is necessary to take into account the direction relative to the promoter, but for gene library it is not necessary. Cutting the cDNA with enzymes from restriction and the plasmid with the same enzymes- transformed a population of bacteria with these plasmids - forms a cDNA library

strands are shown in blue and purple. The letter × marks the position of the mutation.

Procedure in a single reaction tube (2 complementary primers per reaction)

- It's economical
- Cannot be used by multiplex
- Needs analysis if denaturation curve

Sequence specific probes- TaqMan

- The probe is a single-stranded DNA fragment with a fluorescent reporter on a bridge and a “quencher” on the other (The latter absorbs energy from the reporter when together on the same molecule)
- Binds to DNA at a specific location
- After the primers are connected, when the polymerase is copying the chain, its exonuclease activity 5'□3 'removes the nucleotides from the probe, and at the time that it disconnects from DNA the reporter emits fluorescence
- The more strings are copied and so more separate probes, more fluorescence is emitted.
- What prevents the probe from emitting fluorescence is its connection to the quench it when the polymerase acts separates the nucleotides, separating the reporter from the quencher (So it stops absorbing the your energy)
- As we have the probe and the 2 primers to pair this method is extremely specific and not we need to resort to curves of denaturation
- Curve analysis is not required denaturation

TaqMan improves specificity, product quantification and multiplex PCR.

Sometimes it is necessary to have several fluorescences to mark different PCR-reactions can be multiplex.

PCR has become a central tool for DNA analysis across all disciplines of biology and biochemistry

Novel enzymes and instrumentation are creating new applications for PCR

Other advanced PCR methods for research and diagnostic applications:

Start Hot start PCR (specificity)
- Cycling sequencing (DNA sequencing)
- Site-directed mutagenesis PCR
- Colony PCR
- Multiplex-PCR