BASIC METHODS IN MOLECULAR BIOLOGY

MANIPULATIONS WITH DNA:
- DNA cloning in plasmids (restriction endonucleases)
- Sequencing
- Polymerase Chain Reaction (PCR)

ISOLATION OF DNA, RNA:
Phenol extraction
Ethanol precipitation

CONVERSION OF mRNA TO cDNA (complementary DNA)
Enzyme reverse transcriptase
In DNA: A, G, C, T (with 2'-deoxyribose) dA, dG, dC, dT
In RNA: A, G, C, U (with ribose)

DNA structure: phosphodiester bonds
DNA can be easily:
- isolated as a pure nucleic acid, free of proteins and RNA
- cleaved at specific sites with restriction enzymes and recombined
- sequenced

Types of DNA:
- genomic (nuclear): high molecular weight DNA (> 100 kb long)
- cDNA (copy of messenger RNA)

linear (genomic DNA, DNA of some DNA viruses, cleaved circular DNA) or circular (plasmids, E.Coli chromosome,...)

DNA double strand:
5´... T C G C G C T A A A C T C C C T ...3´ = upper strand, the same sequence as in mRNA
3´... A G C G C G A T T T G A G G G A ...5´

when these strands are separated, they have different nucleotide composition
and can be separated (e.g. by electrophoresis)
or
5´... T C G C G C T A A A C T C C C T ...3´
(thecomplementary strand is usually not shown)
in RNA: 5´... U C G C G C U A A A C U C C C U ...3´
<table>
<thead>
<tr>
<th>DNA</th>
<th>RNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STRUCTURE:</strong></td>
<td><strong>FUNCTION:</strong></td>
</tr>
<tr>
<td>2’-deoxyribose ribose</td>
<td>- storage of genetic information - role in the expression of genetic information</td>
</tr>
<tr>
<td>- thymine - uracil</td>
<td>- replication, transcription, translation</td>
</tr>
<tr>
<td>- double helix, higher order structures in the nucleus - single strand with a secondary structure</td>
<td></td>
</tr>
<tr>
<td>Basic processes in which they participate:</td>
<td></td>
</tr>
<tr>
<td>- replication, transcription (ssDNA as template)</td>
<td></td>
</tr>
<tr>
<td><strong>Localization in the cell:</strong></td>
<td><strong>Formation of hybrids:</strong></td>
</tr>
<tr>
<td>- nucleus, (mitochondria) - nucleus, cytoplasm, (mitochondria)</td>
<td>DNA x DNA DNA x RNA RNA x RNA</td>
</tr>
</tbody>
</table>

**Isolation of pure nucleic acids – DNA and/or RNA**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>~ Disintegration of cellular compartments</td>
</tr>
<tr>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>+ sodium dodecylsulphate</td>
<td>~ PROTEIN DENATURATION</td>
</tr>
<tr>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>+ phenol/chloroform</td>
<td>~ PHASE SEPARATION</td>
</tr>
<tr>
<td>↓</td>
<td>DNA and RNA in the water phase</td>
</tr>
<tr>
<td>↓</td>
<td>DNA and RNA in the water phase</td>
</tr>
<tr>
<td>↓</td>
<td>Ethanol precipitation (both DNA and RNA precipitate in the presence of higher salt concentration and 70% final conc. of ethanol)</td>
</tr>
</tbody>
</table>

The method works in a range of nucleic acid concentrations and molecular weight.
Q: Can RNA also form a duplex?

Q: What is the difference in the stability of pure DNA and RNA
CLONING OF DNA INSERTS INTO PLASMIDS

Plasmid: circular DNA, replicates autonomously in bacteria, requires origin of replication and resistance to an antibiotic

Insert: any fragment of ds DNA

Ends of plasmid background and insert are important:
Must be compatible (sticky) or blunt

**Cleavage by restriction endonucleases**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>5' extension</th>
<th>3' extension</th>
<th>Blunt end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eco RI</td>
<td>5' extension</td>
<td>3' extension</td>
<td>Blunt end</td>
</tr>
<tr>
<td>Psi I</td>
<td>5' extension</td>
<td>3' extension</td>
<td>Blunt end</td>
</tr>
<tr>
<td>Dra I</td>
<td>5' extension</td>
<td>3' extension</td>
<td>Blunt end</td>
</tr>
</tbody>
</table>
Detection of nucleic acids:

Hybridization,
“probing”,
Types of probes, labeling of probes
Radioactive phosphates in NTP/dNTP

\[ \gamma^{32}\text{P}-\text{ATP} \quad \alpha^{32}\text{P}-\text{dATP} \]

**Polymerase chain reaction (PCR)**

(≈ “cloning” without bacteria, in the test tube)

Use: DNA diagnostics, forensic medicine, research
CELL CULTURE METHODS
FLOW CYTOMETRY

FACS (Fluorescence Activated Cell Sorting):

Forward Scatter (FSc)
- particle size

Side (Orthogonal) Scatter (SSc)
- Cell surface, granularity

Fluorescent Labeling - emitted light
DNA Analysis
Analýza fází buňč. cyklu pomocí průtokové cytometrie

FLOW CYTOMETRY

Common Laser Lines

PE-TR Conj.
Texas Red
PI
Ethidium
PE
FITC
cis-Parinaric acid
Fluorochrome Dyes Used in Flow Cytometry

Laser Line

Excitation

Emission

Marina Blue®
Alexa Fluor® 350
Cascade Blue®
Cascade Yellow™
Fluorescein
Phycocerythrin
BD CyChrome™
(PE-Cy5)
PerCP™
Texas Red®
Allophycocyanin
APC-Cy7

Wavelength (nm)