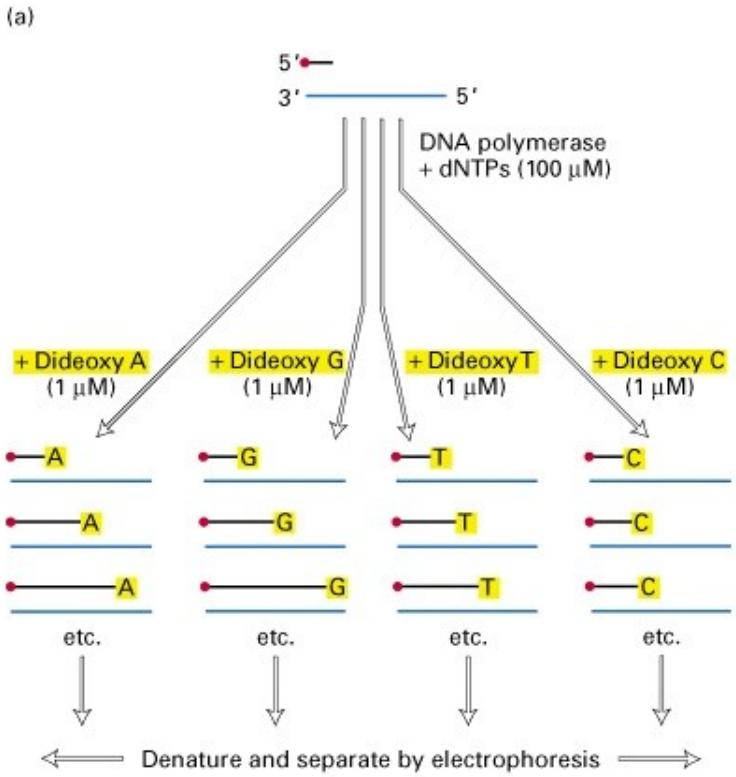
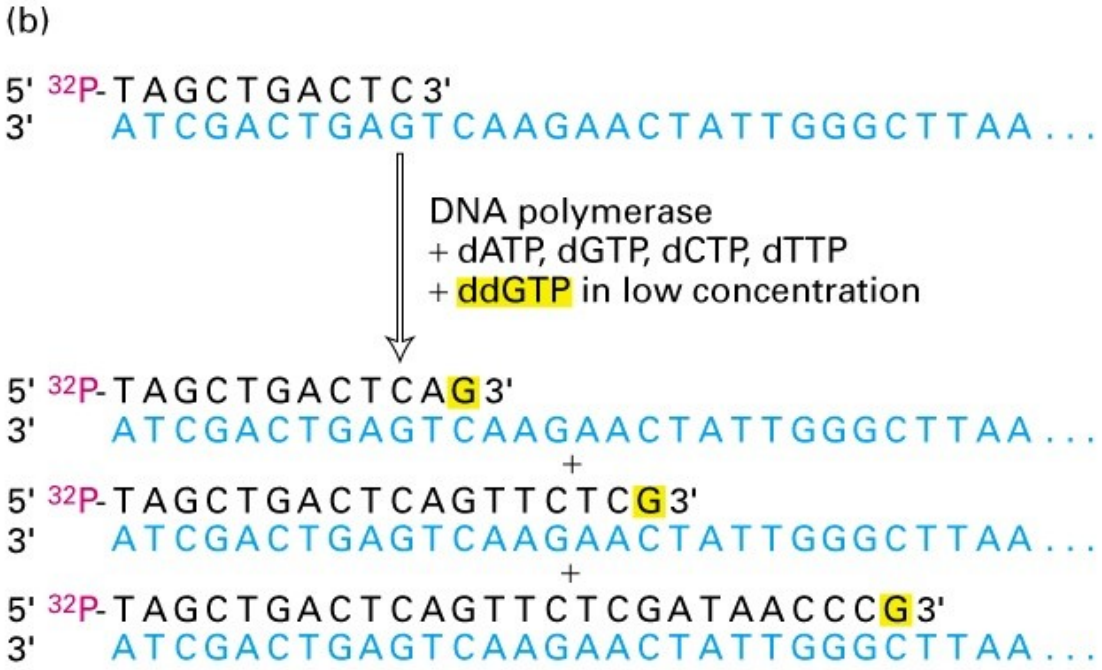
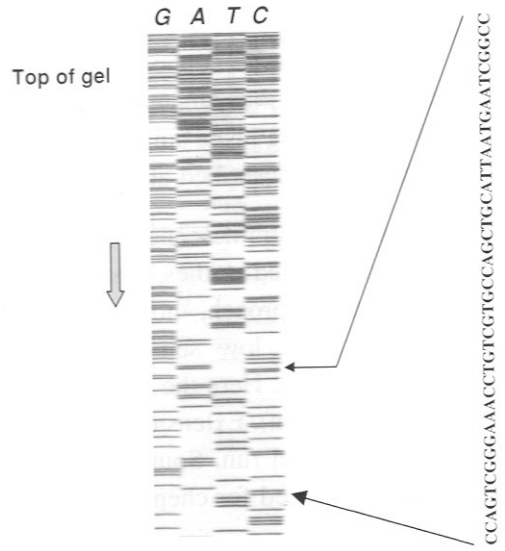
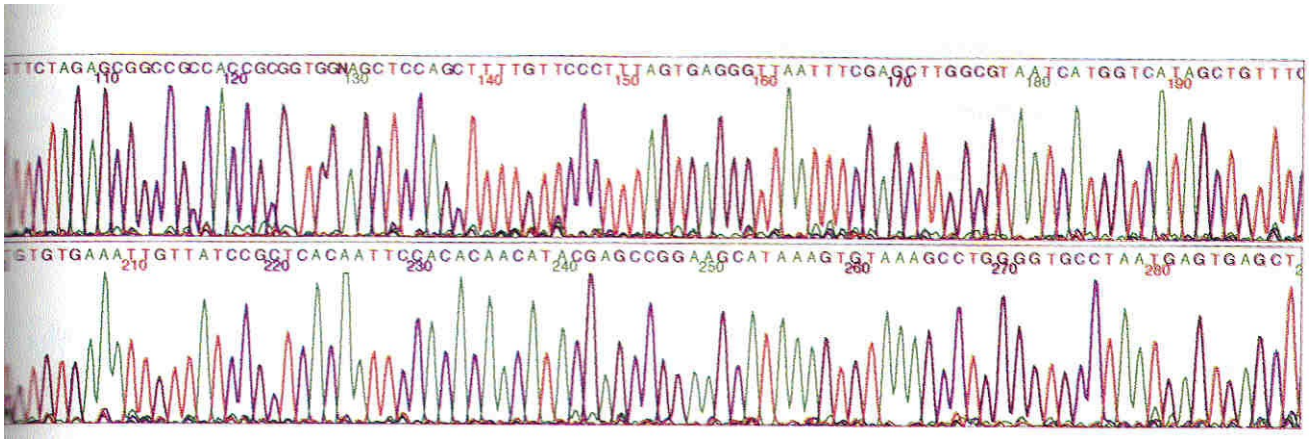
METODY STUDIA

Sekvence DNA – chemická (selektivní štěpení – Maxam-Gilbert), biochemická (syntéza, dideoxy metoda - Sanger)









PCR

Taq polymeráza, dNTP, primery, vzorek-templát

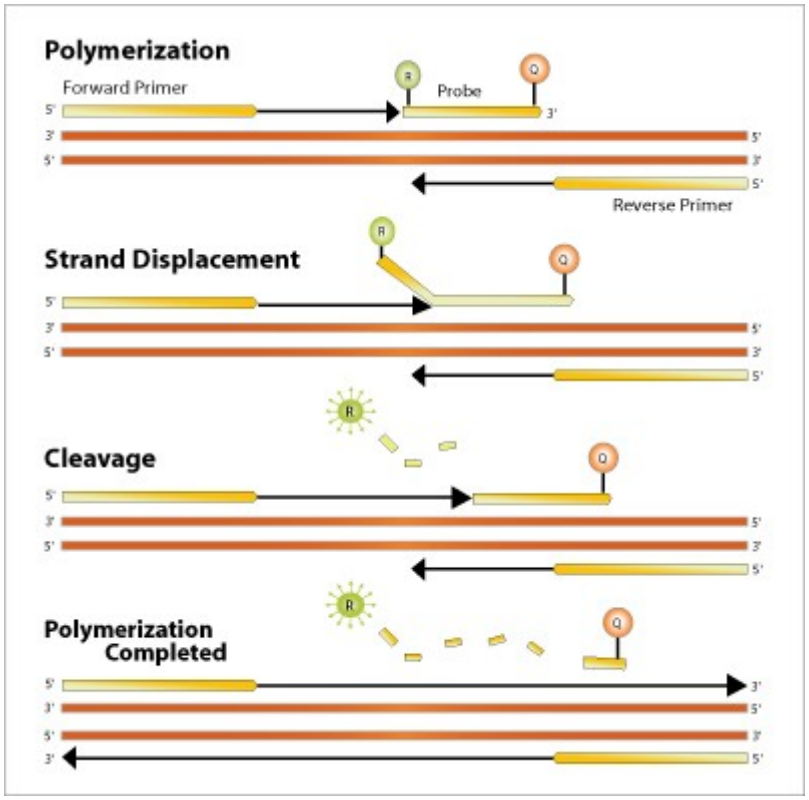
Termocycler - animace

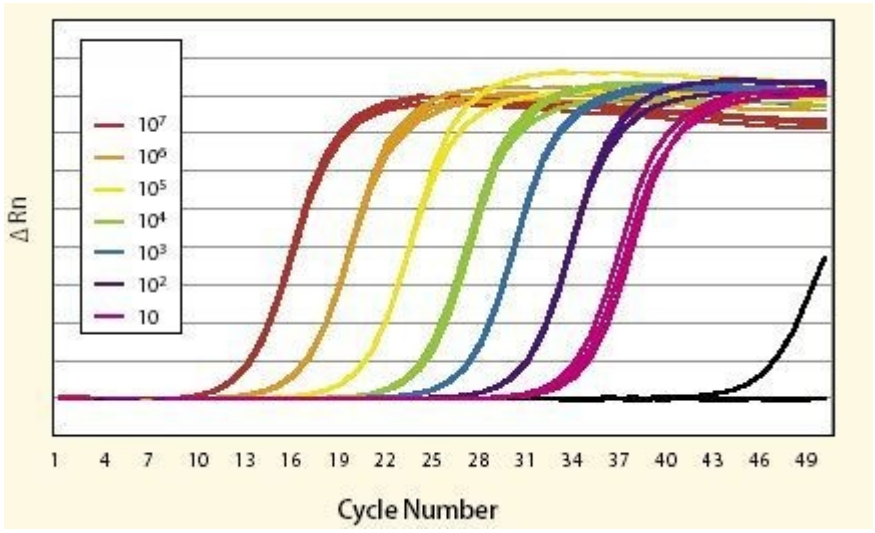
Potřeba primerů

Kvantitativní PCR

Význam – analytický – diagnostika, forenzní analýza,

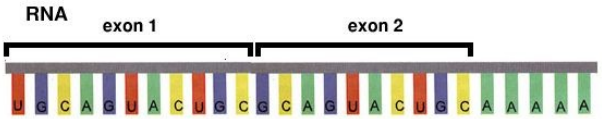
- preparativní - pomnožení materiálů, umělé geny atd.

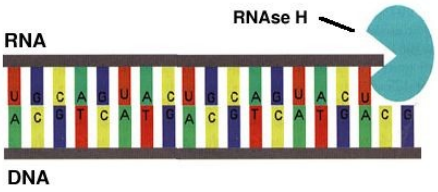

Polymerization
Forward Primer
R
Probe
Q
Reverse Primer
Strand Displacement
Cleavage
Polymerization
Completed

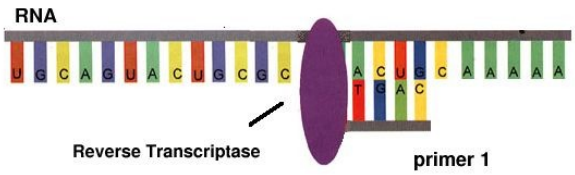

10^7
10^6
10^5
10^4
10^3
10^2
10
ΔRn
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
Cycle Number

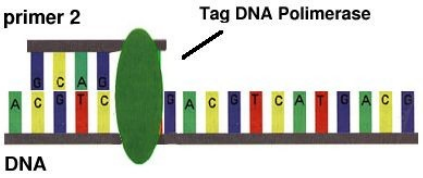
RT-PCR


exon 1
exon 2

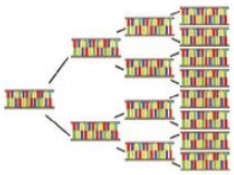

RNA
exon 1
exon 2


RNAse H
RNA
DNA

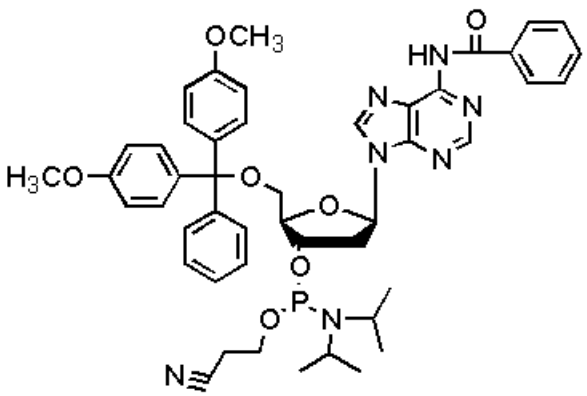

RNA
Reverse Transcriptase
primer 1


primer 2
Tag DNA Polimerase
DNA


RNA
DNA

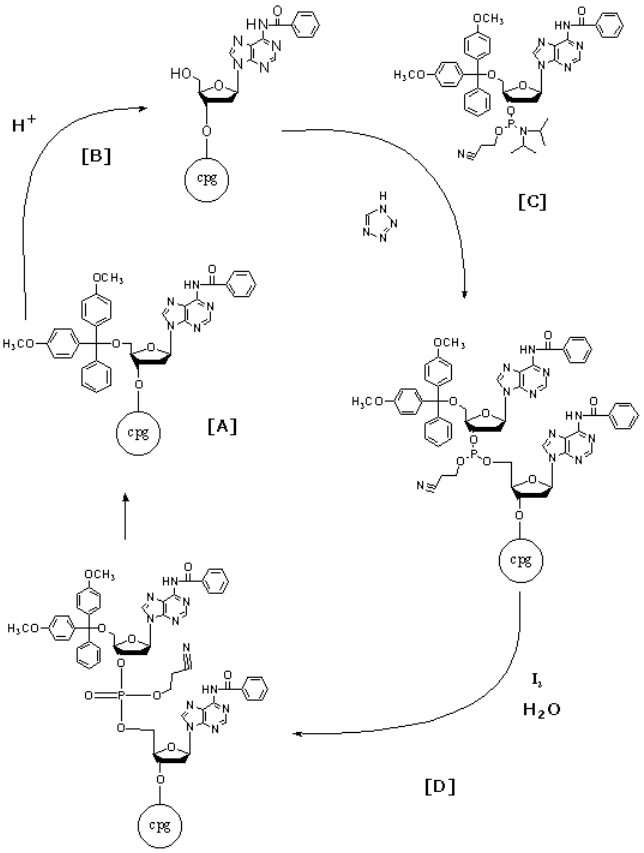


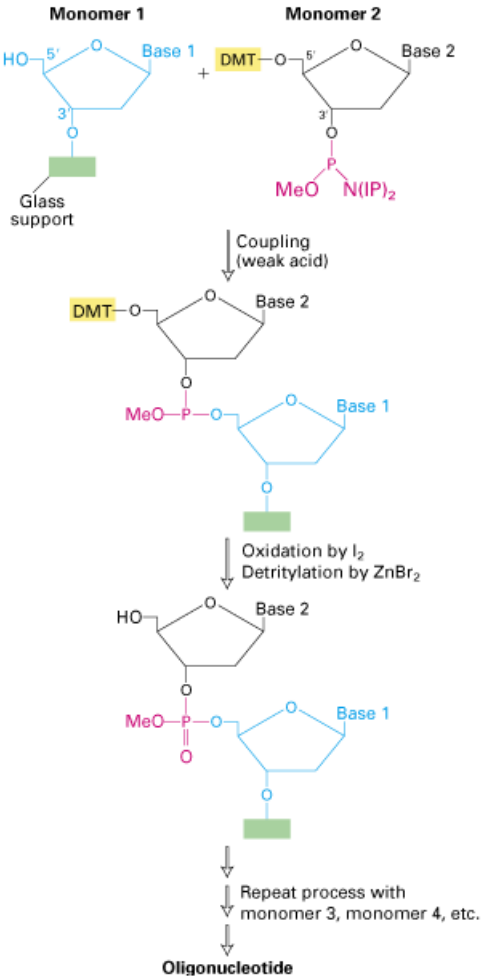
Syntéza oligonukleotidů



N-6-benzoyl-deoxyadenosine phosphoramidite

Cyklický proces – automatizace
Syntéza na pevné fázi





Komerčně dostupné oligonukleotidy – primery Vlastní primery

Syntetizované geny

Table 12.3	
Some Chemically Synthesized Genes	
Gene	Size (bp)
tRNA	126
α-Interferon	542
Secretin	81
γ-Interferon	453
Rhodopsin	1057
Proenkephalin	77
Connective tissue activating peptide III	280
Lysozyme	385
Tissue plasminogen activator	1610
c-Ha-ras	576
RNase T1	324
Cytochrome b_5	330
Bovine intestinal Ca-binding protein	298
Hirudin	226
RNase A	375

Delší geny po částech

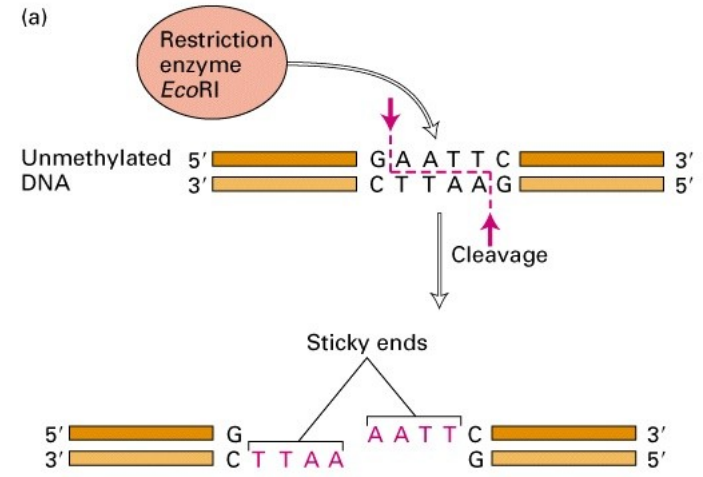
Komerčně dostupné oligonukleotidy

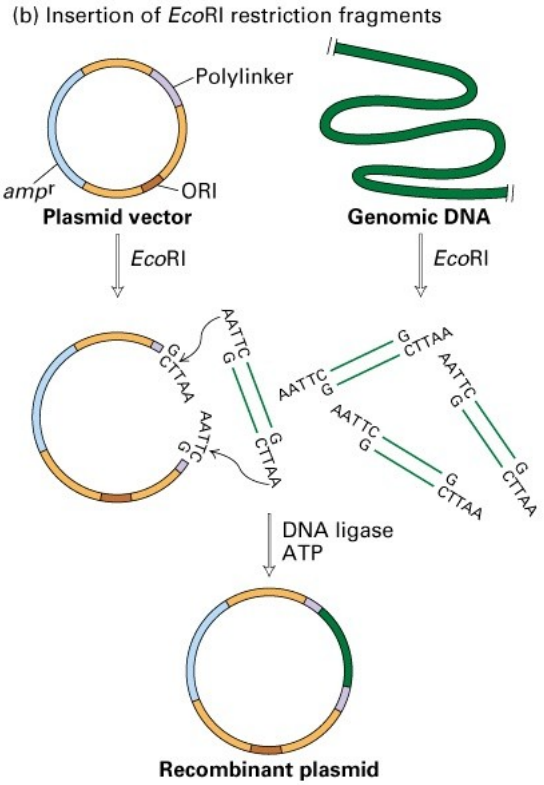
Cílená (site-directed) mutageneze

GENOVÉ MANIPULACE

Gen se inkorporuje do nosiče – vektoru (analogie se strategií virů) a vnese do hostitelské buňky.

Využití restrikčních endonukleáz (palindromové sekvence) event. reversních transkriptáz (vyřeší problém intronů)





Vektor, cílový organizmus, manipulace s vlastními geny (pod jiný promotor)