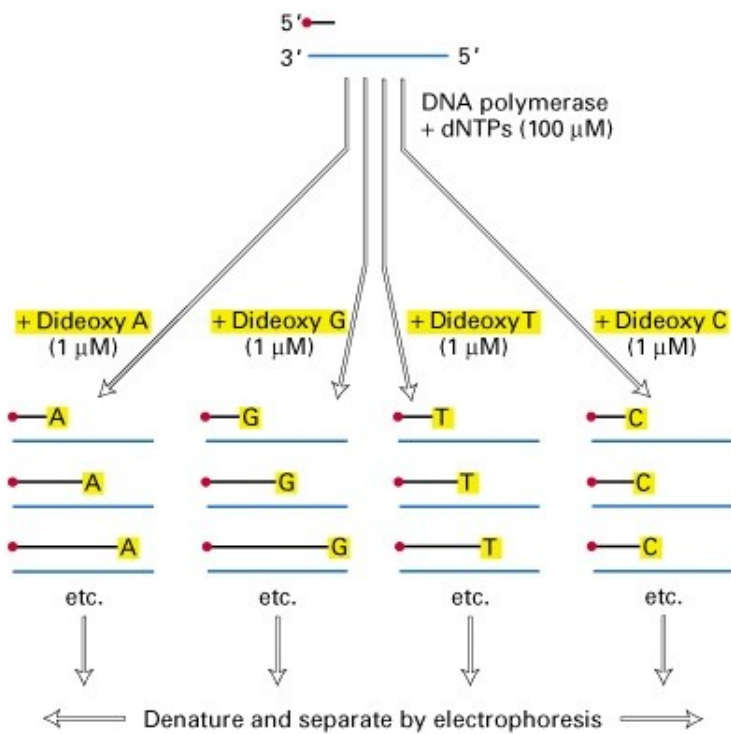


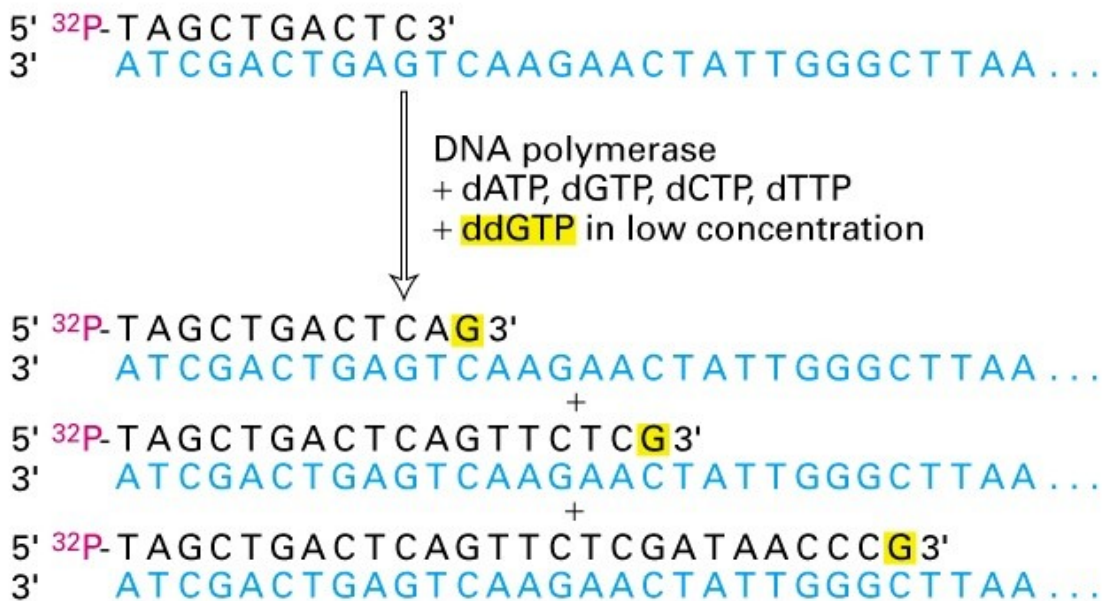
METODY STUDIA

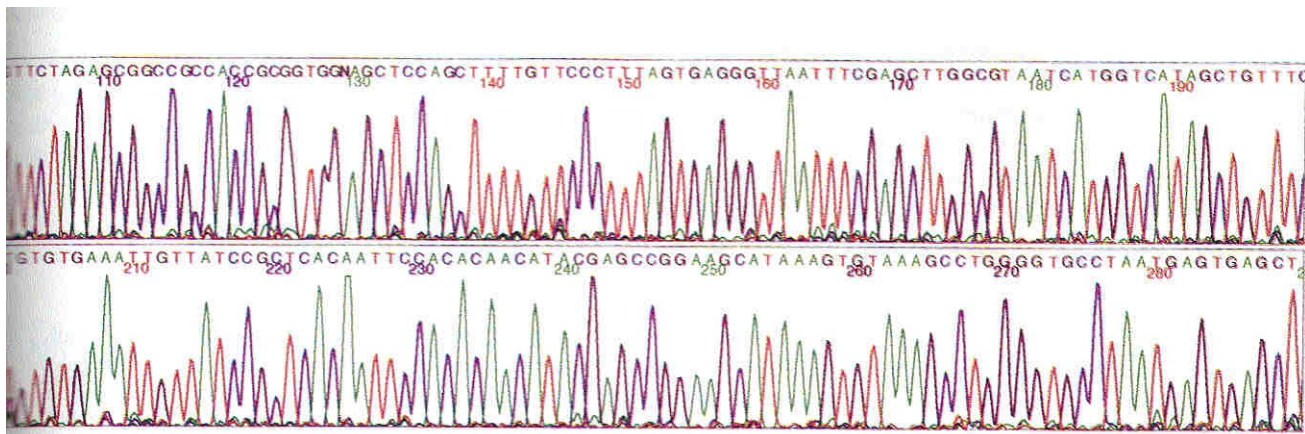
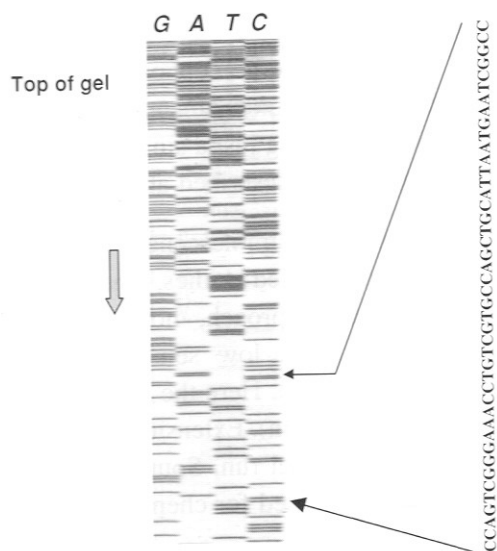
Sekvence DNA – chemická (selektivní štěpení – Maxam-Gilbert), biochemická (syntéza, dideoxy metoda - Sanger)

(a)



(b)





PCR

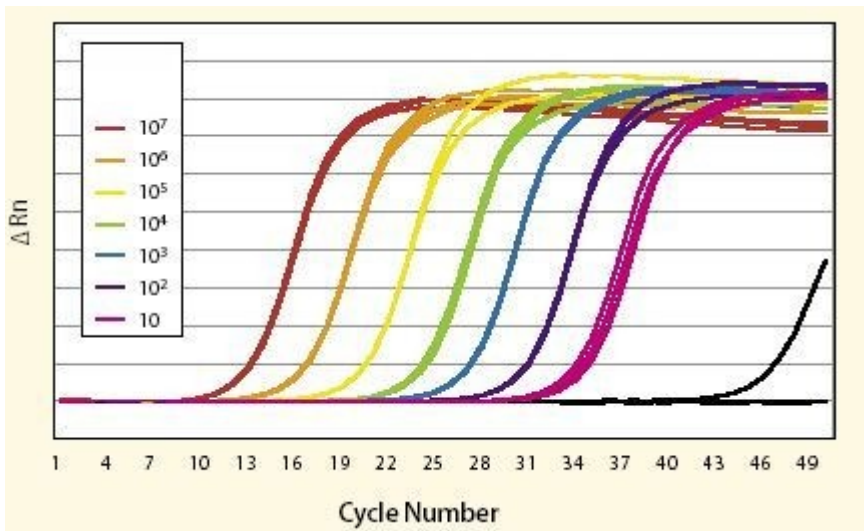
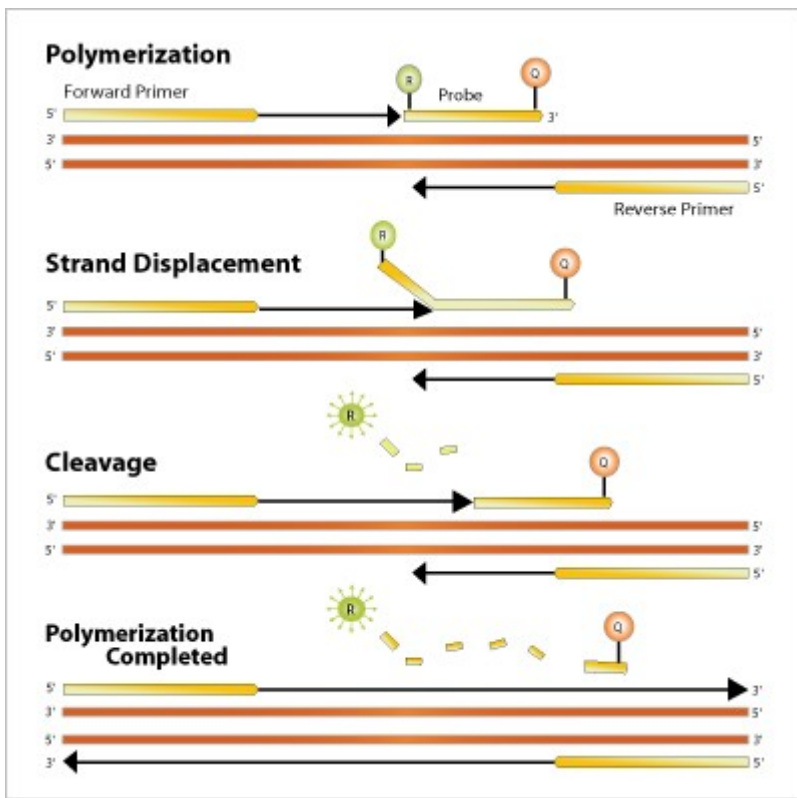
Taq polymeráza, dNTP, primery, vzorek-templát

Termocycler - animace

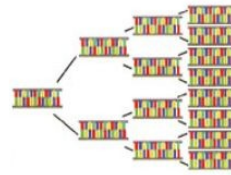
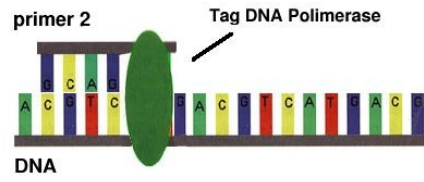
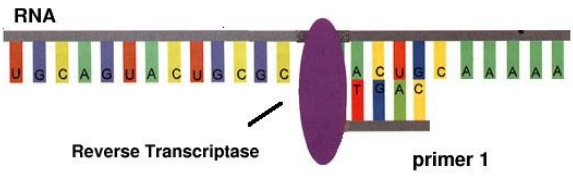
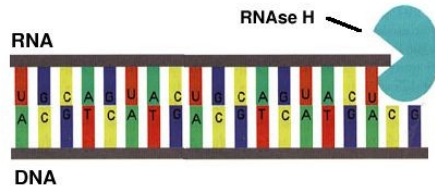
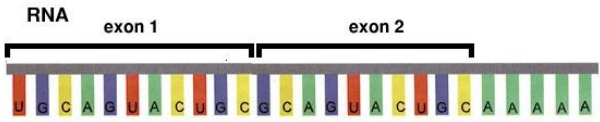
Potřeba primerů

Kvantitativní PCR

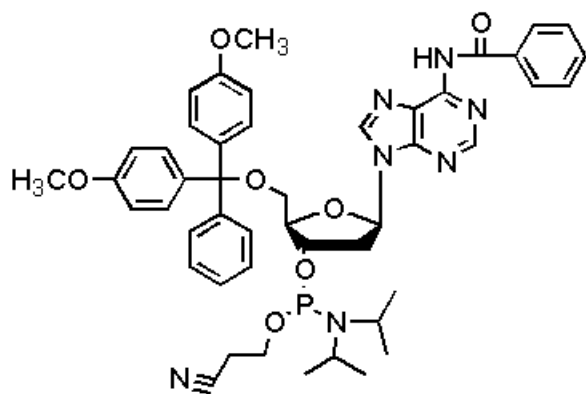
Význam – analytický – diagnostika, forenzní analýza,
 - preparativní - pomnožení materiálů, umělé geny atd.



RT-PCR

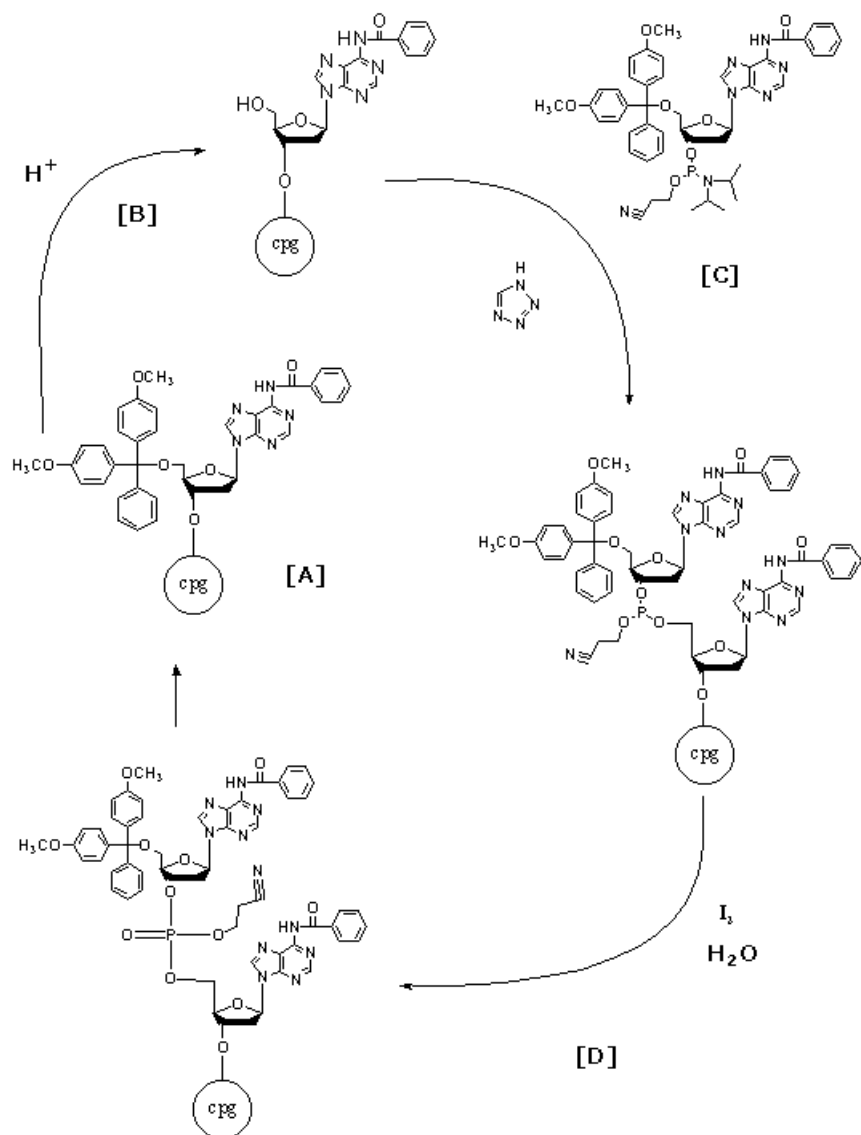


Syntéza oligonukleotidů



N-6-benzoyl-deoxyadenosine
phosphoramidite

Cyklický proces – automatizace
Syntéza na pevné fázi



Syntetizované geny

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 <i>b</i> ₅	330
Bovine intestinal Ca-binding protein	298
Hirudin	226
RNase A	375

Delší geny po částech

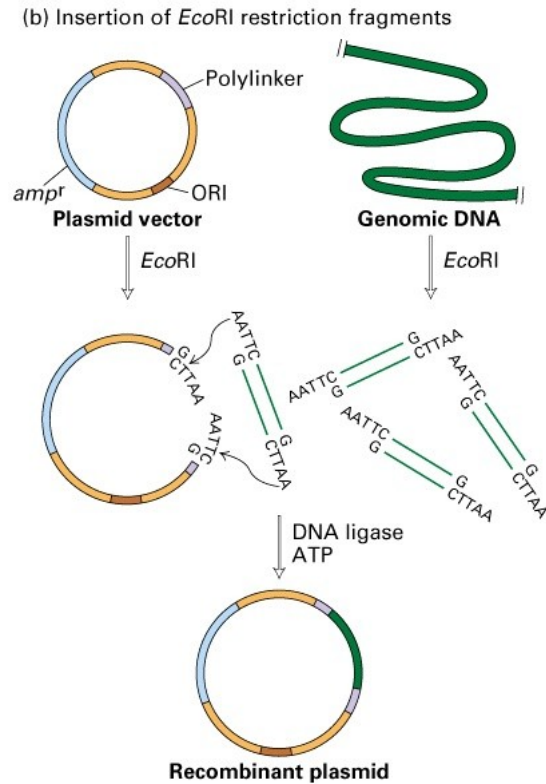
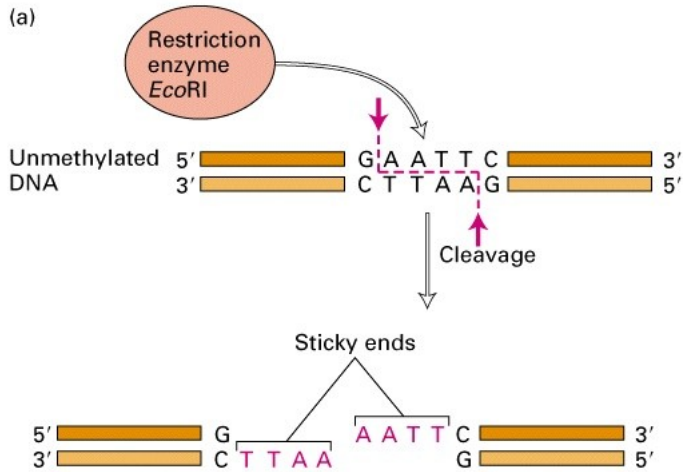
Komerčně dostupné oligonukleotidy

Cílená (site-directed) mutageneze

GENOVÉ MANIPULACE

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

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



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

