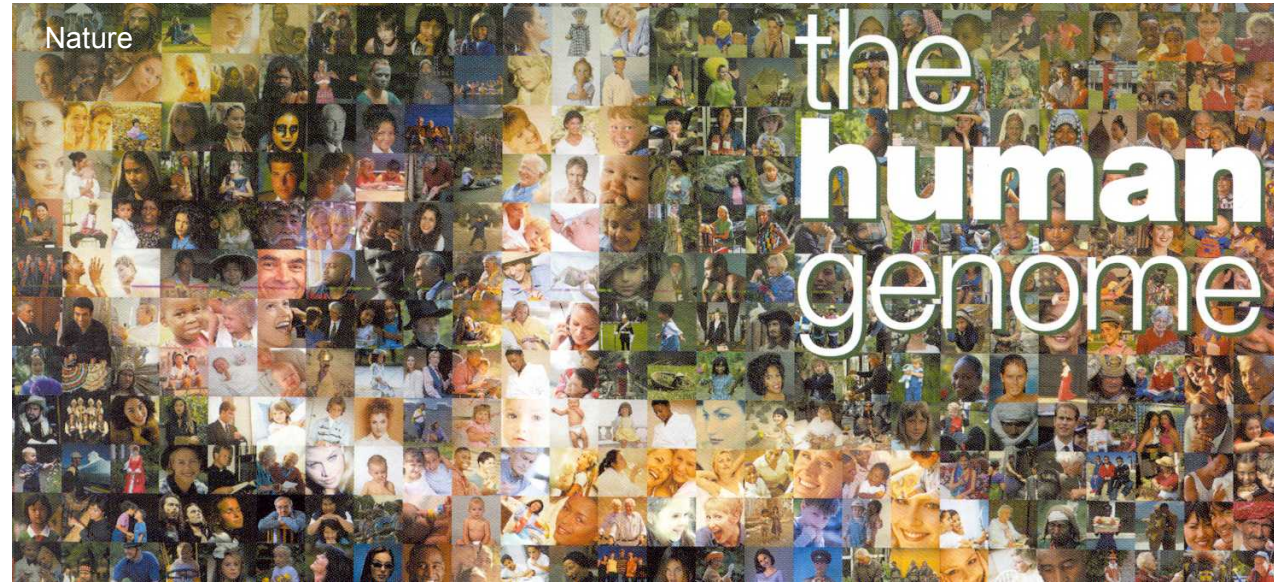
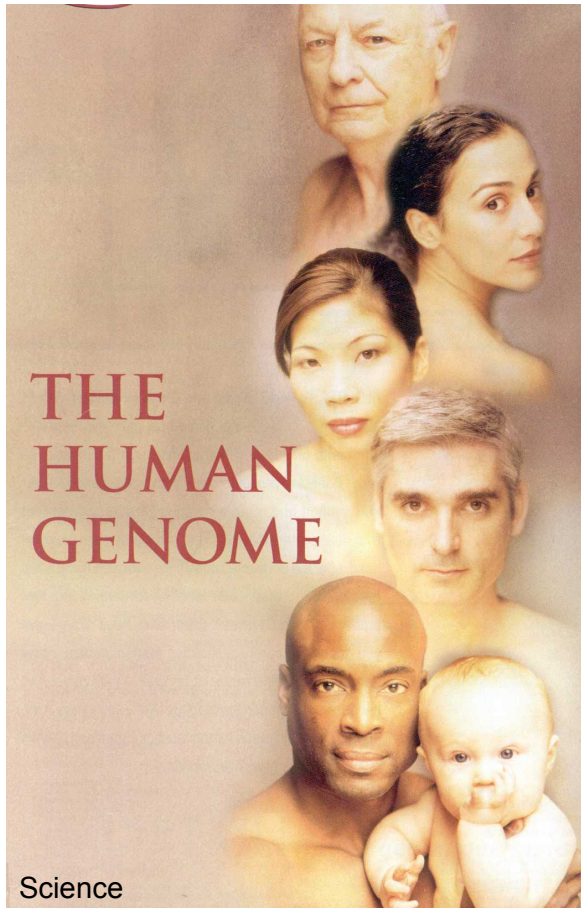
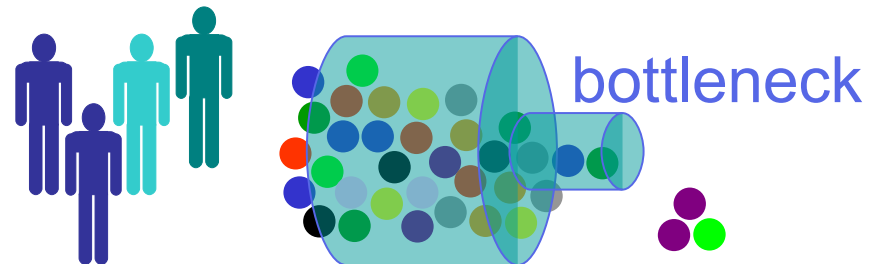


variabilita genomu



genetická diverzita člověka
na úrovni SNP je nízká: asi 0.1%
cca. 90% variace je uvnitř populací
cca. 10% mezi populacemi (kontinenty)



typy variability

bodové substituce: SNP, bodové mutace

malé delece a inserce

krátké tandemové repetice

polymorfismy typu STR, VNTR

dynamické mutace (zejm. expanze trinukleotidů)

polymorfní inserce retroelementů

velké strukturální varianty: delece, duplikace, inverze

(SV - Structural Variants, CNV - Copy Number Variants)

většinou submikroskopické

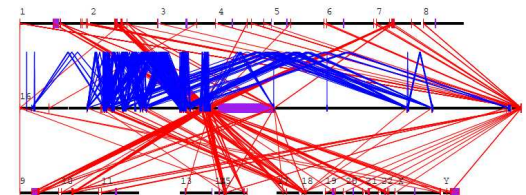
přes 10 tis. lokusů, medián několik kb

mikrodeleční syndromy

často v oblastech segmentálních duplikací

referenční sekvence genomu = ?

klinická interpretace CNV = ?



základní typy mutací

ATG ACC CAG CAG CCA ATG AAA
Met Thr Gln Gln Pro Met Lys

normální sekvence
čtecí rámeček je označen mezerami



ATG **CCC** CAG CAG CCA ATG AAA
Met **Pro** Gln Gln Pro Met Lys

bodová substituce typu missense
(threonin je nahrazen prolinem)



ATG ACC **TAG** CAG CCA ATG AAA
Met Thr **STOP** - - - -

bodová substituce typu nonsense
(předčasné ukončení syntézy proteinu)



ATG **ACA** CAG CAG CCA ATG AAA
Met Thr Gln Gln Pro Met Lys

tichá substituce
(threonin je kódován jiným kodonem)



ATG --- CAG CAG CCA ATG AAA
Met - Gln Gln Pro Met Lys

delece bez posunu čtecího rámce
(chybí jedna aminokyselina)



ATG -CCC AGC AGC CAA TGA AA
Met **Pro Ser Ser Gln STOP** -

delece s posunem čtecího rámce
(jiné aminokyseliny + předčasná terminace)



ATG ACC CAG CAG **CAG CAG CAG** CCA ATG AAA
Met Thr Gln Gln **Gln Gln Gln** Pro Met Lys

expanze trinukleotidové repetice
(vložen polyglutaminový úsek)
dynamické mutace

základní typy polymorfismů DNA

▼
ATGCC**C**AGCAGCCAAT
▼
ATGCC**T**AGCAGCCAAT

polymorfismus typu SNP
(Single Nucleotide Polymorphism)

chromozóm (jedinec) A

chromozóm (jedinec) B

tři možné genotypy

▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCC**CACACACACACAC**AGAAA
▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCC**CACACACACACACACAC**AGAAA
▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCC**CACACACACACACACACACACAC**AGAAA

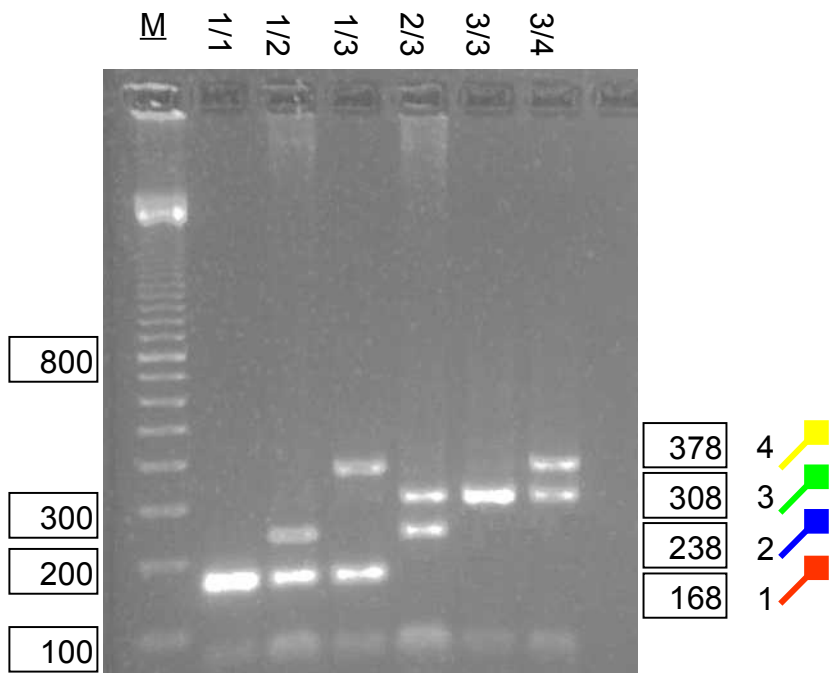
polymorfismus typu STR
(Short Tandem Repeat)

alela A

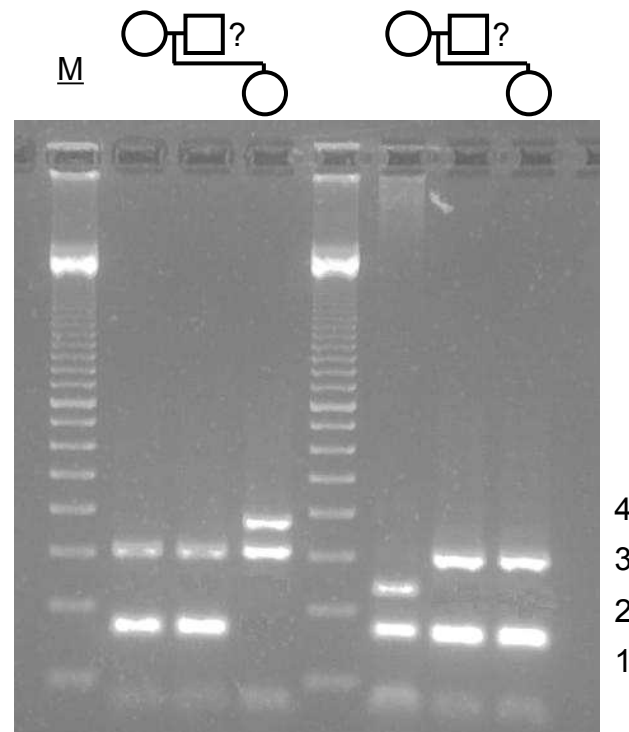
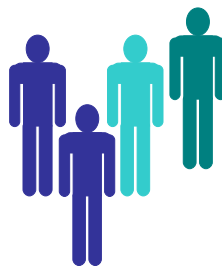
alela B

alela C

mnoho možných genotypů

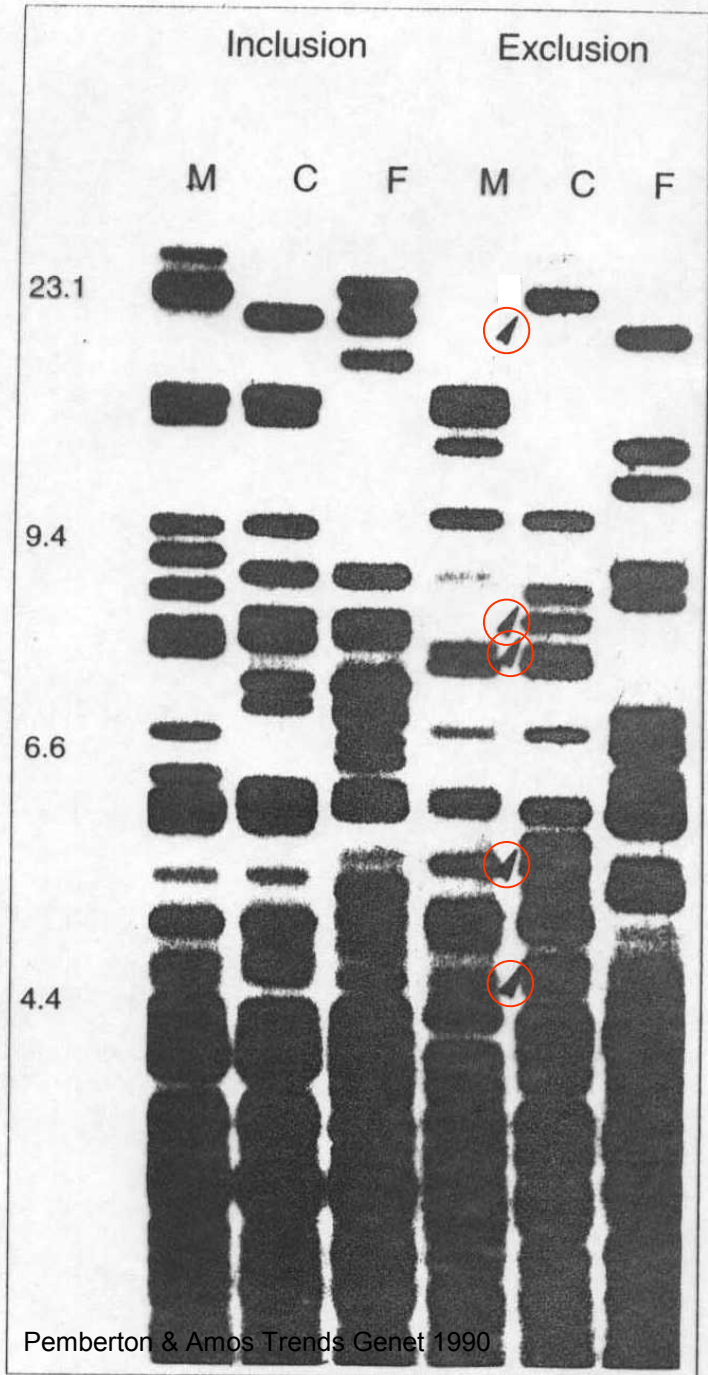


identifikace
osob



paternita

jednolokusové sondy



ISBN 0-86577-958-9



9 780865 779587

ISBN 3-13-100362-6 (Thieme)
ISBN 0-86577-958-9 (TNY)



8 594007 072020 >

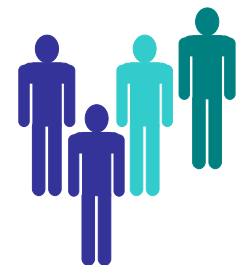
JAN PONDĚLÍK, pekařství a cukrářství Dvorec 307
LINECKÉ KOLÁČKY



8 594007 072044 >

JAN PONDĚLÍK, pekařství a cukrářství Dvorec 307
LINECKÉ TŘENÉ S MARMELÁDOU

multilokusové sondy



hot-spots pro variabilitu

dinukleotid CG (CpG): ^{me}CG mutuje na TG

tandemové repetice: sklouznutí při replikaci (slippage)

rozptýlené repeaty: rekombinace mezi nealelními kopiemi

ATG ACG CAG CAG CCC CCT ATG AAA
Met Thr Gln Gln Pro Pro Met Lys



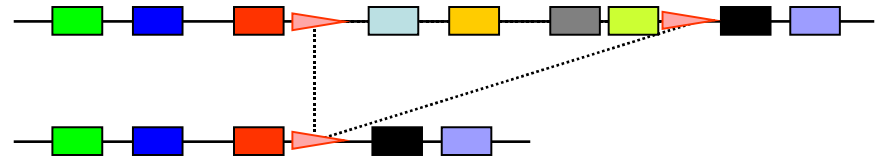
ATG ATG CAG CAG CCC CCT ATG AAA
Met Met Gln Gln Pro Pro Met Lys



ATG ACG CAG CAG CCC CTA TGA AA
Met Thr Gln Gln Pro Leu STOP -



ATG ACG CAG CAG CAG CAG CAG CCC CCT ATG AAA
Met Thr Gln Gln Gln Gln Gln Pro Pro Met Lys

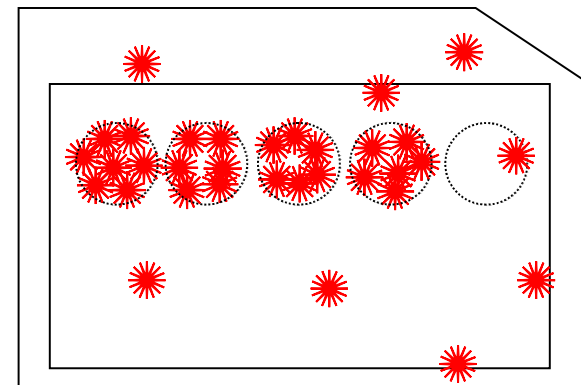
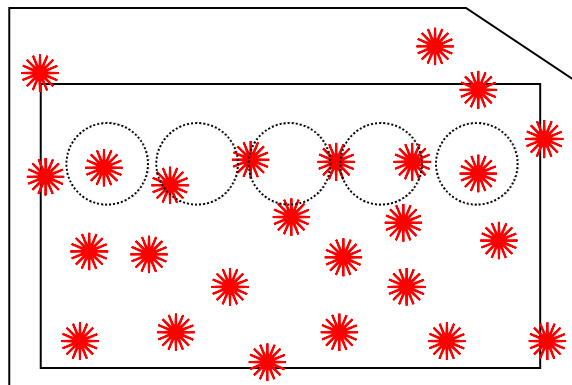
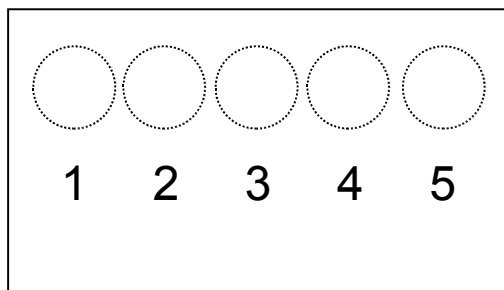




vzorky 1-4:
úplná komplementarita,
sonda hybridizuje

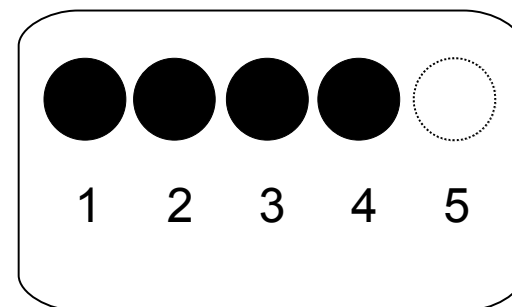
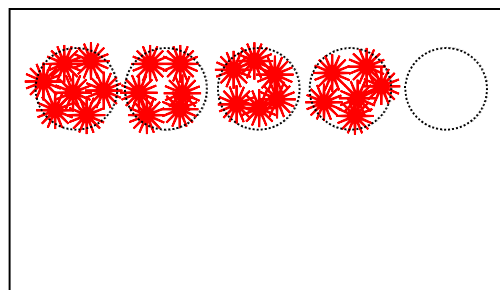


vzorek 5:
neúplná komplementarita,
sonda nehybridizuje



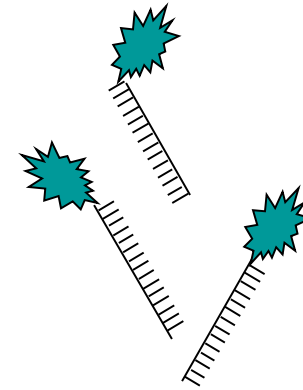
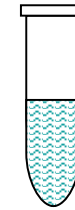
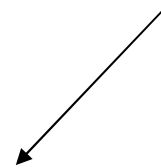
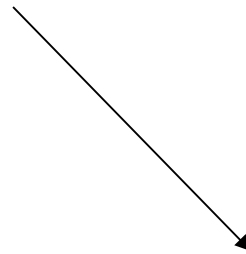
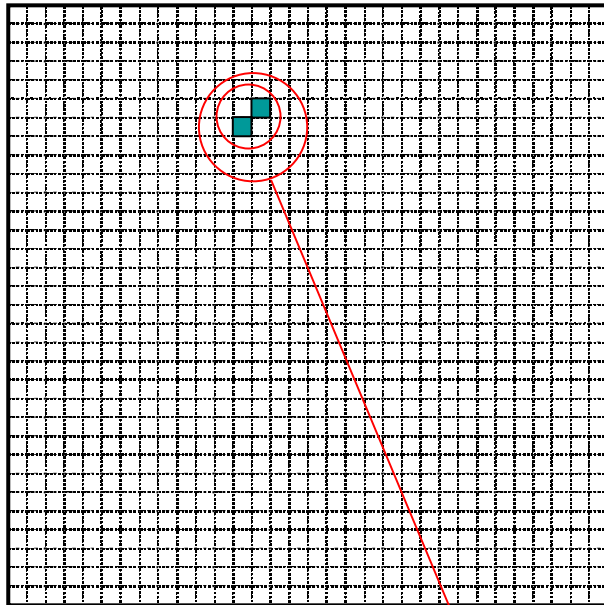
alelově specifická
hybridizace

hybridizace s alelově
specifickými oligonukleotidy
(ASO)



DNA čipové technologie: paralelní hybridizace s mnoha oligonukleotidy
(nebo delšími klonovanými sondami) rozmístěnými hustě na pevném podkladu

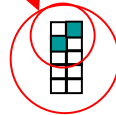
DNA čipové technologie: resekvenace



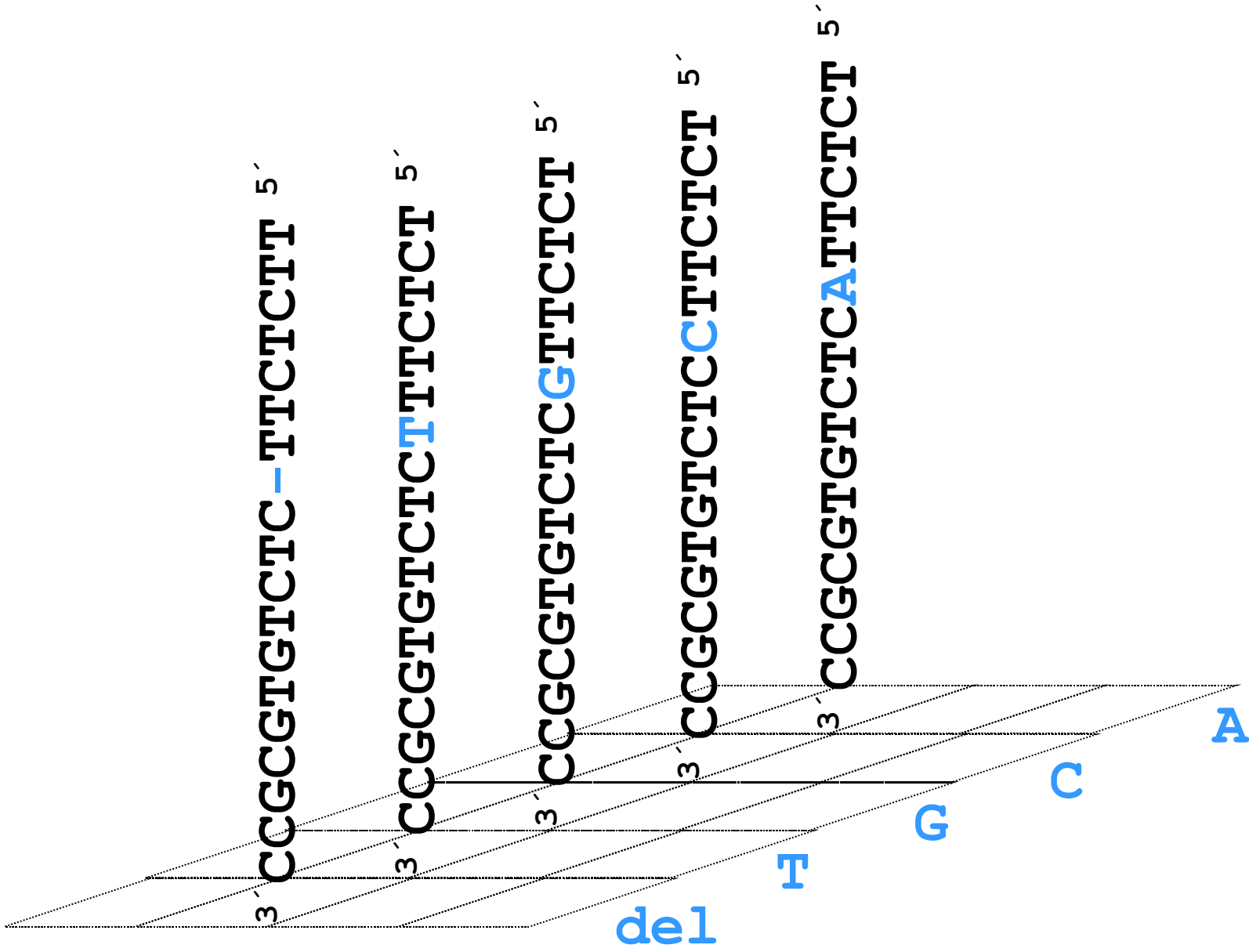
5' GCGGCATGAACCGTAGGCCCATC 3' 

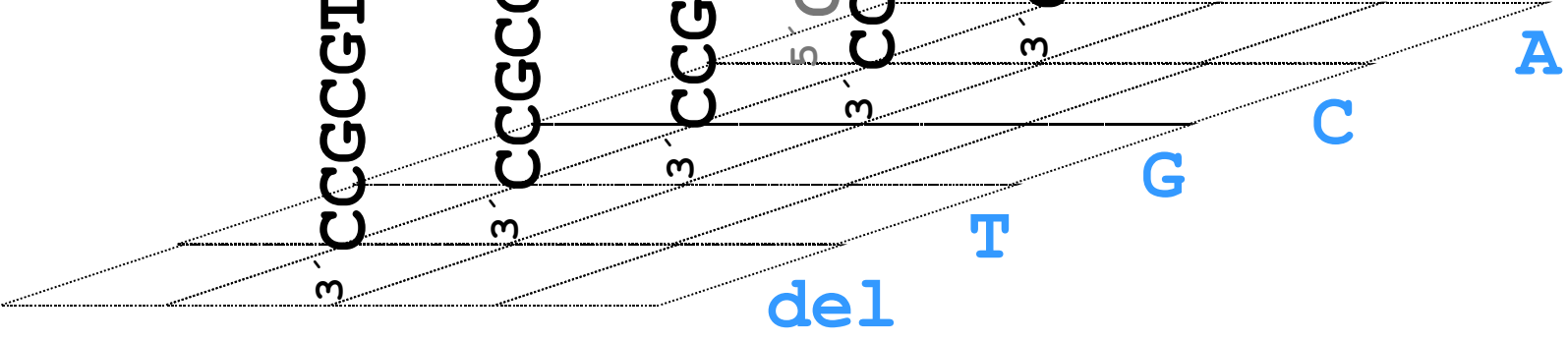
3'  GCCGTACTTGG  CATCCGG 5'
GCCGTACTTGG  ATCCGG
GCCGTACTTGG  TATCCGG
GCCGTACTTGG - ATCCGGG

 CCGTACTTGGC  TCCGGG
CCGTACTTGGC  C TCCGGG
CCGTACTTGGC  G TCCGGG
CCGTACTTGGC  T TCCGGG
CCGTACTTGGC - TCCGGGT



5' CGGCGCACAGAGG AAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGG AAGAGAATCTCCGCAAG 3'





3' CCGCGTGTCTC - TTCTCTT 5'

3' CCGCGTGTCTC TTCTCT 5'

3' CCGCGTGTCTC GTTCTCT 5'

del

5' GCGCACAGAGAAATC 3'

3' CCGCGTGTCTC TTCTCT 5'

H

G

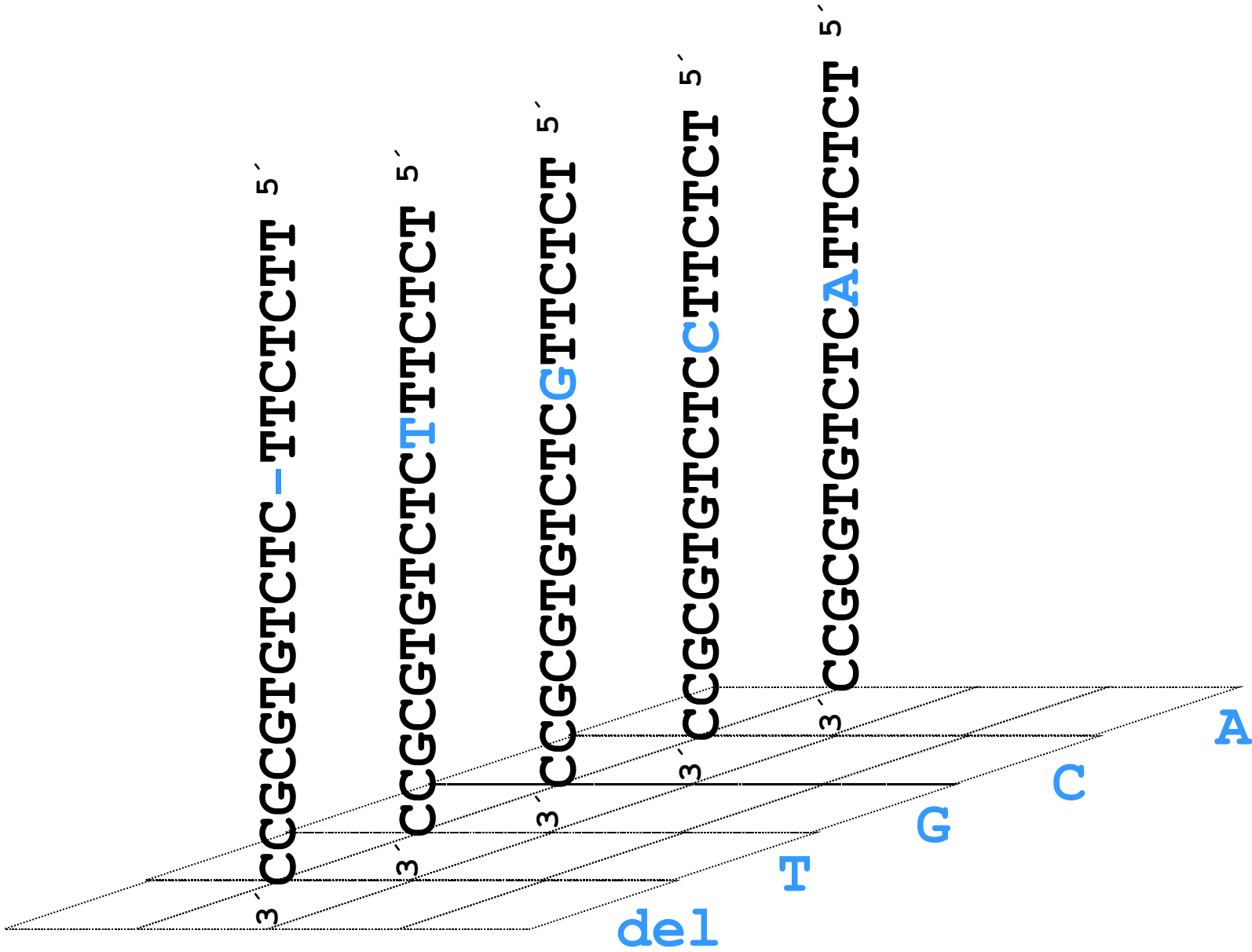
C

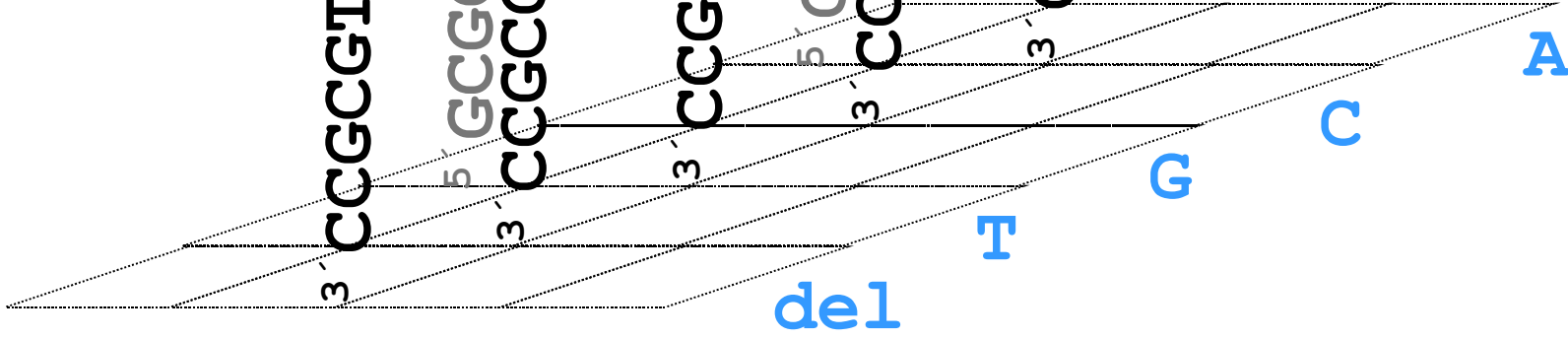
A


3' CCGCGTGTCTCA TTCTCT 5'



5' CGGCGCACAGAG **G**AAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAG **A**AAGAGAATCTCCGCAAG 3'






3' CCGCGTGTCTC - TTCTCTT 5' 

5' GCGCACAGAGAAATC 3'

3' CCGCGTGTCTC TTCTCTT 5'

3' CCGCGTGTCTC GTTCTCT 5' 

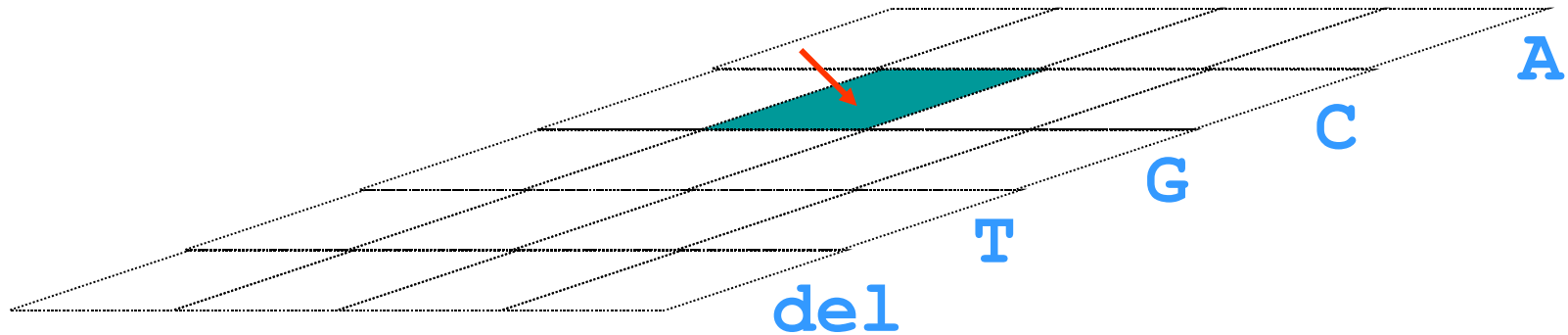
5' GCGCACAGAGAAATC 3'

3' CCGCGTGTCTC TTCTCTT 5'

3' CCGCGTGTCTCA TTCTCT 5'

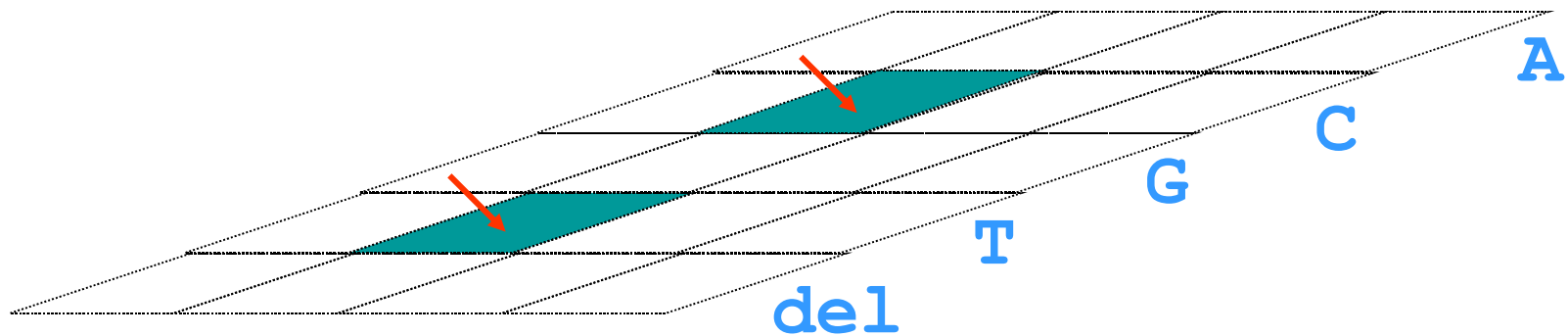
homozygot pro normální sekvenci (GAA = Glu)

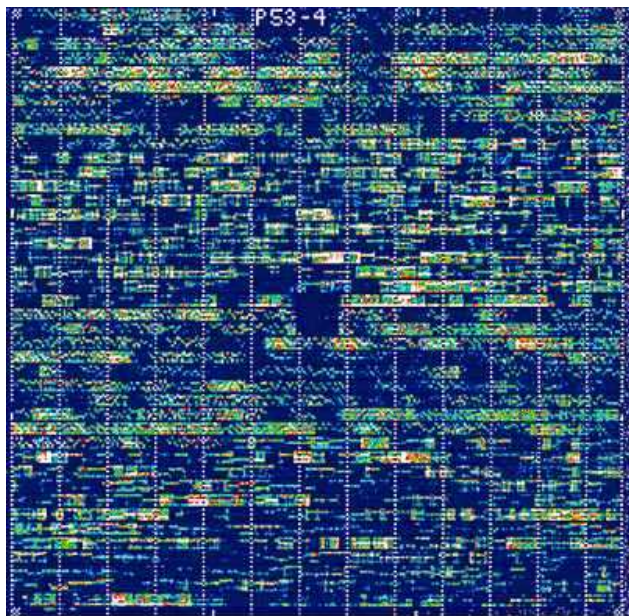
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'



heterozygot pro mutovanou sekvenci (GAA = Glu / AAA = Lys)

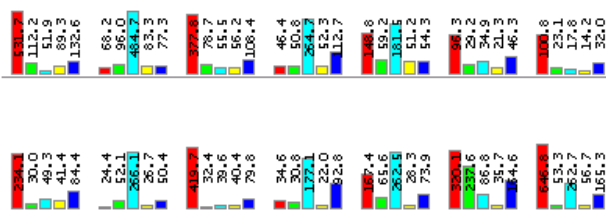
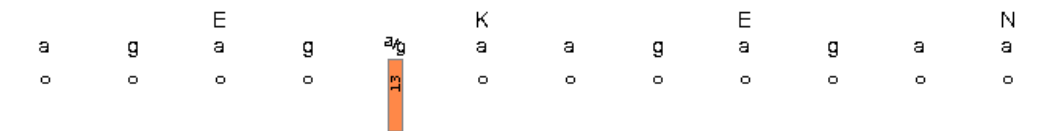
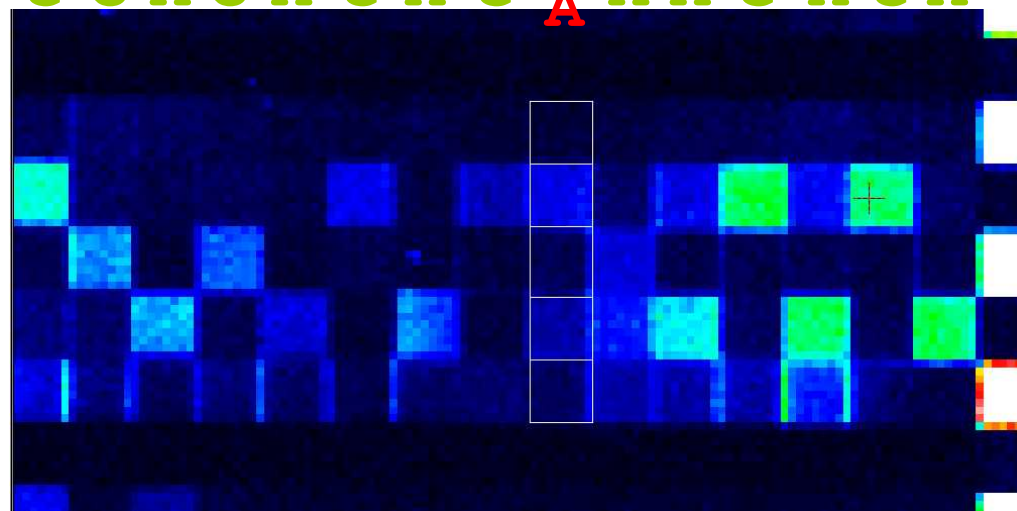
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGAAAGAGAATCTCCGCAAG 3'





G C A C A G A G **G** A A G A G A

A
C
G
T
del



AFFYMETRIX

Microarray Suite ChipReport "e:\kmuehlbauer\p53-ZS- -5-6-02.RPT" 06/05/02 13:37:17

Report Type: p53 Report
Filter Type: Mutations Only
Date: 01:36PM 06/05/2002

Filename: p53-ZS- -5-6-02.CHP
Probe Array Type: GP53
Algorithm: Mixture Detection

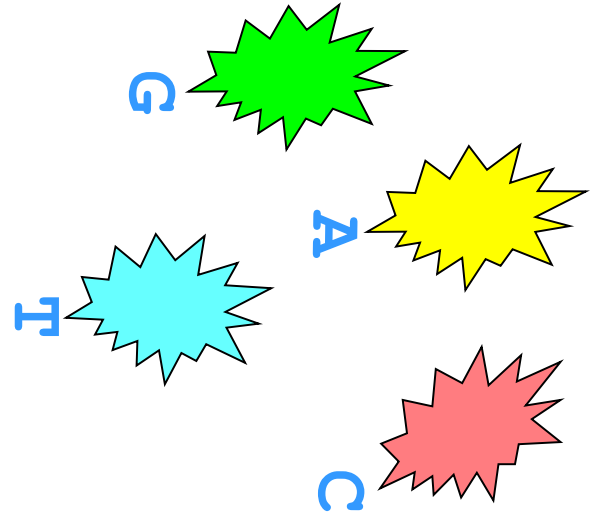
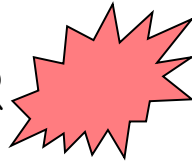
SampleID	Location	AA Change	Codon Change	Score	SAW Reference
p53-ZS-	-5-6-02	p53 exon8E286K	gaa->aaa 13,0,0	1080,1081,1082	



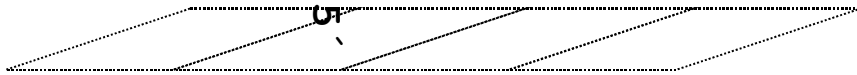
heterozygot pro mutovanou sekvenci TP53 (kodon 286: GAA = Glu / AAA = Lys)

neznačená
testovaná
DNA

5' GAGGAGAGAGATCTCCGCAAG 3'
3' CTTCCTTAGAGGCCGTTCTT 5'



extenze oligonukleotidu
zakotveného v buňce čipu
o jeden značený dideoxynukleotid



homozygot pro normální sekvenci (286 GAA (Glu))

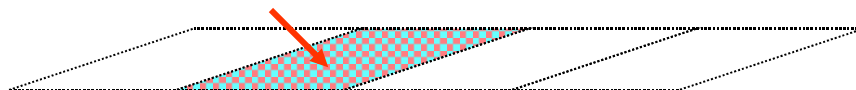
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'



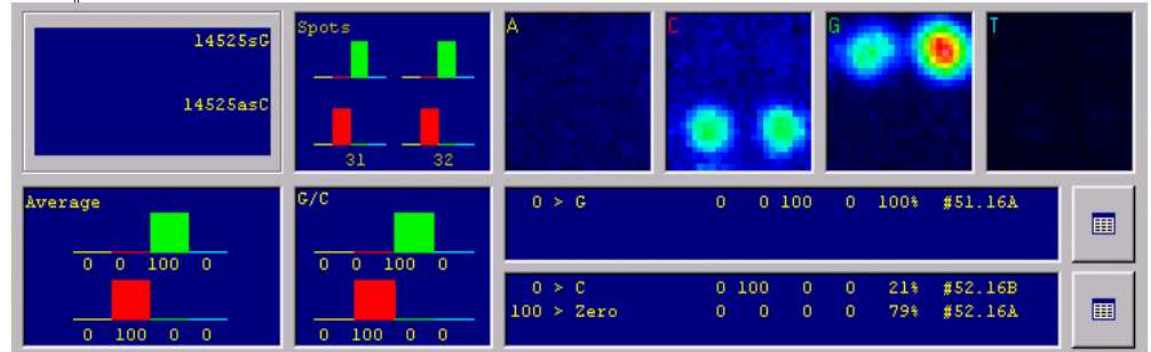
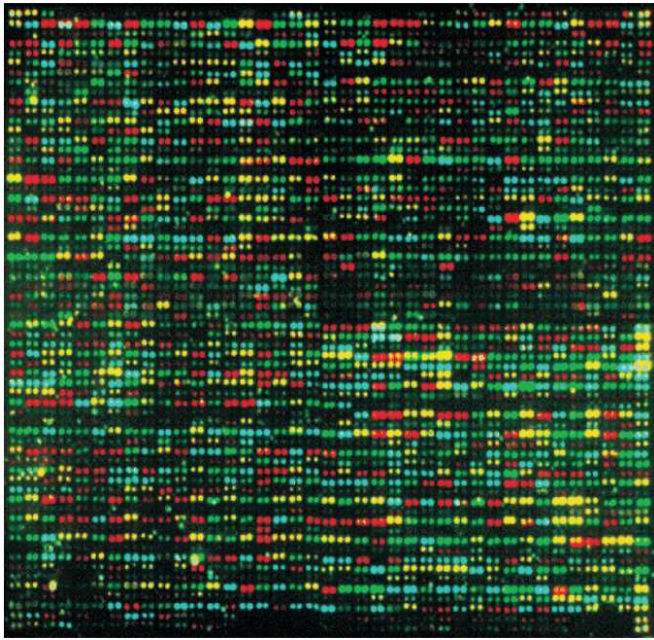
červený fluorescenční signál v buňce B

heterozygot pro mutovanou sekvenci (286 AAA (Lys))

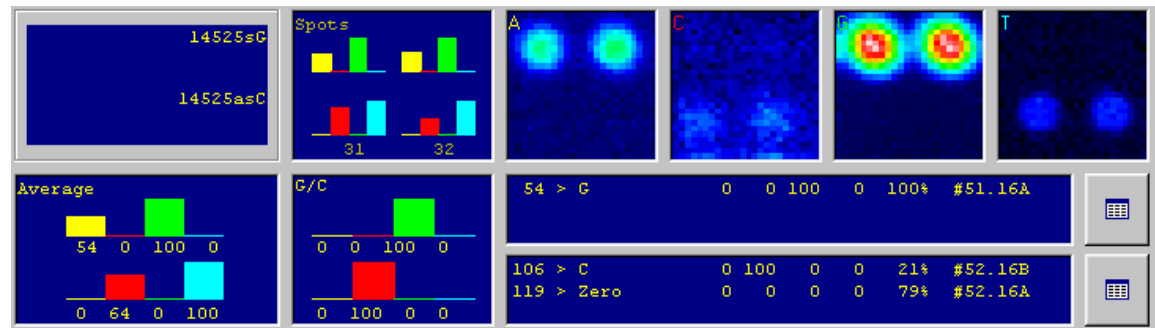
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGAAAGAGAATCTCCGCAAG 3'



červený + modrý fluorescenční signál v buňce B

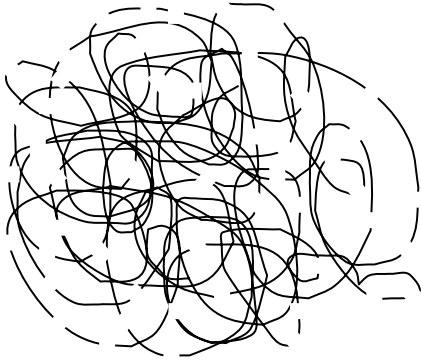


286 Glu/Glu



286 Glu/Lys

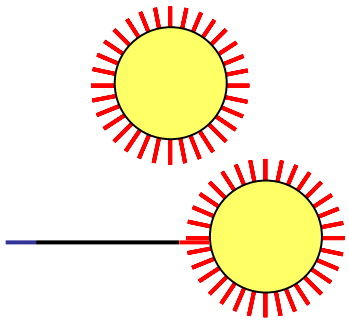
sekvenování nové generace



fragmentace DNA

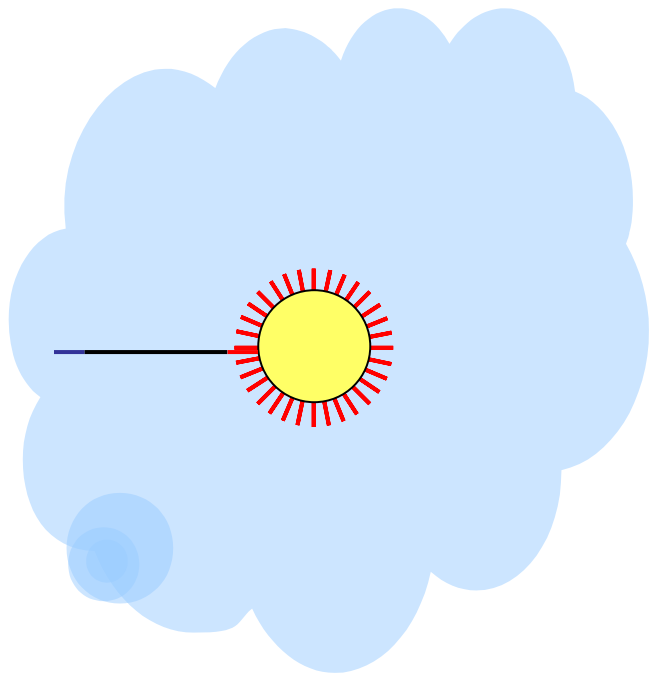


ligace adaptorů, denaturace

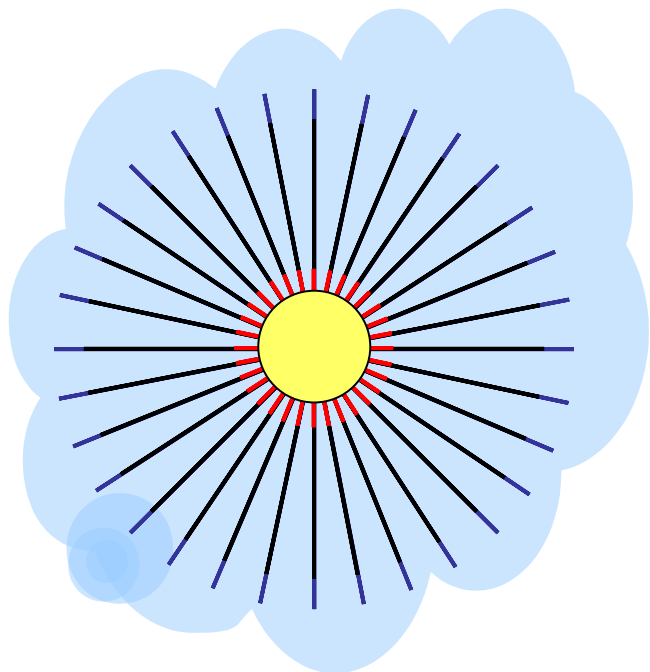


annealing fragmentů na partikule

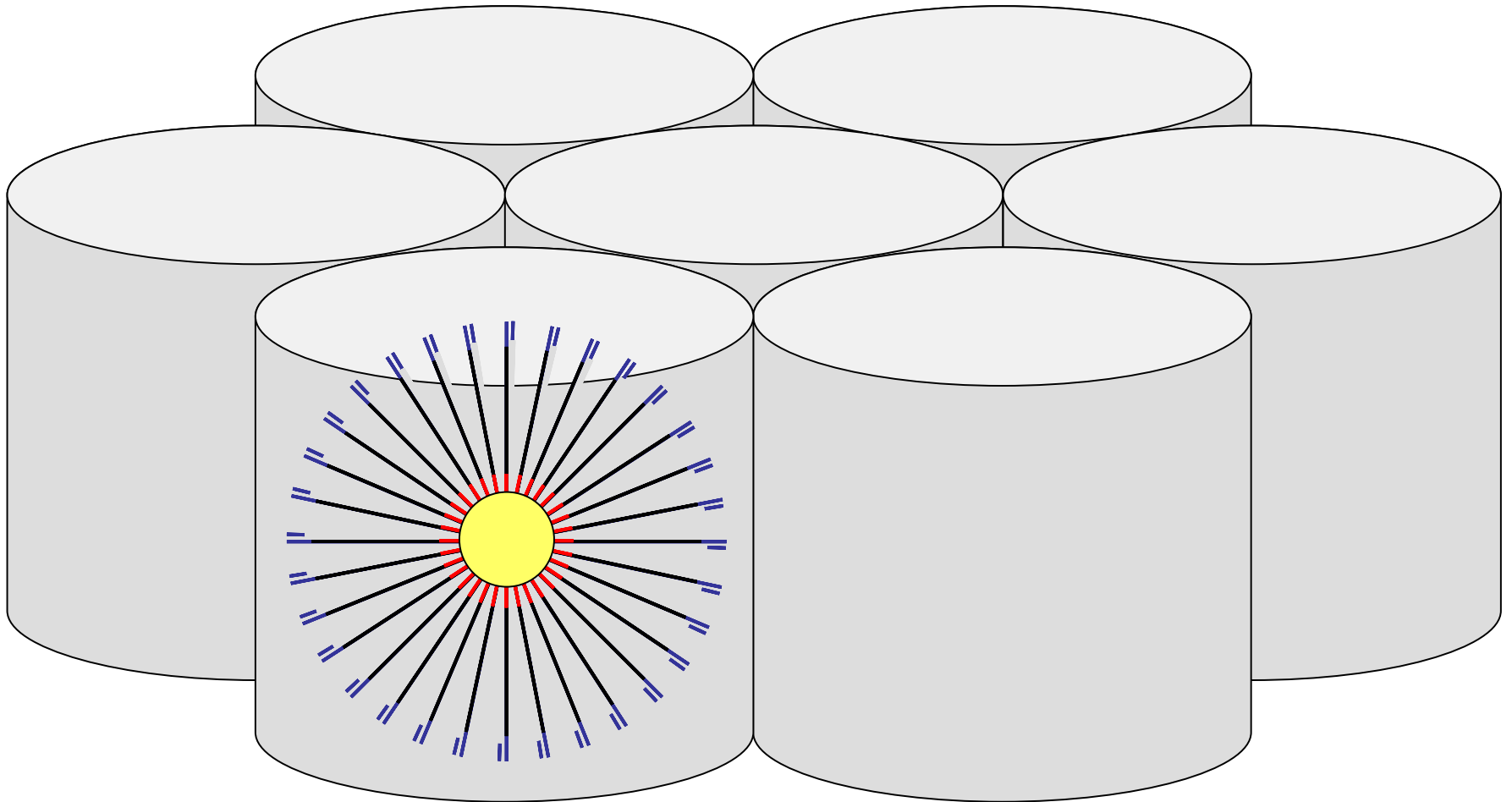
vytvoření emulze



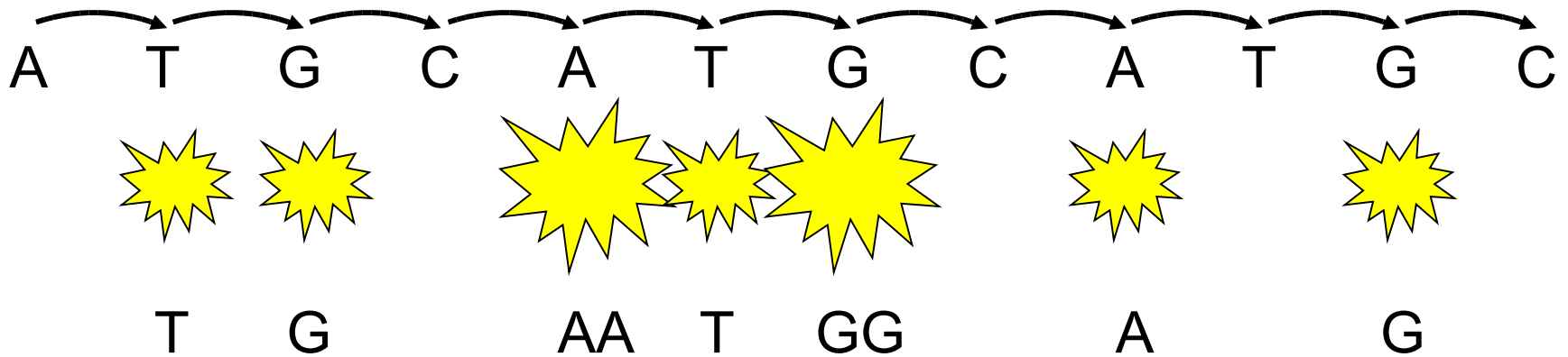
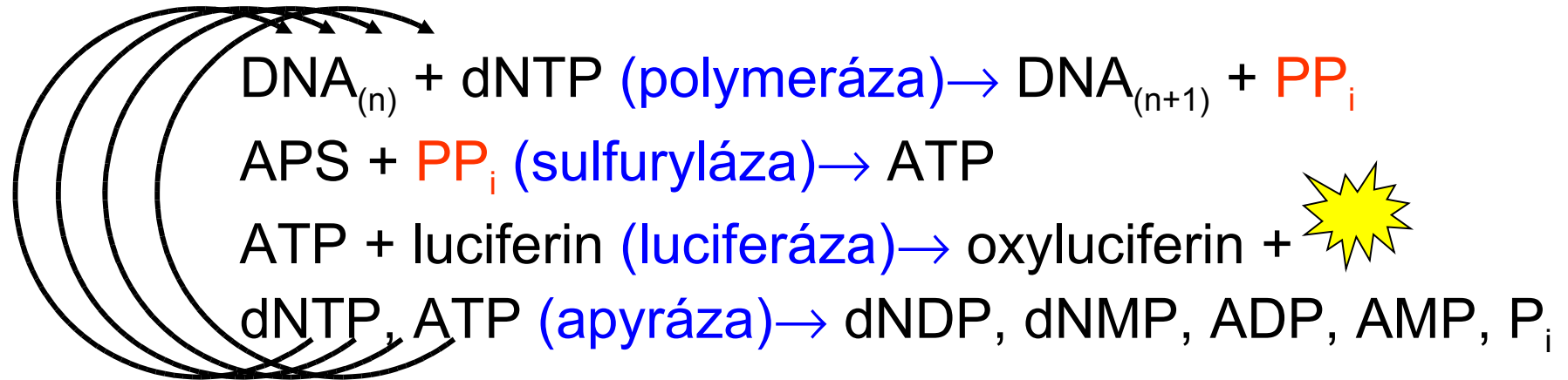
emulzní PCR, denaturace



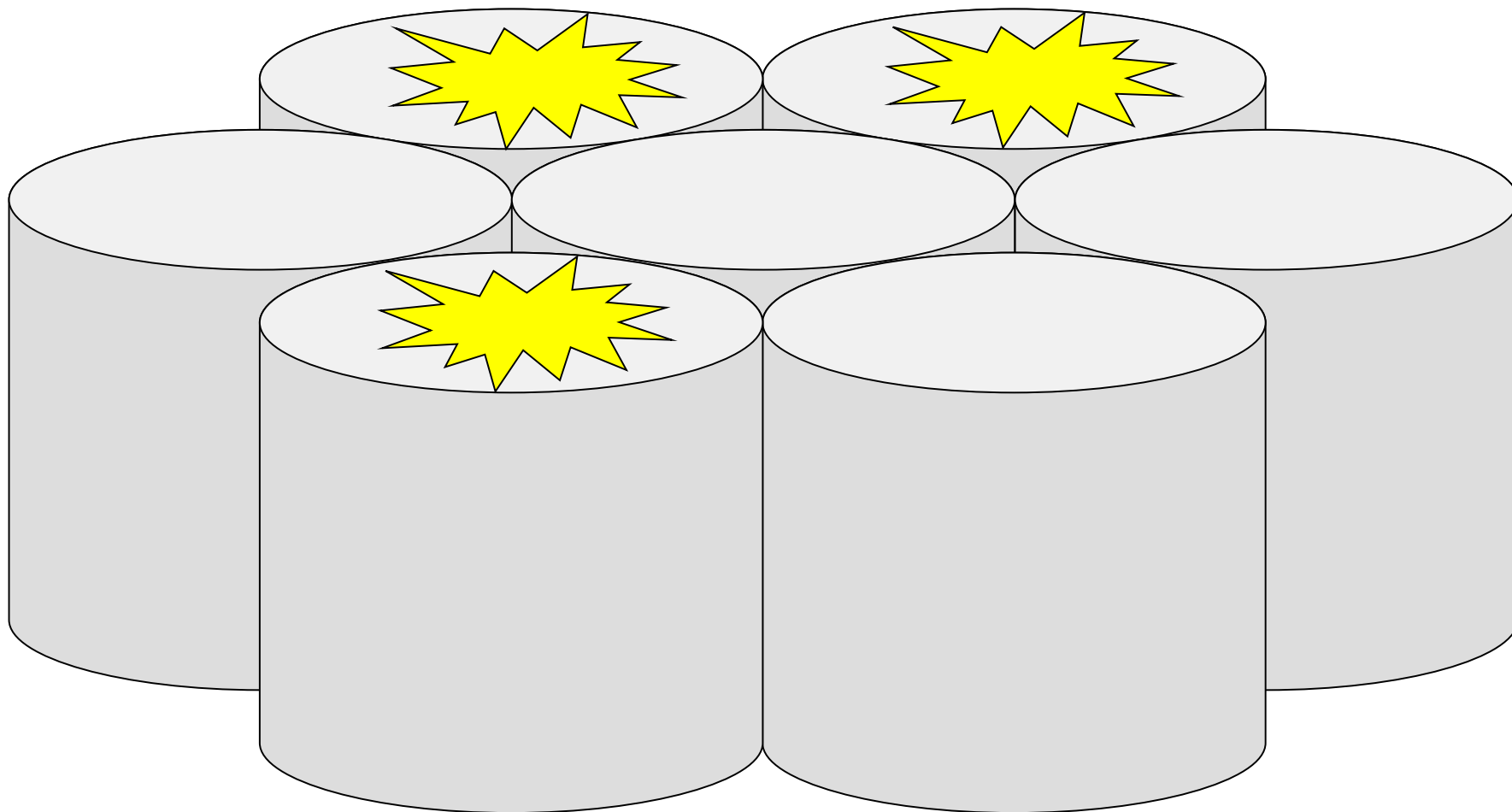
zachycení partikulí v pikotitrační desce



pyrosekvenace a detekce záblesků



pyrosekvenace a detekce záblesků



next generation sequencing

2nd generation: with amplification

3rd generation: single-molecule sequencing

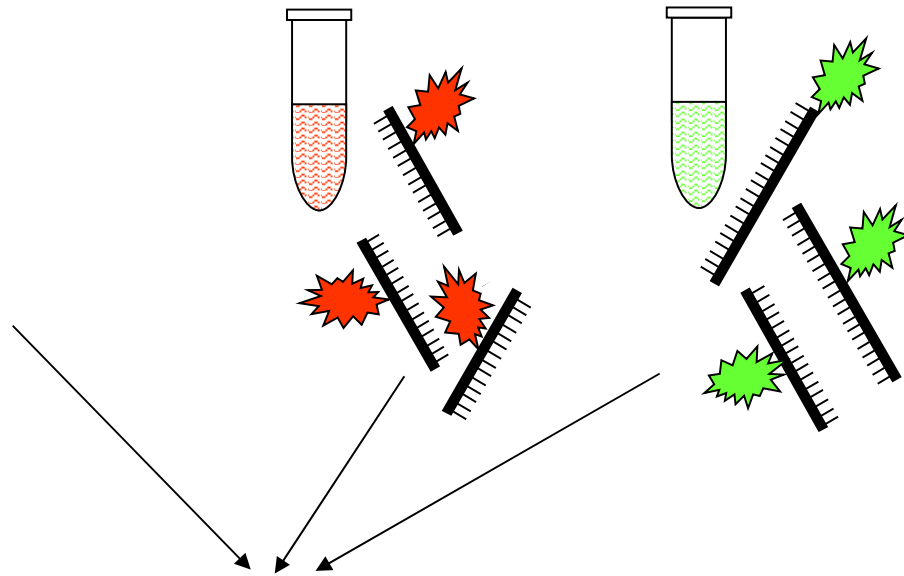
454/Roche	http://www.454.com/
Solexa/Illumina	http://www.illumina.com/
SOLiD/ABI	http://www.appliedbiosystems.com/
Helicos	http://www.helicosbio.com/
Pacific Biosciences	http://www.pacificbiosciences.com/
Complete Genomics	http://www.completegenomicsinc.com/

enrichment of specific regions - exome sequencing

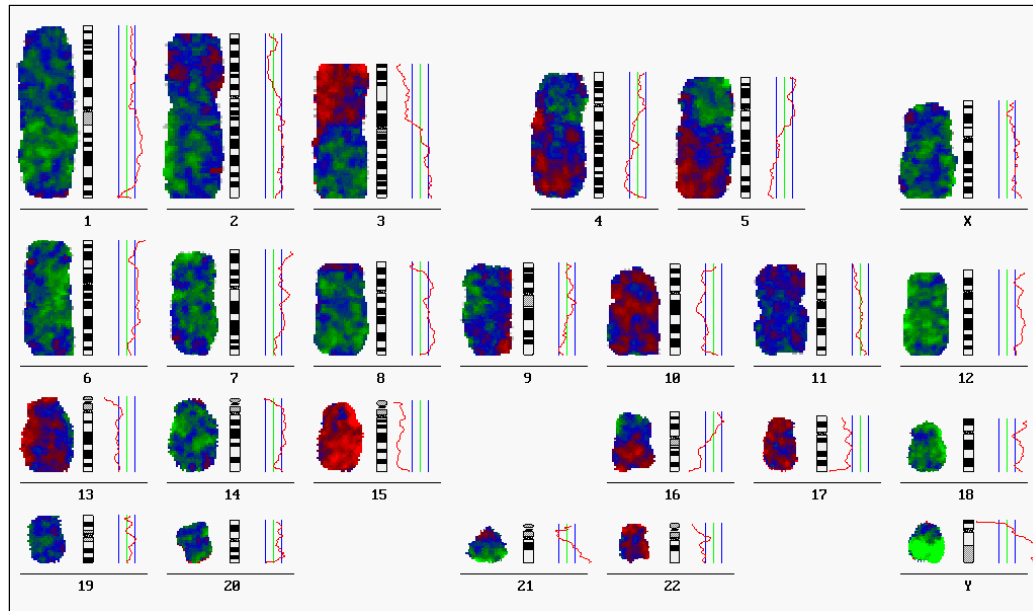


normální DNA

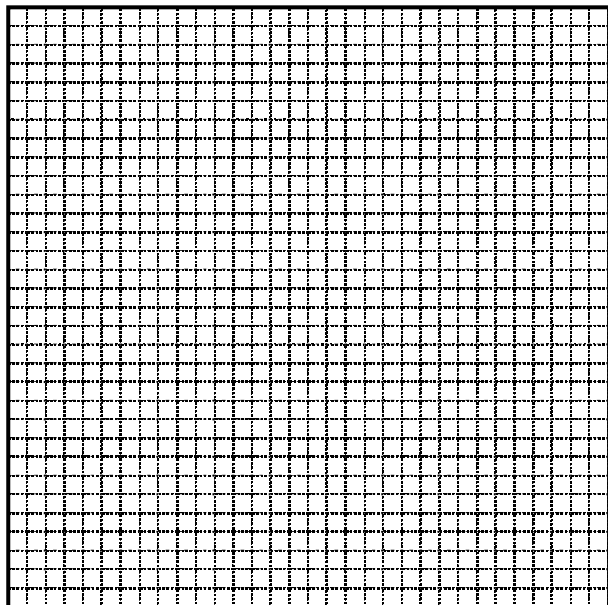
DNA nádoru



CGH
comparative
genome
hybridisation

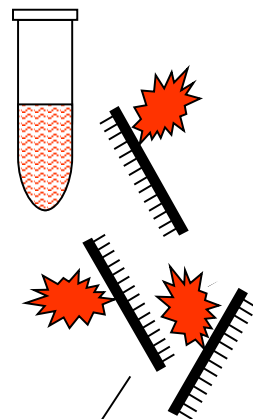


DNA čipové technologie: počet kopií v genomu (copy number)

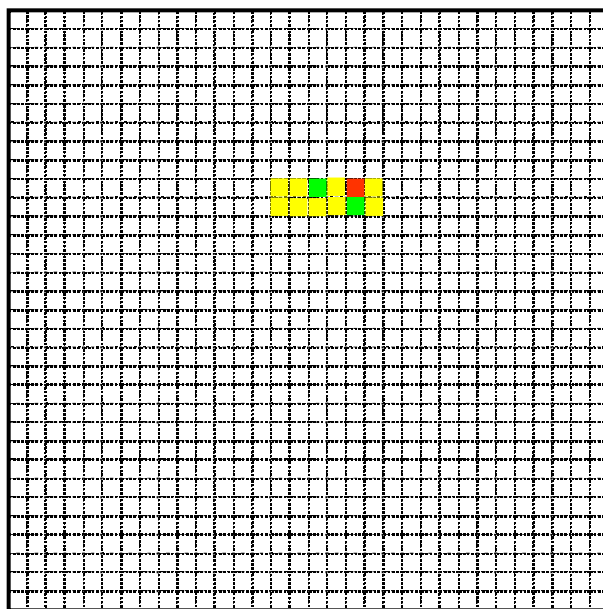
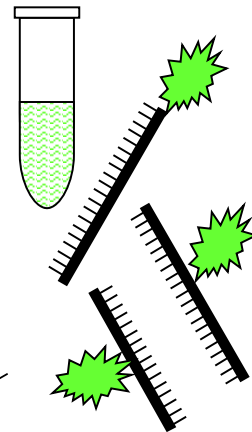


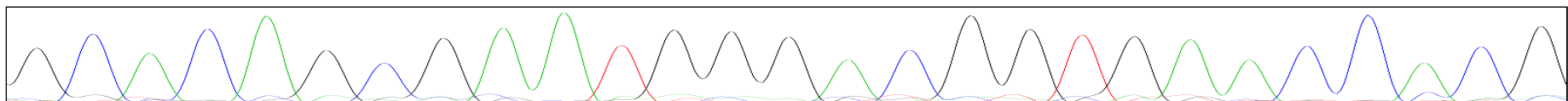
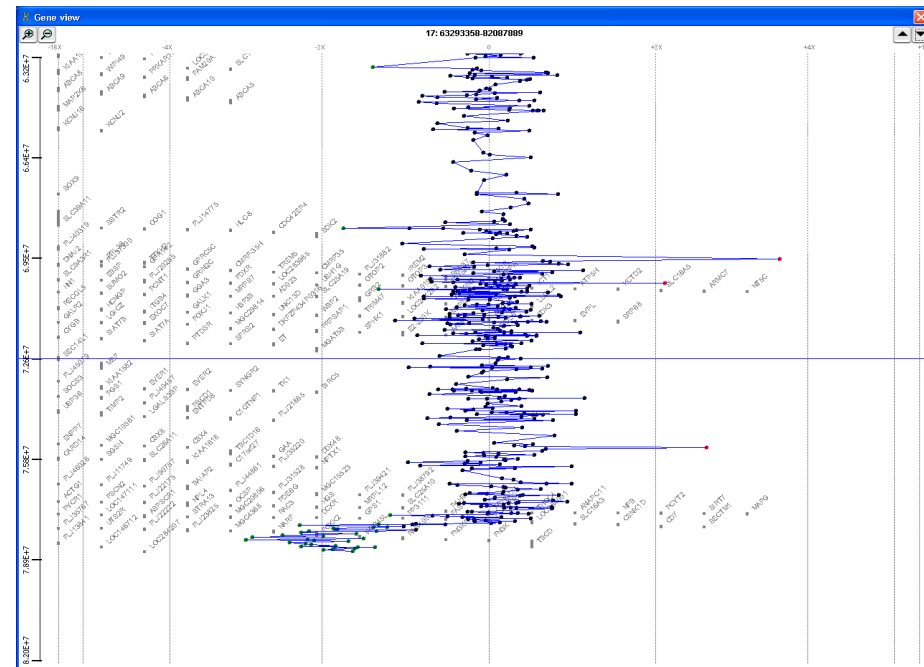
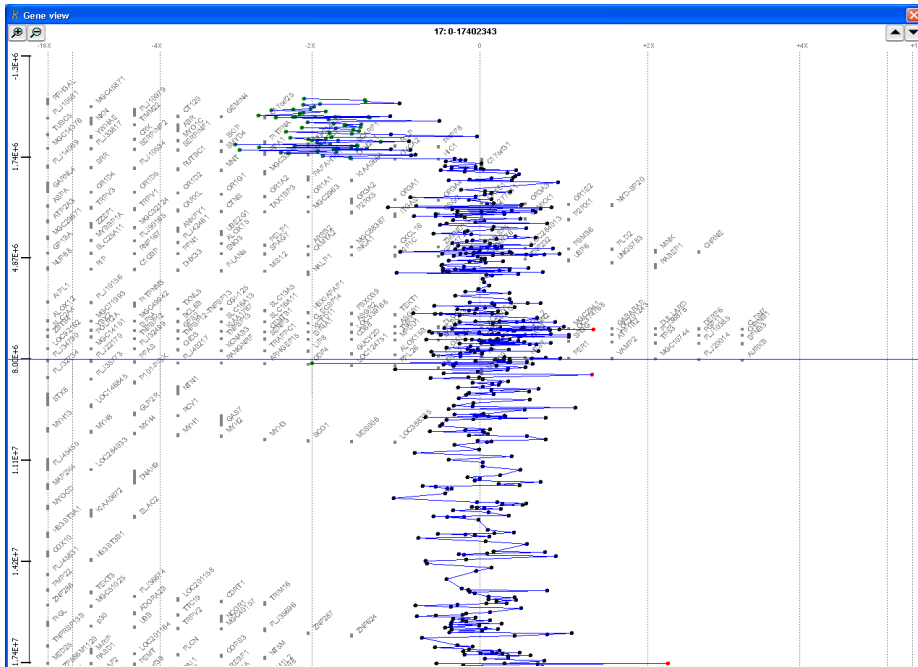
array
CGH

normální DNA



DNA nádoru

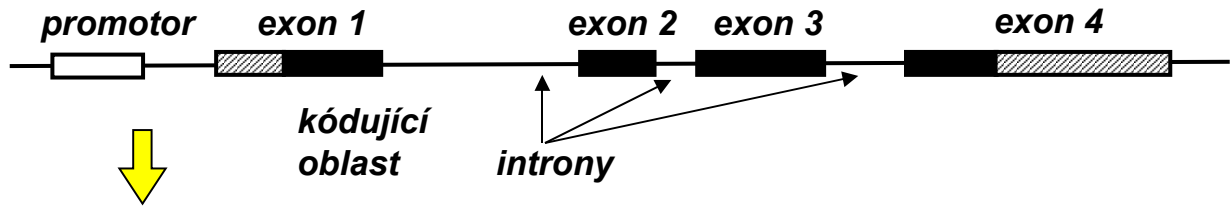




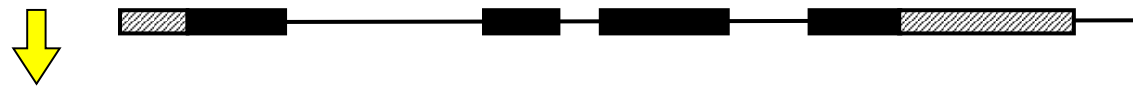
G C A C A G C G A A T G **G G A** C G G T G A A C C A C G

————— 17q ————— **GGACGGTGAAACCACG** ————— 17p —————

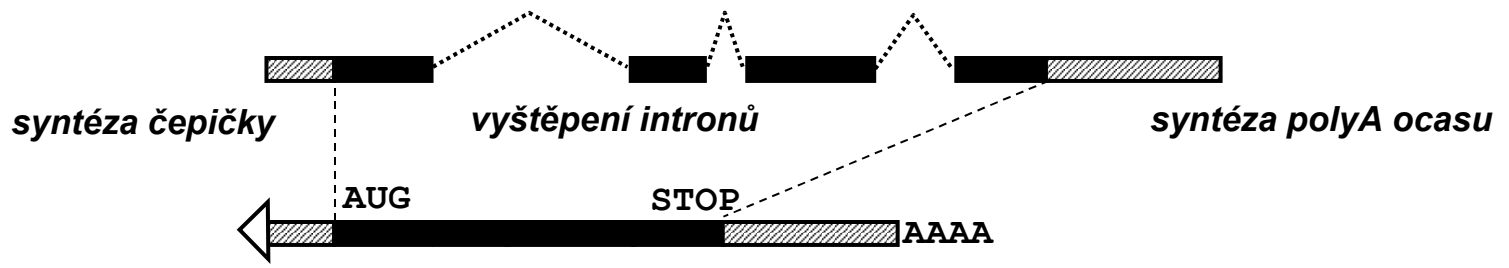
struktura eukaryontního genu v genomové DNA



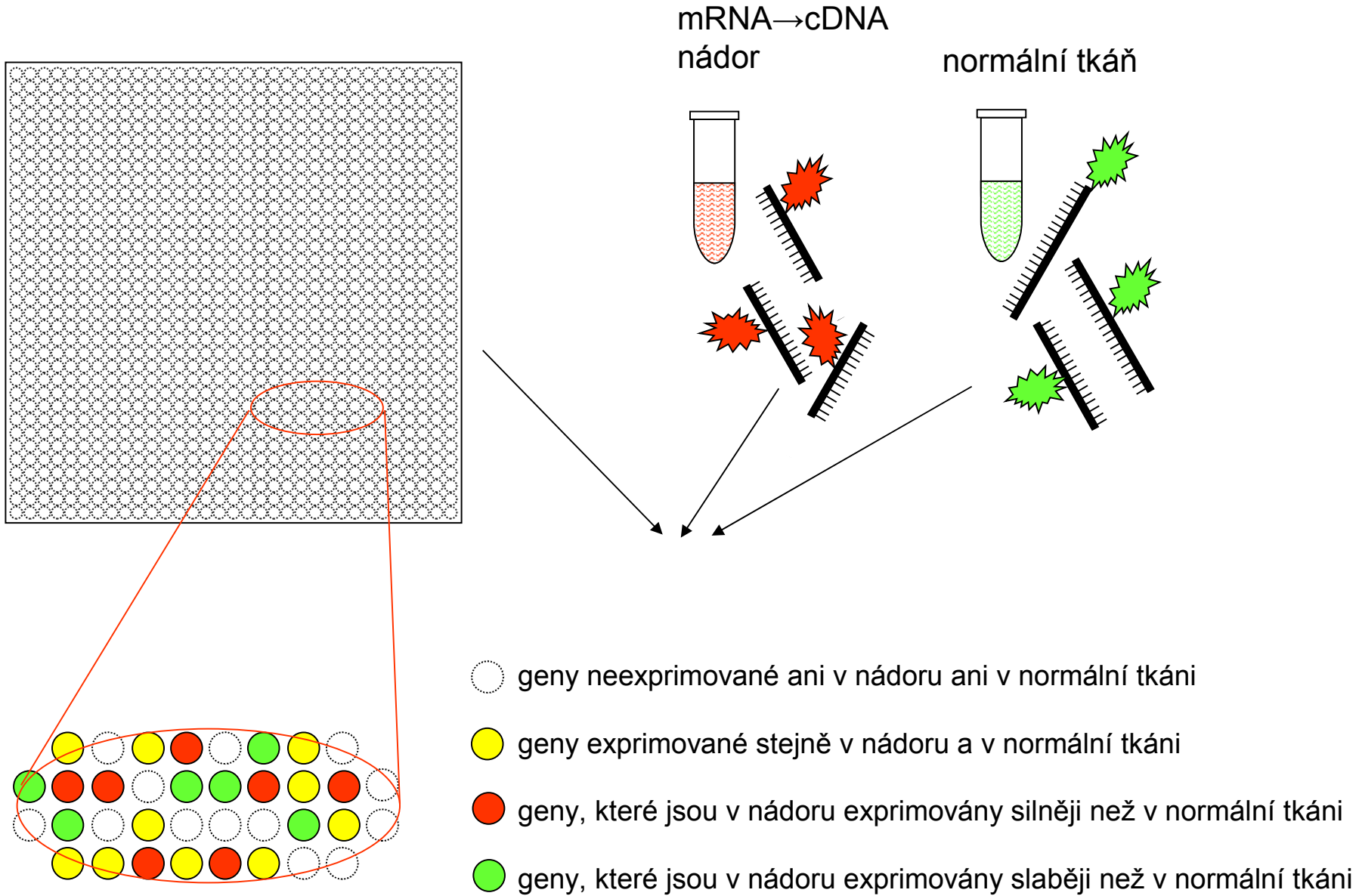
primární transkript (hnRNA, pre-mRNA)



postranskripční úpravy (vznik maturované mRNA)

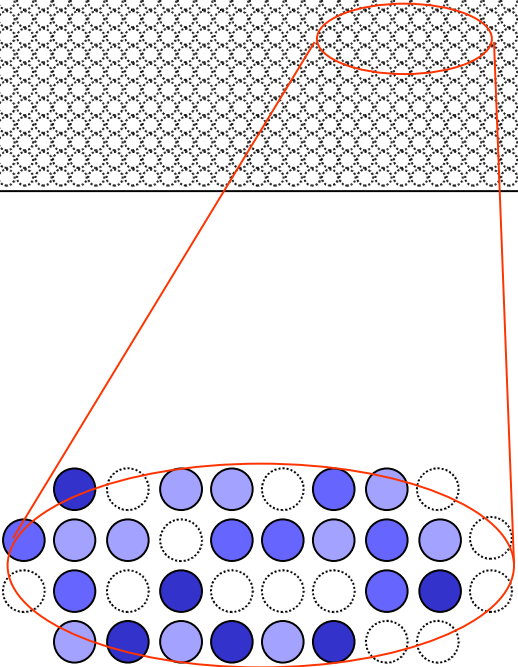
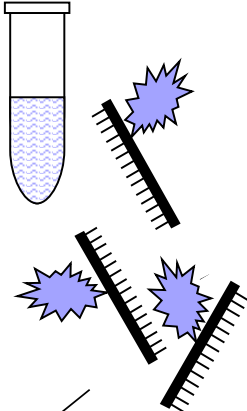
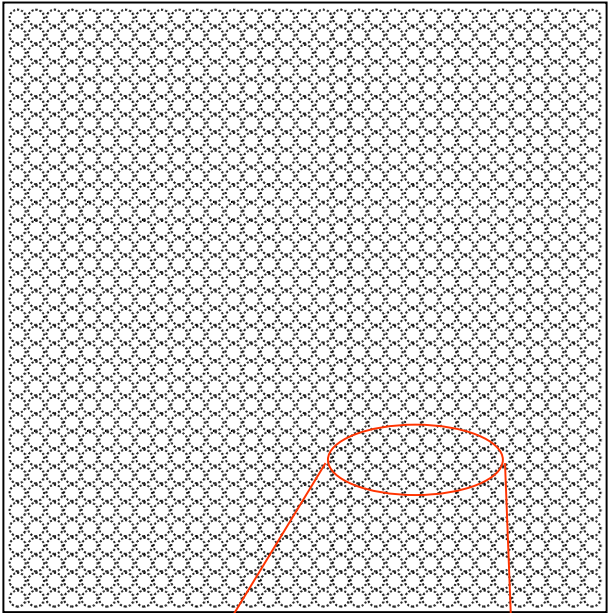


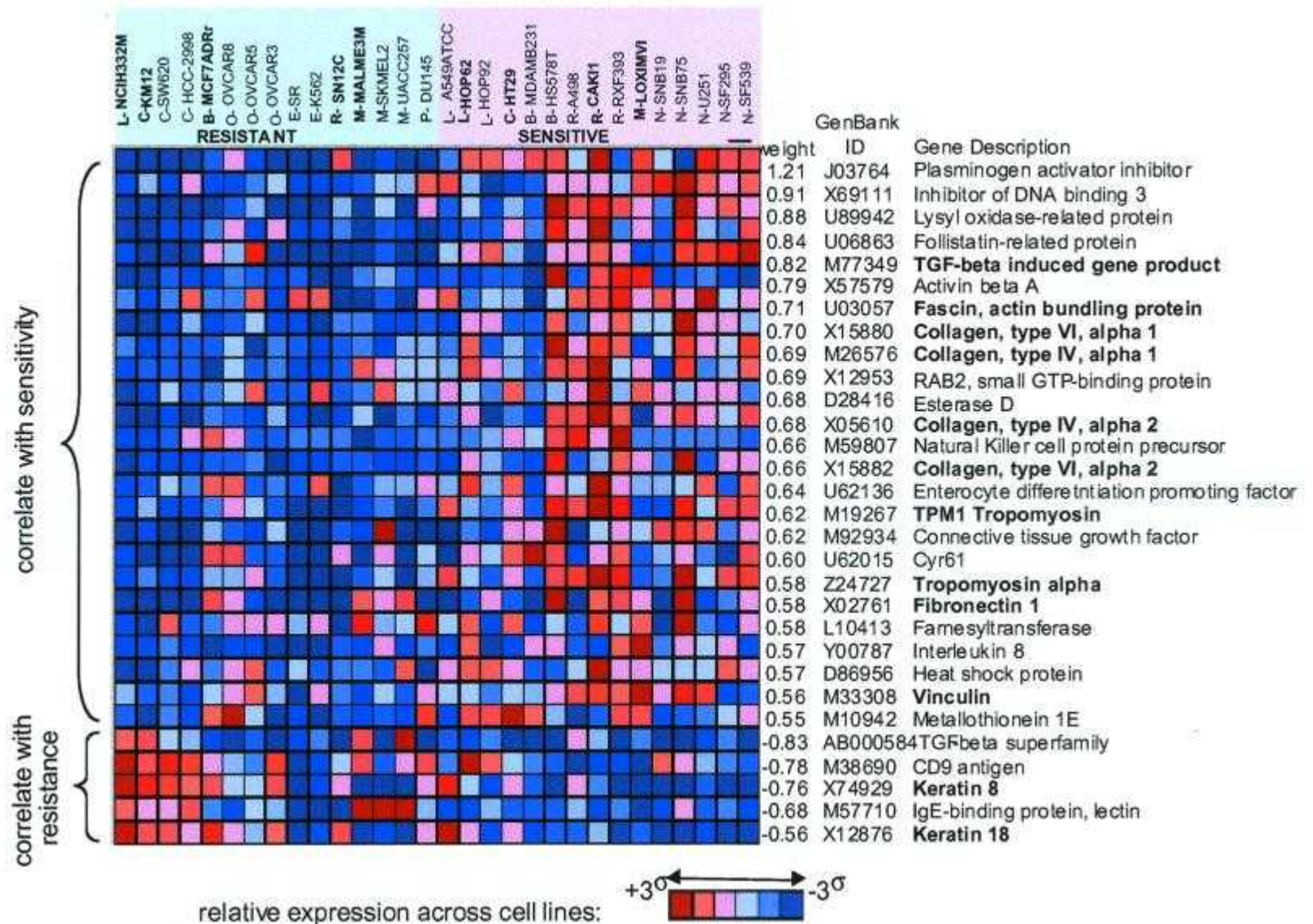
DNA čipové technologie: expresní profilování



DNA čipové technologie: expresní profilování

mRNA → cDNA
nádor





Staunton JE, Slonim DK, Collier HA, Tamayo P, Angelo MJ, Park J, Scherf U, Lee JK, Reinhold WO, Weinstein JN, Mesirov JP, Lander ES, Golub TR. Chemosensitivity prediction by transcriptional profiling. Proc Natl Acad Sci U S A. 2001 Sep 11;98(19):10787-92.