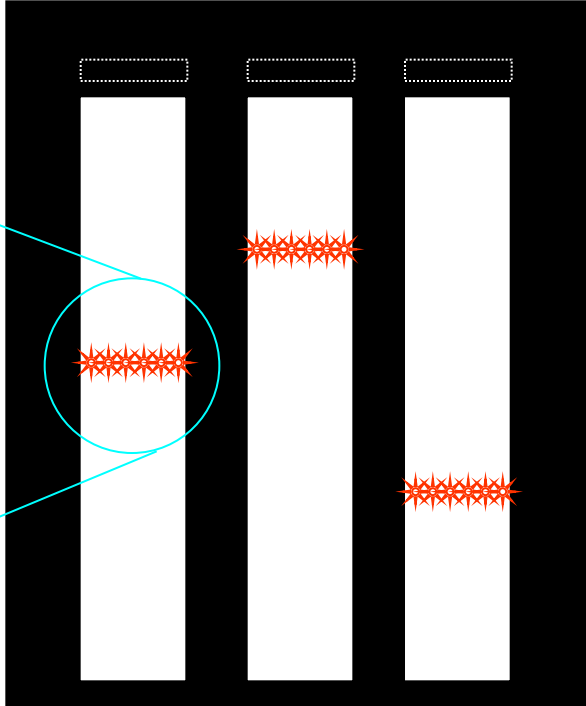
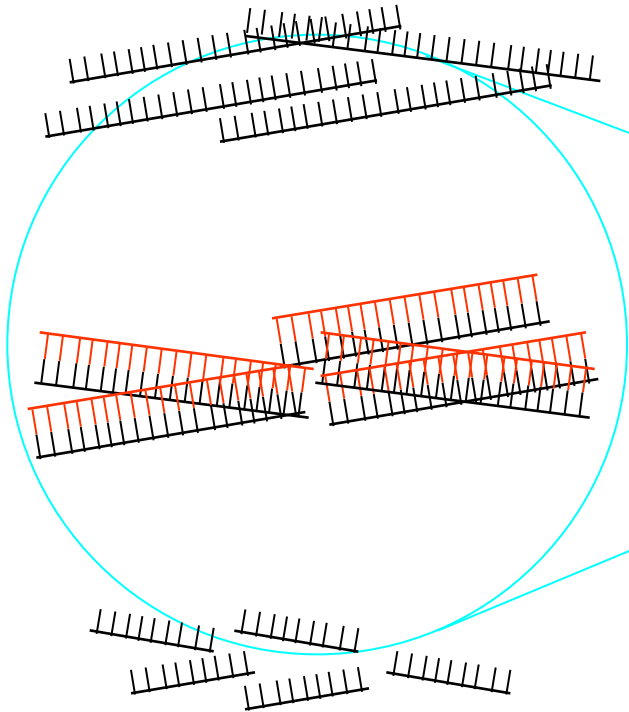


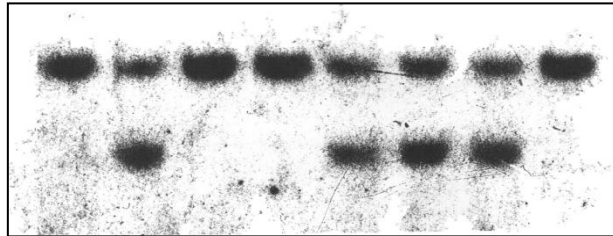
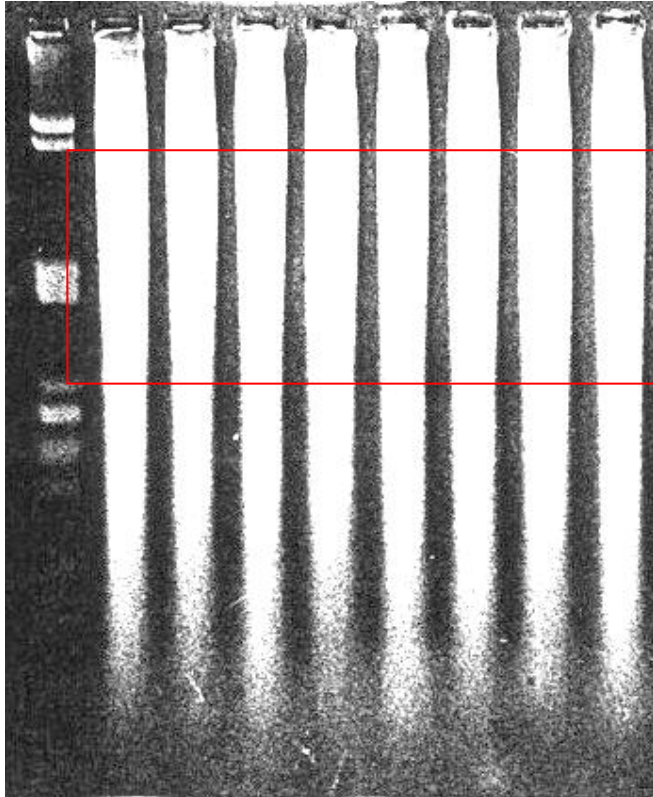
člověk

pacient 1

pacient 2

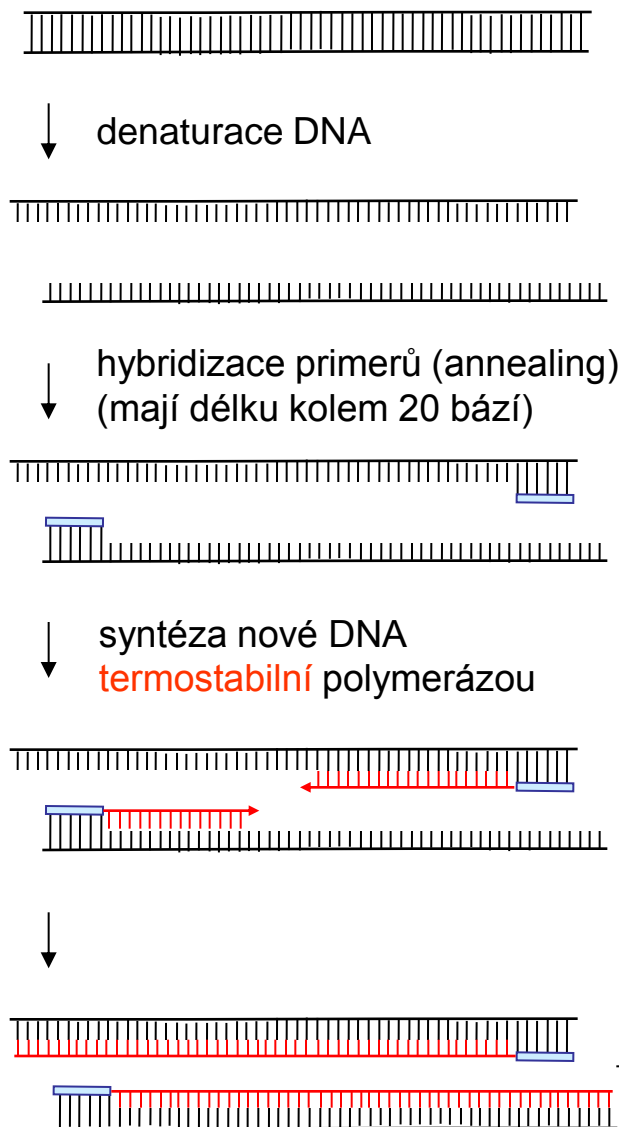
pacient 3





Southern
1975

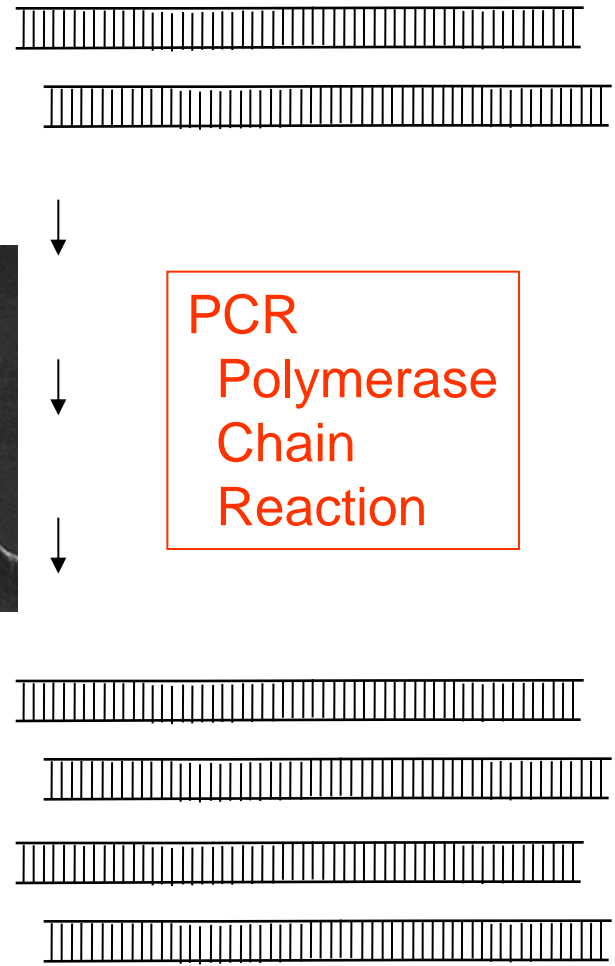
Northern
Western



po proběhnutí jednoho cyklu se jedna molekula DNA zmnoží na dvě



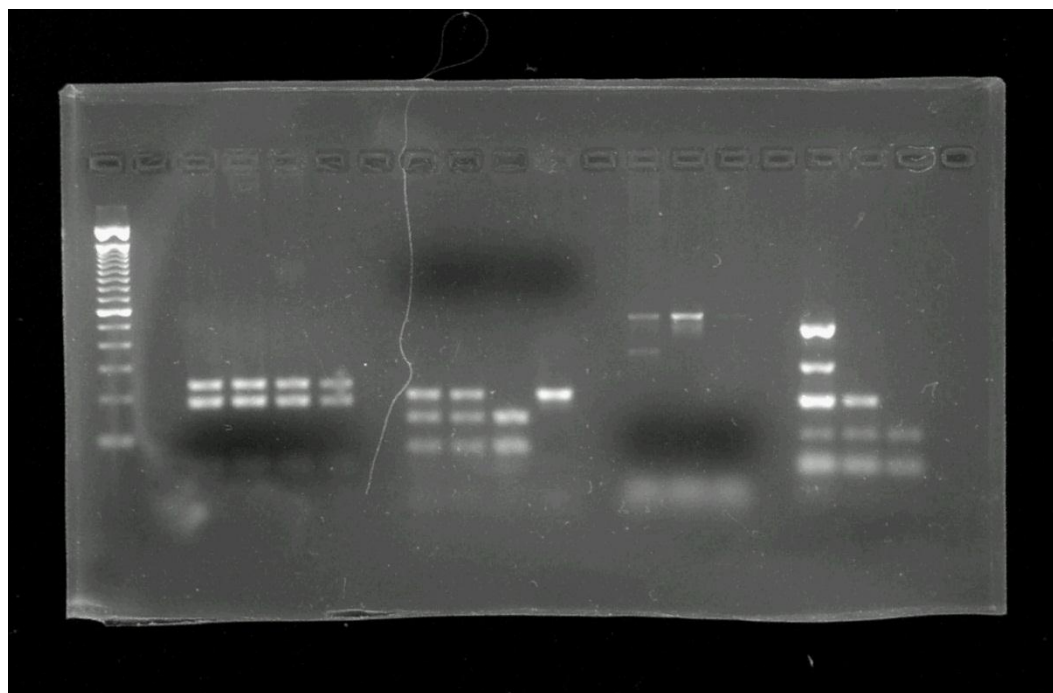
Mullis 1985



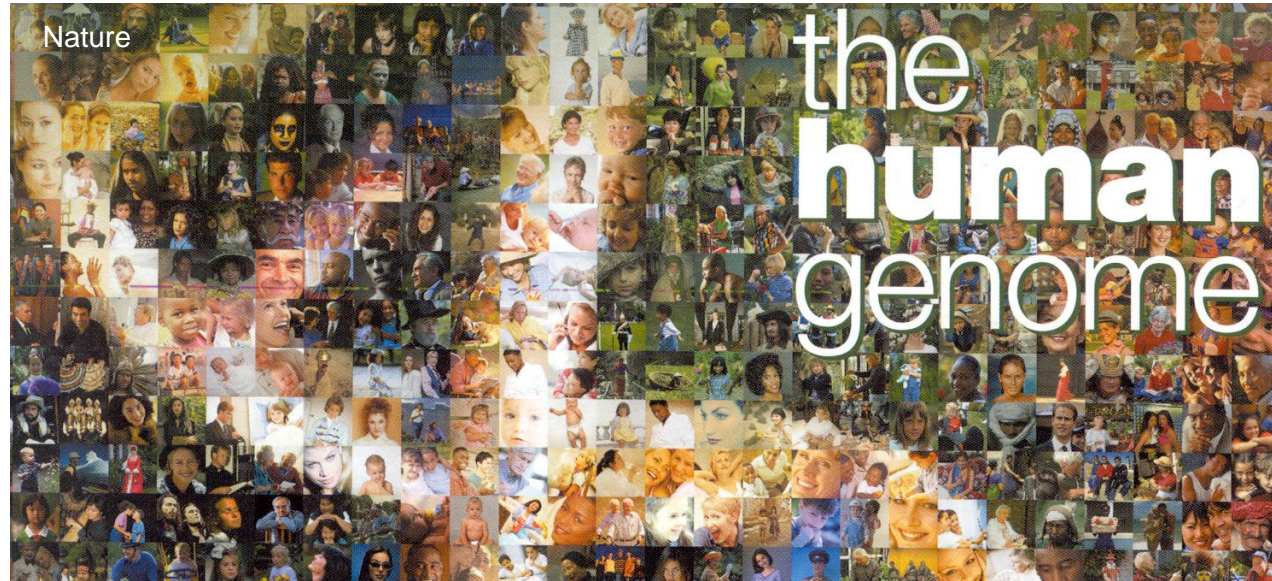
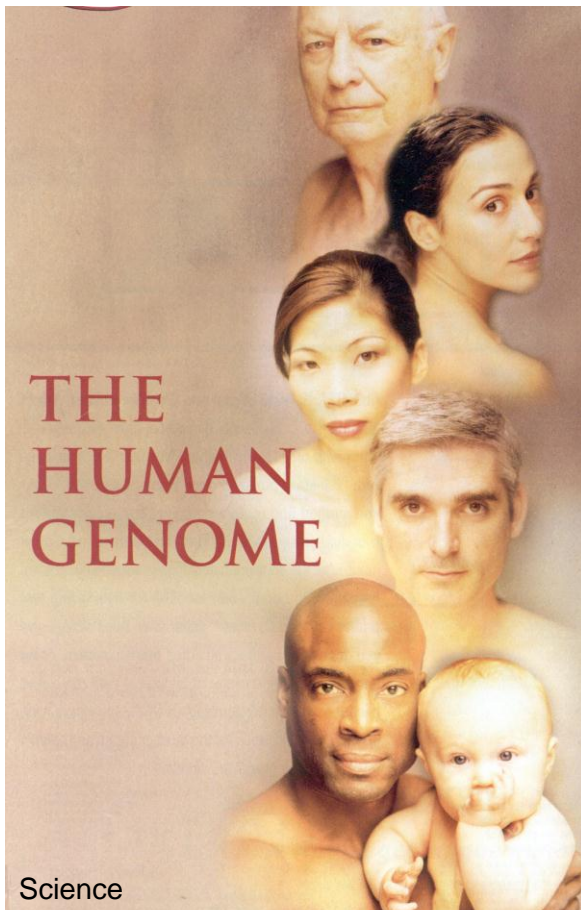
PCR
Polymerase
Chain
Reaction

po proběhnutí dvou cyklů se jedna molekula DNA zmnoží na čtyři

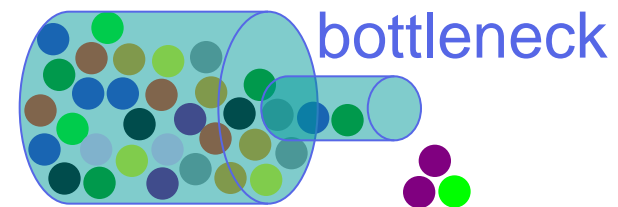
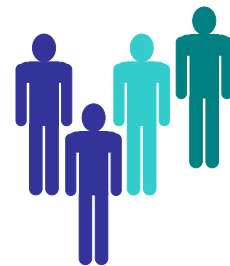
po proběhnutí n cyklů se jedna molekula DNA zmnoží na 2^n molekul



variabilita genomu



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



základní typy mutací

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

normální sekvence
čtecí rámec 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

polymorfismus typu SNP
(Single Nucleotide Polymorphism)

chromozóm (jedinec) A

chromozóm (jedinec) B

tři možné genotypy

polymorfismus typu STR
(Short Tandem Repeat)

alela A

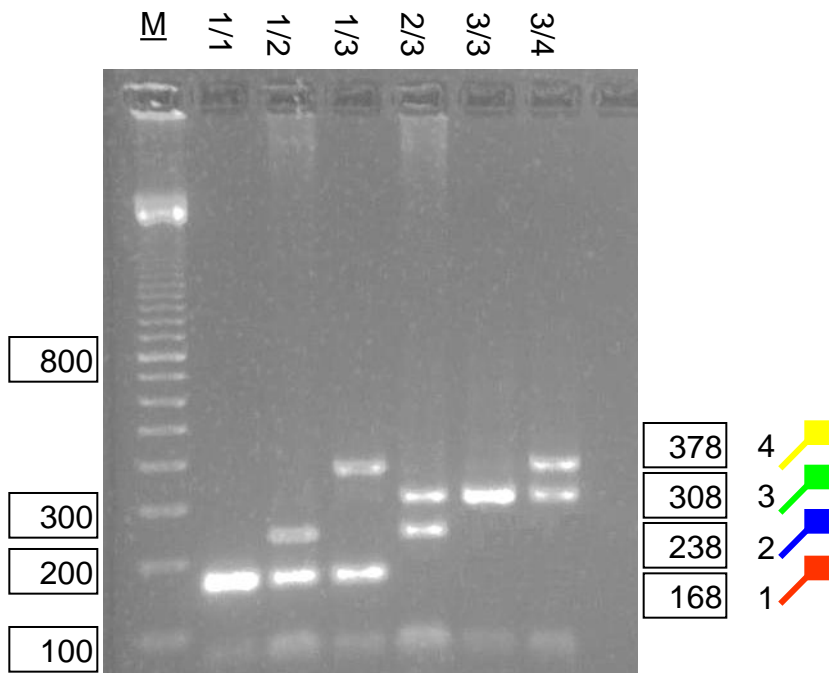
alela B

alela C

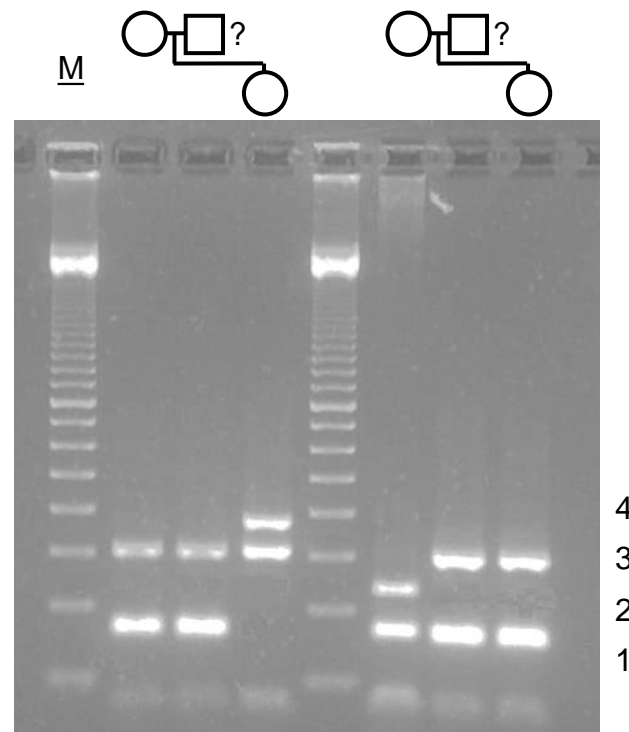
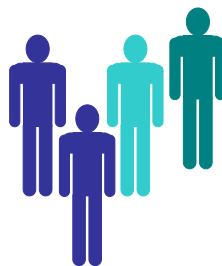
mnoho možných genotypů

▼
ATGCCCCAGCAGCCAAT
▼
ATGCCCTAGCAGCCAAT

▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCCCCACACACACACAGAAA
▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCCCCACACACACACACAGAAA
▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼
ATGCCCCACACACACACACACAGAAA

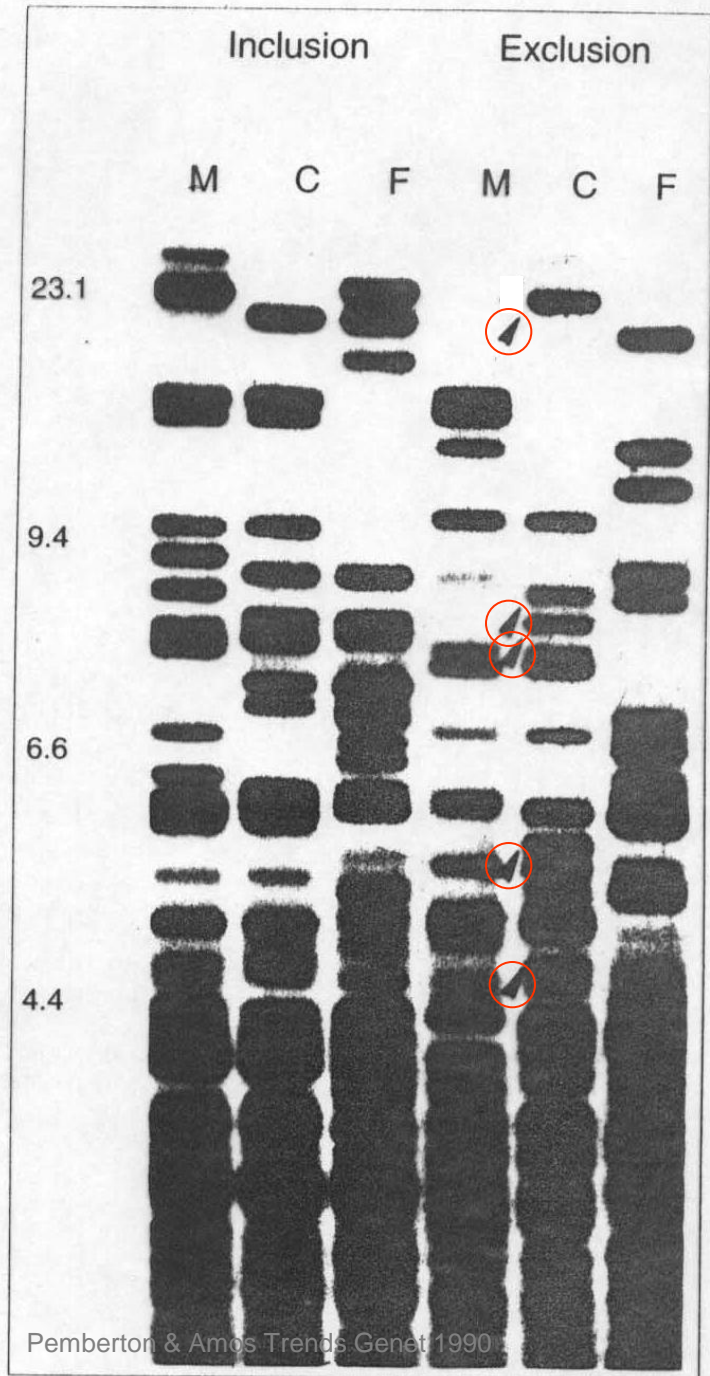


identifikace
osob



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 >

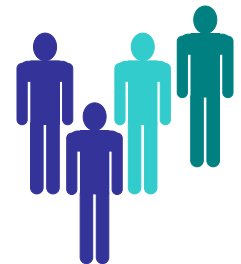
JAN PONDĚLÍK, pekařství a cukrářství Dvorec 307
LINECKÉ KOLÁČKY



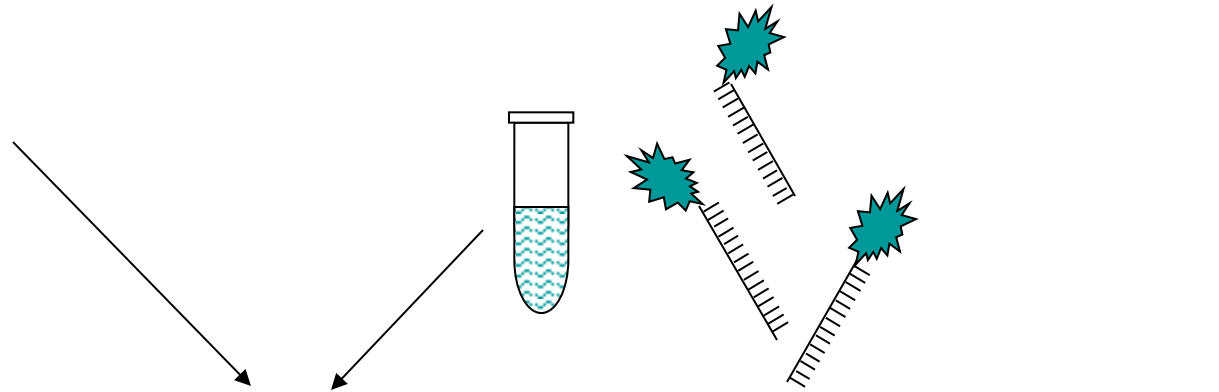
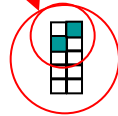
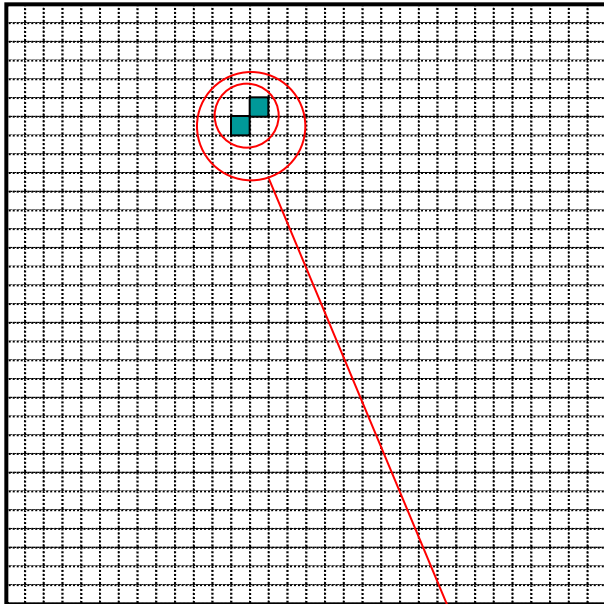
8 594007 072044 >

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

multilokusové sondy



DNA čipové technologie: resekvenace



5' GCGGCATGAACCGTAGGCCCCATC 3'

3' GCCGTACTTGGAAATCCGG 5'
GCCGTACTTGGCATCCGG
GCCGTACTTGGGATCCGG
GCCGTACTTGGTATCCGG
GCCGTACTTGG-ATCCGGG

CCGTACTTGGC(A)TCCGGG
CCGTACTTGGCCTCCGGG
CCGTACTTGGCGTCCGGG
CCGTACTTGGCTTCCGGG
CCGTACTTGGC-TCCGGGT

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

3' CCGCGTGTCTC - TTCTCTT 5'

3' CCGCGTGTCTC **T**TTCTCT 5'

3' CCGCGTGTCTC **G**TTCTCT 5'

3' CCGCGTGTCTC **C**TTCTCT 5'

3' CCGCGTGTCTC **A**TTCTCT 5'

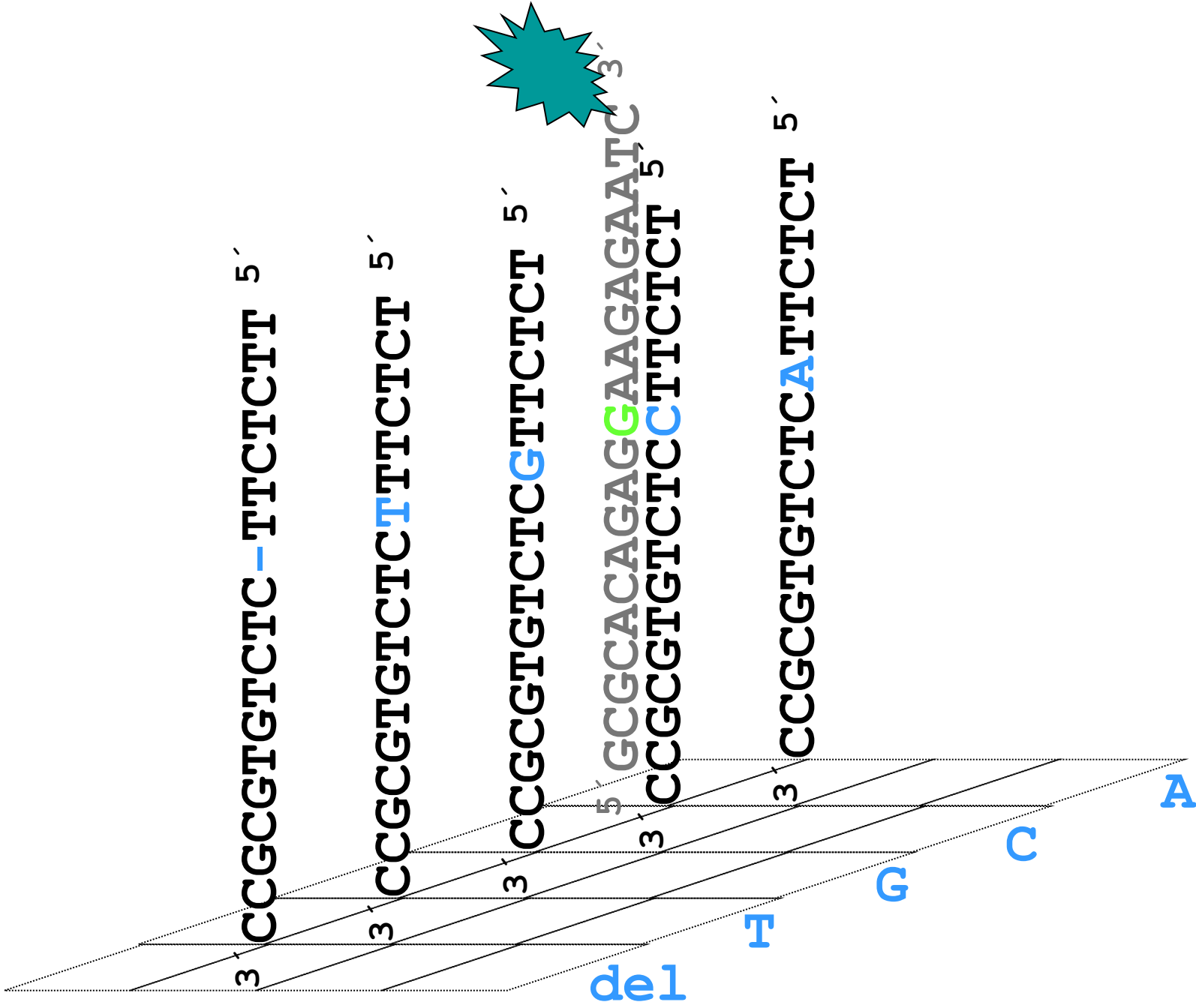
T

H

G

C

A



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

3' CCGCGTGTCTC **T**TCTCTT 5'

3' CCGCGTGTCTC **T**TCTCTT 5'

3' CCGCGTGTCTC **G**TTCTCT 5'

3' CCGCGTGTCTC **C**TTCTCT 5'

3' CCGCGTGTCTC **A**TTCTCT 5'

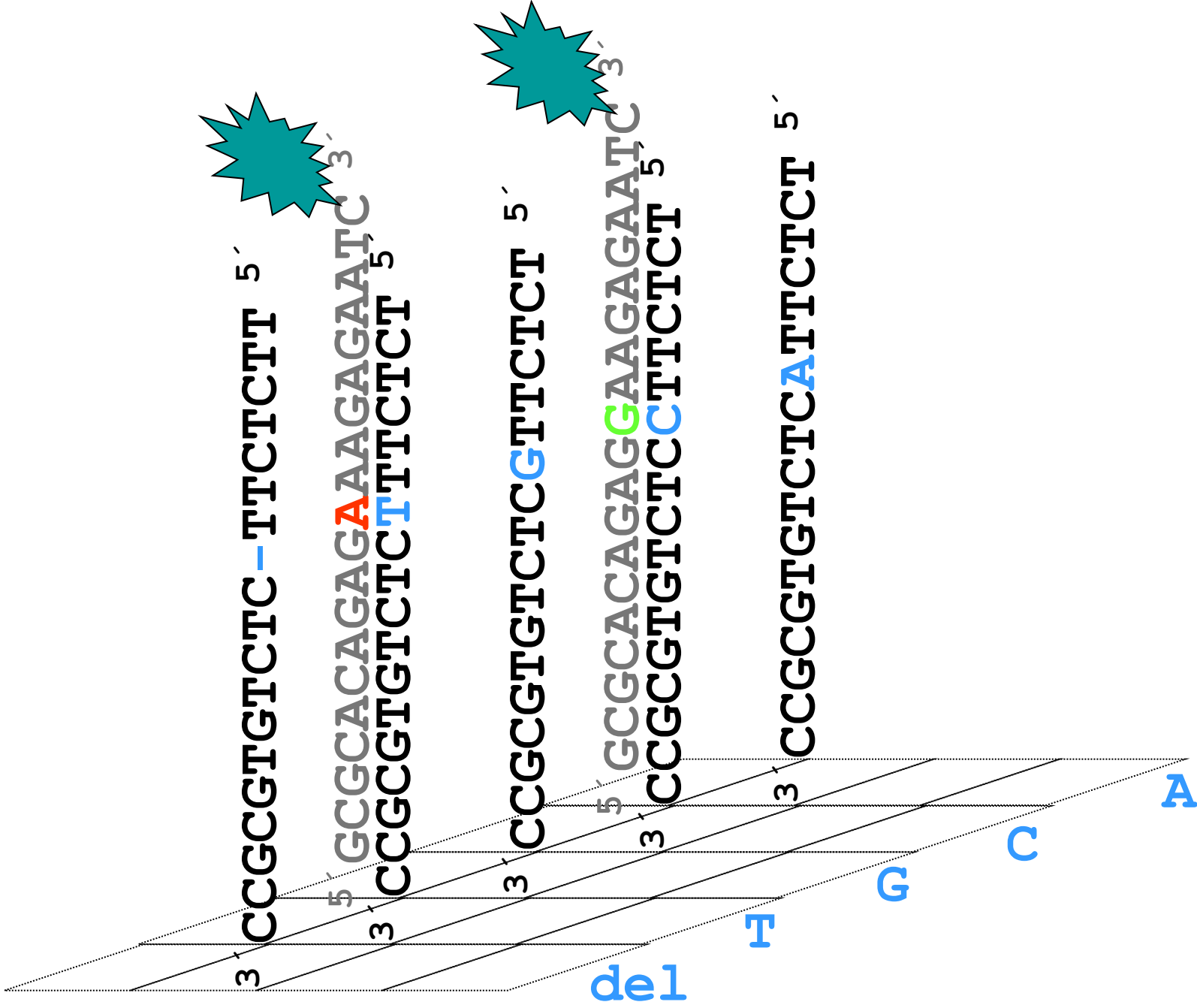
Top

H

G

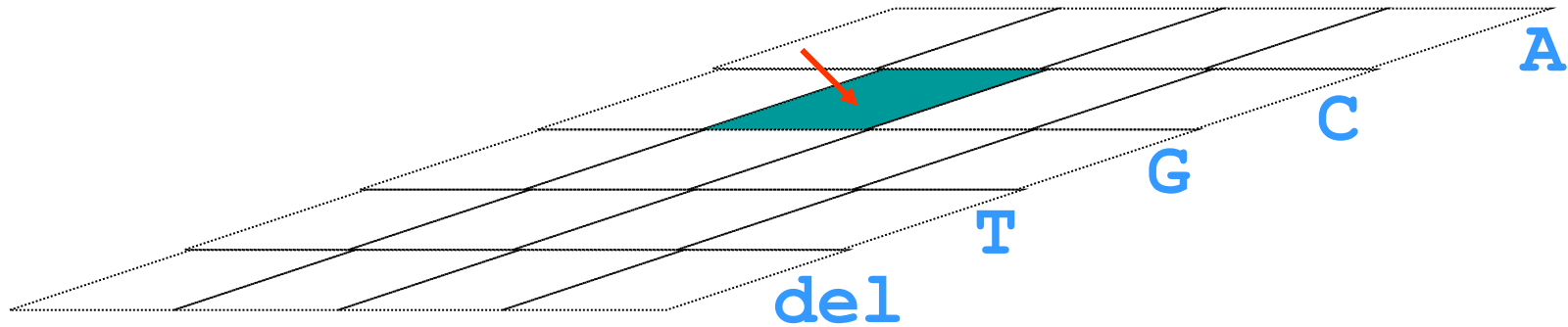
C

A



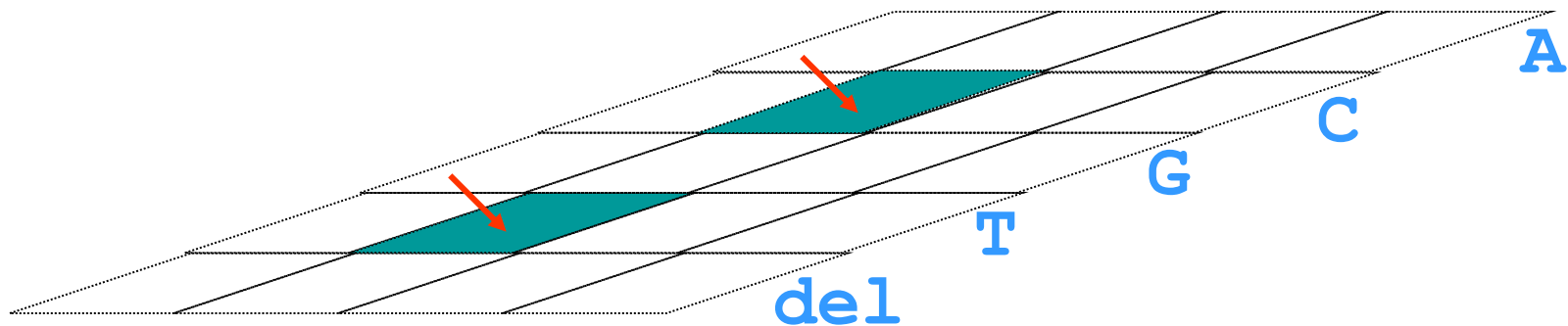
homozygot pro normální sekvenci (GAA = Glu)

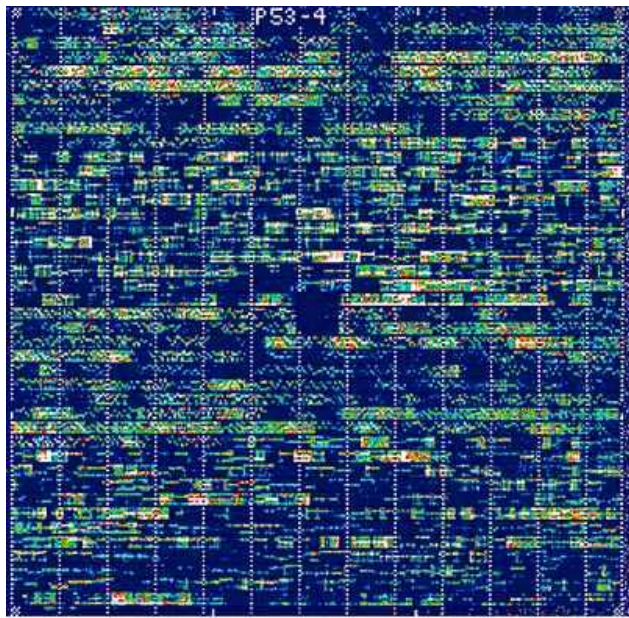
5' CGGCGCACAGAGGAAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGGAAAGAGAATCTCCGCAAG 3'



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

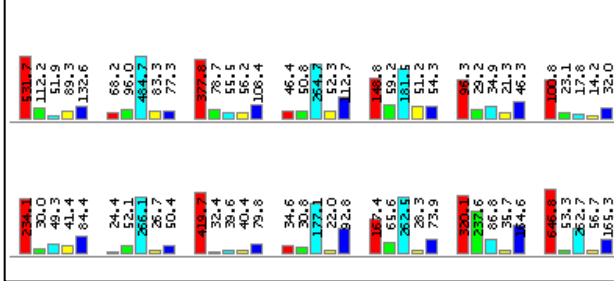
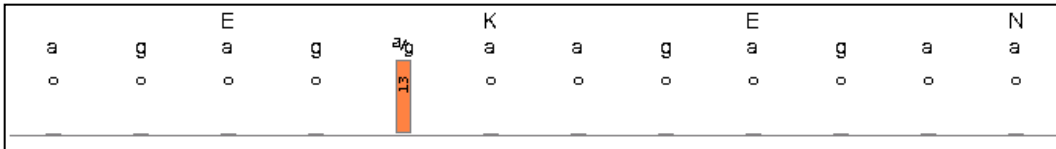
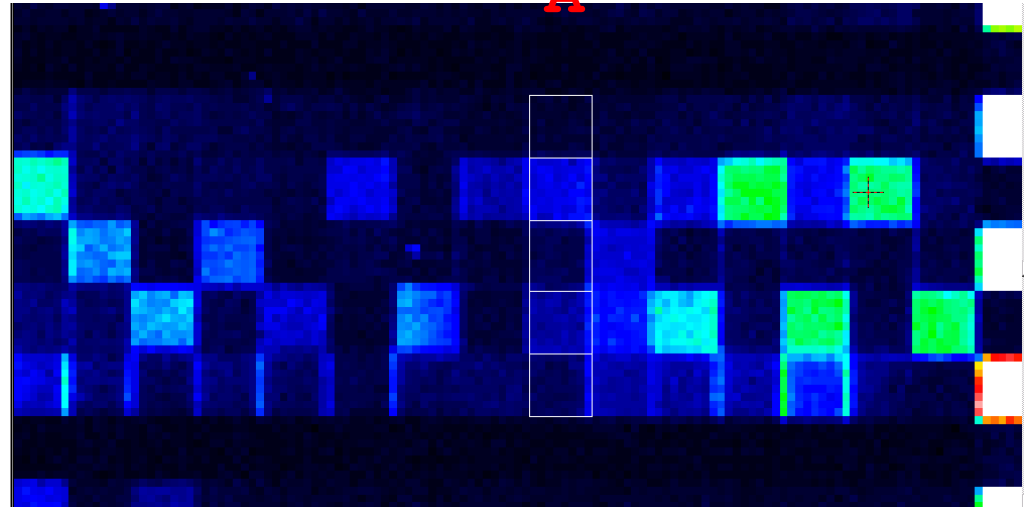
5' CGGCGCACAGAGGAAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGAAAAGAGAATCTCCGCAAG 3'





G C A C A G A G ^G A 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

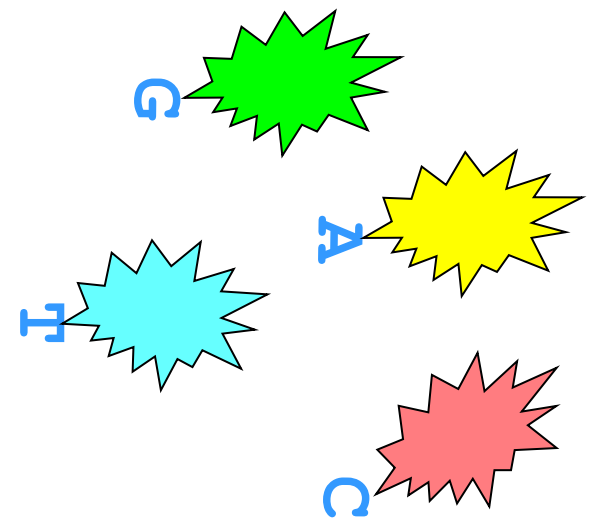
Filename: p53-ZS- -5-6-02.CHP
Probe Array Type: GP53
Algorithm: Mixture Detection

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

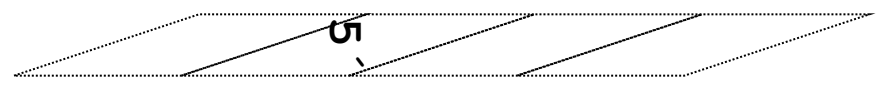
▲ ▲

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

neznačená
testovaná
DNA

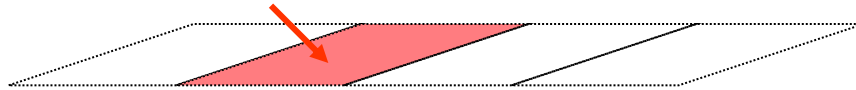


extenze oligonukleotidu
zakotveného v buňce čipu
o jeden značený dideoxynukleotid



homozygot pro normální sekvenci (286 GAA (Glu))

5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'



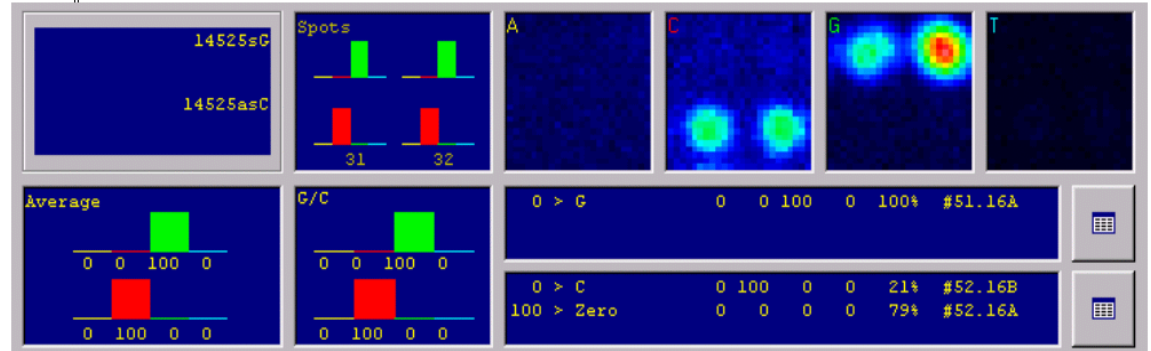
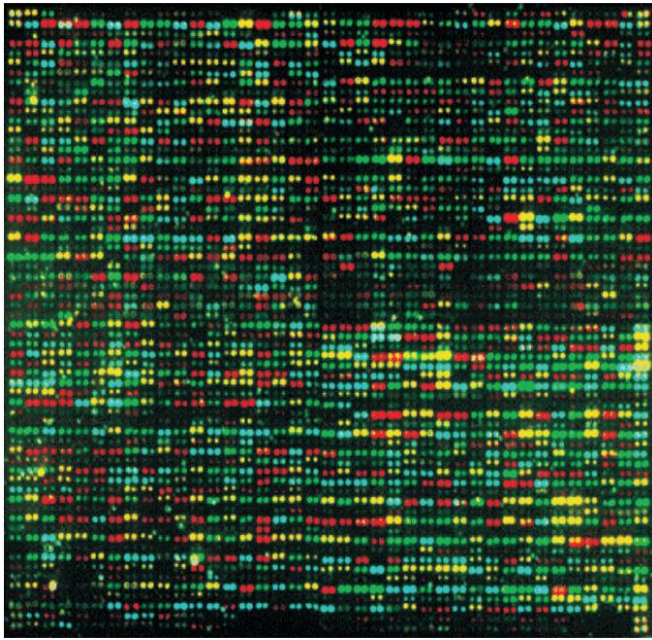
červený fluorescenční signál v buňce B

heterozygot pro mutovanou sekvenci (286 AAA (Lys))

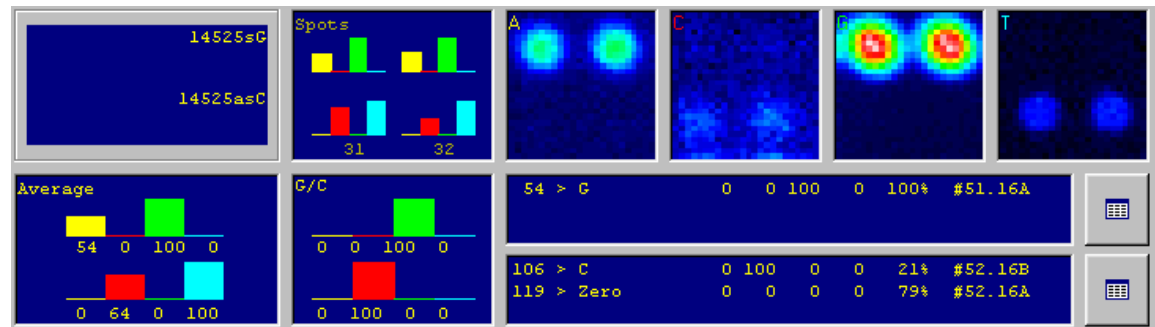
5' CGGCGCACAGAGGAAGAGAATCTCCGCAAG 3'
5' CGGCGCACAGAGAAAGAGAATCTCCGCAAG 3'



červený + modrý fluorescenční signál v buňce B

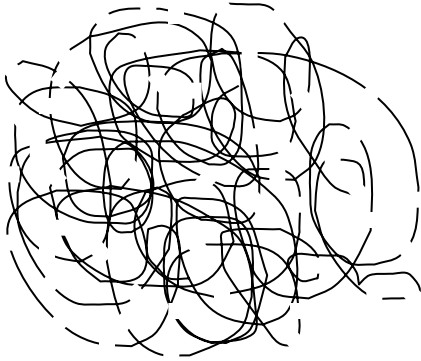


286 Glu/Glu



286 Glu/Lys

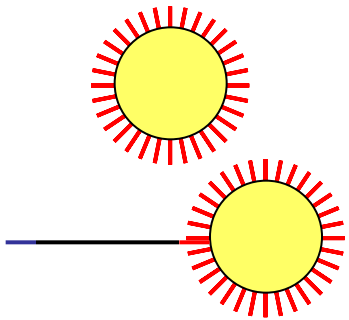
sekvenování nové generace



fragmentace DNA

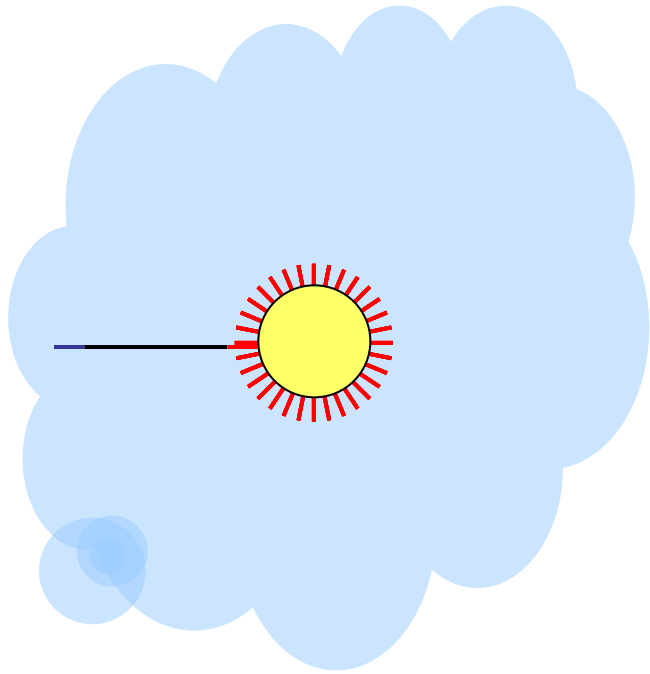


ligace adaptorů, denaturace

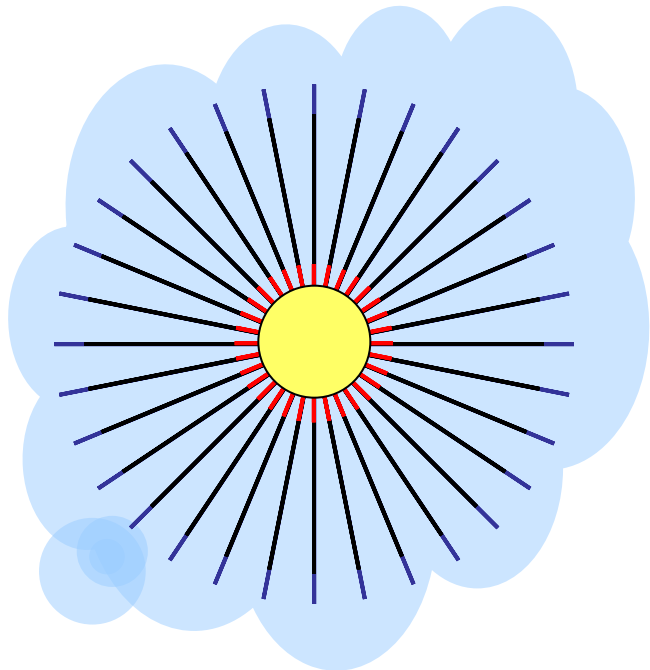


annealing fragmentů na partikule

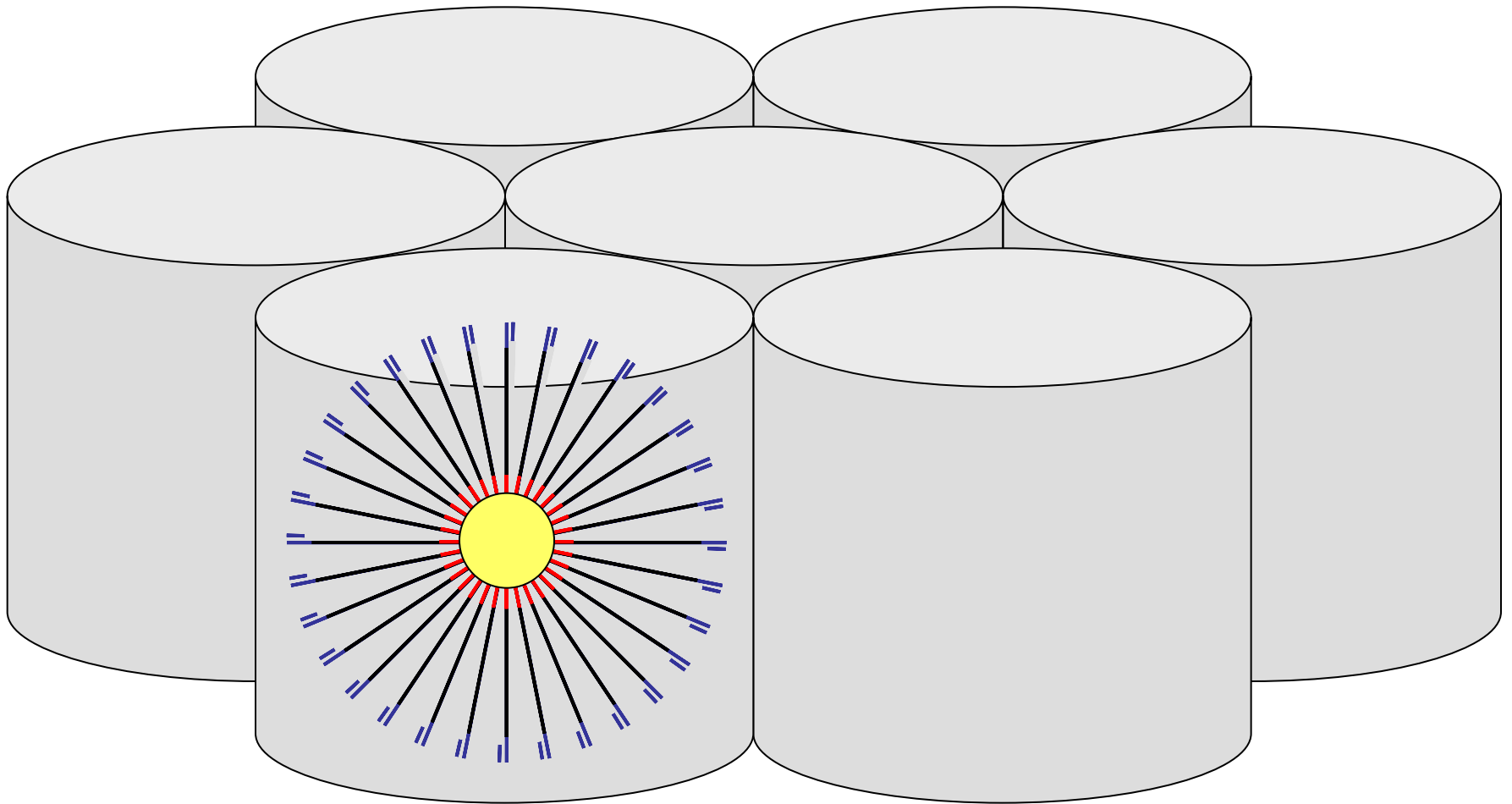
vytvoření emulze



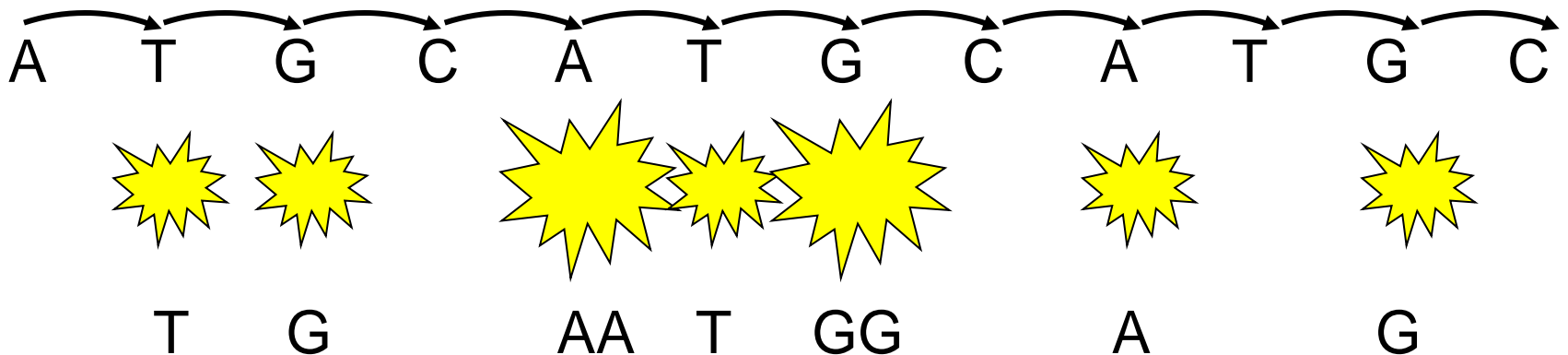
emulzní PCR, denaturace



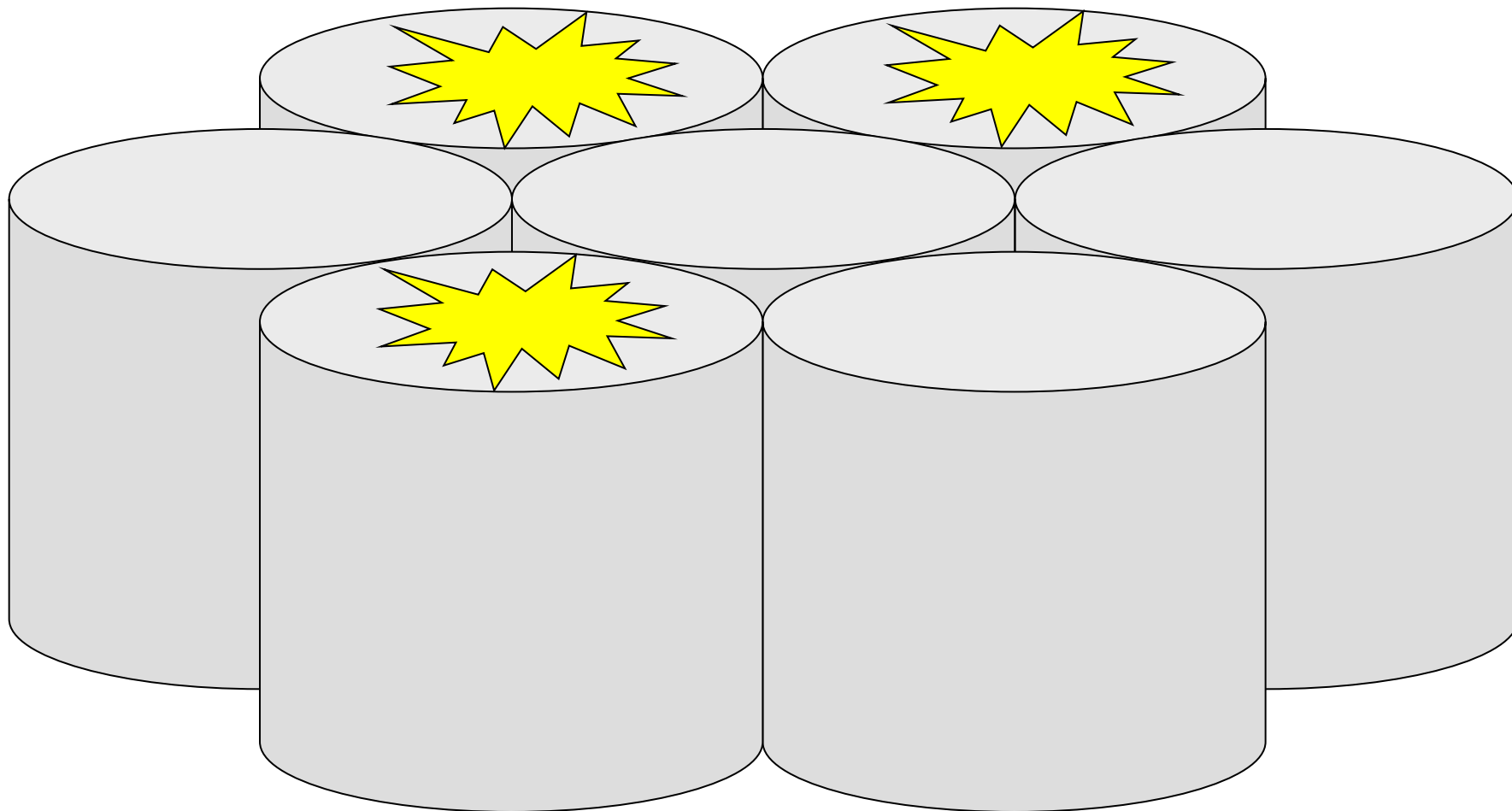
zachycení partikulí v pikotitrační desce



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

GEN (GENY)
v genomu člověka
GENOTYP

EXPRESE GENU

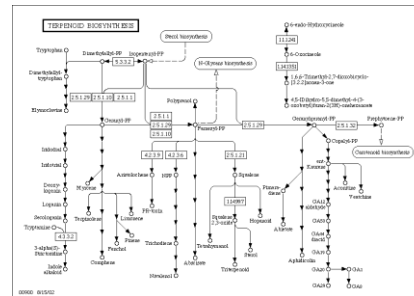
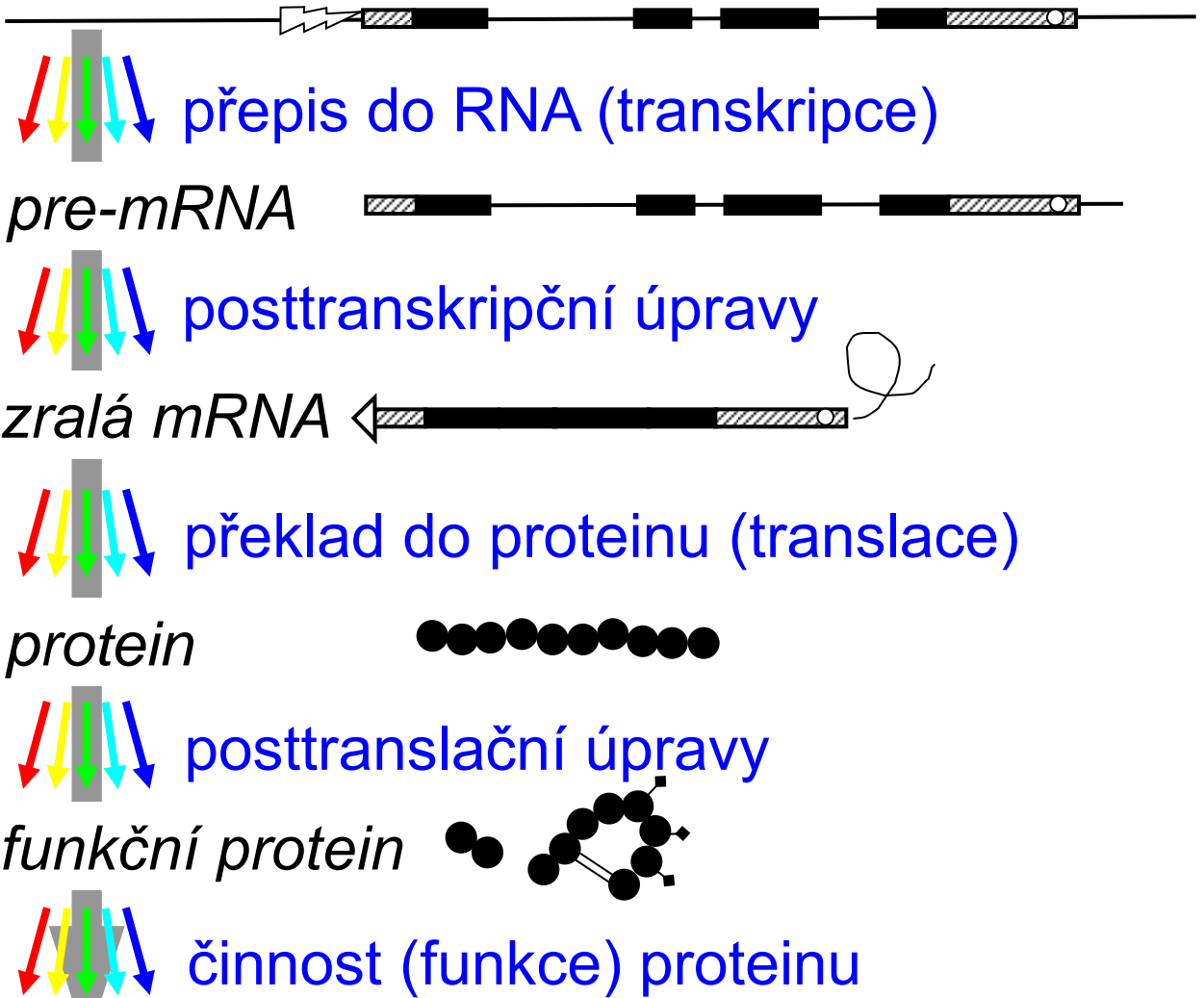
interakce s ost.
geny (proteiny)

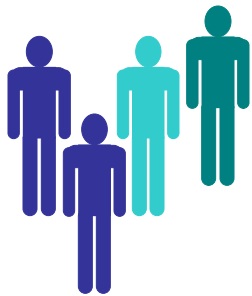
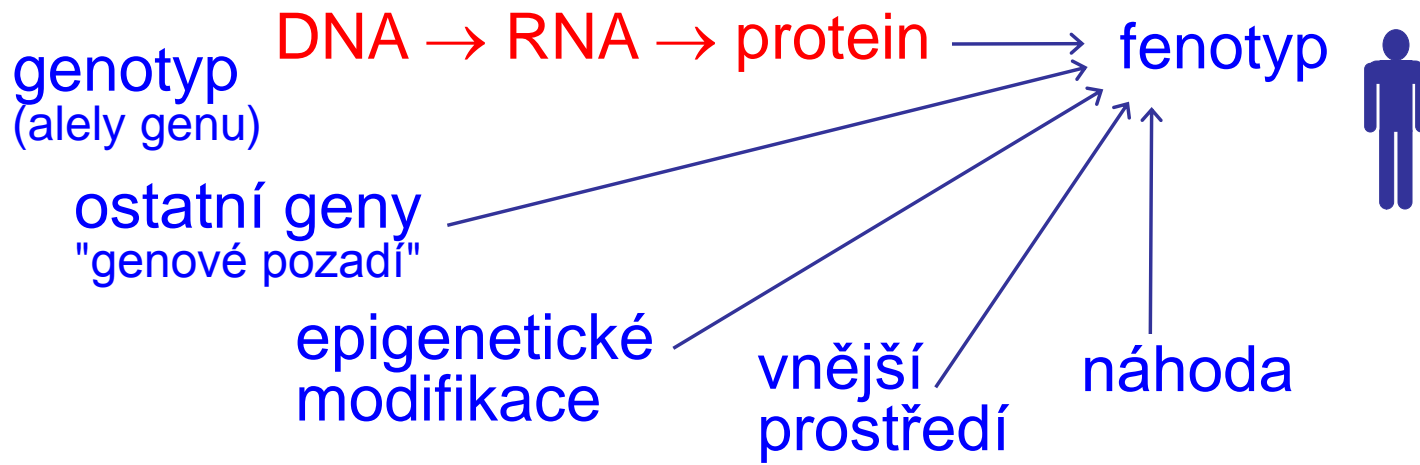
epigenetika

vnější prostředí

náhoda

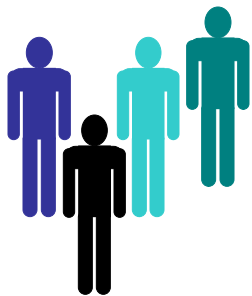
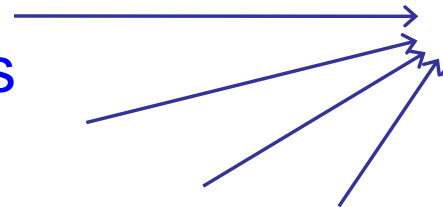
FENOTYP
lidského jedince





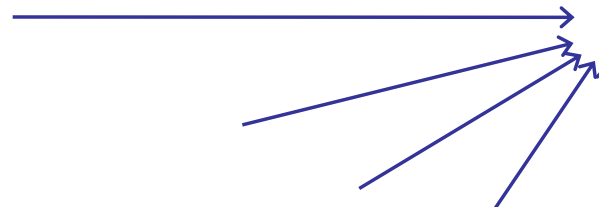
genetický
polymorfismus

fenotypová
variabilita

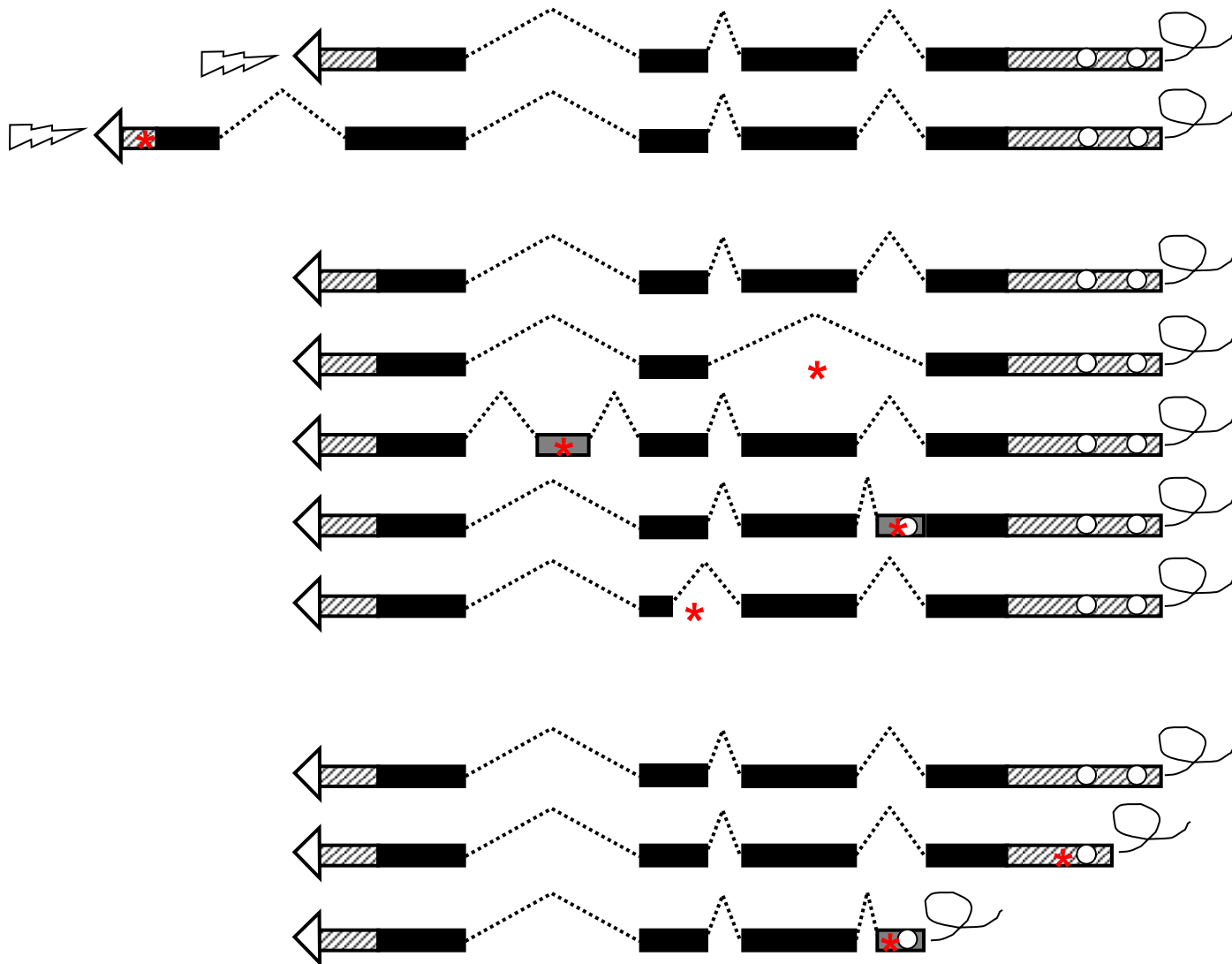


mutace

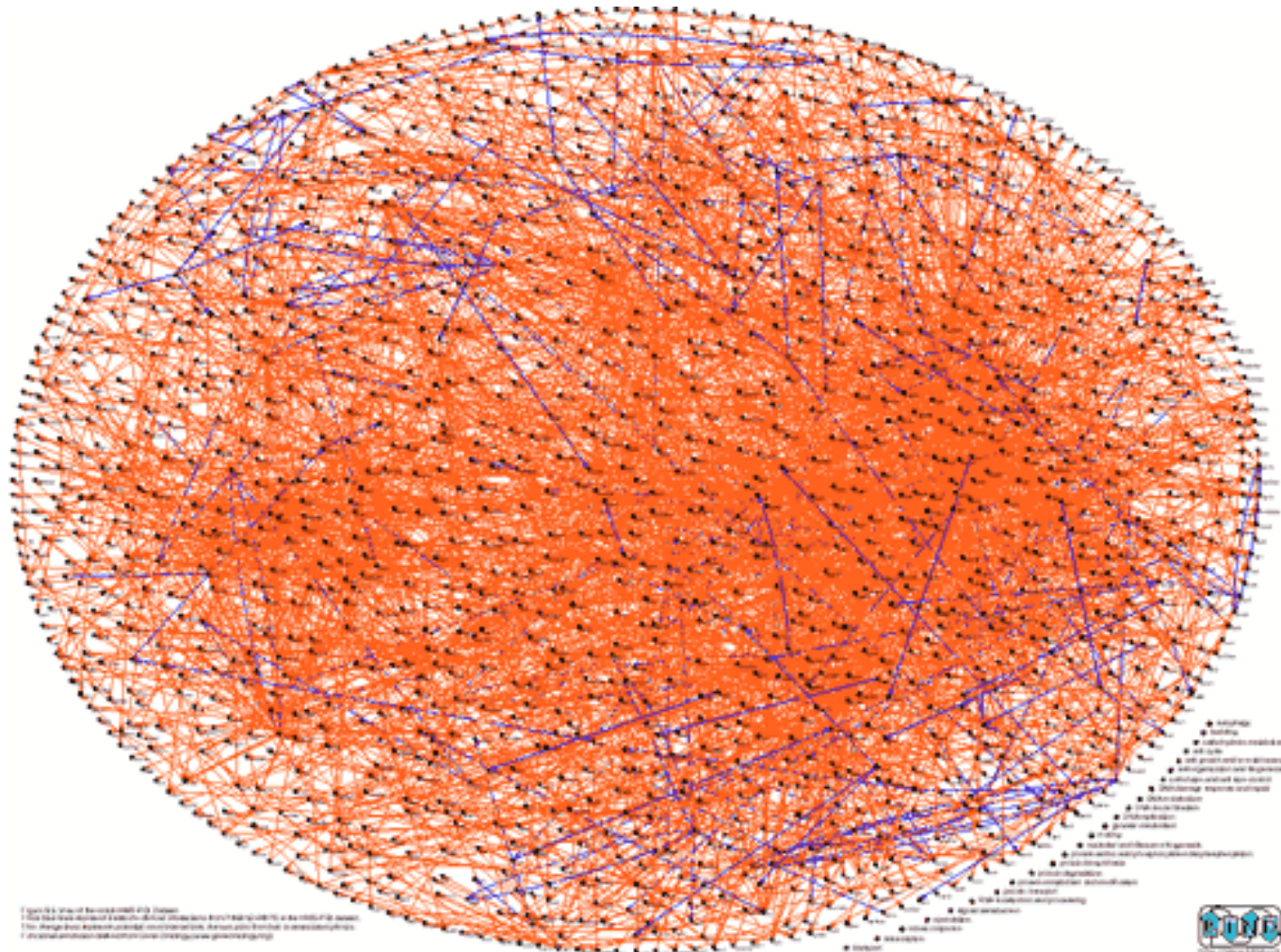
patologický
fenotyp (choroba)



alternativní sestřih transkriptů RNA



interakce genů / genových produktů

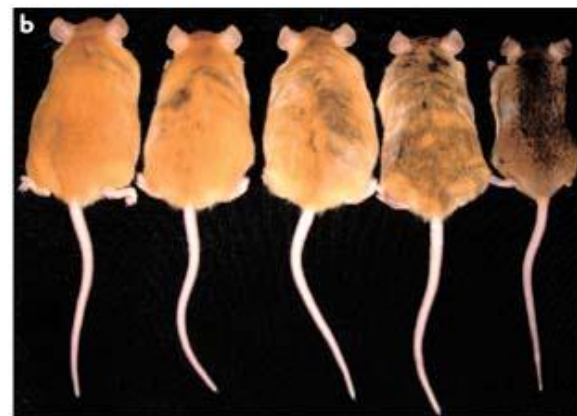
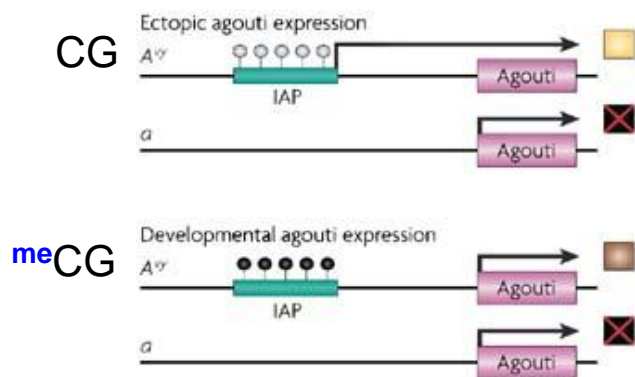


http://gnn.tigr.org/articles/01_02/Yeast_proteins_image3.shtml

epigenetika

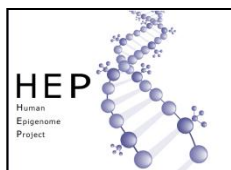
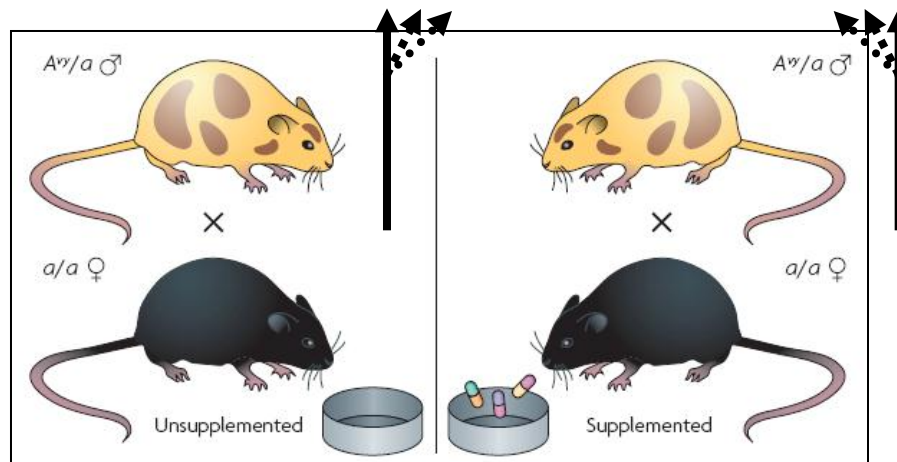
metylace DNA, nekódující RNA, modifikace histonů, ...

gen Agouti - signalizace, folikulární melanocyty produkují žlutý phaeomelanin místo černého eumelaninu
A - exprese je normálně limitována do určité periody vývoje folikulů - pouze žlutý proužek na chlupu
A^{vy} - konstitutivní exprese, žlutá srst, diabetes, obesita a nádory
a - nefunkční (pouze černá)



Nature Reviews | Genetics

časná mateřská péče
časná (prenatální) výživa
jednovaječná dvojčata

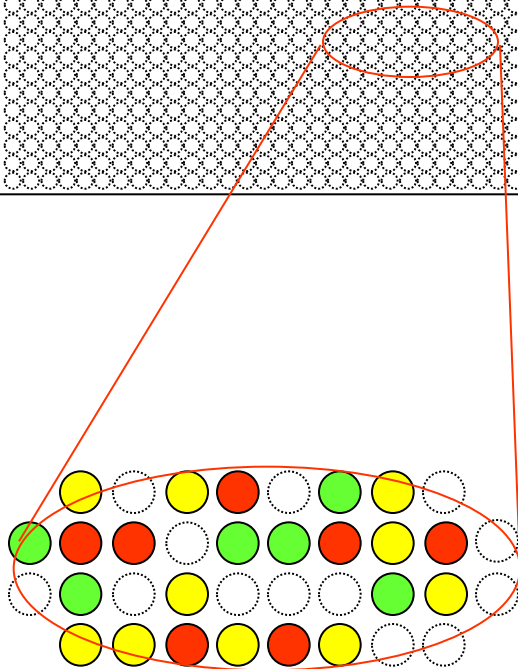
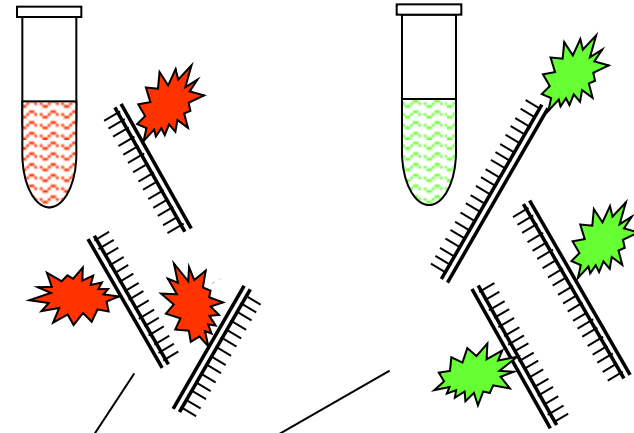
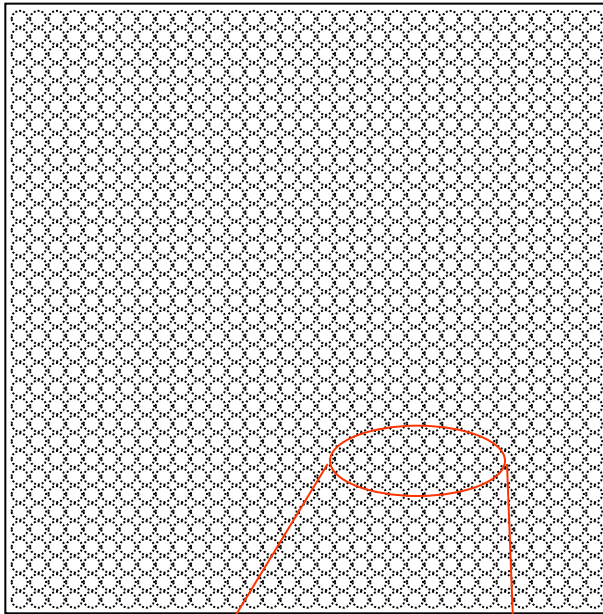


DNA čipové technologie: expresní profilování

mRNA → cDNA

nádor

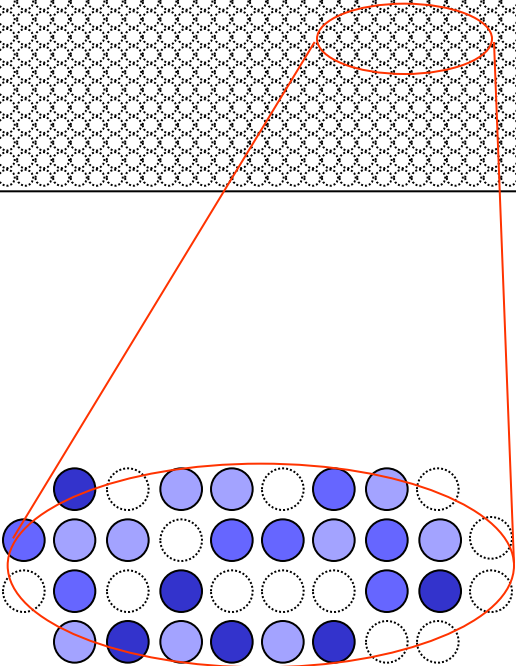
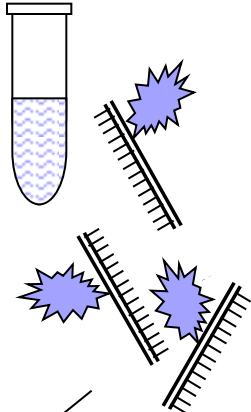
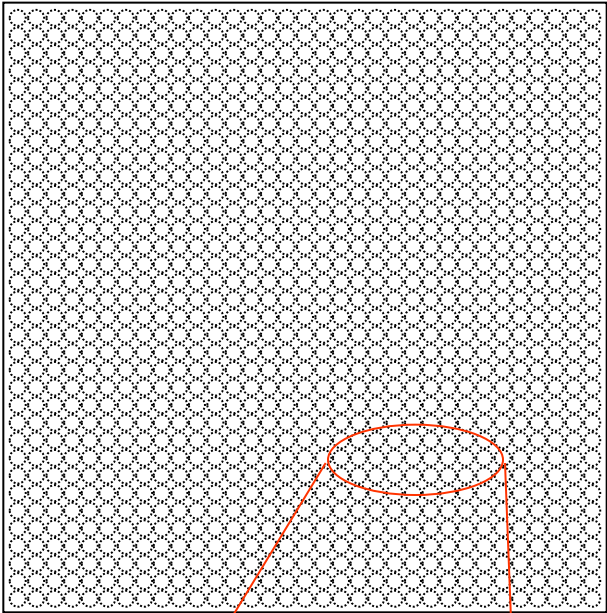
normální tkáň

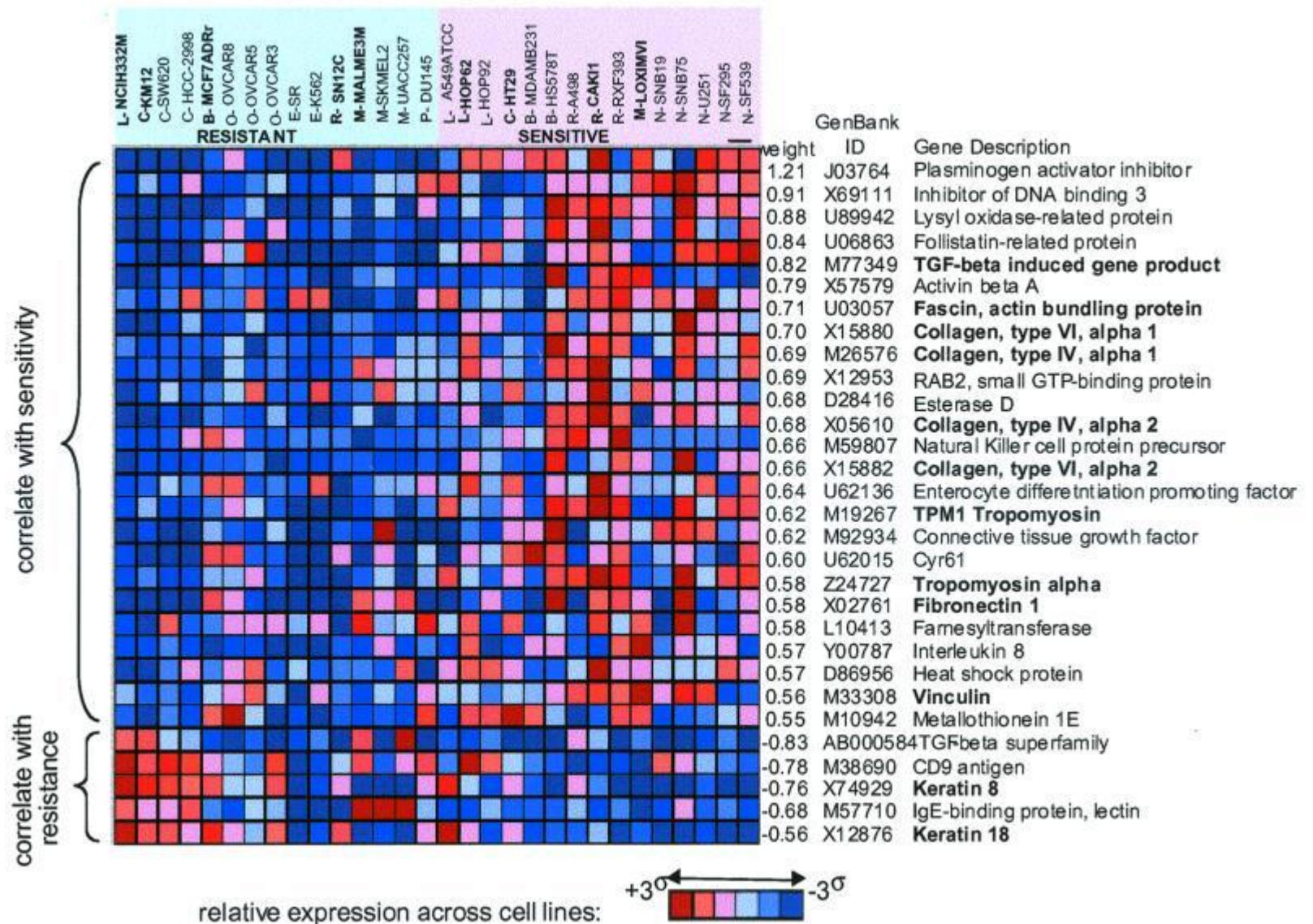


- geny neexprimované ani v nádoru ani v normální tkáni
- geny exprimované stejně v nádoru a v normální tkáni
- geny, které jsou v nádoru exprimovány silněji než v normální tkáni
- geny, které jsou v nádoru exprimovány slaběji než v normální tkáni

DNA čipové technologie: expresní profilování

mRNA → cDNA
nádor





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