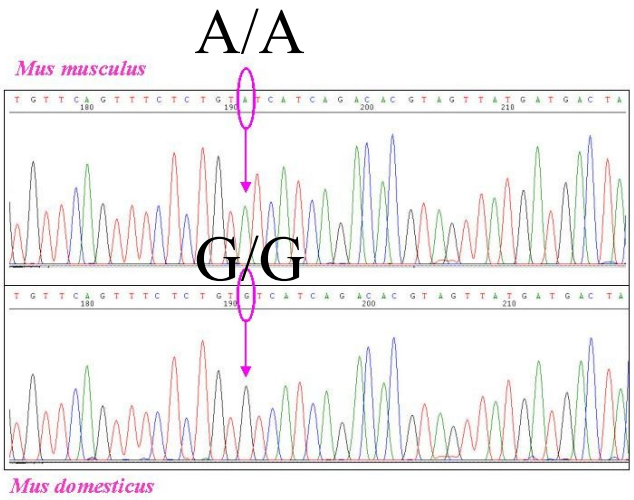
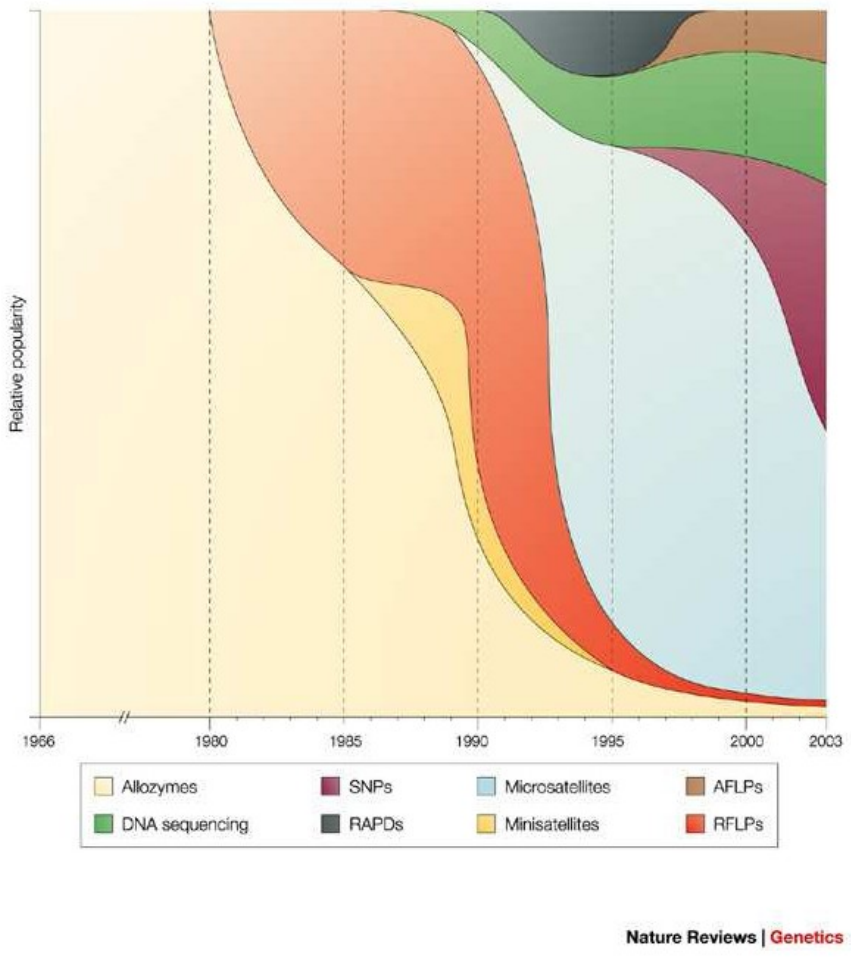
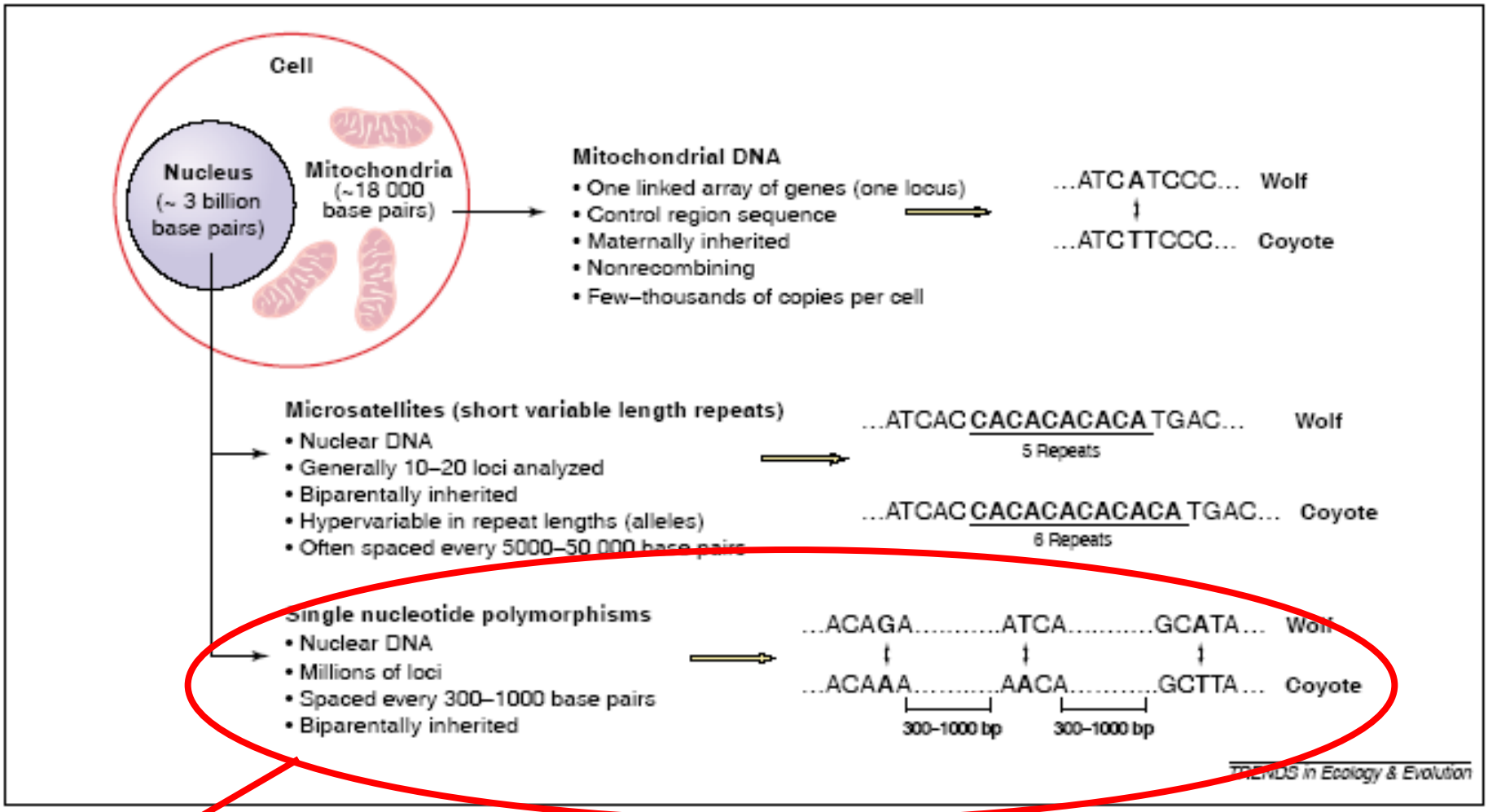
# Single nucleotide polymorphisms (SNPs)



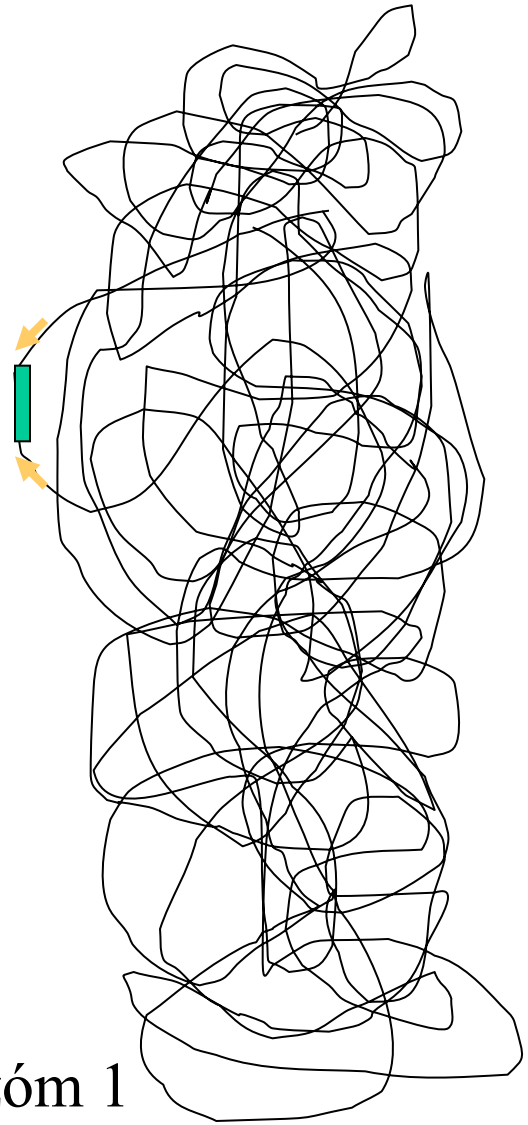
# Single nucleotide polymorphisms (SNPs)




SNPs : nuclear genome (consensus)

# SNPs = single-locus genetic markers

- SNPs (single nucleotide polymorphisms) – sekvenční polymorfismus
- kodominantní – je možné odlišit heterozygota (např. A/T) od homozygota (např. A/A)



| | |
|---|---|
| CA**A**GTA<br>TG**G**ACG | |
| CA**T**GTA<br>TG**C**ACG | |

**A/T**

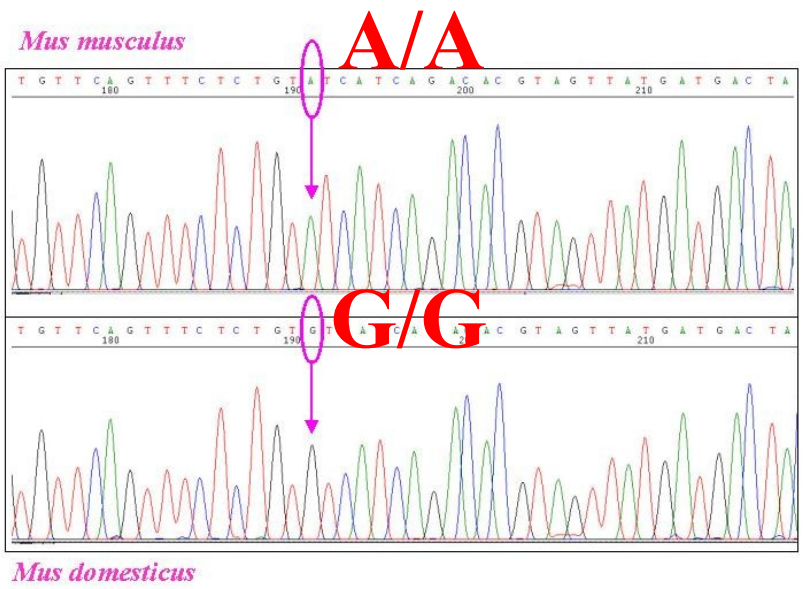
| | |
|---|---|
| CA**A**GTA<br>TG**G**ACG | |
| CA**A**GTA<br>TG**G**ACG | |

**A/A**

Př.: chromozóm 1

# Příklad informativního SNP znaku

- fixovaný polymorfismus (homozygoti) = **diagnostické SNPs** - využití např. při studiu hybridizací (hybridi = heterozygoti)




**Značení heterozygotů**

N = A, C, G, T
V = G, A, C
D = G, A, T
H = A, T, C
B = G, T, C
R = A, G
Y = C, T
M = A, C
K = G, T
S = G, C
W = A, T

**transice**

**A ↔ G**

Synonymní vs. nesynonymní substituce

transition: Pu→Pu or Py→Py

transversion: Pu→Py or Py→Pu

# Využití SNPs znaků

- obdobné jako u mikrosatelitů
- identifikace druhu (nebo genetické skupiny) - studium hybridizace (+ introgrese částí genomu)
- fylogeografie
- populační genetika (genetická variabilita a struktura, tok genů, identifikace jedinců a vztahů mezi nimi, populační velikost a její změny atd.)
- mutace ve funkčních genech – i záměna jedné aminokyseliny může mít fatální dopad
- genome-wide genotyping – asociace s fenotypem

# Výhody

- početné a rozšířené v genomu (v kódujících i nekódujících oblastech) – milióny lokusů
- 1 SNP cca každých 300-1000 bp (v rámci druhu)
- Mendelovská dědičnost (vs. mtDNA)
- evoluce je dobře popsatelná jednoduchým mutačním modelem (vs. microsatellites)
- jsou analyzovány kratší fragmenty DNA – neinvazivní genetika

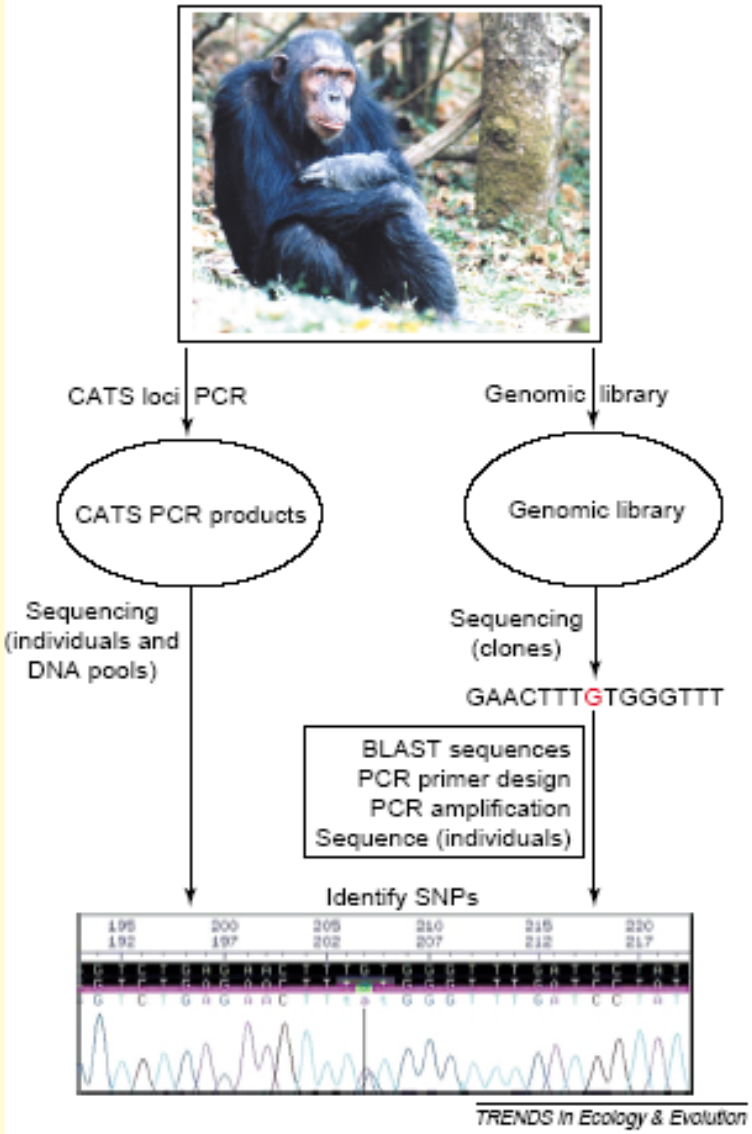
# Nevýhody

- „ascertainment bias“ – výběr informativních znaků se provádí na základě jen malého počtu jedinců a nemusí být reprezentativní
- nízká variabilita na lokus (většinou jen 2 alely)
- pro populační genetiku je vyžadován větší počet lokusů (4-10 krát více než u mikrosatelitů)

# Metody analýzy

1. Nalezení lokusů („ascertainment“)
2. Genotypizace

# 1. Nalezení SNPs

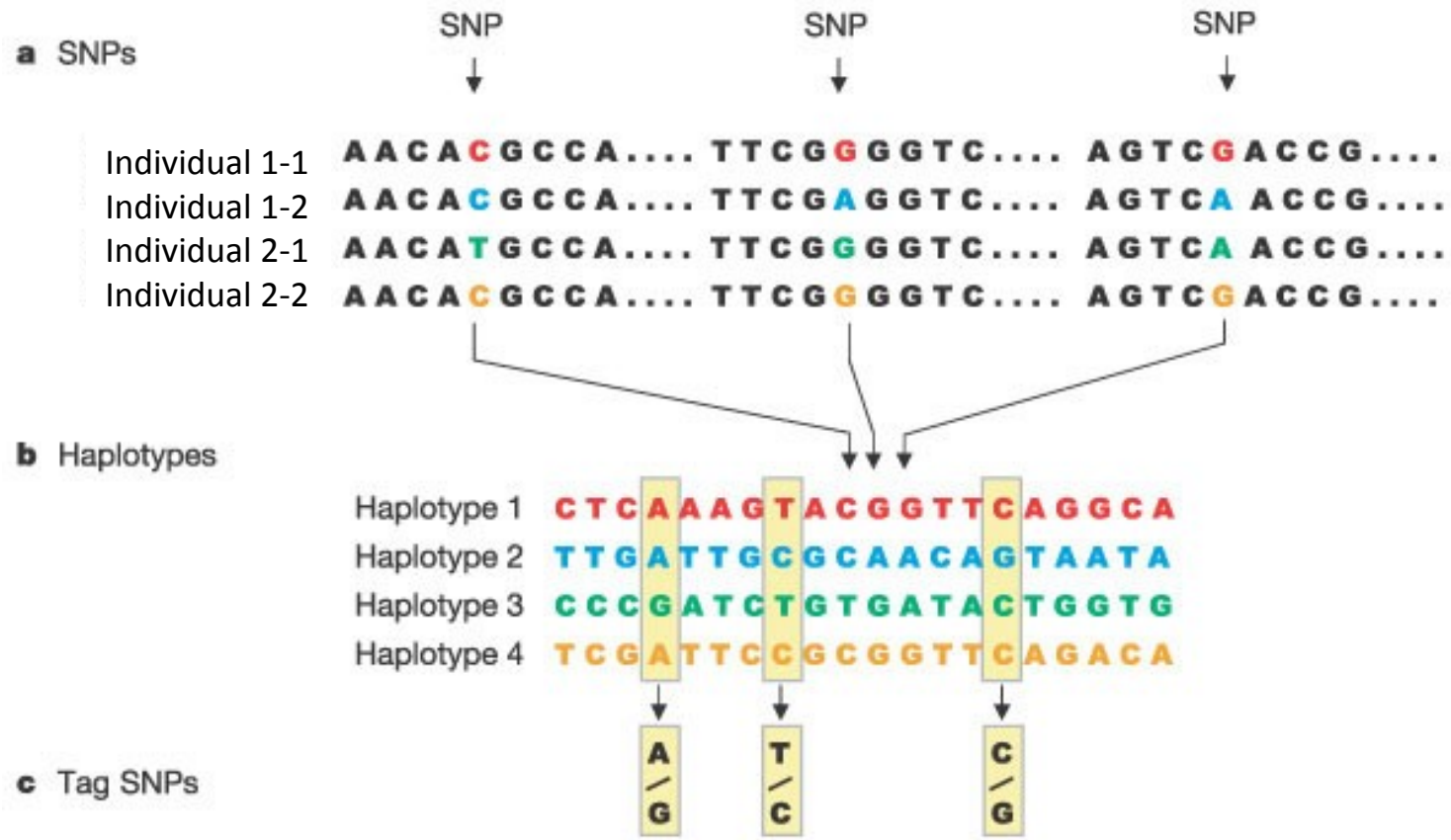



(1) CATS loci = comparative anchor tagged site loci (= cross amplification)

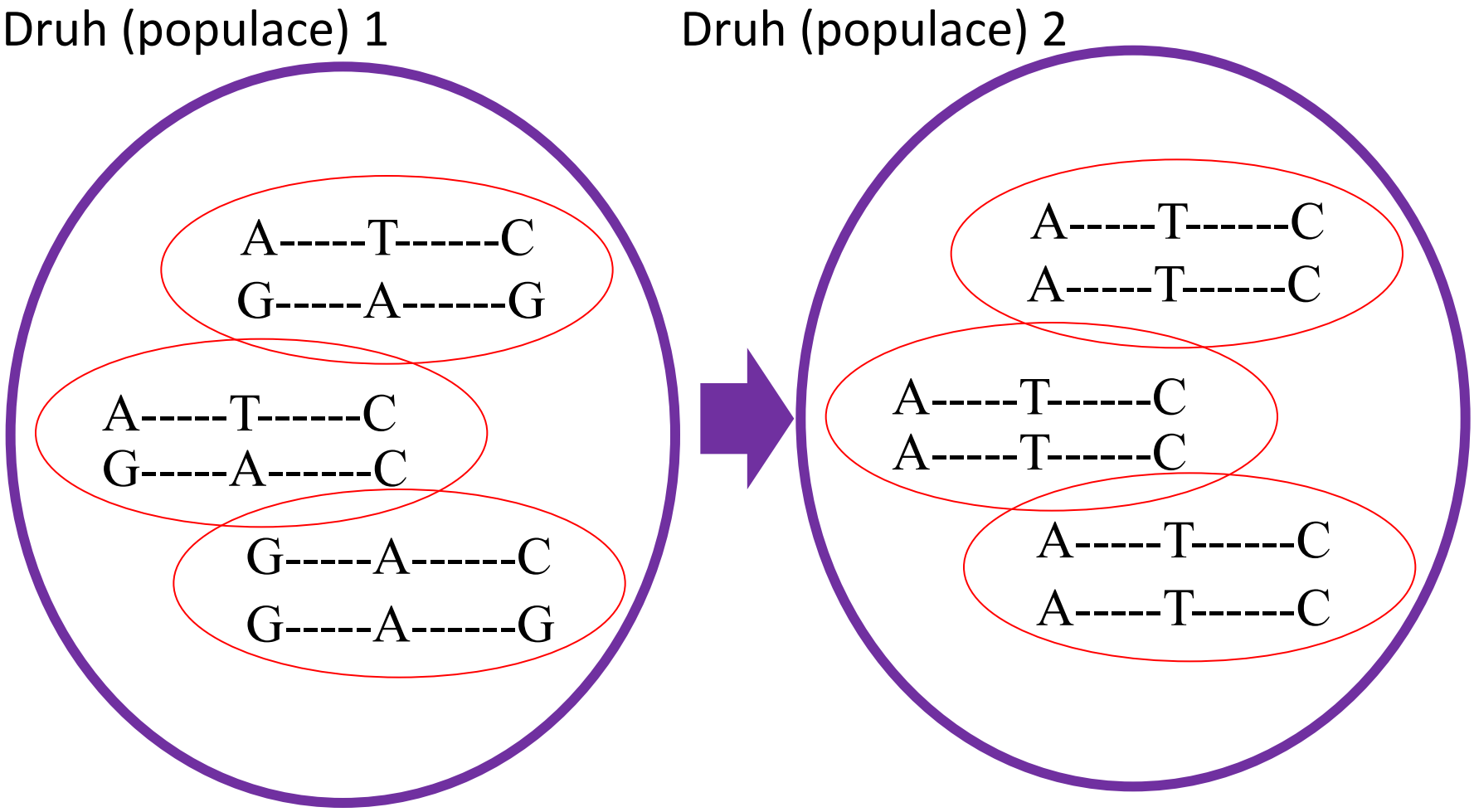
(2) Genomic library = genome restriction + cloning (náhodný výběr klonů – 1 SNP každých 300-1000 bp)

**V současné době: Next-generation sequencing** – sekvenování genomu více jedinců a hledání polymorfismů, např. tzv. RAD sequencing (viz další přednášky)

# Analýza NGS dat:
# Identifikace různých genotypů u různých jedinců (= homologních chromozómů, tj. variabilita alel)



# Ascertainment bias



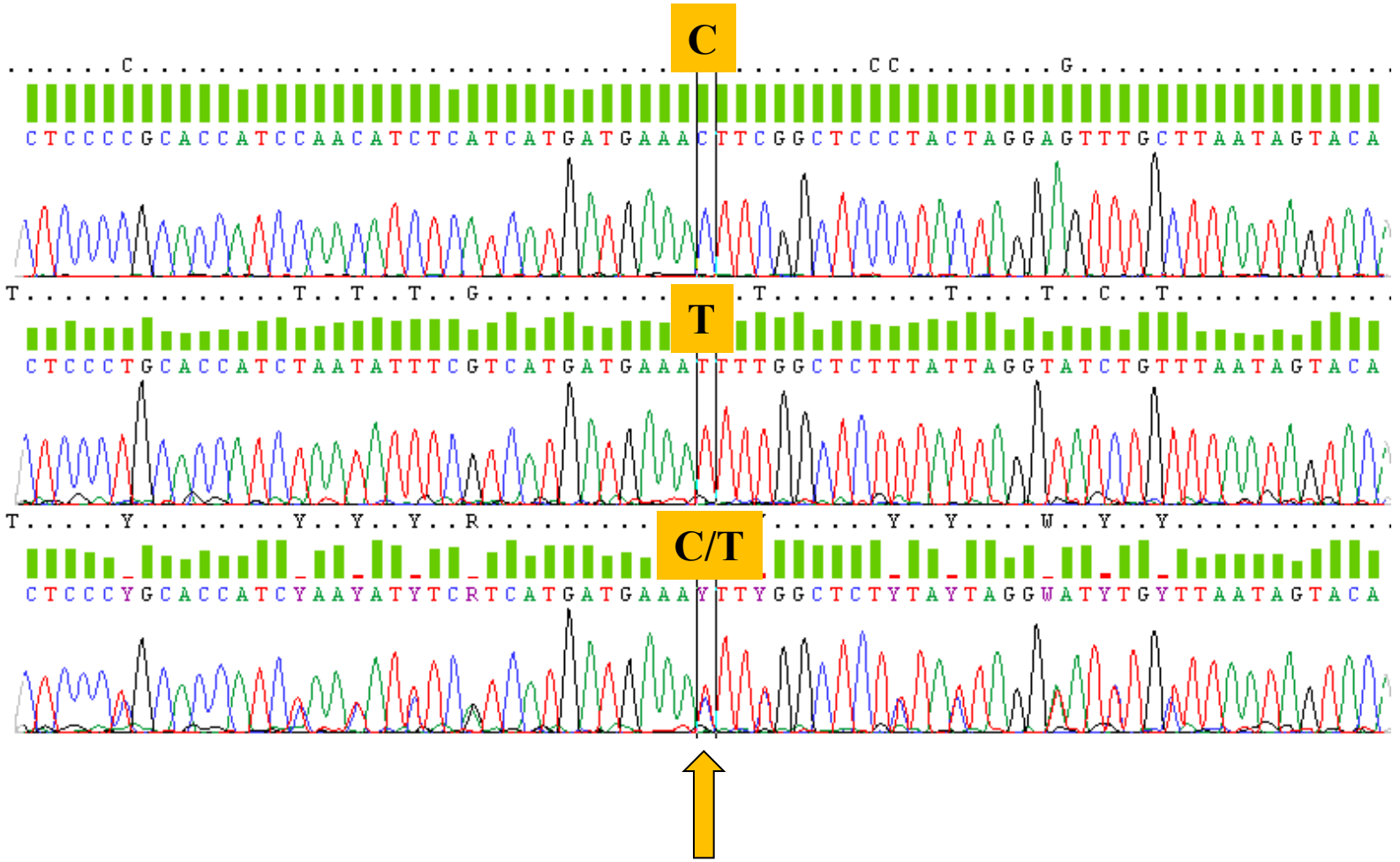

Analýza 3 jedinců u druhu (populace) 1
Tři polymorfní (informativní) SNPs

Polymorfismus daných SNPs je druhově (populačně) specifický

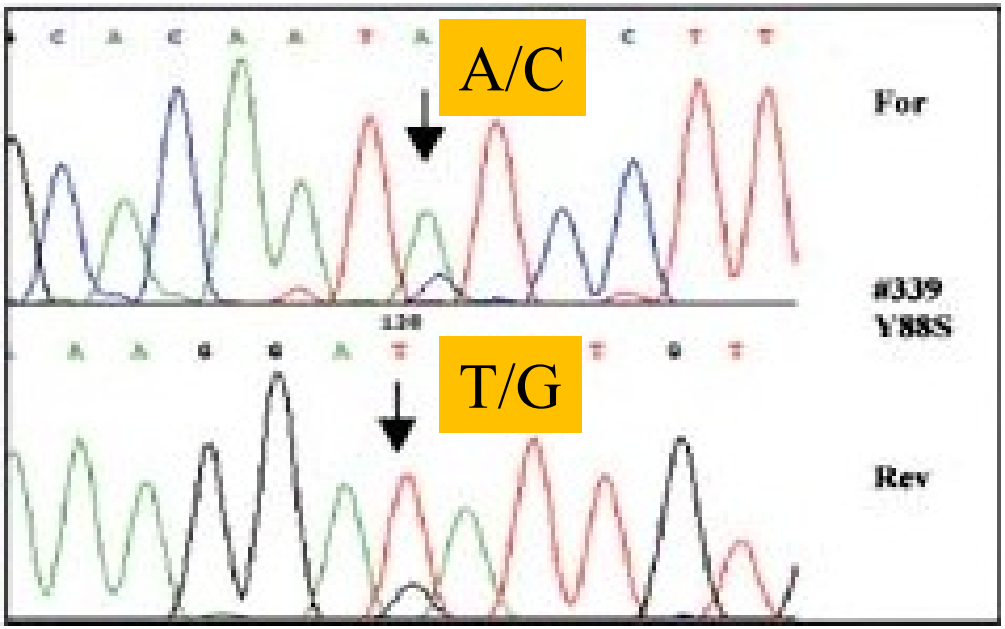
# 2. SNPs genotyping

= zjištění genotypu daného jedince

# SNPs genotyping – sekvenování?
# Je drahé a nejasné u heterozygotů

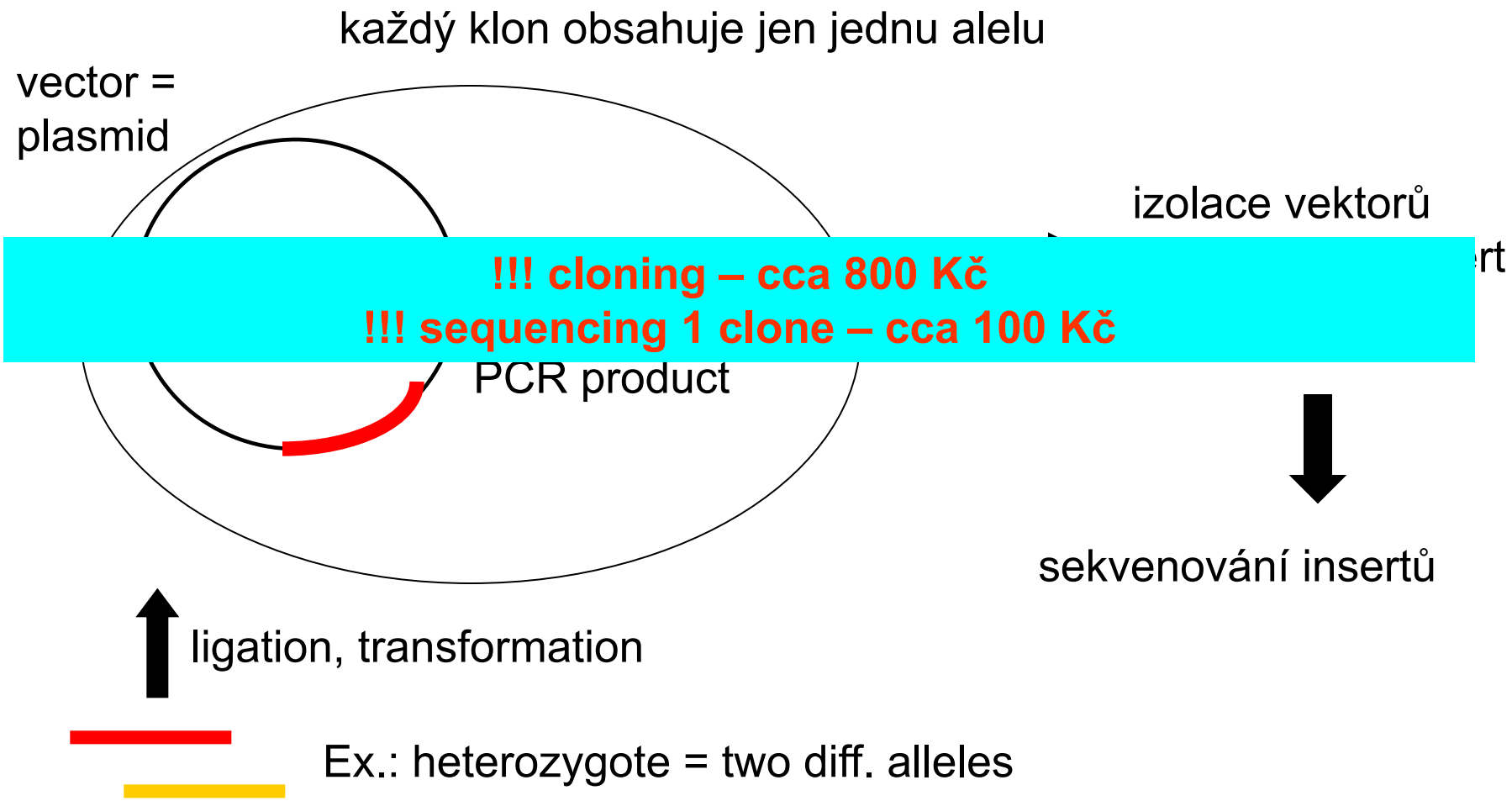


# Heterozygotes?




**Sekvenování z obou stran – are you really sure?**

# SNPs genotyping – klonování a následné sekvenování? - rozdělení dvou alel (či více u duplikovaných genů)

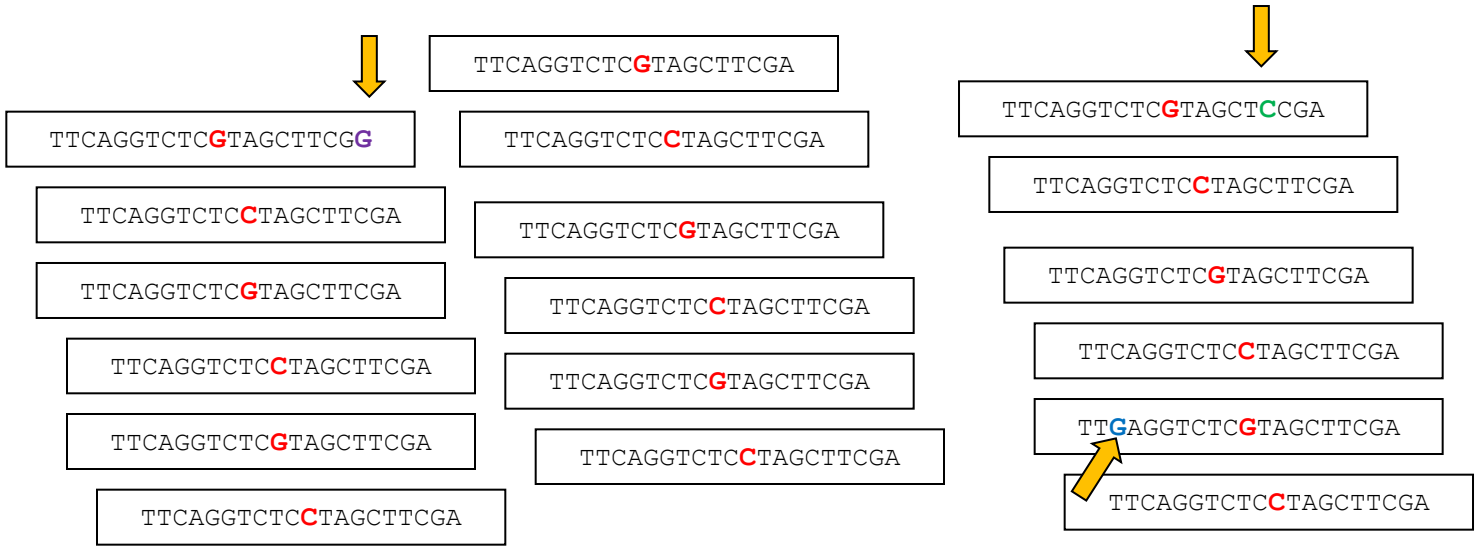


# PCR is making substitution errors that **are visualised by cloning (!)**

TTCAGGTCTCGTAGCTTCGA

TTCAGGTCTCCTAGCTTCGA

... před PCR = heterozygot G/C




PCR artefacts

(= šum při standardním sekvenování, ale velmi jasné při sekvenování klonů)

# SNPs genotyping

1. **Old standards (PCR-based)**

- RFLP:  PCR + štěpení + standardní elfo
- DGGE, TGGE, SSCP: PCR + nestandardní elfo
- původně diagnostika geneticky podmíněných chorob, např. cystická fibróza

2. **New methods (not based on standard PCR)**

- HRM: high-resolution melting (real-time PCR)
- real-time PCR se specifickými sondami (TaqMan, molecular beacon)
- ASPE: allele-specific primer extension
- SBE: single base extension
- SNP microarrays (GeneChip method)

# SNP genotyping - old standards

„Jelenovi pivo nelej“

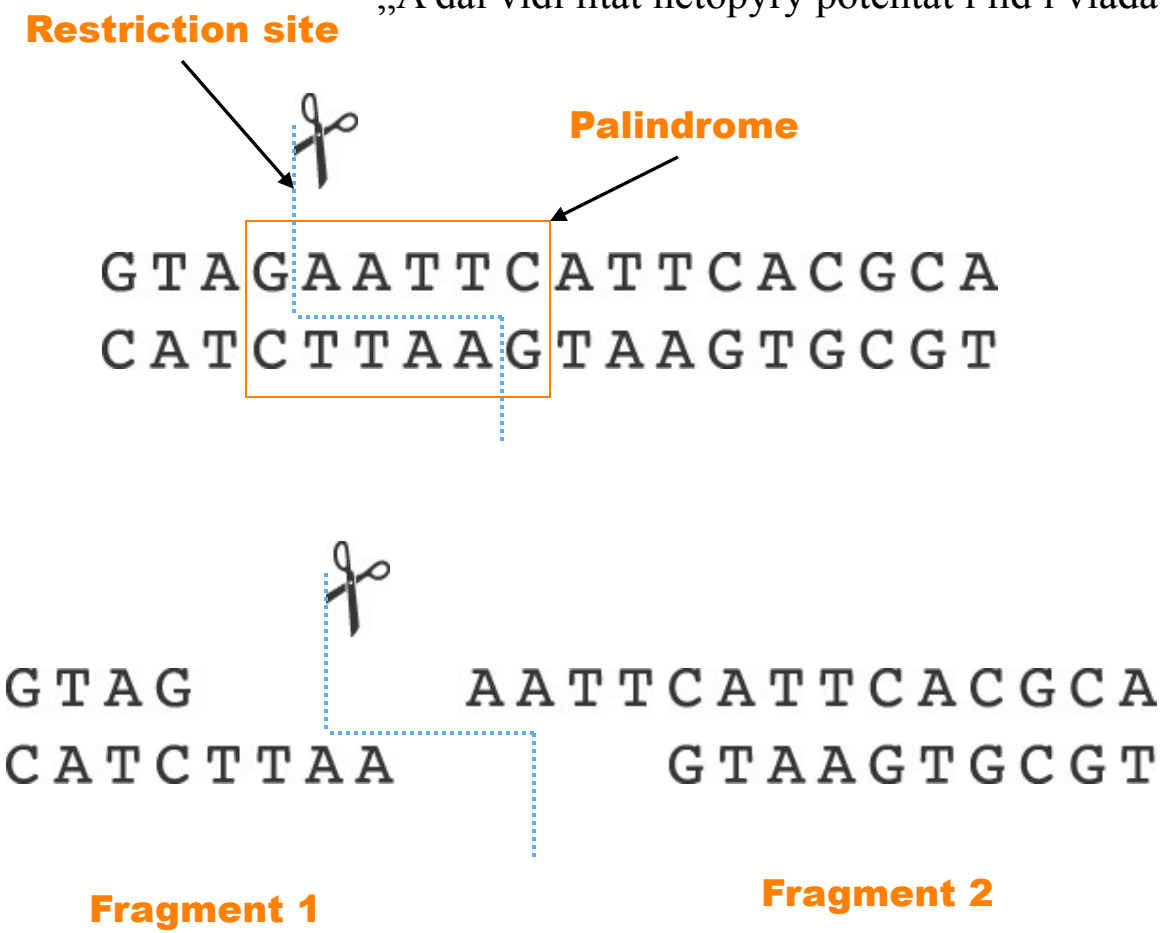
„A dál vidí lítat netopýry potentát i lid i vláda“

## PCR-RFLP

(restriction fragments length polymorphism)

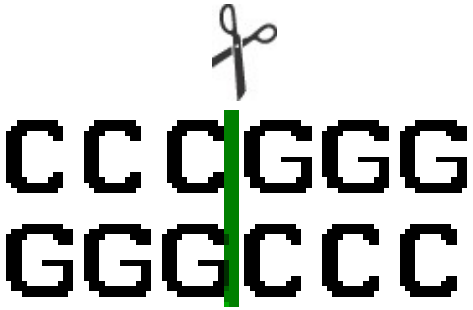
Enzyme Site Recognition

- Each enzyme digests (cuts) DNA at a specific sequence = **restriction site**
- Enzymes recognize 4- or 6- base pair, **palindromic sequences (eg GAATTC)**



# Běžné restrikční enzymy

„blunt ends“




**SmaI**
– blunt end

„sticky ends“


**EcoRI**
– *Eschericha coli*
– 5 prime overhang


**PstI**
– *Providencia stuartii*
– 3 prime overhang

# SNP genotyping - old standards

PCR-RFLP

**Allele A**

CCGATCA**A**TGCGGCAA
GGCTAGT**T**ACGCCGTT

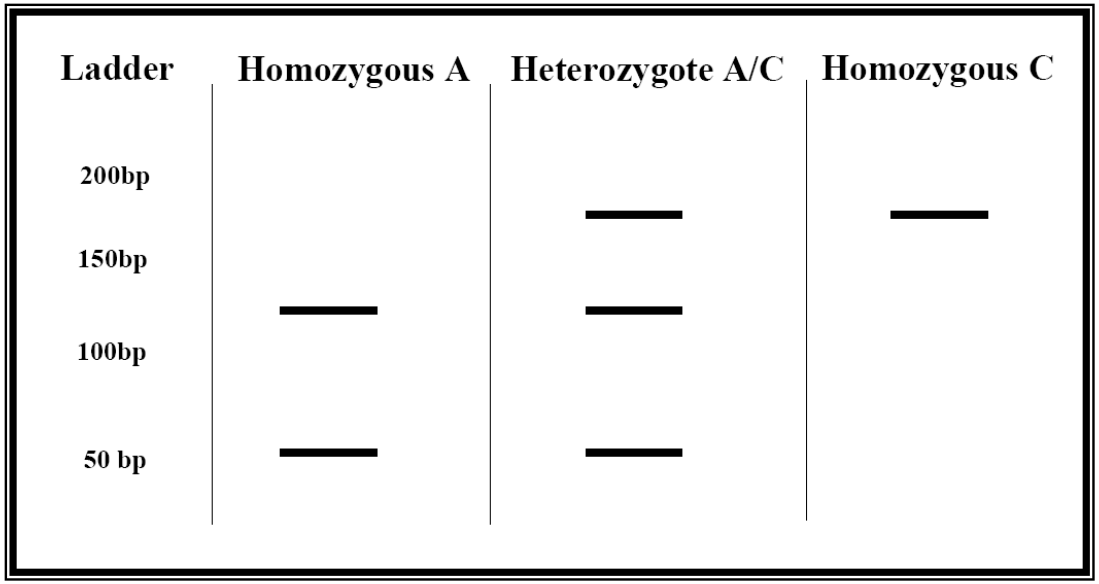
cutting by restriction endonuclease

**Allele C**

CCGATCA**C**TGCGGCAA
GGCTAGT**G**ACGCCGTT

no cut

- neumožní nalézt novou variantu daného SNP (odliší pouze 2 formy daného znaku: +/- )



# SNPs genotyping – old standards electrophoresis methods of mutation detection

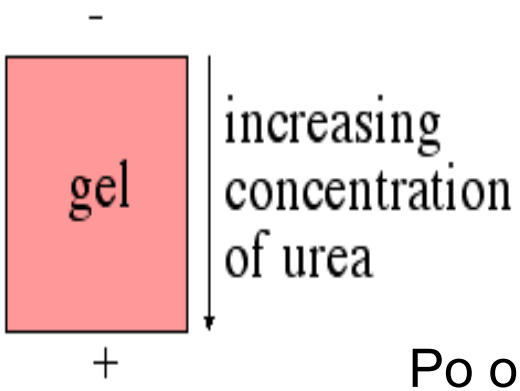
- Thermal gradient gel electrophoresis (**TGGE**)
- Denaturing gradient gel electrophoresis (**DGGE**)
- Single-strand conformation polymorphism (**SSCP**)

= special electrophoresis methods based on differences in mobility of different DNA sequences

# Denaturing gradient gel electrophoresis (DGGE) (TGGE – podobné, ale gradient teploty)

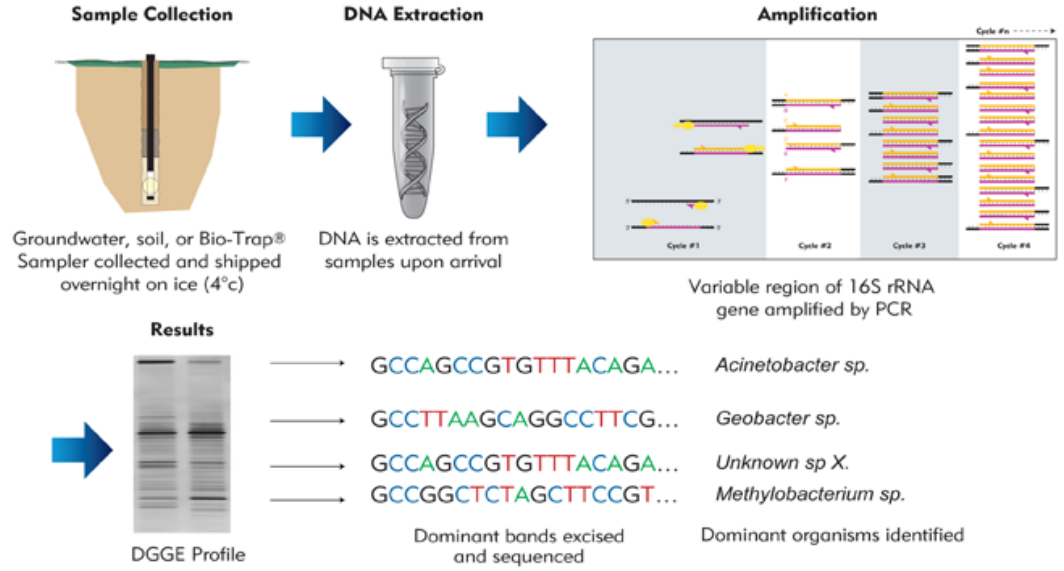
**Krátké PCR fragmenty (200-700 bp) jsou separovány v denaturačním gradientu (PAGE = polyakrylamidový gel)**

→ v určitém bodě začně DNA denaturovat („melting point“) – závisí na sekvenci, tj. každá sekvence denaturuje při jiné koncentraci močoviny



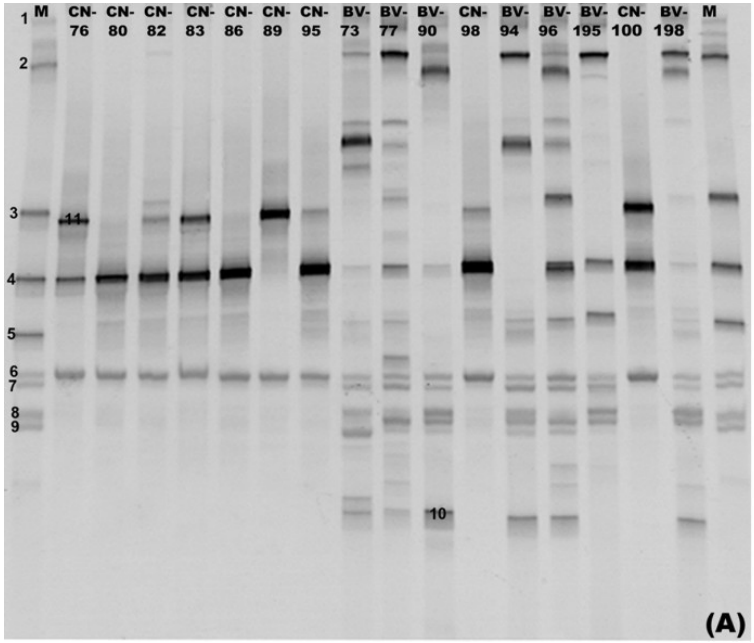

Denaturované fragmenty putují v gelu pomaleji

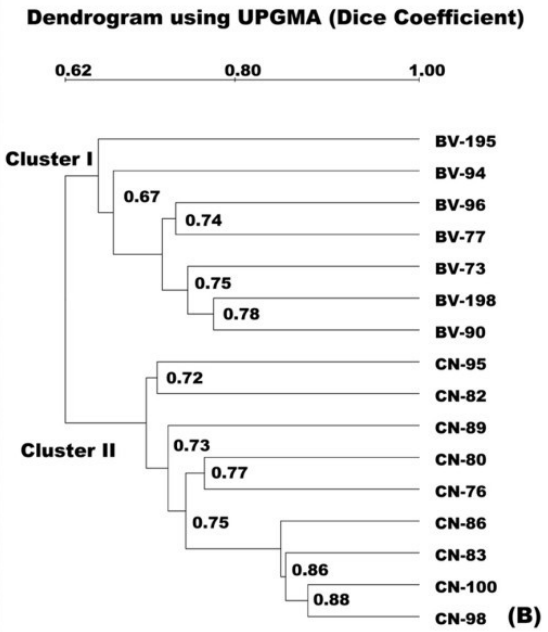
Po obarvení lze vidět rozdílné pozice PCR produktů v závislosti na jejich sekvenci




# DGGE v bakteriální metagenomice

Dnes rychle nahrazováno NGS



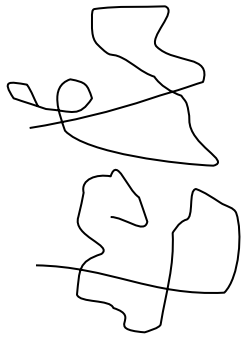



# Single strand conformation polymorphism (SSCP)

**Allele 1 - C**

...CGCTTCAGG ...

...GCGAAGTCC...



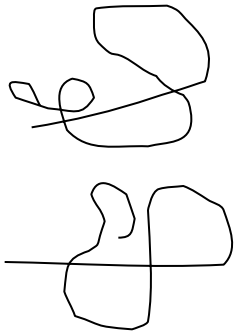
heating - denaturation

snap-cooling → partial renaturation

**Allele 2 - A**

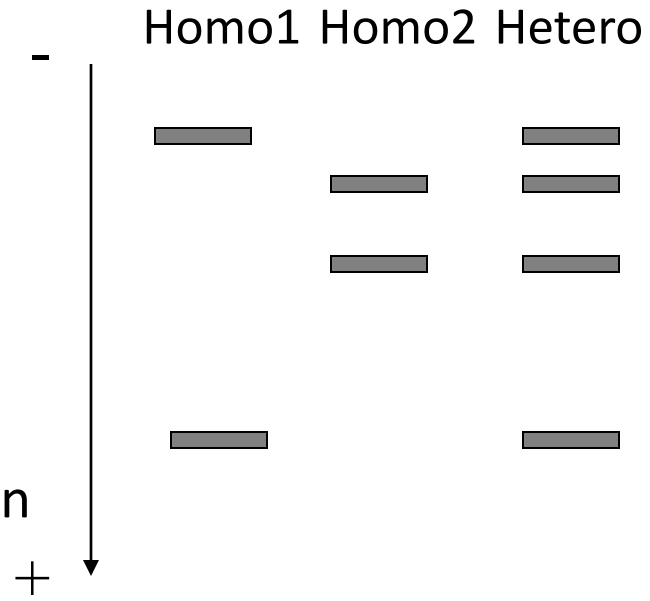
...CGCTTAAGG ...

...GCGAATTCC...



sequence-specific

ssDNA conformations




**!!! non-denaturing PAGE**

radioisotopes

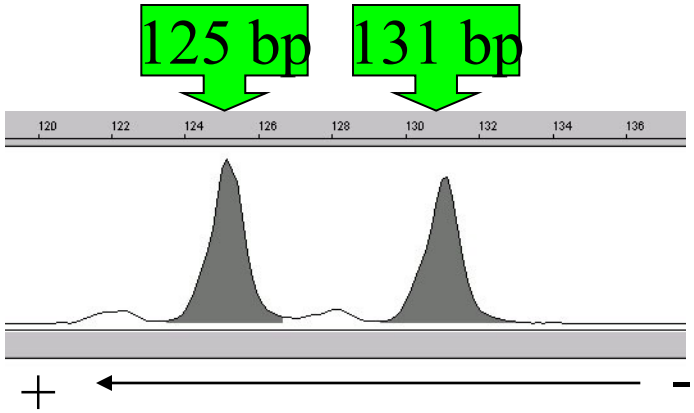
silver-staining

fluorescent dyes (SYBR gold)

# Použití automatických sekvenátorů

(**denaturing** polymer POP7 – ssDNA, e.g. microsatellites – one labelled primer)






Well controlled electrophoresis parameters, high sensitivity

# Použití automatických sekvenátorů

## Why not non-denaturing electrophoresis?
## e.g. CAP (conformation analysis polymer)

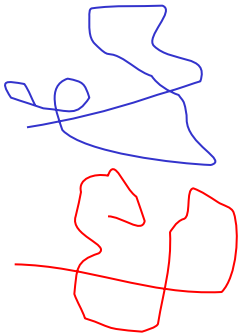
- well controlled electrophoresis

- two fluorescent labels

- high sensitivity

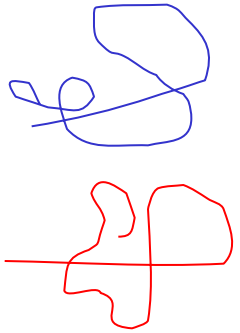
**Allele 1**

***FAM...*** **C**GCTT**C**AGG ...
... GCGAA**G**TC**C** ***...HEX***

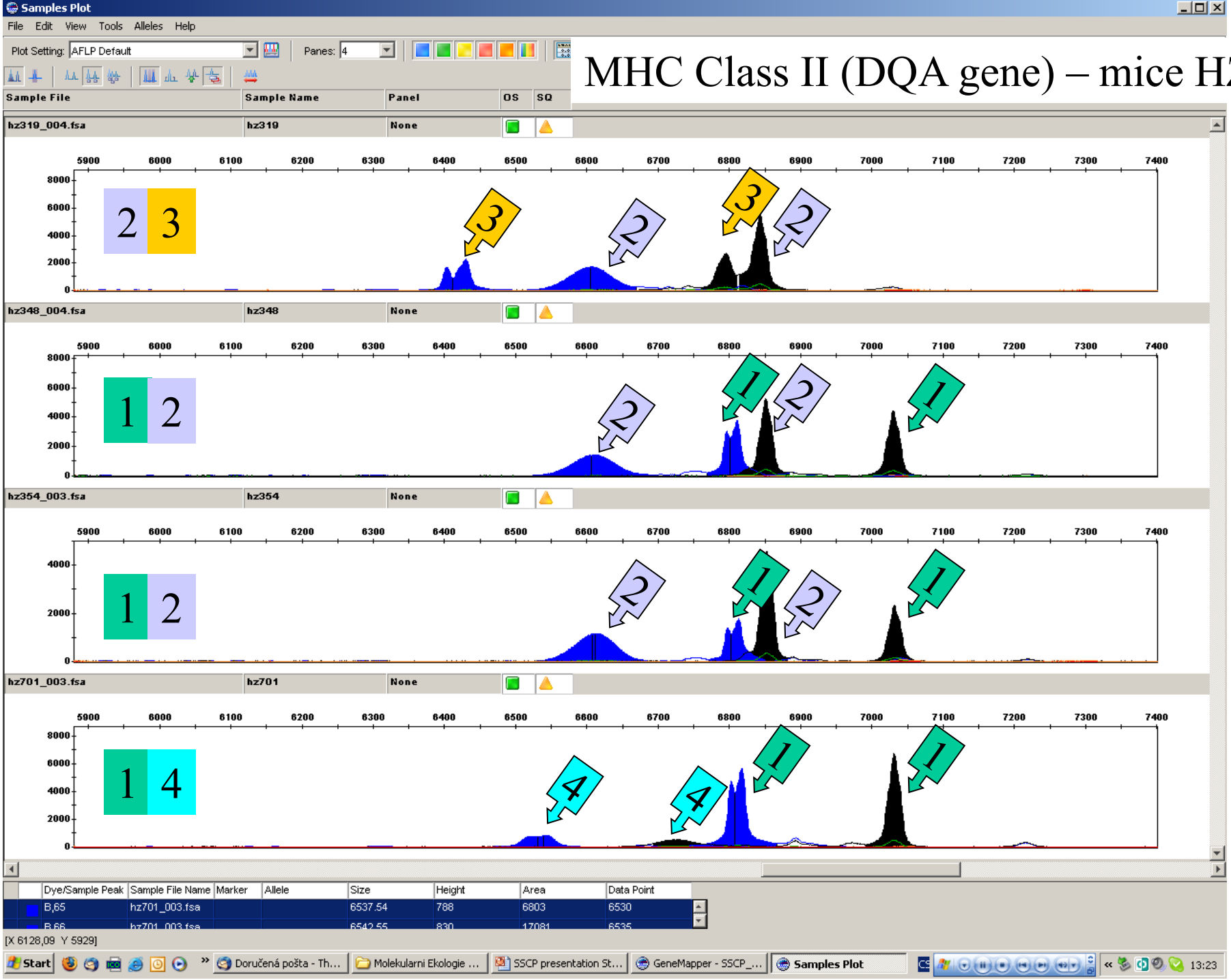


**Allele 2**

***FAM...*** **C**GCTT**A**AGG ...
... GCGAA**T**TC**C** ***...HEX***



# MHC Class II (DQA gene) – mice HZ



# Analýza elektroforetogramů

- např. GeneMapper (Applied Biosystems)
- specifický „Size+Conformation Standard“ pro každou teplotu (konformace závisí na teplotě)
- srovnání více vzorků
- umožňuje detekci krátkých odlišných sekvencí s více SNPs (užitečné např. pro genotypizaci MHC, tj. vysoce variabilních genů)
- opět rychle nahrazováno „next-generation sequencing“

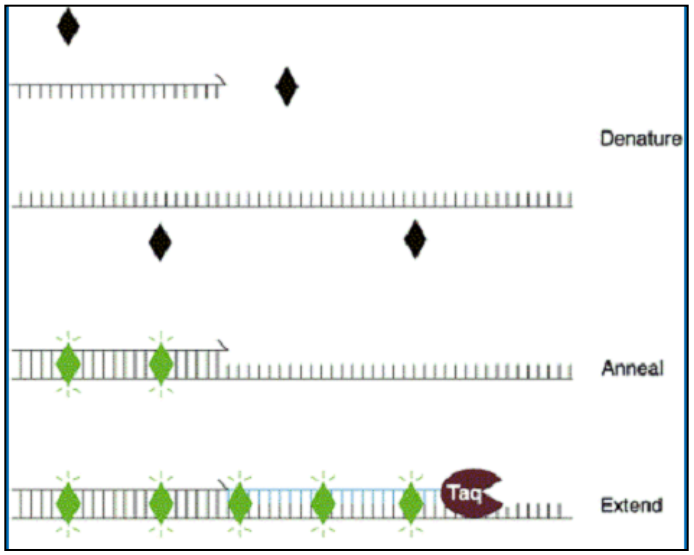
# SNP genotyping – new methods

= not based on standard PCR

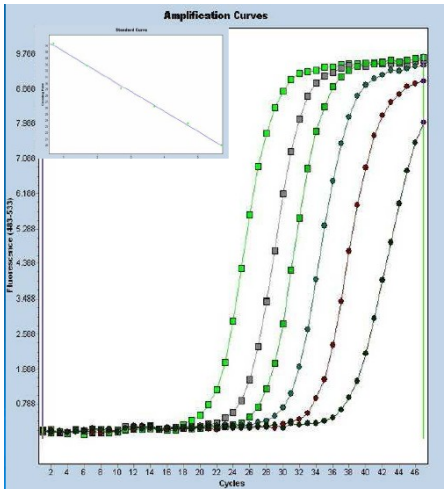
1. high-resolution melting temperature (HRMT)
2. real-time PCR se specifickými sondami (TaqMan, molecular beacon)
3. ASPE: allele-specific primer extension
4. SBE: single base extension
5. Alelově-specifická hybridizace

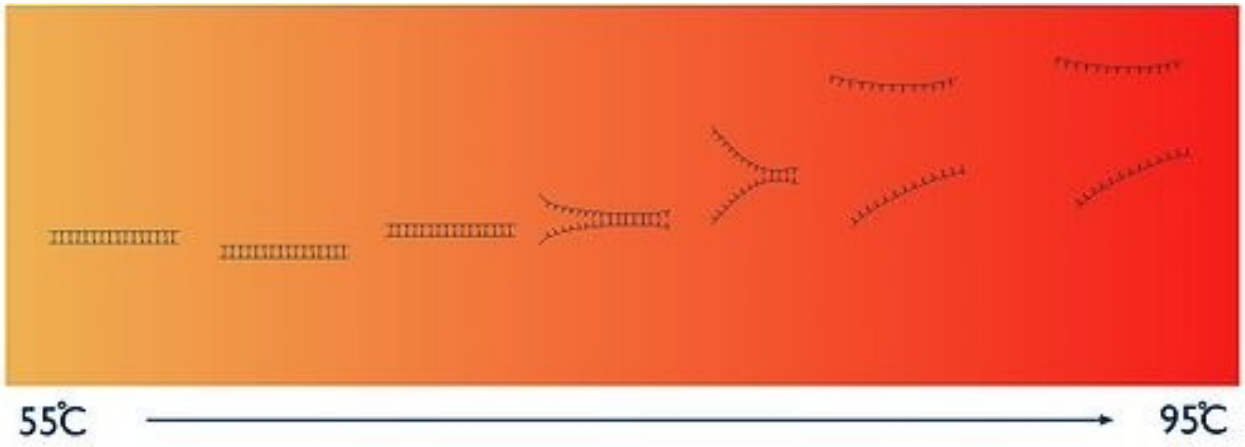
} mohou využívat tzv. microarrays („SNP chips“)

# 1. High-resolution melting temperature (HRMT)



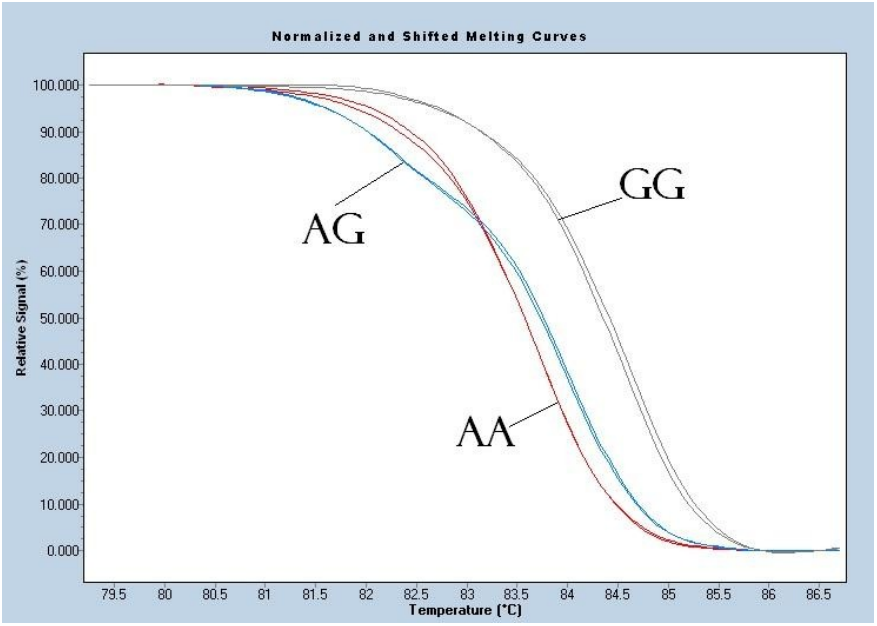

Step 1: real-time PCR = increase of fluorescence

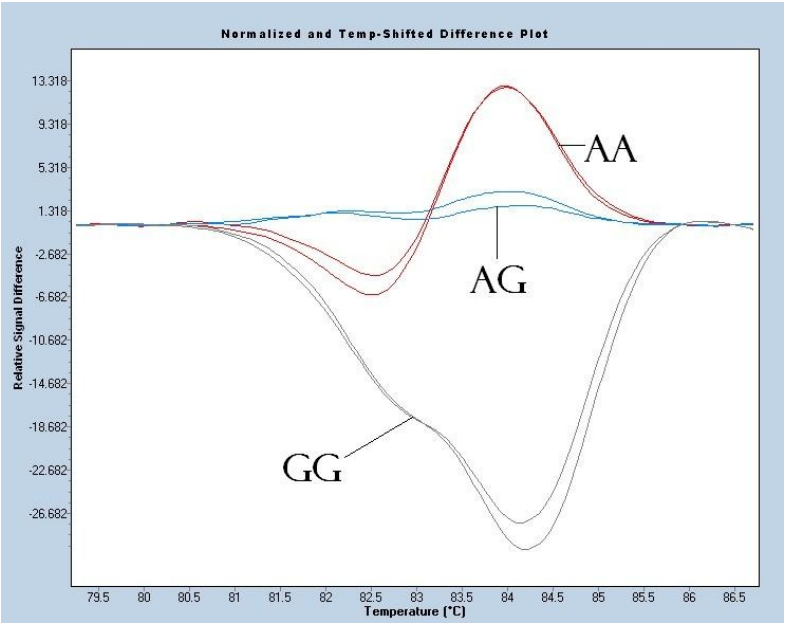



Step 2: measuring melting after PCR = decrease of fluorescence

# HRMT genotyping

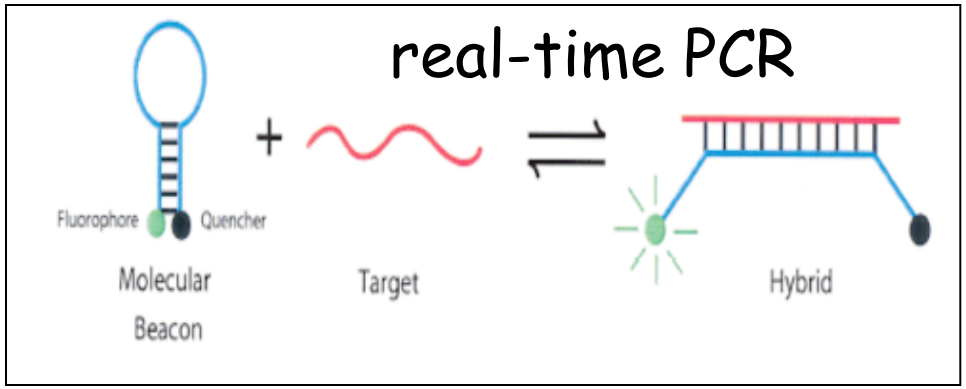


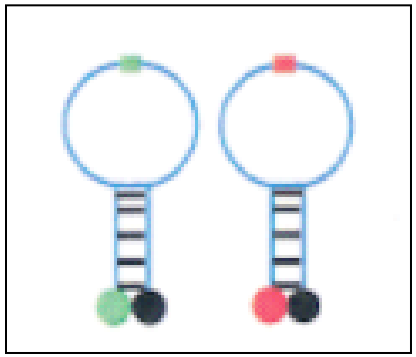

## Detekce heterozygotů

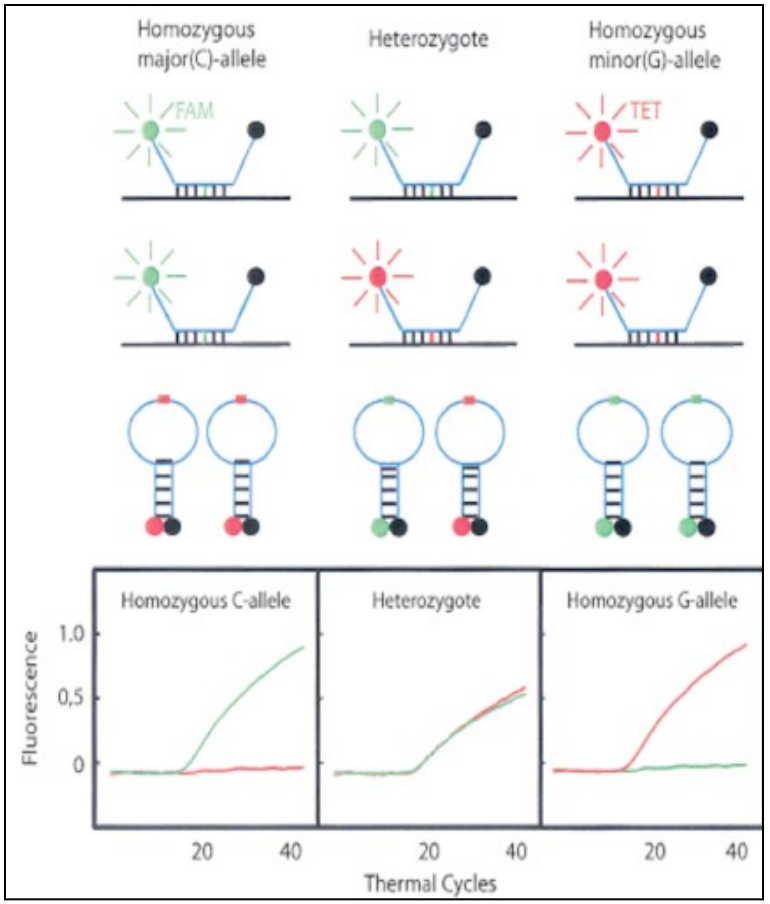
- velmi levná a jednoduchá metoda – v podstatě jen qPCR
- vhodná na genotypizace jednoduchých SNP u velkého množství vzorků

# 2. Real-time PCR se specifickou sondou



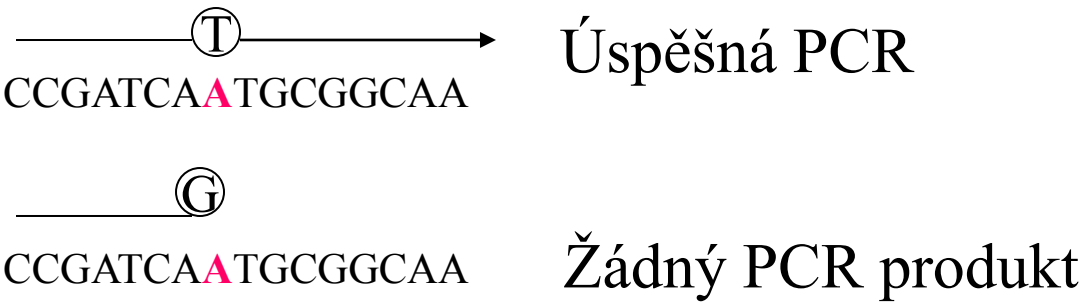

sondy specifické pro jednotlivé alely





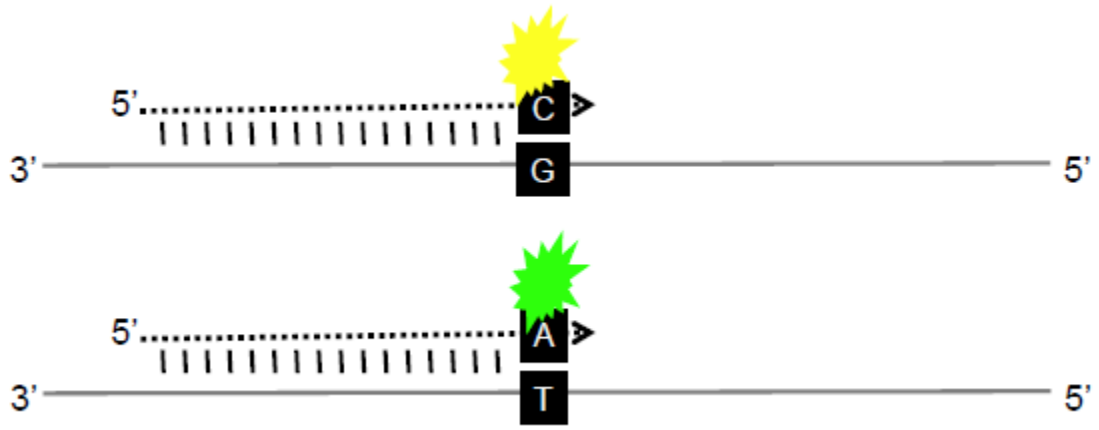

1) TaqMan sondy
2) Molecular Beacons („maják“)

# 3. ASPE: allele-specific primer extension



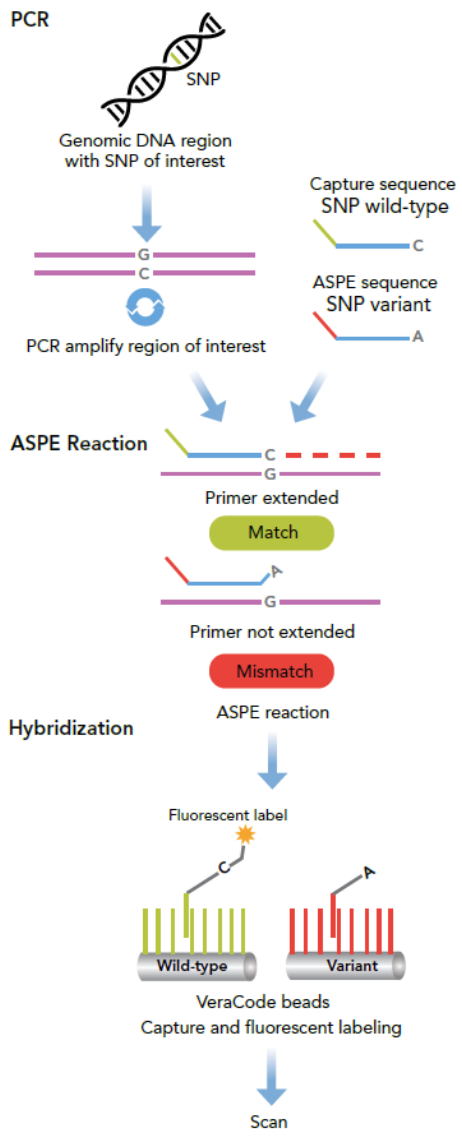

- dvě PCR (každá se specifickými primery k danému SNP)
- 3’ terminální nukleotid na primerech je komplementární k SNP nukleotidu
- alelově-specifická amplifikace je umožněna vysoce specifickou polymerázou

# ASPE: allele-specific primer extension (automatizovaná verze)




- existují zoptimalizované multiplexy pro modelové druhy (např. člověk 1536 SNPs)
- fluorescenční detekce (např. Illumina nebo LGC Genomics)

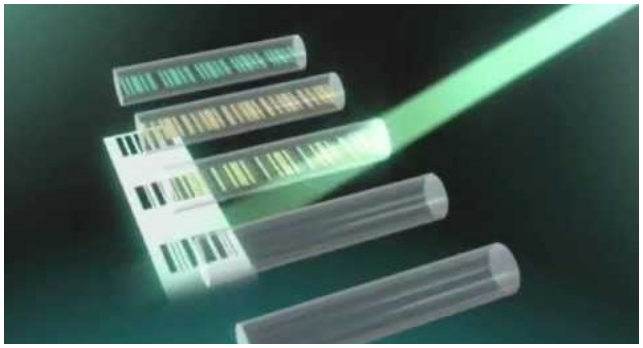
Example: Precise Genotyping Calls Made using ASPE on the VeraCode Platform




# Illumina – GoldenGate ASPE

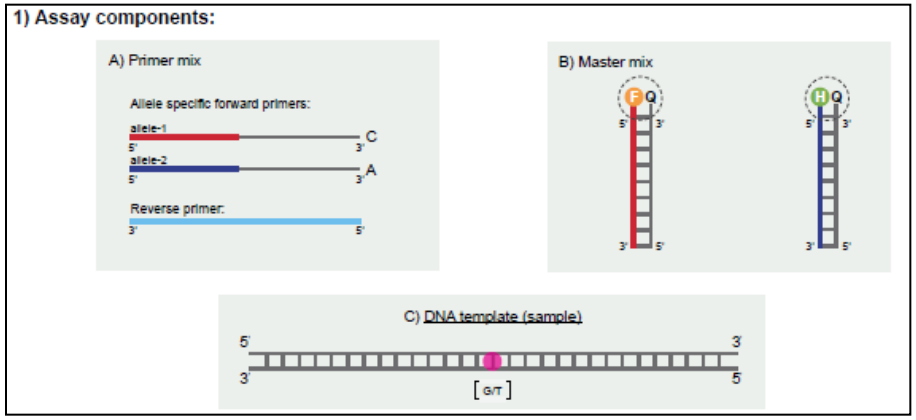
## (web-based VeraCode Assay Designer)

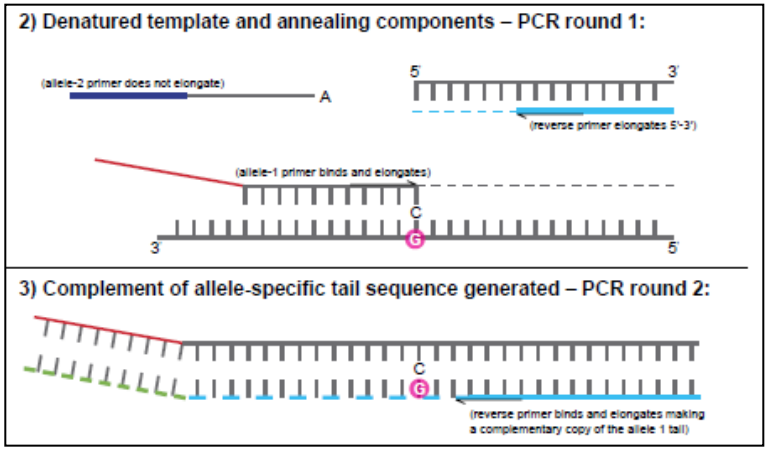
Each VeraCode Capture Bead contains a unique 23-mer oligonucleotide immobilized on its surface. Designing ASPE extension primers that include complementary sequences to these capture oligos allows exclusive targeting of specific beads. Primers that match the targeted sequence will extend preferentially. When a labeled target hybridizes with the complementary sequence on the assigned VeraCode microbead, the target is identified through the embedded holographic digital code.

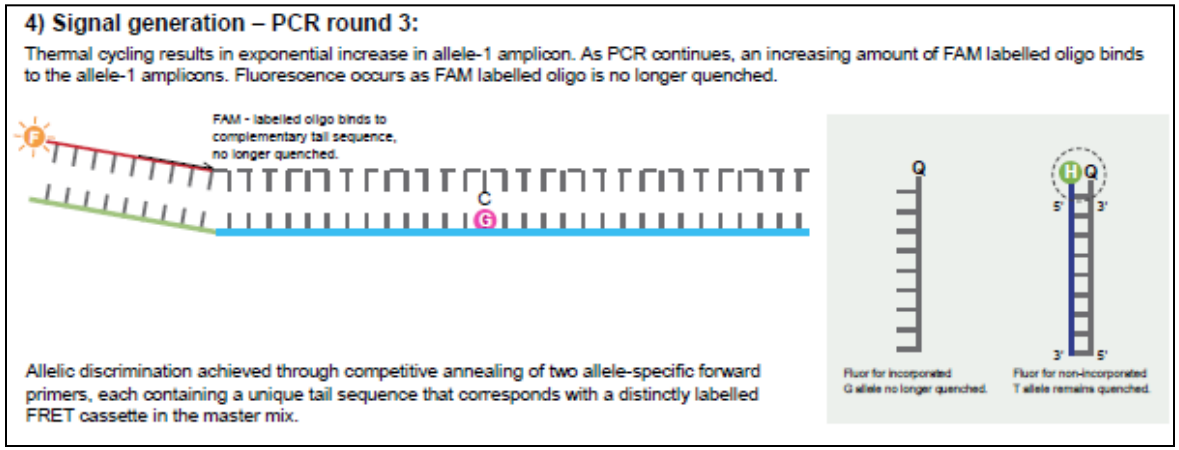


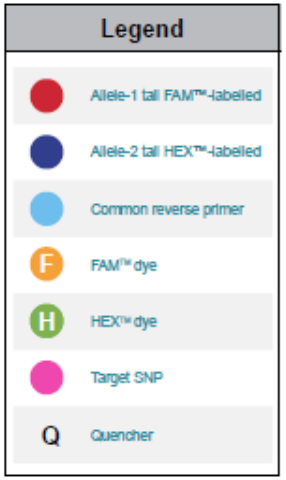
VeraCode Capture Beads
fluorescenční detekce

# Kompetitive Alelle Specific PCR




The KASP™ genotyping assay from **LGC Genomics**

LGC Genomics incorporating biosearch

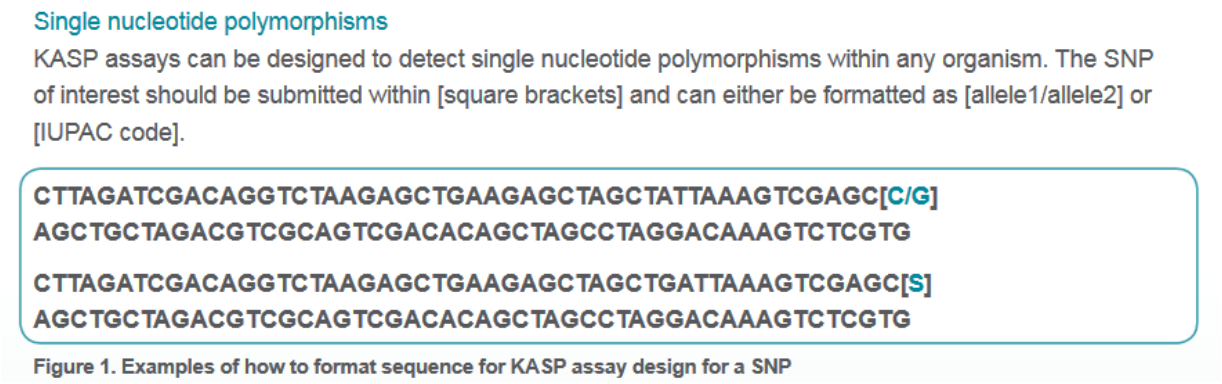
# Cena analýzy

The KASP™ genotyping assay from **LGC Genomics**

LGC Genomics incorporating KBioscience

**Small scale study of 15 SNPs genotyped over 96 samples where no Assay on Demand (an alternative type of assay from ABI) SNP exists**

| | LGC Genomics cost | ABI Taqman® Assay by Design |
|---|---|---|
| SNP assay design costs (validation) | **£1,620.00** | £6,750.00 |
| Genotyping cost | **£701.50** | £388.80 |
| Total | **£2,321.50** | £7,138.80 |



Single nucleotide polymorphisms

KASP assays can be designed to detect single nucleotide polymorphisms within any organism. The SNP of interest should be submitted within [square brackets] and can either be formatted as [allele1/allele2] or [IUPAC code].

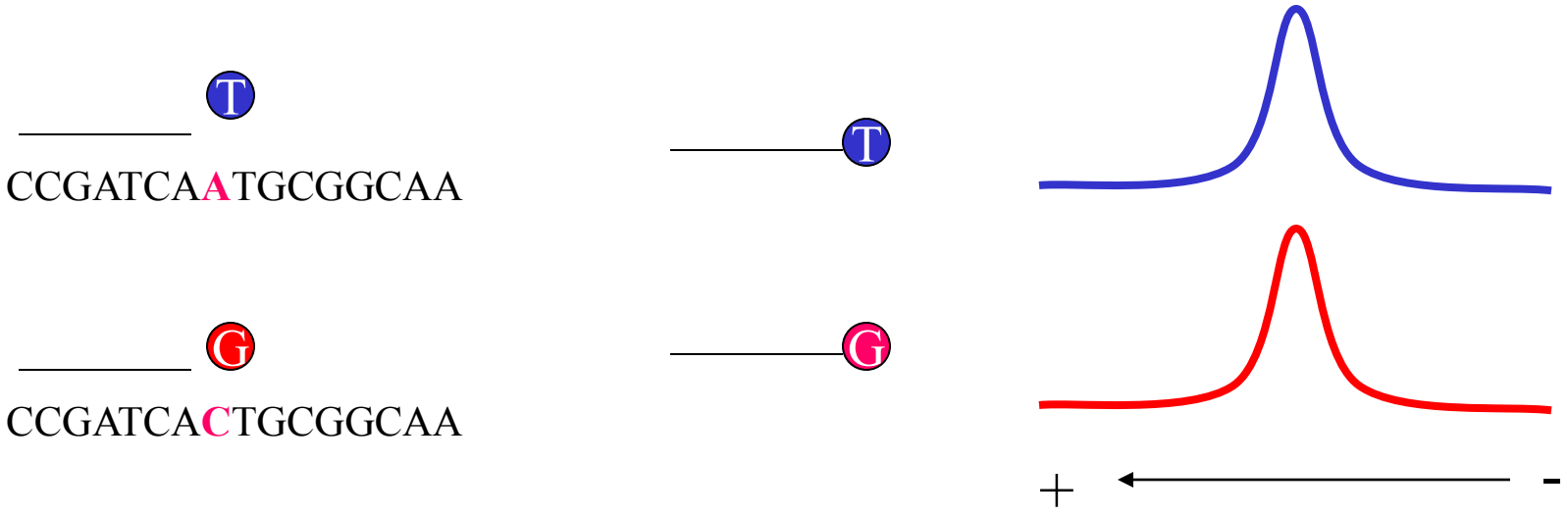
CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTATTAAAGTCGAGC[C/G]
AGCTGCTAGACGTCGCAGTCGACACAGCTAGCCTAGGACAAAGTCTCGTG

CTTAGATCGACAGGTCTAAGAGCTGAAGAGCTAGCTGATTAAAGTCGAGC[S]
AGCTGCTAGACGTCGCAGTCGACACAGCTAGCCTAGGACAAAGTCTCGTG

Figure 1. Examples of how to format sequence for KASP assay design for a SNP

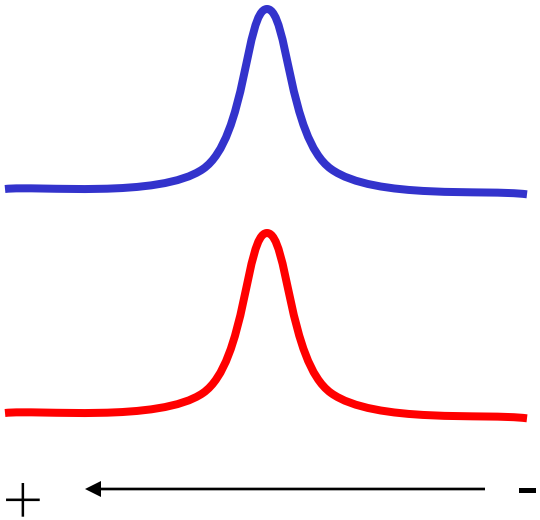
http://www.lgcgroup.com/services/genotyping/cal/

# 4. SBE: single base extension

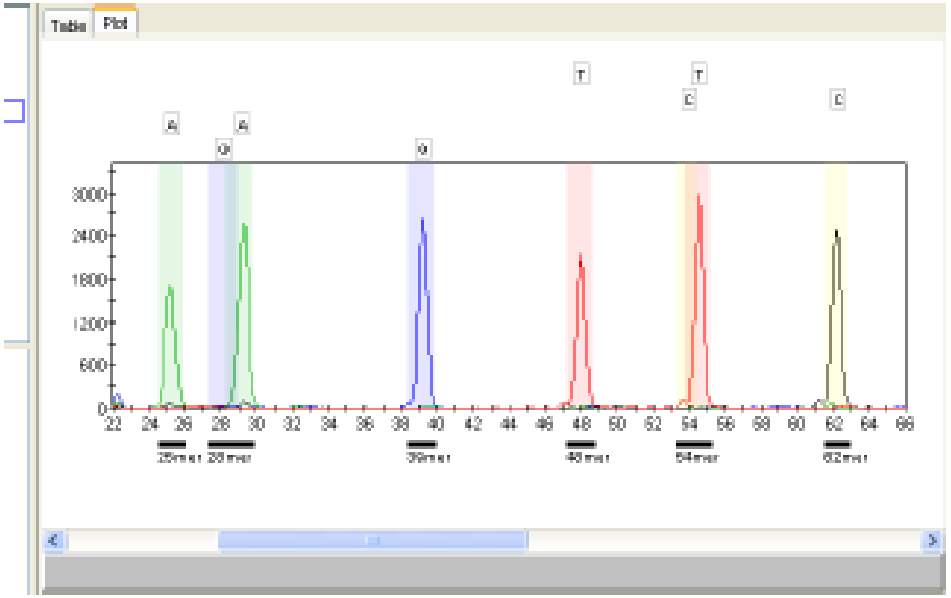



- pouze jeden dideoxynukleotid je přidán k primeru
- detekce různými metodami

# (A) Detekce SBE produktů kapilární elektroforézou




kapilární elektroforéza SNaPShot Multiplex Kit (Life Technologies)

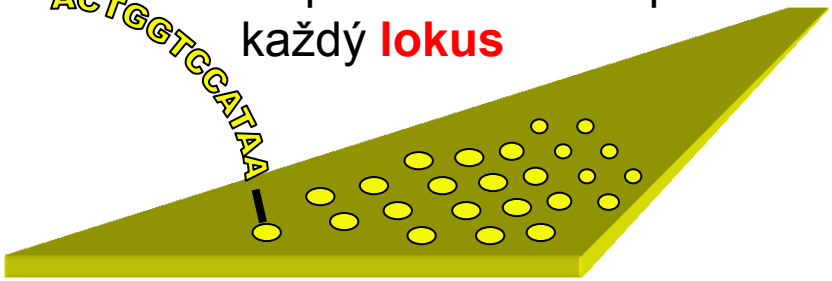



„multiplex version" – různě dlouhé primery, aby bylo možné odlišit různé lokusy

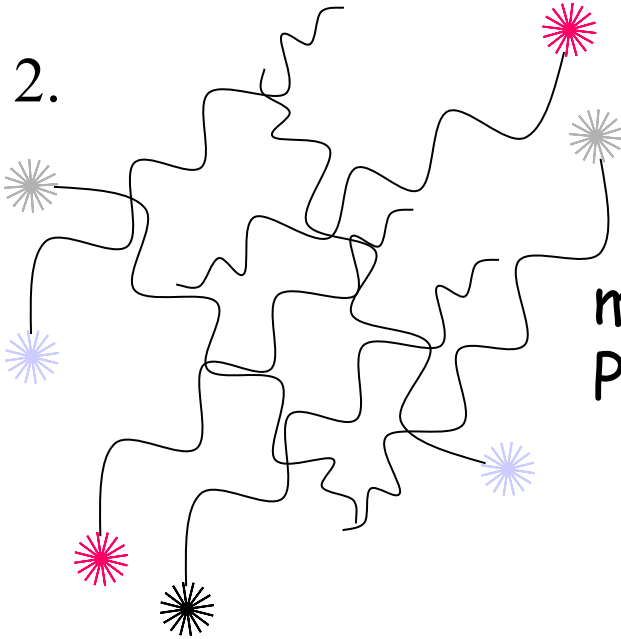
# (B) Detekce SBE produktů přes „microarray" (tj. hybridizace)

1. **tag** – specifický pro každý lokus


3. **tag-complementary probe – specifická sonda pro každý lokus**

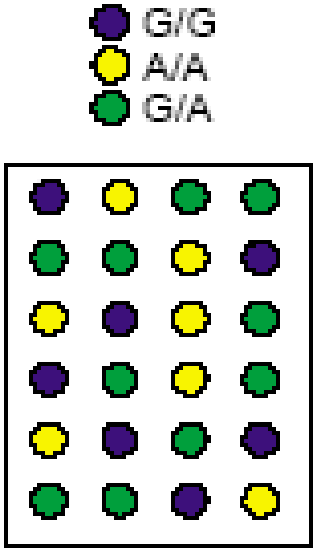



2.



multiplex PCR

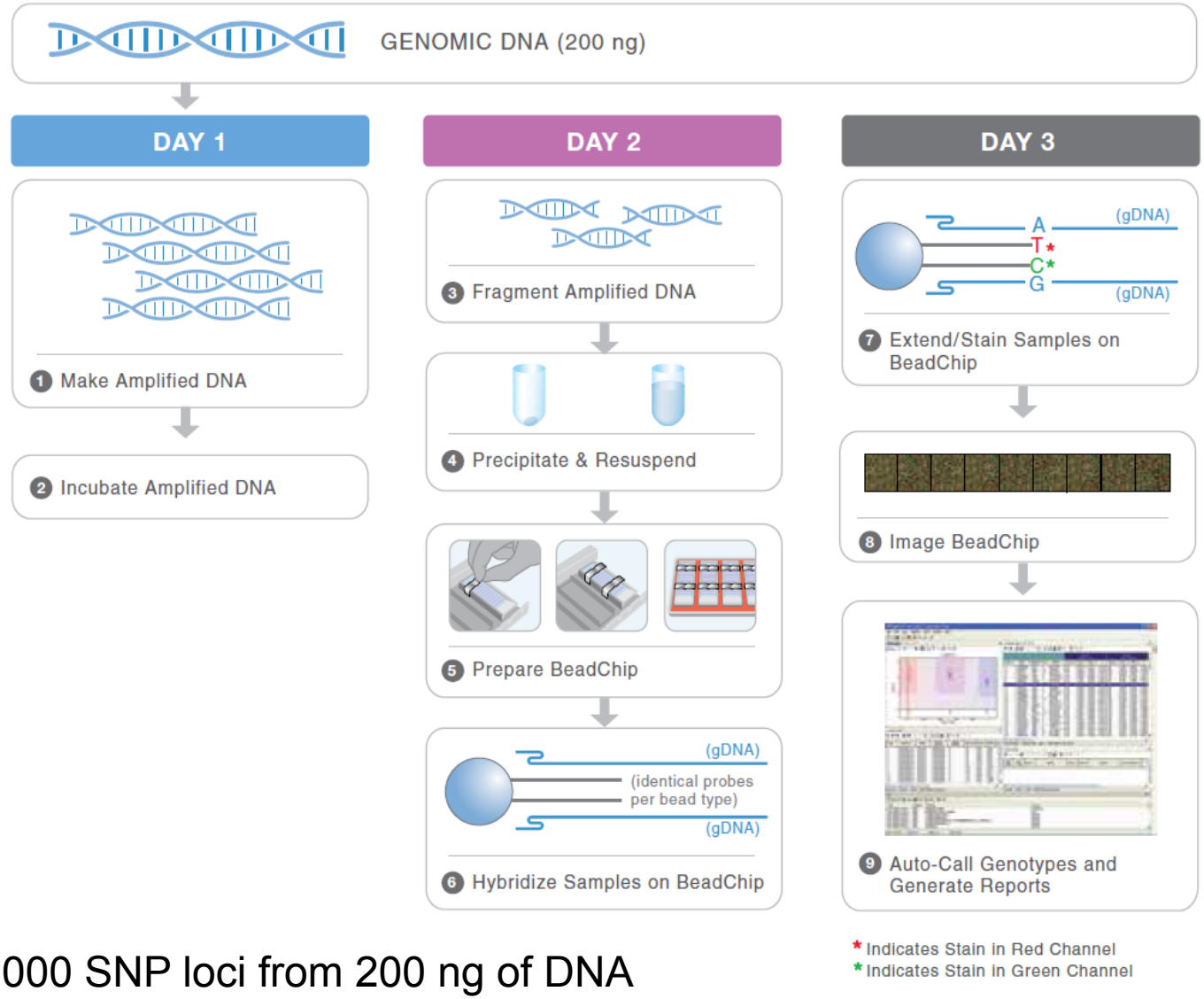
4.




Small-scale "in house" SNP genotyping

multicolor detection (using of 5' oligonucleotide tags on SBE primers)

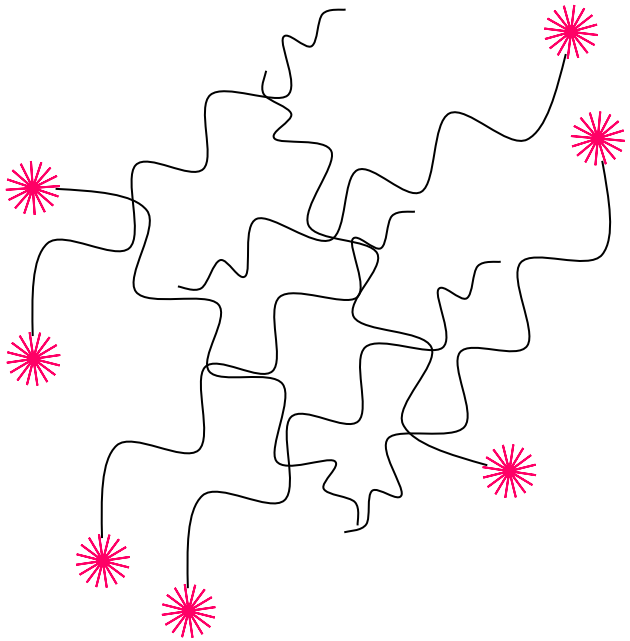
# Illumina Infinium Bead Chip

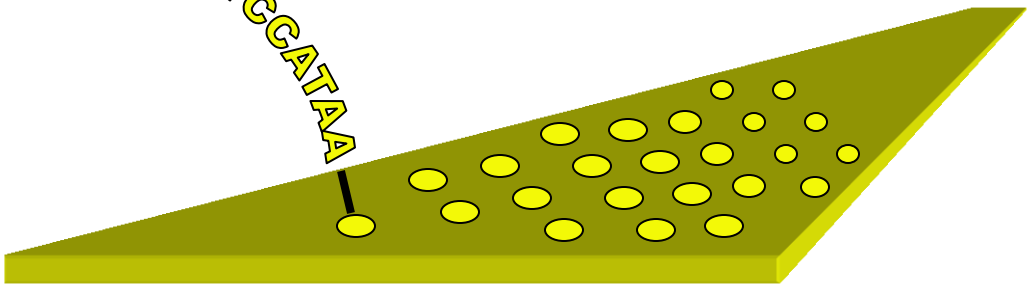



cca 300 000 SNP loci from 200 ng of DNA

# 5. Alelově specifická hybridizace

## Microarrays – SNPs chips

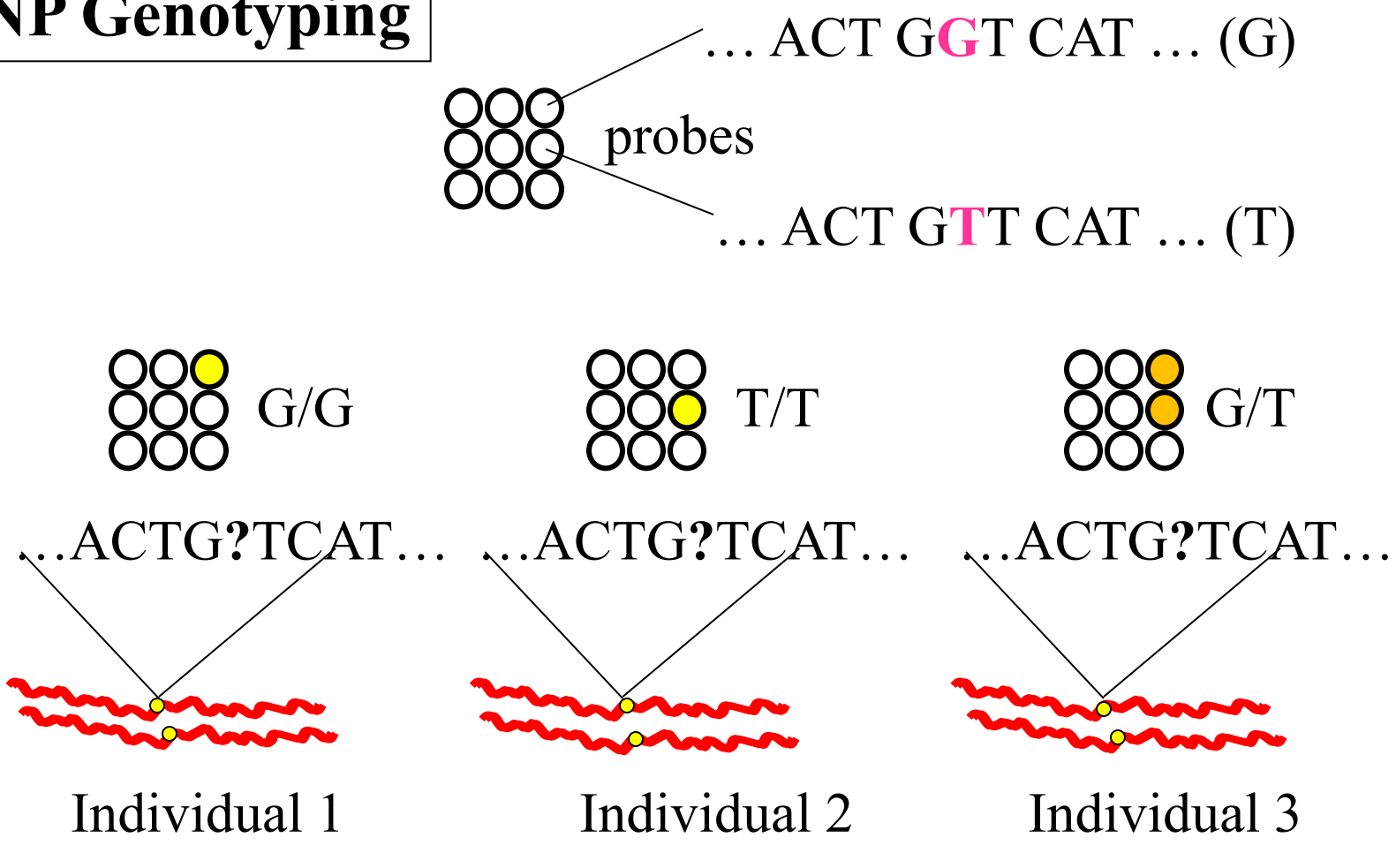




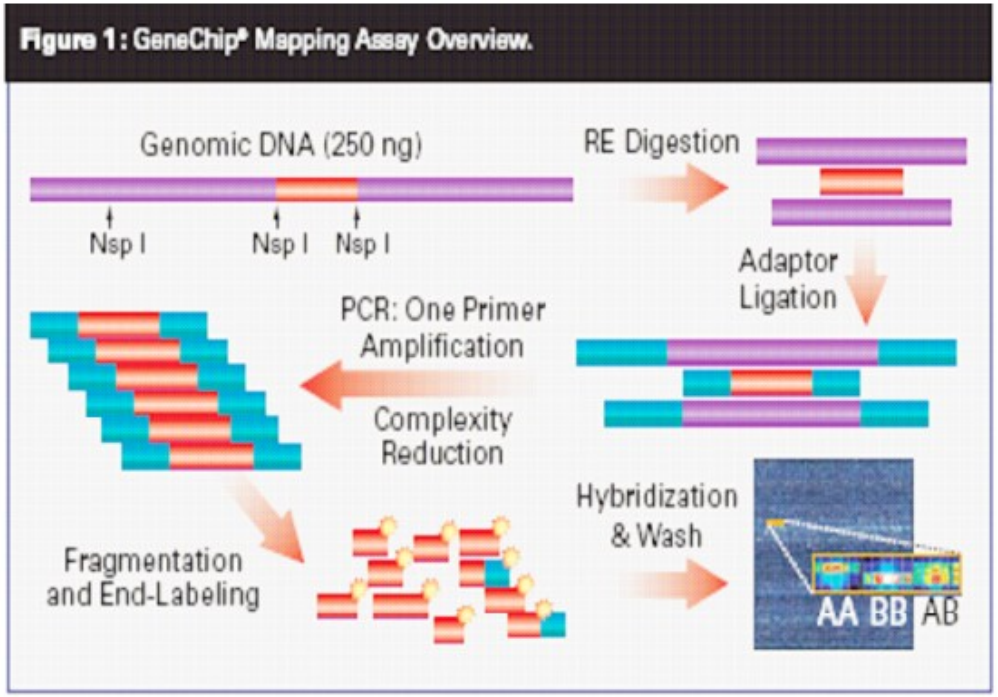

**Target** (genomická DNA rozštěpená restrikčními enzymy)

**Probe**
(specifická sonda pro každou **alelu**)

# Microarray SNP Genotyping



# Detekce: např. Affymetrix





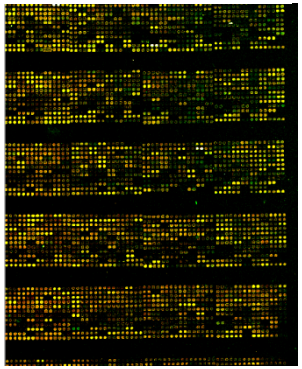
Affymetrix® Mouse Diversity Genotyping Array

- 10 tisíc – 1 milión SNP znaků najednou – „chip technology“

- např. Mouse Diversity Genotyping Array – 623 tisíc SNPs (je známa pozice každého z nich na genomu)

- je možné si navrhnout vlastní Array

Affymetrix®
Revolutionize life



| Fees - Whole Genome Genotyping | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Platform | SNP multiplex | # samples per array | # genotypes | array $ | reagent $ | core fee $ | Project price per sample | Project price per genotype | volume discount bins |
| **Affymetrix 10K** | 10,000 | 1 | 10,000 | 185 | 50 | 255 | **$490.00** | $0.0490 | |
| **Affymetrix 50K** | 50,000 | 1 | 50,000 | 210 | 50 | 255 | **$515.00** | $0.0103 | |
| **Affymetrix 100K (50K x2)** | 100,000 | 1 | 100,000 | 420 | 100 | 510 | **$920.00** | $0.0092 | |
| **Affymetrix 250K** | 250,000 | 1 | 250,000 | 470 | 55 | 255 | **$780.00** | $0.0031 | |
| **Affymetrix 500K (250K x2)** | 500,000 | 1 | 500,000 | 940 | 110 | 510 | **$1,560.00** | $0.0031 | |
| **Affymetrix 500K (250K x2)** | 500,000 | 1 | 500,000 | 800 | 110 | 510 | **$1,420.00** | $0.0028 | 1000-2000 samples |
| **Affymetrix 500K (250K x2)** | 500,000 | 1 | 500,000 | 700 | 110 | 510 | **$1,320.00** | $0.0026 | 2001-5000 samples |
| | | | | | | | | | |
| **Illumina Human-1** | 109,000 | 1 | 109,000 | 800 | na | 110 | **$910.00** | $0.0083 | 1-256 samples |
| **Illumina Human-1** | 109,000 | 1 | 109,000 | 720 | na | 110 | **$830.00** | $0.0076 | 257-496 samples |
| **Illumina Human-1** | 109,000 | 1 | 109,000 | 640 | na | 110 | **$750.00** | $0.0069 | 497-736 samples |
| **Illumina Human-1** | 109,000 | 1 | 109,000 | 560 | na | 110 | **$670.00** | $0.0061 | 737-976 samples |
| **Illumina Human-1** | 109,000 | 1 | 109,000 | 480 | na | 110 | **$590.00** | $0.0054 | 977+ samles |
| **Illumina HumanHap300** | 317,000 | 1 | 317,000 | 1100 | na | 110 | **$1,210.00** | $0.0038 | 1-256 samples |
| **Illumina HumanHap300** | 317,000 | 1 | 317,000 | 990 | na | 110 | **$1,100.00** | $0.0035 | 257-496 samples |
| **Illumi** | | | | | | | | | ples |
| **Illumi** | | | | | | | | | ples |
| **Illumi** | | | | | | | | | mles |
| **Illumi** | | | | | | | | | ples |
| **Illumi** | | | | | | | | | ples |
| **Illumi** | | | | | | | | | ples |
| **Illumina HumanHapS** | 240,000 | 1 | 240,000 | 700 | na | 110 | **$810.00** | $0.0034 | 737-976 samples |
| **Illumina HumanHapS** | 240,000 | 1 | 240,000 | 600 | na | 110 | **$710.00** | $0.0030 | 977+ samles |
| **Illumina HumanHap550** | 550,000 | 1 | 550,000 | 1600 | na | 110 | **$1,710.00** | $0.0031 | 1-256 samples |
| **Illumina HumanHap550** | 550,000 | 1 | 550,000 | 1440 | na | 110 | **$1,550.00** | $0.0028 | 257-496 samples |
| **Illumina HumanHap550** | 550,000 | 1 | 550,000 | 1280 | na | 110 | **$1,390.00** | $0.0025 | 497-736 samples |
| **Illumina HumanHap550** | 550,000 | 1 | 550,000 | 1120 | na | 110 | **$1,230.00** | $0.0022 | 737-976 samples |
| **Illumina HumanHap550** | 550,000 | 1 | 550,000 | 960 | na | 110 | **$1,070.00** | $0.0019 | 977+ samles |
| **HumanHap300 + HumanHapS** | 550,000 | 1 | 550,000 | 1750 | na | 220 | **$1,970.00** | $0.0036 | 1-256 samples |
| **HumanHap300 + HumanHapS** | 550,000 | 1 | 550,000 | 1575 | na | 220 | **$1,795.00** | $0.0033 | 257-496 samples |
| **HumanHap300 + HumanHapS** | 550,000 | 1 | 550,000 | 1400 | na | 220 | **$1,620.00** | $0.0029 | 497-736 samples |
| **HumanHap300 + HumanHapS** | 550,000 | 1 | 550,000 | 1225 | na | 220 | **$1,445.00** | $0.0026 | 737-976 samples |
| **HumanHap300 + HumanHapS** | 550,000 | 1 | 550,000 | 1050 | na | 220 | **$1,270.00** | $0.0023 | 977+ samles |

Použití u příbuzných druhů je možné, ale je tam velmi silný „ascertainment bias“

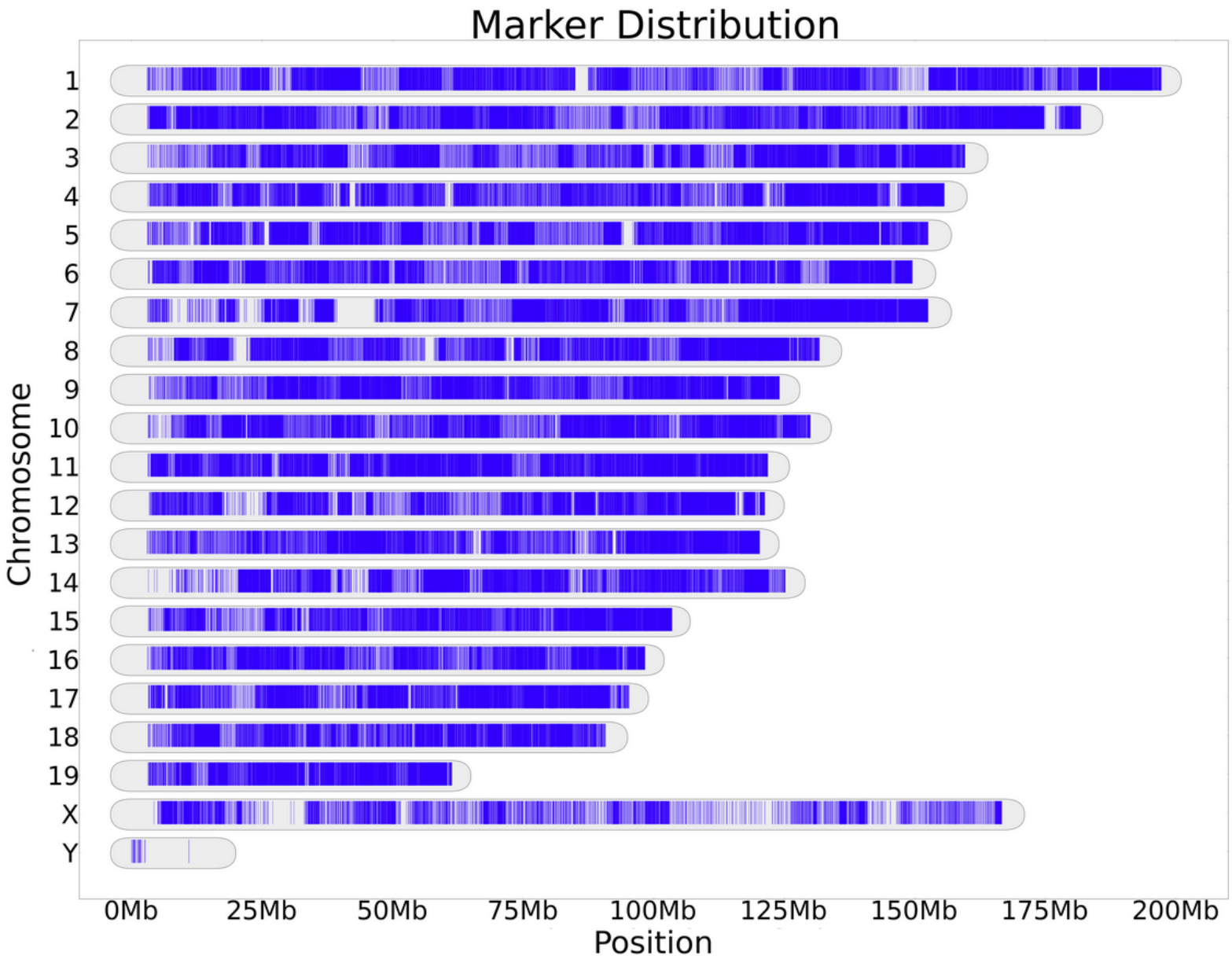
# Př. ascertainment bias: MegaMUGA chips


## Performance

- The MegaMUGA array provides robust calls for over 74,000 SNP markers in classical strains with greater than 99.9% concordance (based on over 300 controls).
- Initial analysis in classical inbred strains shows that on average the number of informative SNPs in pairwise combinations is ~20,000. MegaMUGA allows to discriminate between closely related sister strains (e.g. C57BL/6J and C57BL/6CR) and between related wild derived strains of similar origin (e.g. PWK/PhJ and PWD/PhJ; ZALENDE/EiJ and TIRANO/EiJ).
- Genotypes for many inbred strains can be viewed at the UNC Systems Genetics Core Facility website (http://www.csbio.unc.edu/CCstatus/index.py?run ).

**Up to 77,800 SNPS**
**$90/$100**
**Per DNA Sample**

Využívá Illumina Infinium technologii (single base extension)

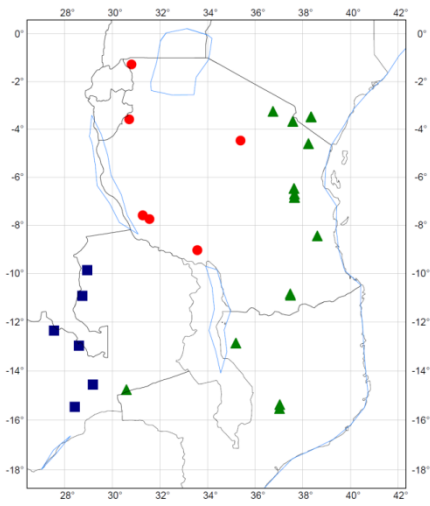

Marker Distribution
Chromosome
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Y
0Mb
25Mb
50Mb
75Mb
100Mb
125Mb
150Mb
175Mb
200Mb
Position

# Použití pro nemodelové druhy („cross-genotyping“)


| | | | MOZ27 | MOZ27 | VM121 | VM121 | RS0847 | RS0847 | JS052 | JS052 | KE55 | KE55 | 24645 | 24645 | T8_414 | T8_414 | TA452 | TA452 | TA453 | TA453 | TA171 | TA171 | TA536 | TA536 | RS1173 | RS1173 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | 42818H | |
| | | | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 | Apr 22 2014 | |
| | | | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_M | Mega_Muga_15035012_A | |
| | | | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716 | 9305716002_R06C02 | |
| | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | | | calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_(calls | Score_Call | |
| Locus | Chromosom | Position | | | | | | | | | | | | | | | | | | | | | | | | | |
| UNC6 | 1 | 3000355 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | B | 0.5009 | BBBBBBBBBBBB |
| JAX00000010 | 1 | 3125499 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0.0015 | U | 0.0105 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0.0069 | UUUUUUUUUUUU |
| JAX00240603 | 1 | 3242877 | U | 0 | U | 0.0106 | U | 0.0154 | U | 0.0146 | U | 0 | U | 0.0174 | U | 0.0152 | U | 0 | U | 0 | U | 0 | U | 0.0041 | U | 0 | UUUUUUUUUUUU |
| JAX00240610 | 1 | 3256689 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | B | 0.444 | BBBBBBBBBBBB |
| JAX00240613 | 1 | 3313481 | U | 0.0318 | U | 0 | U | 0.0081 | U | 0.0087 | U | 0.011 | U | 0.0085 | U | 0.0067 | U | 0.015 | U | 0.0069 | U | 0.0228 | U | 0.0315 | U | 0.0212 | UUUUUUUUUUUU |
| JAX00240636 | 1 | 3379644 | B | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | H | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | B | 0.9343 | BBBBHBBBBBBB |
| JAX00240649 | 1 | 3445065 | U | 0.0038 | U | 0.093 | U | 0.0941 | U | 0.0699 | U | 0 | U | 0.0957 | B | 0.1629 | B | 0.6778 | B | 0.8011 | B | 0.3681 | B | 0.4583 | U | 0.102 | UUUUUUBBBBBU |
| JAX00000040 | 1 | 3534900 | U | 0.0863 | U | 0.0372 | U | 0.0317 | U | 0 | U | 0.0508 | U | 0.0722 | U | 0.1401 | U | 0.0702 | U | 0.1155 | U | 0.0592 | U | 0.0218 | U | 0 | UUUUUUUUUUUU |
| UNC010515443 | 1 | 3658709 | U | 0.0525 | A | 0.9521 | A | 0.9521 | A | 0.9521 | A | 0.9521 | A | 0.9235 | A | 0.9521 | U | 0.0438 | A | 0.9521 | A | 0.9521 | A | 0.9521 | A | 0.9521 | UAAAAAAUAAAA |
| UNC9371 | 1 | 3882799 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | B | 0.9228 | BBBBBBBBBBBB |
| UNC010001943 | 1 | 3967211 | U | 0.0673 | U | 0.0191 | U | 0.067 | U | 0.0271 | H | 0.4843 | H | 0.3235 | U | 0.0226 | H | 0.5626 | U | 0.1401 | U | 0.0153 | U | 0.0338 | U | 0.0396 | UUUUHHUUUUUU |
| UNC11461 | 1 | 4014866 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | B | 0.8933 | BBBBBBBBBBBB |
| UNC12838 | 1 | 4126115 | U | 0.0059 | U | 0.0186 | U | 0 | U | 0 | U | 0.0146 | U | 0 | U | 0 | U | 0.0199 | U | 0 | U | 0 | U | 0.0149 | U | 0 | UUUUUUUUUUUU |
| JAX00000089 | 1 | 4185434 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | B | 0.7857 | BBBBBBBBBBBB |
| UNC15185 | 1 | 4347901 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | A | 0.7989 | AAAAAAAAAAAA |
| UNC010515539 | 1 | 4420704 | A | 0.3613 | A | 0.4559 | A | 0.7183 | A | 0.5208 | A | 0.3029 | A | 0.3067 | A | 0.3725 | H | 0.5522 | A | 0.482 | A | 0.2231 | A | 0.3333 | A | 0.3274 | AAAAAAAHAAAA |
| UNC16386 | 1 | 4436818 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | U | 0 | UUUUUUUUUUUU |
| UNC010515556 | 1 | 4521110 | B | 0.5607 | B | 0.5607 | B | 0.5607 | B | 0.5607 | B | 0.5607 | B | 0.5607 | B | 0.5607 | B | 0.5607 | U | 0.0183 | B | 0.5607 | U | 0.0142 | B | 0.5607 | BBBBBBBBUBUB |
| UNC19217 | 1 | 4658505 | U | 0.0681 | U | 0.0489 | U | 0.038 | U | 0.0253 | U | 0.0468 | U | 0.056 | U | 0.0317 | U | 0.0412 | U | 0.098 | U | 0.1393 | U | 0.0312 | U | 0.0359 | UUUUUUUUUUUU |
| UNC21023 | 1 | 4799357 | U | 0.0294 | U | 0.0205 | B | 0.3533 | U | 0.0227 | U | 0 | U | 0.0246 | U | 0.1149 | U | 0.0392 | B | 0.2556 | U | 0.0203 | U | 0.0054 | U | 0 | UUBUUUUUBUUU |
| UNC23507 | 1 | 5022167 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | B | 0.8236 | BBBBBBBBBBBB |
| UNC23995 | 1 | 5123463 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | B | 0.9264 | BBBBBBBBBBBB |
| UNC24700 | 1 | 5214659 | A | 0.9205 | A | 0.9205 | A | 0.9069 | A | 0.5187 | A | 0.9205 | A | 0.9205 | A | 0.9205 | A | 0.9205 | A | 0.9205 | A | 0.9205 | A | 0.9205 | A | 0.9024 | AAAAAAAAAAAA |
| UNC25932 | 1 | 5460949 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | A | 0.2893 | U | 0.0357 | A | 0.2893 | A | 0.2893 | AAAAAAAAAUAA |
| UNC26205 | 1 | 5624267 | A | 0.8824 | A | 0.3919 | A | 0.927 | A | 0.5961 | A | 0.927 | A | 0.927 | A | 0.927 | A | 0.927 | A | 0.9141 | U | 0.1074 | A | 0.927 | A | 0.927 | AAAAAAAAAUAA |
| UNC26932 | 1 | 5772370 | U | 0.0349 | U | 0.0119 | U | 0.0172 | U | 0.0157 | U | 0.0522 | U | 0.0064 | U | 0.0203 | U | 0.0267 | U | 0.0114 | U | 0.0147 | U | 0.014 | U | 0.0095 | UUUUUUUUUUUU |
| UNC28841 | 1 | 5934873 | U | 0.0271 | U | 0.0154 | U | 0.0104 | U | 0.0399 | U | 0.0403 | U | 0.0094 | U | 0.0393 | U | 0.0556 | U | 0.0395 | U | 0.0339 | U | 0.016 | U | 0.0129 | UUUUUUUUUUUU |
| UNC010516347 | 1 | 6010860 | A | 0.2088 | U | 0.0421 | A | 0.364 | A | 0.1876 | U | 0.0147 | A | 0.4013 | A | 0.3915 | U | 0.0335 | U | 0.0802 | A | 0.4013 | A | 0.3984 | U | 0.0174 | AUAAUAAUUAAU |
| UNC30322 | 1 | 6053779 | B | 0.2848 | U | 0.038 | U | 0.0969 | U | 0.0569 | B | 0.2002 | B | 0.1682 | U | 0.0182 | B | 0.4365 | B | 0.2283 | U | 0.0406 | U | 0.0733 | U | 0.1213 | BUUUBBUBBUUU |
| UNC34218 | 1 | 6347559 | U | 0.0886 | U | 0.0545 | U | 0.061 | U | 0 | U | 0.0168 | B | 0.2264 | U | 0.0122 | B | 0.2666 | B | 0.1678 | U | 0 | U | 0.124 | U | 0.031 | UUUUUBUBBUUU |
| UNC36398 | 1 | 6519513 | U | 0 | B | 0.899 | B | 0.899 | B | 0.899 | U | 0.0557 | U | 0.0532 | B | 0.899 | U | 0 | U | 0.0058 | U | 0.0545 | U | 0.0788 | B | 0.899 | UBBBUUBUUUUB |
| UNC38030 | 1 | 6661408 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2145 | A | 0.2128 | A | 0.2145 | A | 0.2145 | AAAAAAAAAAAA |
| JAX00000280 | 1 | 6737521 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | B | 0.928 | BBBBBBBBBBBB |
| JAX00241205 | 1 | 6746102 | B | 0.7869 | B | 0.8506 | B | 0.8506 | B | 0.8506 | B | 0.8506 | B | 0.8506 | B | 0.8506 | B | 0.6516 | B | 0.8506 | B | 0.8506 | B | 0.8506 | B | 0.8506 | BBBBBBBBBBBB |

jedinec 1

jedinec 2




Studium kontaktní zóny *Mus minutoides* ve východní Africe

1620 informativních lokusů z 5598 lokusů na chromozómu 1 (60 SNPs fixovaných pro dané subpopulace v celém genomu)

# Dnes široká škála komerčních možností SNP genotyping pro nemodelové druhy – př. Illumina

Figure 2: Illumina Custom Genotyping Options

| Number of Markers | Illumina Assay or Product |
|---|---|
| 3K–1M | Infinium iSelectHD |
| 1K–500K | Infinium Semi-Custom and Add-On Content |
| 96–3,072 | GoldenGate on BeadArray |
| 1–384 | GoldenGate on VeraCode |
| 48 | qPCR on Eco Real-Time PCR System |

Illumina products enable a wide range of genotying experimental designs, depending upon the number of markers.




| Platform | iScan System | HiScanSQ System | BeadXpress Reader | Eco Real-Time PCR System |
|---|---|---|---|---|
| Technology | BeadArray | | VeraCode | Real-Time PCR |
| Assay | InfiniumHD<br>GoldenGate | | GoldenGate<br>ASPE | Allelic Discrimination/<br>High Resolution Melt (HRM) |
| Product | iSelectHD BeadChips;<br>Custom and Semi-Custom Add-On;<br>GoldenGate Genotyping Assay Kit | | VeraCode GoldenGate<br>Genotyping Assay Kit;<br>Universal Capture Beads | Open Platform |

ASPE: Allele-Specific Primer Extension

| | | | |
|---|---|---|---|
| No. of loci: | 3 000 – 1 milión | 48-384 | 48 |
| Samples/day | 288 | 288 | 384 |