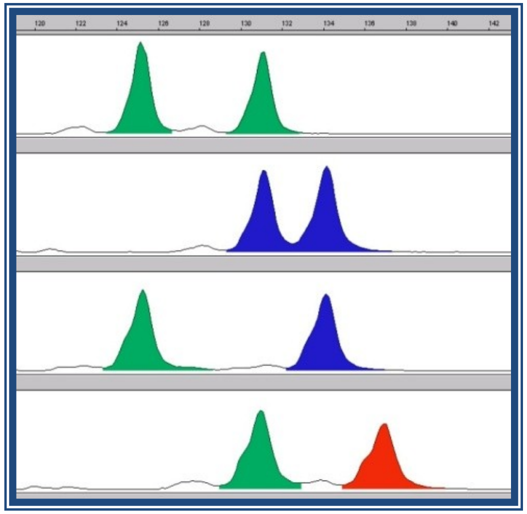
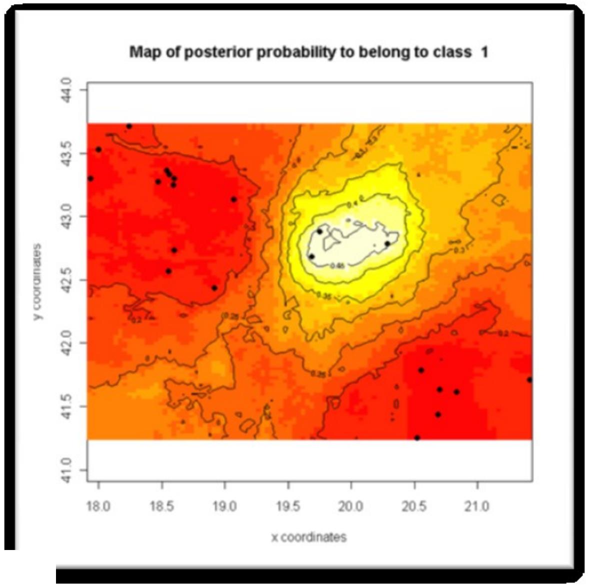
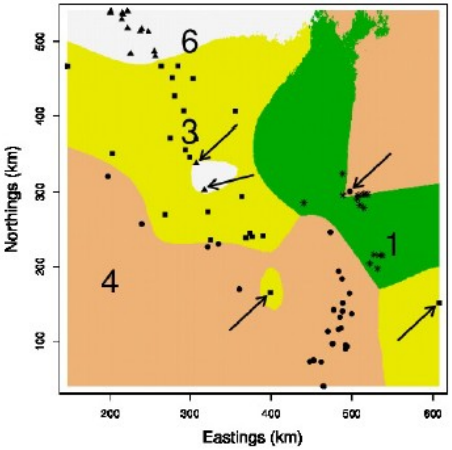
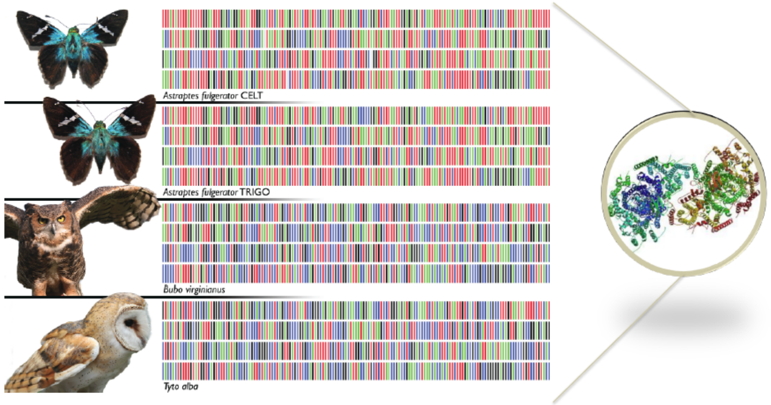
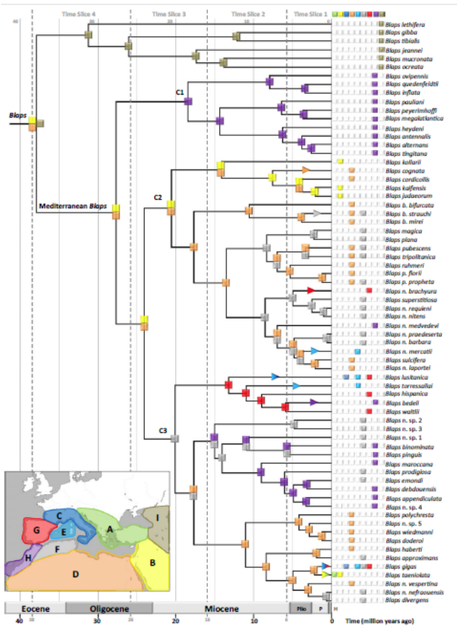








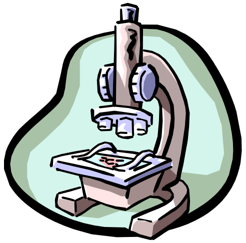


Molekulární ekologie

J. Bryja, M. Macholán, A. Konečný (+ hosté)

What is molecular ecology?

Artificially delimited discipline – ecological and evolutionary questions are answered by molecular-genetic approaches.



Classical problems and questions of ecology
(zoology, evolutionary biology)

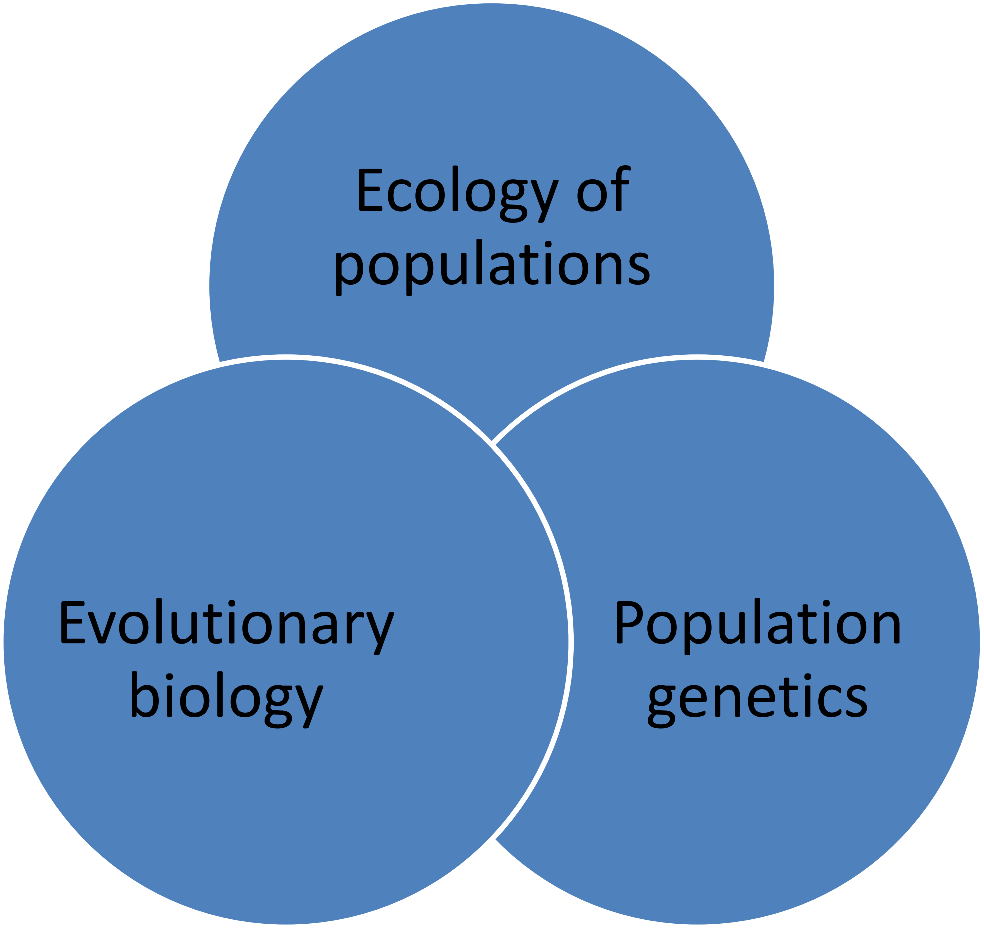
+

Molecular-genetic data and population-genetic analyses

It works on different levels of DNA variability (genome, individuals, populations, groups of populations or closely related species)

It is in fact applied population genetics – analyses and interprets molecular-genetic data

Interdisciplinary



Ecology of populations

Evolutionary biology

Population genetics

Molecular ecology

Mechanisms of microevolution

(theory)

+

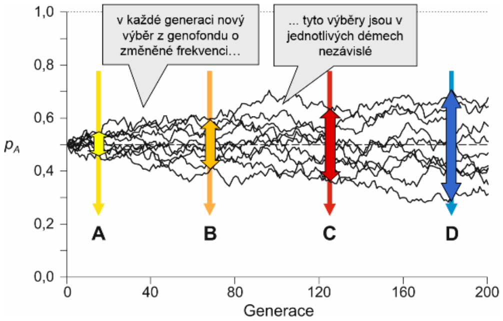
Genetic methods in zoology

(data)

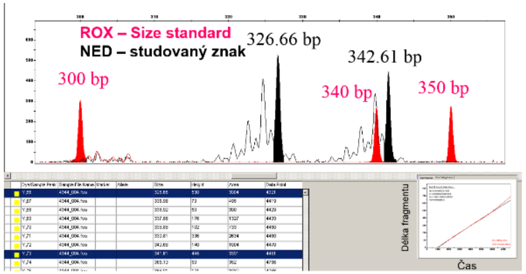
=

Molecular ecology

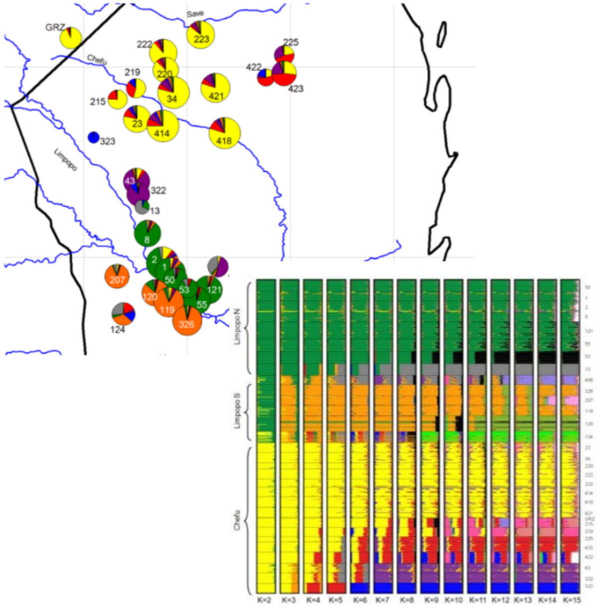
(analysis and interpretation of results)



Závěr 9: Drift vede k divergenci mezi démy.



Genotyp mikrosatelitu na lokusu NED = 326/342 nebo 327/343
Programy: GeneMapper, Genotyper, Geneious, GeneMarker, ...



It is popular ...

- journal Molecular Ecology (from 1992) – now 24 issues/year
- Textbooks

MOLECULAR ECOLOGY

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Online ISSN: 1365-294X
Print ISSN: 0962-1083
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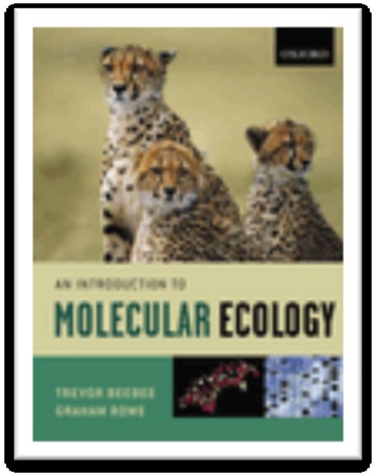
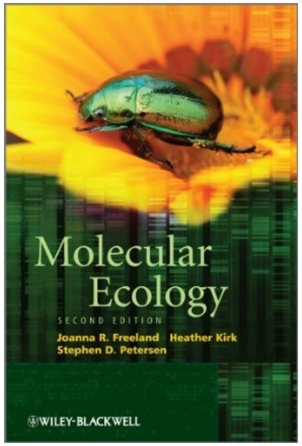
Latest issue

Volume 34, Issue 18
September 2025





MOLECULAR ECOLOGY RESOURCES

Editors-in-Chief: Shawn Narum and Ben Sibbett
Online ISSN: 1755-0998
Print ISSN: 1755-098X
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Latest issue
Volume 25, Issue 7
October 2025

- *Molecular Ecology Resources* encourages development of broad resources for the community including **computer programs**, **statistical and molecular advances**, and **extensive molecular tools**. The journal is a vehicle for dissemination of these resources, targeting a wide audience of researchers in the fields of evolution, ecology, and conservation. Articles in *Molecular Ecology Resources* are intended to facilitate studies addressing consequential questions in these fields.
- In addition to novel resource articles, *Molecular Ecology Resources* publishes relevant Reviews, Opinions, and Comments. The journal also publishes occasional Special Issues that emphasize development of resources in a particular area.

- **Experimental eDNA work:** Testing the impact of physico-chemical factors (e.g. natural biogeochemistry and PCR pollutants) on eDNA, degradation, transport, shedding and detection rate, comparing detectionand abundance estimate with conventional methods.
- **Trophic and community ecology:** Ecosystem dynamics, functional diversity, predator-prey interactions (e.g. diet analysis), host-associated microbiota.
- **Palaeo-environments:** Past species and community diversity and abundance measurements, inference in space and time.
- **Biomonitoring, conservation biology:** Single- and multi-species detection, comprehensive biodiversity at different scales, abundance estimates, detection of rare, cryptic and endangered species, non-invasive sampling, management (e.g. fisheries), occurrence and detection estimates.
- **Invasion biology:** Early species detection at low abundance, passive surveillance, impacts on ecosystems, vectors and pathways of dispersal.
- **Environmental assessment:** Impacts of pollutants and other environmental disturbance on species and communities, microbial source tracking (fecal bacteria or pathogens).
- **Physical eDNA properties:** Uptake and transformation based on geochemistry, particles, organic chemistry or microbial community.
- **Techniques and methods:** Engineering development, developing, testing and evaluating eDNA biotechnology and biostatistical approaches.
- **Applications in citizen science and biodiversity education**

Why use genetic methods in ecology?

- **No other choice**
- cryptic species
- paternity – mating is elusive and not always lead to fertilization
- identification (species, sex, individual) from feaces or feathers
- barriers to gene flow – not obvious from other data
- number of migrants
- genetic basis of adaptations (e.g. immune genes)

Why use genetic methods in ecology?

- ecological, evolutionary, and population genomics
- **population structure and phylogeography**
- landscape genomics
- **community ecology and coevolution**
- **reproductive strategies**
- **relatedness and kin selection**
- sex allocation
- population genetic theory
- analytical methods development
- **conservation genetics**
- **speciation and hybridization**
- microbial biodiversity
- evolutionary dynamics of QTLs
- ecological interactions
- **molecular adaptation and environmental genomics**
- impact of genetically modified organisms
- indigenous perspectives/methodologies

MOLECULAR ECOLOGY

How to obtain genetic data – viz Genetic methods in zoology

Genotyping – analysis of genotypes

- identification of specific form of particular DNA molecule/fragment (=alele or haplotype) - selection of the marker type depends on the level of genetic variability in population/species

1) DNA extraction from tissues
2) PCR amplification (in PCR-based methods)
3) analysis of marker (lokus) variability

„Molecular genetic“ methods

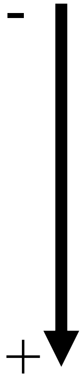
- analysis of DNA polymorphism
- length polymorfism (e.g. microsatellites)

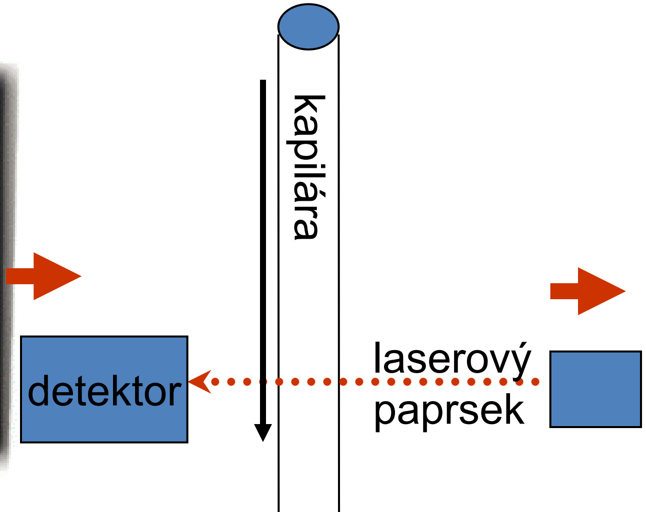
CGCACATCTCTAGCTTCGATTCAGGAA

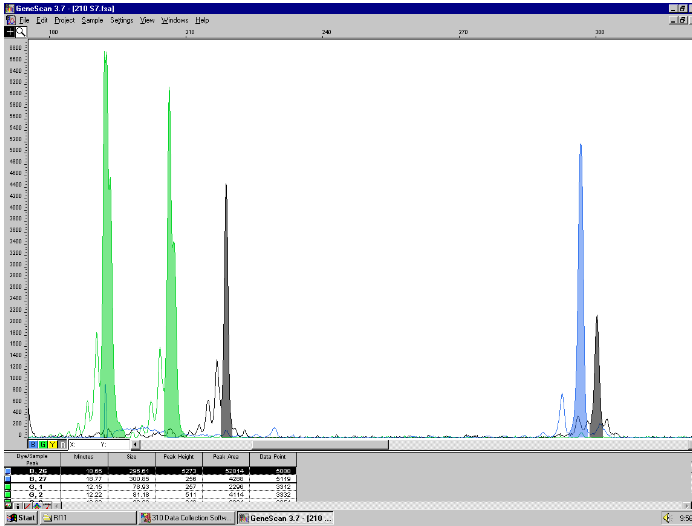
CGCATCTCTAGCTTTGATTCAGGAA

Separation of DNA fragments according their size

- Agarosa – up to 15 bp difference
- Polyacrylamide – more precise (4 bp)
- Capillary electrophoresis – even 1bp differences (fluorescently-labelled fragments)







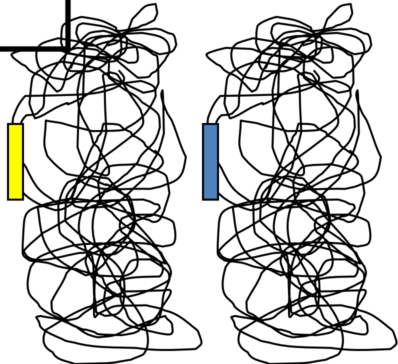
Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
B, 26	18.66	296.61	5273	52814	5088
B, 27	18.77	300.85	256	4288	5119
G, 1	12.15	78.93	257	2296	3312
G, 2	12.22	81.18	511	4114	3332

„Molecular-genetic“ methods

- analysis of DNA polymorphism
- sequence polymorphism („SNPs“):

CGCATCTCTAGCTTCGATTCAGGAA

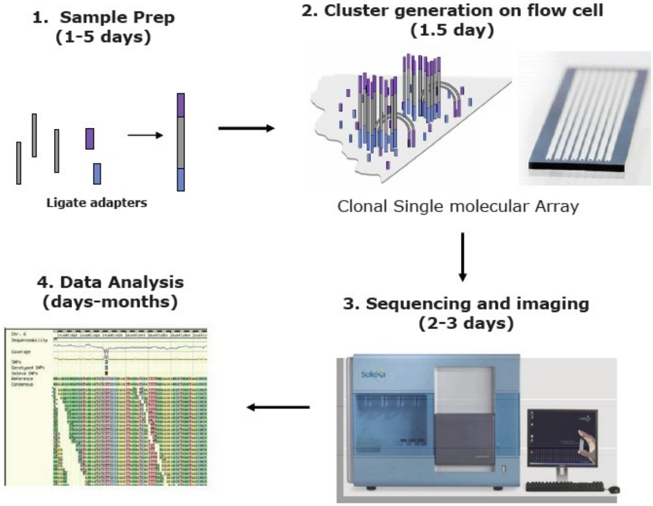
CGCATCTCTAGCTTTGATTCAGGAA

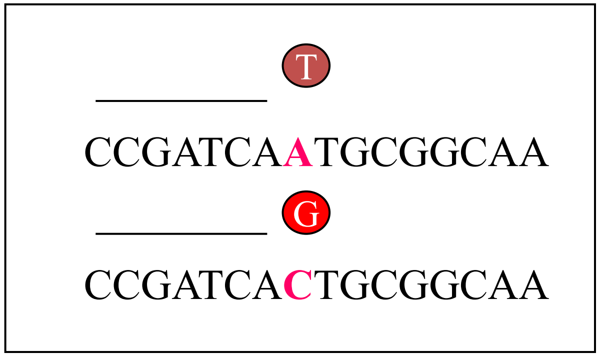


diploid genotype: **C/T**

Methods for analysis of sequence variability

- Sanger sequencing (very good for mtDNA, less for nDNA problém with heterozygotes)
- SNP („single nucleotide polymorphism“) genotyping – např. RFLP, SSCP, microarrays – chips, atd.
- high-throughput sequencing





Population-genetic datasets

microsatellites

Ind	Marker 1	Marker 2
Ind_1	170/172	133/136
Ind_2	168/172	133/139
Ind_3	168/168	136/139

SNPs

Ind	Marker 1	Marker 2
Ind_1	A/T	C/T
Ind_2	A/T	T/T
Ind_3	T/T	C/T

SINE

Ind	Marker 1	Marker 2
Ind_1	+/-	-/-
Ind_2	+/+	+/+
Ind_3	-/-	+/-

AFLP

Ind	Marker 1	Marker 2
Ind_1	+	-
Ind_2	+	+
Ind_3	-	-

Types of data – codomiant markers

no. loci

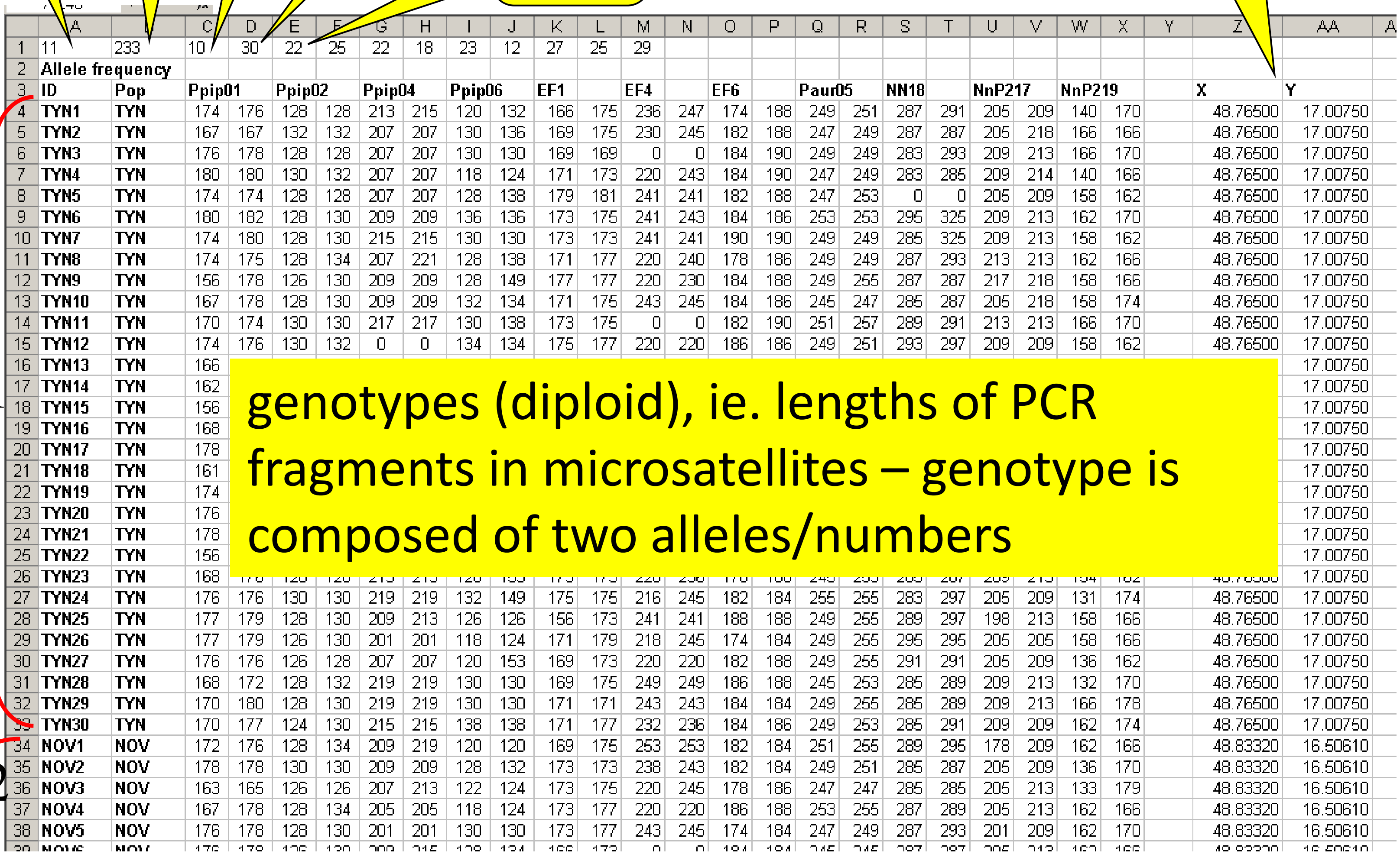
no. individuals

no. populations

no. of samples in pop1

no. of samples in pop, etc.

GPS coordinates



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2	**Allele frequency**																										
3	**ID**	**Pop**	**Ppip01**		**Ppip02**		**Ppip04**		**Ppip06**		**EF1**		**EF4**		**EF6**		**Paur05**		**NN18**		**NnP217**		**NnP219**			**X**	**Y**
4	**TYN1**	**TYN**	174	176	128	128	213	215	120	132	166	175	236	247	174	188	249	251	287	291	205	209	140	170		48.76500	17.00750
5	**TYN2**	**TYN**	167	167	132	132	207	207	130	136	169	175	230	245	182	188	247	249	287	287	205	218	166	166		48.76500	17.00750
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18	**TYN15**	**TYN**	156																								17.00750
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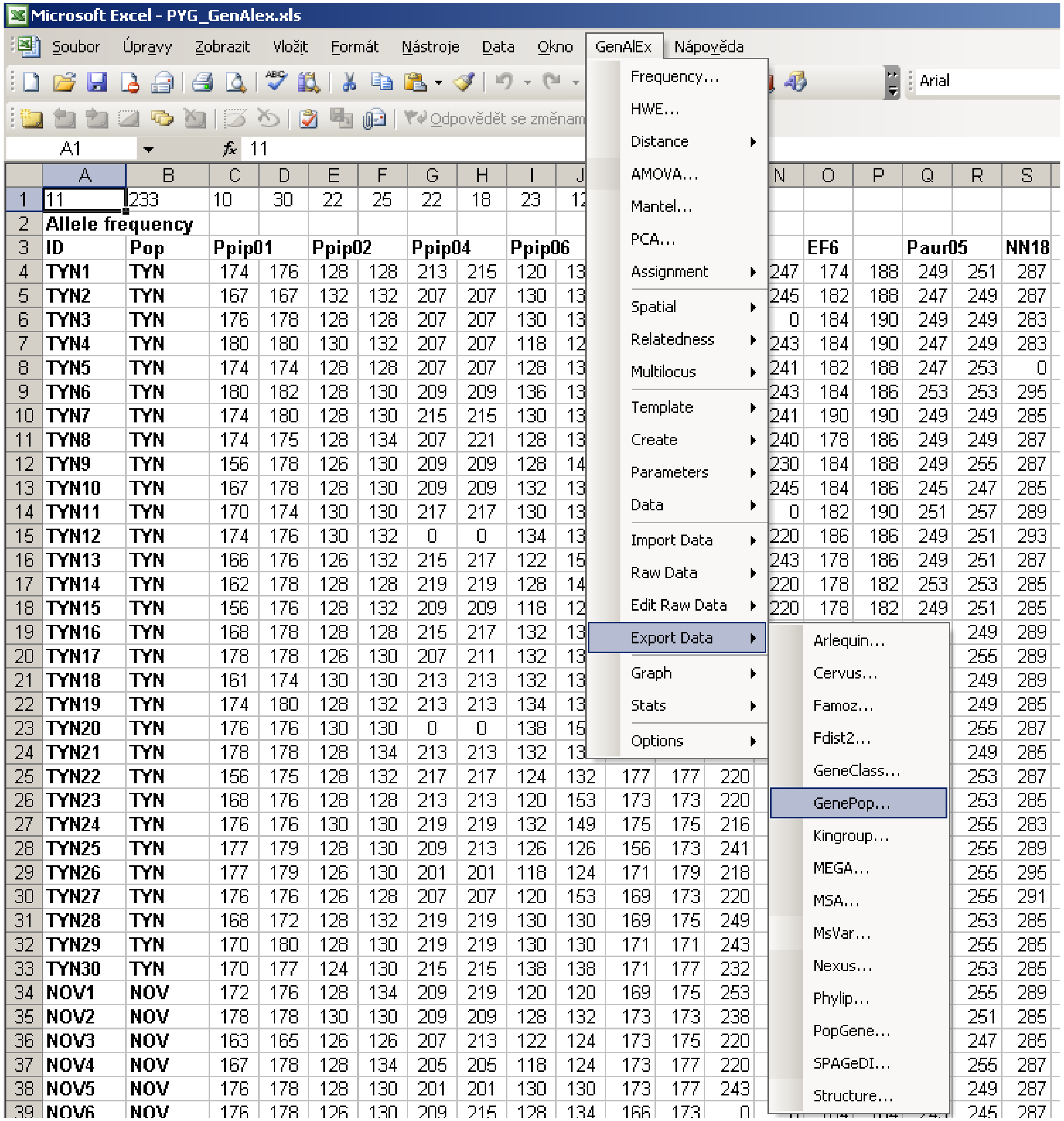
pop1

pop2

genotypes (diploid), ie. lengths of PCR fragments in microsatellites – genotype is composed of two alleles/numbers

GenAlex

GenAlEx®
Genetic Analysis in Excel
Version 6.1



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2	**Allele frequency**																		
3	**ID**	**Pop**	**Ppip01**		**Ppip02**		**Ppip04**		**Ppip06**						**EF6**		**Paur05**		**NN18**
4	**TYN1**	**TYN**	174	176	128	128	213	215	120					247	174	188	249	251	287
5	**TYN2**	**TYN**	167	167	132	132	207	207	130					245	182	188	247	249	287
6	**TYN3**	**TYN**	176	178	128	128	207	207	130					0	184	190	249	249	283
7	**TYN4**	**TYN**	180	180	130	132	207	207	118					243	184	190	247	249	283
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37	**NOV4**	**NOV**	167	178	128	134	205	205	118	124	173	177	220					255	287
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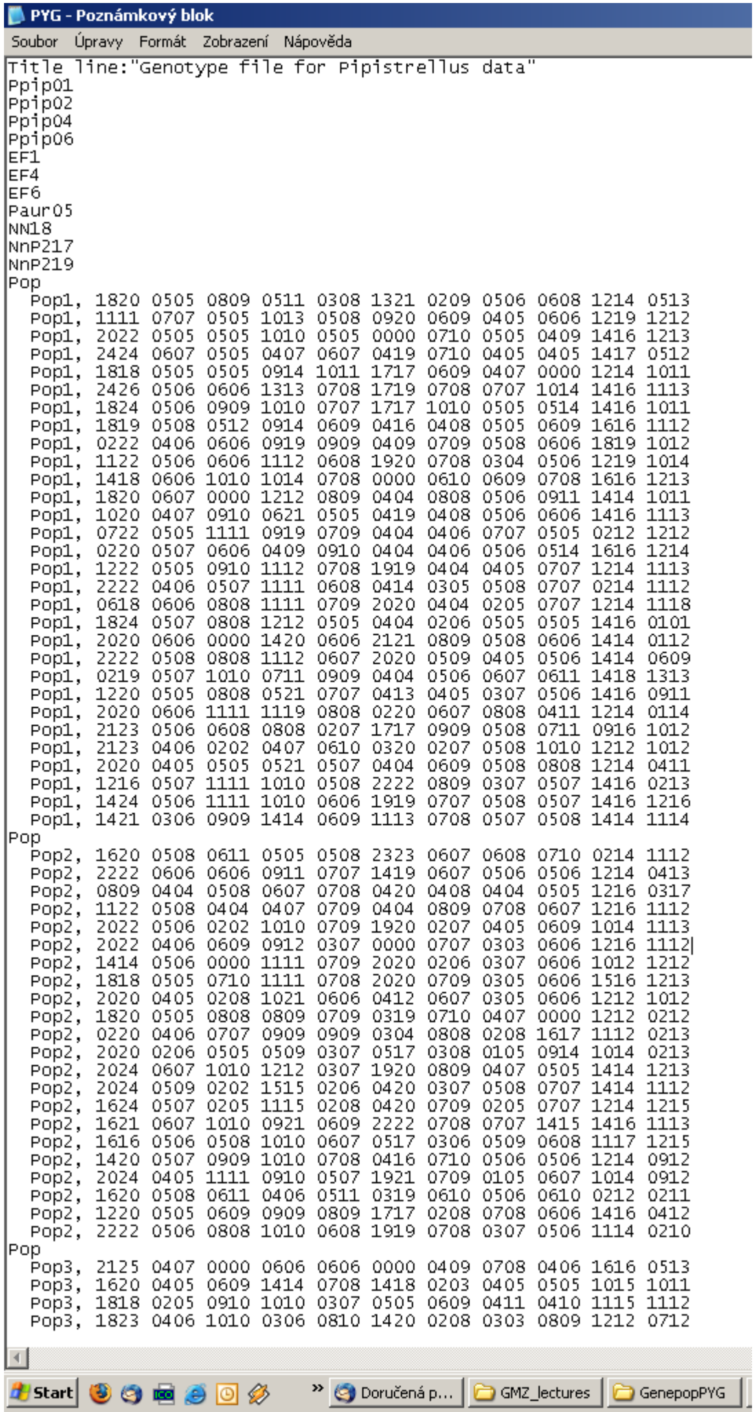
http://www.anu.edu.au/BoZo/GenAlEx/



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Ppip02
Ppip04
Ppip06
EF1
EF4
EF6
Paur05
NN18
NnP217
NnP219
Pop
   Pop1, 1820 0505 0809 0511 0308 1321 0209 0506 0608 1214 0513
   Pop1, 1111 0707 0505 1013 0508 0920 0609 0405 0606 1219 1212
   Pop1, 2022 0505 0505 1010 0505 0000 0710 0505 0409 1416 1213
   Pop1, 2424 0607 0505 0407 0607 0419 0710 0405 0405 1417 0512
   Pop1, 1818 0505 0505 0914 1011 1717 0609 0407 0000 1214 1011
   Pop1, 2426 0506 0606 1313 0708 1719 0708 0707 1014 1416 1113
   Pop1, 1824 0506 0909 1010 0707 1717 1010 0505 0514 1416 1011
   Pop1, 1819 0508 0512 0914 0609 0416 0408 0505 0609 1616 1112
   Pop1, 0222 0406 0606 0919 0909 0409 0709 0508 0606 1819 1012
   Pop1, 1122 0506 0606 1112 0608 1920 0708 0304 0506 1219 1014
   Pop1, 1418 0606 1010 1014 0708 0000 0610 0609 0708 1616 1213
   Pop1, 1820 0607 0000 1212 0809 0404 0808 0506 0911 1414 1011
   Pop1, 1020 0407 0910 0621 0505 0419 0408 0506 0606 1416 1113
   Pop1, 0722 0505 1111 0919 0709 0404 0406 0707 0505 0212 1212
   Pop1, 0220 0507 0606 0409 0910 0404 0406 0506 0514 1616 1214
   Pop1, 1222 0505 0910 1112 0708 1919 0404 0405 0707 1214 1113
   Pop1, 2222 0406 0507 1111 0608 0414 0305 0508 0707 0214 1112
   Pop1, 0618 0606 0808 1111 0709 2020 0404 0205 0707 1214 1118
   Pop1, 1824 0507 0808 1212 0505 0404 0206 0505 0505 1416 0101
   Pop1, 2020 0606 0000 1420 0606 2121 0809 0508 0606 1414 0112
   Pop1, 2222 0508 0808 1112 0607 2020 0509 0405 0506 1414 0609
   Pop1, 0219 0507 1010 0711 0909 0404 0506 0607 0611 1418 1313
   Pop1, 1220 0505 0808 0521 0707 0413 0405 0307 0506 1416 0911
   Pop1, 2020 0606 1111 1119 0808 0220 0607 0808 0411 1214 0114
   Pop1, 2123 0506 0608 0808 0207 1717 0909 0508 0711 0916 1012
   Pop1, 2123 0406 0202 0407 0610 0320 0207 0508 1010 1212 1012
   Pop1, 2020 0405 0505 0521 0507 0404 0609 0508 0808 1214 0411
   Pop1, 1216 0507 1111 1010 0508 2222 0809 0307 0507 1416 0213
   Pop1, 1424 0506 1111 1010 0606 1919 0707 0508 0507 1416 1216
   Pop1, 1421 0306 0909 1414 0609 1113 0708 0507 0508 1414 1114
Pop
   Pop2, 1620 0508 0611 0505 0508 2323 0607 0608 0710 0214 1112
   Pop2, 2222 0606 0606 0911 0707 1419 0607 0506 0506 1214 0413
   Pop2, 0809 0404 0508 0607 0708 0420 0408 0404 0505 1216 0317
   Pop2, 1122 0508 0404 0407 0709 0404 0809 0708 0607 1216 1112
   Pop2, 2022 0506 0202 1010 0709 1920 0207 0405 0609 1014 1113
   Pop2, 2022 0406 0609 0912 0307 0000 0707 0303 0606 1216 1112
   Pop2, 1414 0506 0000 1111 0709 2020 0206 0307 0606 1012 1212
   Pop2, 1818 0505 0710 1111 0708 2020 0709 0305 0606 1516 1213
   Pop2, 2020 0405 0208 1021 0606 0412 0607 0305 0606 1212 1012
   Pop2, 1820 0505 0808 0809 0709 0319 0710 0407 0000 1212 0212
   Pop2, 0220 0406 0707 0909 0909 0304 0808 0208 1617 1112 0213
   Pop2, 2020 0206 0505 0509 0307 0517 0308 0105 0914 1014 0213
   Pop2, 2024 0607 1010 1212 0307 1920 0809 0407 0505 1414 1213
   Pop2, 2024 0509 0202 1515 0206 0420 0307 0508 0707 1414 1112
   Pop2, 1624 0507 0205 1115 0208 0420 0709 0205 0707 1214 1215
   Pop2, 1621 0607 1010 0921 0609 2222 0708 0707 1415 1416 1113
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   Pop2, 1420 0507 0909 1010 0708 0416 0710 0506 0506 1214 0912
   Pop2, 2024 0405 1111 0910 0507 1921 0709 0105 0607 1014 0912
   Pop2, 1620 0508 0611 0406 0511 0319 0610 0506 0610 0212 0211
   Pop2, 1220 0505 0609 0909 0809 1717 0208 0708 0606 1416 0412
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Pop
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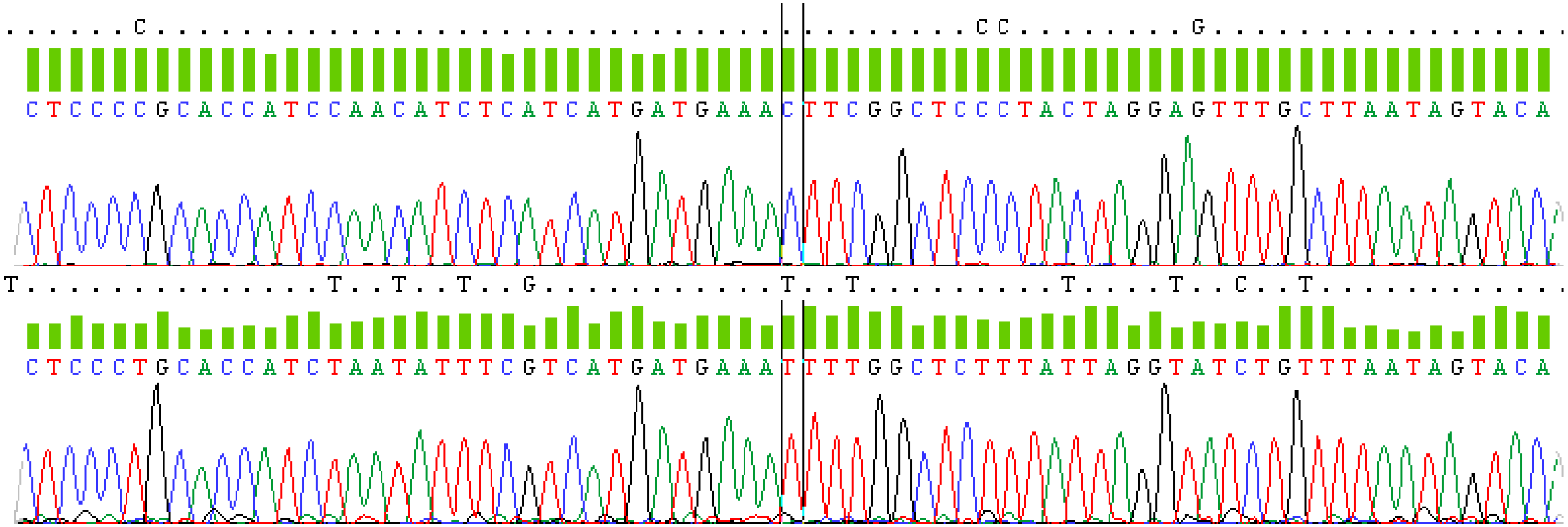
   Pop3, 1818 0205 0910 1010 0307 0505 0609 0411 0410 1115 1112
   Pop3, 1823 0406 1010 0306 0810 1420 0208 0303 0809 1212 0712
```

Genepop file format – simple ASCI .txt

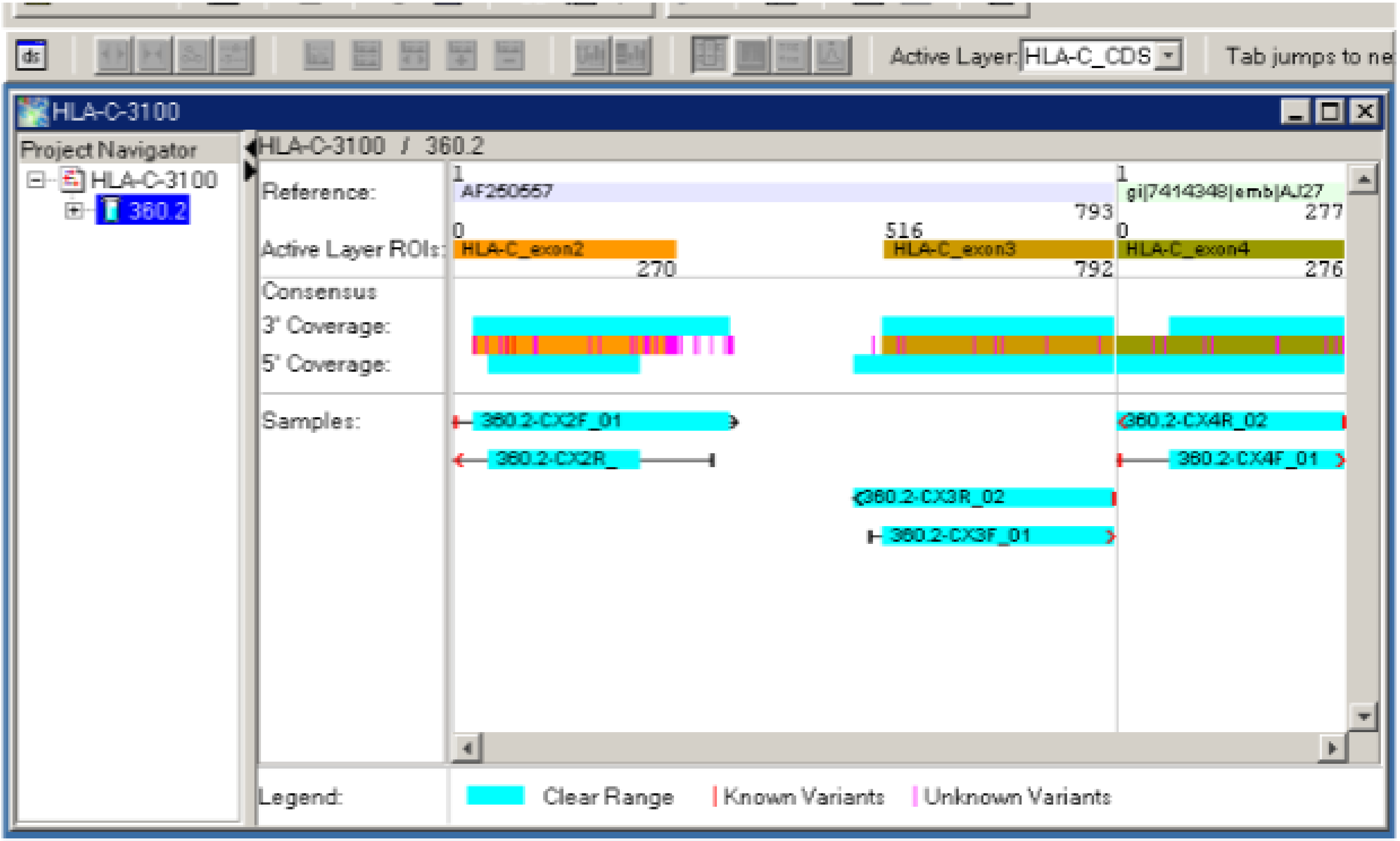
- alely for each locus are ranked according size and renumbered
- eg. 128/130 is transformed to 10/11

Sanger sequencing



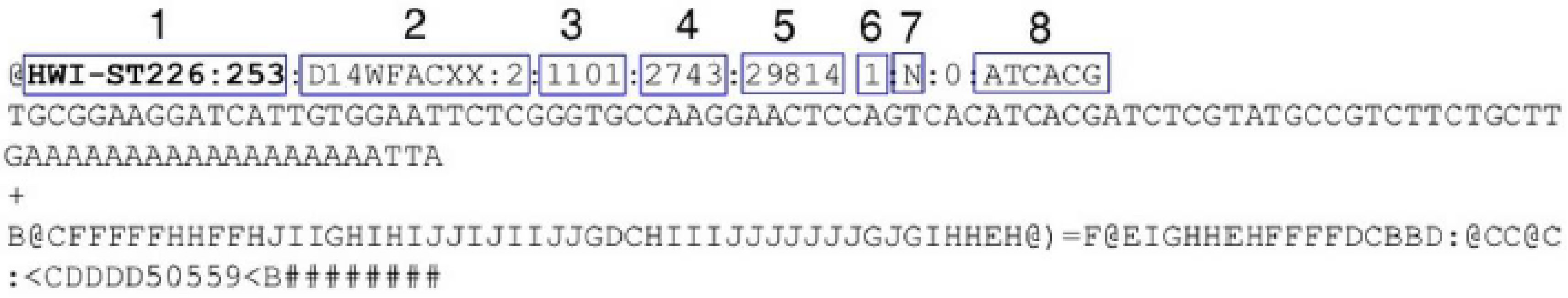
DNA sequences

„Alignment“ → contig (different sequencing reactions from multiple PCR from the same specimen)



Active Layer: HLA-C_CDS

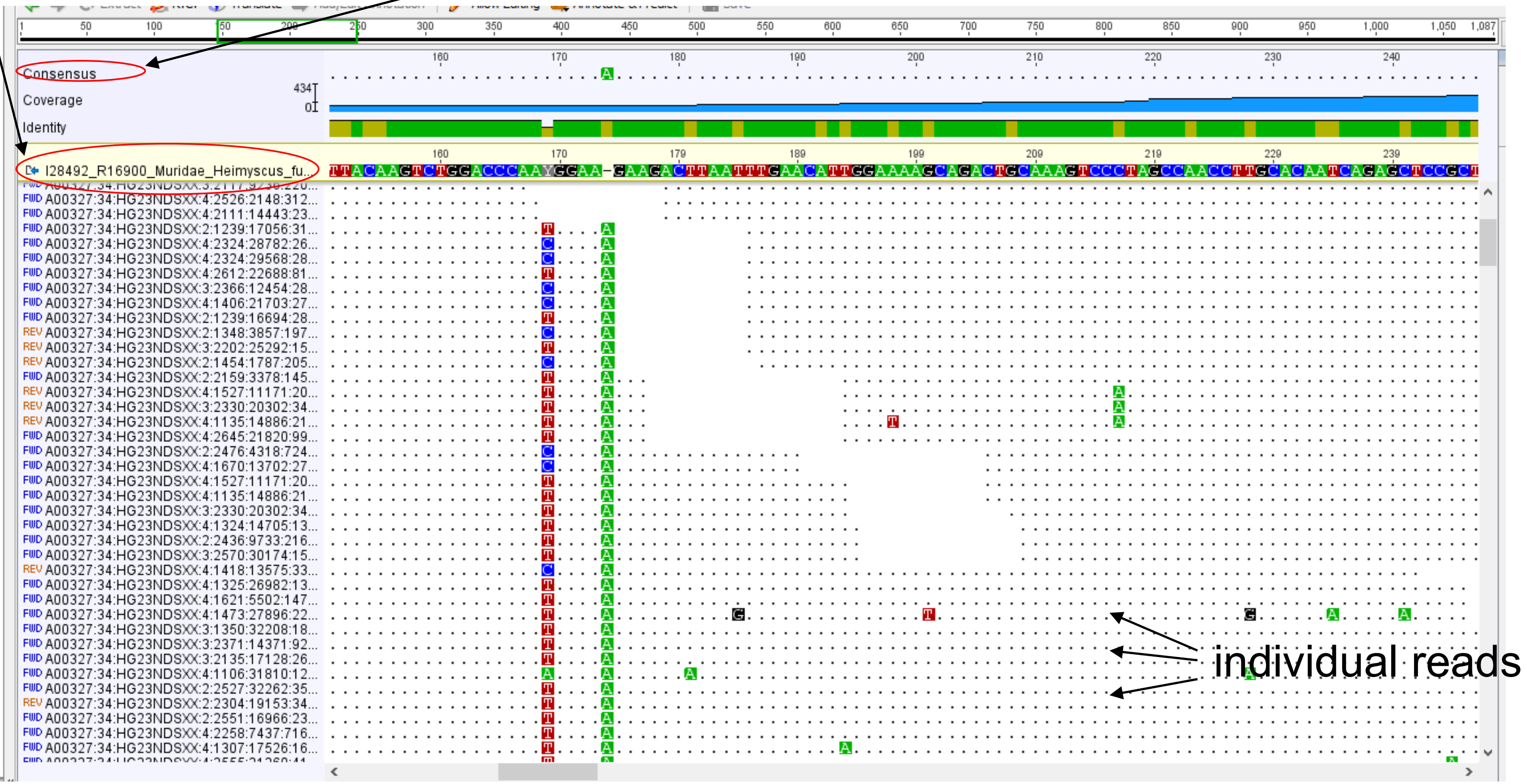
Illumina *fastq* format



```
@HWI-ST226:253:D14WFACXX:2:1101:2743:29814 1:N:0:ATCACG
TGCGGAAGGATCATTGTGGAATTCTCGGGTGCCAAGGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTT
GAAAAAAAAAAAAAAAAAATTA
+
B@CFFFFFHHFFHJIIGHIHIJJIJIIJJGDCHIIIJJJJJJGJGIHHEH@)=F@EIGHHEHFFFFDCBBD:@CC@C
:<CDDDD50559<B########
```

1. unique instrument ID and run ID
2. Flow cell ID and lane
3. tile number within the flow cell lane
4. 'x'-coordinate of the cluster within the tile
5. 'y'-coordinate of the cluster within the tile
6. the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*
7. N if the read passes filter, Y if read fails filter otherwise
8. Index sequence

Contig from „reads“ obtained by HTS (high-throughput sequencing)



```
I28492_R16900_Muridae_Heimyscus_fu...   TTACAAGTCTGGACCCAAYGGAA-GAAGACTTAATTTGAACATTGGAAAAGCAGACTGCAAAGTCCCTAGCCAACCTTGCACAATCAGAGCTCCGCT
FWD A00327:34:HG23NDSXX:4:2526:2148:312...
FWD A00327:34:HG23NDSXX:4:2111:14443:23...
FWD A00327:34:HG23NDSXX:2:1239:17056:31...
FWD A00327:34:HG23NDSXX:4:2324:28782:26...
FWD A00327:34:HG23NDSXX:4:2324:29568:28...
FWD A00327:34:HG23NDSXX:4:2612:22688:81...
FWD A00327:34:HG23NDSXX:3:2366:12454:28...
FWD A00327:34:HG23NDSXX:4:1406:21703:27...
FWD A00327:34:HG23NDSXX:2:1239:16694:28...
REV A00327:34:HG23NDSXX:2:1348:3857:197...
REV A00327:34:HG23NDSXX:3:2202:25292:15...
REV A00327:34:HG23NDSXX:2:1454:1787:205...
FWD A00327:34:HG23NDSXX:2:2159:3378:145...
REV A00327:34:HG23NDSXX:4:1527:11171:20...
REV A00327:34:HG23NDSXX:3:2330:20302:34...
REV A00327:34:HG23NDSXX:4:1135:14886:21...
FWD A00327:34:HG23NDSXX:4:2645:21820:99...
FWD A00327:34:HG23NDSXX:2:2476:4318:724...
FWD A00327:34:HG23NDSXX:4:1670:13702:27...
FWD A00327:34:HG23NDSXX:4:1527:11171:20...
FWD A00327:34:HG23NDSXX:4:1135:14886:21...
FWD A00327:34:HG23NDSXX:3:2330:20302:34...
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FWD A00327:34:HG23NDSXX:3:2570:30174:15...
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FWD A00327:34:HG23NDSXX:4:1621:5502:147...
FWD A00327:34:HG23NDSXX:4:1473:27896:22...
FWD A00327:34:HG23NDSXX:3:1350:32208:18...
FWD A00327:34:HG23NDSXX:3:2371:14371:92...
FWD A00327:34:HG23NDSXX:3:2135:17128:26...
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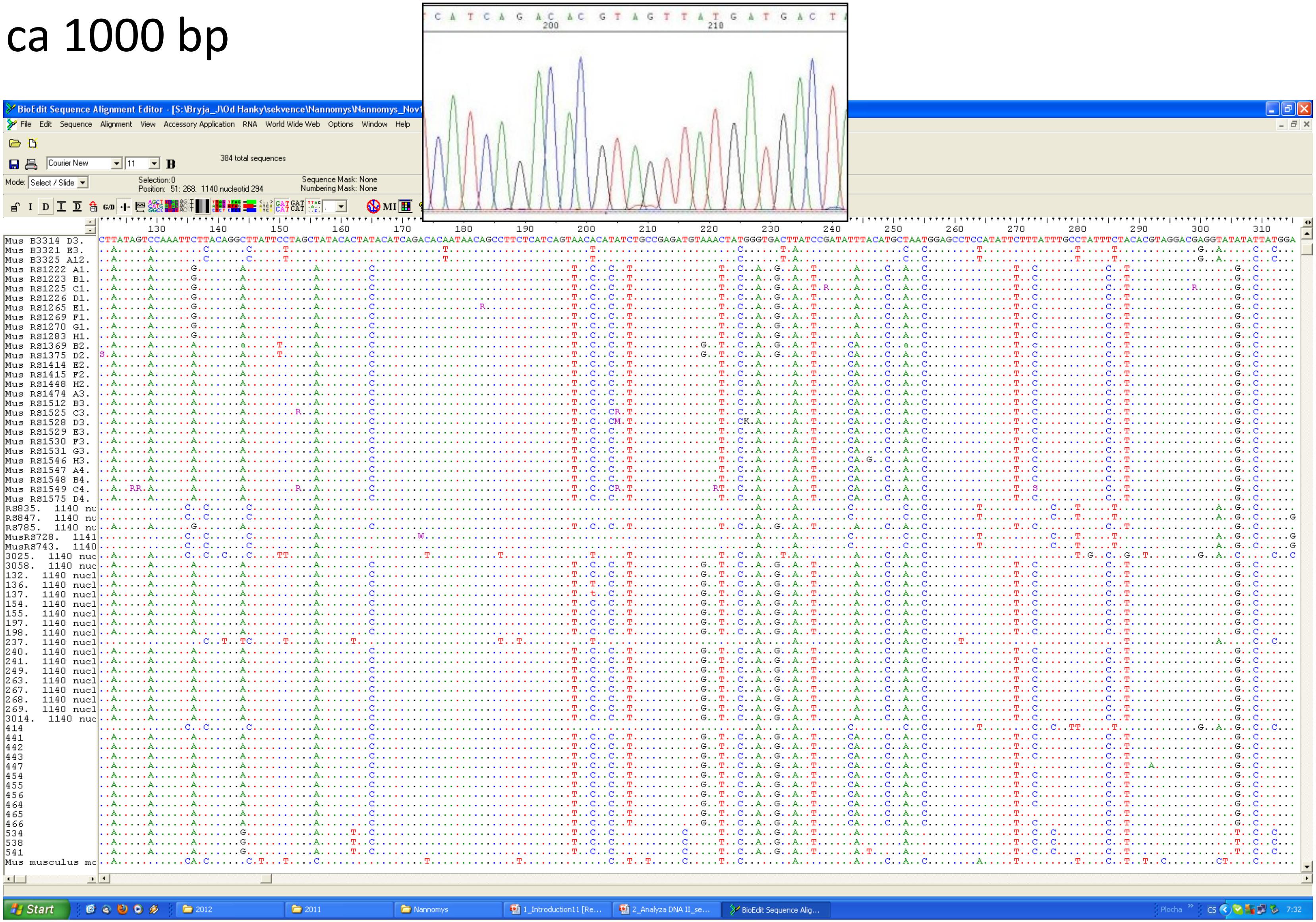
FWD A00327:34:HG23NDSXX:2:2527:32262:35...
REV A00327:34:HG23NDSXX:2:2304:19153:34...
FWD A00327:34:HG23NDSXX:2:2551:16966:23...
FWD A00327:34:HG23NDSXX:4:2258:7437:716...
FWD A00327:34:HG23NDSXX:4:1307:17526:16...
```

Comparison of sequences between different individuals

PCR + Sanger
= ca 1000 bp



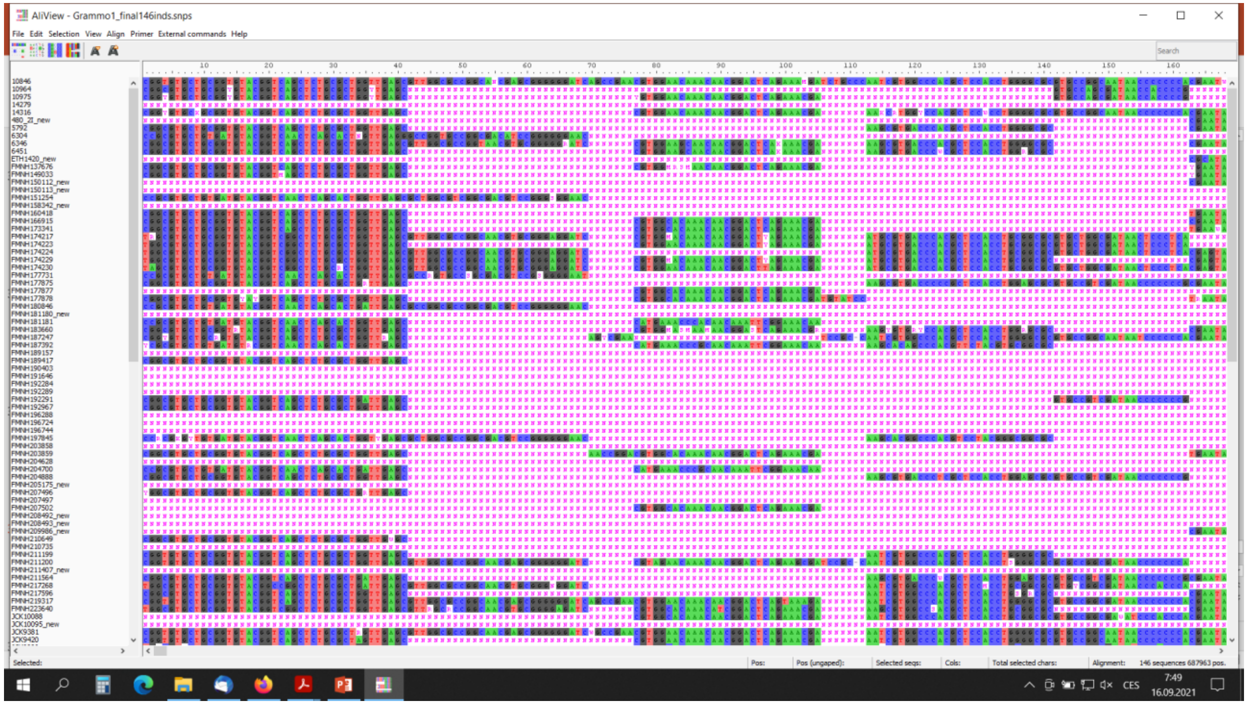
BioEdit Sequence Alignment Editor - [S:\Bryja_J\Od Hanky\sekvence\Nannomys\Nannomys_Nov1

384 total sequences

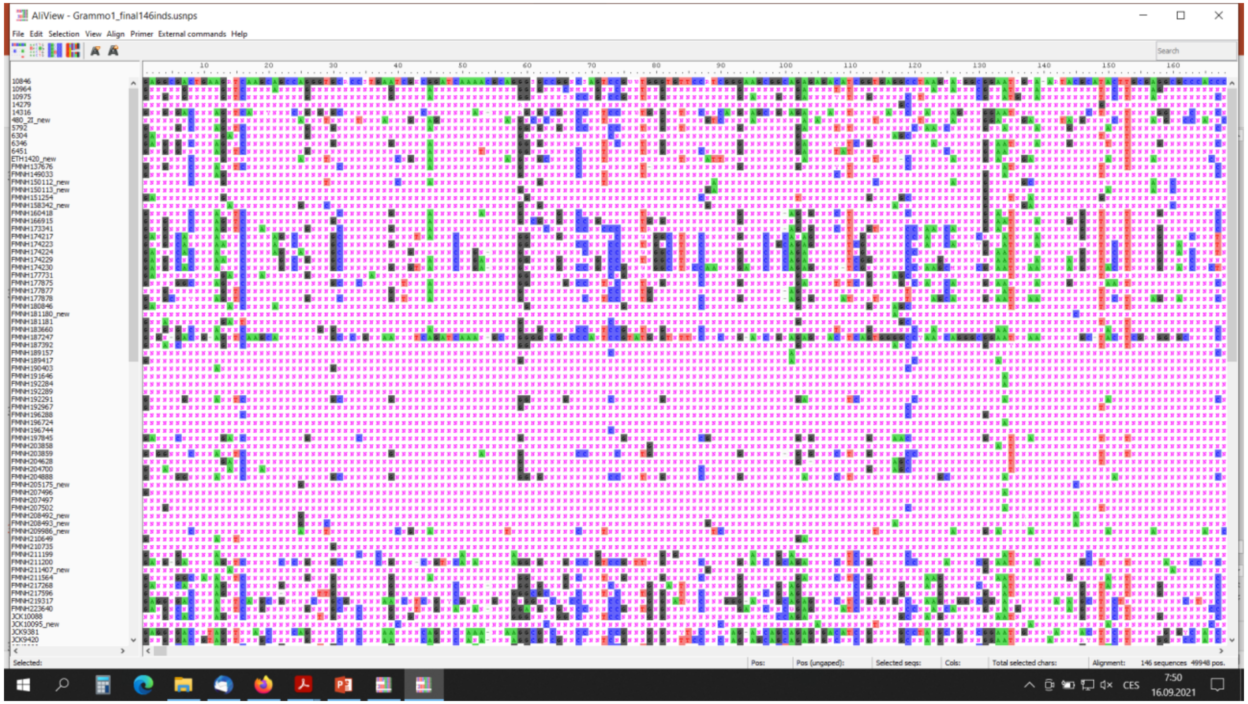
Mode: Select / Slide

Selection: 0
Position: 51: 268. 1140 nucleotid 294

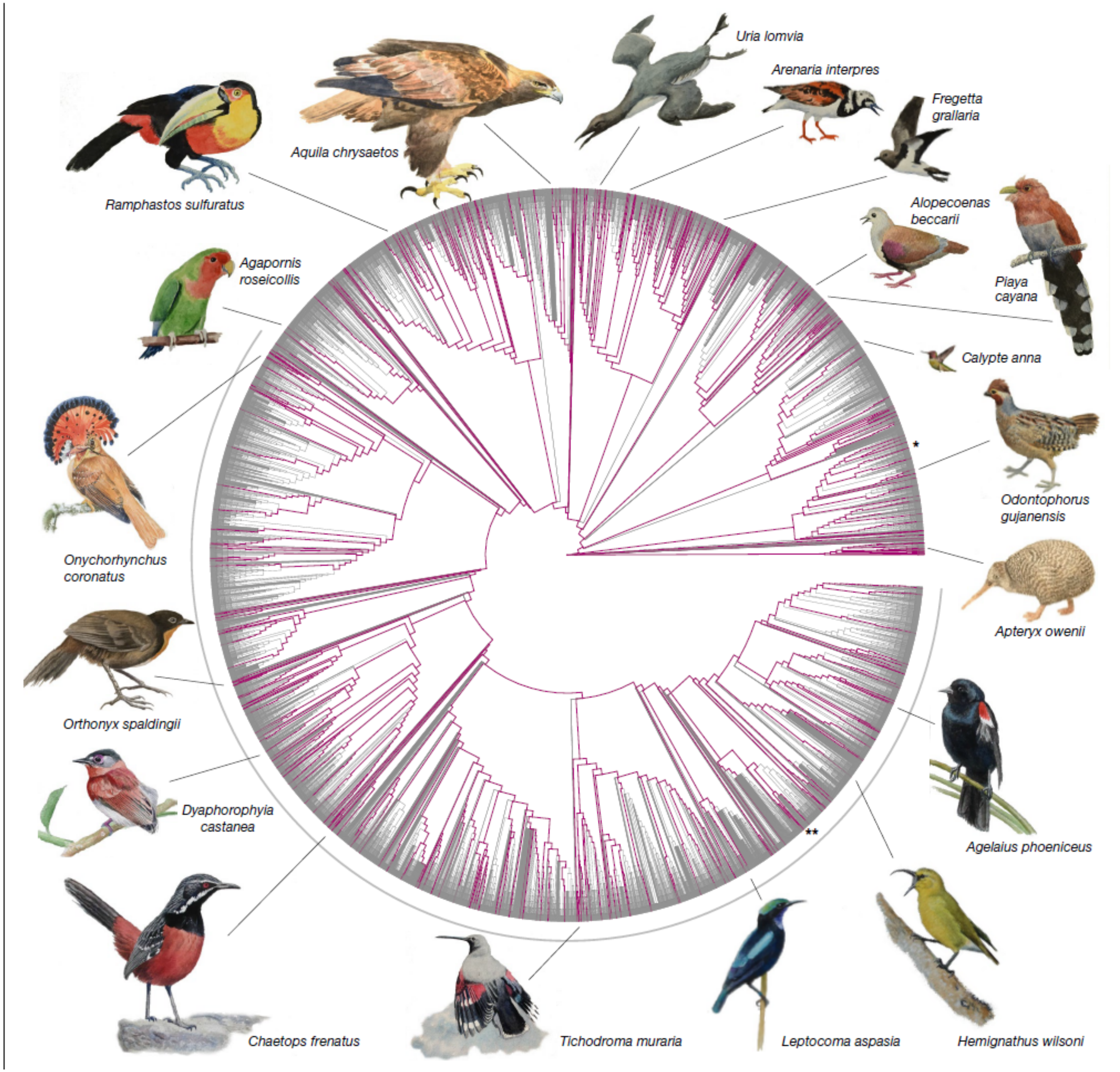
Sequence Mask: None
Numbering Mask: None



ddRAD
(„snps“ file)
= 500 000 bp (závisí na intenzitě sekvenování etc.)
1 row = 1 specimen



ddRAD
(„usnps“ file)
= 1 SNP/locus
= 50 000 SNPs



complete genomes (Feng et al. 2020 Nature)

So, what now with these datasets …