Find the PDF with the presentations here:

bioinformatics.uni-muenster.de/graid/education/presentations

MinION sequencing

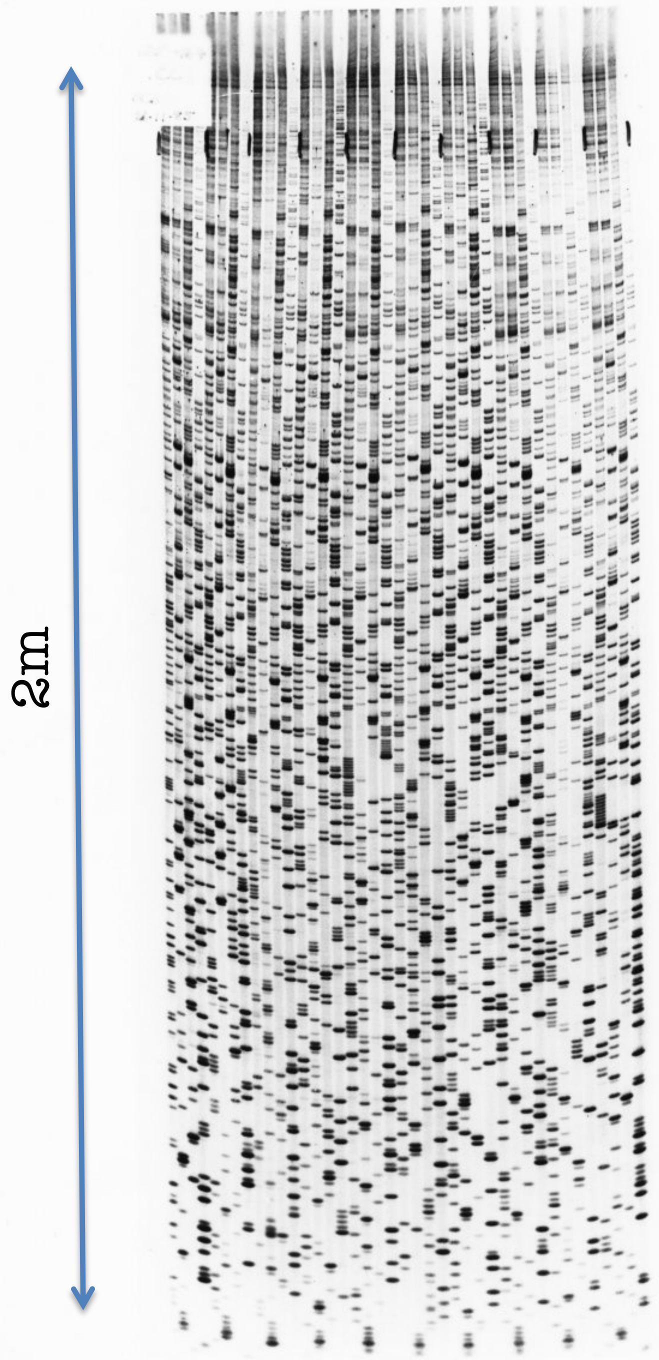
What’s next?

Sapporo, July 7-10 2019

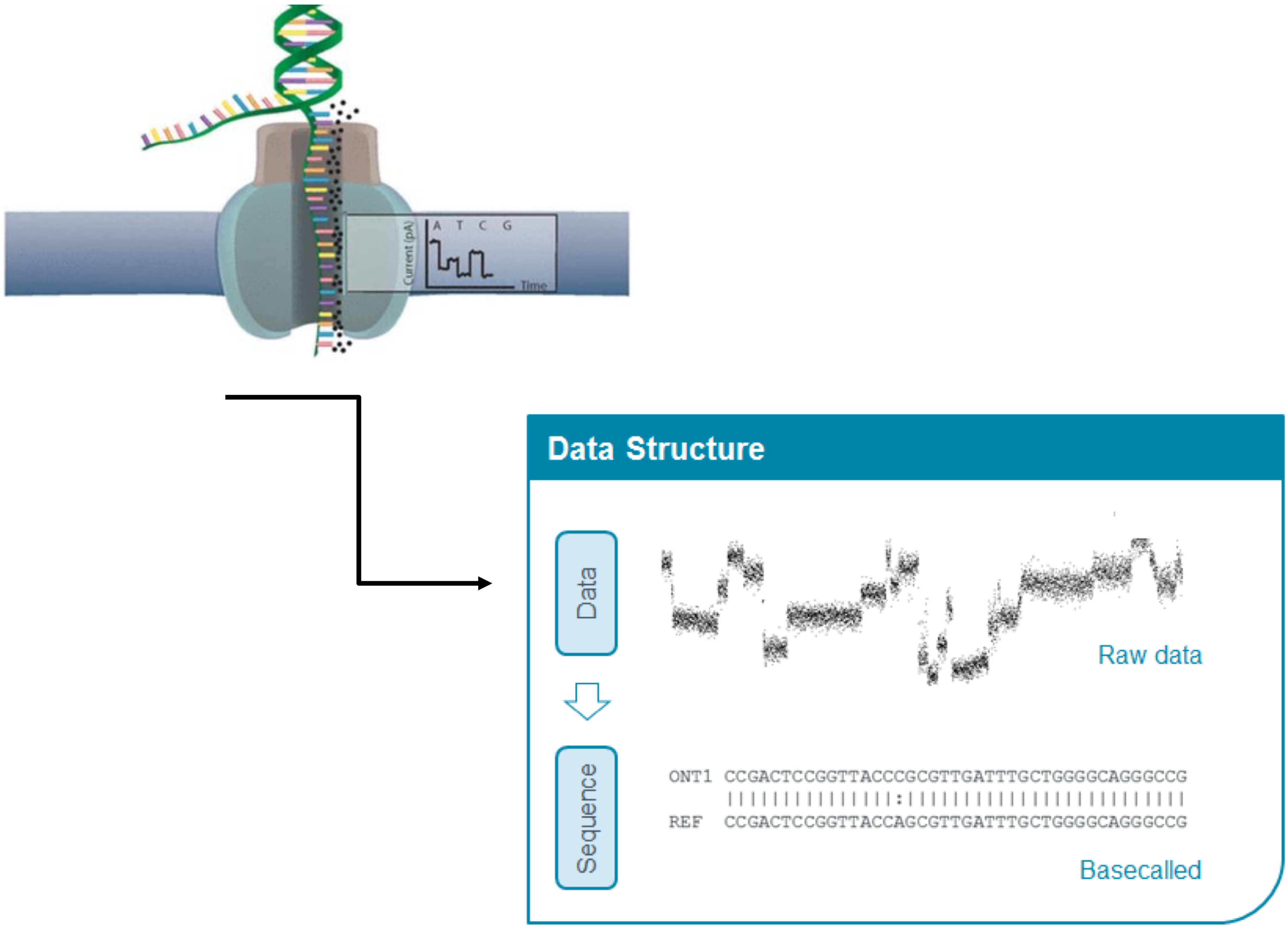
Victoria Shabardina

Elegance of the technical progress ;)

1977

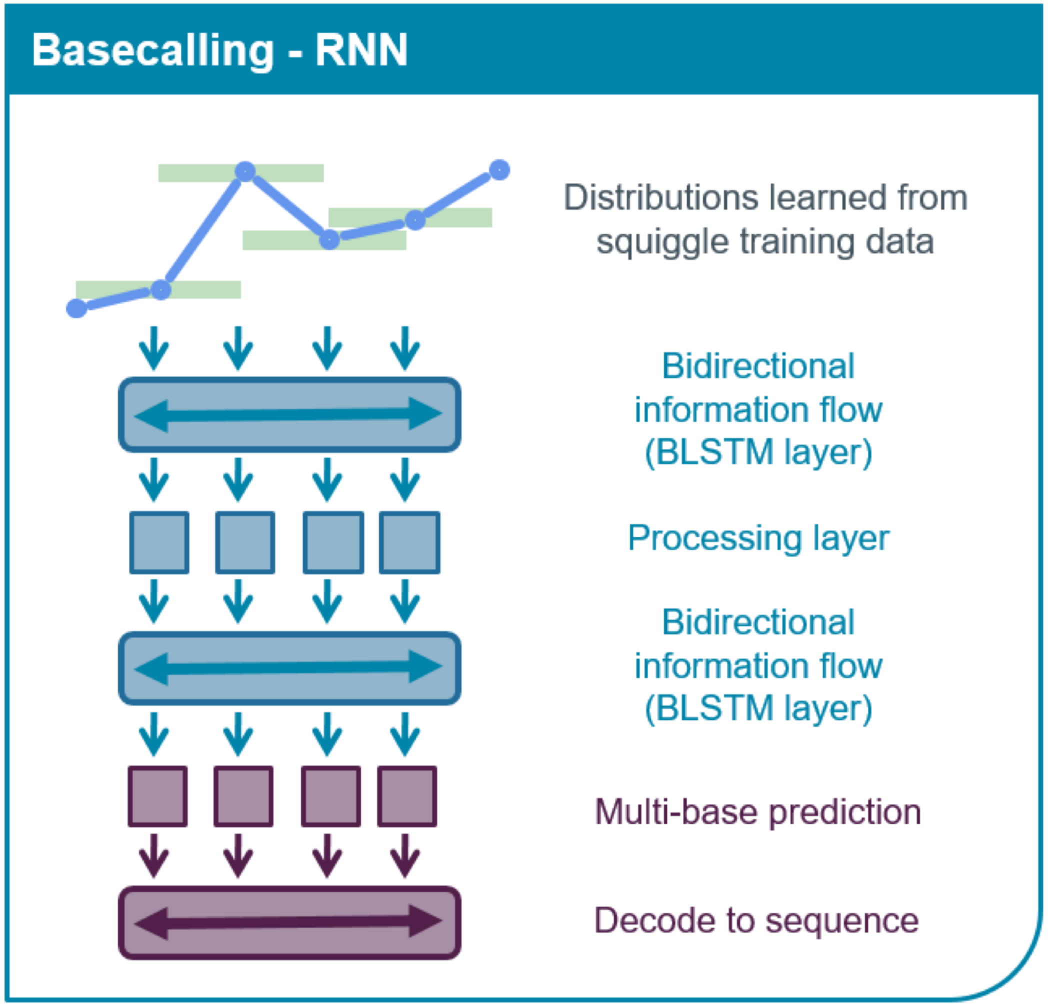


2017

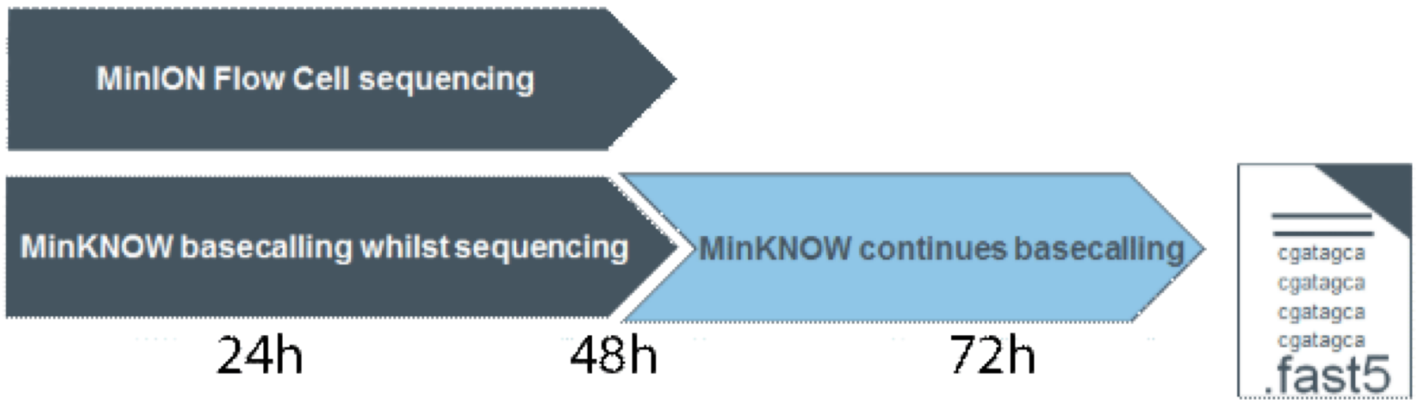


```
ONT1 CCGACTCCGGTTACCCGCGTTGATTTGCTGGGGCAGGGCCG
     ||||||||||||||:||||||||||||||||||||||||||
REF  CCGACTCCGGTTACCAGCGTTGATTTGCTGGGGCAGGGCCG
```

Reccurent Neural Network (RNN) – works like your brain! It can learn on the previous data and improve its performance on new data


Basecalling - RNN
Distributions learned from squiggle training data
Bidirectional information flow (BLSTM layer)
Processing layer
Bidirectional information flow (BLSTM layer)
Multi-base prediction
Decode to sequence

Nanopore basecallers are trained on many sequenced data, so you can run it on your data even if you are sequencing first time


MinION Flow Cell sequencing
MinKNOW basecalling whilst sequencing
MinKNOW continues basecalling
24h
48h
72h
cgatagca
cgatagca
cgatagca
cgatagca
.fast5

MinION sequencing is controlled by MinKNOW software → MinKNOW runs basecalling in parralel → If you stop sequencing MinKNOW stops basecalling !!!

Alternative: Guppy software (command line)

Mind gaps in command line! underscore_saves_from_blanks

or dots.saves.too.for.example

Otherwise the program may be confused…

Basecalling with Guppy, an ONT produced tool-kit

Guppy can do 4 different jobs:	Commands:
1 1D basecalling	*guppy_basecaller*
2 1D2 basecalling	*guppy_basecaller_1d2*
3 Debarcoding (demultiplexing)	*guppy_barcoder*
4 Alignment	*guppy_aligner*

Guppy can be used on Windows, Mac OS, and Linux

Basecalling with Guppy, an ONT produced tool-kit

Guppy can do 4 different jobs:	Commands:
1 1D basecalling (calibration strand)	*guppy_basecaller*
2 1D2 basecalling	*guppy_basecaller_1d2*
3 Debarcoding (demultiplexing)	*guppy_barcoder*
4 Alignment	*guppy_aligner*

Guppy can be used on Windows, Mac OS, and Linux

Basecalling with Guppy

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1 1D basecalling	*guppy_basecaller*
2 1D2 basecalling	*guppy_basecaller_1d2*
3 Debarcoding (demultiplexing)	*guppy_barcoder*
4 Alignment	*guppy_aligner*

Workflow:

guppy_basecaller / MinKNOW → *guppy_barcoder*

guppy_basecaller / MinKNOW → *guppy_aligner*

Basecalling with Guppy

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1 1D basecalling	*guppy_basecaller*
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Workflow:

guppy_basecaller / MinKNOW → *guppy_barcoder*

guppy_basecaller / MinKNOW → *guppy_aligner*

Basecalling with Guppy

How to use your computer efficiently?

Concider: RAM (random-access memory) and number of CPUs (central processing unit).

Guppy_basecaller (1D) uses 1GB per 1 CPU + 4 GB

4 CPUs: 1x4 + 4 = 8 GB of RAM

Guppy_basecaller_1d2 uses 2GB per 1 CPU + 4GB

Basecalling with Guppy

```
guppy_basecaller --help
```

Basecalling with Guppy

```
guppy_basecaller --help
```

One line:

```
guppy_basecaller –i input/reads.fast5  -s output/reads.fastq --flowcell FLO-MIN107
--kit SQK-LSK108 --qscore_filtering –q 0 --num_callers 1 --cpu_threads_per_caller 1 -r
```

Basecalling with Guppy

One line:

```
guppy_basecaller –i input/reads.fast5  -s output/reads.fastq --flowcell FLO-MIN107
--kit SQK-LSK108 --qscore_filtering –q 0 --num_callers 1 --cpu_threads_per_caller 1 -r
```

-i (where is your input files)
-s (where you want to save the output)
--flowcell
--kit
--qscore_filtering (sorts reads into ‘pass‘ and ‘fail‘ folders, min qscore is 7 by default)
-q 0 (writes all reads per run in one FASTQ file, default is 4000 reads per file)
-r – recursive (will go through all files in the folder)
--num_callers and *--cpu_threads_per_caller* tell how much of your computer power to use

--fast5_out (output FAST5 and FASTQ files, default – only FASTQ)
--compress_fastq (generates gzip output file)

RNAseq:
--reverse_sequence (RNA strain goes through the pore backwards)
--u_substitution (T → U)

Basecalling with Guppy

```
guppy_basecaller --help
```

One line:

```
guppy_basecaller –i input/reads.fast5  -s output/reads.fastq --flowcell FLO-MIN107
--kit SQK-LSK108 --qscore_filtering –q 0 --num_callers 1 --cpu_threads_per_caller 1 -r
```

```
guppy_basecaller --print_workflows
```

De-barcoding with Guppy

```
guppy_barcoder --help
```

One line:

```
guppy_barcoder –i input/reads.fastq  -s output/reads.fastq --config configuration.cfg
--barcode_kits EXP-NBD103  -r
```

--config configuration.cfg (default). When using own barcodes – change config file!
--barcode_kits (optional, shortens the time of search)

Output files: de-barcoded reads in several folders, barcoding summary

NanoPipe – interactive tool for MinION sequencing analysis

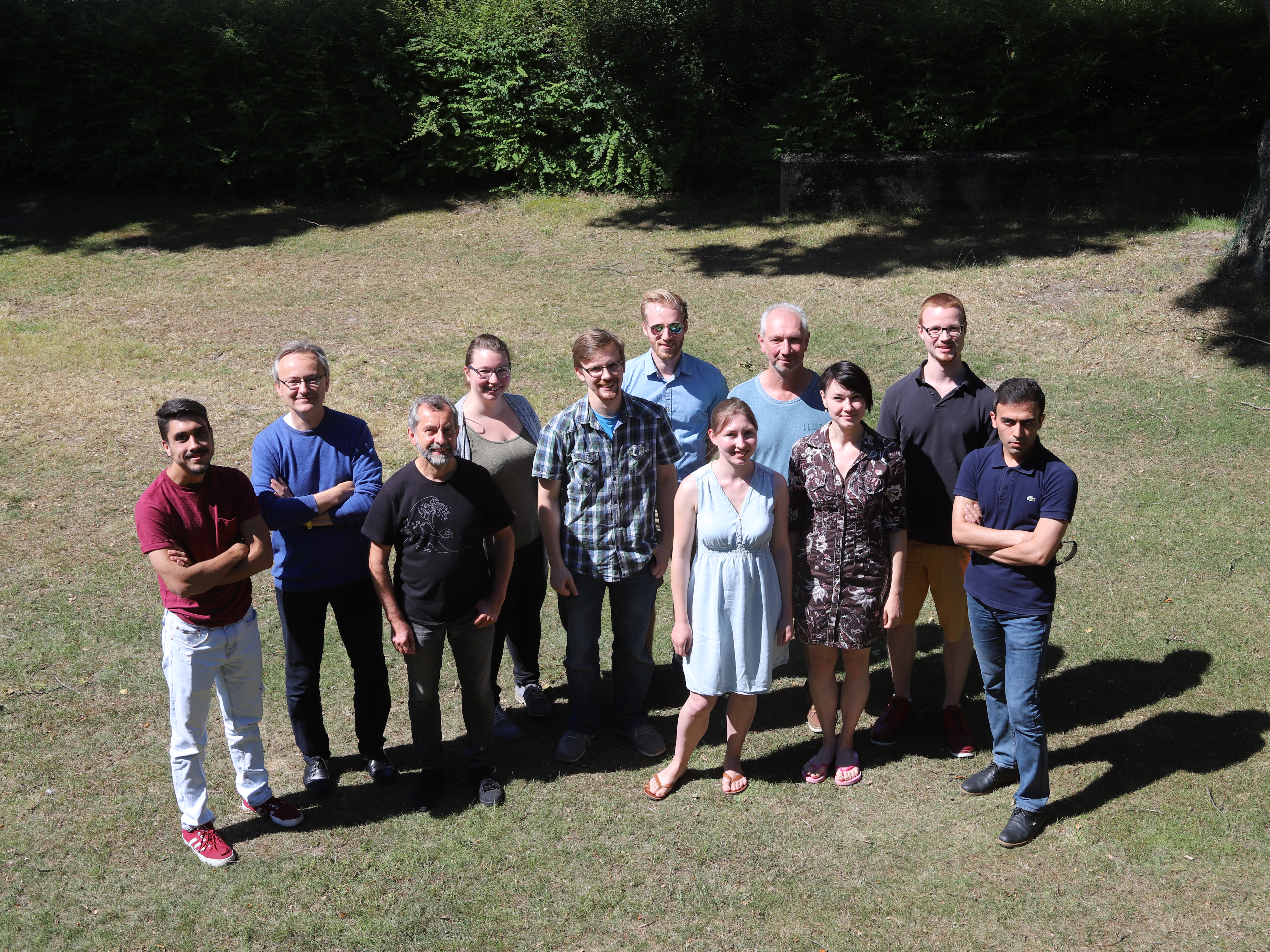
Sapporo, July 7-10 2019

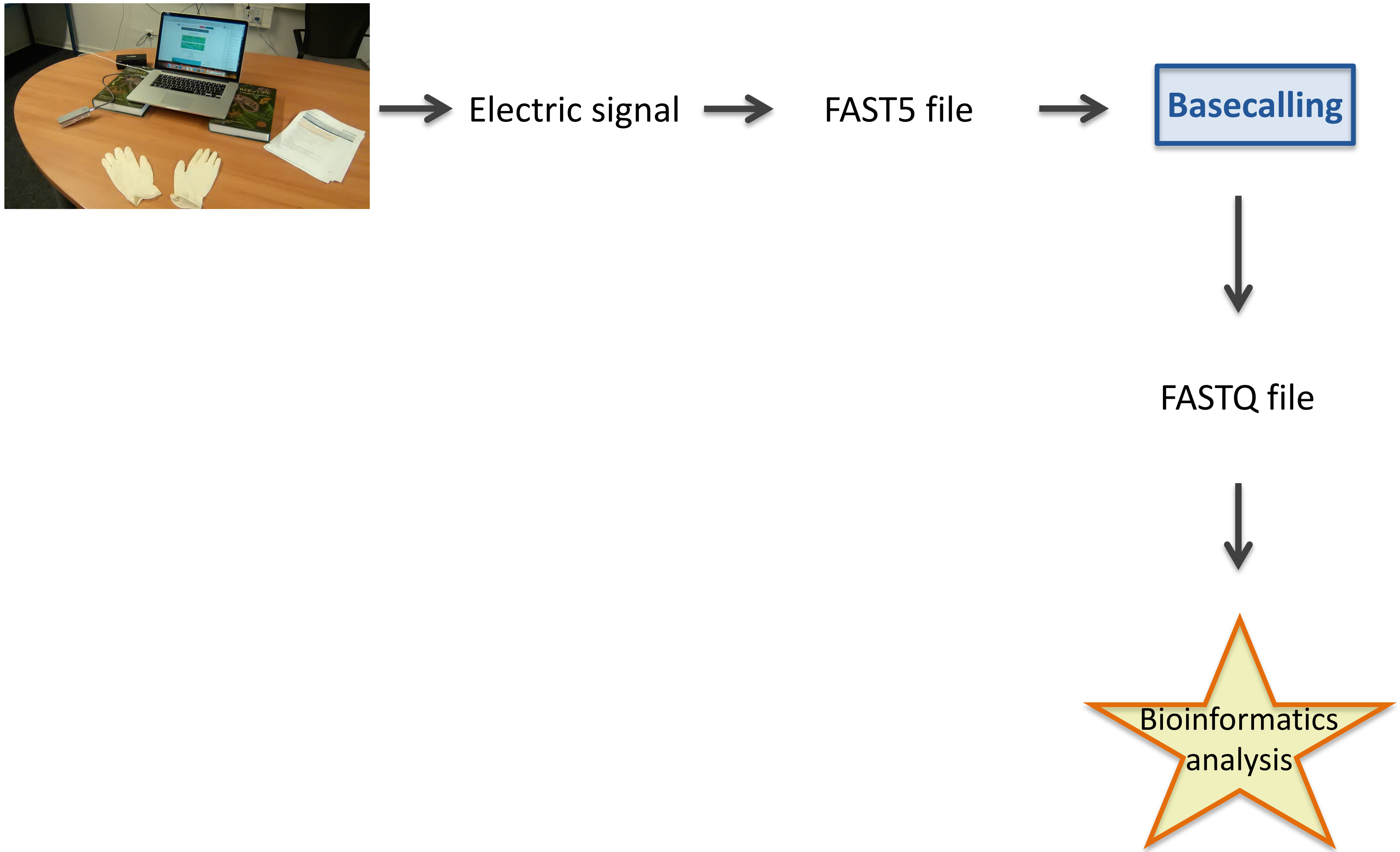
Victoria Shabardina

Nanopipe is a **web-based** tool for **fast and easy** processing

and analysis of the **MinION** sequencing data.

Created in the Institute of Bioinformatics, University of Münster, Germany




Electric signal
FAST5 file
Basecalling
FASTQ file
Bioinformatics analysis

FAST5 file format

FAST5 is a type of the Hierarchical Data Format (HDF) and designed for the storage of big datasets

It is binary – not readable by human

HDFview – the tool to see HGF files:

FASTQ file format

Each entry consists of 4 lines:

1 @header_name_of_sequence/read
2 sequence
3 +
4 quality (coded by ASCII symbols: https://en.wikipedia.org/wiki/FASTQ_format#Encoding)

Poretools is the tool developed for MinION to convert .fasta5 files to .fastq or .fasta files

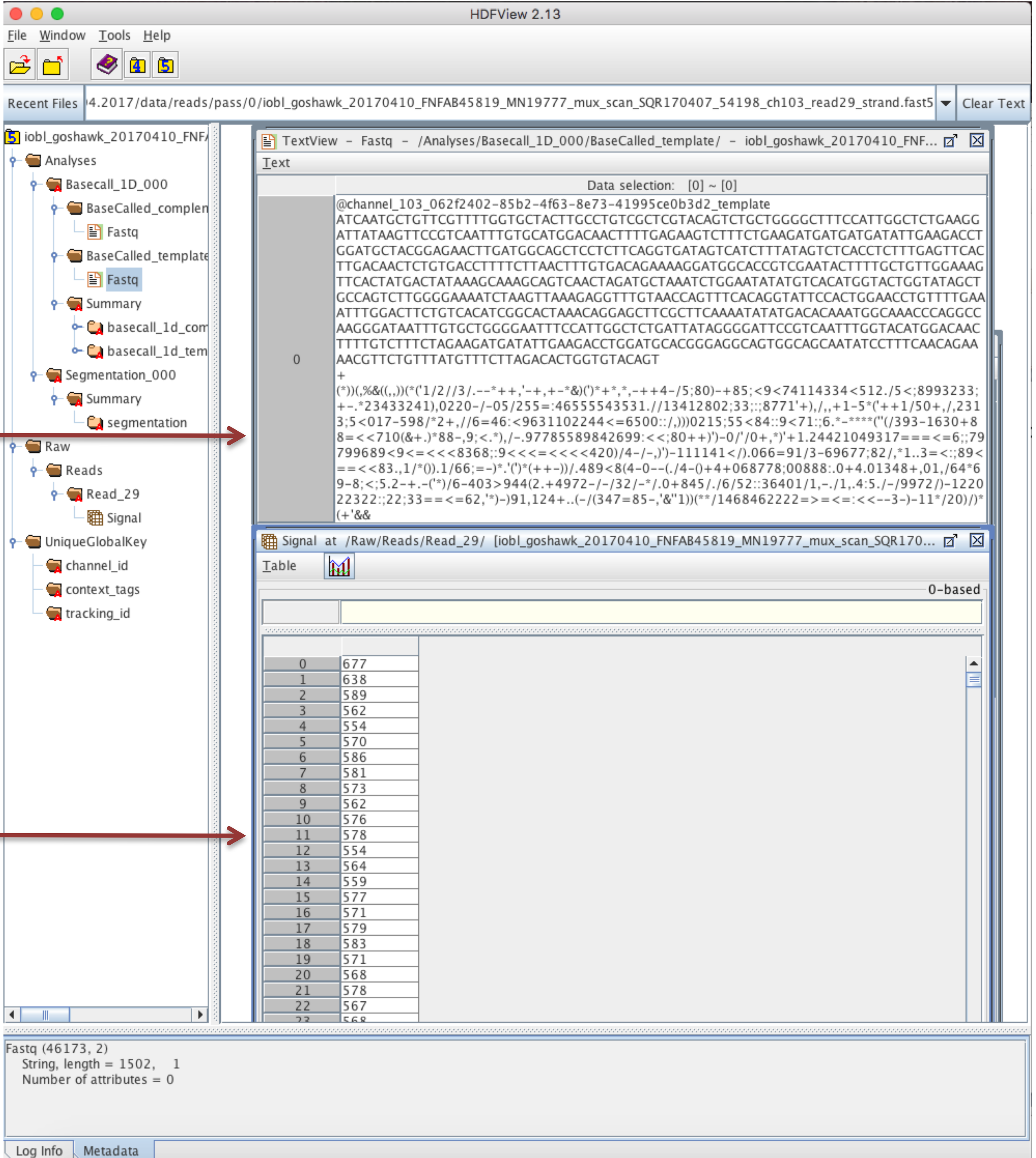
Guppy basecaller allows generating directly .fast5 or .fastq files ☺

HDFviewer window:

(https://support.hdfgroup.org)

FASTQ file

Raw signal
(current from a MinION channel)


HDFView 2.13
File Window Tools Help
Recent Files
4.2017/data/reads/pass/0/iobl_goshawk_20170410_FNFAB45819_MN19777_mux_scan_SQR170407_54198_ch103_read29_strand.fast5
Clear Text
iobl_goshawk_20170410_FNF
Analyses
Basecall_1D_000
BaseCalled_complen
Fastq
BaseCalled_template
Fastq
Summary
basecall_1d_com
basecall_1d_tem
Segmentation_000
Summary
segmentation
Raw
Reads
Read_29
Signal
UniqueGlobalKey
channel_id
context_tags
tracking_id
TextView – Fastq – /Analyses/Basecall_1D_000/BaseCalled_template/ – iobl_goshawk_20170410_FNF...
Text
Data selection: [0] ~ [0]
0
Signal at /Raw/Reads/Read_29/ [iobl_goshawk_20170410_FNFAB45819_MN19777_mux_scan_SQR170...
Table
0-based
Log Info
Metadata

```
@channel_103_062f2402-85b2-4f63-8e73-41995ce0b3d2_template
ATCAATGCTGTTCGTTTTGGTGCTACTTGCCTGTCGCTCGTACAGTCTGCTGGGGCTTTCCATTGGCTCTGAAGG
ATTATAAGTTCCGTCAATTTGTGCATGGACAACTTTTGAGAAGTCTTTCTGAAGATGATGATGATATTGAAGACCT
GGATGCTACGGAGAACTTGATGGCAGCTCCTCTTCAGGTGATAGTCATCTTTATAGTCTCACCTCTTTGAGTTCAC
TTGACAACTCTGTGACCTTTTCTTAACTTTGTGACAGAAAAGGATGGCACCGTCGAATACTTTTGCTGTTGGAAAG
TTCACTATGACTATAAAGCAAAGCAGTCAACTAGATGCTAAATCTGGAATATATGTCACATGGTACTGGTATAGCT
GCCAGTCTTGGGGAAAATCTAAGTTAAAGAGGTTTGTAACCAGTTTCACAGGTATTCCACTGGAACCTGTTTTGAA
ATTTGGACTTCTGTCACATCGGCACTAAACAGGAGCTTCGCTTCAAAATATATGACACAAATGGCAAACCCAGGCC
AAGGGATAATTTGTGCTGGGGAATTTCCATTGGCTCTGATTATAGGGGATTCCGTCAATTTGGTACATGGACAAC
TTTTGTCTTTCTAGAAGATGATATTGAAGACCTGGATGCACGGGAGGCAGTGGCAGCAATATCCTTTCAACAGAA
AACGTTCTGTTTATGTTTCTTAGACACTGGTGTACAGT
+
(*))(,%&((,,))(*('1/2//3/.--*++,'-+,+-*&)(')*+*,*,-++4-/5;80)-+85;<9<74114334<512./5<;8993233;
+-.*23433241),0220-/-05/255=:46555543531.//13412802;33;:;8771'+),/,,+1-5*('++1/50+,/,231
3;5<017-598/*2+,//6=46:<9631102244<=6500::/,)))0215;55<84::9<71:;6.*-****("(/393-1630+8
8=<<710(&+.)*88-,9;<.*),/-.97785589842699:<<;80++)')-0/'/0+,*)'+1.24421049317===<=6;;79
799689<9<=<<<8368;:9<<<=<<<<420)/4-/-,)')-111141</).066=91/3-69677;82/,*1..3=<:;89<
==<<83.,1/*()).1/66;=-)*.'(')*(++-))/.489<8(4-0--(./4-()+4+068778;00888:.0+4.01348+,01,/64*6
9-8;<;5.2-+.-('*)/6-403>944(2.+4972-/-/32/-*/.0+845/./6/52::36401/1,-./1,.4:5./-/9972/)-1220
22322:;22;33==<=62,'*)-)91,124+..(-/(347=85-,'&"1))(**/1468462222=>=<=:<<--3-)-11*/20)/)*
(+'&&
```

0	677
1	638
2	589
3	562
4	554
5	570
6	586
7	581
8	573
9	562
10	576
11	578
12	554
13	564
14	559
15	577
16	571
17	579
18	583
19	571
20	568
21	578
22	567

```
Fastq (46173, 2)
    String, length = 1502,    1
    Number of attributes = 0
```

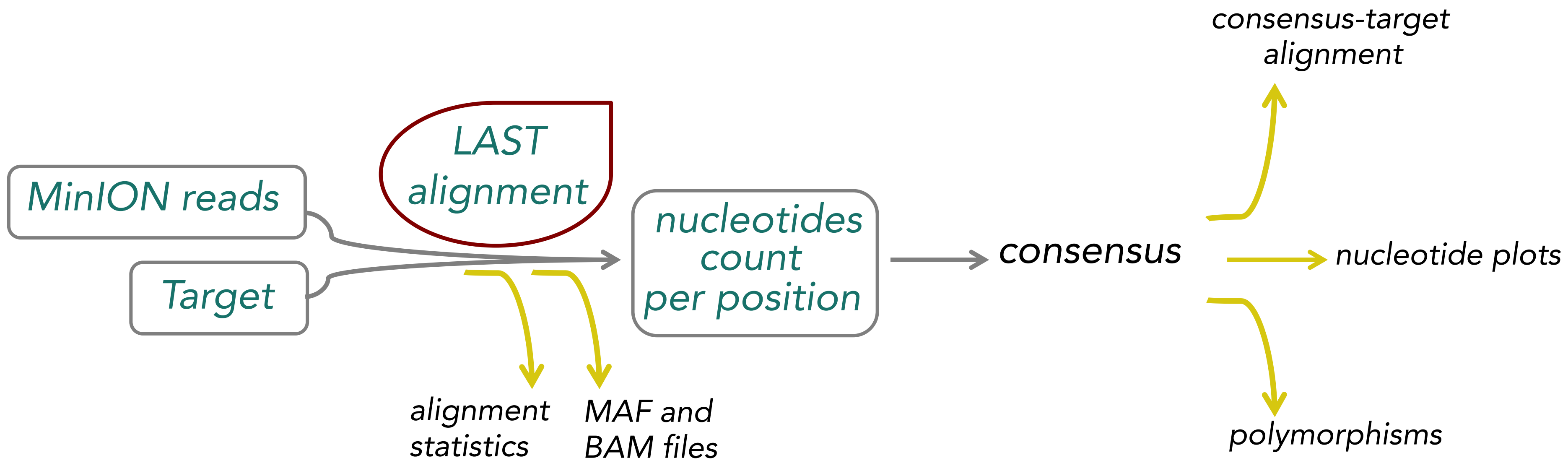
FASTA file format

contains 2 lines:
1 >header
2 sequence

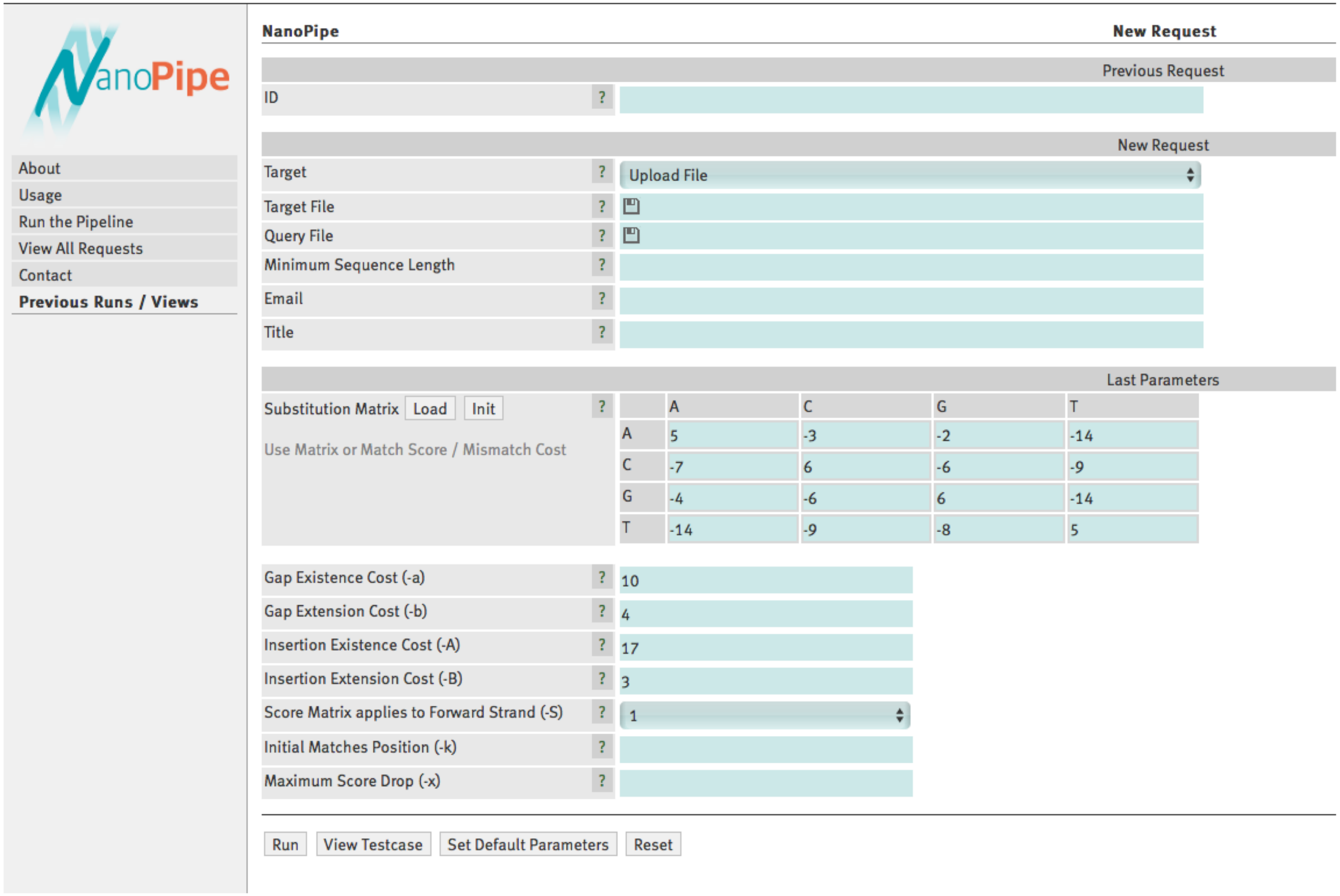
```
>ff6c98dd-bce9-4a2b-bf13-081841413c94_Basecall_2D_minion_20170511_Ae_aegypti
GCGCTGGTTCAGTTACATATTGCTAGGGTTAAGCAGTGGTGACCACAGATTTTTATGATTTATGGATT
CTTTTCTTCTGGCTACATTACTGGAACAGAGCCTGCTTCTCAACAGTGTTCTTATGAACGCTTCAGCTTA
GTATAAAGGC
>4c8a2487-0e13-41a6-ac7b-6cd2fbf5eb95_Basecall_2D_minion_20170511_Ae_aegypti
TACGCGGTGACAAAAACGTGCGTACCGGCAACCGCATGTTGAAACAGGAAAACGTACAAAGGACCC
TCGCAAAATGCGCGACAAAATCTGCAACGTACAACATGCGATAAACGTGCGTGAGGAGATC
>…..
………..
```

> sign is convenient marker to browse through the FASTA files

NanoPipe workflow: what does “pipe” mean?



Web interface



NanoPipe **New Request**

Previous Request

ID ?	

New Request

Target ?	Upload File
Target File ?	
Query File ?	
Minimum Sequence Length ?	
Email ?	
Title ?	

Last Parameters

Substitution Matrix Load Init ?

Use Matrix or Match Score / Mismatch Cost

	A	C	G	T
A	5	-3	-2	-14
C	-7	6	-6	-9
G	-4	-6	6	-14
T	-14	-9	-8	5

Gap Existence Cost (-a) ?	10
Gap Extension Cost (-b) ?	4
Insertion Existence Cost (-A) ?	17
Insertion Extension Cost (-B) ?	3
Score Matrix applies to Forward Strand (-S) ?	1
Initial Matches Position (-k) ?	
Maximum Score Drop (-x) ?	

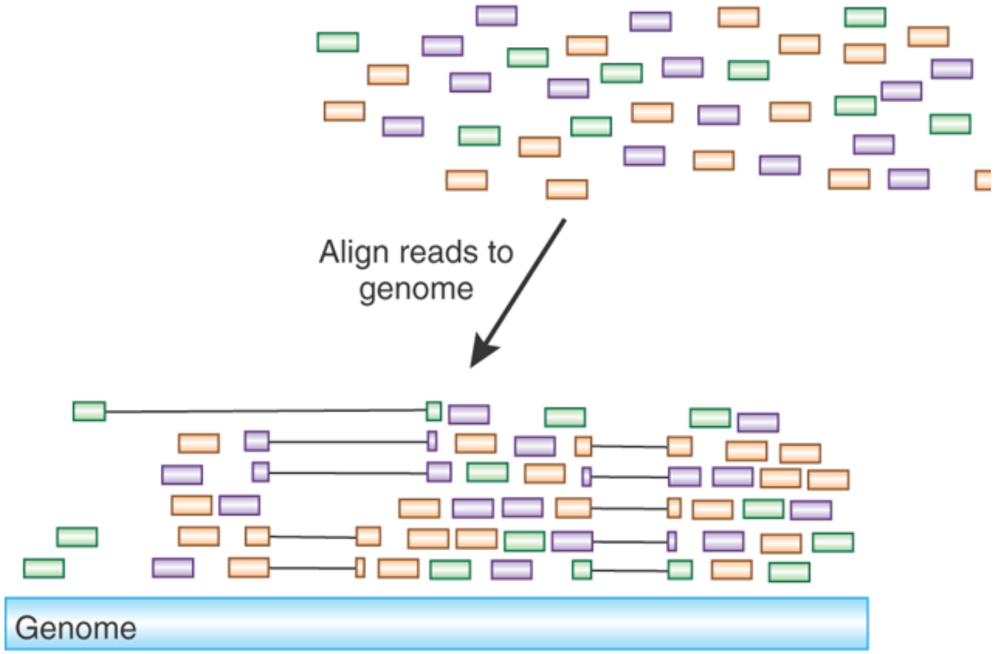
Key steps of NanoPipe: LAST

LAST sequence aligner maps MinIon-produced reads to a target (selected region, exon, gene, genome)

Martin Frith
University of Tokyo,
Division of Biosciences

Query

Target

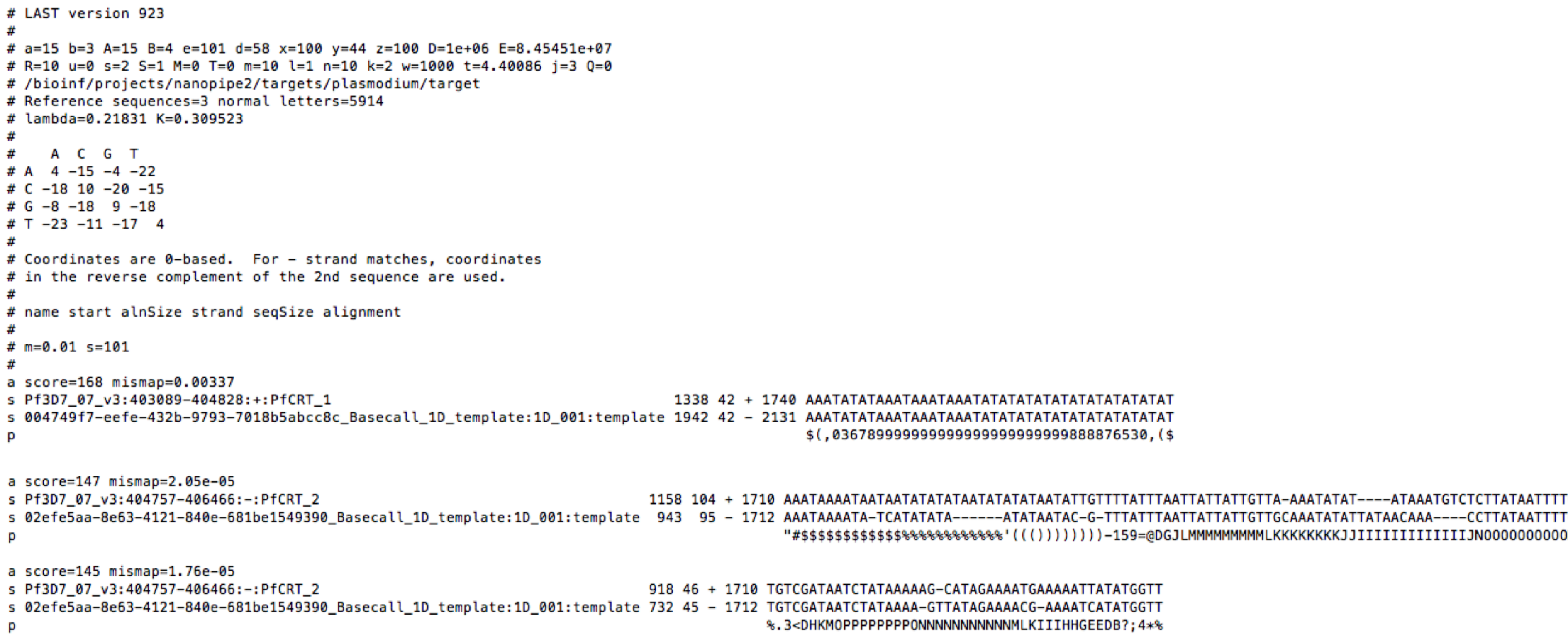


Other aligners: BLAST (psi-BLAST, delta-BLAST), HMMER, MUSCLE, MAFFT…

File formats that are important to know when working with mapped reads:

- .maf
- .sam
- .bam

.maf file format – shows pairs of aligned sequences with the coordinates

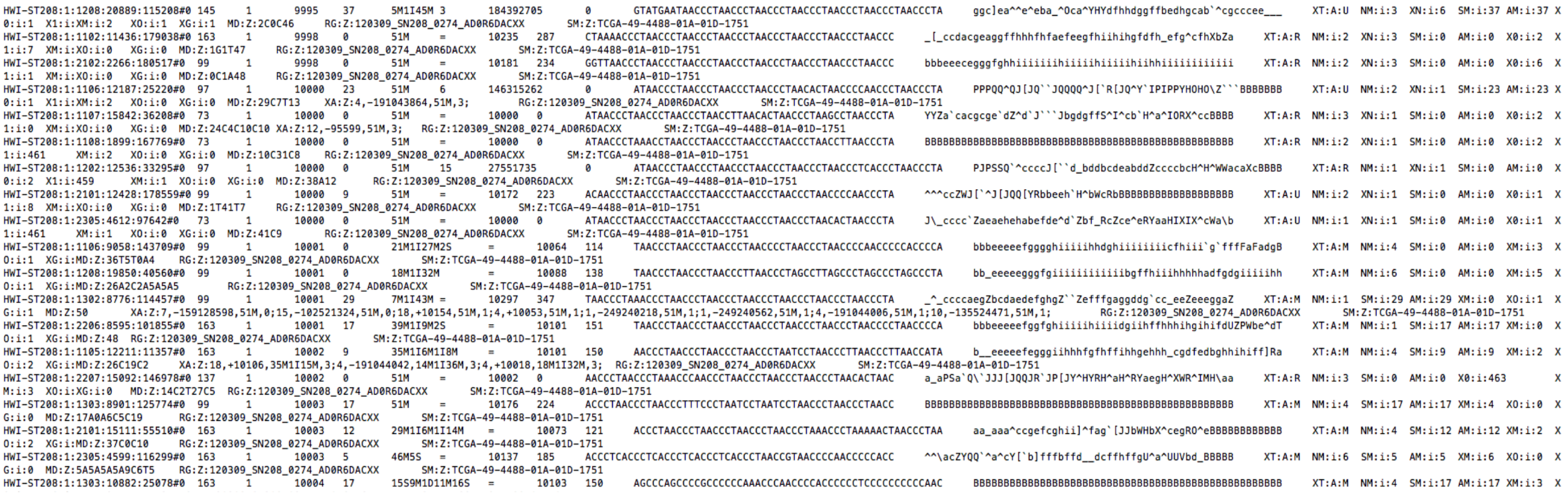


```
# LAST version 923
#
# a=15 b=3 A=15 B=4 e=101 d=58 x=100 y=44 z=100 D=1e+06 E=8.45451e+07
# R=10 u=0 s=2 S=1 M=0 T=0 m=10 l=1 n=10 k=2 w=1000 t=4.40086 j=3 Q=0
# /bioinf/projects/nanopipe2/targets/plasmodium/target
# Reference sequences=3 normal letters=5914
# lambda=0.21831 K=0.309523
#
#    A   C   G   T
# A  4 -15  -4 -22
# C -18 10 -20 -15
# G -8 -18   9 -18
# T -23 -11 -17   4
#
# Coordinates are 0-based.  For - strand matches, coordinates
# in the reverse complement of the 2nd sequence are used.
#
# name start alnSize strand seqSize alignment
#
# m=0.01 s=101
#
a score=168 mismap=0.00337
s Pf3D7_07_v3:403089-404828:+:PfCRT_1                                         1338 42 + 1740 AAATATATAAATAAATAAATATATATATATATATATATATAT
s 004749f7-eefe-432b-9793-7018b5abcc8c_Basecall_1D_template:1D_001:template 1942 42 - 2131 AAATATATAAATAAATAAATATATATATATATATATATATAT
p                                                                                           $(,0367899999999999999999999988876530,($


a score=147 mismap=2.05e-05
s Pf3D7_07_v3:404757-406466:-:PfCRT_2                                        1158 104 + 1710 AAATAAAATAATAATATATATAATATATATAATATTGTTTTATTTAATTATTATTGTTA-AAATATAT----ATAAATGTCTCTTATAATTTT
s 02efe5aa-8e63-4121-840e-681be1549390_Basecall_1D_template:1D_001:template  943  95 - 1712 AAATAAAATA-TCATATATA------ATATAATAC-G-TTTATTTAATTATTATTGTTGCAAATATATTATAACAAA----CCTTATAATTTT
p                                                                                              "#$$$$$$$$$$$$%%%%%%%%%%%%%'((()))))))))-159=@DGJLMMMMMMMMMLKKKKKKKKJJIIIIIIIIIIIIIJNOOOOOOOOOO

a score=145 mismap=1.76e-05
s Pf3D7_07_v3:404757-406466:-:PfCRT_2                                        918 46 + 1710 TGTCGATAATCTATAAAAAG-CATAGAAAATGAAAAATTATATGGTT
s 02efe5aa-8e63-4121-840e-681be1549390_Basecall_1D_template:1D_001:template 732 45 - 1712 TGTCGATAATCTATAAAA-GTTATAGAAAACG-AAAATCATATGGTT
p                                                                                          %.3<DHKMOPPPPPPPPPONNNNNNNNNNNNMLKIIIHHGEEDB?;4*%
```

.bam and .sam file format – informative about alignment of sequences to a target

.bam is a binary file (humans can not read), it contains a lot of information about the alignment.
Important part is the HEADER and FLAGs specifications
(https://broadinstitute.github.io/picard/explain-flags.html)

.sam format is the readable version of .bam (some info is casted away):



```
HWI-ST208:1:1208:20889:115208#0 145     1       9995    37      5M1I45M 3       184392705       0       GTATGAATAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTA     ggc]ea^^e^eba_^Oca^YHYdfhhdggffbedhgcab`^cgcccee___     XT:A:U  NM:i:3  XN:i:6  SM:i:37 AM:i:37 X
0:i:1  X1:i:XM:i:2   XO:i:1  XG:i:1  MD:Z:2C0C46     RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1102:11436:179038#0 163     1       9998    0       51M     =       10235   287     CTAAAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC     _[_ccdacgeaggffhhhfhfaefeegfhiihihgfdfh_efg^cfhXbZa     XT:A:R  NM:i:2  XN:i:3  SM:i:0  AM:i:0  X0:i:2  X
1:i:7  XM:i:XO:i:0   XG:i:0  MD:Z:1G1T47     RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2102:2266:180517#0  99      1       9998    0       51M     =       10181   234     GGTTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC     bbbeeecegggfghhiiiiiihiiiiihiiiiihiihhiiiiiiiiiiii     XT:A:R  NM:i:2  XN:i:3  SM:i:0  AM:i:0  X0:i:6  X
1:i:1  XM:i:XO:i:0   XG:i:0  MD:Z:0C1A48     RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1106:12187:25220#0  97      1       10000   23      51M     6       146315262       0       ATAACCCTAACCCTAACCCTAACCCTAACACTAACCCCAACCCTAACCCTA     PPPQQ^QJ[JQ``JQQQQ^J[`R[JQ^Y`IPIPPYHOHO\Z```BBBBBBB     XT:A:U  NM:i:2  XN:i:1  SM:i:23 AM:i:23 X
0:i:1  X1:i:XM:i:2   XO:i:0  XG:i:0  MD:Z:29C7T13    XA:Z:4,-191043864,51M,3;        RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1107:15842:36208#0  73      1       10000   0       51M     =       10000   0       ATAACCCTAACCCTAACCCTAACCTTAACACTAACCCTAAGCCTAACCCTA     YYZa`cacgcge`dZ^d`J```JbgdgffS^I^cb`H^a^IORX^ccBBBB     XT:A:R  NM:i:3  XN:i:1  SM:i:0  AM:i:0  X0:i:2  X
1:i:0  XM:i:XO:i:0   XG:i:0  MD:Z:24C4C10C10 XA:Z:12,-95599,51M,3;   RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1108:1899:167769#0  73      1       10000   0       51M     =       10000   0       ATAACCCTAAACCTAACCCTAACCCTAACCCTAACCCTAACCTTAACCCTA     BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB     XT:A:R  NM:i:2  XN:i:1  SM:i:0  AM:i:0  X0:i:2  X
1:i:461      XM:i:2  XO:i:0  XG:i:0  MD:Z:10C31C8    RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1202:12536:33295#0  97      1       10000   0       51M     15      27551735        0       ATAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTCACCCTAACCCTA     PJPSSQ`^ccccJ[``d_bddbcdeabddZccccbcH^H^WWacaXcBBBB     XT:A:R  NM:i:1  XN:i:1  SM:i:0  AM:i:0  X
0:i:2  X1:i:459      XM:i:1  XO:i:0  XG:i:0  MD:Z:38A12      RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2101:12428:178559#0 99      1       10000   9       51M     =       10172   223     ACAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCAACCCTA     ^^^ccZWJ[`^J[JQQ[YRbbeeh`H^bWcRbBBBBBBBBBBBBBBBBBBB     XT:A:U  NM:i:2  XN:i:1  SM:i:0  AM:i:0  X0:i:1  X
1:i:8  XM:i:XO:i:0   XG:i:0  MD:Z:1T41T7     RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2305:4612:97642#0   73      1       10000   0       51M     =       10000   0       ATAACCCTAACCCTAACCCTAACCCTAACCCTAACACTAACCCTA     J\_cccc`Zaeaehehabefde^d`Zbf_RcZce^eRYaaHIXIX^cWa\b     XT:A:U  NM:i:1  XN:i:1  SM:i:0  AM:i:0  X0:i:1  X
1:i:461      XM:i:1  XO:i:0  XG:i:0  MD:Z:41C9       RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1106:9058:143709#0  99      1       10001   0       21M1I27M2S      =       10064   114     TAACCCTAACCCTAACCCTAACCCCTAACCCTAACCCCAACCCCCACCCCA     bbbeeeeefggghiiiiihhdghiiiiiiiicfhiii`g`fffFaFadgB     XT:A:M  NM:i:4  SM:i:0  AM:i:0  XM:i:3  X
O:i:1  XG:i:MD:Z:36T5T0A4    RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1208:19850:40560#0  99      1       10001   0       18M1I32M        =       10088   138     TAACCCTAACCCTAACCCTTAACCCTAGCCTTAGCCCTAGCCCTAGCCCTA     bb_eeeeegggfgiiiiiiiiiiibgffhiiihhhhhadfgdgiiiiihh     XT:A:M  NM:i:6  SM:i:0  AM:i:0  XM:i:5  X
O:i:1  XG:i:MD:Z:26A2C2A5A5A5        RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1302:8776:114457#0  99      1       10001   29      7M1I43M =       10297   347     TAACCCTAAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTA     _^_ccccaegZbcdaedefghgZ``Zefffgaggddg`cc_eeZeeeggaZ     XT:A:M  NM:i:1  SM:i:29 AM:i:29 XM:i:0  XO:i:1  X
G:i:1  MD:Z:50       XA:Z:7,-159128598,51M,0;15,-102521324,51M,0;18,+10154,51M,1;4,+10053,51M,1;1,-249240218,51M,1;1,-249240562,51M,1;4,-191044006,51M,1;10,-135524471,51M,1;          RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2206:8595:101855#0  163     1       10001   17      39M1I9M2S       =       10101   151     TAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCTAACCCCA     bbbeeeeefggfghiiiiihiiiidgiihffhhhihgihifdUZPWbe^dT     XT:A:M  NM:i:1  SM:i:17 AM:i:17 XM:i:0  X
O:i:1  XG:i:MD:Z:48  RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1105:12211:11357#0  163     1       10002   9       35M1I6M1I8M     =       10101   150     AACCCTAACCCTAACCCTAACCCTAATCCTAACCCTTAACCCTTAACCATA     b__eeeeefegggiihhhfgfhffihhgehhh_cgdfedbghhihiff]Ra     XT:A:M  NM:i:4  SM:i:9  AM:i:9  XM:i:2  X
O:i:2  XG:i:MD:Z:26C19C2     XA:Z:18,+10106,35M1I15M,3;4,-191044042,14M1I36M,3;4,+10018,18M1I32M,3;  RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2207:15092:146978#0 137     1       10002   0       51M     =       10002   0       AACCCTAACCCTAAACCCAACCCTAACCCTAACCCTAACACTAAC     a_aPSa`Q\`JJJ[JQQJR`JP[JY^HYRH^aH^RYaegH^XWR^IMH\aa     XT:A:R  NM:i:3  SM:i:0  AM:i:0  X0:i:463        X
M:i:3  XO:i:XG:i:0   MD:Z:14C2T27C5  RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1303:8901:125774#0  99      1       10003   17      51M     =       10176   224     ACCCTAACCCTAACCCTTTCCCTAATCCTAATCCTAACCCTAACCCTAACC     BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB     XT:A:M  NM:i:4  SM:i:17 AM:i:17 XM:i:4  XO:i:0  X
G:i:0  MD:Z:17A0A6C5C19      RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2101:15111:55510#0  163     1       10003   12      29M1I6M1I14M    =       10073   121     ACCCTAACCCTAACCCTAACCCTAACCCTAAACCCTAAAAACTAACCCTAA     aa_aaa^ccgefcghii]^fag`[JJbWHbX^cegRO^eBBBBBBBBBBB     XT:A:M  NM:i:4  SM:i:12 AM:i:12 XM:i:2  X
O:i:2  XG:i:MD:Z:37C0C10     RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:2305:4599:116299#0  163     1       10003   5       46M5S   =       10137   185     ACCCTCACCCTCACCCTCACCCTCACCCTAACCGTAACCCCAACCCCCACC     ^^\acZYQQ`^a^cY[`b]fffbffd__dcffhffgU^a^UUVbd_BBBBB     XT:A:M  NM:i:6  SM:i:5  AM:i:5  XM:i:6  XO:i:0  X
G:i:0  MD:Z:5A5A5A5A9C6T5    RG:Z:120309_SN208_0274_AD0R6DACXX       SM:Z:TCGA-49-4488-01A-01D-1751
HWI-ST208:1:1303:10882:25078#0  163     1       10004   17      15S9M1D11M16S   =       10103   150     AGCCCAGCCCCGCCCCCCAAACCCAACCCCACCCCCCTCCCCCCCCCAAC     BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB     XT:A:M  NM:i:4  SM:i:17 AM:i:17 XM:i:3  X
```

Summary: files' formats

Files' formats used for storing sequences:

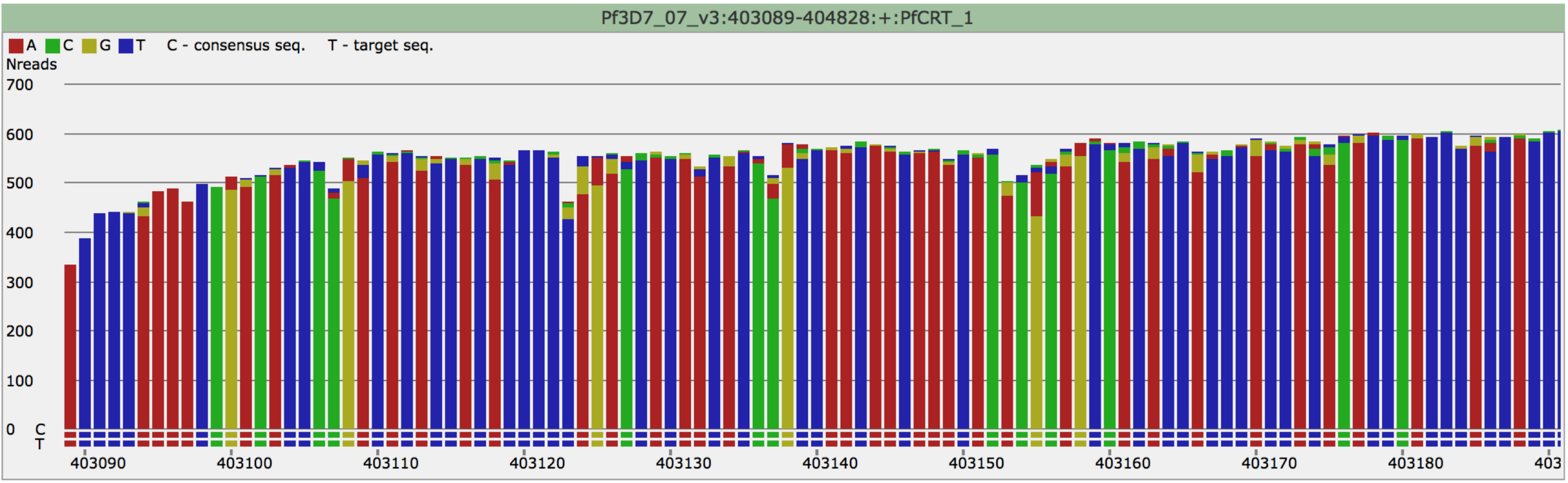
FAST5– big, binary (cant read), contain a lot of metadata
FASTQ– readable by human, contains sequences and sequence quality
FASTA– readable, contains sequences

Files' formats used for storing results of sequence alignment:

maf – contains pairs of aligned sequences with the alignment's coordinates; for example, used by LAST
bam – binary format, includes aligned sequences, coordinates, information about bioinformatics processing, quality, …
sam – human readable version of .bam, much bigger in size

FASTQ, FASTA and bam files are widely used in all DNA/RNA bioinformatics analysis

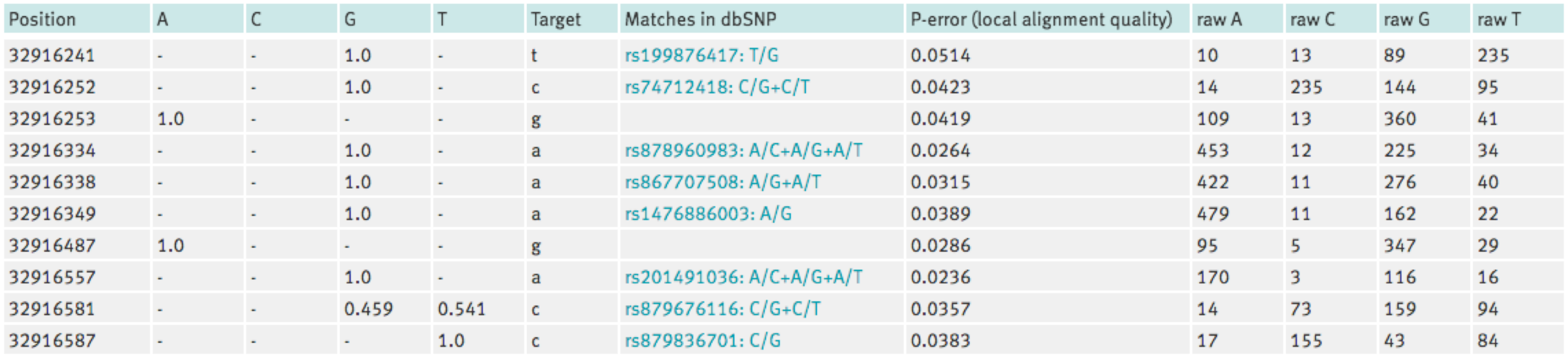
Key Steps of NanoPipe: Consensus Sequence



– majority rule, i.e. position coverage should be >50%

Key steps of Nanopipe: SNP detection

NanoPipe traces potential mutations: SNP analysis, the rule of 20%



Position	A	C	G	T	Target	Matches in dbSNP	P-error (local alignment quality)	raw A	raw C	raw G	raw T
32916241	-	-	1.0	-	t	rs199876417: T/G	0.0514	10	13	89	235
32916252	-	-	1.0	-	c	rs74712418: C/G+C/T	0.0423	14	235	144	95
32916253	1.0	-	-	-	g		0.0419	109	13	360	41
32916334	-	-	1.0	-	a	rs878960983: A/C+A/G+A/T	0.0264	453	12	225	34
32916338	-	-	1.0	-	a	rs867707508: A/G+A/T	0.0315	422	11	276	40
32916349	-	-	1.0	-	a	rs1476886003: A/G	0.0389	479	11	162	22
32916487	1.0	-	-	-	g		0.0286	95	5	347	29
32916557	-	-	1.0	-	a	rs201491036: A/C+A/G+A/T	0.0236	170	3	116	16
32916581	-	-	0.459	0.541	c	rs879676116: C/G+C/T	0.0357	14	73	159	94
32916587	-	-	-	1.0	c	rs879836701: C/G	0.0383	17	155	43	84

NanoPipe helps us to…

- See if our sequencing worked: how many reads were mapped to the target and where exactly, what part of each read mapped (Alignments length distribution)

- Detect insertions/deletions and single nucleotide variations

- Visualization of the experiment in NanoPipe and in IGV-viewer (bam file)

- FASTA file with the consensus sequence

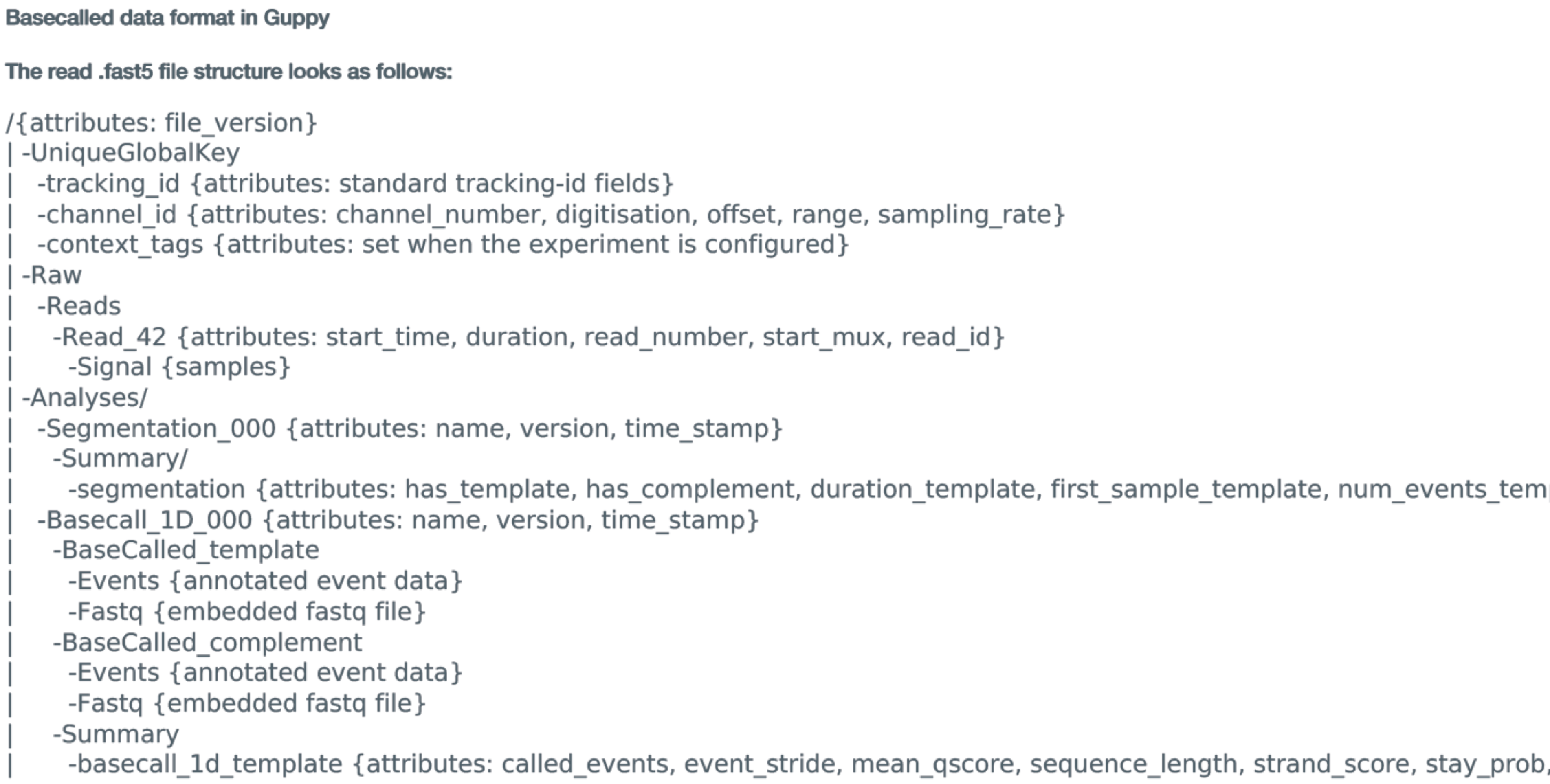
Practical session

Go to http://bioinformatics.uni-muenster.de/tools/nanopipe

http://bioinformatics.uni-muenster.de/tools/nanopipe/generate/register.pl

bioinformatics.uni-muenster.de/graid/education/presentations

FAST5 file structure of a basecalled read



Basecalled data format in Guppy

The read .fast5 file structure looks as follows:

```
/{attributes: file_version}
| -UniqueGlobalKey
|   -tracking_id {attributes: standard tracking-id fields}
|   -channel_id {attributes: channel_number, digitisation, offset, range, sampling_rate}
|   -context_tags {attributes: set when the experiment is configured}
| -Raw
|   -Reads
|     -Read_42 {attributes: start_time, duration, read_number, start_mux, read_id}
|       -Signal {samples}
| -Analyses/
|   -Segmentation_000 {attributes: name, version, time_stamp}
|     -Summary/
|       -segmentation {attributes: has_template, has_complement, duration_template, first_sample_template, num_events_tem
|   -Basecall_1D_000 {attributes: name, version, time_stamp}
|     -BaseCalled_template
|       -Events {annotated event data}
|       -Fastq {embedded fastq file}
|     -BaseCalled_complement
|       -Events {annotated event data}
|       -Fastq {embedded fastq file}
|     -Summary
|       -basecall_1d_template {attributes: called_events, event_stride, mean_qscore, sequence_length, strand_score, stay_prob,
```