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Assembly of MHC with hybrid data

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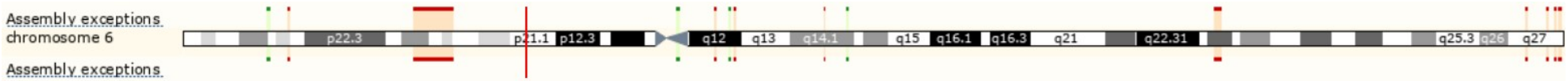
group of Dr. Alexander Dilthey
joint project with Prof. Dr. Birgit Henrich

Group Seminar
22.05.2019

MHC (Major Histocompatibility Complex)

Human genome: chromosome 6



ensembl.org

Length: 171 Mbp
MHC locus: ~ 28.7 – 33.4 Mbp

Goal

=> Get a better assembly of the haplotype of the MHC (Major Histocompatibility Complex) region for different cell lines

cell line	**origin**
QBL	dutch, blood (EBV-transformed lymphoblastoid)
SSTO	amish, blood
MANN/MOU	danish, blood
DBB	amish, blood
APD	not specified
MCF7	caucasian, breast adenocarcinoma
COX	south african, unknown
PGF	english, blood

Goal

=> Get a better assembly of the haplotype of the MHC (Major Histocompatibility Complex) region for different cell lines

cell line

QBL

SSTO

MANN/MOU

DBB

APD

MCF7

~~COX~~

~~PGF~~

Already assembled

Goal

=> Get a better assembly of the haplotype of the MHC (Major Histocompatibility Complex) region for different cell lines

cell line

QBL

SSTO

MANN/MOU

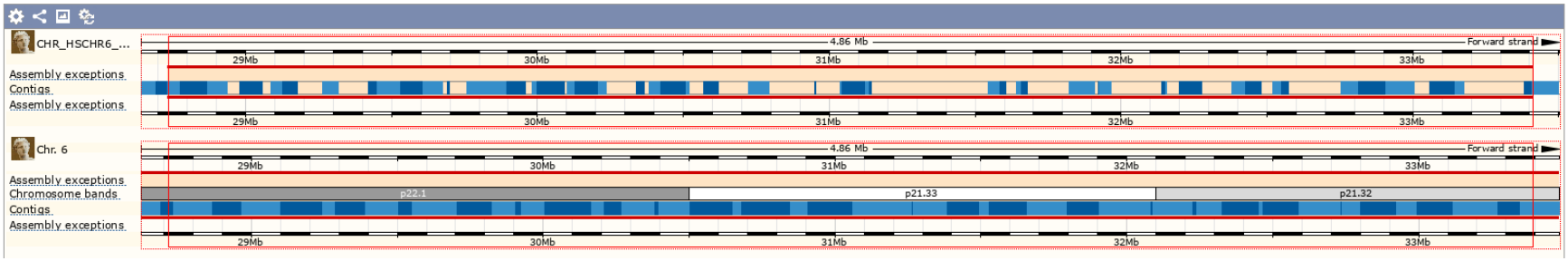
DBB

APD

MCF7

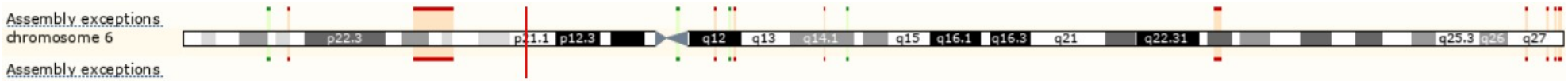
~~COX~~

~~PGF~~



Goal

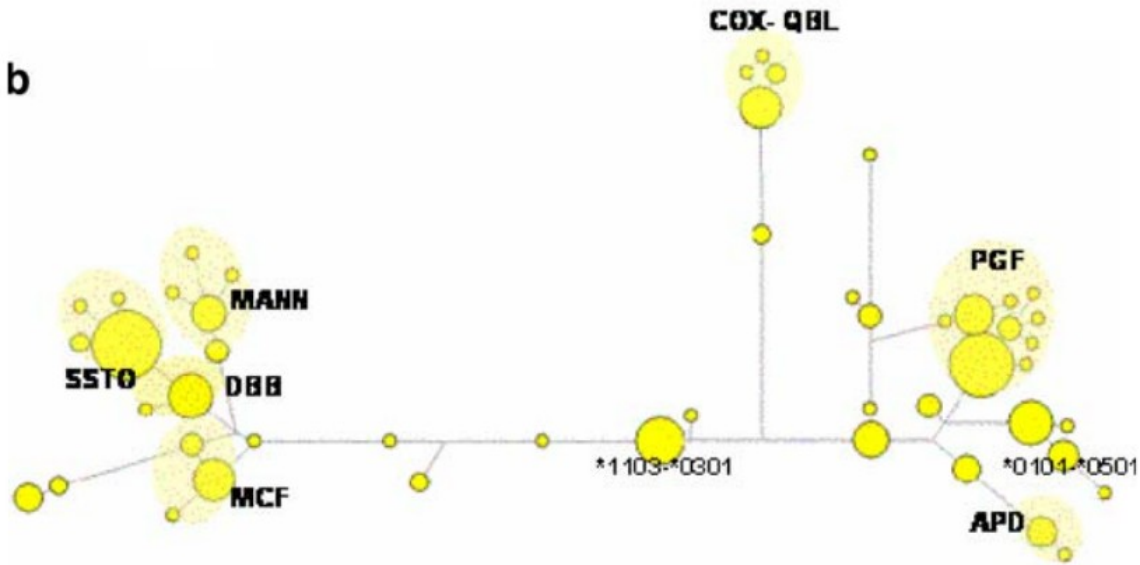
Human genome: chromosome 6



ensembl.org

Length: 171 Mbp
MHC locus: ~ 28.7 – 33.4 Mbp

"… were selected on the basis of conferring either protection against or susceptibility to two autoimmune diseases, type 1 diabetes and multiple sclerosis, and that represented common haplotypes in European populations"

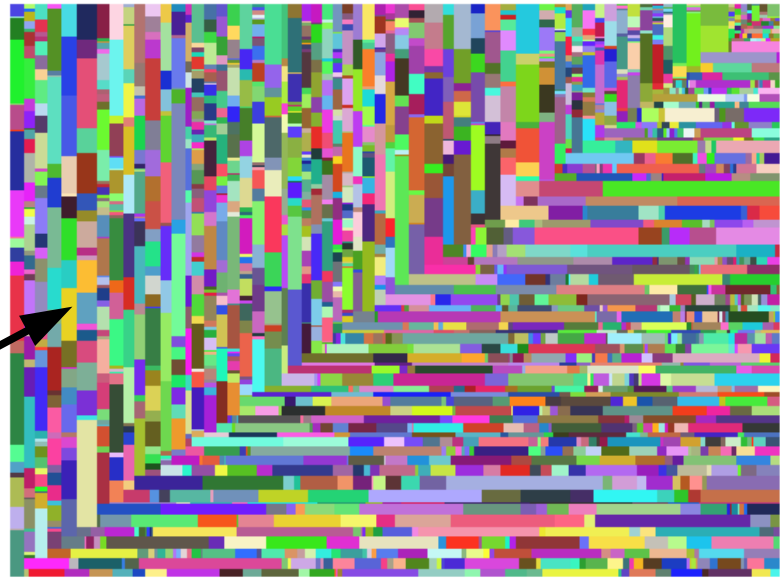


2008 "Variation analysis and gene annotation of eight MHC haplotypes: The MHC Haplotype Project" doi: 10.1007/s00251-007-0262-2

Short Read data

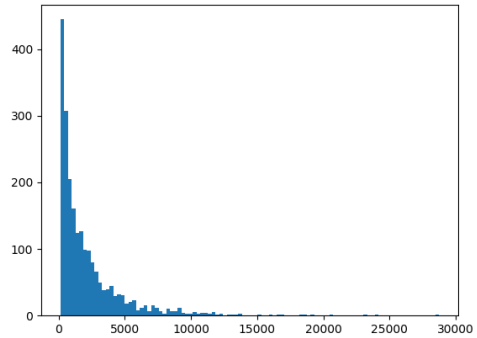
contig 1 ATGGAATCGTGTTG**CTCTCTCTCTCTCTCTCTCT**TAGGTCGCTCCAGTAG **contig 2**

The contig length distribution of the APD cell line



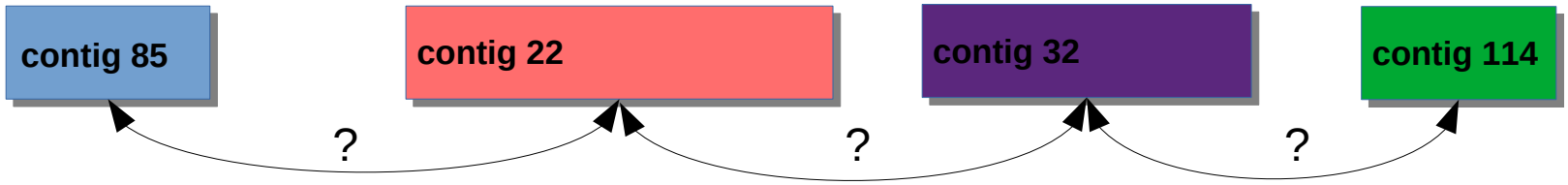
each rectangle
depicts size of one contig

APD
2219 contigs



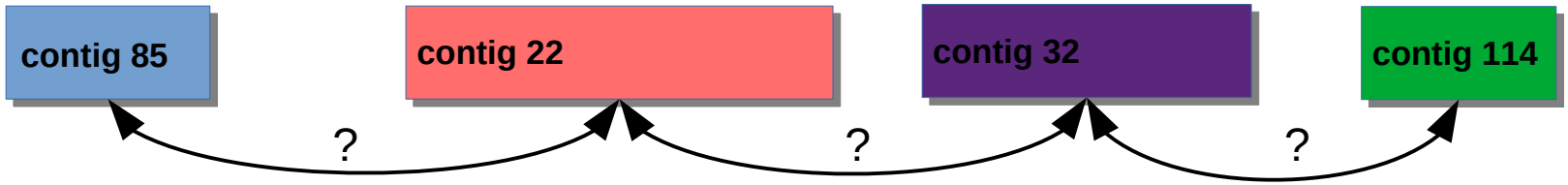
base pairs

Contig Arrangement Problem



- How are the contigs arranged (direction)?

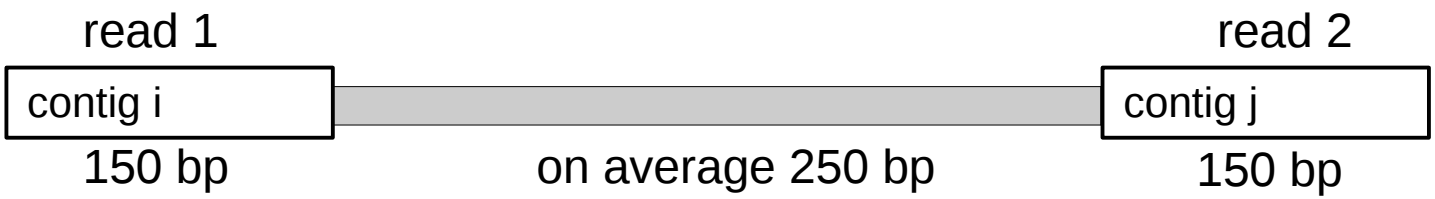
Contig Arrangement Problem

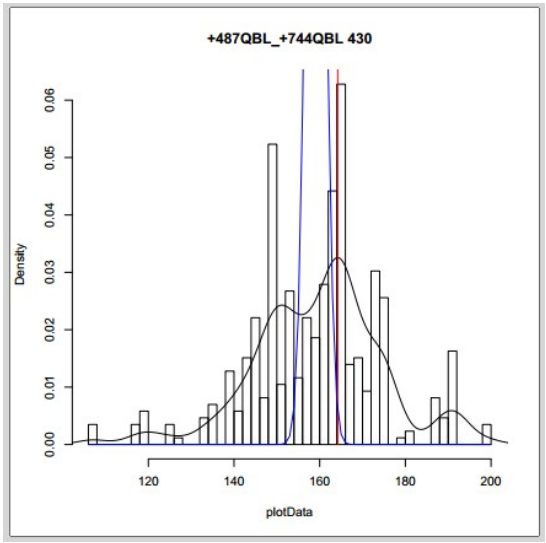


- How are the contigs arranged (direction)?

- Distances between the contigs?
- What is the sequence between the contigs?

Short Read data



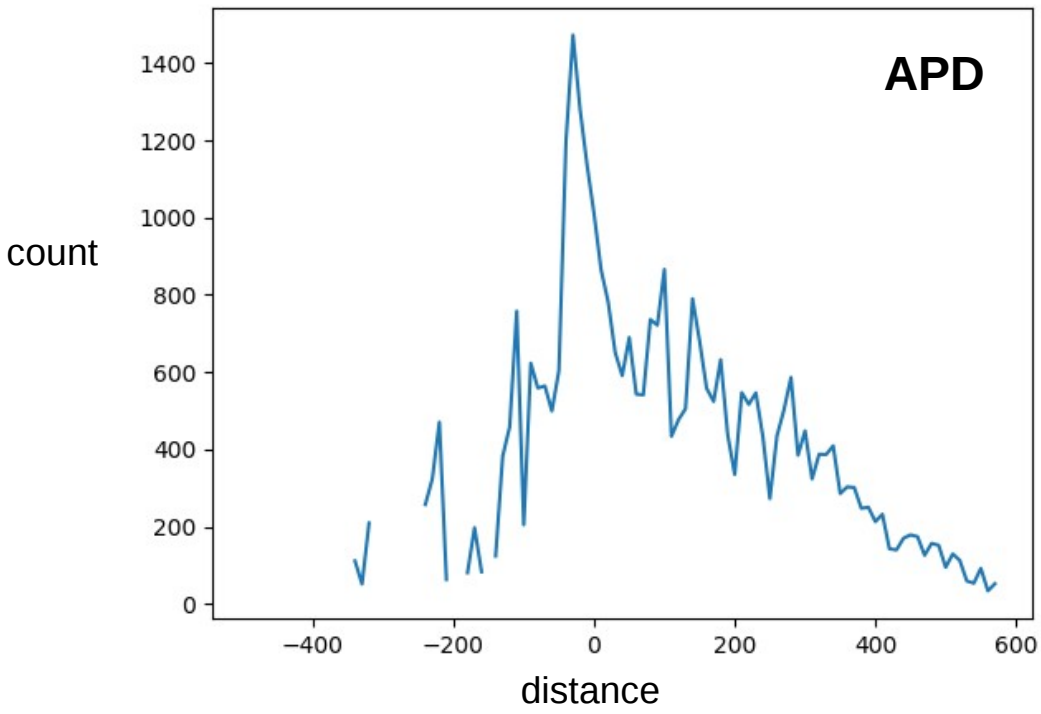


→ distance between 487QBL and 744QBL :

162 – 166 bp

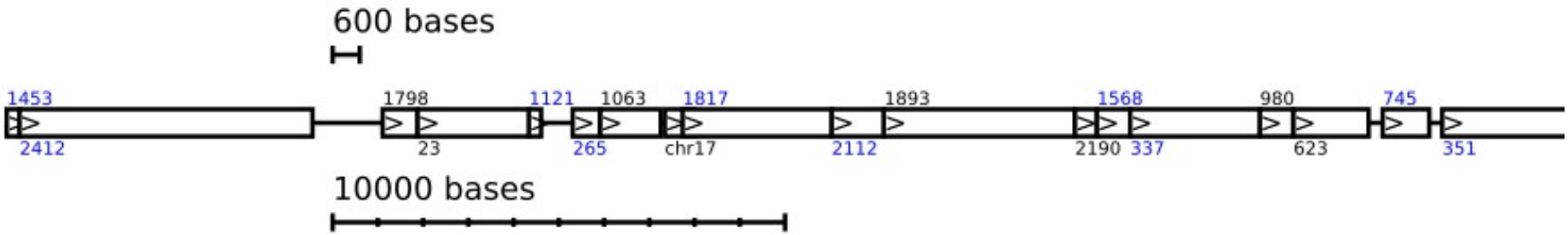
distances between all pairs of contigs summary

→ up to 600 bp



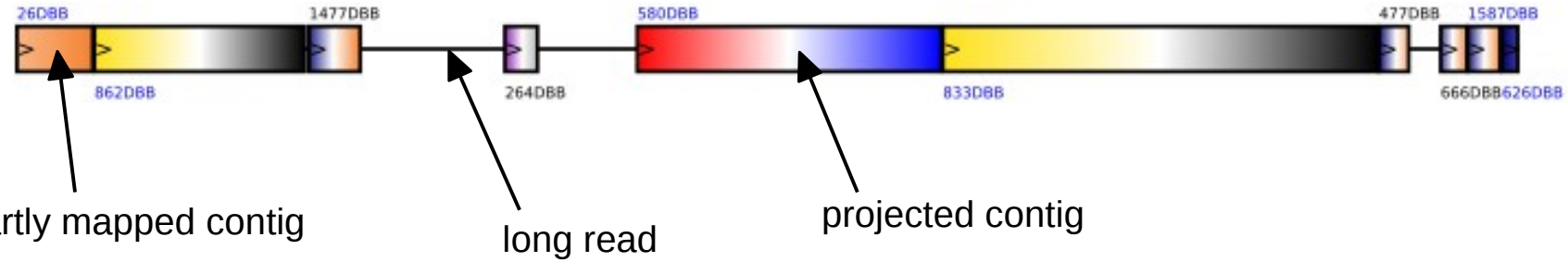
Long Read data

Data from 3rd generation sequencing **Nanopore**
→ - high error rate (15%), - expensive, + long



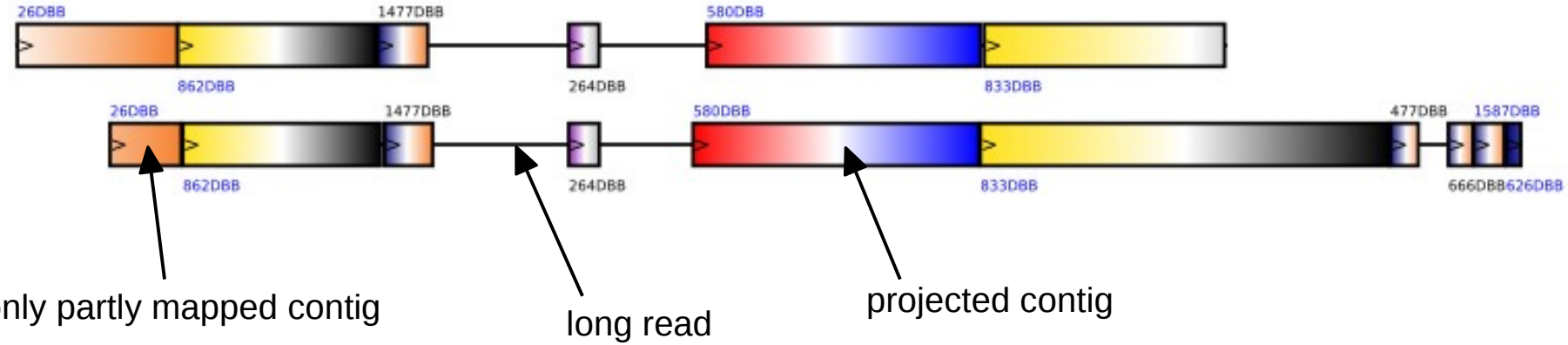
The Idea

long read – contig signature



The Idea – Aligning Signatures

long read – contig signatures fit together



Code Improvements

Modularized Scaffolds

Pseudolongreads
- consist of pieced together real longreads

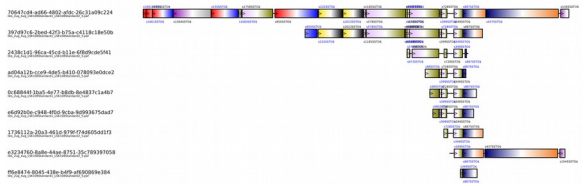
Pseudoalignments
- diminishes problem of paralogs greatly
- automatize assembly (in principle)

Colors

Modularized Scaffolds

less_naive_scaffolding

automatized assembly



alignment2SVG

clusters reads and draws them

class Longreads

- initialize with *.paf, *.erate fileS(!)
  - track origin of each longread
- alternative constructor from dictionary
  - allows merging of longreads
- pseudoalign_all
  - N^2 alignments between all N longreads

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Automatic Assembly

- always yields a result
- but problems can arise
  caused by “wrong” longreads: dsDNA, unexplainable long/short regions
  regions with high variability and/or low coverage

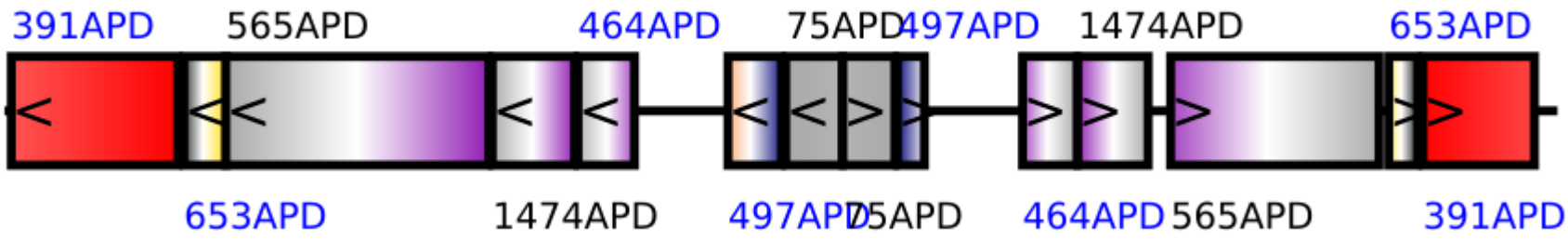
Semi-Automatic Curated Assembly

- takes a little longer
- guarantees there are no long range mistakes

Problematic Reads

“Fake Hairpins” - dsDNA that is seperated at the pore and sequenced in the same read?

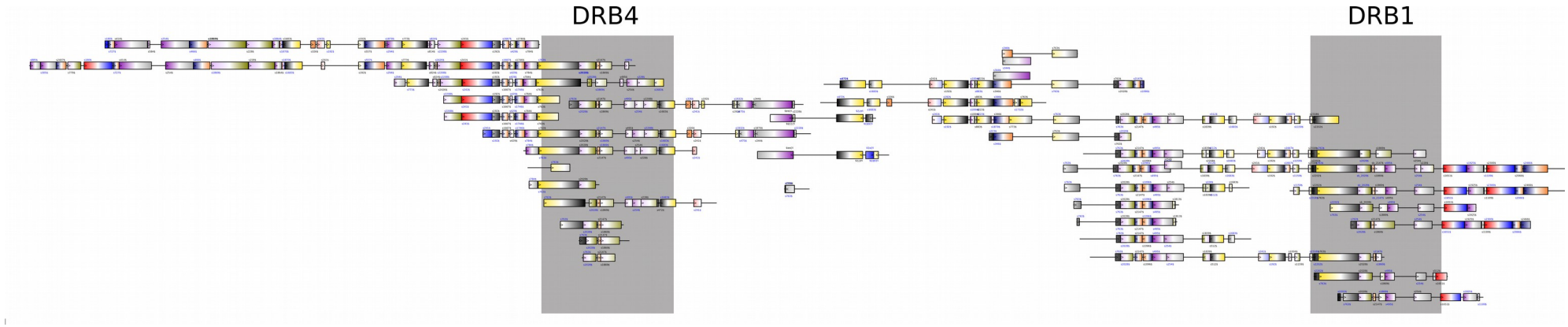
~1% of data



DRB region of cell line DBB

with genes DRB4 and DRB1
- paraloges
→ very similar sequence
→ very similar contig structure

=> difficult to assemble, a strong case for manually curated assembly



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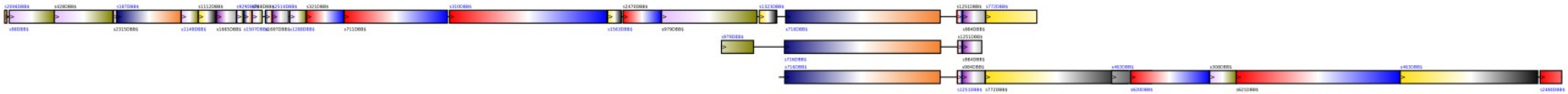
Semi-Automatic Curated Assembly

1. Collect good representatives (good long reads) of a section of the MHC
2. Connect them via contigs



Assembly of MHC with hybrid data

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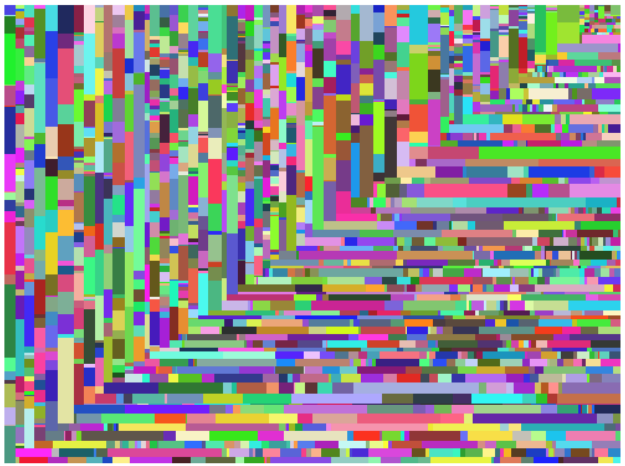
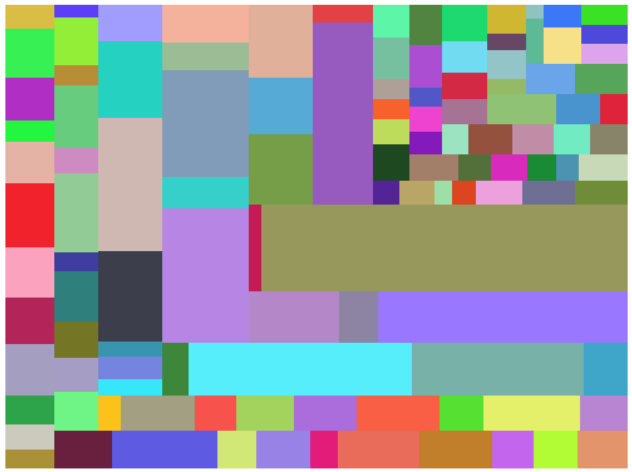
Semi-Automatic Curated Assembly

1. Collect good representatives of a section of the MHC
2. Connect them via contigs

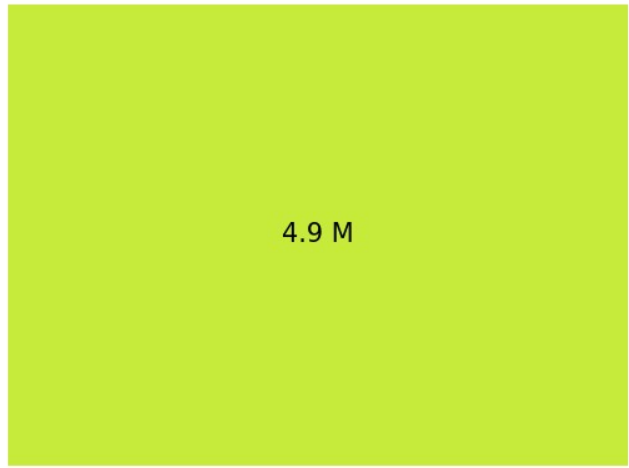
contigs



representative longreads



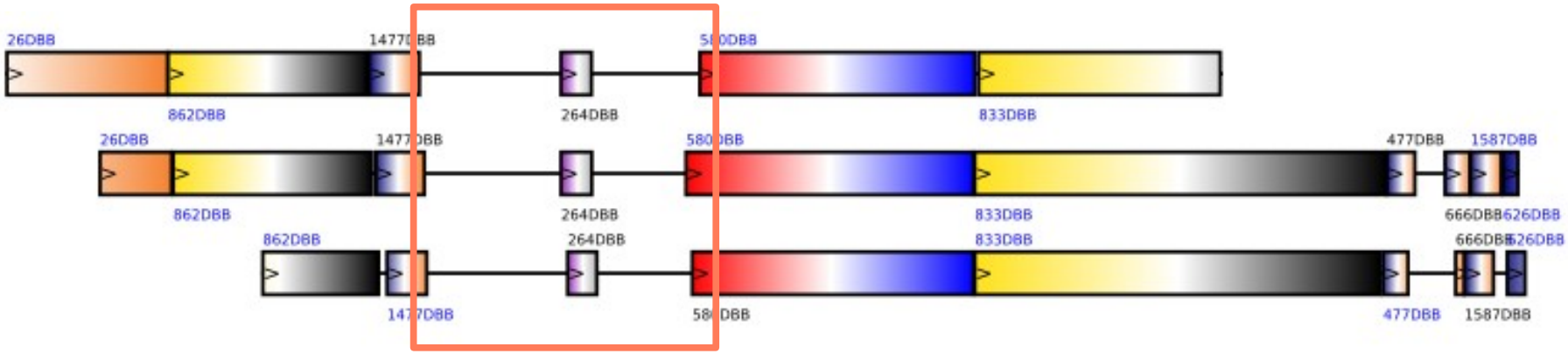
complete assembly



APD

DBB

Refinement / Polishing



error rate of longreads, ~ 15%

Refinement / Polishing

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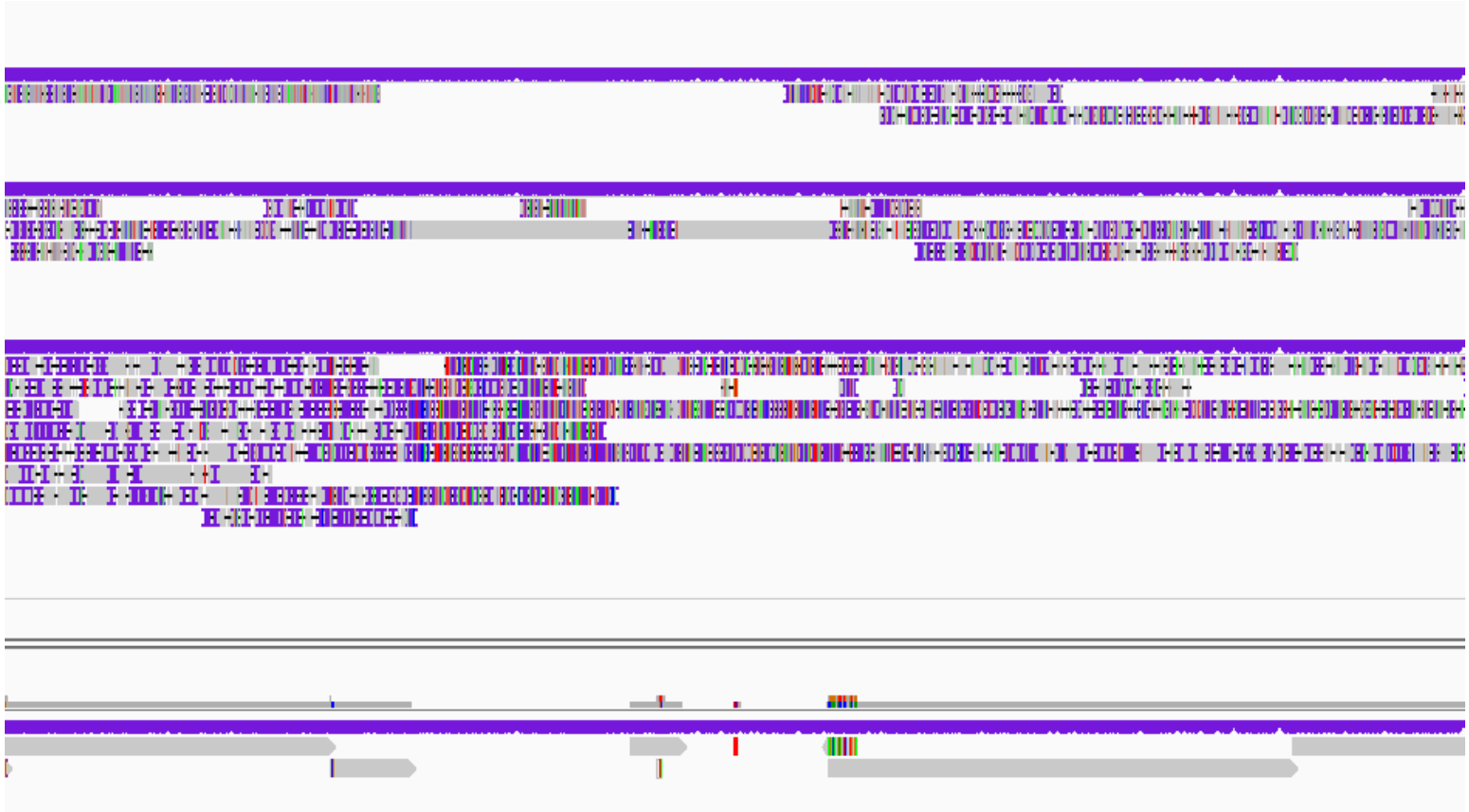
data from three different flow cells



Refinement / Polishing

data from three different flow cells

→ the more colorful, the more alignment errors
→ where there are no errors: these are the representatives for the reference

Refinement / Polishing

Ref. built from contigs → less errors → sequencing errors
Ref. built from longread-representatives → more errors → seqeuncing errors, errors in the reference

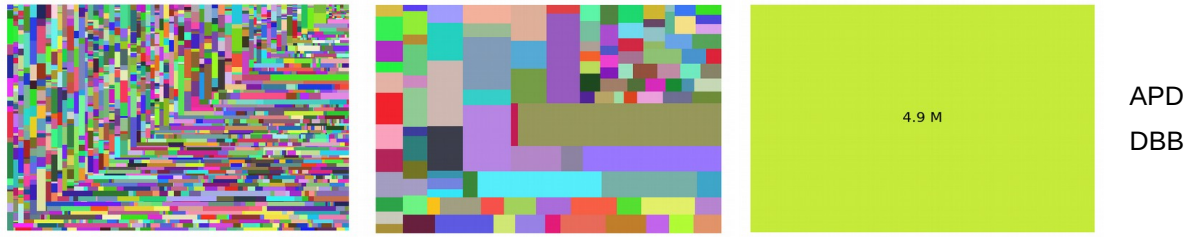
Refinement / Polishing

Taking a representative → Multiple sequence alignment ; Polish with Illumina WGS data

Nanopolish Seqan Samtools Pileup

Summary and Outlook

- Assembly with hybrid data successful



- Finish Missing Cell Lines
    - SSTO, longreads quite short makes assembly tedious, in progress
    - MANN, cells at BMFZ
    - MCF7, cell line with cooperation partner
    - QBL, does not grow, extracted DNA available

- Enhance Sequence Between Contigs
    1. multiple sequence alignment with Seqan
    2. nanopolish (default coverage: 20x, heuristics suitable for lower coverage?)
    3. samtools pileup consensus

- Graph Based Reference Project

Acknowledgments

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Dana Belick

Prof. Dr. Birgit Henrich

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University of Cambridge

Dr. James Traherne

BMFZ

Dr. Tobias Lautwein

HHU Algorithmische Bioinformatik

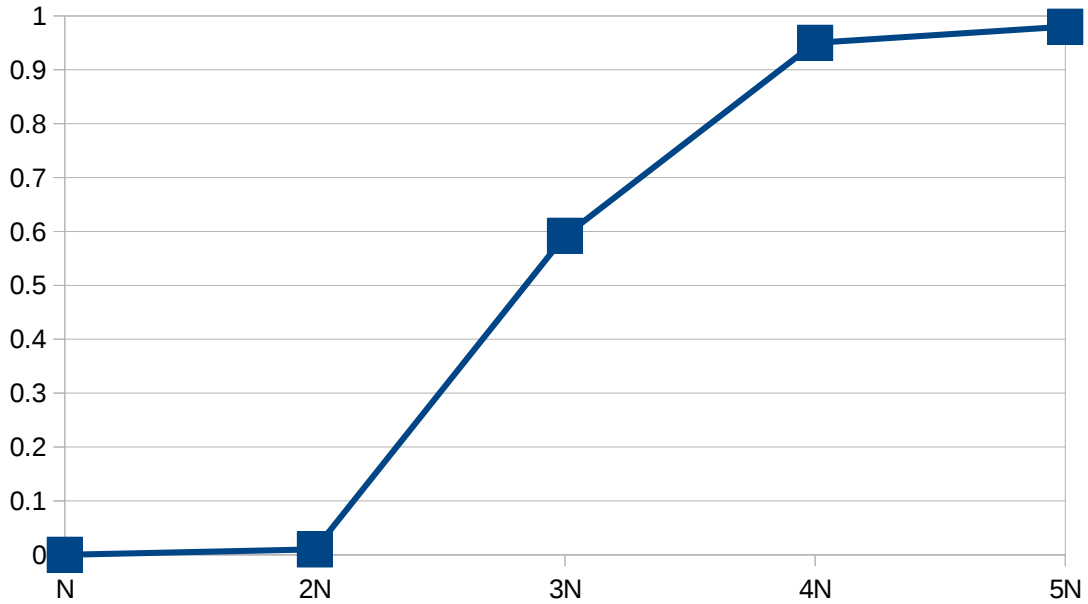
Prof. Dr. Gunnar Klau

Cost Estimation

simulate_read_distribution.py

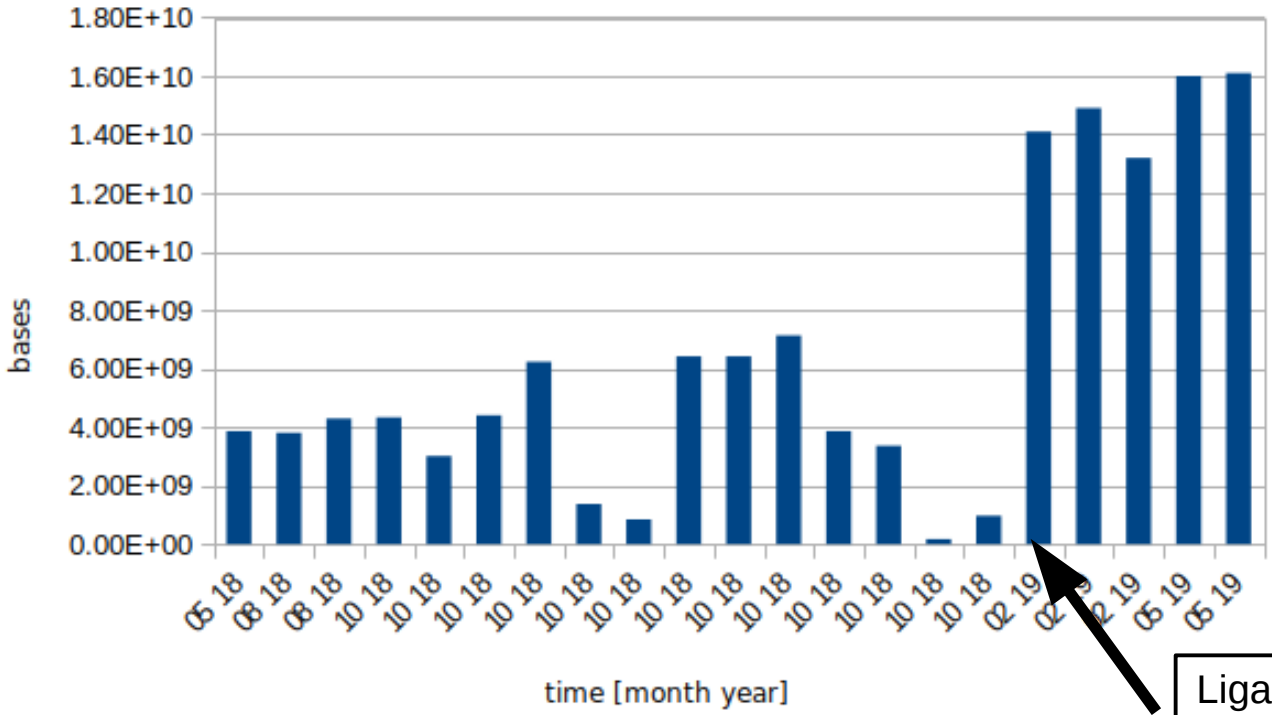
N = 39,000 reads → MHC covered completely 0 times

N ~ 1.5 flowcells
4N ~ 6 flowcells ~ 3600 - 6000€



https://github.com/DiltheyLab/ContigAnalysisScripts/

Quantity Improvements of Nanopore at BMFZ



=> 2-3 Flowcells
per cellline

Ligation Sequencing Kit 109
Flowcell revD
shearing at 75kb with Megaruptor

weighted histogram