Swiss Institute of Bioinformatics

INTRODUCTION TO SEQUENCING DATA ANALYSIS

Quality control + database retrieval

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Adapted from previous year courses

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Why Quality control?

1. How is the base quality?
2. What is the read length?
3. Are there adapters/barcodes in my sequences?
4. Are there overrepresented sequences?

Dedicated software

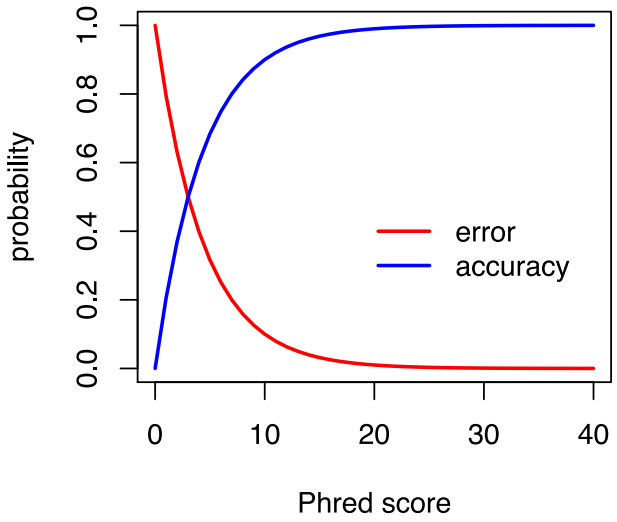
- Manufacturer Tools
  - Built-in QC during base calling and run monitoring
- Commonly Used QC Software:
  - Illumina:
  - FastQC – General QC (per-base quality, GC content, adapter detection)
  - MultiQC – Aggregates FastQC and other reports for batch analysis
- Oxford Nanopore (ONT):
  - pycoQC – Run-level metrics from basecalling (yield, read length, quality)
  - NanoPlot – Read length and quality visualization (FASTQ, BAM, etc.)
- ONT & PacBio:
  - NanoStat – Summary statistics from long-read sequencing files

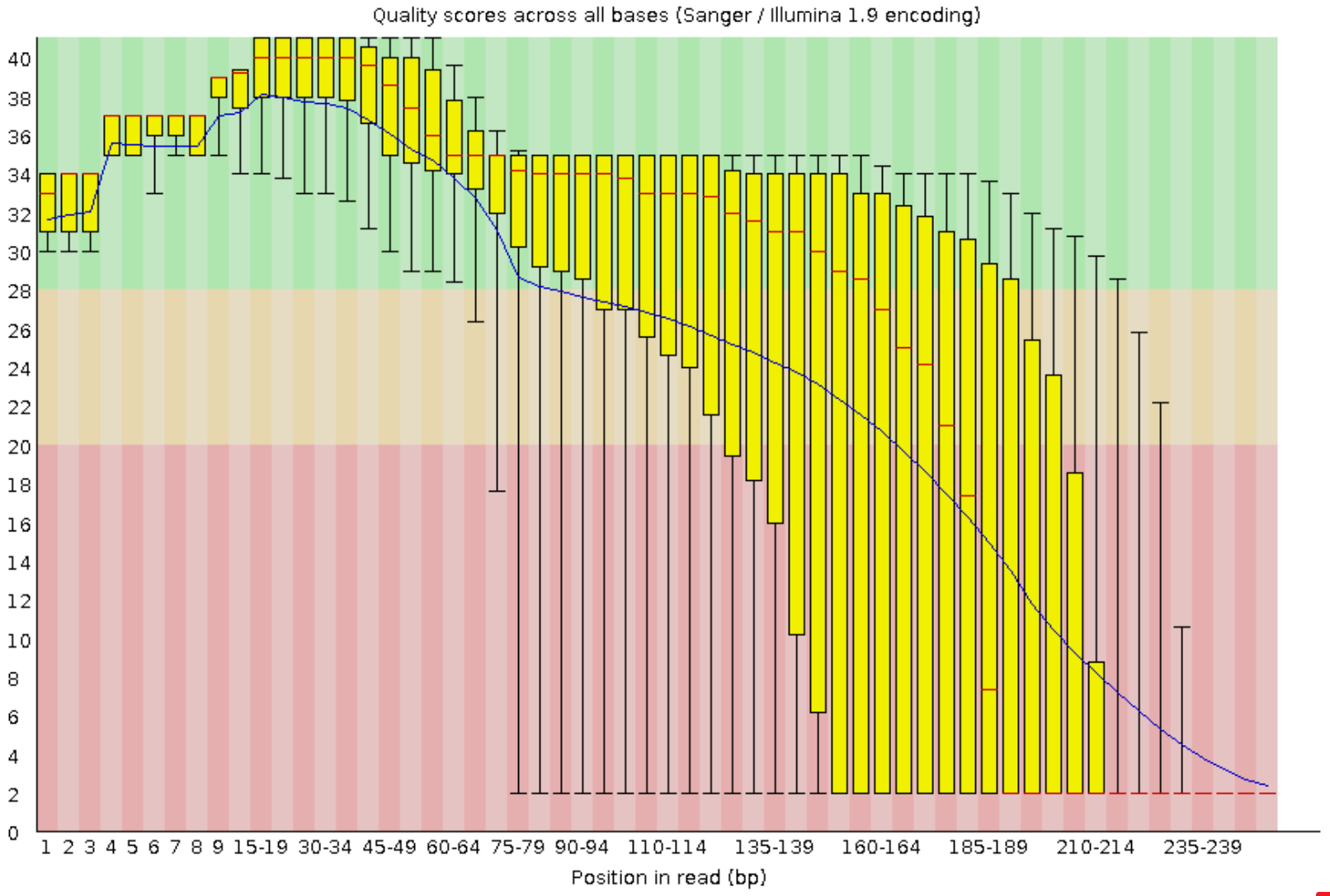
fastq

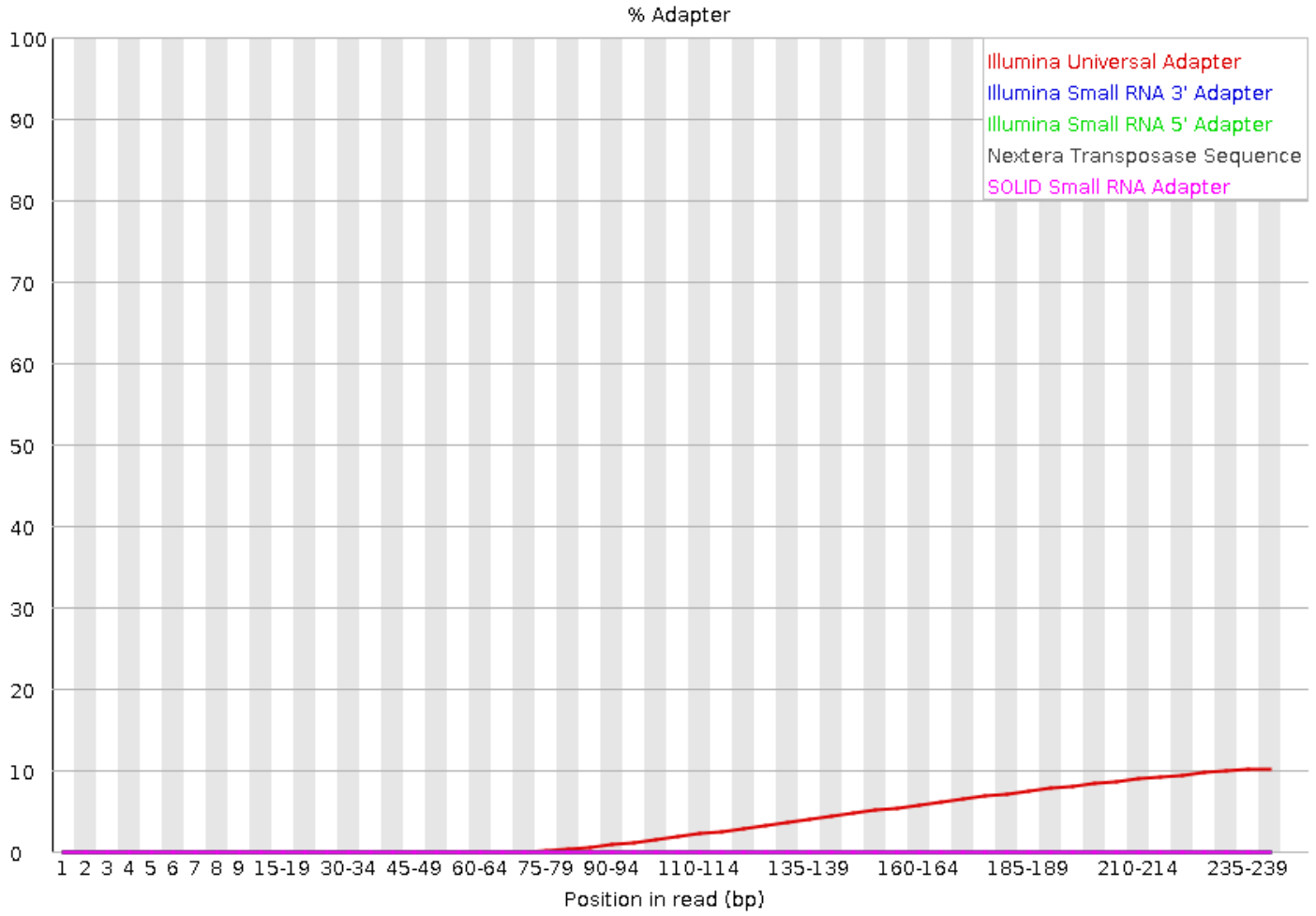
fasta + basequality (fasta + q = fastq)

$$BASEQ = -10log_{10}\Pr\{base\ is\ wrong\}$$

$$-10log_{10}(0.01) = 20$$
$$-10log_{10}(0.1) = 10$$
$$-10log_{10}(0.5) = 3$$




Quality scores across all bases (Sanger / Illumina 1.9 encoding)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 15-19 30-34 45-49 60-64 75-79 90-94 110-114 135-139 160-164 185-189 210-214 235-239
Position in read (bp)


% Adapter
100
90
80
70
60
50
40
30
20
10
0
Illumina Universal Adapter
Illumina Small RNA 3' Adapter
Illumina Small RNA 5' Adapter
Nextera Transposase Sequence
SOLID Small RNA Adapter
1 2 3 4 5 6 7 8 9 15-19 30-34 45-49 60-64 75-79 90-94 110-114 135-139 160-164 185-189 210-214 235-239
Position in read (bp)

Trimming

Find and remove:

- Regions or reads with low base quality
- Adapter sequences
- poly G sequences (e.g. with NovaSeq 6000)

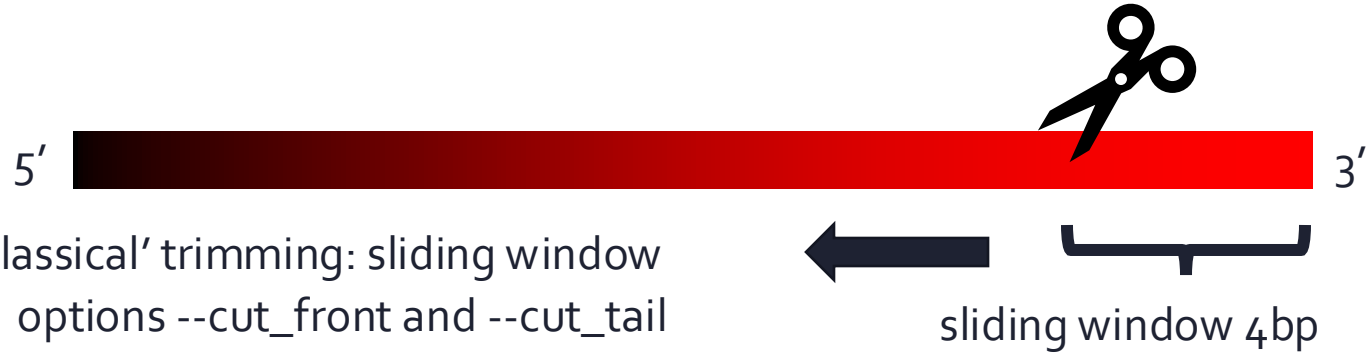
Software: fastp (or cutadapt, trimmomatic, trim_galore, bbduk ..)

Articles on frequently occurring issues: sequencing.qcfail.com

Quality trimming with fastp

Default:

- Remove reads with >40% bases <Q15
- Trim poly N (and poly G)
- Autodetect adapters in R1, for both:
  --detect_adapter_for_pe



'Classical' trimming: sliding window

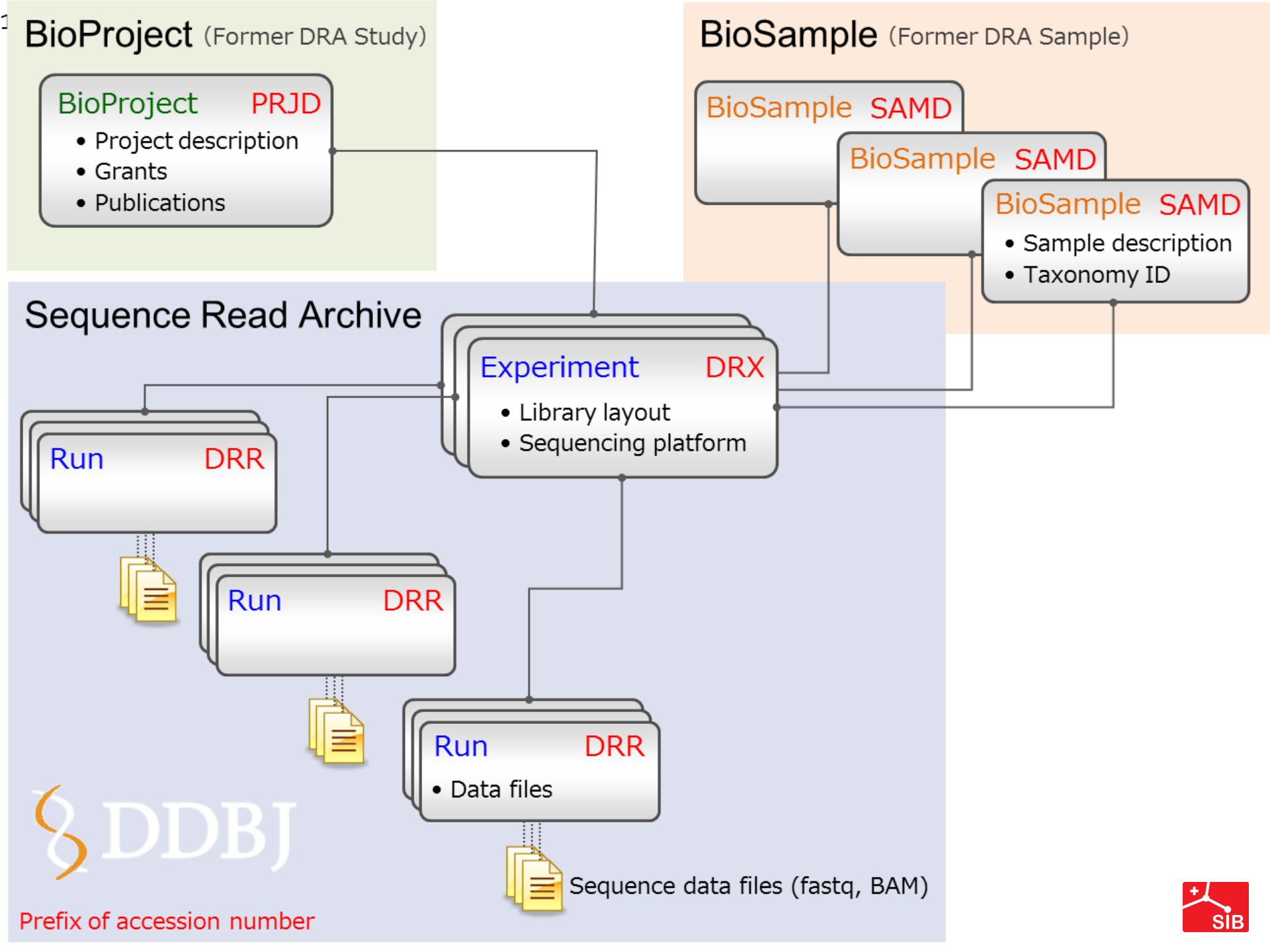
- options --cut_front and --cut_tail

Databases

INSDC: **I**nternational **N**ucleotide **S**equence **D**atabase **C**ollaboration

What is INSDC?

- INSDC = International Nucleotide Sequence Database Collaboration
- Founded to ensure free and open access to nucleotide sequence data worldwide
- Three partners:
  - DDBJ (Japan, Asia)
  - ENA (Europe)
  - GenBank (USA, North America)
- Synchronized daily to maintain a shared global repository


BioProject (Former DRA Study)
BioProject PRJD
• Project description
• Grants
• Publications
BioSample (Former DRA Sample)
BioSample SAMD
BioSample SAMD
BioSample SAMD
• Sample description
• Taxonomy ID
Sequence Read Archive
Experiment DRX
• Library layout
• Sequencing platform
Run DRR
Run DRR
Run DRR
• Data files
DDBJ
Sequence data files (fastq, BAM)
Prefix of accession number
SIB

Display Settings: Send to:

Multi-omics profiling of living human pancreatic islet donors reveals heterogeneous beta cell trajectories toward type 2 diabetes (human)

Accession: PRJNA690574 ID: 690574

To gain insight into the history of islet cell deterioration along the progression from normal glycemic regulation to T2D, we collected surgical pancreatic tissue samples from 133 metabolically phenotyped pancreatectomized patients (PPP). More...

Accession	PRJNA690574; GEO: GSE164416
Data Type	Transcriptome or Gene expression
Scope	Multiisolate
Organism	**Homo sapiens** [Taxonomy ID: 9606] Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo; Homo sapiens
Publications	Wigger L *et al.*, "Multi-omics profiling of living human pancreatic islet donors reveals heterogeneous beta cell trajectories towards type 2 diabetes.", *Nat Metab*, 2021 Jul;3(7):1017-1031
Submission	Registration date: 7-Jan-2021 **Genomic Technologies Facility, University of Lausanne**
Relevance	Medical

See Genome Information for Homo sapiens

NAVIGATE ACROSS
88717 additional projects are related by organism.

Project Data:

Resource Name	Number of Links
SEQUENCE DATA	
SRA Experiments	133
PUBLICATIONS	
PubMed	1
OTHER DATASETS	
BioSample	133
GEO DataSets	1

GEO Data Details

Parameter	Value
Data volume, Supplementary Mbytes	36

SRA Data Details

Parameter	Value
Data volume, Gbases	349
Data volume, Tbytes	0.23

Related information
BioSample
Genome
GEO DataSets
PubMed
SRA
Taxonomy

Recent activity
Turn Off Clear
Multi-omics profiling of living human pancreatic islet donors reveals heter BioProject
SRP300812 (133) SRA
SRP021519 (8) SRA
Entrez Direct: E-utilities on the Unix Command Line - Entrez Programming
SRR519926 (1) SRA
See more...

identifier	PRJNA690574
type	bioproject
sameAs	**GEO** GSE164416
organism	Homo sapiens
title	Multi-omics profiling of living human pancreatic islet donors reveals heterogeneous beta cell trajectories toward type 2 diabetes
description	To gain insight into the history of islet cell deterioration along the progression from normal glycemic regulation to T2D, we collected surgical pancreatic tissue samples from 133 metabolically phenotyped pancreatectomized patients (PPP). Gene expression profiles of islets isolated by laser capture microdissection (LCM) from resected and snap-frozen pancreas samples were assessed by RNA sequencing.Overall design: This study includes RNA-Seq samples from pancreatic islets of 133 human donors, stratified into four groups based on their diabetes status: 18 were non-diabetic (ND), 41 had impaired glucose tolerance (IGT), 35 had Type 3c diabetes (T3cD), and 39 had Type 2 diabetes (T2D). The group assignments are based on thresholds defined in the guidelines of the American Diabetes Association.For data analysis, a subset of 92 pancreatic islet samples was defined, which included only those samples in which the gene INS showed the highest expression (i.e., highest normalized counts value). Statistical analyses were performed both on the complete transcriptomics data set and on this restricted data set.
data type	Transcriptome or Gene expression
organization	
publication	34183850
external link	

For sensitive human data

EUROPEAN
GENOME-PHENOME
ARCHIVE

See also:
https://ega-archive.org/about/projects-and-funders/federated-ega/

EGA

- EGA = European Genome-phenome Archive (EMBL-EBI, Europe)
- Designed for controlled access to human data with privacy concerns
- Ideal for:
  - Clinical studies
  - Patient phenotypes
  - Genomic variants
- Access requires data access committee (DAC) approval

Summary

- INSDC enables global sharing of nucleotide sequence data
- GEO / SRA are part of this ecosystem, focusing on expression/functional data
- For sensitive human datasets, EGA is the recommended platform
- Choosing the right repository ensures data availability and compliance

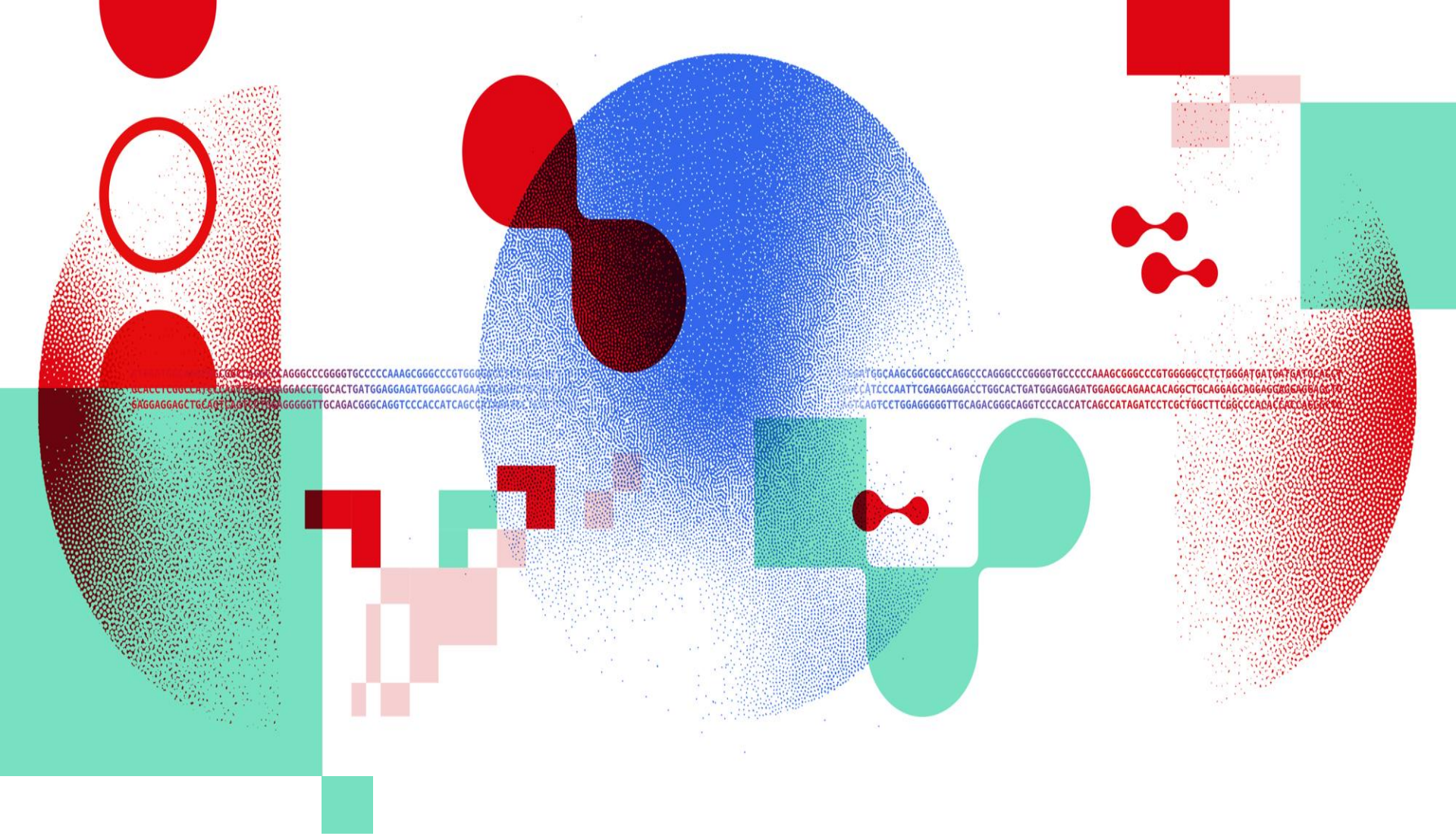
Command line tools

Retrieve raw data: SRA-tools

- prefetch
- fastq-dump

Retrieve sequences: Entrez Direct

- esearch
- efetch



Thank you

DATA SCIENTISTS FOR LIFE

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