UPPSALA
UNIVERSITET

Experimental design in genomics

Olga Vinnere Pettersson, Uppsala University

Scientific Lead of Planetary Biology Capability, SciLifeLab

ERGA Vice-chair

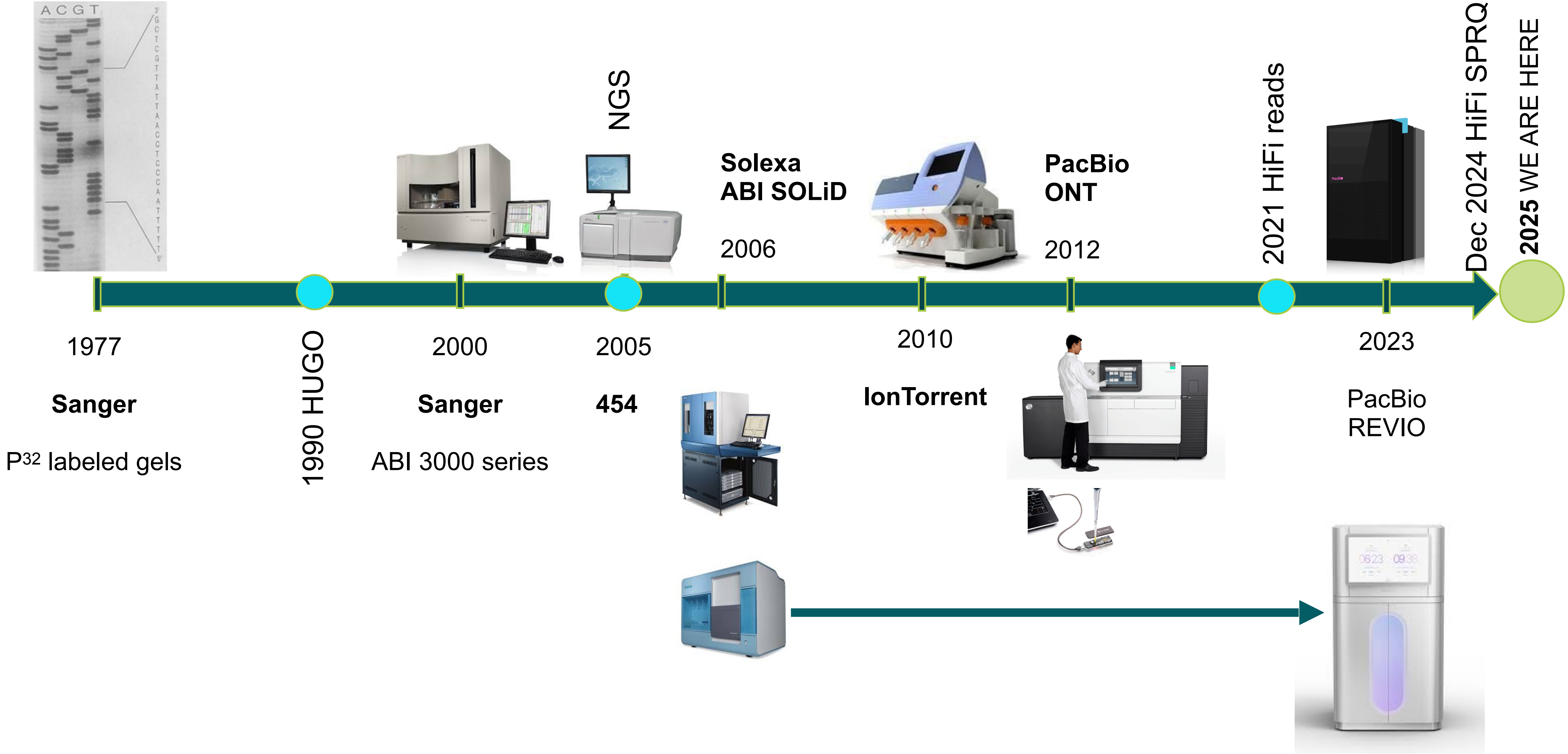
Český Krumlov
2026-01-16

Outline

- What to think about BEFORE planning a sequencing project (aka Project Design)
- Sequencing applications and experiment design specifics:
  - Whole-genome sequencing
  - Targeted sequencing
  - Transcriptome sequencing
  - Shotgun metagenomics
  - Reference genome sequencing + optimal project workflow example

- Sampling and sample quality requirements
- What every facility wish you knew before sending your samples
- Some perspective

An outcome of HUGO – Genomic Revolution



1977

Sanger

P32 labeled gels

1990 HUGO

2000

Sanger

ABI 3000 series

NGS

2005

454

Solexa
ABI SOLiD

2006

2010

IonTorrent

PacBio
ONT

2012

2021 HiFi reads

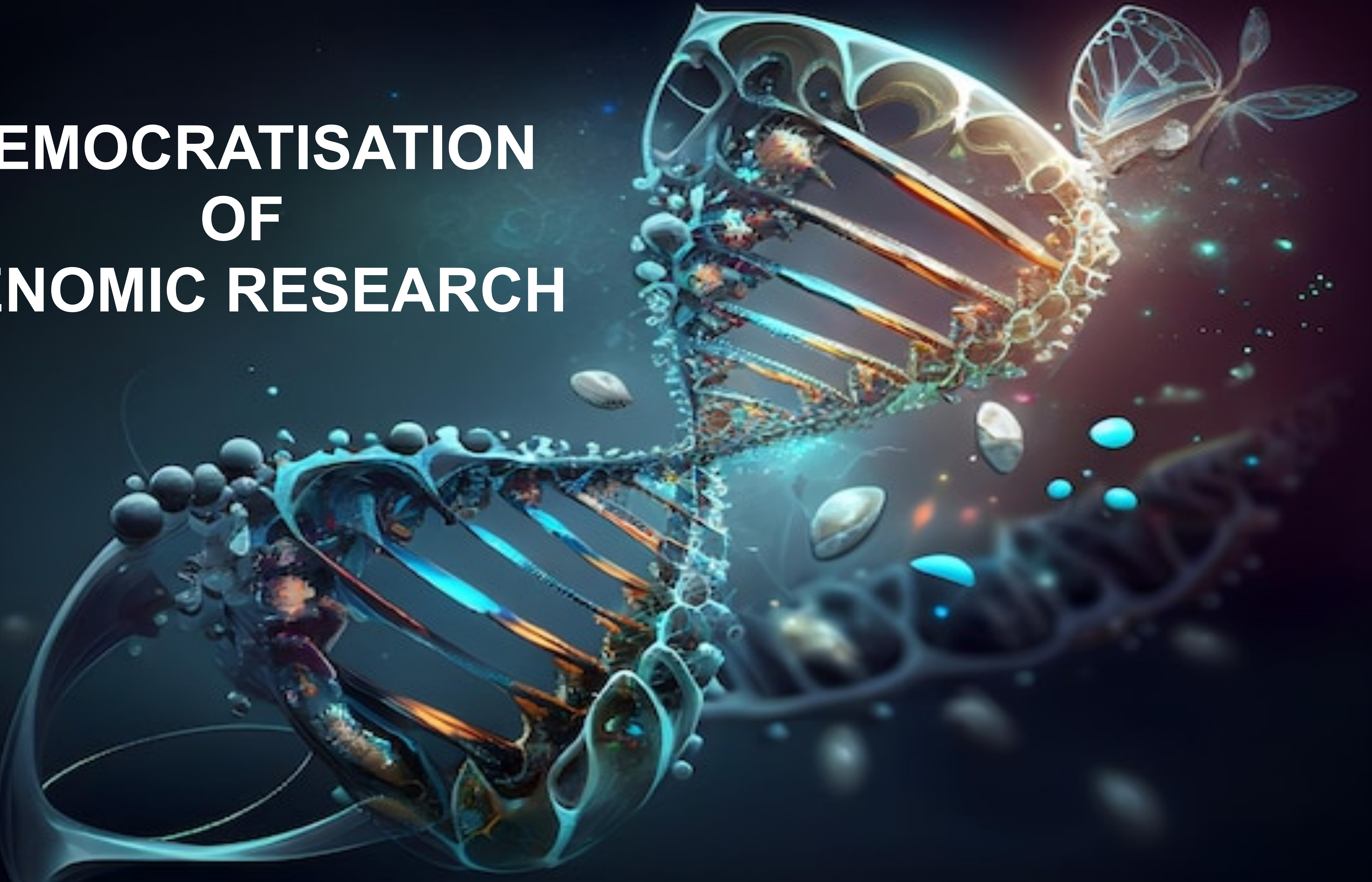
2023

PacBio
REVIO

Dec 2024 HiFi SPRQ

2025 WE ARE HERE

DEMOCRATISATION OF GENOMIC RESEARCH



Drawback of genomic revolution: how to stay up to date?

- Immense speed of technological progress

- Inspiration from papers? HA!
  - Design + gathering material + conducting experiments (1 month - years)
  - Sequencing (1-6 months)
  - Analysis (…)
  - Writing paper (month - year)
  - Paper submission (weeks)
  - Reviewer #3 (weeks - months)
    - Design -> published paper 1-3 years

Drawback of genomic revolution: how to stay up to date?

- Immense speed of technological progress

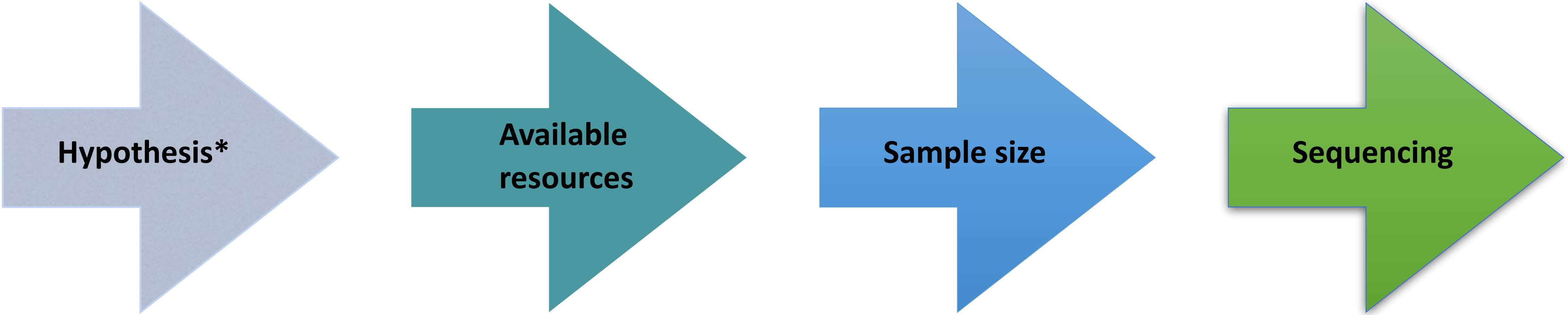
- Inspiration from papers? HA!
  - Design + gathering material + conducting experiments (1 month - years)
  - Sequencing (1-6 months)
  - Analysis (…)
  - Writing paper (month - year)
  - Paper submission (weeks)
  - Reviewer #3 (weeks - months)
    - Design -> published paper 1-3 years

SOLUTION: talk to a sequencing center near you

Planning the sequencing project

WHAT IS MY QUESTION?

- Qualitative or quantitive?
  - Avoid: “let’s sequence and see what happens”
  - Frequent scenario: “here is what I have, do your best/worst”



Hypothesis* → Available resources → Sample size → Sequencing

**or creating a resource (e.g. variant database, reference, etc)*

Available resources

- €€€
- **Store, compute, back-up**
- Who will analyse the data?
- Who will biologically interpret the data?
- Sample availability (number, quality and amount of material)

Sample size

- Statistical sensitivity: #of samples, #of replicates, #of reads (sequencing depth)

Sequencing

- Choice of application and technology

Early planning before writing a grant proposal is highly recommended. Get advise!

When planning a sequencing experiment, most think only of the **sequencing** cost

The REAL sequencing project cost =

= collection + sample processing + nucleic acid extraction + shipment* +

+ *sequencing* + data storage + data compute + data analysis + data back-up +

+ work hours

Sequencing is the **CHEAPEST** part

💡 ***(Never EVER believe the sequencing technology vendor prices!)***

Data Management:

The Most Underestimated Step in Genomics Experiments

Modern sequencers produce Tbs of data per day

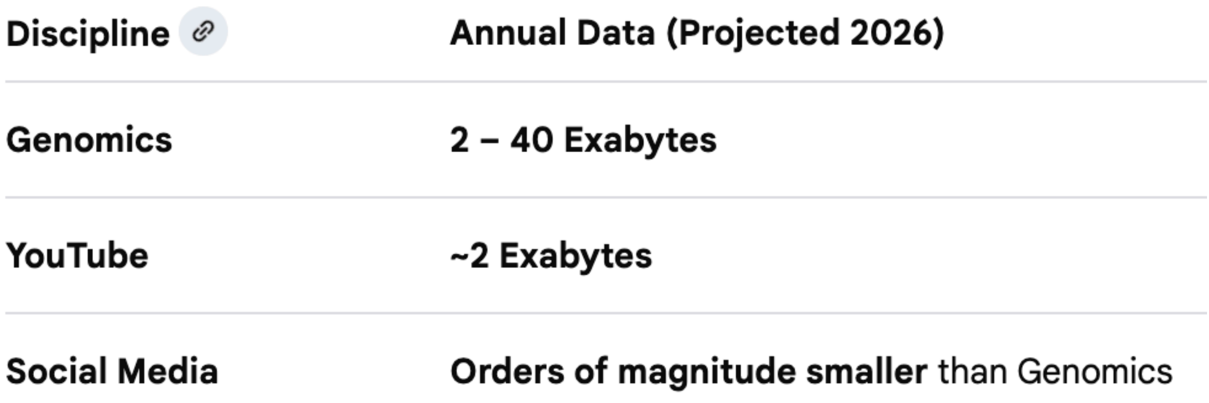
Whole-genome sequencing (human, 30×)

- Raw FASTQ: ~100–150 GB per sample
- Aligned BAM/CRAM: ~30–80 GB
- Intermediate files: often **2–5× raw data size**

RNA-seq / single-cell experiments

- Many small files → metadata + backups become painful

10–100 samples quickly becomes **multiple terabytes**



Discipline	**Annual Data (Projected 2026)**
Genomics	**2 – 40 Exabytes**
YouTube	**~2 Exabytes**
Social Media	**Orders of magnitude smaller** than Genomics

AI projection based on https://doi.org/10.1371/journal.pbio.1002195

💡 *Intermediate files often dwarf the final results*

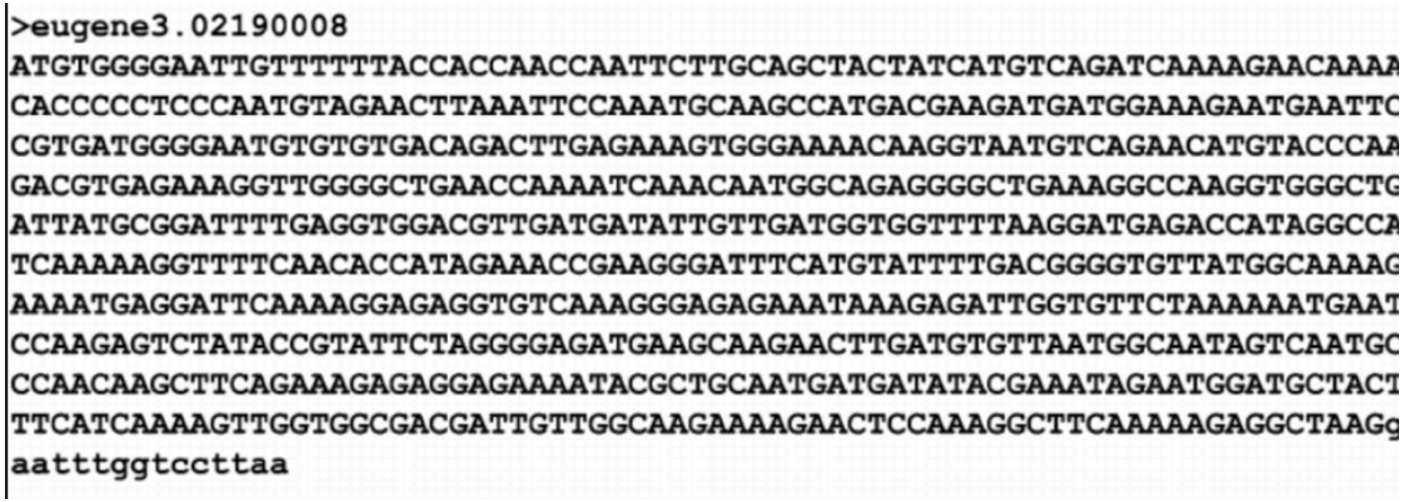
Before planning the sequencing experiment

Data management

Contact your university IT and bioinformaticians (preferably as early as in the grant-writing stage):

Data management plan
Compute and store estimation
Are you **allowed** to use Cloud?
Guidelines for back-up
Guidelines for data storage duration (years - permanent)

Data without context loose value



```
>eugene3.02190008
ATGTGGGGAATTGTTTTTTACCACCAACCAATTCTTGCAGCTACTATCATGTCAGATCAAAAGAACAAAA
CACCCCCTCCCAATGTAGAACTTAAATTCCAAATGCAAGCCATGACGAAGATGATGGAAAGAATGAATTC
CGTGATGGGGAATGTGTGTGACAGACTTGAGAAAGTGGGAAAACAAGGTAATGTCAGAACATGTACCCAA
GACGTGAGAAAGGTTGGGGCTGAACCAAAATCAAACAATGGCAGAGGGGCTGAAAGGCCAAGGTGGGCTG
ATTATGCGGATTTTGAGGTGGACGTTGATGATATTGTTGATGGTGGTTTTAAGGATGAGACCATAGGCCA
TCAAAAAGGTTTTCAACACCATAGAAACCGAAGGGATTTCATGTATTTTGACGGGGTGTTATGGCAAAAG
AAAATGAGGATTCAAAAGGAGAGGTGTCAAAGGGAGAGAAATAAAGAGATTGGTGTTCTAAAAAATGAAT
CCAAGAGTCTATACCGTATTCTAGGGGAGATGAAGCAAGAACTTGATGTGTTAATGGCAATAGTCAATGC
CCAACAAGCTTCAGAAAGAGAGGAGAAAATACGCTGCAATGATGATATACGAAATAGAATGGATGCTACT
TTCATCAAAAGTTGGTGGCGACGATTGTTGGCAAGAAAAGAACTCCAAAGGCTTCAAAAAGAGGCTAAGg
aatttggtccttaa
```

?

FAIR: Making data reusable by humans and machines

F : findable <- clear identifiers, searchable

A : accessible <- retrievable

I : interoperable <- uses standard formats and vocabularies

R : reusable <- rich metadata + clear provenance

💡 *FAIR is not just for people — it’s for algorithm*

FAIR data enable future AI applications

Machine learning needs:

- many datasets
- consistent labels
- known experimental conditions

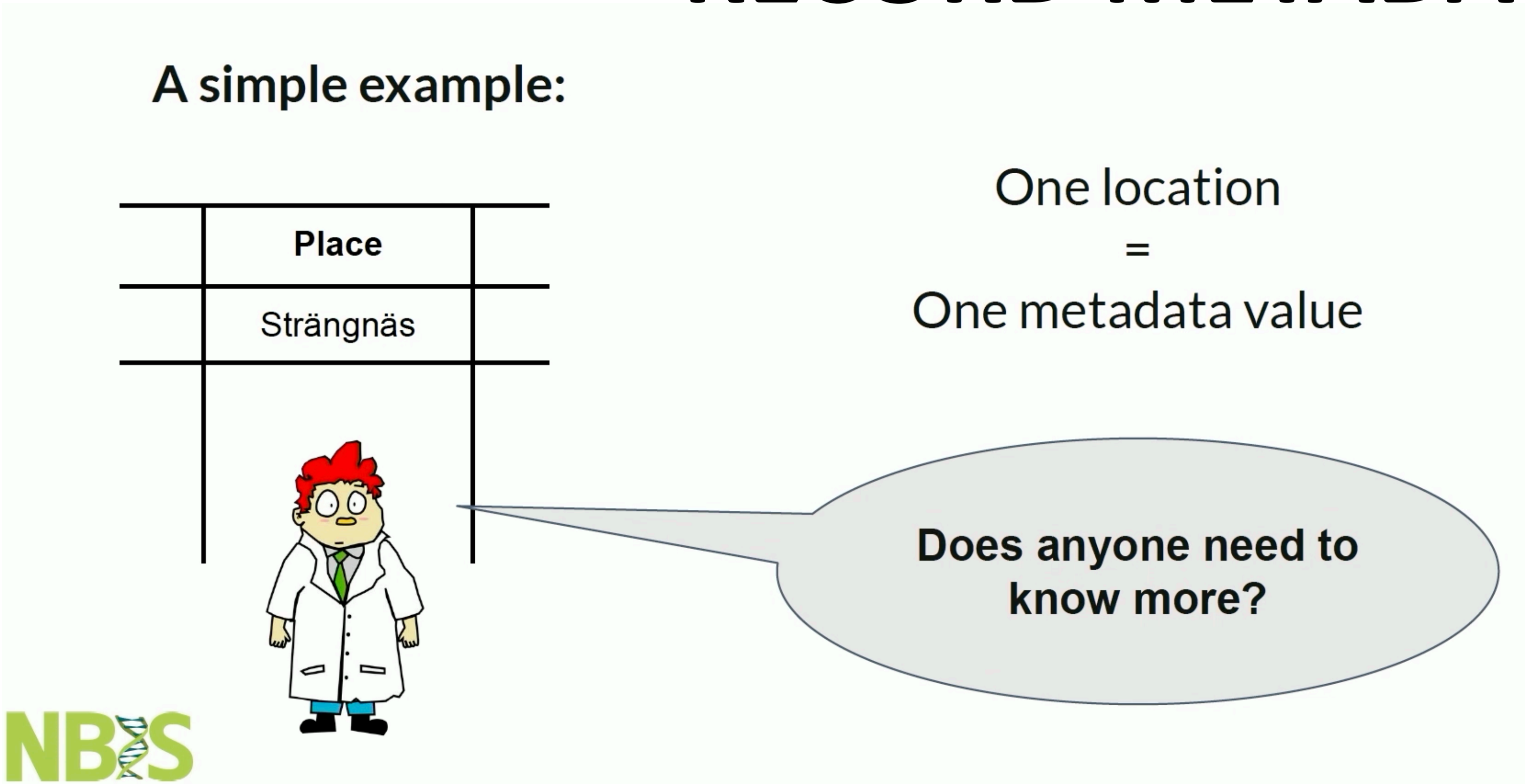
Metadata captures:

- sample attributes
- protocols & parameters
- batch effects & technical variation

Poor metadata = biased or unusable models

💡 *You are not just generating data for this experiment—
you are creating training data for future models you haven't imagined yet.*

RECORD METADATA ASAP!!!

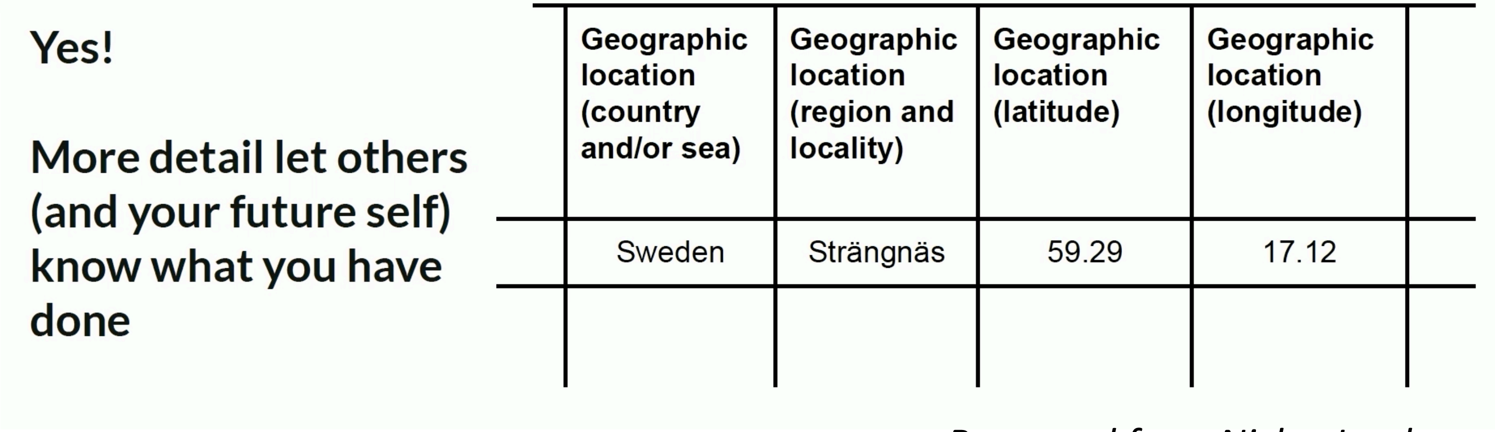


A simple example:

Place
Strängnäs

One location
=
One metadata value

Does anyone need to know more?

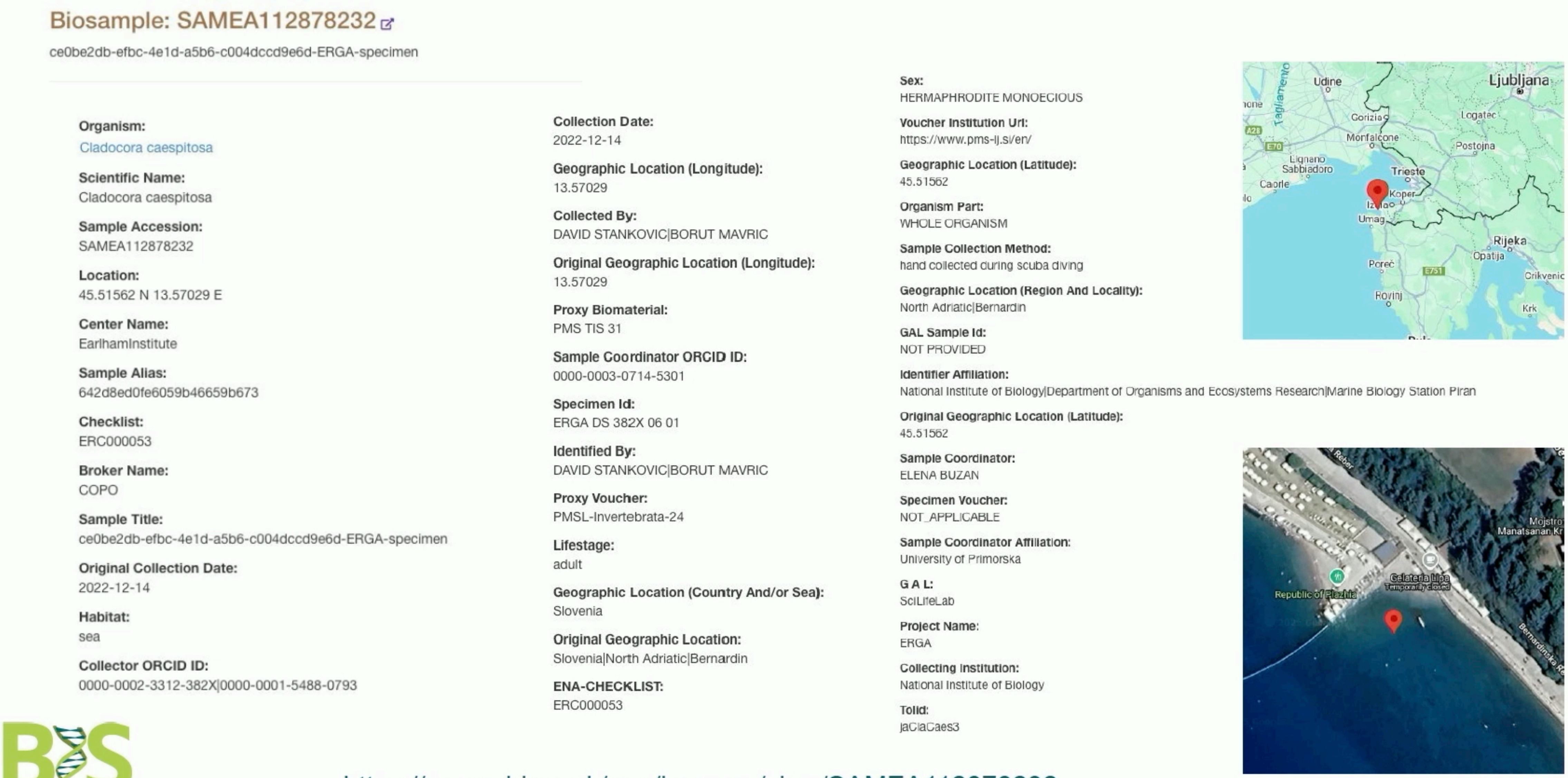


Yes!

More detail let others (and your future self) know what you have done

Geographic location (country and/or sea)	Geographic location (region and locality)	Geographic location (latitude)	Geographic location (longitude)
Sweden	Strängnäs	59.29	17.12

Borrowed from Niclas Jareborg



Biosample: SAMEA112878232

ce0be2db-efbc-4e1d-a5b6-c004dccd9e6d-ERGA-specimen

Organism:
Cladocora caespitosa

Scientific Name:
Cladocora caespitosa

Sample Accession:
SAMEA112878232

Location:
45.51562 N 13.57029 E

Center Name:
EarlhamInstitute

Sample Alias:
642d8ed0fe6059b46659b673

Checklist:
ERC000053

Broker Name:
COPO

Sample Title:
ce0be2db-efbc-4e1d-a5b6-c004dccd9e6d-ERGA-specimen

Original Collection Date:
2022-12-14

Habitat:
sea

Collector ORCID ID:
0000-0002-3312-382X|0000-0001-5488-0793

Collection Date:
2022-12-14

Geographic Location (Longitude):
13.57029

Collected By:
DAVID STANKOVIC|BORUT MAVRIC

Original Geographic Location (Longitude):
13.57029

Proxy Biomaterial:
PMS TIS 31

Sample Coordinator ORCID ID:
0000-0003-0714-5301

Specimen Id:
ERGA DS 382X 06 01

Identified By:
DAVID STANKOVIC|BORUT MAVRIC

Proxy Voucher:
PMSL-Invertebrata-24

Lifestage:
adult

Geographic Location (Country And/or Sea):
Slovenia

Original Geographic Location:
Slovenia|North Adriatic|Bernardin

ENA-CHECKLIST:
ERC000053

Sex:
HERMAPHRODITE MONOECIOUS

Voucher Institution Url:
https://www.pms-lj.si/en/

Geographic Location (Latitude):
45.51562

Organism Part:
WHOLE ORGANISM

Sample Collection Method:
hand collected during scuba diving

Geographic Location (Region And Locality):
North Adriatic|Bernardin

GAL Sample Id:
NOT PROVIDED

Identifier Affiliation:
National Institute of Biology|Department of Organisms and Ecosystems Research|Marine Biology Station Piran

Original Geographic Location (Latitude):
45.51562

Sample Coordinator:
ELENA BUZAN

Specimen Voucher:
NOT_APPLICABLE

Sample Coordinator Affiliation:
University of Primorska

G A L:
SciLifeLab

Project Name:
ERGA

Collecting Institution:
National Institute of Biology

Tolid:
jaClaCaes3

https://www.ebi.ac.uk/ena/browser/view/SAMEA112878232

Let’s dive into it

NGS Technologies and Applications

NGS technologies

Short read NGS

Long-read NGS

Whole genome re-sequencing
RNA-seq
Targeted re-seq
Panels
Amplicons up to 600 bp

De novo genome sequencing
Whole-transcript sequencing
Structural variant resolving
Targeted re-seq
Amplicons up to 13 kb

Whole genome sequencing (WGS)

Re-sequencing or de novo?

- Re-sequencing (WGS):
  - Pre-requisite: a reference genome to map to.

    Population studies (genotyping, variant discovery, allele frequency, etc)

    SNPs only? Short reads.

    SVs? Long reads.

    SNPs and SVs? PacBio **HiFi** (January 2026).

- *De novo* (Reference genome sequencing):
  - Creating a genomic reference from scratch
  - Long reads (sometimes coupled with short-read skims)

WGS sequencing depth

Population sequencing: individual libraries or pools of many individuals

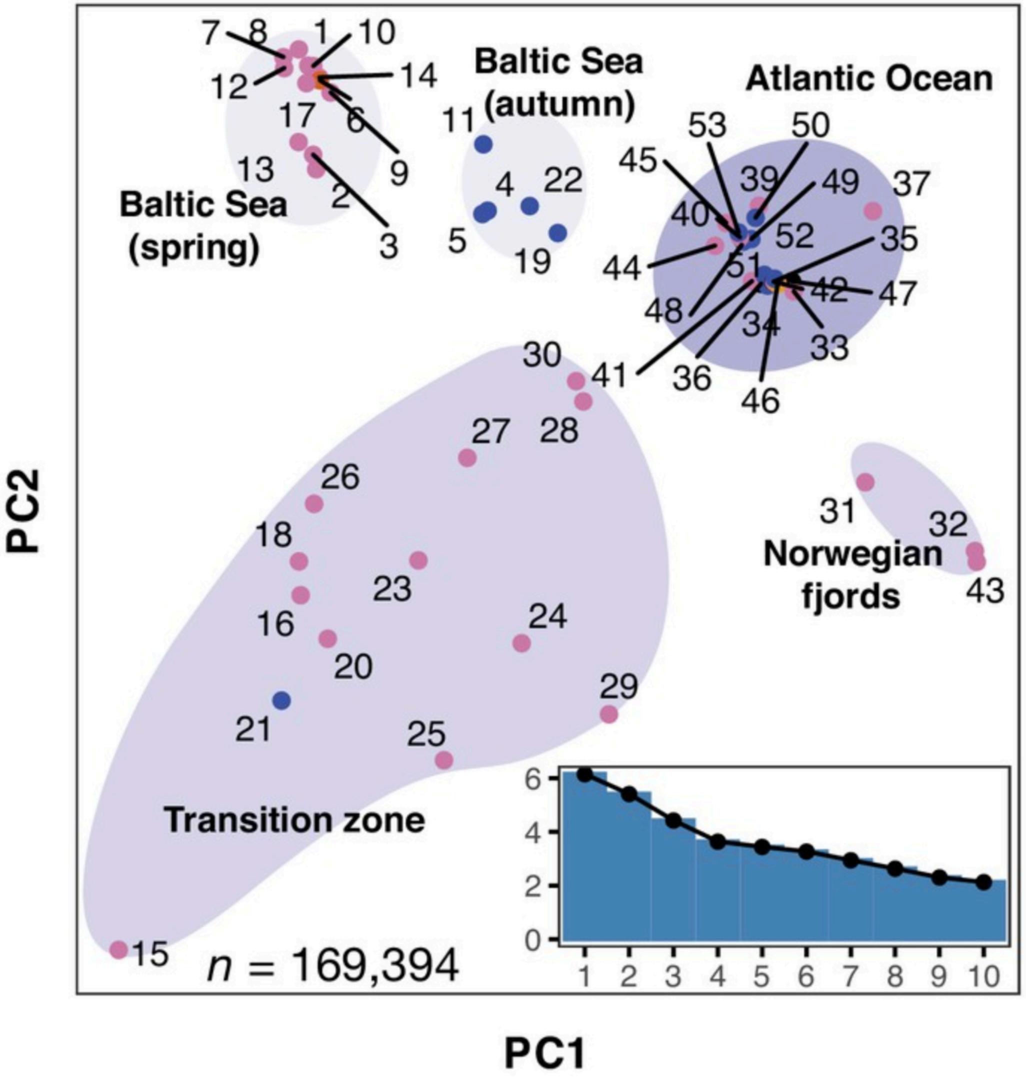
Type of Experiment	Coverage Required
Haploid SNPs/divergence	≥ 10 x
Diploid SNPs/divergence	≥ 30 x
Aneuploid/somatic mutations	≥ 50 x
Population sequencing	≥ 200 x

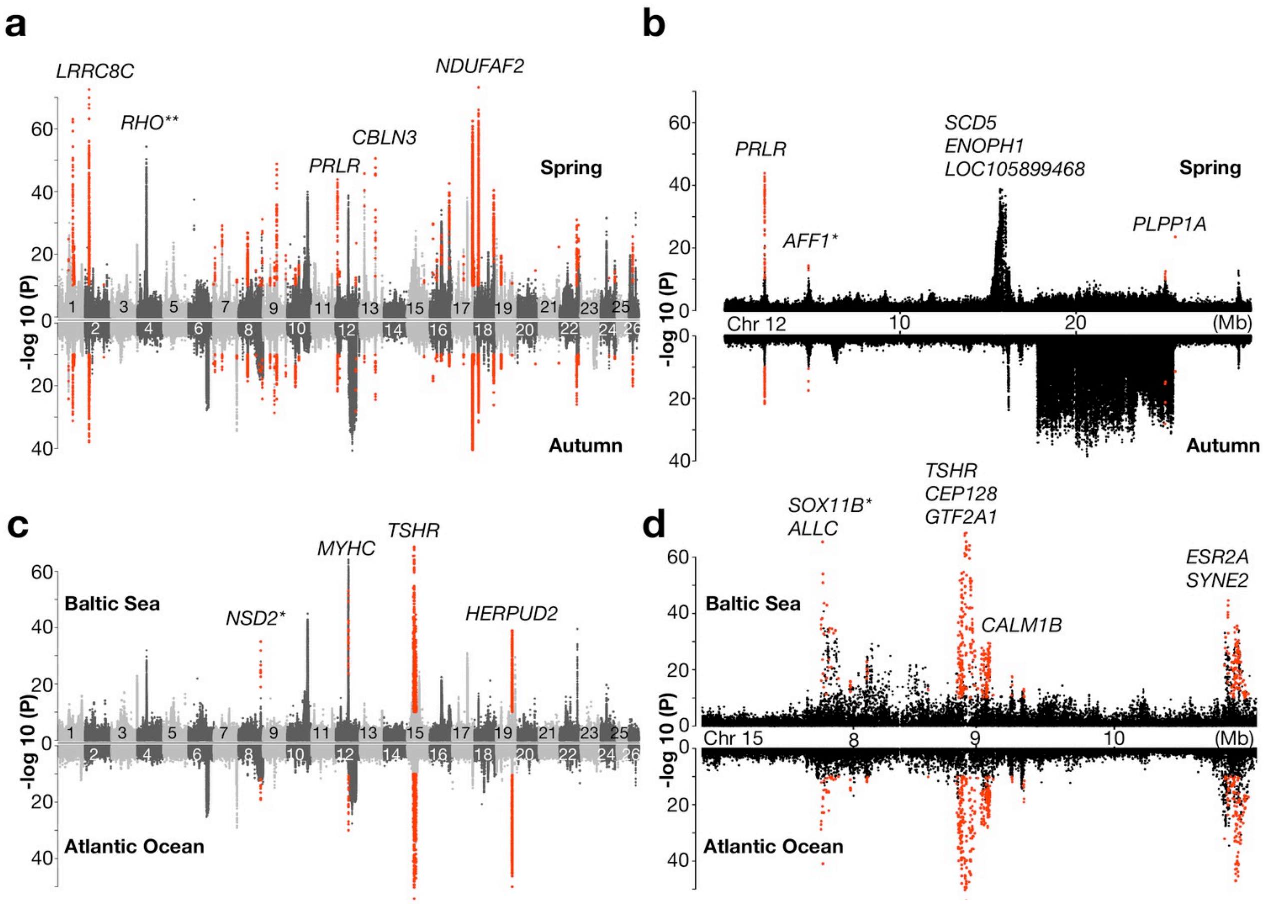
Borrowed from Mike Zody

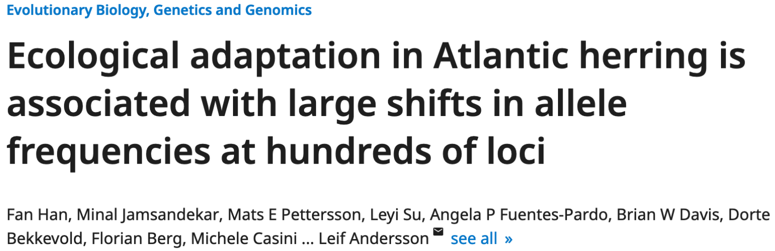
Individual libraries give better resolution, but are more expensive
Pool sequencing: will pick up main trends (e.g. loci under selection)

Caution not to over sequence: sequencing errors vs true biology

WGS, examples







Evolutionary Biology, Genetics and Genomics

Ecological adaptation in Atlantic herring is associated with large shifts in allele frequencies at hundreds of loci

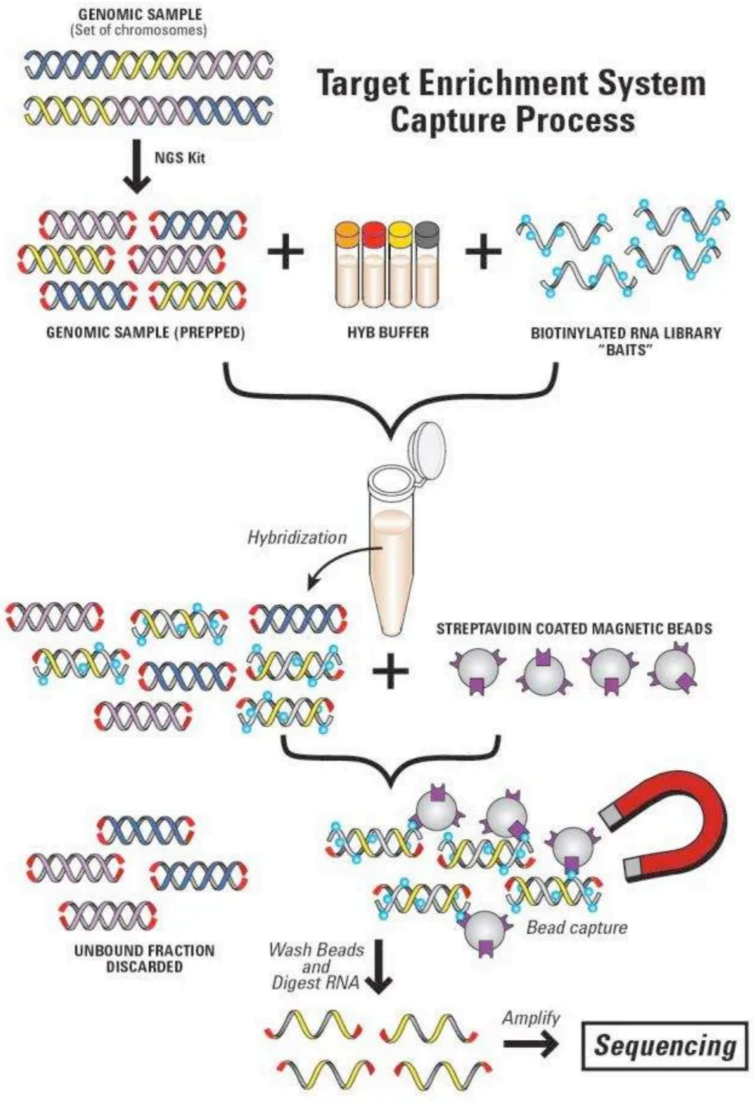
Fan Han, Minal Jamsandekar, Mats E Pettersson, Leyi Su, Angela P Fuentes-Pardo, Brian W Davis, Dorte Bekkevold, Florian Berg, Michele Casini ... Leif Andersson see all »

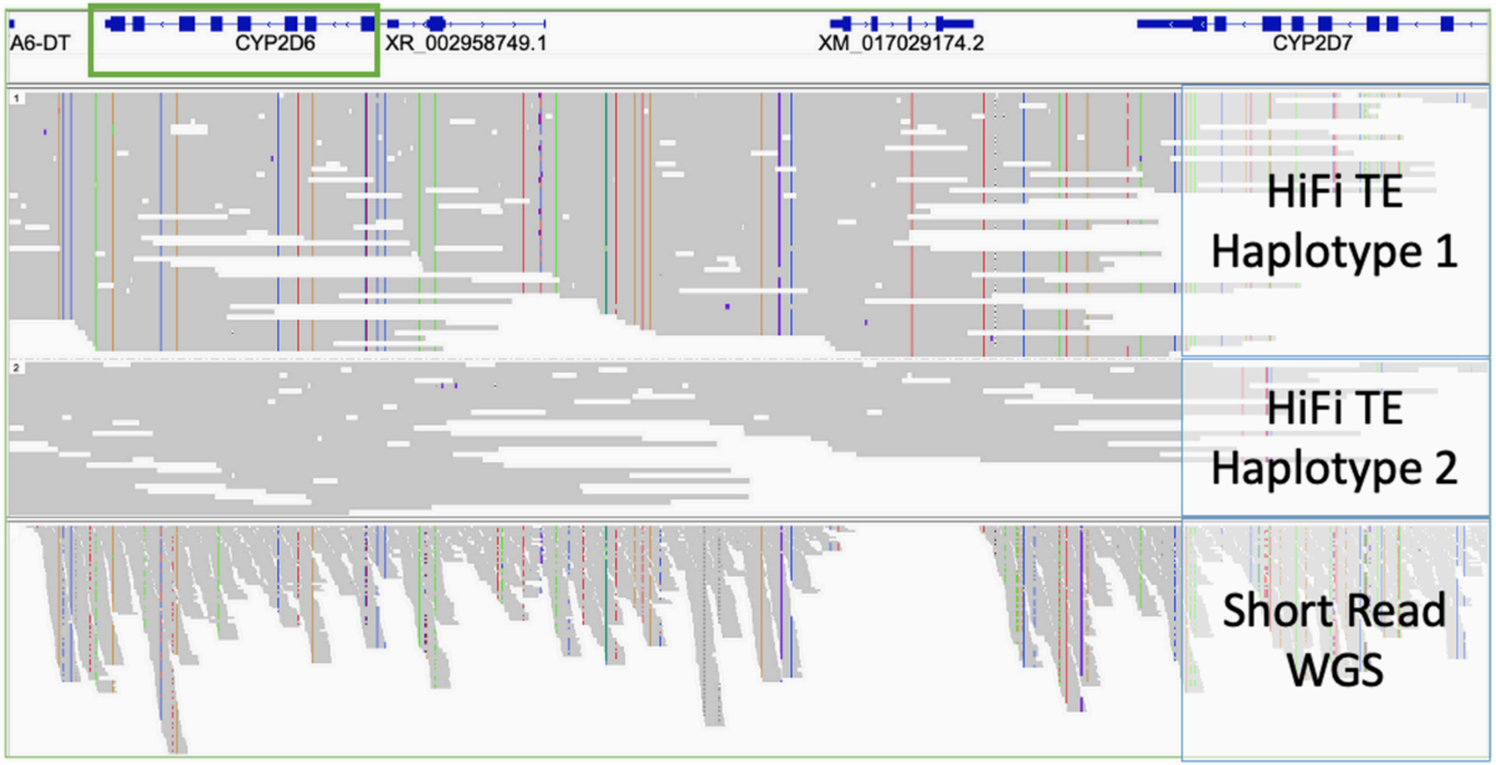
Targeted sequencing

Zooming into portions of a genome - a cost effective screening alternative

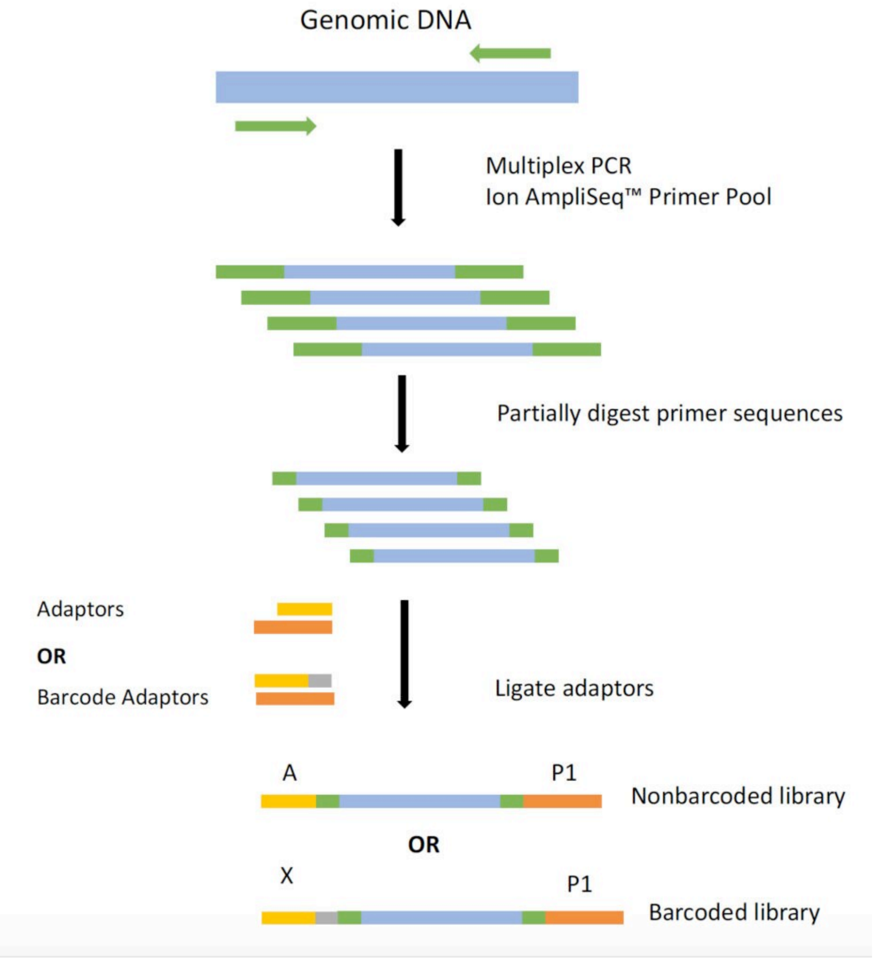
- **Capture with probes**
  - Panels or custom (Agilent, ThermoFisher, Twist, etc)
  - Specificity varies
  - Material requirements (good quality and quantity)
  - Cost
- **PCR**
  - Own primers or custom panels (e.g. Ion AmpliSeq)
  - High sensitivity and specificity
  - Prior knowledge of sequence is needed
  - Bias and product length limitations
- **CRISPR-Cas9**
  - Prior knowledge of sequence is needed
  - Requires a lot of DNA
  - Off-target effects reported

Targeted sequencing: capture with probes

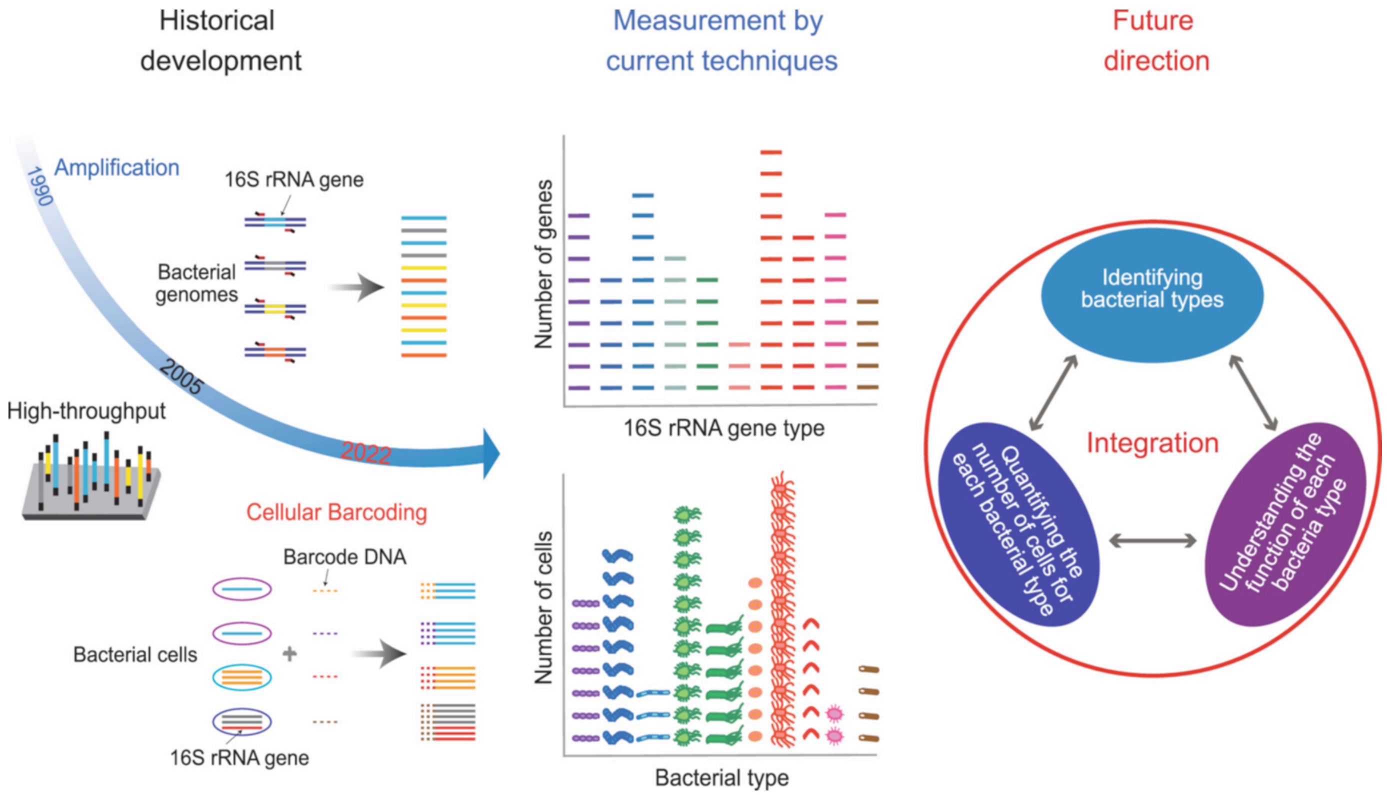




Targeted sequencing: PCR (amplicon seq)



Disease panels with multiple targets (e.g. Ion AmpliSeq)



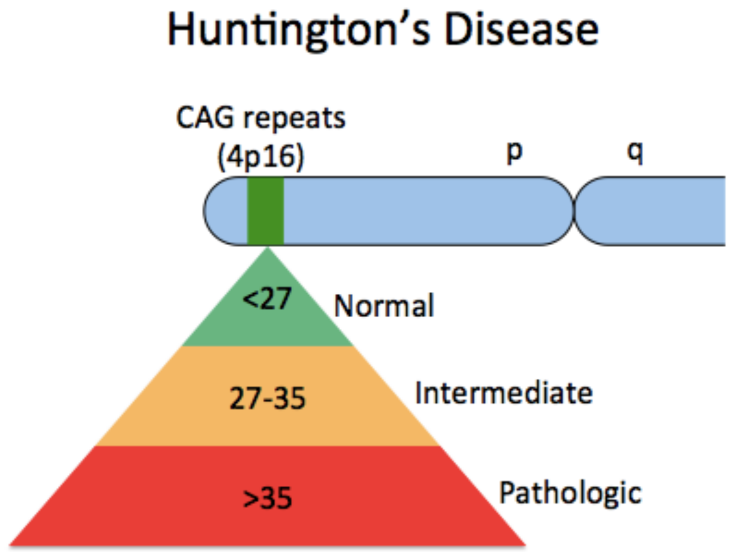
REVIEW ARTICLE | Open Access |

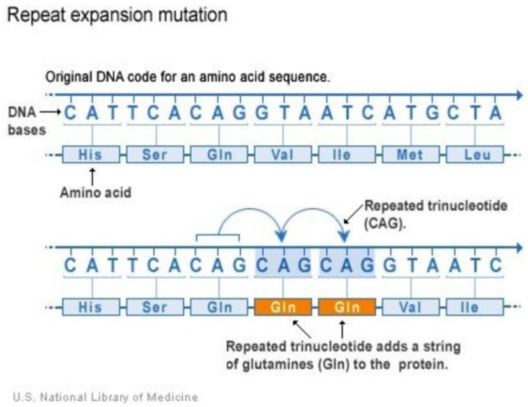
Long journey of 16S rRNA-amplicon sequencing toward cell-based functional bacterial microbiota characterization

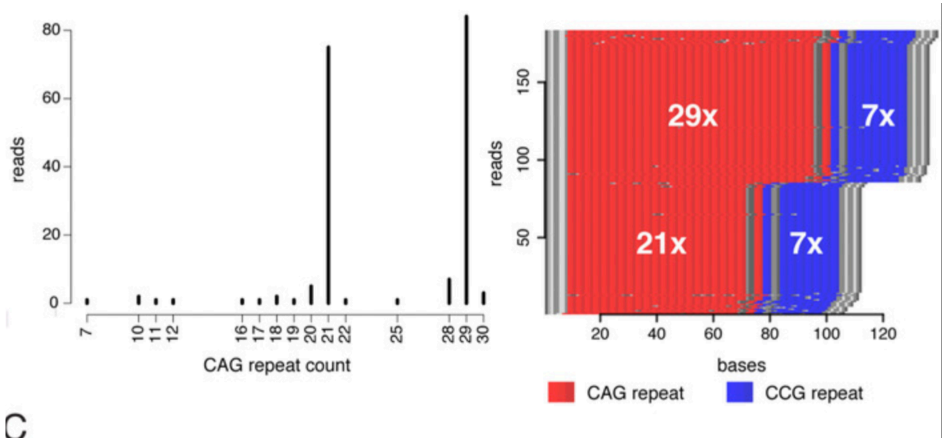
Jianshi Jin, Xiongduo Liu, Katsuyuki Shiroguchi

First published: 11 July 2024 | https://doi.org/10.1002/imo2.9 | Citations: 2

Targeted sequencing: CRISPR-Cas9




Repeat expansion mutation
Original DNA code for an amino acid sequence.
DNA bases
C A T T C A C A G G T A A T C A T G C T A
His Ser Gln Val Ile Met Leu
Amino acid
Repeated trinucleotide (CAG).
C A T T C A C A G C A G C A G G T A A T C
His Ser Gln Gln Gln Val Ile
Repeated trinucleotide adds a string of glutamines (Gln) to the protein.
U.S. National Library of Medicine



Huntington's disease:

- Inherited disorder resulting in brain cell death
- Decline of motor and cognitive functions
- Common onset: 30-50 years of age
- No cure
- Causative genetic variant: CAG-repeat expansion in *HTT* gene

Problem: polymerase slippage – low complexity regions

PCR-based methods do not work

Detailed analysis of *HTT* repeat elements in human blood using targeted amplification-free long-read sequencing

Ida Höijer, Yu-Chih Tsai, Tyson A. Clark, Paul Kotturi, Niklas Dahl, Eva-Lena Stattin, Marie-Louise Bondeson, Lars Feuk, Ulf Gyllensten, Adam Ameur

First published: 22 June 2018 | https://doi.org/10.1002/humu.23580 | Citations: 62

Targeted sequencing: technology & depth?

Talk to different vendors: technology-specific solutions

Run a pilot! (e.g. known truth vs real-life)

Hierarchical data reduction to determine sequencing depth

Ask for advise from your sequencing provider

Transcriptome Sequencing (RNA-seq)

TOTAL RNA

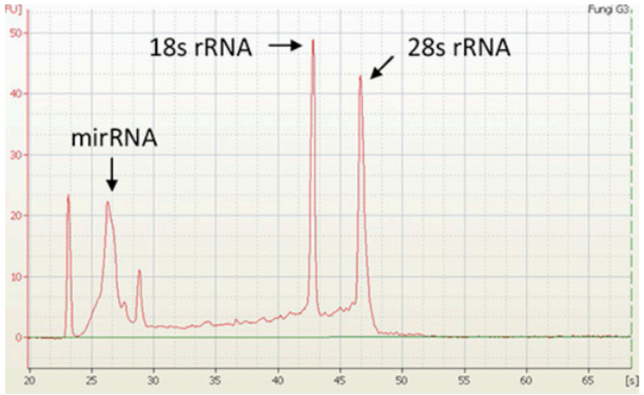
mRNA

- **Differential expression**
- Annotation

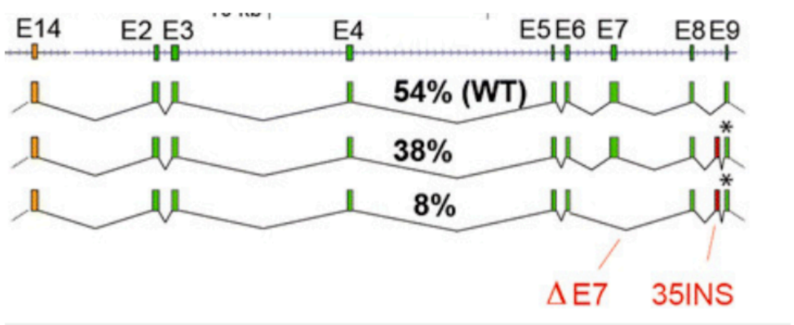
Non-codingRNA

- Transcriptional regulation

miRNA



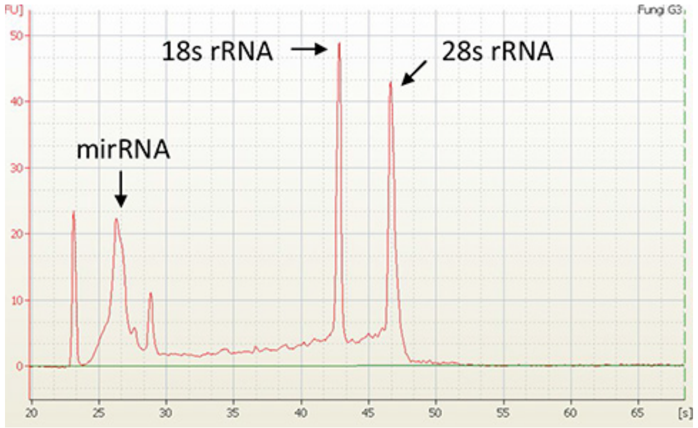
Splice isoforms

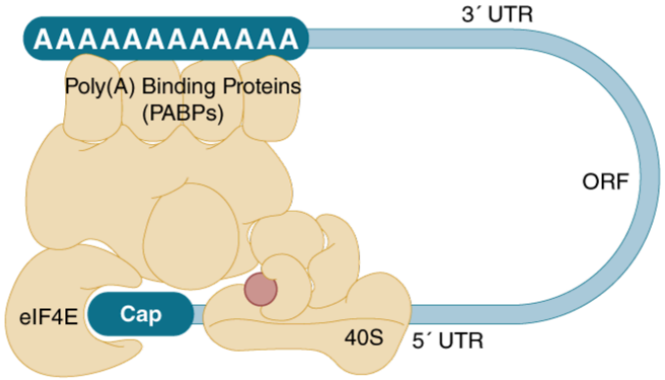


Transcriptome Sequencing (RNA-seq)

One must get rid or rRNA. To PolyA or not to PolyA?

Method	Pros	Cons
rRNA depletion	• Captures on-going transcription • Picks up non-coding RNAs	• Does not get rid of all rRNA • Messy Dif.Ex. profile
polyA selection	• Gives a clean Dif.Ex. Profile • Looses all non-polyA RNAs	• Does not pick many non-coding RNAs





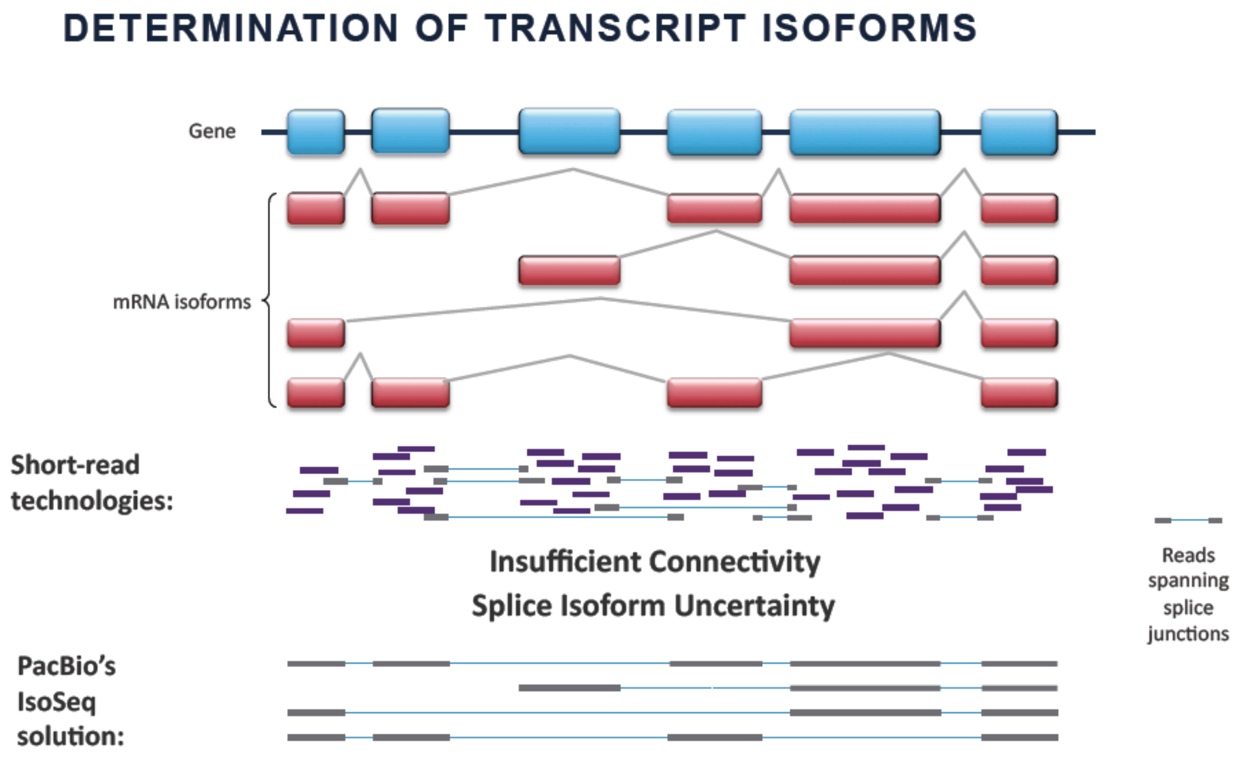
Number of reads

Differential expression with a **good*** reference: 5+M PE reads (up to 100M for rare transcripts)

Annotation: a minimum of 50M PE reads of mixed tissue (rather 100M per tissue)

*well-annotated

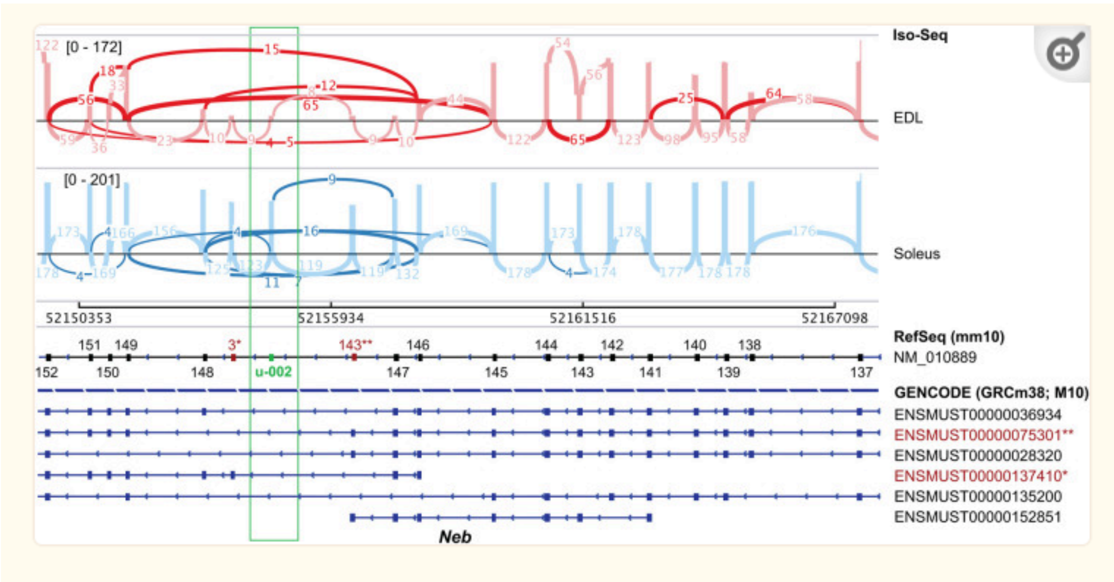
RNA-seq with log reads



DETERMINATION OF TRANSCRIPT ISOFORMS

Insufficient Connectivity
Splice Isoform Uncertainty

Full-length cDNA Sequence Reads
Splice Isoform Certainty – No Assembly Required



Genome Res. 2020 Jun; 30(6): 885–897.

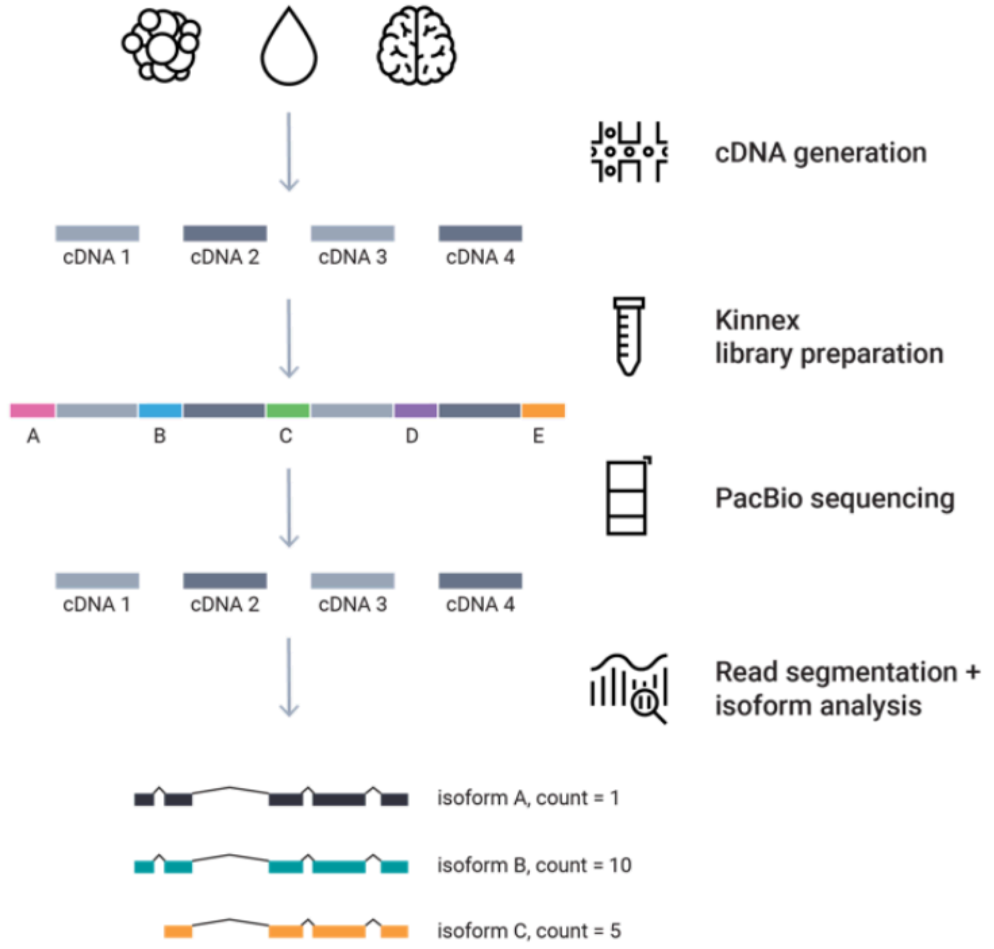
NEB:

one of the biggest protein coding genes in vertebrates (22kb mRNA, 183 exons)

Codes for nebulin, muscle protein

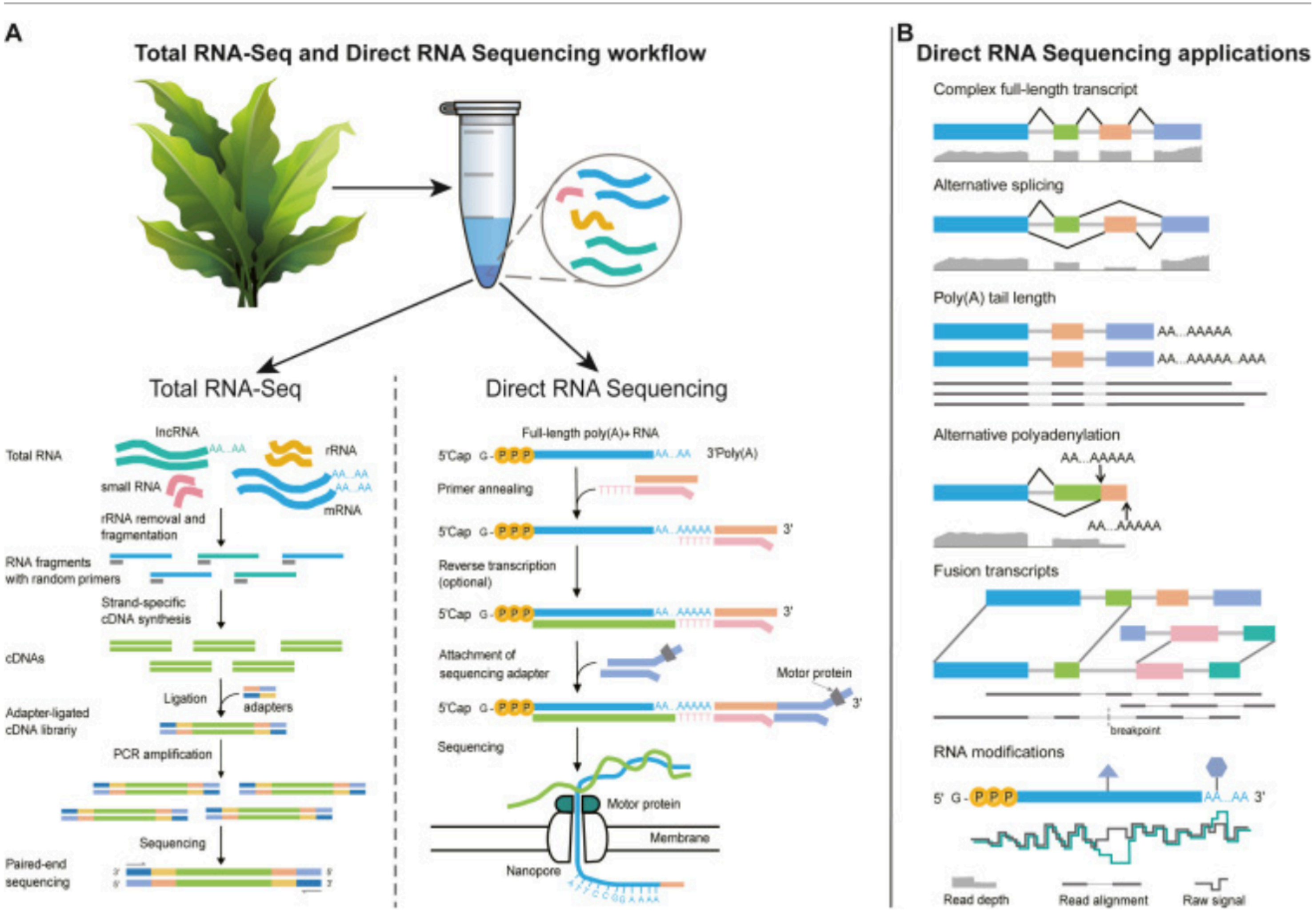
Differential expression with long reads

- Best of both worlds: expression values AND isoform information
- Prior to Q2 2024 - ONT only (polyA or CAP-selected)
- Now: both ONT and PacBio Kinnex



RNA-seq: direct RNA sequencing on ONT

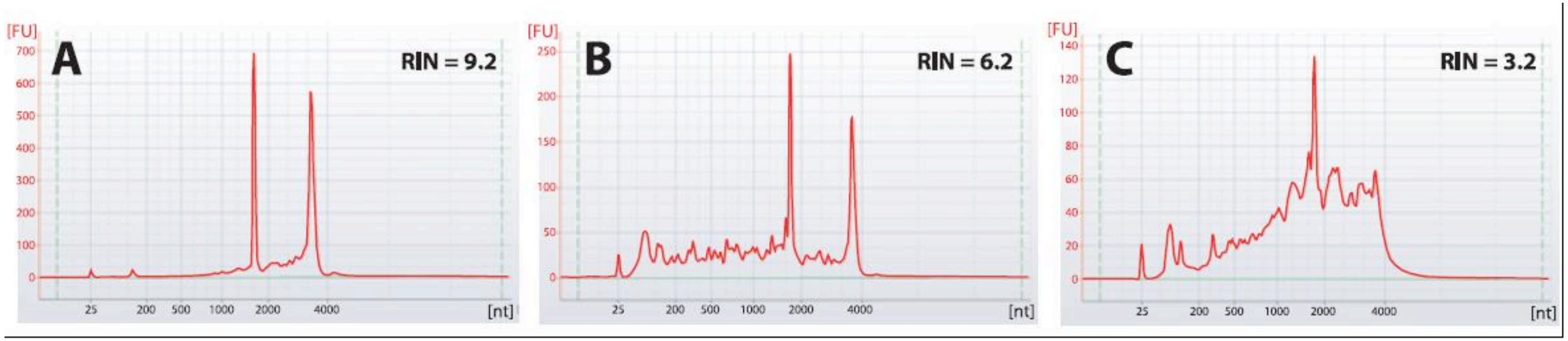
Do not get too excited



One cannot use
direct RNA-sequencing
for differential gene expression
experiments!
(January 2026)

RNA-seq considerations

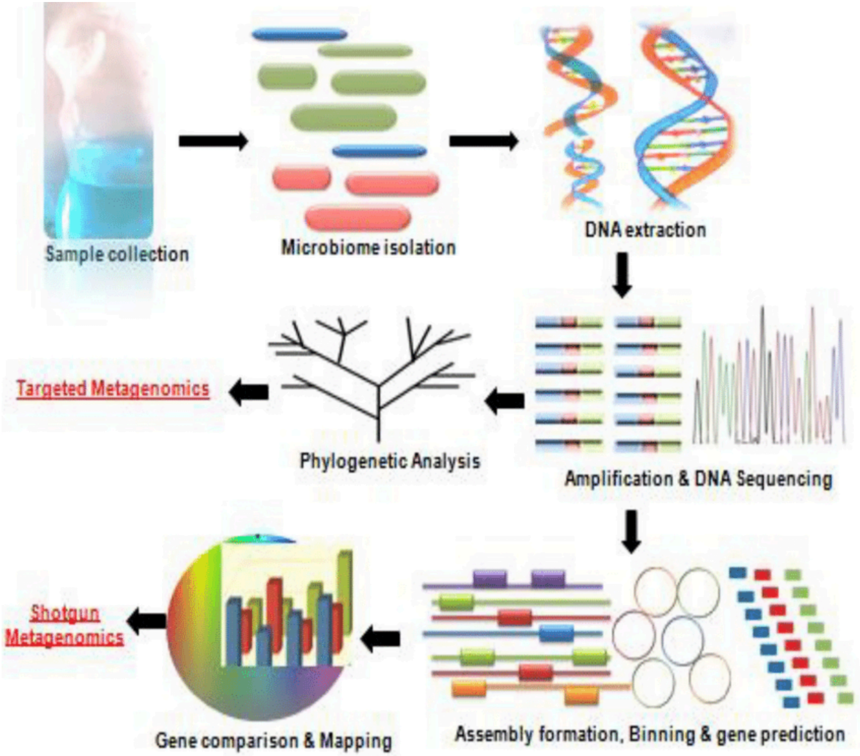
- mRNA only: use any kit (for annotation and long-reads we recommend TRIZOL)
- mRNA **and** miRNA: only specialized kits
- Always use DNase!
- RIN value above 8

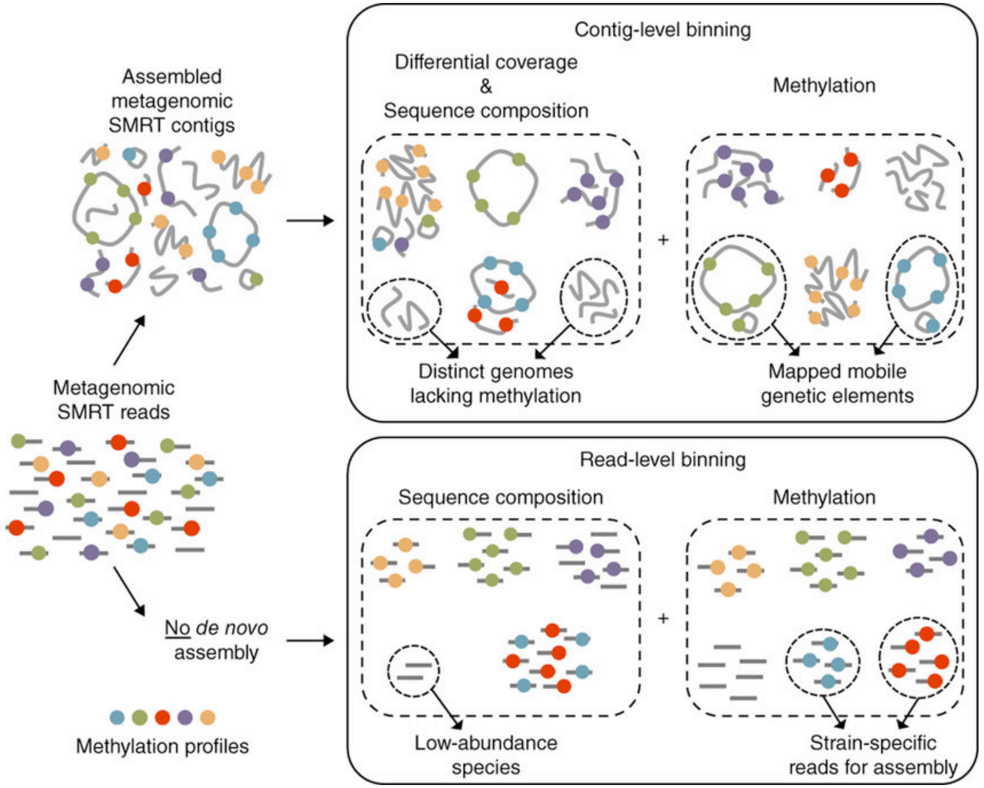


- CONTROL vs experimental conditions
- Biological replicates: a minimum of 4 is strongly recommended

Shotgun metagenomics

- Strongly recommend a pilot + hierarchical data reduction to determine the sequencing depth
- Can be done with both short and long reads
- If with long reads - consider utilizing epigenetic signature for plasmid assignment / OTU binning





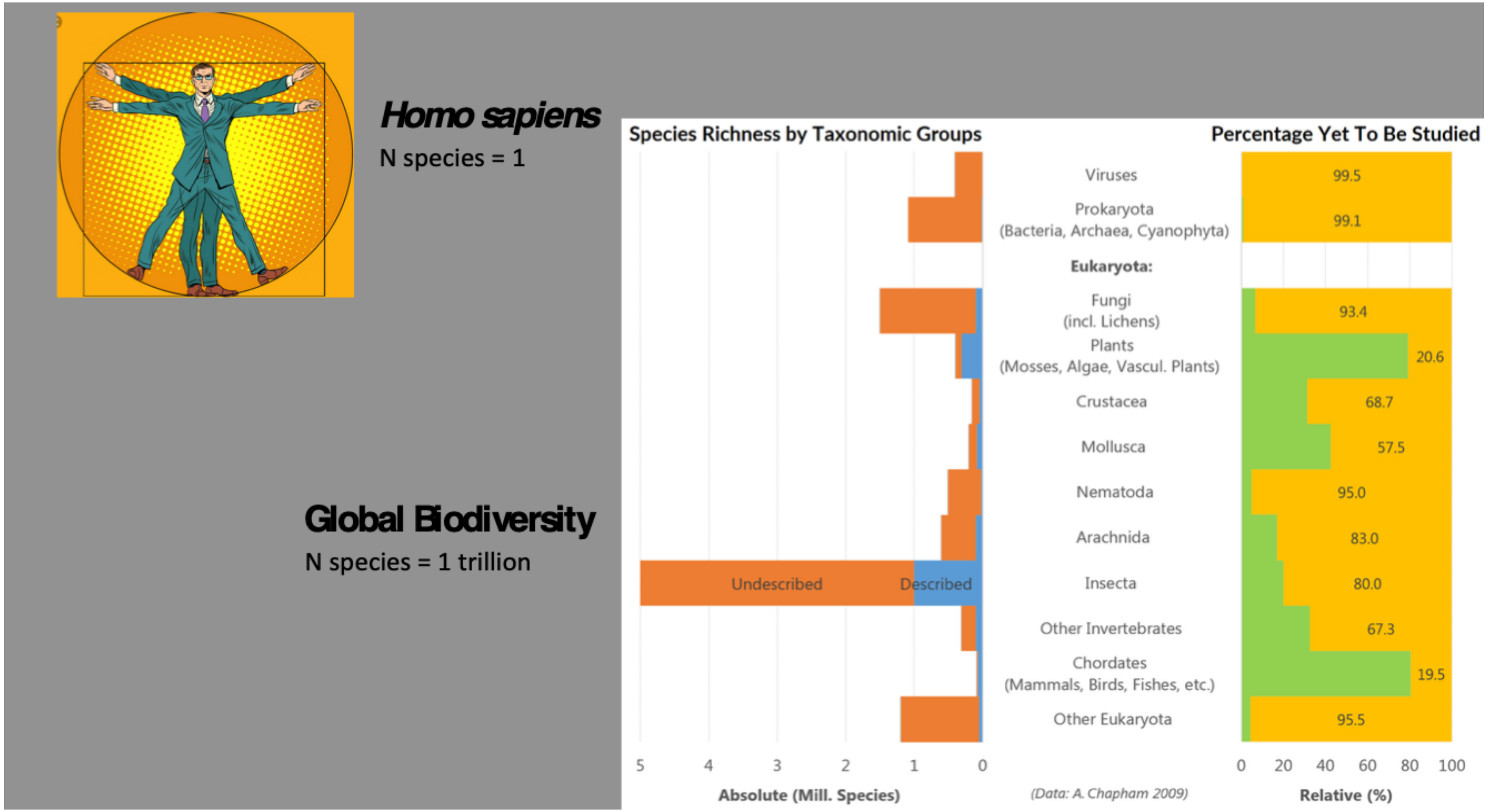
Assembled metagenomic SMRT contigs
Metagenomic SMRT reads
No de novo assembly
Methylation profiles
Contig-level binning
Differential coverage & Sequence composition
Methylation
+
Distinct genomes lacking methylation
Mapped mobile genetic elements
Read-level binning
Sequence composition
Methylation
+
Low-abundance species
Strain-specific reads for assembly

Diving into deep: reference genome sequencing of non-models

Coffee break?

De novo (reference genome) sequencing

- Sequencing a genome without a prior reference



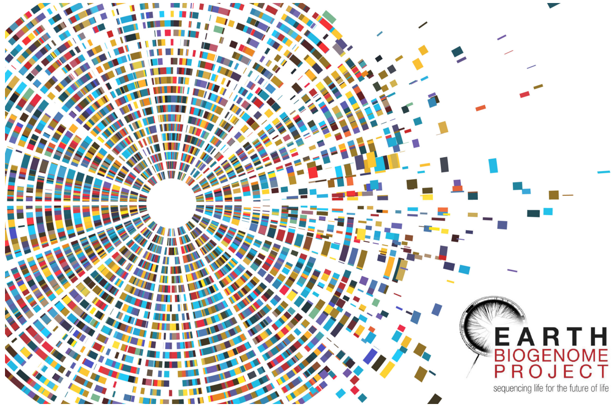
Reference genome sequencing (RefGen)

- Only closely related taxa can be used for alignment

- Always done with long reads
- Enable any kind of downstream genetic analysis

- Generating a chromosome-scale reference genome is a life-time investment. But it is costly.

- Sit tight, it is going to be a long one



ERGA
EUROPEAN REFERENCE GENOME ATLAS

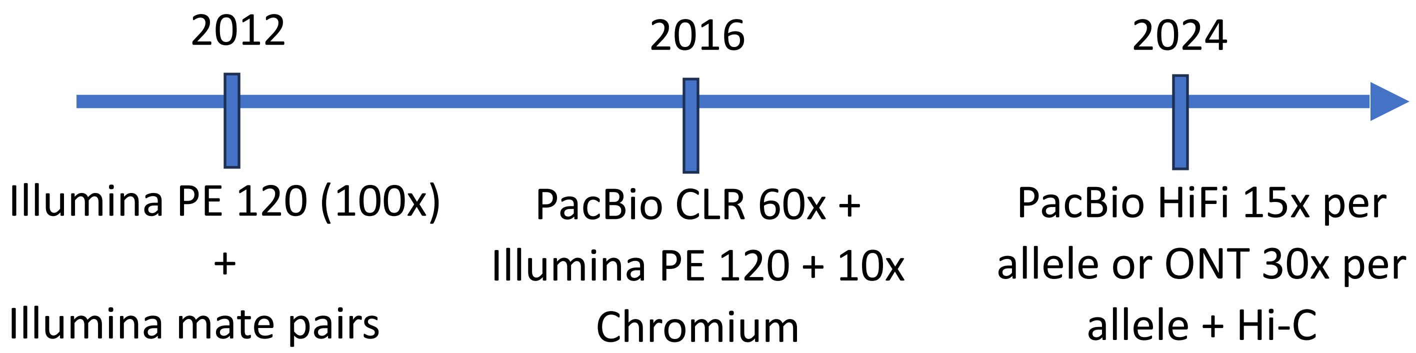
Biodiversity
Genomics
Europe

S

Optimal technology combination for RefGen is a moving target

Technology is constantly developing

So do the methods of analysis



2012

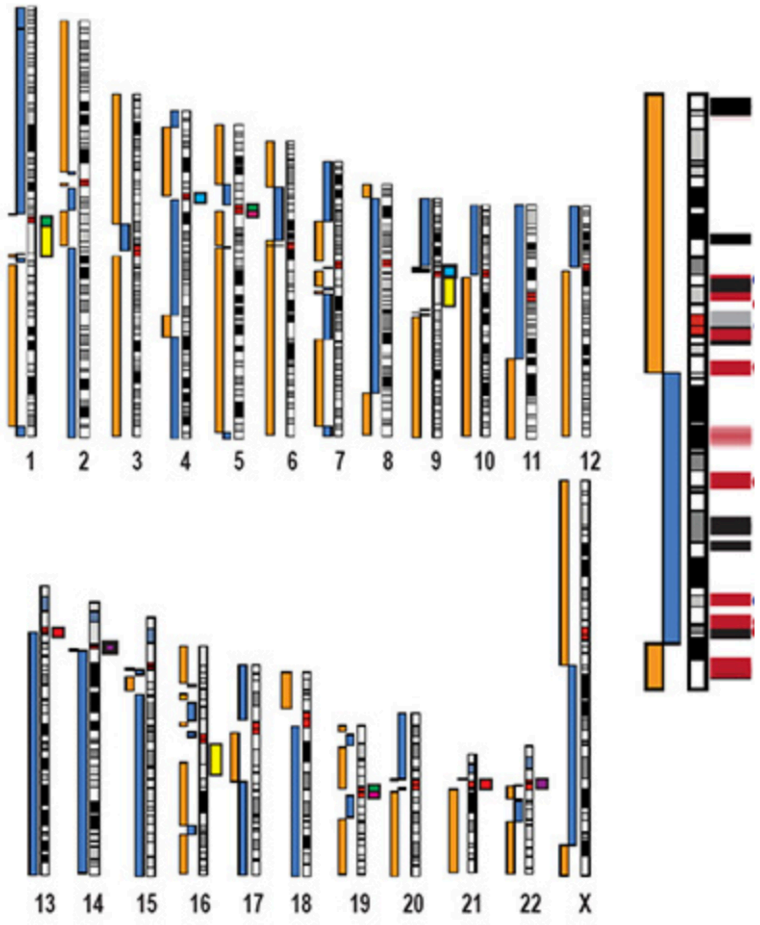
Illumina PE 120 (100x)
+
Illumina mate pairs

2016

PacBio CLR 60x +
Illumina PE 120 + 10x
Chromium

2024

PacBio HiFi 15x per
allele or ONT 30x per
allele + Hi-C



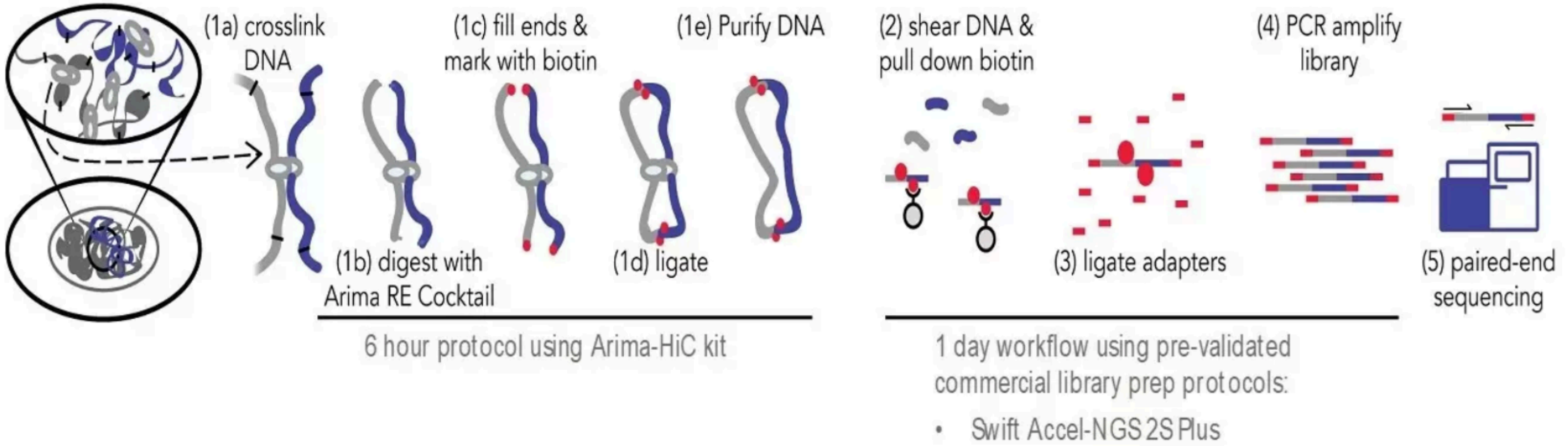
Where to check? ERGA, DToL or EBP SOPs

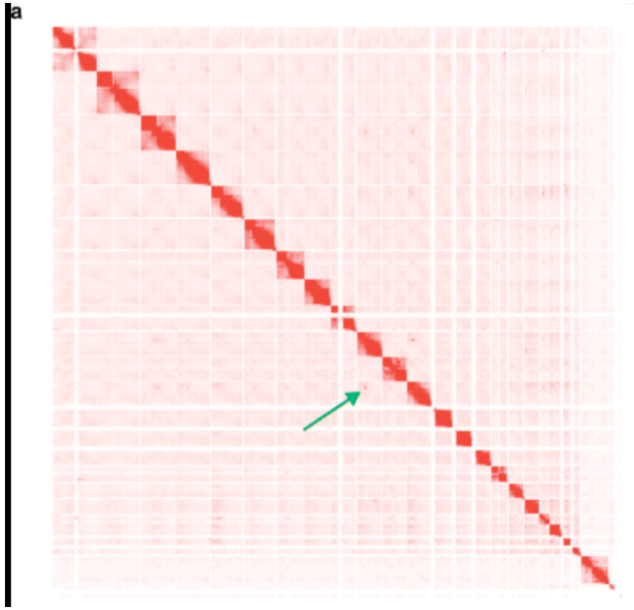
RefGen recipe, details

- 15x PacBio HiFi per allele / ONT 30x per allele + low-coverage Illumina /or ONT 20x per allele with R10 pores
- HiC 50M reads per Gb of genome (Arima HiC or DoveTail OmniC)
- RNA-seq for annotation
  - 50M reads per tissue mix (EBP standard)
  - Rather 100M reads per tissue, use as many tissues as possible

HiC library principle: DNA arrangement in chromosomes

Invaluable for chromosome reconstruction


(1a) crosslink DNA
(1c) fill ends & mark with biotin
(1e) Purify DNA
(2) shear DNA & pull down biotin
(4) PCR amplify library
(1b) digest with Arima RE Cocktail
(1d) ligate
(3) ligate adapters
(5) paired-end sequencing
6 hour protocol using Arima-HiC kit
1 day workflow using pre-validated commercial library prep protocols:
• Swift Accel-NGS 2S Plus



FAQ: What data should I add to improve my existing fragmented assembly?

A: Do not waste your time and just do it from scratch.

Time of a bioinformatician is more expensive than sequencing.

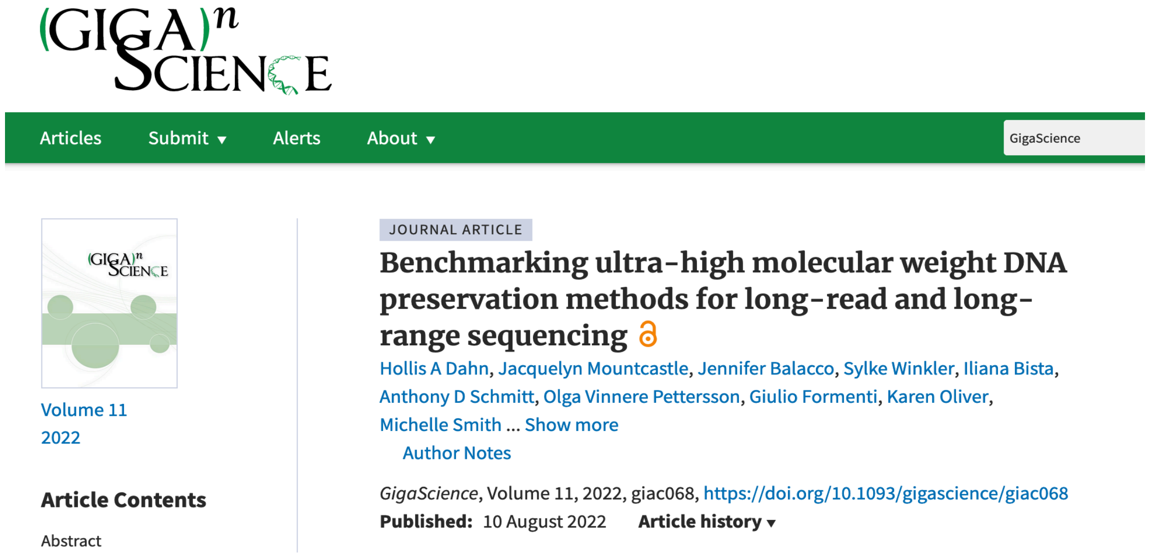
Long-read technologies nowadays is not what they used to be 5 years ago.

Do not “polish” your HiFi reads with Illumina - you will just introduce errors.

Important to keep in mind while planning reference genome sequencing

RefGen: sampling methodology is IMPORTANT

Taxon-specific sampling and preservation



(GIGA)^n SCIENCE

Articles | Submit ▾ | Alerts | About ▾ | GigaScience

Volume 11
2022

Article Contents

Abstract

JOURNAL ARTICLE

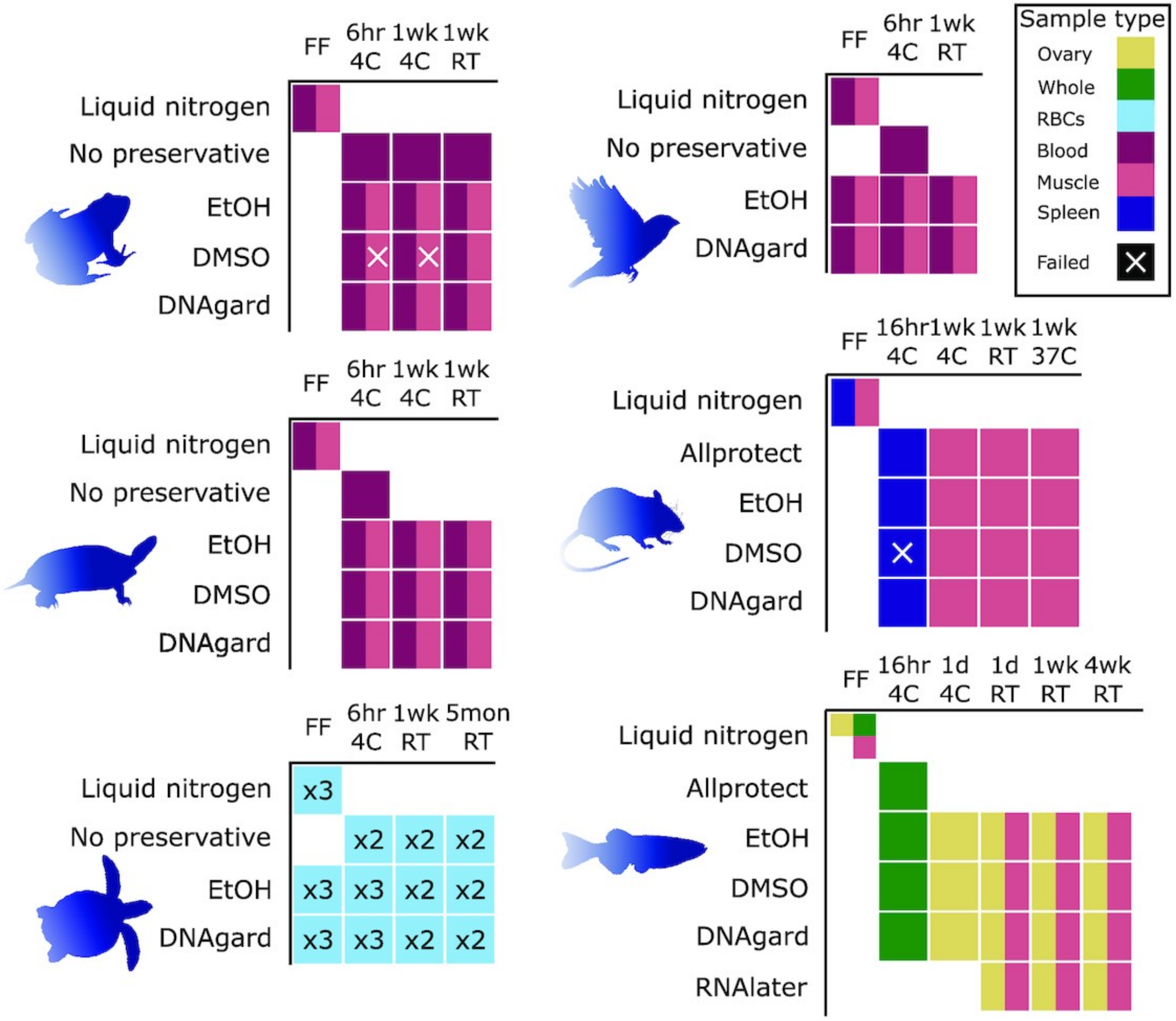
Benchmarking ultra-high molecular weight DNA preservation methods for long-read and long-range sequencing

Hollis A Dahn, Jacquelyn Mountcastle, Jennifer Balacco, Sylke Winkler, Iliana Bista, Anthony D Schmitt, Olga Vinnere Pettersson, Giulio Formenti, Karen Oliver, Michelle Smith ... Show more

Author Notes

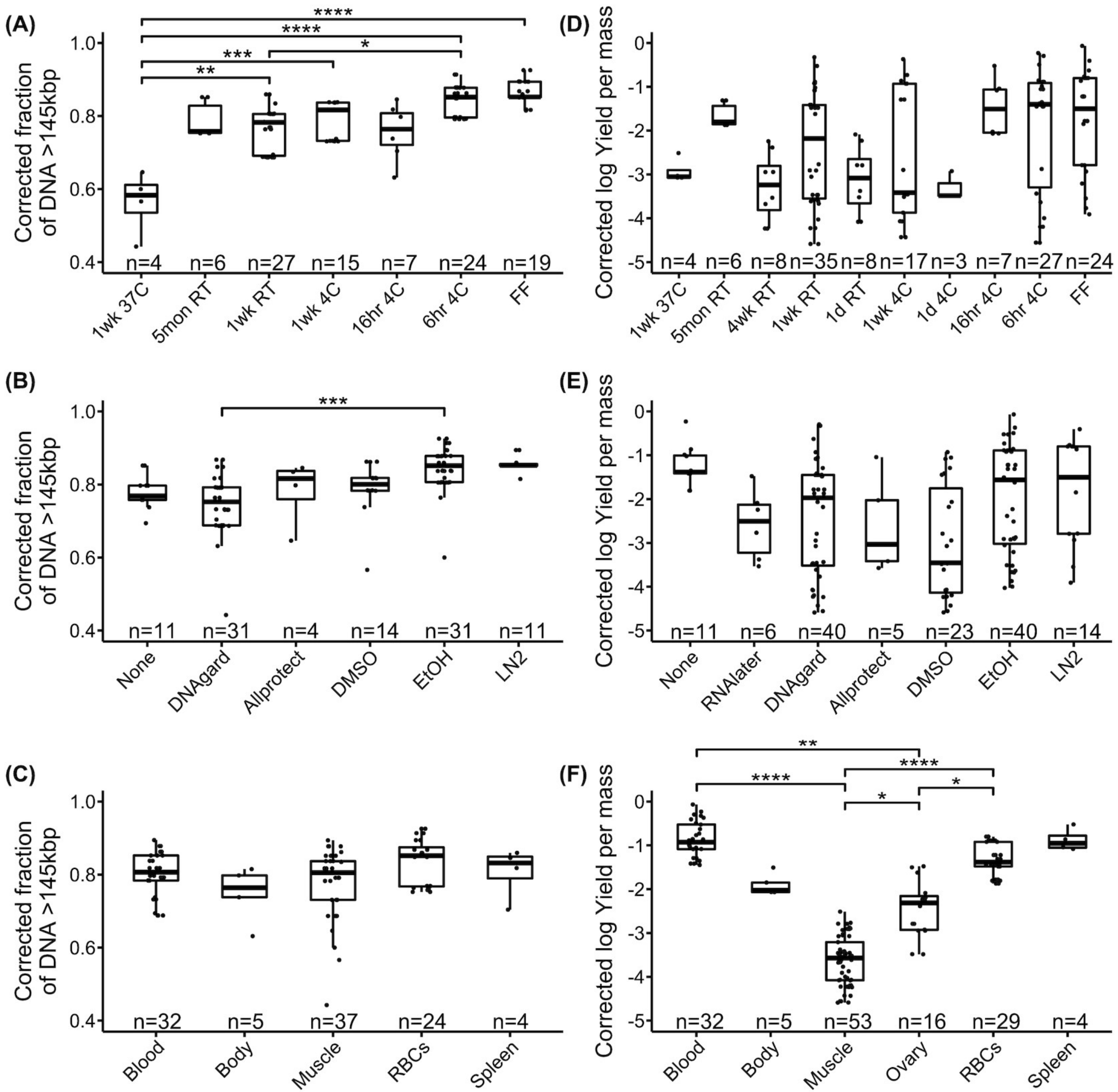
GigaScience, Volume 11, 2022, giac068, https://doi.org/10.1093/gigascience/giac068

Published: 10 August 2022 **Article history** ▾



Dahn et al, 2022

RefGen: Sampling parameters matter



The most important one – TEMPERATURE after collection

Dahn et al, 2022

RefGen: Sample processing in the lab or field

- Optimally – keep the organism alive as long as possible
  - Do not freeze before dissection

- If large organism:
  - Dissect on ice
  - **Lentil-size** tissue parts are the best

  Weight the sample if possible

Picture by Mara Lawnizak

- Place the parts into **pre-chilled** separate (barcoded) tubes
  - Make sure the tubes are suitable for -70°C

Please, do not send us a whole frozen mammal…..

RefGen: Correct taxonomic identification

- Make sure that the specimen is correctly identified!
- Ask for second opinion if unsure

  Note: the heterogametic sex is always preferred

- Sanger-sequence DNA barcodes
  - Will help ID
  - Will be used as a tracker to safeguard against sample mix-up

- Take a picture including a **measurement instrument** (=digital voucher)

  (if possible – include a colour chart)

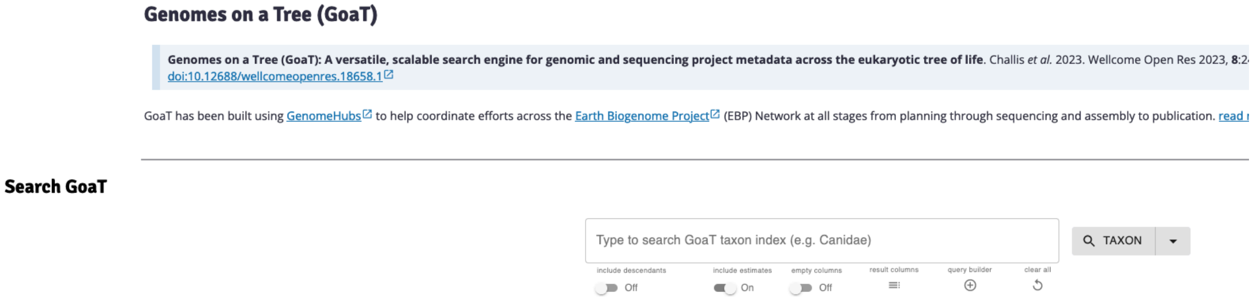
Before going to the field, check list

- How much material is needed? How many individuals?
- What should I bring to the field if the sample must be processed there? Can we invest in a dry shipper or a portable fridge?
- Can my sample be preserved in ethanol (check with seq center and literature!)
- If the genome is supposed to be annotated – bring along RNALater or TRIZOL for the dedicated sample

- Record metadata (FAIR):
  - living stage of the organism
  - sex
  - body / organism part
  - time and temperature between sampling and preservation
  - GPS coordinates

How to check if someone is already sequencing the species you are interested in?

- **Talk to the GoaT!** https://goat.genomehubs.org/



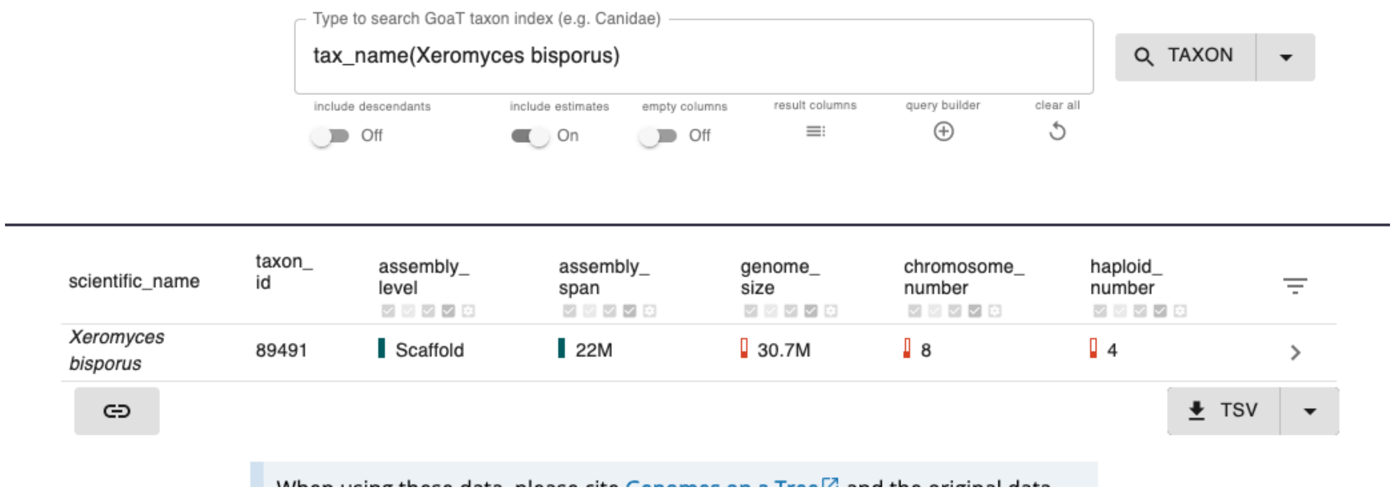
Genomes on a Tree (GoaT)

> **Genomes on a Tree (GoaT): A versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic tree of life**. Challis *et al.* 2023. Wellcome Open Res 2023, **8**:2
> doi:10.12688/wellcomeopenres.18658.1

GoaT has been built using GenomeHubs to help coordinate efforts across the Earth Biogenome Project (EBP) Network at all stages from planning through sequencing and assembly to publication. read r

Search GoaT

Type to search GoaT taxon index (e.g. Canidae)

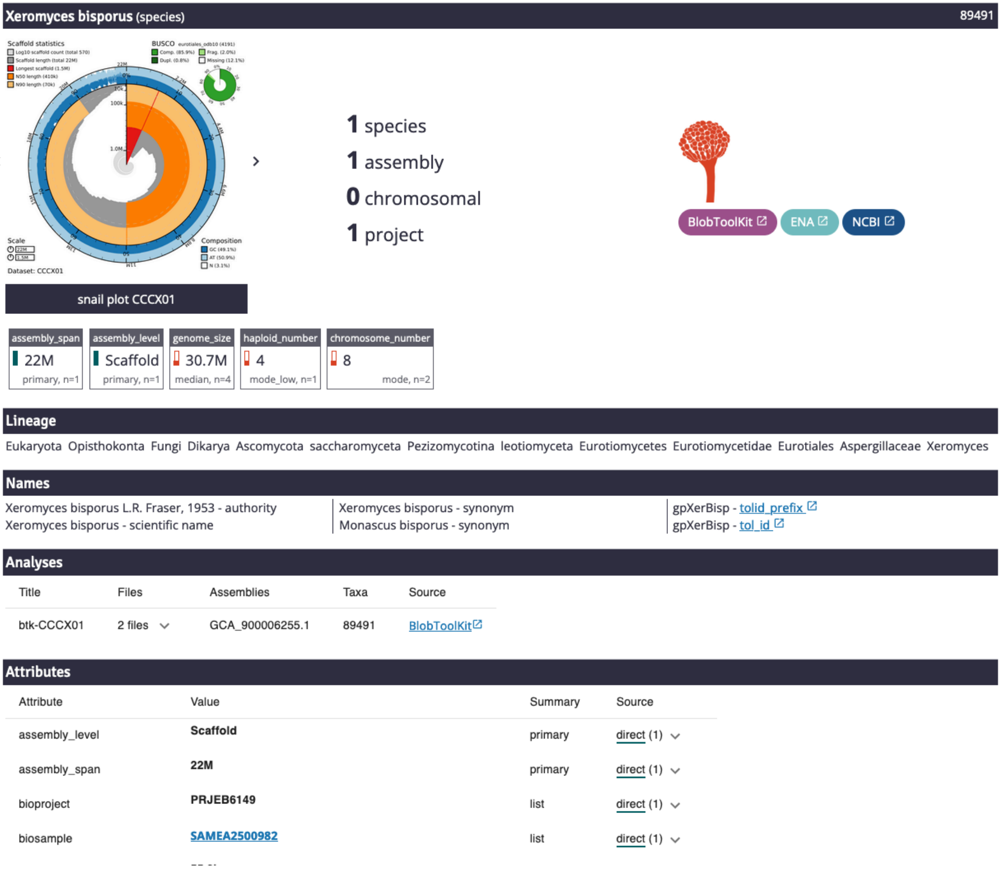


Type to search GoaT taxon index (e.g. Canidae)

tax_name(Xeromyces bisporus)

scientific_name	taxon_id	assembly_level	assembly_span	genome_size	chromosome_number	haploid_number
Xeromyces bisporus	89491	Scaffold	22M	30.7M	8	4

When using these data, please cite Genomes on a Tree and the original data



Xeromyces bisporus (species) 89491

1 species
1 assembly
0 chromosomal
1 project

assembly_span	assembly_level	genome_size	haploid_number	chromosome_number
22M primary, n=1	Scaffold primary, n=1	30.7M median, n=4	4 mode_low, n=1	8 mode, n=2

Lineage

Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Eurotiomycetidae Eurotiales Aspergillaceae Xeromyces

Names

Xeromyces bisporus L.R. Fraser, 1953 - authority
Xeromyces bisporus - scientific name
Xeromyces bisporus - synonym
Monascus bisporus - synonym
gpXerBisp - tolid_prefix
gpXerBisp - tol_id

Analyses

Title	Files	Assemblies	Taxa	Source
btk-CCCX01	2 files	GCA_900006255.1	89491	BlobToolKit

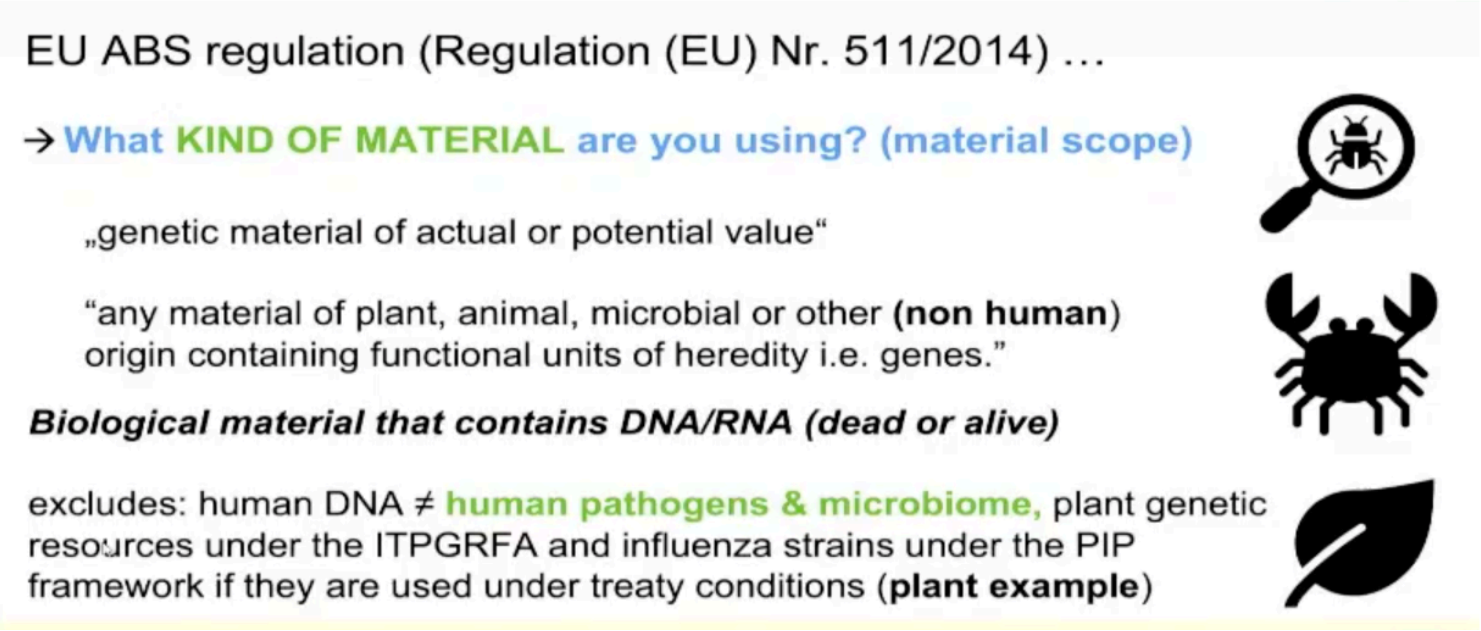
Attributes

Attribute	Value	Summary	Source
assembly_level	**Scaffold**	primary	direct (1)
assembly_span	**22M**	primary	direct (1)
bioproject	**PRJEB6149**	list	direct (1)
biosample	**SAMEA2500982**	list	direct (1)

Not just RefGen: Legal issues - Nagoya & CITES

TAKE IT SERIOUSLY

Non-compliance: jail sentence, fine, paper retraction, etc



EU ABS regulation (Regulation (EU) Nr. 511/2014) …

→ **What KIND OF MATERIAL are you using? (material scope)**

„genetic material of actual or potential value"

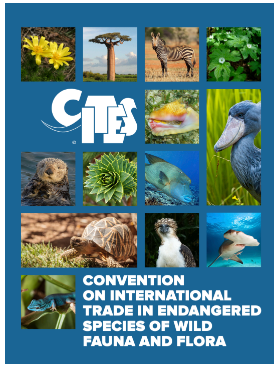
"any material of plant, animal, microbial or other **(non human)** origin containing functional units of heredity i.e. genes."

Biological material that contains DNA/RNA (dead or alive)

excludes: human DNA ≠ **human pathogens & microbiome,** plant genetic resources under the ITPGRFA and influenza strains under the PIP framework if they are used under treaty conditions (**plant example**)

Courtesy: Scarlett Sett, Kiel University, Germany

CITES - trade with endangered species (cites.org)


CITES
CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA

A paperwork nightmare from HELL
Get in touch with your governmental authorities at least 4 months prior to intended shipment

And do not forget all other "normal" import/export permits for shipment of biological material!

Sample quality requirements

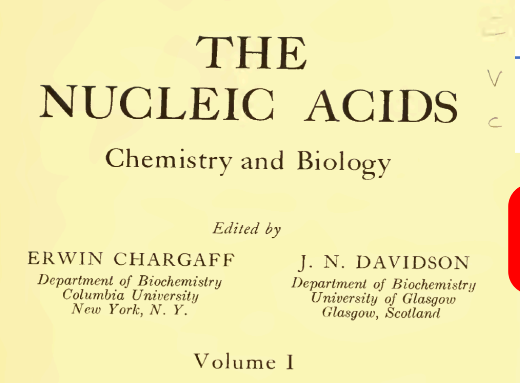
Garbage in – garbage out:

Sequencing success **always** depends on the sample quality

NGS-quality DNA and
PCR-quality DNA
are two completely different things

Especially for long-read sequencing

Considering DNA extractions...



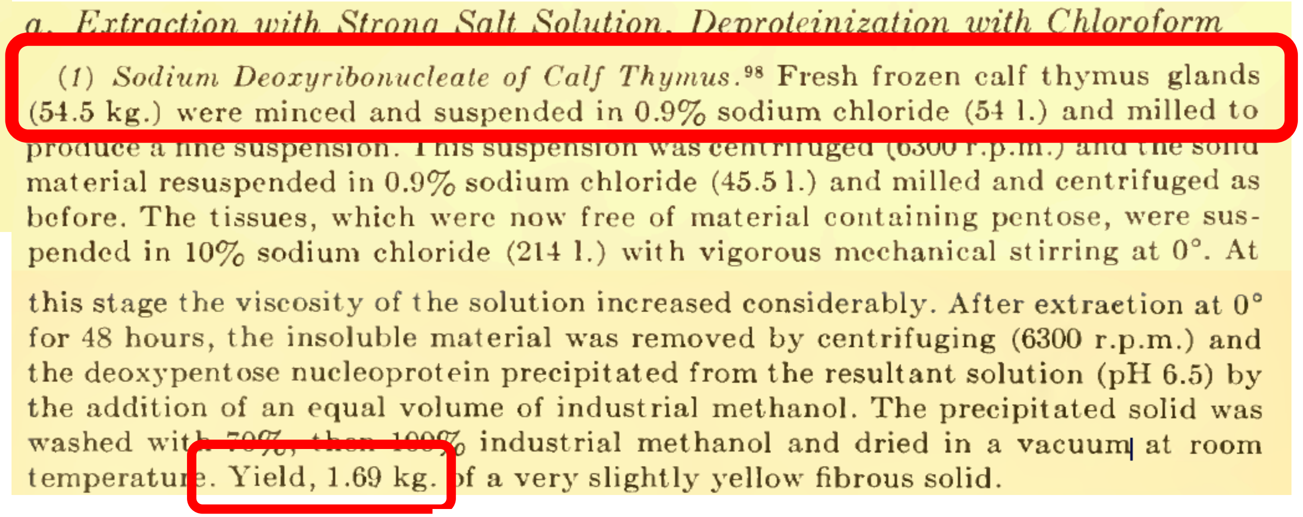
THE
NUCLEIC ACIDS
Chemistry and Biology

Edited by

ERWIN CHARGAFF
Department of Biochemistry
Columbia University
New York, N. Y.

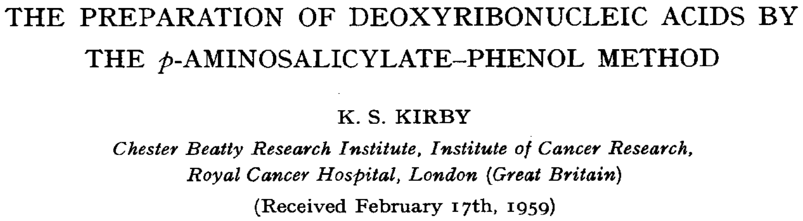
J. N. DAVIDSON
Department of Biochemistry
University of Glasgow
Glasgow, Scotland

Volume I



a. Extraction with Strong Salt Solution. Deproteinization with Chloroform

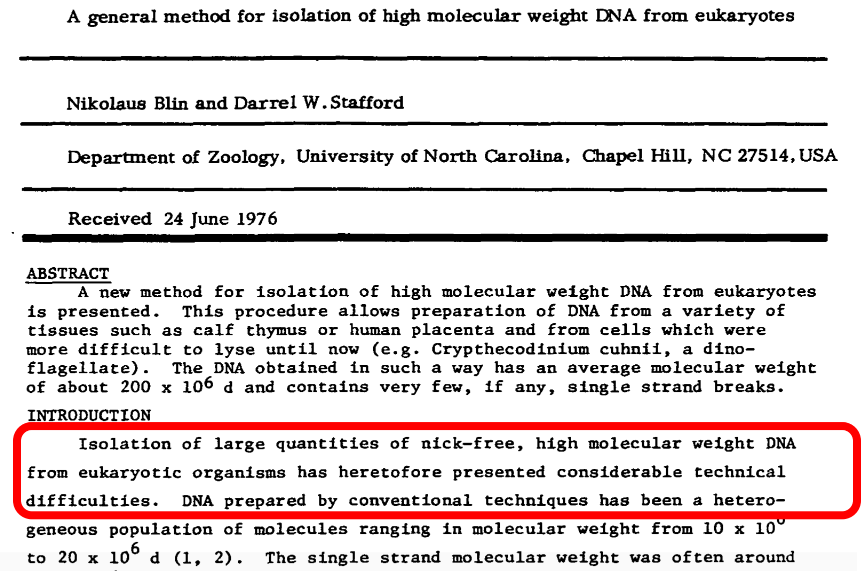
(1) *Sodium Deoxyribonucleate of Calf Thymus.*[98] Fresh frozen calf thymus glands (54.5 kg.) were minced and suspended in 0.9% sodium chloride (54 l.) and milled to produce a fine suspension. This suspension was centrifuged (6300 r.p.m.) and the solid material resuspended in 0.9% sodium chloride (45.5 l.) and milled and centrifuged as before. The tissues, which were now free of material containing pentose, were suspended in 10% sodium chloride (214 l.) with vigorous mechanical stirring at 0°. At this stage the viscosity of the solution increased considerably. After extraction at 0° for 48 hours, the insoluble material was removed by centrifuging (6300 r.p.m.) and the deoxypentose nucleoprotein precipitated from the resultant solution (pH 6.5) by the addition of an equal volume of industrial methanol. The precipitated solid was washed with 70%, then 100% industrial methanol and dried in a vacuum at room temperature. Yield, 1.69 kg. of a very slightly yellow fibrous solid.



THE PREPARATION OF DEOXYRIBONUCLEIC ACIDS BY THE *p*-AMINOSALICYLATE–PHENOL METHOD

K. S. KIRBY
Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London (Great Britain)

(Received February 17th, 1959)



A general method for isolation of high molecular weight DNA from eukaryotes

Nikolaus Blin and Darrel W. Stafford

Department of Zoology, University of North Carolina, Chapel Hill, NC 27514, USA

Received 24 June 1976

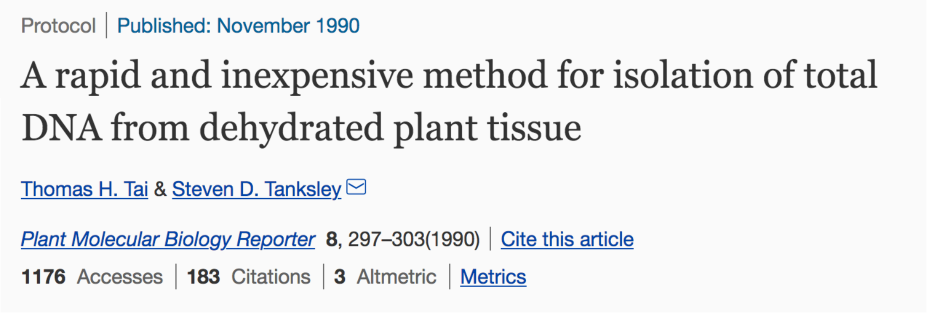
ABSTRACT

A new method for isolation of high molecular weight DNA from eukaryotes is presented. This procedure allows preparation of DNA from a variety of tissues such as calf thymus or human placenta and from cells which were more difficult to lyse until now (e.g. Crypthecodinium cuhnii, a dinoflagellate). The DNA obtained in such a way has an average molecular weight of about 200 x 10^6 d and contains very few, if any, single strand breaks.

INTRODUCTION

Isolation of large quantities of nick-free, high molecular weight DNA from eukaryotic organisms has heretofore presented considerable technical difficulties. DNA prepared by conventional techniques has been a heterogeneous population of molecules ranging in molecular weight from 10 x 10^6 to 20 x 10^6 d (1, 2). The single strand molecular weight was often around

1983: PCR



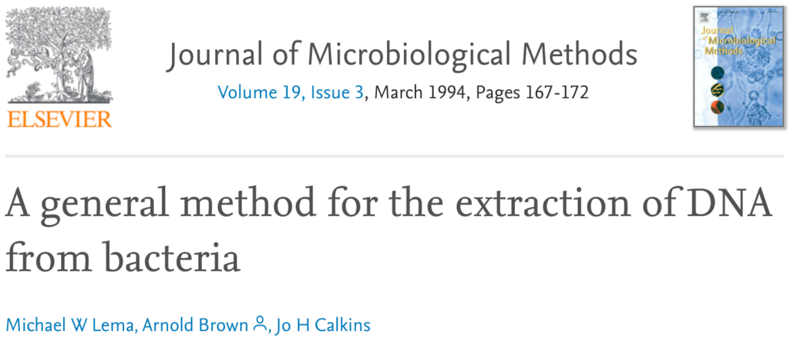
Protocol | Published: November 1990

A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue

Thomas H. Tai & Steven D. Tanksley ✉

Plant Molecular Biology Reporter **8**, 297–303(1990) | Cite this article

1176 Accesses | **183** Citations | **3** Altmetric | Metrics



ELSEVIER

Journal of Microbiological Methods

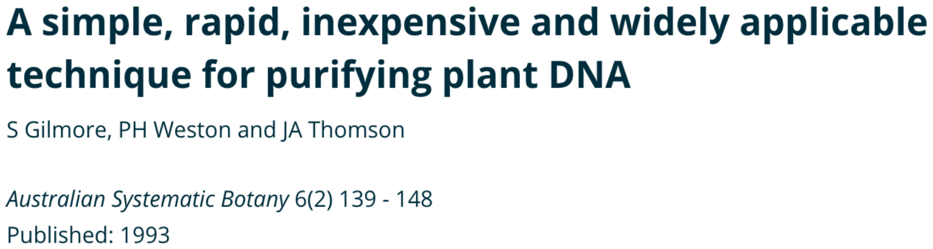
Volume 19, Issue 3, March 1994, Pages 167-172

A general method for the extraction of DNA from bacteria

Michael W Lema, Arnold Brown, Jo H Calkins

⊞ Show more

https://doi.org/10.1016/0167-7012(94)90066-3

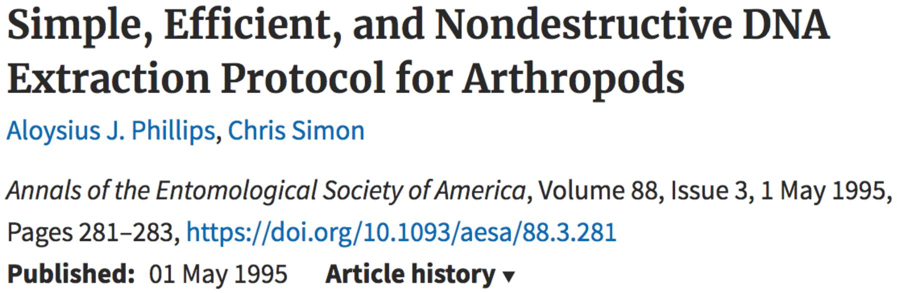


A simple, rapid, inexpensive and widely applicable technique for purifying plant DNA

S Gilmore, PH Weston and JA Thomson

Australian Systematic Botany 6(2) 139 - 148

Published: 1993



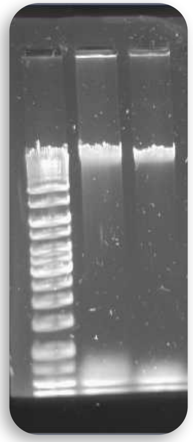
Simple, Efficient, and Nondestructive DNA Extraction Protocol for Arthropods

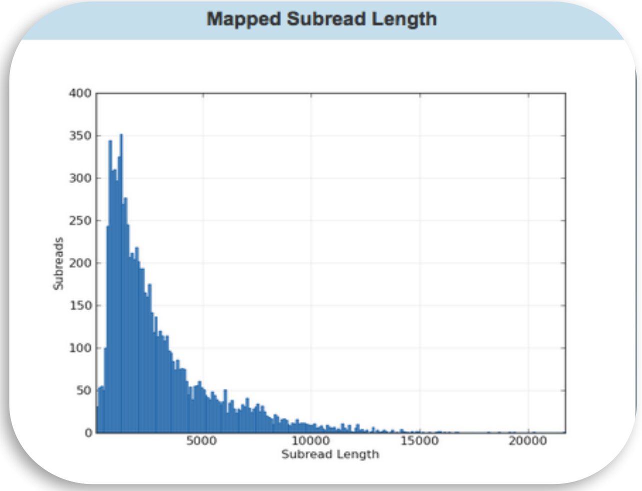
Aloysius J. Phillips, Chris Simon

Annals of the Entomological Society of America, Volume 88, Issue 3, 1 May 1995, Pages 281–283, https://doi.org/10.1093/aesa/88.3.281

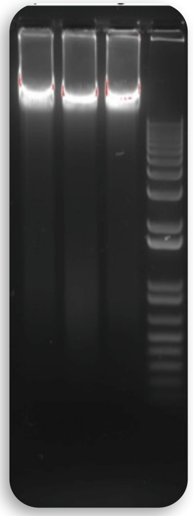
Published: 01 May 1995 **Article history ▾**

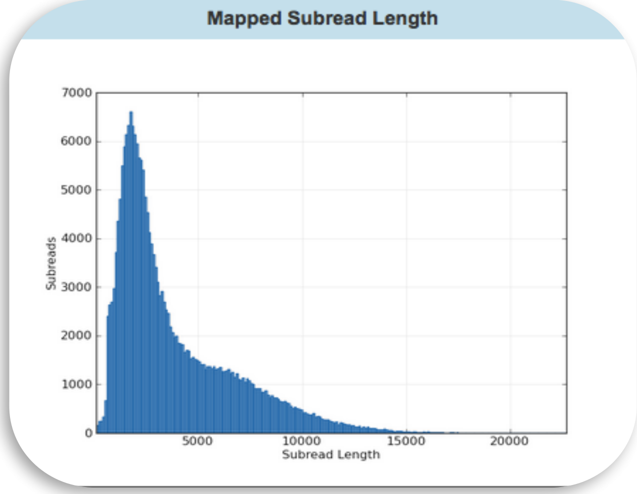
2013: a wake-up call





Polished Contigs	223	Max Contig Length	36,298
N50 Contig Length	2,932	Sum of Contig Lengths	480,087





Polished Contigs	9	Max Contig Length	1,508,929
N50 Contig Length	1,353,702	Sum of Contig Lengths	7,813,244

For Long Reads one needs to have *long and pure* DNA

SciLifeLab

DNA quality and inhibition of sequencing

Short-read technologies: PCR inhibition
Long-read technologies are PCR-free, but one sequences native DNA “as is”.

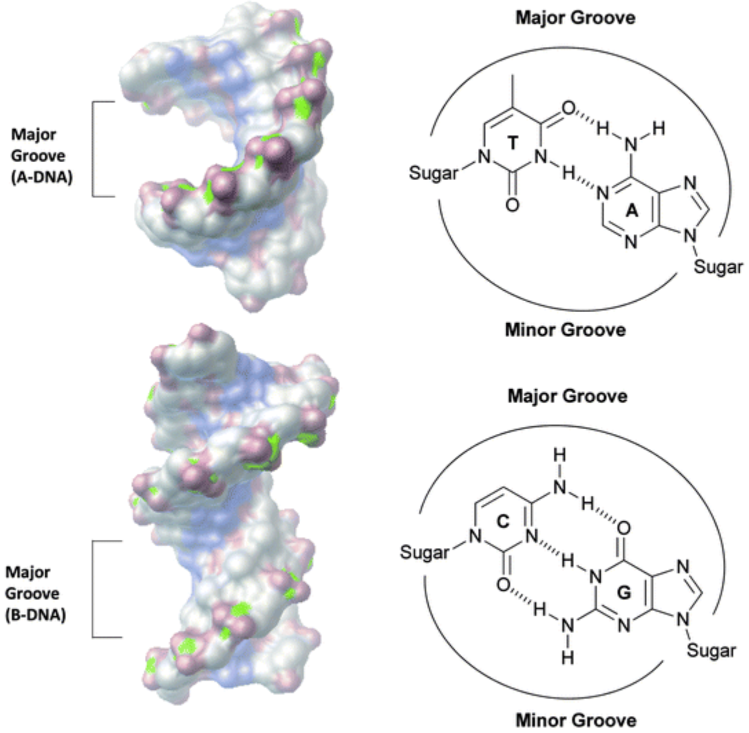
DNA-binders:

- Proteins
- Polyphenols
- Secondary metabolites (e.g. toxins)
- Pigments
- Polysaccharides

Polymerase inhibitors:

- Salts
- Phenol
- Alcohols

Physical inhibiting factors – debris



*[Hamilton & Arya, Nat. Prod. Rep.](), 2012, **29**, 134-143*

What do absorption ratios tell us?

Pure DNA 260/280: 1.8 – 2.0

< 1.8:

Too little DNA compared to other components of the solution; presence of organic contaminants: proteins and phenol; glycogen - **absorb at 280 nm**.

> 2.0:

High share of RNA.

Pure DNA 260/230: 2.0 – 2.2

<2.0:

Salt contamination, humic acids, peptides, aromatic compounds, polyphenols, urea, guanidine, thiocyanates (latter three are common kit components) – **absorb at 230 nm**.

>2.2:

High share of RNA, very high share of phenol, **high turbidity,** dirty instrument, wrong blank.

Photometrically active contaminants:
phenol, polyphenols, EDTA, thiocyanate, protein,
RNA, nucleotides (fragments below 5 bp)

Help! My absorption values are bad!!!

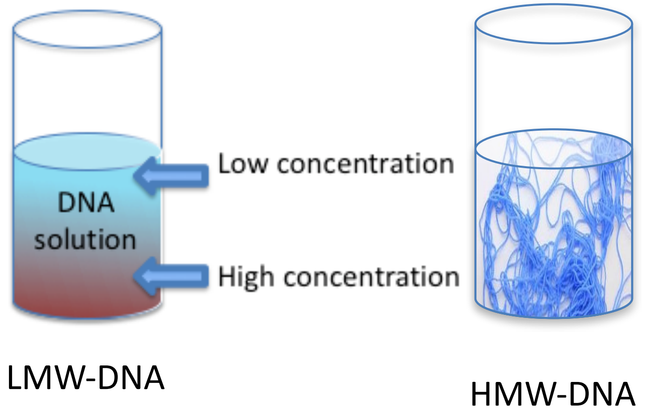
Qiagen DNeasy Power Cleanup Pro

Zymogen gDNA cleanup and concentrator

Besides: AMPure beads, phenol-chloroform-CTAB, etc

Check protocols.io!

How to make a correct DNA measurement



- Thaw DNA completely
- Mix gently (**never vortex!**)
- Put the sample on a thermoblock: 37°C, 15-30 min
- Mix gently
- **Dilute 1:100** (if HMW)
- Mix gently
- Make a measurement with an appropriate blank

- **NANODROP is Bad**. Point.
- Use Qubit, or PicoGreen.
- Nanodrop value : Qubit value ≤ 50%

Causes of DNA degradation/damage

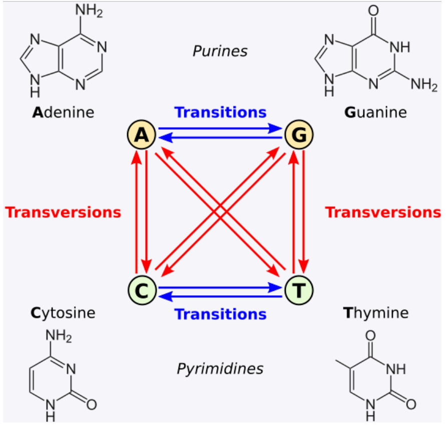
Mechanical damage during tissue homogenization.

Wrong pH and ionic strength of extraction buffer (-> hydrolysis).

Incomplete removal / contamination with **nucleases**.

Phenol: too old, or inappropriately buffered (**pH 7.8 – 8.0**); incomplete removal.

Wrong pH of the **DNA solvent** (acidic water).
Recommended: Low TE for short-term storage, 1xTE for long-term storage.



Vigorous pipetting (wide-bore pipet tips).

Vortexing of DNA in high concentrations.

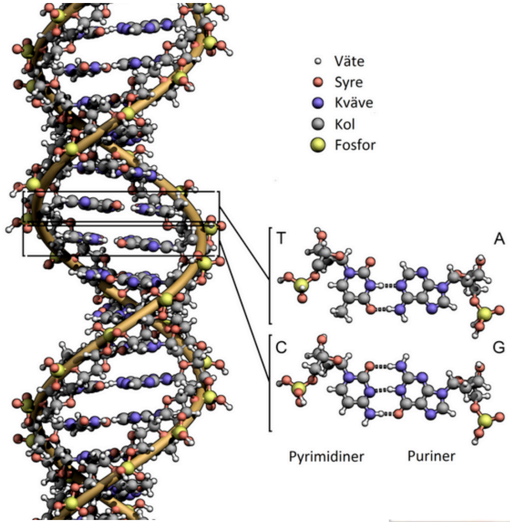
Too many **freeze-thaw** cycles (*we tested 5, still Ok*).

Sequence-dependency: depurination, deamination, T-C transitions…

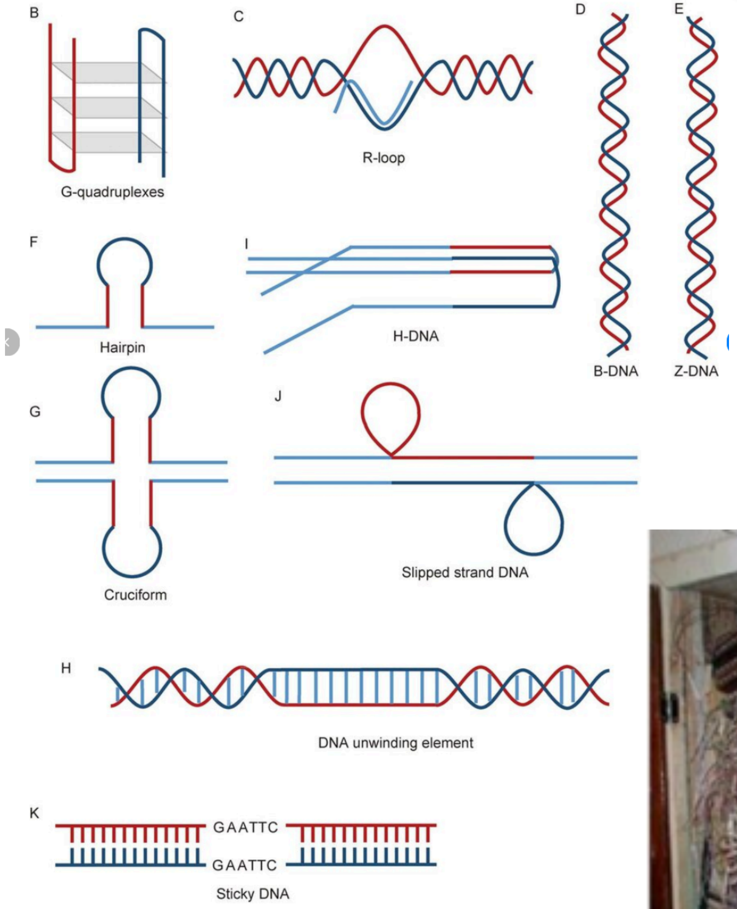
https://www.biorxiv.org/content/10.1101/254276v3

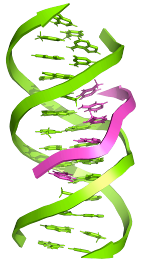
To keep in mind

There are lots of surprises, more so in non-model organisms



What textbook tells you





Brutal reality

Do not forget:
DNA in solution behaves differently

What every sequencing facility wished you knew before starting your project

Sequencing facilities and their sample requirements

Two types: commercial and non-profit (university-based)

Some can do DNA / RNA extractions, some do not

Number of samples and tissues vary (from 1 to 20)

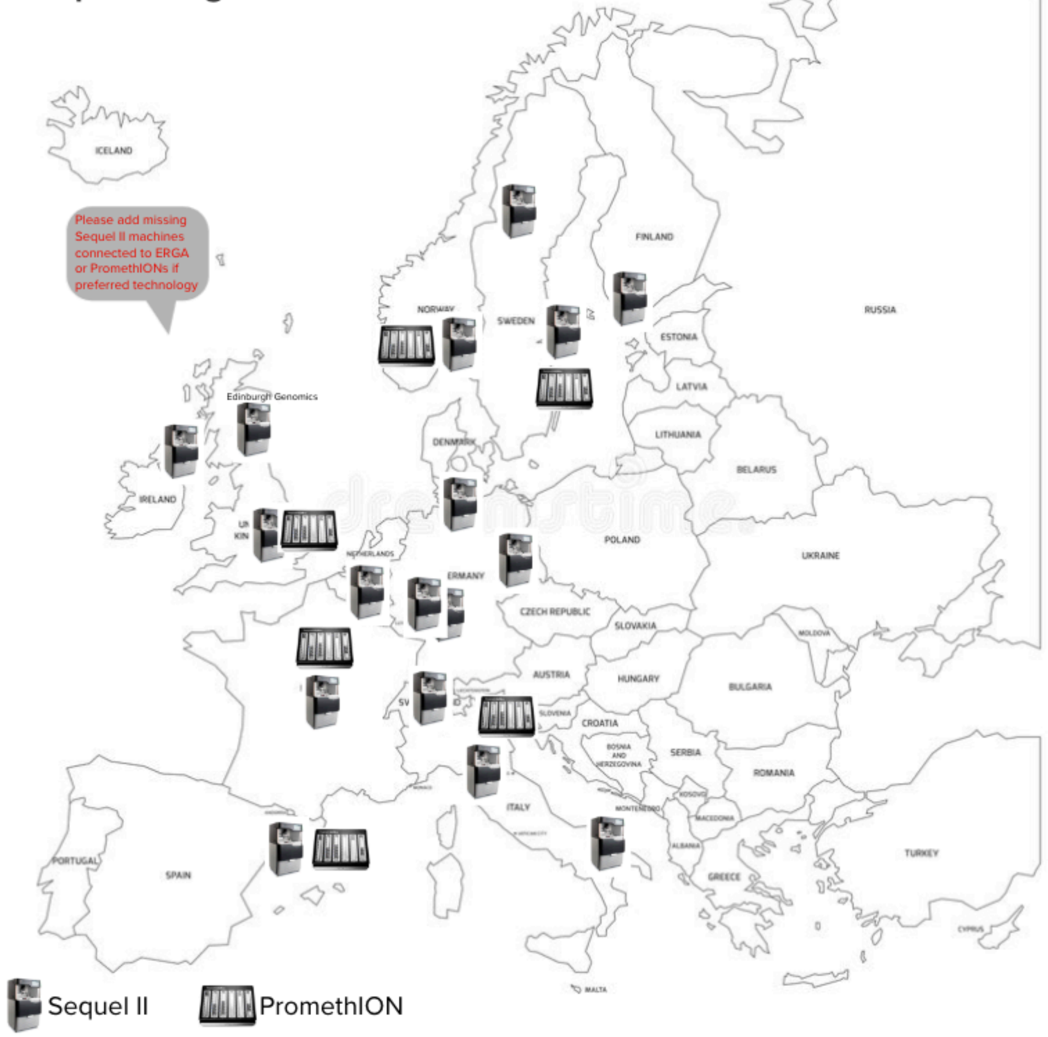
Amount of tissue / DNA vary a lot

Some accept ethanol-preserved samples, some do not

Proximity is important

No-one can answer how long time it will take

Sequencing centers *(connected to ERGA)*



Please add missing Sequel II machines connected to ERGA or PromethIONs if preferred technology

Shipment…

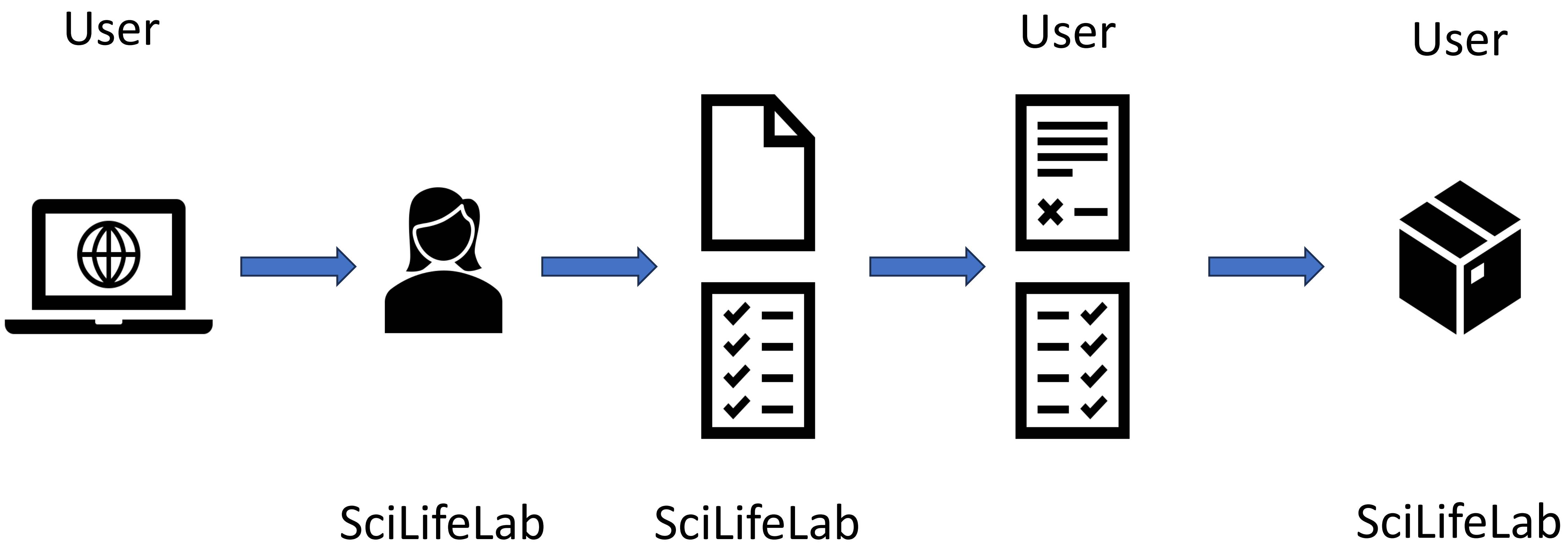
- ALWAYS solid-frozen on dry ice
- Ask for dry-ice top-up
- DHL, FedEx, UPS – all have issues, unfortunately
- BIOCAIR – used to ship human transplants. Expensive, but worth it.
- WorldCourier is very good as well. Not cheap either.

Budget for shipment cost already in grant writing stage!

Sequencing facility documentation

Paperwork is a necessary evil.

SciLifeLab example:



Optimal project workflow

1. Check the latest sequencing recipe / application
2. Get in touch with the sequencing center(s), ask for their sample requirements and necessary paperwork
3. Study papers on similar taxonomic groups – check how were the samples collected and preserved
4. Check **ALL THE PERMITS** (ethics, collection, ABS, Nagoya, CITES, import / export)
5. Collect in the field / request from biobank / assess own stock
6. **Record metadata**
7. *For RefGen:* ID the sample (use DNA barcodes for non-models)
8. *For RefGen*: Deposit a voucher / biobank accession
9. Arrange all documentation required by the sequencing facility
10. Get in touch with a courier company
11. Ship to the sequencing center

Considering costs

Sequencing project cost = collection + sample processing + nucleic acid extraction + shipment* + sequencing + data storage + data compute + data analysis + work hours

** Shipment field-lab, lab-sequencing facility, lab-vouchering collection (left-over material?)*

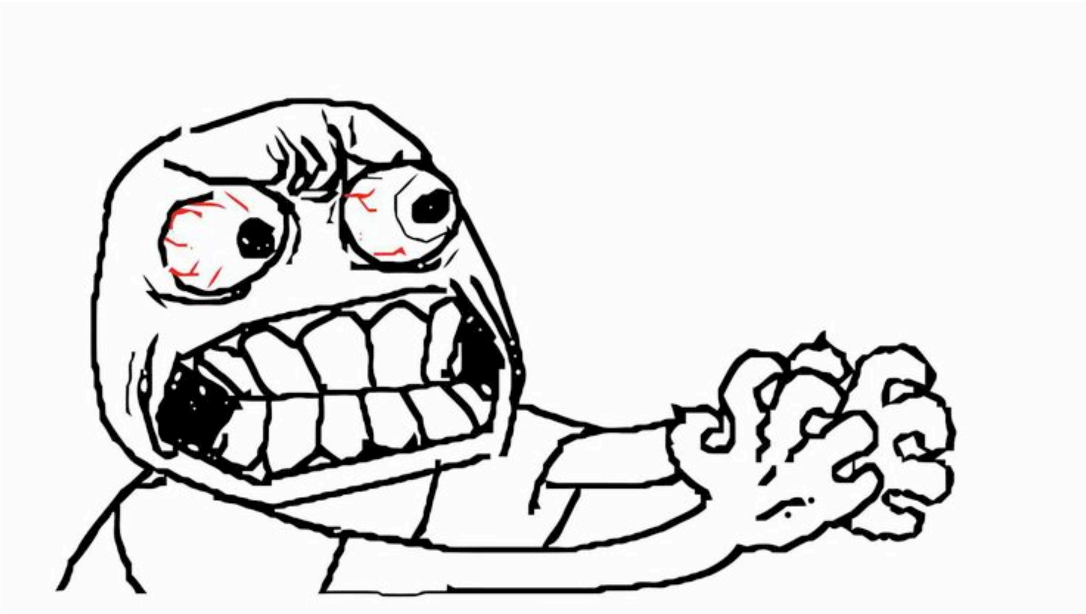
Collection + your & bioinformatican's salaries = **MOST EXPENSIVE** part of the project

Sequencing is the **CHEAPEST** part

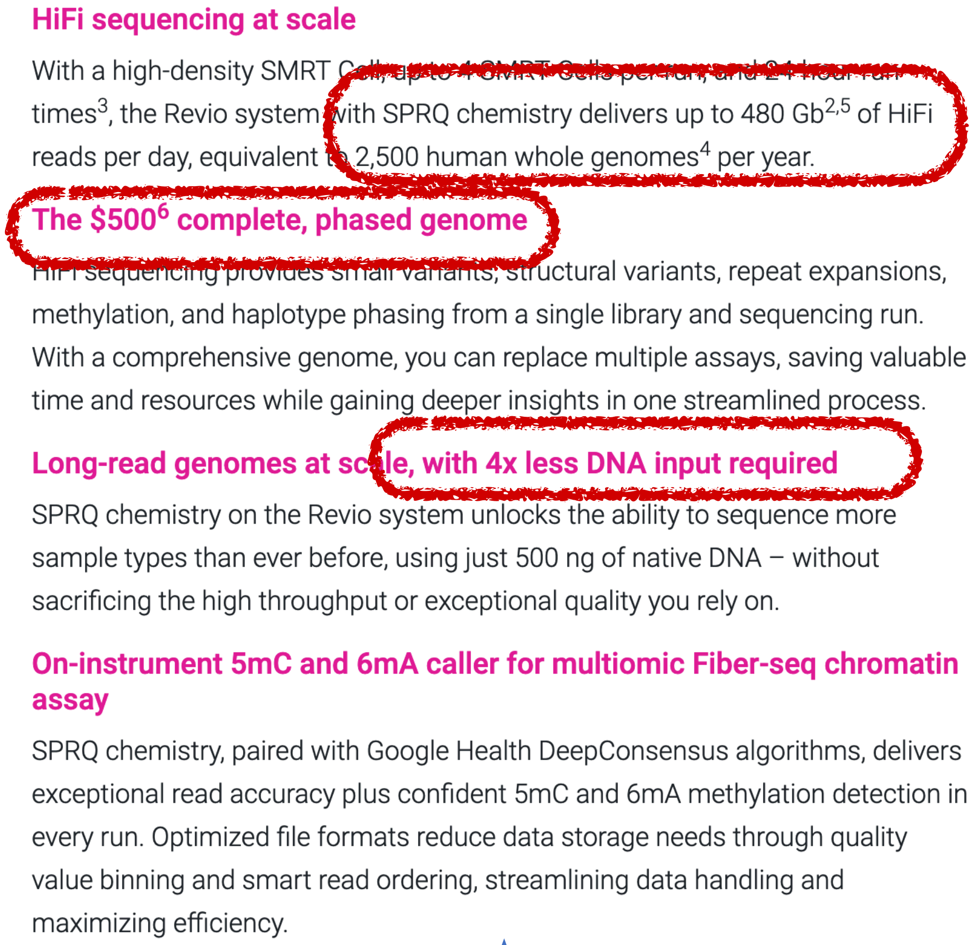
(Never EVER believe the sequencing technology vendor prices!)

Reagent Cost PacBio, Sequel		Price per kit (SEK)	Units per kit	Price / Unit	Units	Cost (SEK)	Price* / Unit	Units	Cost (SEK)	Cost (SEK)
Sample preparation										
HiFi SMRTbell Express Template Prep Kit 2.0+ Enz Clean Up	Cost per library	16279	9	1809	1	**1809**	2532	1	**2532**	**2532**
AMPure Clean-up	Cost per library	2898	20	145	1	**145**	203	1	**203**	**203**
QC and Size Selection										
Megaruptor DNA shearing 2-20 kb fragments	Per sample up to 10 µg DNA	N/A	N/A	105	2	**210**	155	1	**155**	**155**
SageELF	Cost per library			378	1	**378**	612	1	**612**	**612**
Femto PFGE	Input QC, 1-11 samples	N/A	N/A	960	4	**3840**	1350	4	**5400**	**5400**
Consuambles tubes, tips, Ampure Beads	Cost per library	N/A	N/A	220	1	**220**	220	1	**220**	**220**
Sequencing Reagents										
Sequel™ SMRT® Cell 8M v3 Tray (4 cells)	Per SMRT cell	40801	4	10200	1	**10200**	14280	1	**14280**	**14280**
Sequel Sequencing Kit 2.0 Bundle 4rxn	Per SMRT cell	46292	20	2315	1	**2315**	3240	1	**3240**	**3240**
Sequel Sequencing consumables	Per run (4 SMRT cells)	N/A		850	0,25	**213**	927	0,25	**232**	**232**
Reagent Cost, SEK						**19329**			**26875**	**26875**
Additional cost, SEK										
Instrument related cost, run time (HiFi30, CLR15, IsoSeq24)	Per hour / Per SMRT cell	I		300	0	**Paid**	300	30	**9000**	**9000**
Work hour cost (external users only)	Per hour			400	0	**Paid**	400	32	**0**	**12800**
TOTAL project cost excl OH									**35875**	**48675**
University overhead	Per project 29%								**0**	**29 %**
TOTAL project cost incl OH						19329			35875	62790
***Including costs of re-run, auxilliary equipment, other reagents, etc.**						**1.9 k€**			**3.5 k€**	**6.3 k€**

Expectations vs reality★



HUMAN CELL LINES ONLY!!!



HiFi sequencing at scale

With a high-density SMRT Cell, up to 4 SMRT Cells per run, and 24-hour run times[3], the Revio system with SPRQ chemistry delivers up to 480 Gb[2,5] of HiFi reads per day, equivalent to 2,500 human whole genomes[4] per year.

The $500[6] complete, phased genome

HiFi sequencing provides small variants, structural variants, repeat expansions, methylation, and haplotype phasing from a single library and sequencing run. With a comprehensive genome, you can replace multiple assays, saving valuable time and resources while gaining deeper insights in one streamlined process.

Long-read genomes at scale, with 4x less DNA input required

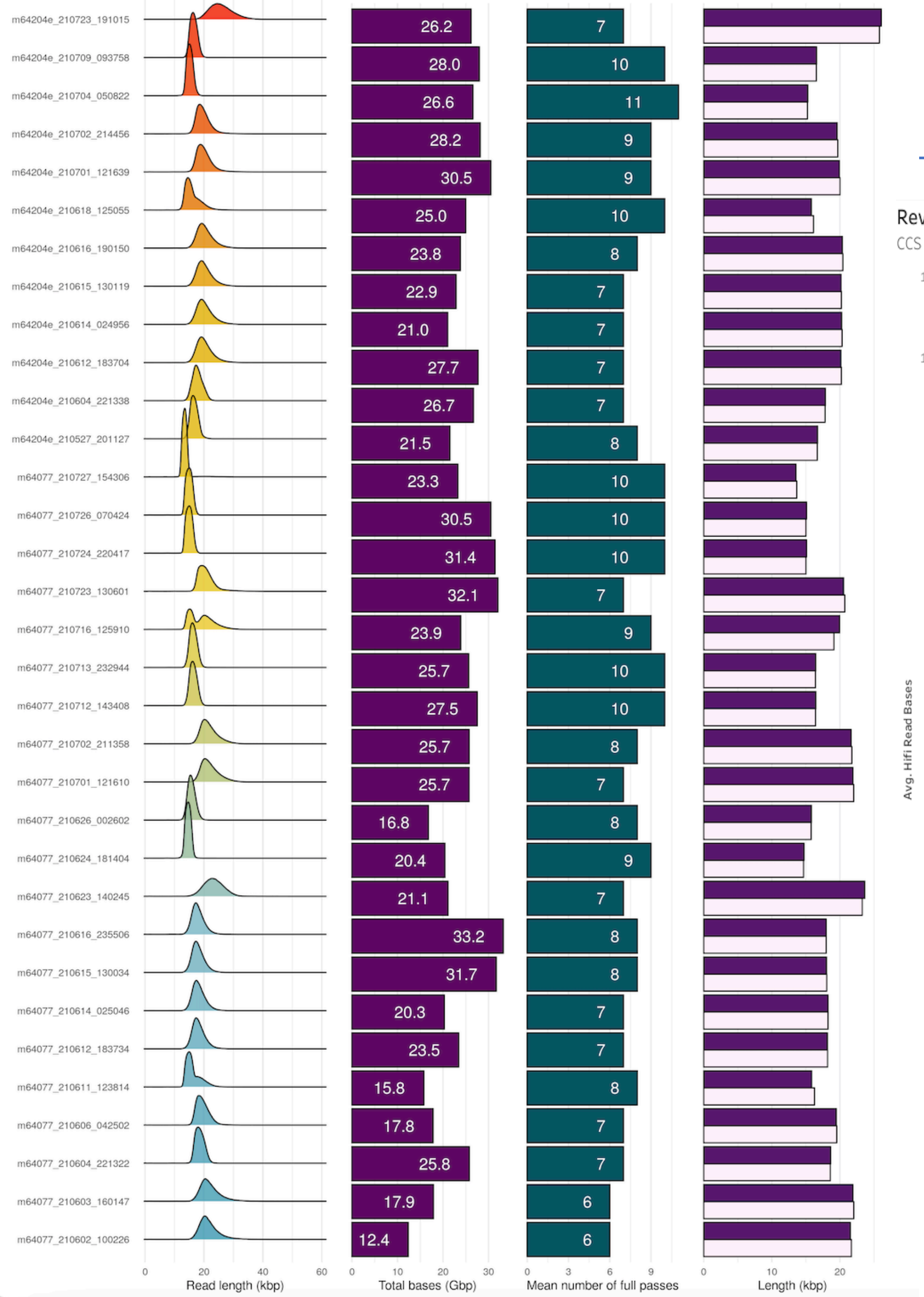
SPRQ chemistry on the Revio system unlocks the ability to sequence more sample types than ever before, using just 500 ng of native DNA – without sacrificing the high throughput or exceptional quality you rely on.

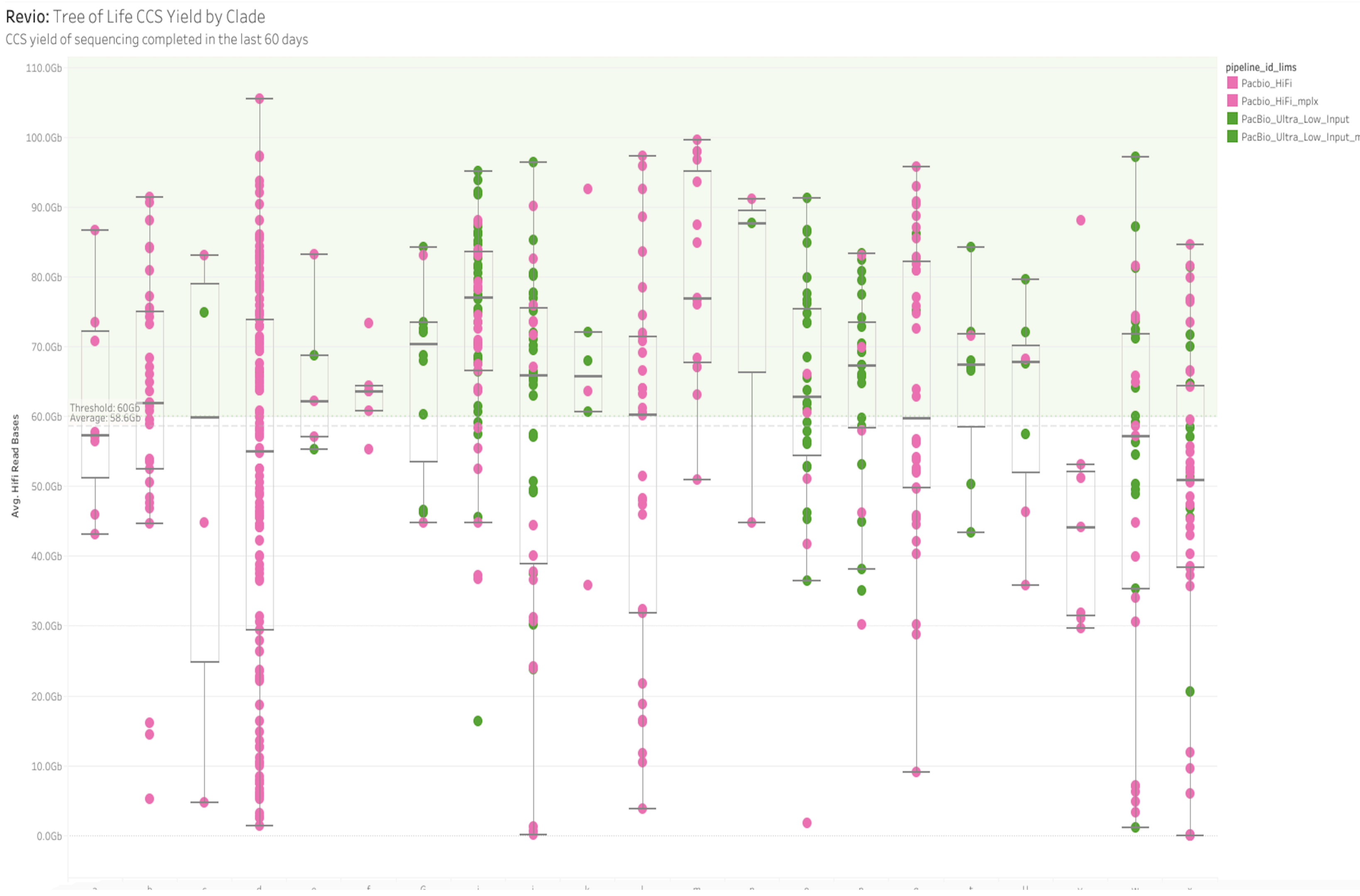
On-instrument 5mC and 6mA caller for multiomic Fiber-seq chromatin assay

SPRQ chemistry, paired with Google Health DeepConsensus algorithms, delivers exceptional read accuracy plus confident 5mC and 6mA methylation detection in every run. Optimized file formats reduce data storage needs through quality value binning and smart read ordering, streamlining data handling and maximizing efficiency.

★ Applicable to ANY vendor

Courtesy: Ignas Bunkis, NGI-SciLifeLab




Revio: Tree of Life CCS Yield by Clade

CCS yield of sequencing completed in the last 60 days

Courtesy: James Watt, Sanger Institute (DToL)

Finally: Recognize the sequencing facility personnel

Shift from specialized labs to sequencing facilities

Sequencing of non-model organisms, especially for RefGen generation:

Heavily reliant on pure, HMW-DNA

High failure rate both for PacBio and ONT

Everything is non-model
Every project is practically R&D
Some projects require weeks of full-time expert lab engineer

Wrapping it all up:

- Some perspective
- What to think about BEFORE planning a sequencing project (aka Project Design)
- Sequencing applications and experiment design specifics:
    - Whole-genome sequencing
    - Targeted sequencing
    - Transcriptome sequencing
    - Shotgun metagenomics
    - Reference genome sequencing + optimal project workflow example
    - Single-cell sequencing -> *Arnaud's lecture next week*

- Sampling and sample quality requirements
- What every facility wish you knew before sending your samples

Experimental design in genomics – take home

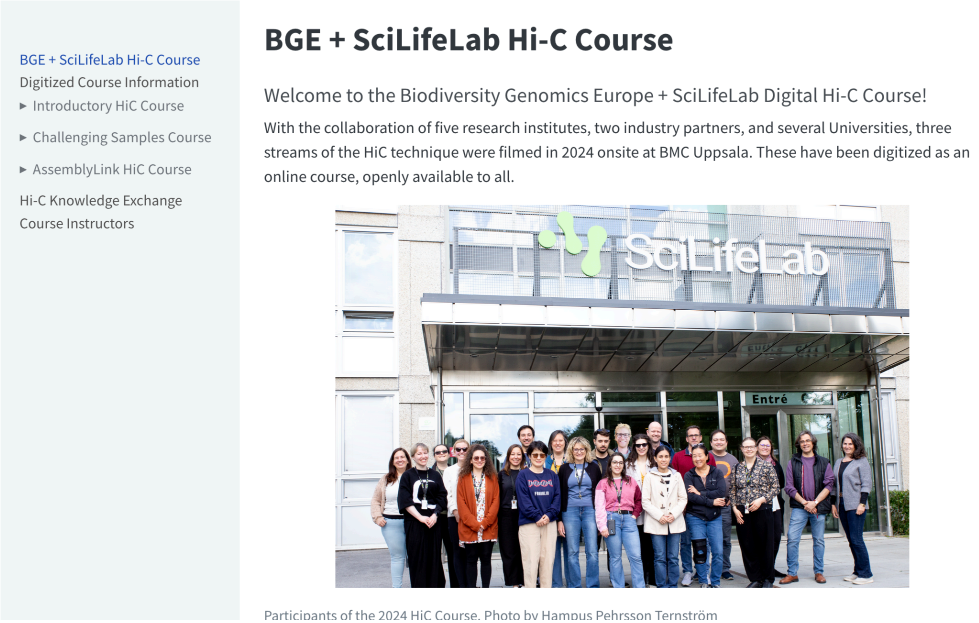
VERY fast development

Difficult to keep oneself updated

Ask your sequencing service provider about the latest updates

Sequencing itself is the CHEAPEST part of the project

ERGA / BGE / SciLifeLab resources



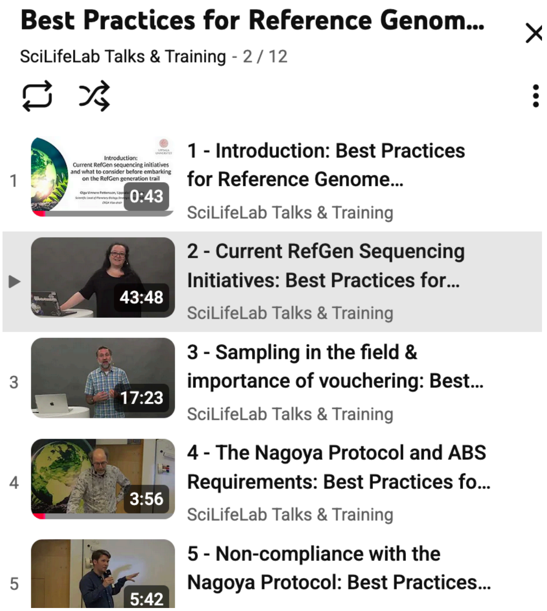
BGE + SciLifeLab Hi-C Course
Digitized Course Information
▸ Introductory HiC Course
▸ Challenging Samples Course
▸ AssemblyLink HiC Course
Hi-C Knowledge Exchange
Course Instructors

BGE + SciLifeLab Hi-C Course

Welcome to the Biodiversity Genomics Europe + SciLifeLab Digital Hi-C Course!

With the collaboration of five research institutes, two industry partners, and several Universities, three streams of the HiC technique were filmed in 2024 onsite at BMC Uppsala. These have been digitized as an online course, openly available to all.

Participants of the 2024 HiC Course. Photo by Hampus Pehrsson Ternström



Best Practices for Reference Genom...

SciLifeLab Talks & Training - 2 / 12

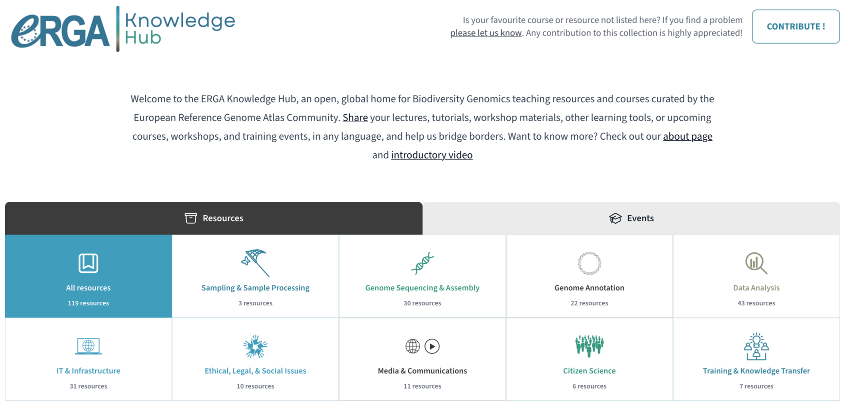
1 - Introduction: Best Practices for Reference Genome... (0:43)
SciLifeLab Talks & Training

2 - Current RefGen Sequencing Initiatives: Best Practices for... (43:48)
SciLifeLab Talks & Training

3 - Sampling in the field & importance of vouchering: Best... (17:23)
SciLifeLab Talks & Training

4 - The Nagoya Protocol and ABS Requirements: Best Practices fo... (3:56)
SciLifeLab Talks & Training

5 - Non-compliance with the Nagoya Protocol: Best Practices... (5:42)



ERGA Knowledge Hub

Is your favourite course or resource not listed here? If you find a problem please let us know. Any contribution to this collection is highly appreciated!

CONTRIBUTE !

Welcome to the ERGA Knowledge Hub, an open, global home for Biodiversity Genomics teaching resources and courses curated by the European Reference Genome Atlas Community. Share your lectures, tutorials, workshop materials, other learning tools, or upcoming courses, workshops, and training events, in any language, and help us bridge borders. Want to know more? Check out our about page and introductory video

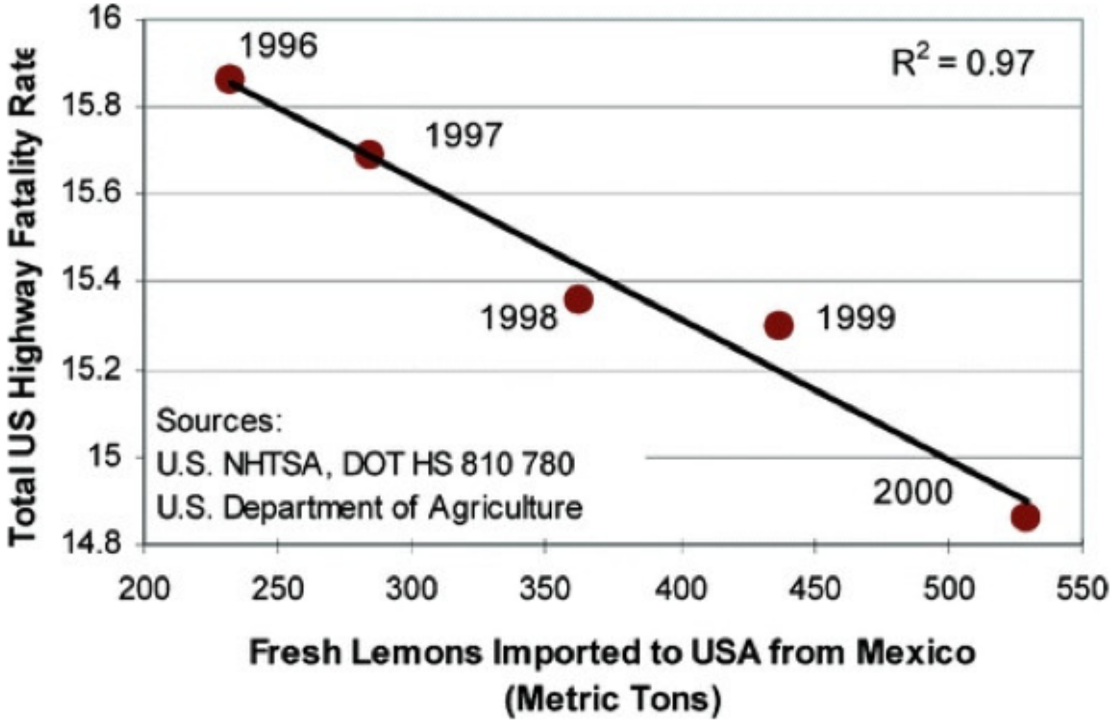
Resources | Events

All resources 119 resources	Sampling & Sample Processing 3 resources	Genome Sequencing & Assembly 30 resources	Genome Annotation 22 resources	Data Analysis 43 resources
IT & Infrastructure 31 resources	Ethical, Legal, & Social Issues 10 resources	Media & Communications 11 resources	Citizen Science 6 resources	Training & Knowledge Transfer 7 resources

GoAT walk-though: 2nd video, 34:10

Finally, 2 minutes of philosophy

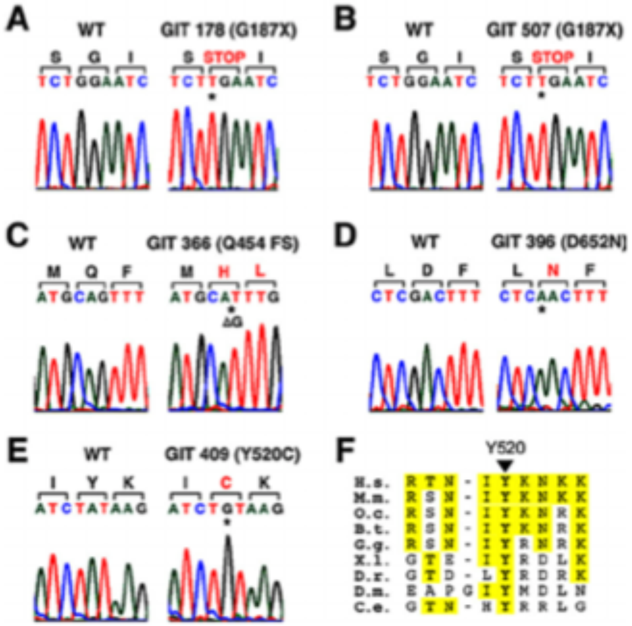
Never forget: Correlation vs Causation



Sources:
U.S. NHTSA, DOT HS 810 780
U.S. Department of Agriculture

Reduction in export of fresh lemons from Mexico causes significant reduction of highway traffic fatality rates in the US!

Genome is not a linear string of bases!!



Mutations in coding regions only

↓

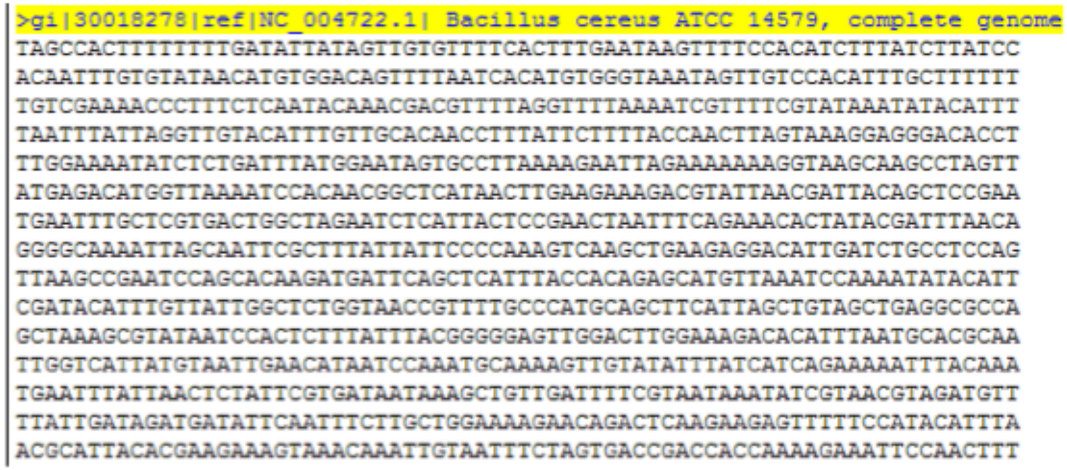
Transcriptional & post-transcriptional regulation

↓

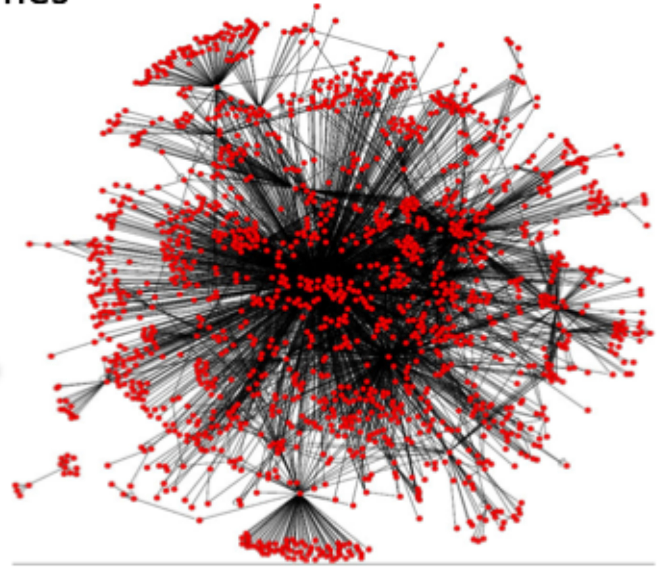
Epigenetics

↓

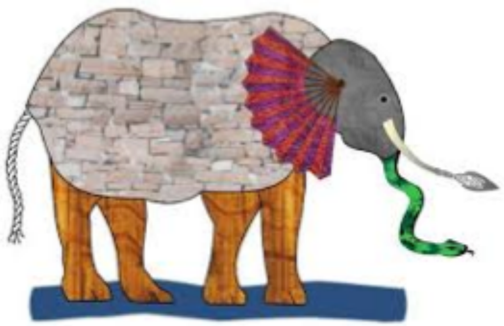
Proximity in chromosomes

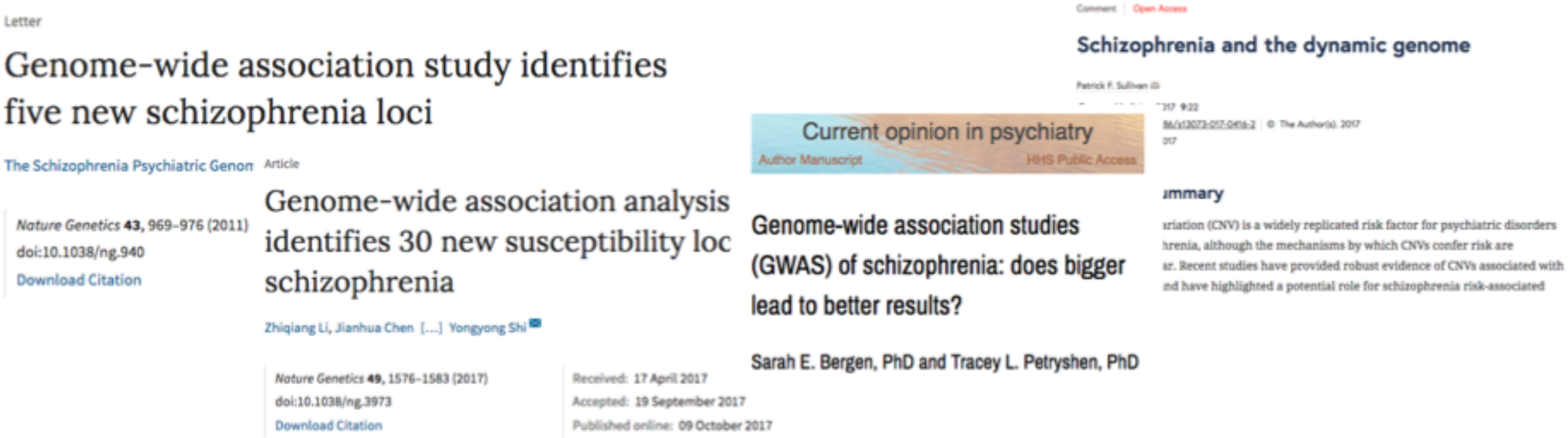


```
>gi|30018278|ref|NC_004722.1| Bacillus cereus ATCC 14579, complete genome
TAGCCACTTTTTTTTGATATTATAGTTGTGTTTTCACTTTGAATAAGTTTTCCACATCTTTATCTTATCC
ACAATTTGTGTATAACATGTGGACAGTTTTAATCACATGTGGGTAAATAGTTGTCCACATTTGCTTTTTT
TGTCGAAAACCCTTTCTCAATACAAACGACGTTTTAGGTTTTAAAATCGTTTTCGTATAAATATACATTT
TAATTTATTAGGTTGTACATTTGTTGCACAACCTTTATTCTTTTACCAACTTAGTAAAGGAGGGACACCT
TTGGAAAATATCTCTGATTTATGGAATAGTGCCTTAAAAGAATTAGAAAAAAAGGTAAGCAAGCCTAGTT
ATGAGACATGGTTAAAATCCACAACGGCTCATAACTTGAAGAAAGACGTATTAACGATTACAGCTCCGAA
TGAATTTGCTCGTGACTGGCTAGAATCTCATTACTCCGAACTAATTTCAGAAACACTATACGATTTAACA
GGGGCAAAATTAGCAATTCGCTTTATTATTCCCCAAAGTCAAGCTGAAGAGGACATTGATCTGCCTCCAG
TTAAGCCGAATCCAGCACAAGATGATTCAGCTCATTTACCACAGAGCATGTTAAATCCAAAATATACATT
CGATACATTTGTTATTGGCTCTGGTAACCGTTTTGCCCATGCAGCTTCATTAGCTGTAGCTGAGGCGCCA
GCTAAAGCGTATAATCCACTCTTTATTTACGGGGGAGTTGGACTTGGAAAGACACATTTAATGCACGCAA
TTGGTCATTATGTAATTGAACATAATCCAAATGCAAAAGTTGTATATTTATCATCAGAAAAATTTACAAA
TGAATTTATTAACTCTATTCGTGATAATAAAGCTGTTGATTTTCGTAATAAATATCGTAACGTAGATGTT
TTATTGATAGATGATATTCAATTTCTTGCTGGAAAAGAACAGACTCAAGAAGAGTTTTTCCATACATTTA
ACGCATTACACGAAGAAAGTAAACAAATTGTAATTTCTAGTGACCGACCACCAAAAGAAATTCCAACTTT
```



Blind men & an elephant





Letter

Genome-wide association study identifies five new schizophrenia loci

The Schizophrenia Psychiatric Genon

Nature Genetics **43**, 969–976 (2011)
doi:10.1038/ng.940
Download Citation

Article

Genome-wide association analysis identifies 30 new susceptibility loc schizophrenia

Zhiqiang Li, Jianhua Chen [...] Yongyong Shi

Nature Genetics **49**, 1576–1583 (2017)
doi:10.1038/ng.3973
Download Citation

Received: 17 April 2017
Accepted: 19 September 2017
Published online: 09 October 2017

Current opinion in psychiatry

Author Manuscript HHS Public Access

Genome-wide association studies (GWAS) of schizophrenia: does bigger lead to better results?

Sarah E. Bergen, PhD and Tracey L. Petryshen, PhD

Comment | Open Access

Schizophrenia and the dynamic genome

Patrick F. Sullivan

017 9:22

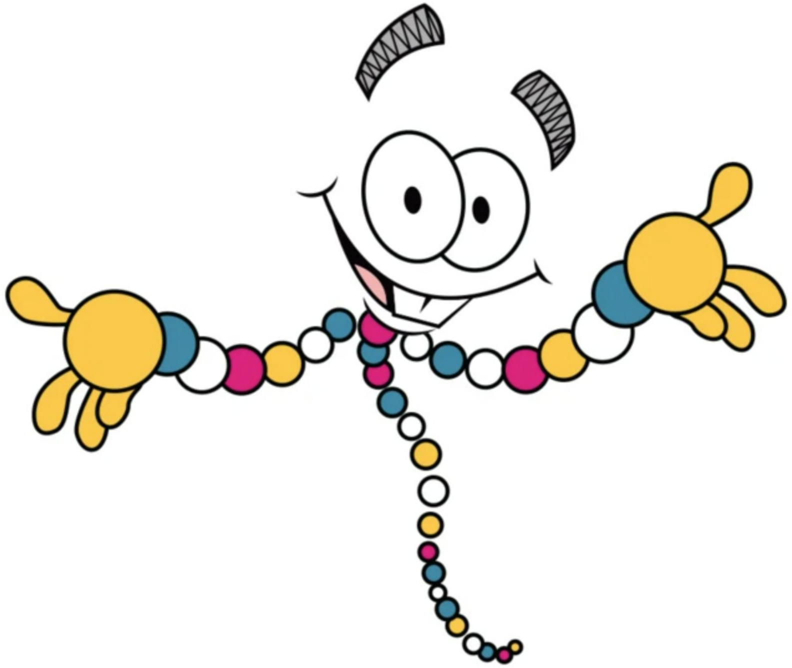
86/s13073-017-0416-2 | © The Author(s). 2017

017

ımmary

ıriation (CNV) is a widely replicated risk factor for psychiatric disorders
hrenia, although the mechanisms by which CNVs confer risk are
ar. Recent studies have provided robust evidence of CNVs associated with
nd have highlighted a potential role for schizophrenia risk-associated

Thank you!



Swedish Research Council

UPPSALA UNIVERSITET

SciLifeLab

European Commission

Horizon Europe 2021-2027