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Bioinformatics Summer School

Long-reads Transcriptomics

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LongTREC - The Long-reads TRanscriptome European Consortium
Marie Skłodowska-Curie grant agreement No 101072892

Section 3

Single-cell analysis pipeline

Overview of commercial pipelines available

Commercial pipelines available

PacBio

Iso-Seq

Scalable
De Novo
Isoform Discovery
from PacBio HiFi Reads

https://isoseq.how

Oxford NANOPORE Technologies

EPI2ME

epi2me-labs / wf-single-cell Public

https://github.com/epi2me-labs/wf-single-cell

Pipelines overview

PacBio

CCS → Segmentation

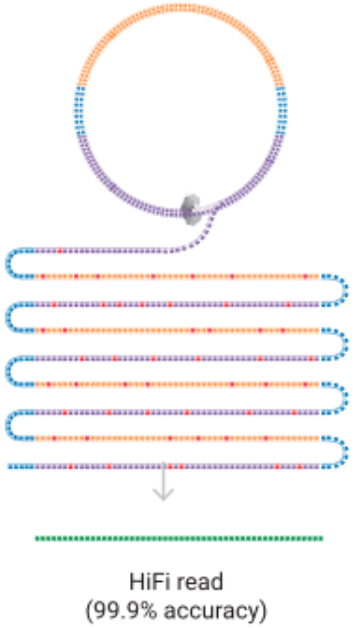
Oxford NANOPORE Technologies

Consensus sequence and segmentation of PacBio CCS reads

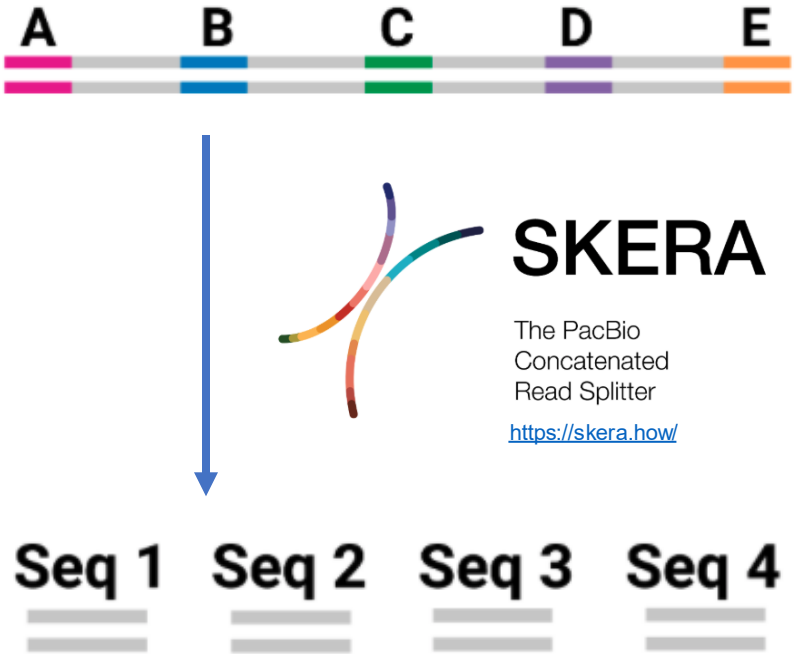
PacBio only

CCS https://ccs.how/

Generate Highly Accurate Single-Molecule Consensus Reads



Adapted from https://ccs.how/



Adapted from https://skera.how/read-segments.html

Pipelines overview

PacBio

Oxford NANOPORE Technologies

Primers removal and barcodes extraction – Iso-Seq

lima

The PacBio
Barcode Demultiplexer &
Primer Remover

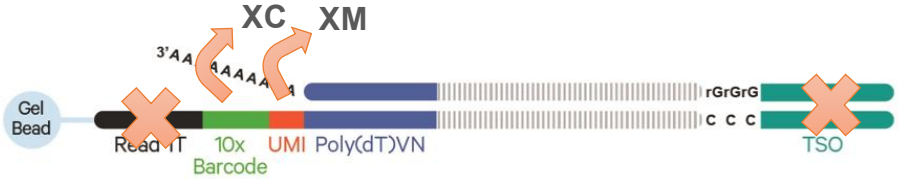
https://lima.how

cDNA from poly-adenylated mRNA

Adapted from https://cdn.10xgenomics.com/image/upload/v1710230393/support-documents/CG000731_ChromiumGEM-X_SingleCell3_ReagentKits_v4_UserGuide_RevA.pdf

Iso-Seq

Scalable
De Novo
Isoform Discovery
from PacBio HiFi Reads



cDNA from poly-adenylated mRNA

Pipelines overview

PacBio

CCS → Segmentation → Primer removal → Barcodes extraction

Oxford NANOPORE Technologies

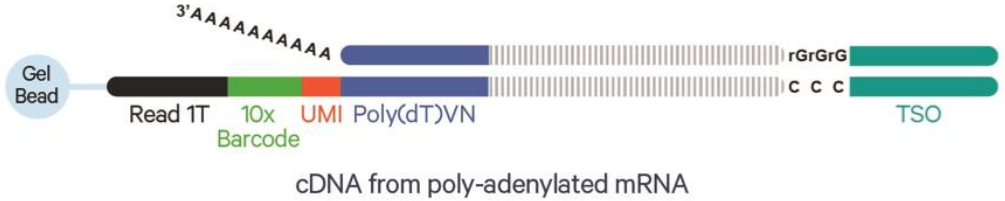
Primer removal → Barcodes extraction

Primers removal and barcodes extraction – wf-single-cell

torognes / vsearch Public

https://github.com/torognes/vsearch

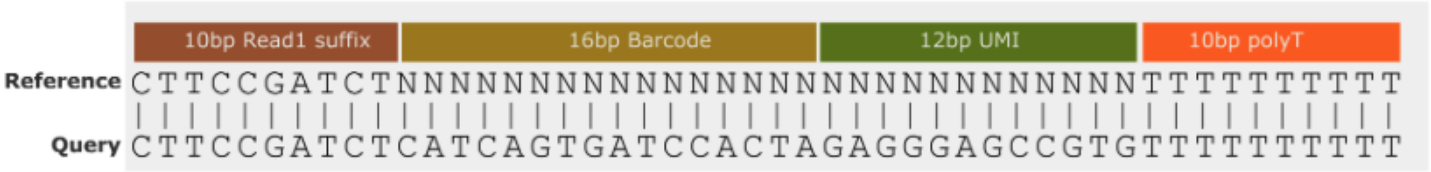
Identification of adaptors



jeffdaily / parasail Public

https://github.com/jeffdaily/parasail

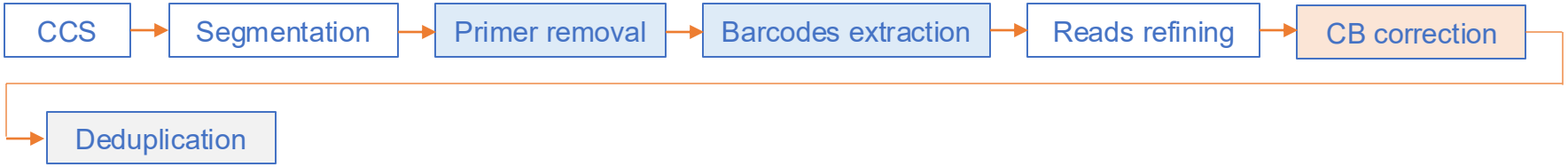
Probes to extract CBs and UMIs



Adapted from https://github.com/epi2me-labs/wf-single-cell

Pipelines overview

PacBio



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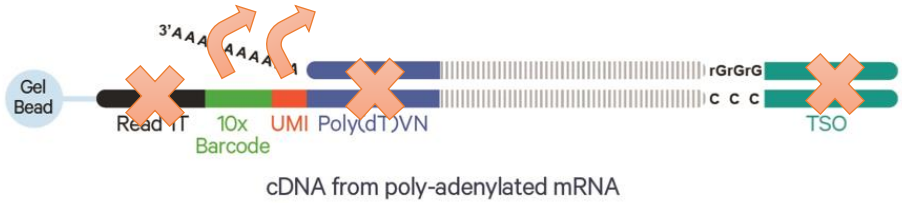
Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming



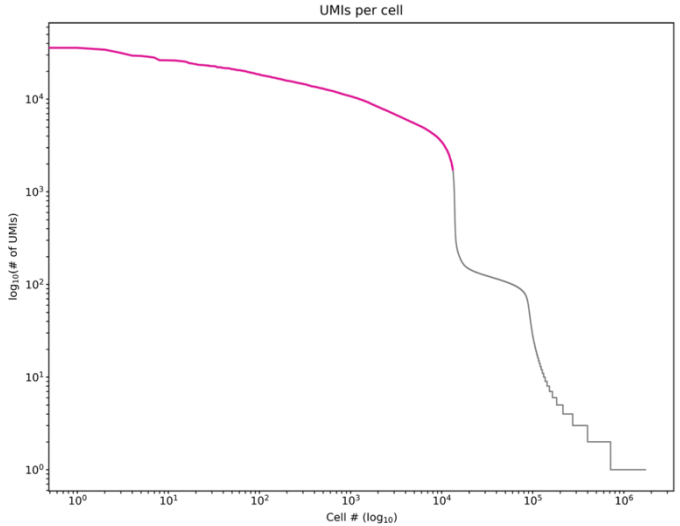
Iso-Seq

Scalable
De Novo
Isoform Discovery
from PacBio HiFi Reads

Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming
- **Cell barcode correction**

- Cell barcodes are known (10x whitelist)
- Cell barcodes are corrected using Hamming distance <=2 (2 substitutions maximum)
- Low quality cells, empty droplets or ambient RNA are also filtered



Adapted from https://downloads.pacbcloud.com/public/dataset/Kinnex-single-cell-RNA/DATA-Revio-Kinnex-PBMC-10x3p/SL-Revio-PBMC-10x3p-ReadSegSingleCell.pdf

Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming
- Cell barcode correction
- **Deduplication – removing PCR artifacts using UMIs**

Adapted from https://isoseq.how/umi/high-level-workflow.html

Iso-Seq

Scalable
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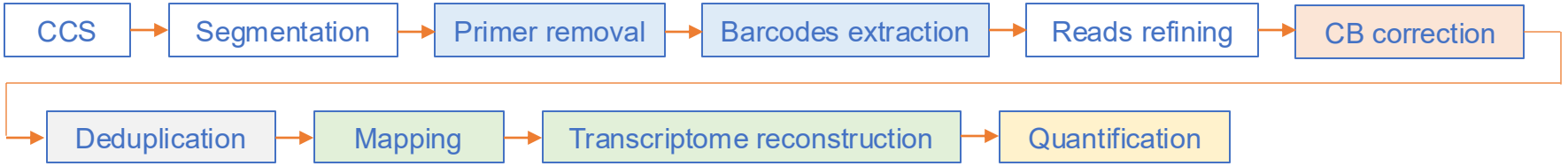
Pipelines overview

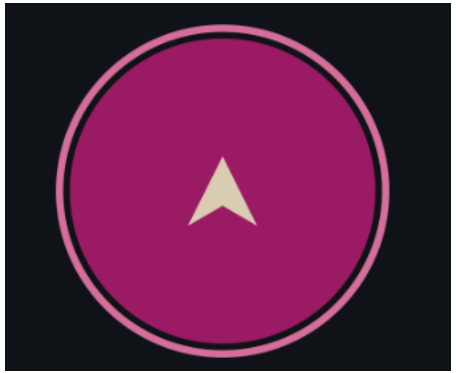
PacBio



Oxford NANOPORE Technologies

Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming
- Cell barcode correction
- Deduplication – removing PCR artifacts with UMIs
- **Mapping to genome reference**



Pbmm2

- PacBio's C++ wrapper of minimap2

Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming
- Cell barcode correction
- Deduplication – removing PCR artifacts with UMIs
- Mapping to genome reference
- **Transcriptome reconstruction and curation**

PIGEON

PacBio
Transcript Toolkit

https://isoseq.how/classification/pigeon.html

Iso-seq pipeline (cont.)

- Reads refinement – polyA trimming
- Cell barcode correction
- Deduplication – removing PCR artifacts with UMIs
- Mapping to genome reference
- Transcriptome reconstruction and curation
- **Expression matrices**

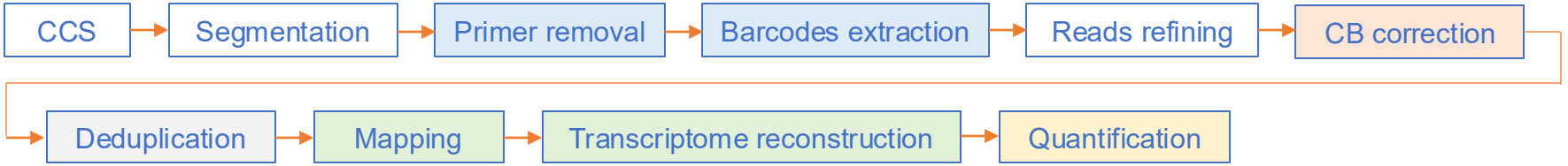
PIGEON

PacBio
Transcript Toolkit

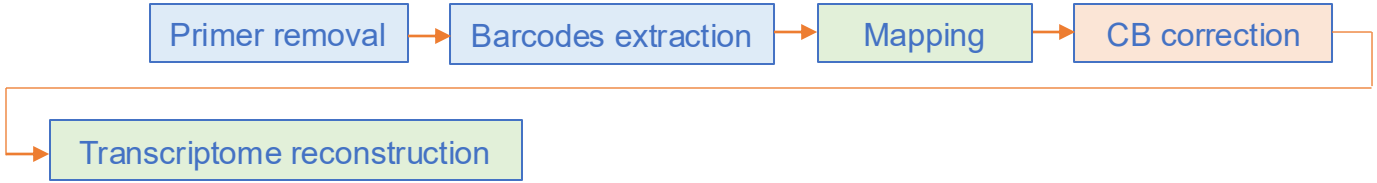
https://isoseq.how/classification/pigeon.html

Pipelines overview

PacBio



Oxford NANOPORE Technologies



wf-single-cell pipeline (cont.)

- Mapping to genome reference

https://github.com/lh3/minimap2

wf-single-cell pipeline (cont.)

- Mapping to genome reference
- **Cell barcode correction**
  - Cell barcodes are known (10x whitelist)
  - Cell barcodes from the sample with high quality are shortlisted
  - Cell barcodes are corrected using Levenshtein distance <=2 (2 indels and/or substitutions maximum)
  - Low quality cells, empty droplets or ambient RNA are also filtered

wf-single-cell pipeline (cont.)

- Mapping to genome reference
- Cell barcode correction
- **Transcriptome reconstruction**

https://github.com/gpertea/stringtie

- Stringtie2
- Using long read mode (-L)
- At least 2 transcripts to call a transcript

https://github.com/gpertea/gffcompare

- Resulting transcriptome is annotated with the reference annotation

wf-single-cell pipeline (cont.)

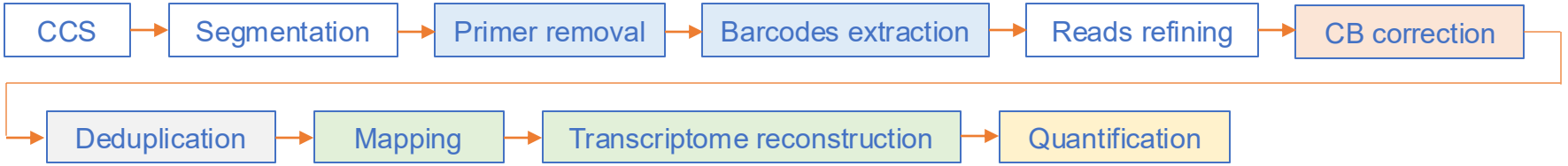
- Mapping to genome reference
- Cell barcode correction
- **Transcriptome reconstruction**
  - Reads are mapped to the resulting transcriptome
  - Transcript assignment similar to FLAMES criteria

https://github.com/LuyiTian/FLAMES

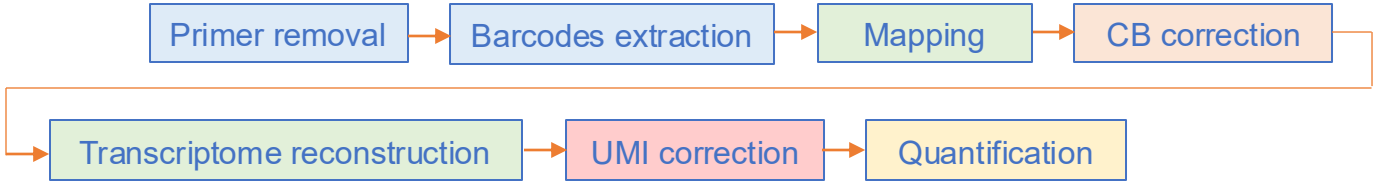
  - Intronic transcripts set to unknown
  - Uniquely mapped reads are assigned to transcripts
  - Ambiguously mapped reads are assigned or not depending on alignment score and coverage

Pipelines overview

PacBio



Oxford NANOPORE Technologies



wf-single-cell pipeline (cont.)

- Mapping to genome reference
- Cell barcode correction
- Transcriptome reconstruction
- **UMI correction**

- UMI barcodes are NOT known (random sequences)
- To reduce searching space, reads are clustered to assigned genes (or genomic interval if not assigned)

UMI-tools

https://github.com/CGATOxford/UMI-tools

- UMIs are corrected using Levenshtein distance <=2 (2 indels and/or substitutions maximum)

wf-single-cell pipeline (cont.)

- Mapping to genome reference
- Cell barcode correction
- Transcriptome reconstruction
- UMI correction
- **Expression matrices**

- Corrected UMIs are collapsed and feature counts are summed
- Expression matrices are further processed
  - Cell with counts below 200 genes/transcripts (default)
  - Genes expressed in fewer than 3 cells (default)
  - Cells with more than 20% mitochondrial expression (default)
- Counts are normalized to 10.000 reads/cell (default) and log10 transformed

Select tools appropriate for your question

Registered Report | Open access | Published: 07 June 2024

Systematic assessment of long-read RNA-seq methods for transcript identification and quantification

Francisco J. Pardo-Palacios, Dingjie Wang, Fairlie Reese, Mark Diekhans, Sílvia Carbonell-Sala, Brian Williams, Jane E. Loveland, Maite De María, Matthew S. Adams, Gabriela Balderrama-Gutierrez, Amit K. Behera, Jose M. Gonzalez Martinez, Toby Hunt, Julien Lagarde, Cindy E. Liang, Haoran Li, Marcus Jerryd Meade, David A. Moraga Amador, Andrey D. Prjibelski, Inanc Birol, Hamed Bostan, Ashley M. Brooks, Muhammed Hasan Çelik, Ying Chen, ... Angela N. Brooks + Show authors

Nature Methods **21**, 1349–1363 (2024) | Cite this article

50k Accesses | **103** Citations | **111** Altmetric | Metrics

- ***For transcriptome reconstruction***

SQANTI3

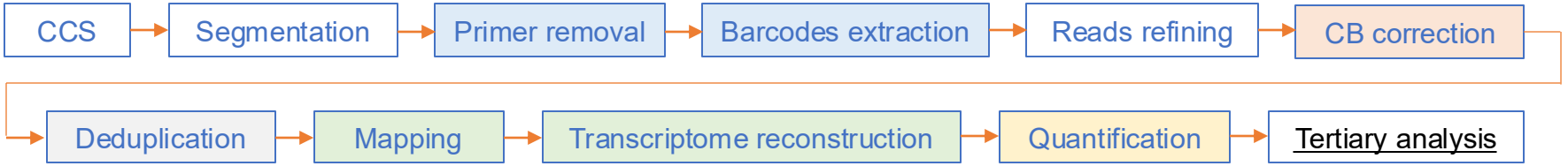
- ***For transcriptome curation***

Question

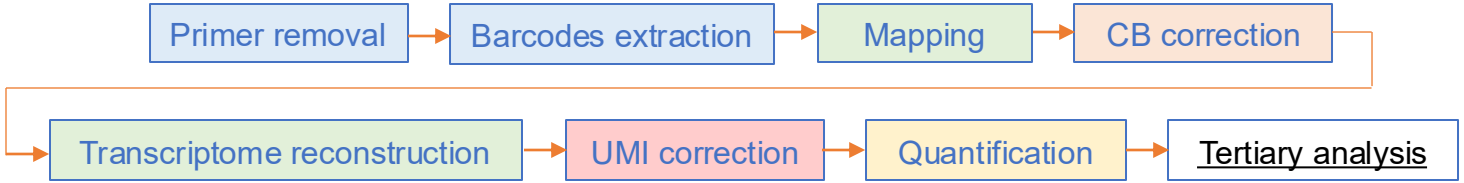
- ***Which one is the unique step of the wf-single-cell pipeline?***

Pipelines overview

PacBio



Oxford NANOPORE Technologies



Thank You!

For more information about the LongTREC Summer School:

https://longtrec.eu