Introduction to Accelerated Genomics

Applied Machine Learning for Biological Data - Module 2, Day 5

- Date: June 6, 2025
- Time: 09:00-16:00

⚙ Prerequisites

- BioNT course - A practical introduction to bioinformatics or equivalent knowledge
- Introduction to Docker
- Introduction to GPU

Learning outcomes

- Gain a foundational understanding of the NVIDIA Parabricks software suite
- Acquire practical experience in using Parabricks tools for basic germline variant calling workflow

Overall schedule

- Introduction to NGS
- Basic CPU-native NGS data processing workflow
- Introduction to NVIDIA Parabricks
- Parabricks Hands-on
- Parabricks vs CPU-native NGS data processing

Introduction to NGS

Next Generation Sequencing (NGS)

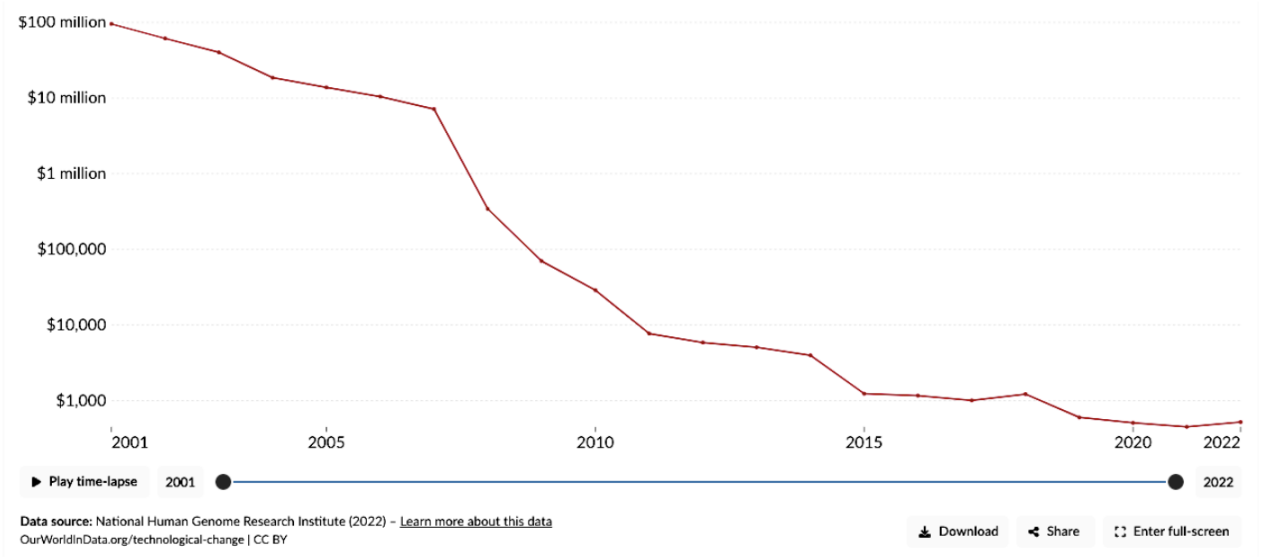
- DNA Sequencing process
  - Process of determining the order of nucleotides in a genome (genome codes the complete set of instructions for cellular functions)
- Next Generation Sequencing (NGS)
  - Increasing the throughput by massively parallelising the sequencing process

Sequencing throughput and sequencing cost

- Today, we are no longer limited by the availability of sequencing data due to the rapidly decreasing cost

Sequencing cost over the years

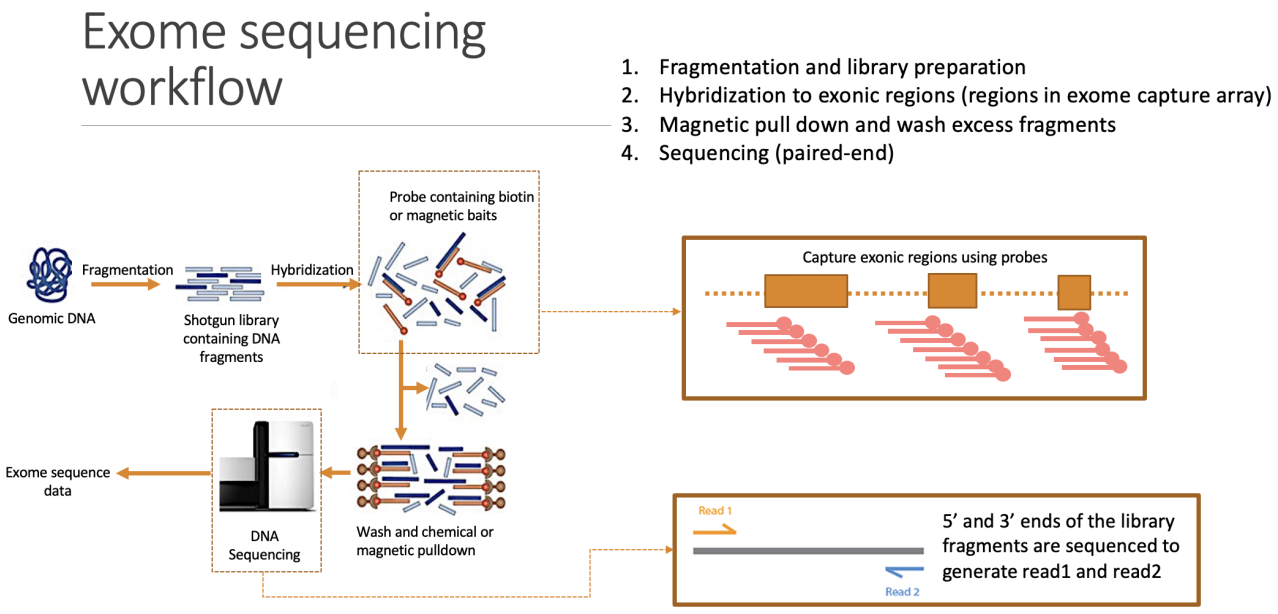
Sequencing throughput over the years



https://ourworldindata.org/grapher/cost-of-sequencing-a-full-human-genome

Sequencing tech used in the Human Genome Project

Main steps of DNA sequencing



NGS data analysis (Germline variant calling)

Main steps in NGS analysis

```
    graph TD;
i1(Raw data) --> 1
i2(Reference data) --> 1
1(Mapping raw data onto the reference)
1 -- Alignment file --> 2
2(Optimization & fine-tuning of the alignment file)
2 -- Optimised & fine-tuned alignment file --> 3
3(Identify DNA changes)

classDef highlight fill:#99ccff;
classDef white fill:#ffffff;
class 1,2,3, highlight;
```

Precision genomics

- Tailoring disease prevention and treatment based on the person's genetic makeup
- Here, we analyse raw sequencing data and identify changes in DNA that could lead to a diseases

Precision genomics in routine clinical practice

- Sequence analysis workflows are routinely used in current clinical setting
- However they all (a large majority) use CPUs for the computing power

Basic CPU-native NGS data processing workflow

- Simplest NGS data processing workflows have two main stages
  - Read mapping and alignment refinement
  - Variant calling
- **Test data:** `https://s3.amazonaws.com/parabricks.sample/parabricks_sample.tar.gz`
- **Source of the test data -** `NVIDIA tutorials` `<https://docs.nvidia.com/clara/parabricks/4.3.0/tutorials/gettingthesampledata.html>`_

Stage 1: Read mapping and alignment refinement

Step 1: BWA read mapping and sorting

```
#!/bin/bash

SAMPLE_NAME="pb_sample"

FASTA="Ref/Homo_sapiens_assembly38.fasta"
READ1="Data/sample_1.fq.gz"
READ2="Data/sample_2.fq.gz"
BAM_OUT="${SAMPLE_NAME}_CPU.bam"

## Run BWA MEM
### Source: https://github.com/nf-core/sarek/blob/f034b737630972e90aeae851e236f9d4292b9a4f/modules/nf-core/bwa/mem/main.nf#L30
bwa mem \
-t 64 \
-R "@RG\tID:rg1\tLB:lib1\tPL:bar\tSM:SM1\tPU:SM1_rg1" \
${FASTA} \
${READ1} ${READ2} \
| samtools     sort     -@64     -o ${BAM_OUT} -

samtools index -@64 -b ${BAM_OUT}
```

Step 2: GATK MarkDuplicates

```
#!/bin/bash

SAMPLE_NAME="pb_sample"
BAM_IN="${SAMPLE_NAME}_CPU.bam"
BAM_OUT="${SAMPLE_NAME}_markdup.bam"

## Mark duplicated reads in BAM file
gatk --java-options  "-Xss3m -XX:-UsePerfData" MarkDuplicates \
--VALIDATION_STRINGENCY SILENT \
-I ${BAM_IN} \
-O ${BAM_OUT} \
-M metrics.txt \
--TMP_DIR temp_dir

samtools index ${BAM_OUT}
```

Step 3: GATK BaseRecalibrator

```
#!/bin/bash

FASTA="Ref/Homo_sapiens_assembly38.fasta"
KNOWN_SITES="Ref/Homo_sapiens_assembly38.known_indels.vcf.gz"

SAMPLE_NAME="pb_sample"
BAM_IN="${SAMPLE_NAME}_markdup.bam"
RECAL_FILE="CPU_recal.txt"

## Generate BQSR Report
gatk --java-options  "-XX:-UsePerfData" BaseRecalibrator \
--input ${BAM_IN} \
--output ${RECAL_OUT} \
--known-sites ${KNOWN_SITES} \
--reference ${FASTA}
```

Step 4: GATK ApplyBQSR

```
#!/bin/bash

FASTA=GRCh38"/"GCA_000001405.15_GRCh38_no_alt_analysis_set.fna
BAM_IN="${SAMPLE_NAME}_markdup.bam"
RECAL_FILE="CPU_recal.txt"
BAM_OUT="${SAMPLE_NAME}_CPU_markdup_BQSR.bam"

## Run ApplyBQSR Step
gatk --java-options  "-XX:-UsePerfData" ApplyBQSR \
-R ${FASTA} \
-I ${BAM_IN} \
--bqsr-recal-file ${RECAL_FILE} \
-O ${BAM_OUT}
```

Stage 2: Variant calling via HaplotypeCaller

```
#!/bin/bash

set -euo pipefail

SAMPLE_NAME="pb_sample"
FASTA="Ref/Homo_sapiens_assembly38.fasta"
BAM_IN="${SAMPLE_NAME}_CPU_markdup_BQSR.bam"

## Variant calling with HaplotypeCaller
gatk --java-options  "-XX:-UsePerfData" HaplotypeCaller \
--input ${BAM_IN} \
--output ${BAM_IN}.vcf \
--reference ${FASTA} \
--native-pair-hmm-threads 64
```

NVIDIA Parabricks

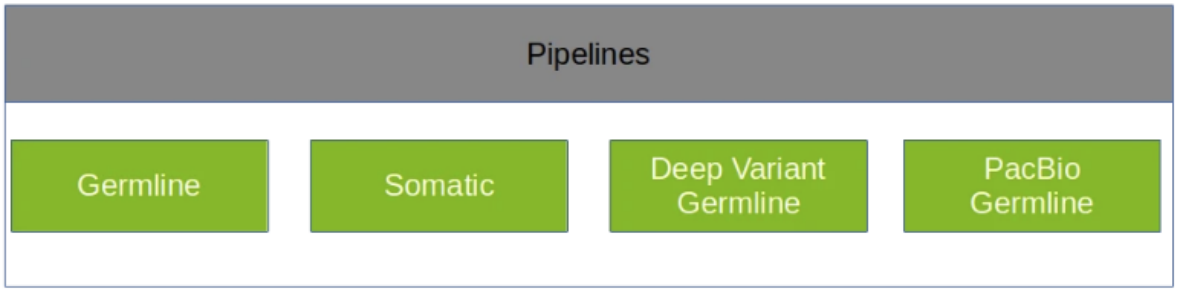
- Parabricks offers range of accelerated NGS data processing tools/commands
- Parabricks documentation:

"Parabricks was built from the ground up by GPU computing and Deep Learning experts who wanted to develop the fastest and most efficient possible implementation of common genomics algorithms."

- We can use these commands and create a simple NGS data processing workflows with two main stages
  - Read mapping and alignment refinement
  - Variant calling

Parabricks supports the tools shown below:

Additionally, the Parabricks tool suite provides a number of variant calling pipelines that are each a combination of several individual tools, combining into one tool what would otherwise be a multi-step process. These pipelines are:

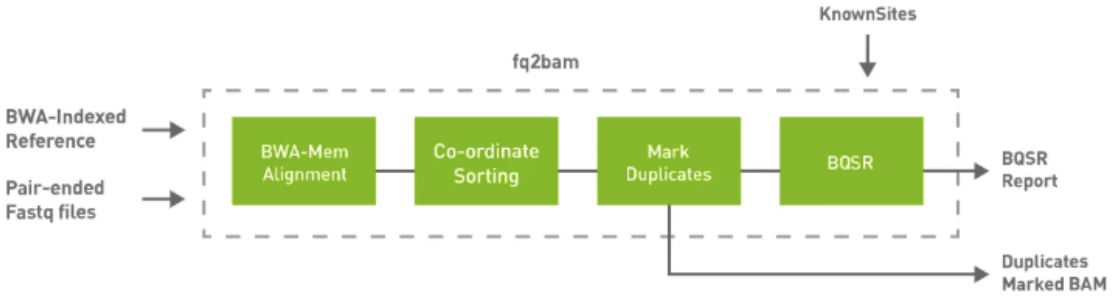


Ref:

Parabricks workflow (basic workflow)

Stage 1: Read mapping and alignment refinement

Step 1: fq2bam



Source

Step 2: applybqsr

- Compatible GATK4 Command: `gatk ApplyBQSR`

Stage 2: Variant calling

Parabricks HaplotypeCaller

Source

Parabricks Hands-on

Objectives

- Run Basic Parabricks pipeline
- Time: 30 minutes

Step 01: Checklist

Checklist

1. Login to the VM assigned to you (Follow the instructions given previously)
2. Check for docker image - `nvcr.io/nvidia/clara/clara-parabricks:4.3.0-1`

Commands:

```
# List Docker images in the VM
docker images
# Move the home directory
cd $HOME
# Check current working-directory (check if you are in $HOME)
echo current working directory: $(pwd)
```

✔ Output

```
REPOSITORY                                TAG       IMAGE ID       CREATED
SIZE
nvcr.io/nvidia/clara/clara-parabricks   4.3.0-1   1a84efd2eedf   14 months ago
3.23GB

current working directory: /home/ubuntu
```

Step 02: Generate Parabricks run-scripts

Step 02.1: fq2bam

Note

```
echo '#!/bin/bash

FASTA="/data/ngs/ref/Homo_sapiens_assembly38.fasta"
KNOWN_SITES="/data/ngs/ref/Homo_sapiens_assembly38.known_indels.vcf.gz"

READ1="/data/ngs/fastq/dw_sample_R1.fastq.gz"
READ2="/data/ngs/fastq/dw_sample_R2.fastq.gz"

pbrun fq2bam \
--ref ${FASTA} \
--in-fq ${READ1} ${READ2} \
--num-gpus 1 \
--knownSites ${KNOWN_SITES} \
--out-bam pbrun_fq2bam_GPU.bam \
--out-recal-file pbrun_recal_gpu.txt \
--logfile fq2bam.log \
--tmp-dir .'  > fq2bam.sh
```

Step 02.2: applybqsr

Note

```
echo '#!/bin/bash

FASTA="/data/ngs/ref/Homo_sapiens_assembly38.fasta"

pbrun applybqsr \
--ref ${FASTA} \
--in-bam pbrun_fq2bam_GPU.bam \
--num-gpus 1 \
--in-recal-file pbrun_recal_gpu.txt \
--logfile applybqsr.log \
--out-bam  pbrun_fq2bam_GPU_applybqsr.bam ' > applybqsr.sh
```

Step 02.3: haplotypecaller

Note

```
echo '#!/bin/bash

FASTA="/data/ngs/ref/Homo_sapiens_assembly38.fasta"

pbrun haplotypecaller \
--ref ${FASTA} \
--num-gpus 1 \
--in-bam pbrun_fq2bam_GPU_applybqsr.bam \
--logfile hc.log \
--out-variants  pbrun_fq2bam_GPU.bam_applybqsr.vcf ' > hc.sh
```

Step 3: Run docker in interactive mode

- Run docker in interactive mode and enter the docker container

Note

```
docker run \
-it \
--rm \
--gpus all \
-v /data:/data \
-v $PWD:$PWD \
-w $PWD \
nvcr.io/nvidia/clara/clara-parabricks:4.3.0-1 bash
```

Step 4: Execute run_scripts

Now you are inside the docker container

```
# If you are inside docker; you'll see `root@712558e5c91f:/home/ubuntu#`
# Execute bash scripts you created

# Run fq2bam.sh
bash fq2bam.sh

# Run applybqsr.sh
bash applybqsr.sh

# Run hc.sh
bash hc.sh
```

- Exit the docker container with `exit` command

Step 5: Inspect results

Note

```
ls -l
total 1041856
-rw-r--r-- 1 root root      1707 Jun  5 21:42 applybqsr.log
-rw-rw-r-- 1 1000 1000       255 Jun  5 21:16 applybqsr.sh
drwxrwxr-x 3 1000 1000      4096 Jun  2 10:49 data
-rw-r--r-- 1 root root   2551016 Jun  5 13:37 deepvariant.vcf
-rw-r--r-- 1 root root      1893 Jun  5 13:37 dv.log
-rw-rw-r-- 1 1000 1000        33 Jun  4 13:25 file-with-numbers.txt
-rw-r--r-- 1 root root      4976 Jun  5 21:42 fq2bam.log
-rw-rw-r-- 1 1000 1000       443 Jun  5 21:15 fq2bam.sh
-rw-r--r-- 1 root root      1941 Jun  5 21:44 hc.log
-rw-rw-r-- 1 1000 1000       235 Jun  5 21:17 hc.sh
-rw-r--r-- 1 root root 516112463 Jun  5 21:42 pbrun_fq2bam_GPU.bam
-rw-r--r-- 1 root root   5447024 Jun  5 21:42 pbrun_fq2bam_GPU.bam.bai
-rw-r--r-- 1 root root   4719876 Jun  5 21:44 pbrun_fq2bam_GPU.bam_applybqsr.vcf
-rw-r--r-- 1 root root 532038424 Jun  5 21:42 pbrun_fq2bam_GPU_applybqsr.bam
-rw-r--r-- 1 root root   5447024 Jun  5 21:42 pbrun_fq2bam_GPU_applybqsr.bam.bai
-rw-r--r-- 1 root root     86706 Jun  5 21:42 pbrun_fq2bam_GPU_chrs.txt
-rw-r--r-- 1 root root    392014 Jun  5 21:42 pbrun_recal_gpu.txt
-rw-r--r-- 1 root root         0 Jun  4 16:59 test
```

Benchmarking accelerated next-generation sequencing analysis pipelines

Our Accelerated NGS analysis benchmark paper

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JOURNAL ARTICLE

Benchmarking accelerated next-generation sequencing analysis pipelines

Pubudu Saneth Samarakoon ✉ , Ghislain Fournous , Lars T Hansen , Ashen Wijesiri , Sen Zhao , Rodriguez Alex A , Tarak Nath Nandi , Ravi Madduri , Alexander D Rowe , Gard Thomassen ... Show more

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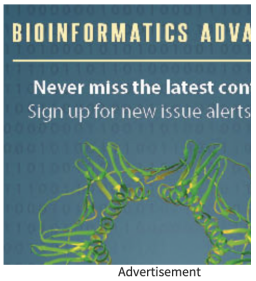
https://doi.org/10.1093/bioadv/vbaf085
Published: 15 May 2025 **Article history** ▾

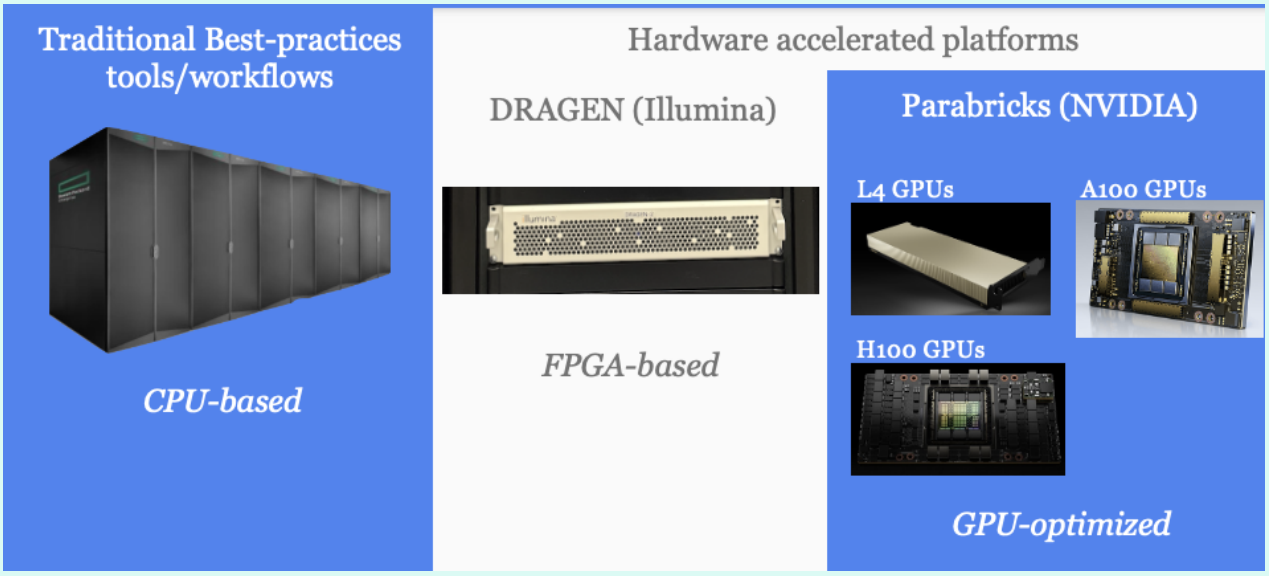


Advertisement

- Link to the paper

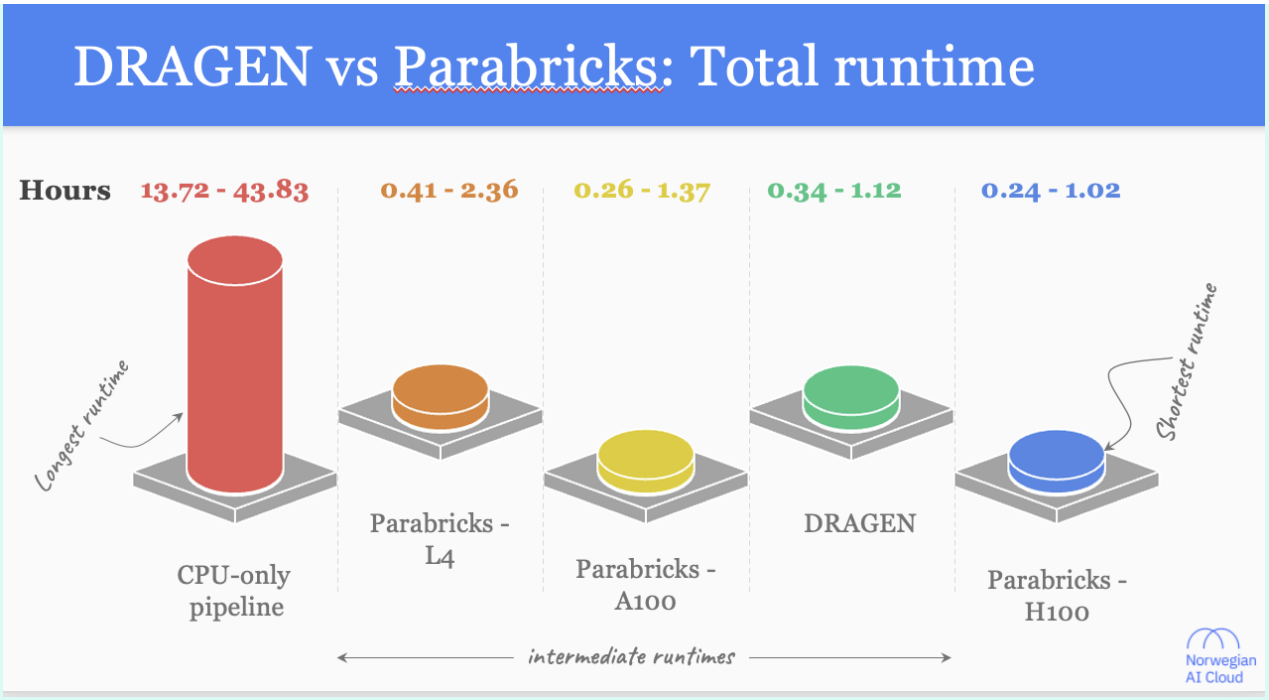
Hardware Platforms for NGS analysis

✔ Hardware accelerated platforms



Significant performance gain can be achieved with any accelerated platform

✔ Runtime comparison

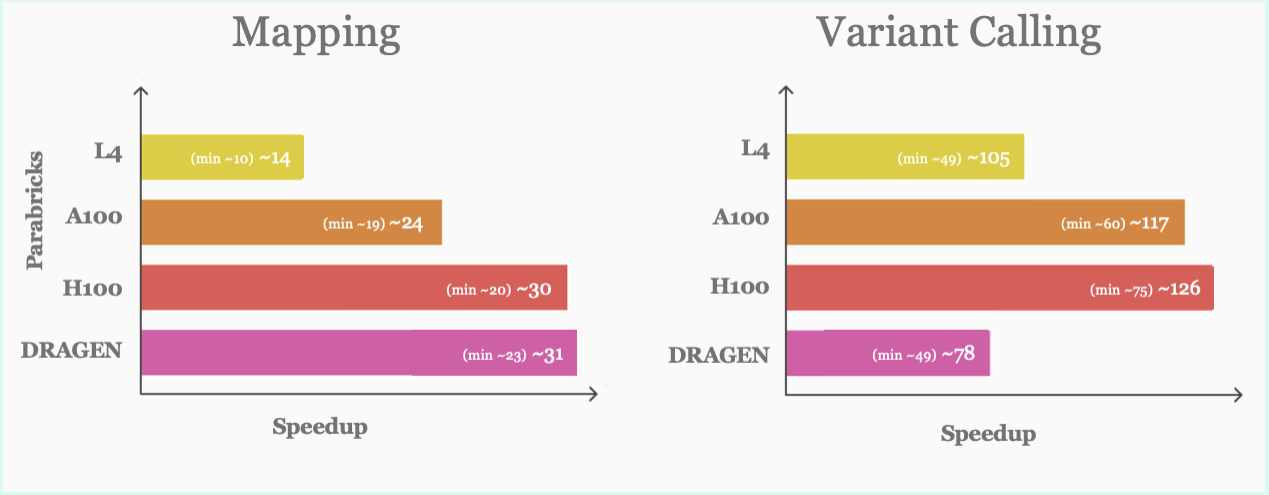


Above is a cartoon figure to highlight the patters the original paper contains correct figures

Speedup ## Quantify performance improvements (Speedup = CPU-only runtime ÷ Accelerated runtime)

Variant calling showed higher speedup than Mapping

✔ Speedup comparison



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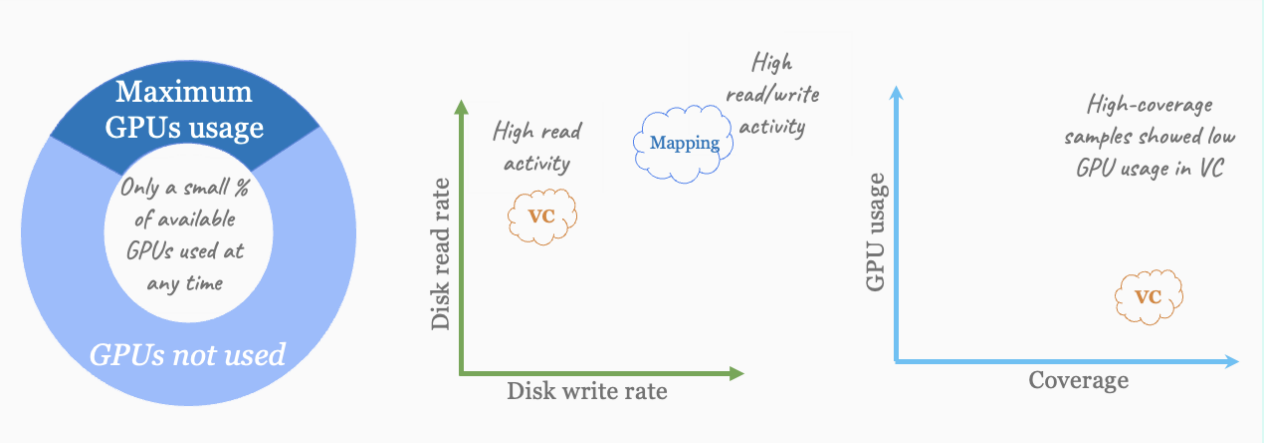
Parabricks resource usage provided new insights

✔ Profiling Parabricks



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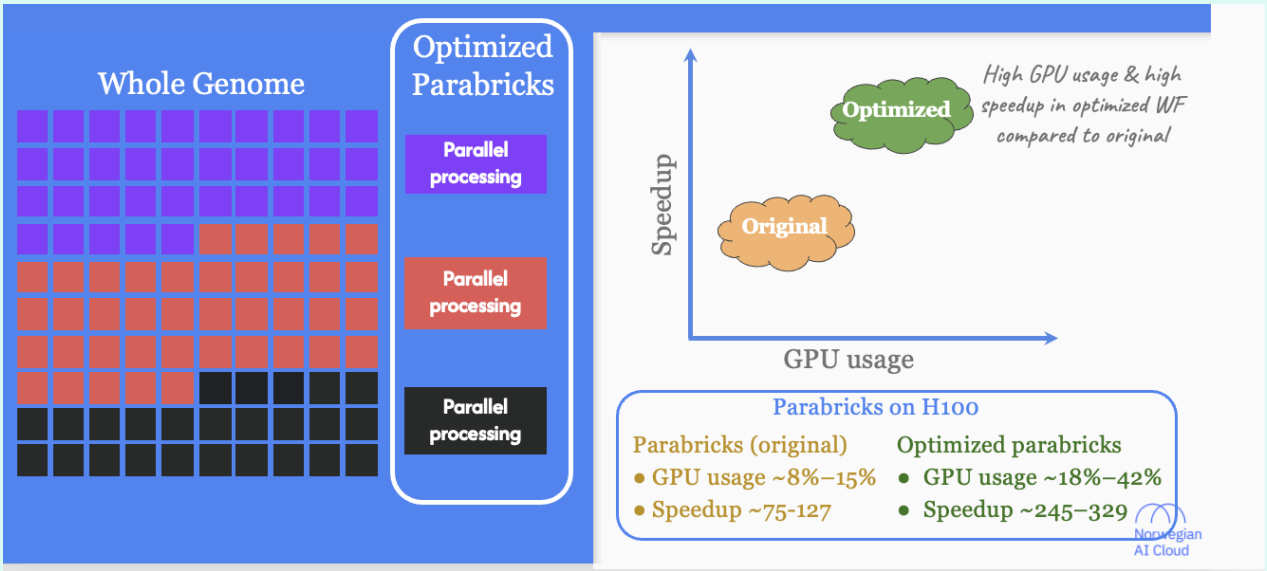
Optimizing Parabricks for further performance gains

✔ Solution



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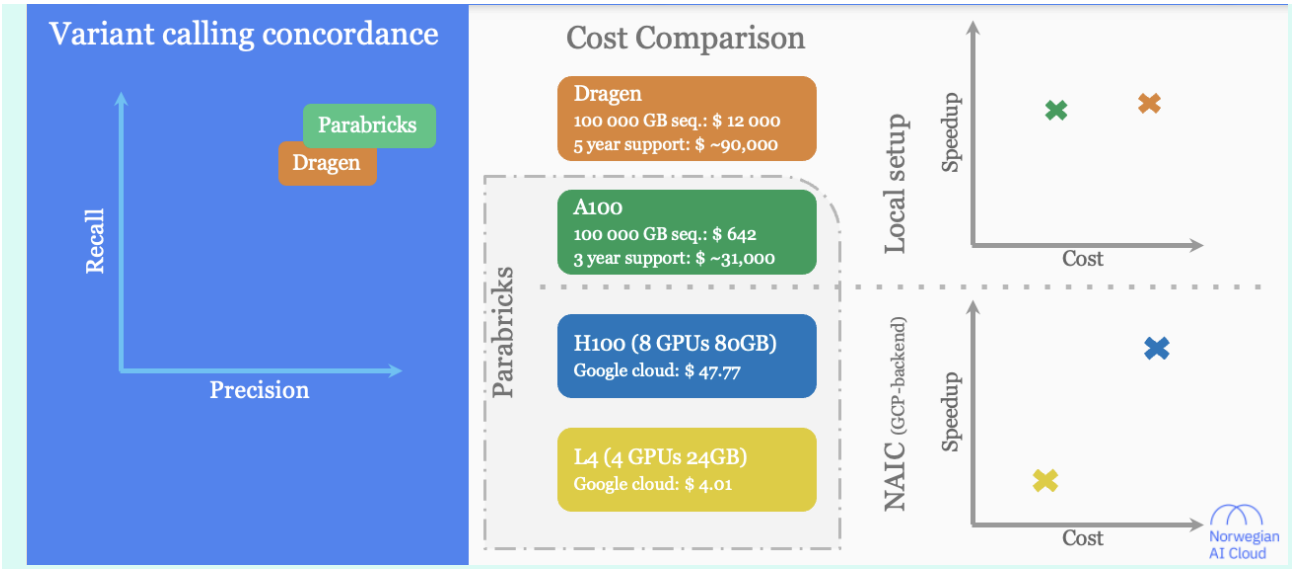
Parabricks and DRAGEN, speedup and cost consideration

✔ Practical Selection Framework



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Accelerated NGS analysis NextFlow pipeline