DENOThe Meeting 2019

Sequenziamento Nanopore applicazioni in onco-ematologia

Simone Romagnoli

(UR12 – Prof. Vannucchi)

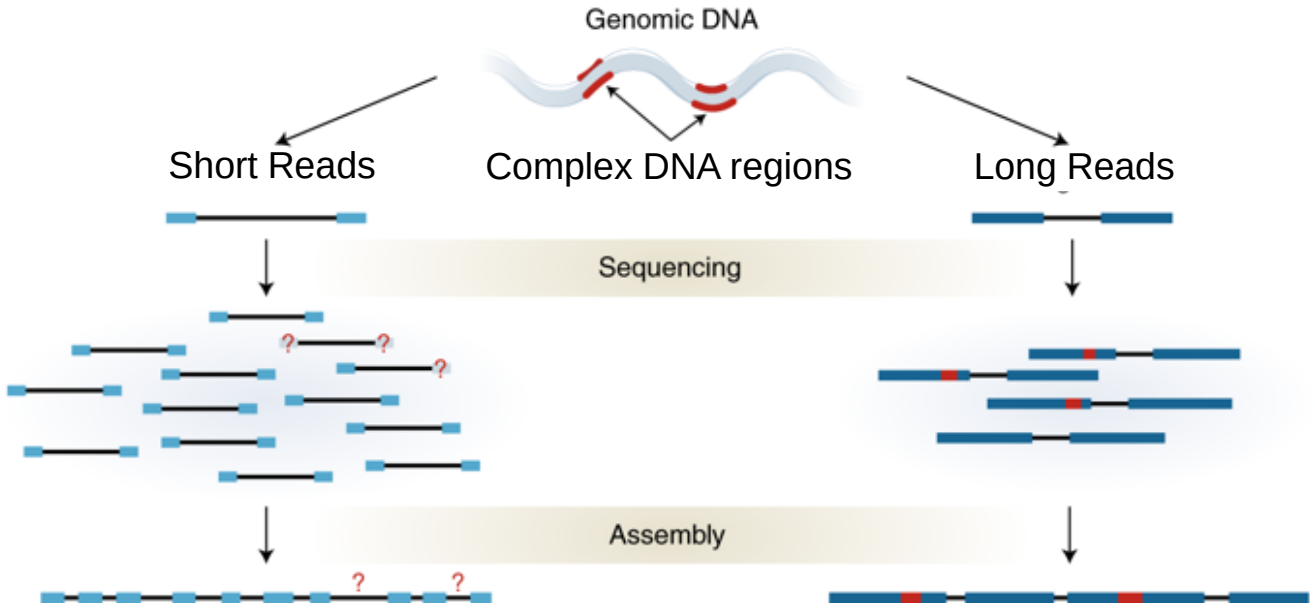
Email:
romagnoli.simone@unifi.it

Third Generation Sequencing

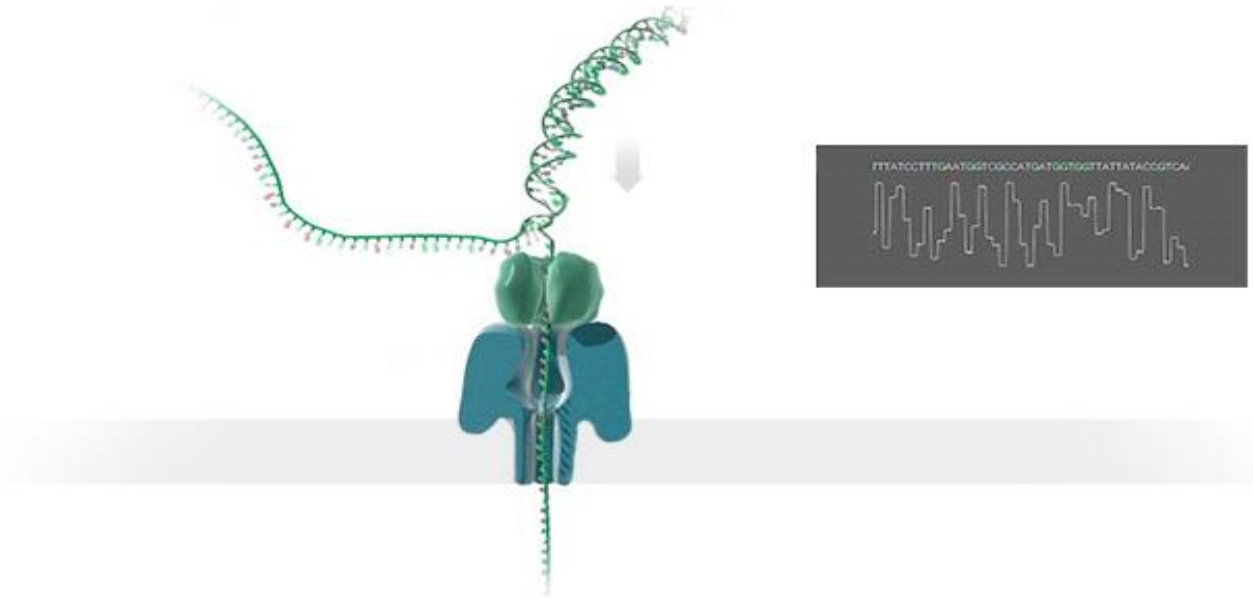
- The majority of cancers are driven by genomic alterations defined as structural variants (SVs), including translocations, inversions and copy number variations (CNVs).

- SVs detection tools rely on the so-called Second-Generation Sequencing (SGS) platforms, these technologies face inaccuracy and limitations in detecting some classes of SVs.

- In this scenario, the advent of new long reads (up to 20 Kb) -based Third-Generation Sequencing (TGS) technologies might allow to explore those genomic regions uncovered by SGS.

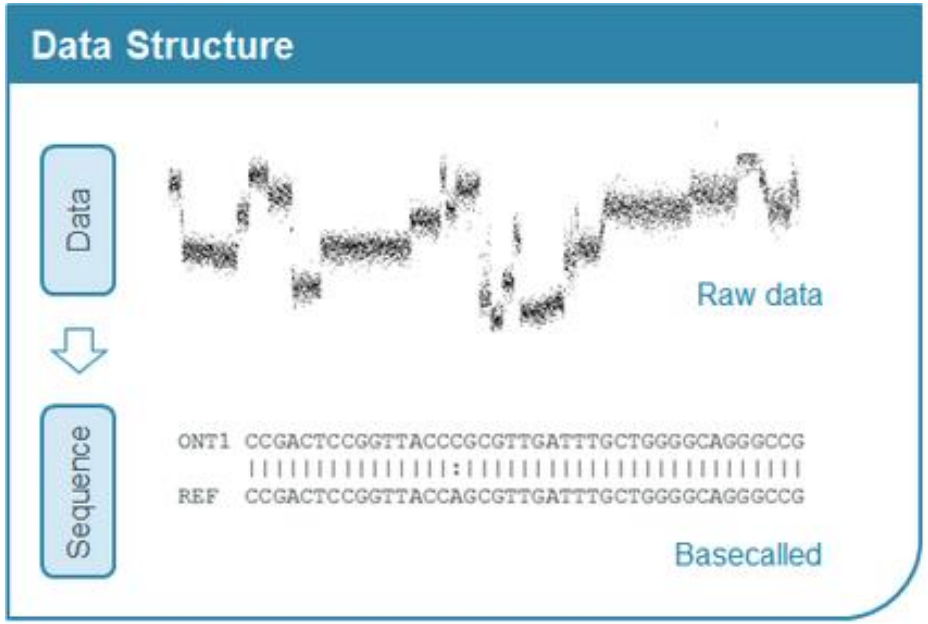


Nanopore Sequencing – How it works?



For DNA sequencing, Oxford Nanopore uses a strand sequencing method, in which intact DNA strands are processed by the nanopores and analysed in real-time

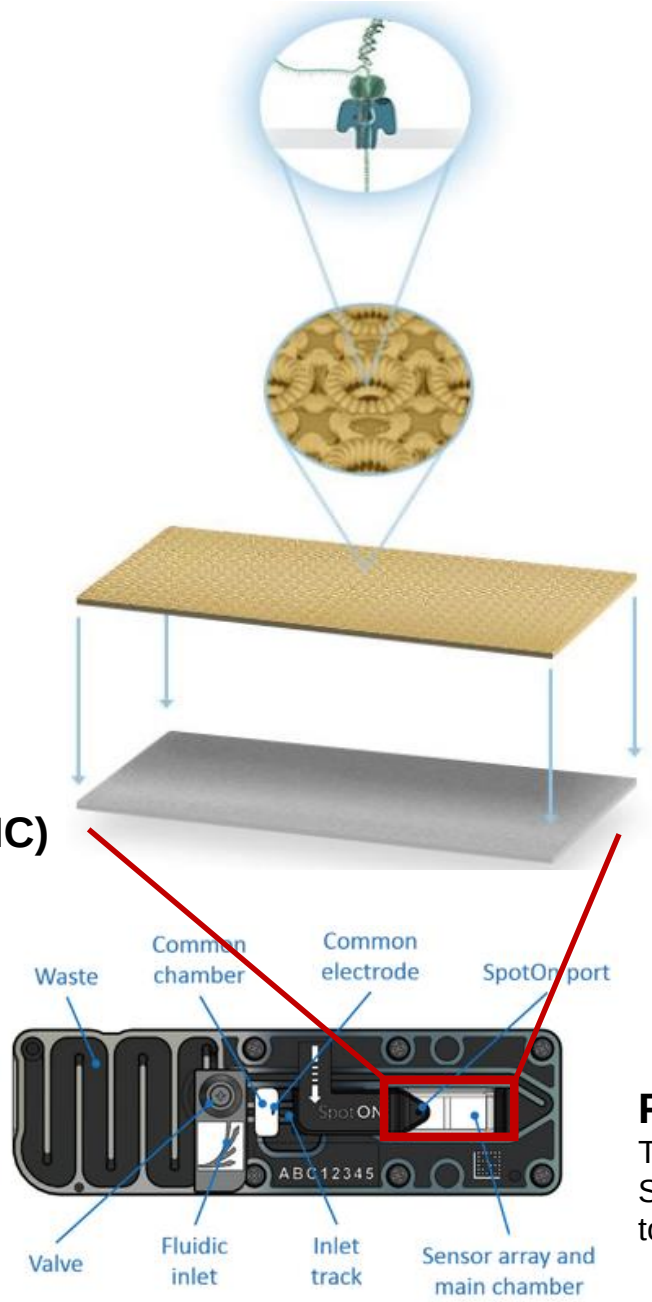
In Oxford Nanopore's strand sequencing method, the speed of translocation is controlled by the inclusion of a Motor protein.



Raw data - a direct measurement of the changes in ionic current as a DNA/RNA strand passes through the pore, which are recorded by the MinKNOW software.

Basecalling - the raw signal is further processed by the basecalling algorithm to generate the base sequence of the read.

Nanopore Sequencing – How it works?



Nanopore

A sampling ionic current is applied through each pore by setting a voltage across this membrane, the translocation of a strand of DNA through the nanopore creates a characteristic disruption in current as the oligonucleotides pass through the pore in different combinations.

Array of Microscaffolds

An array of microscaffols holds the membrane in which the nanopore are embedded. This keep the membrane stable during shipping and usage.

Array Chip

Each microscaffold on the sensor array chip contains an individual electrode, allowing for multiple nanopore experiments to be performed in parallel.

Application Specific Integrated Circuit (ASIC)

Each nanopore is controlled and measured by an individual channel on a corresponding, bespoke ASIC.

Flow-cell 9.4.1 RevD

The Flow Cells contain the proprietary sensor array, Application-Specific Integrated Circuit (ASIC) and nanopores that are needed to perform a complete single-molecule sensing experiment.

Nanopore Platforms

Overview	MinION	GridION
Yield per flow cell, DNA, cDNA	50 Gb	50 Gb
Number of Flow cell per device	1	5
Yield per device	50 Gb	250 Gb
Application	Whole Genome/Exomes Targeted Sequencing Whole Transciptomes (cDNA) Smaller transcriptomes (direct RNA)	Larger Genome Whole Transciptomes (cDNA or direct RNA)

- **De novo AML**
- **Secondary AML**
- **MPN**
- **Prenatal Diagnosis**

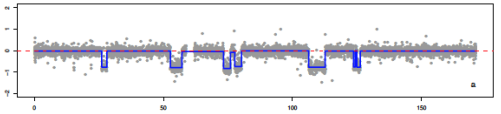
Nanopore Sequencing – Workflow

Samples collection

gDNA extraction

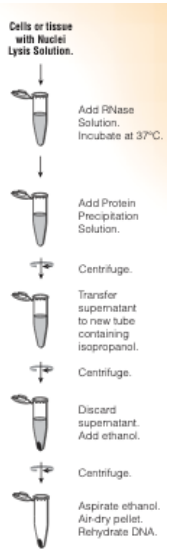
Library prep.

GridION sequencing

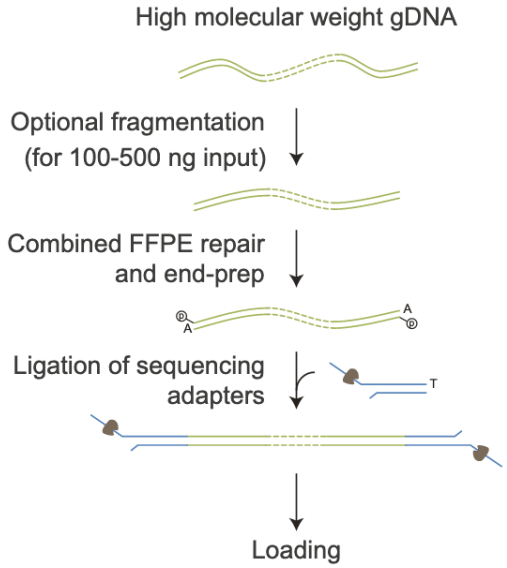


Nano-GLADIATOR analysis

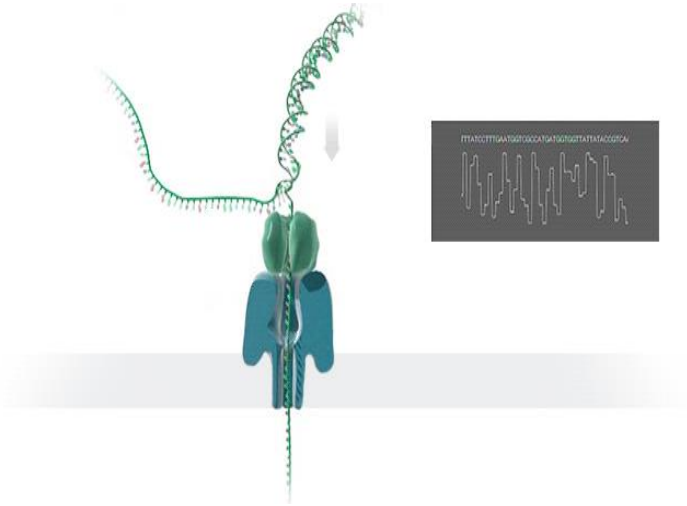
- **De novo AML**
- **Secondary AML**
- **TN MPN**
- **Prenatal Diagnosis**



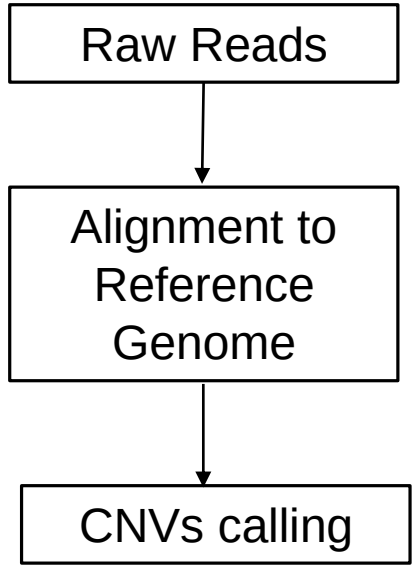
90 min



60 min



48 h



2 h

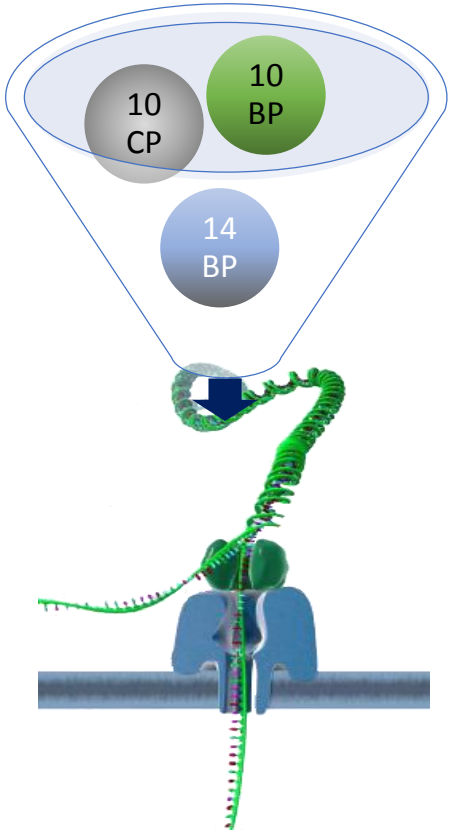
Nanopore Sequencing – Advantages and Limitation

- Low cost
- Sequencing of native DNA molecules – no bias and loss of valuable information
- Sequencing of Repetitive DNA regions
- Time

- Error Rate ~ 5%
- DNA quantity ~ 700 ng

WORK IN PROGRESS …

Nanopore Sequencing – Application on sAML



paired samples

10 MPNs in Chronic Phase (**CP**)
(6 PMF, 3 ET, 1 PV)

10 sAML in Blastic Phase (**BP**)
PB blast count > 20%

CP and BP samples were separated by a mean of 77 months

14 sAML **BP**

CNAs identification and categorization [1,2,3]

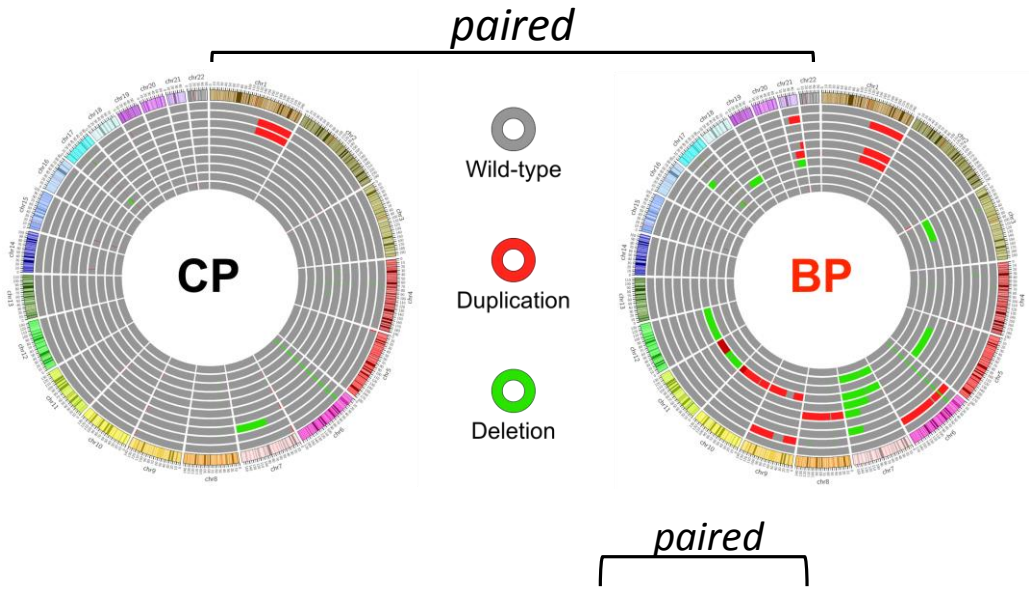
- Focal CNAs < 1 Mb
- CNAs > 1 Mb
- CNAs > 30 Mb
- Chromosome arm
- Whole Chromosome

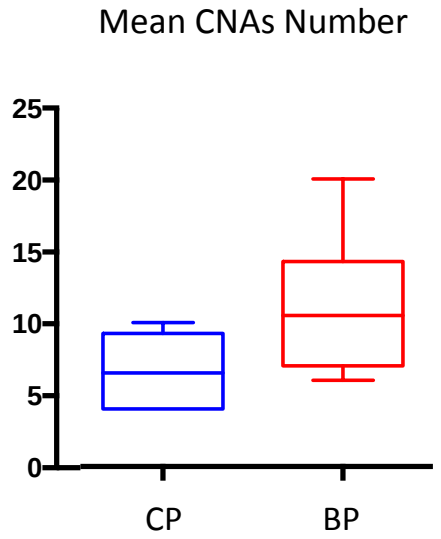
→ **Recurrent alterations**
Involved genes and pathways

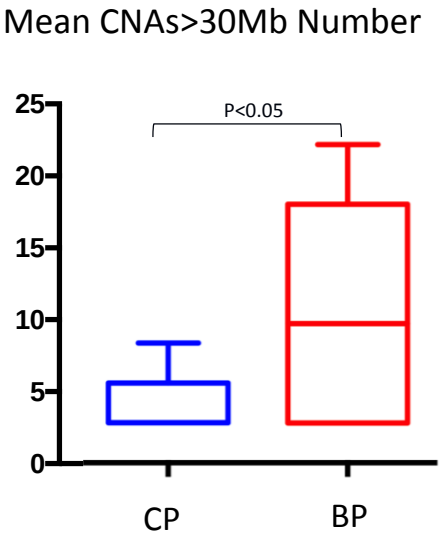
→ **Hallmark of sAML evolution**
Evolution biomarkers

[1] Davoli T, Science, 2017 [2] Maser RS, Science, 2002. [3] Negrini S, Nat Rew., 2010

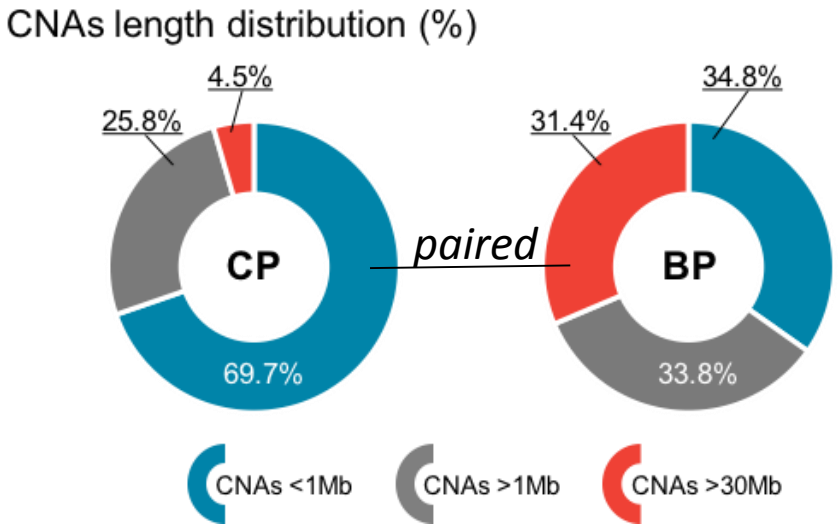
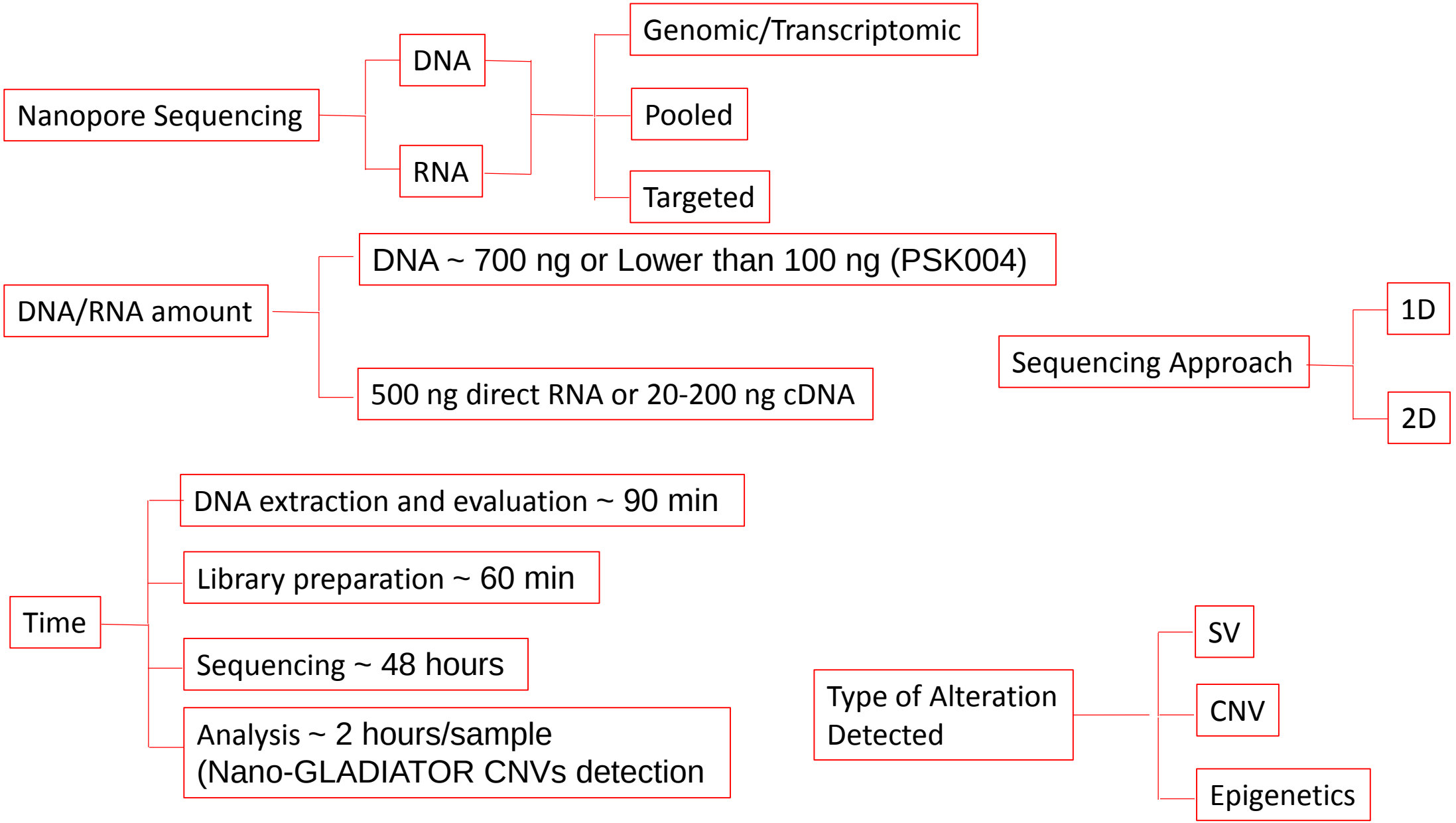
Nanopore Sequencing– sAML Preliminary Results







Variable	CP	BP
Total CNAs mean number	6.6±2.6	11.1±4.4
Focal CNAs (%)	69.7	34.8
>1Mb CNAs (%)	25.8	33.8
>30 Mb CNAs (%)	4.5	31.4
Samples with ≥ 1 arm altered	20% (2 of 10)	40% (4 of 10)
Samples with ≥ 1 chr altered	none	40%
Mean VAF	0.81	0.79
Mean bp involved	$7.07x10^6$	$16.64x10^6$



Nanopore Sequencing – To Sum Up