

8<sup>e</sup> ÉDITION

# JOURNÉES DU GFCO 2022

Biomarqueurs et analyses moléculaires en oncologie

Avec la participation  
scientifique du



# NOUVELLES TECHNOLOGIES

## Séquençage de 3<sup>ème</sup> génération

Julien Masliah Planchon, Institut Curie, Paris

[julien.masliahplanchon@curie.fr](mailto:julien.masliahplanchon@curie.fr)

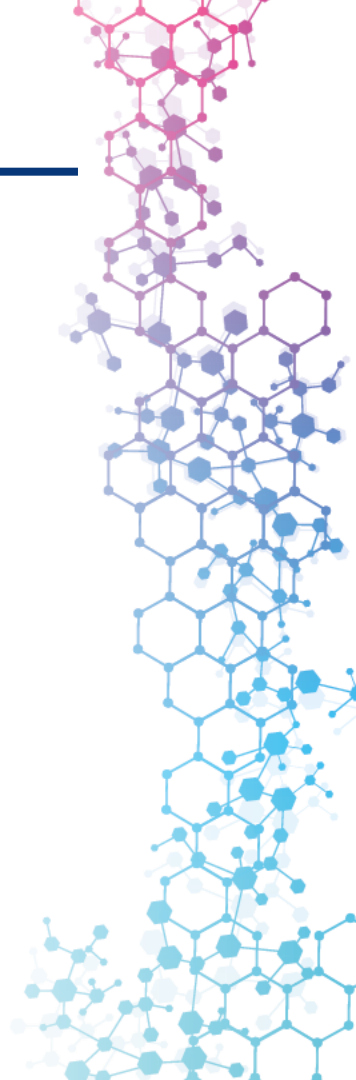
Modérateur : Alexandre Harlé, Nancy



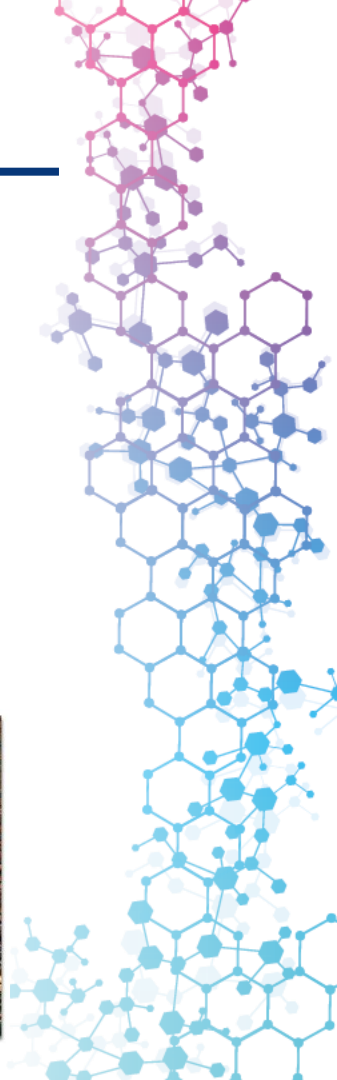
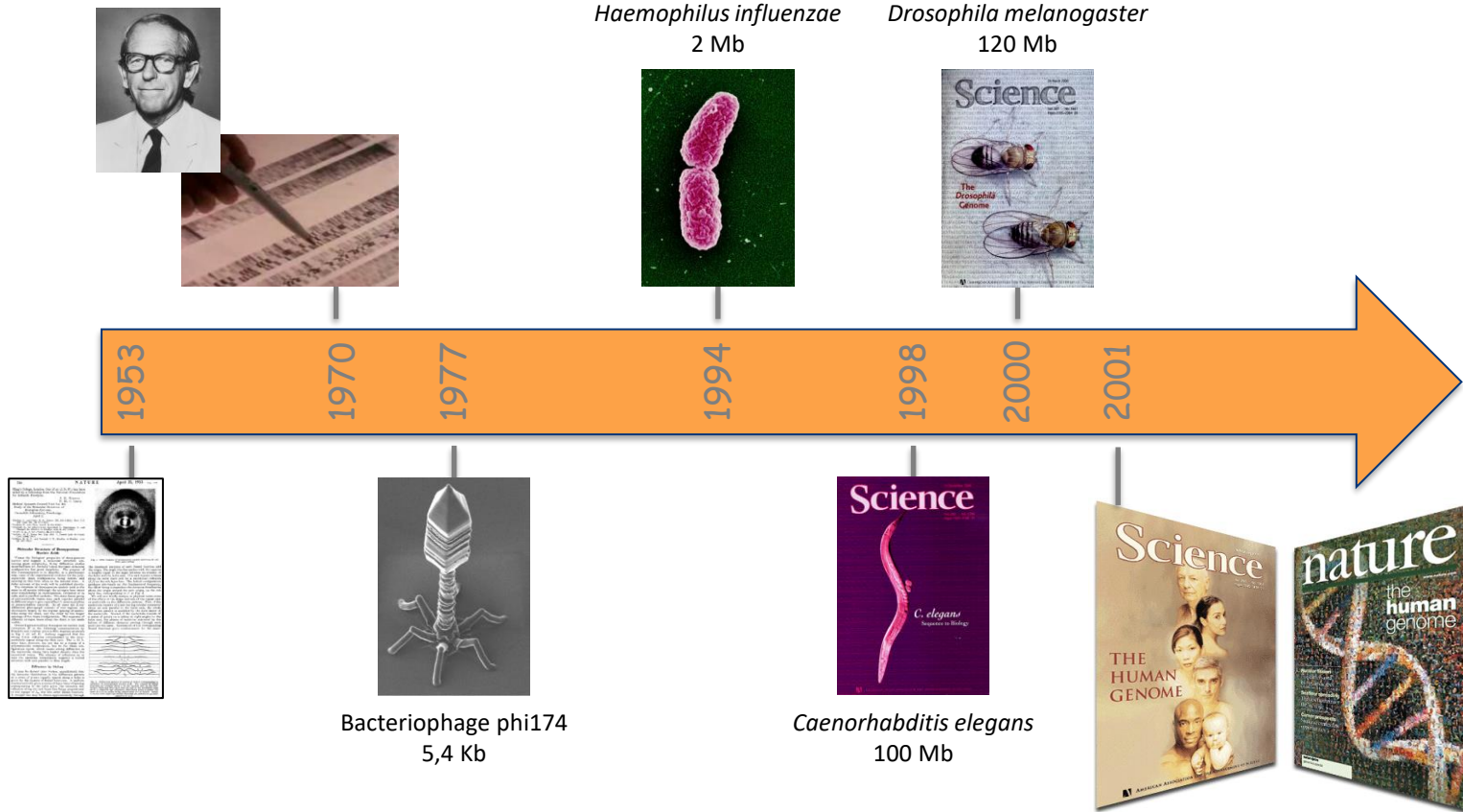
# LIENS D'INTÉRÊT

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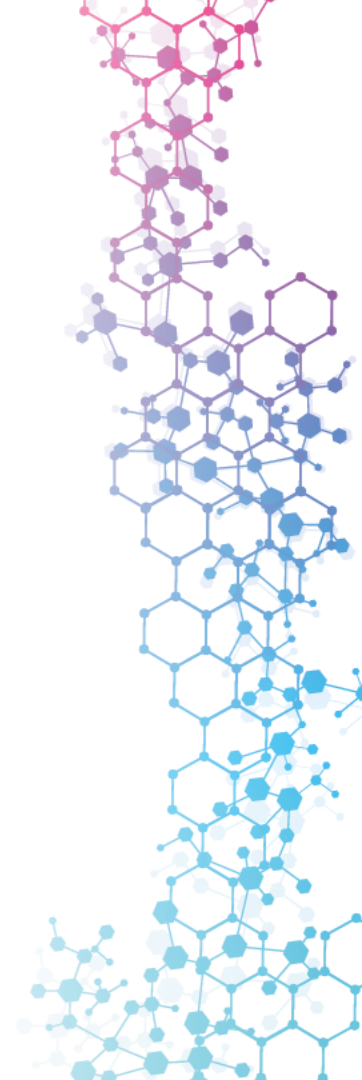
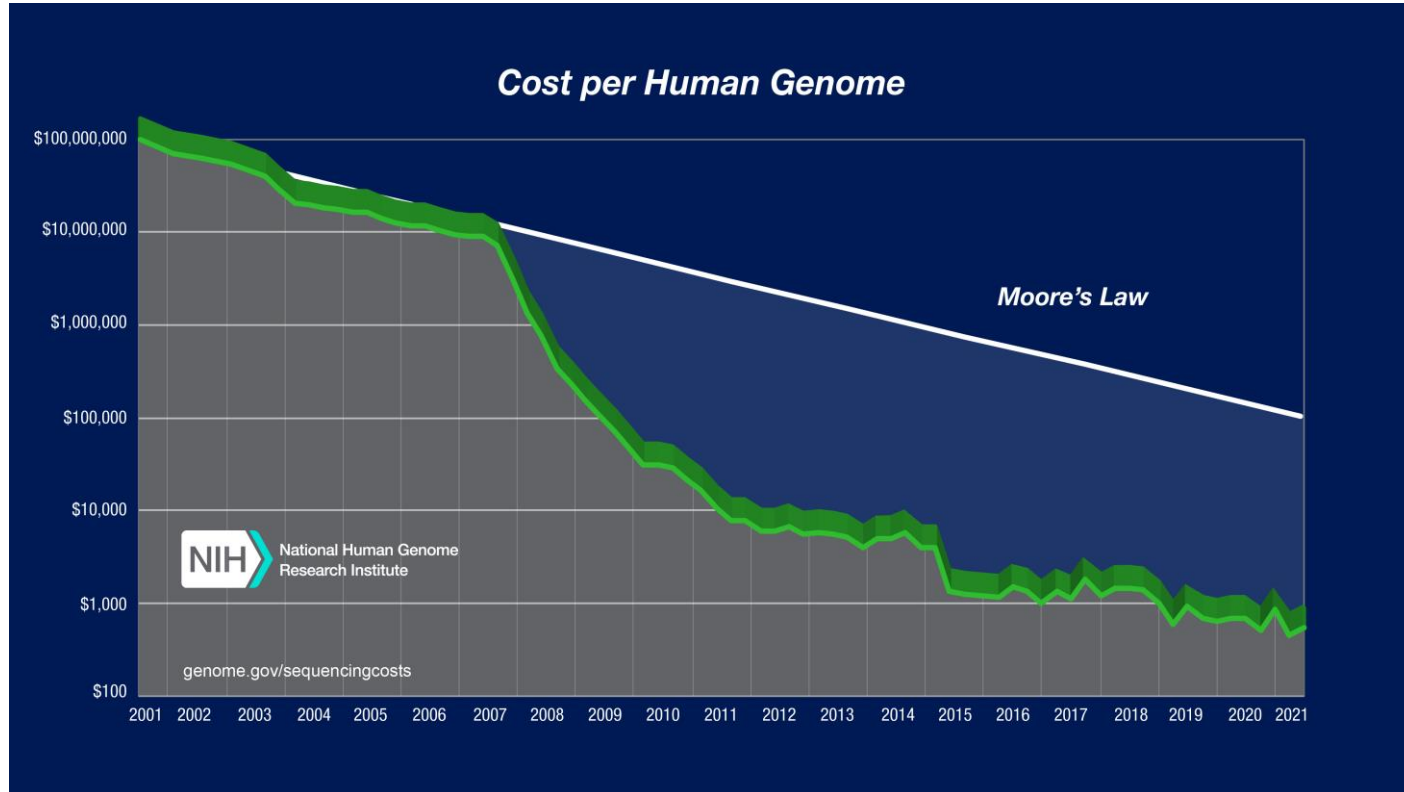
- Pas de conflit d'intérêt



# Historique de la génomique moderne



# Le NGS : une révolution technologique



# 3<sup>ème</sup> génération de séquenceur

Séquençage de  
1<sup>ère</sup> génération



Sanger

Séquençage de  
2<sup>ème</sup> génération

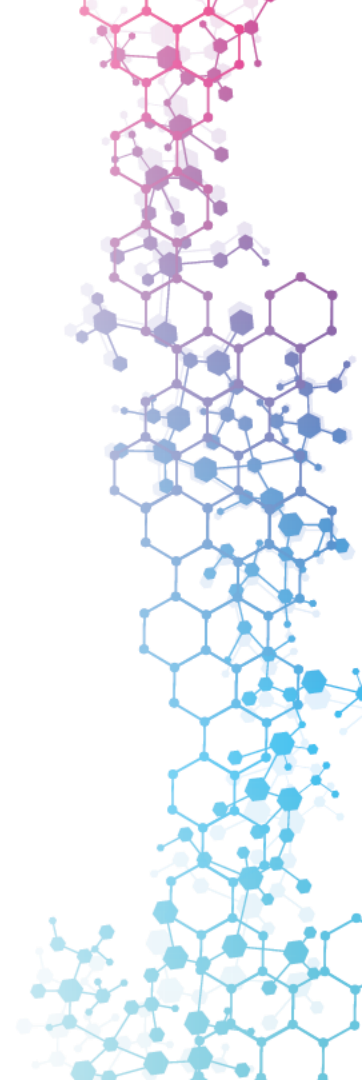


NGS

Séquençage de  
3<sup>ème</sup> génération



Nanopore





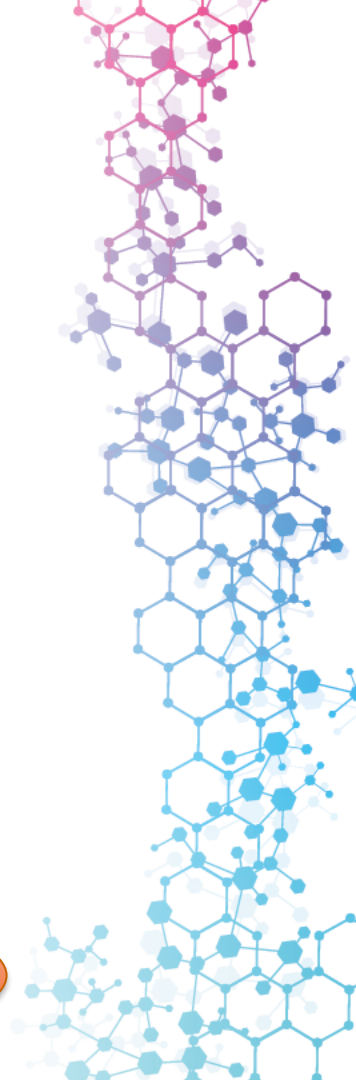
# 3<sup>ème</sup> génération de séquençage



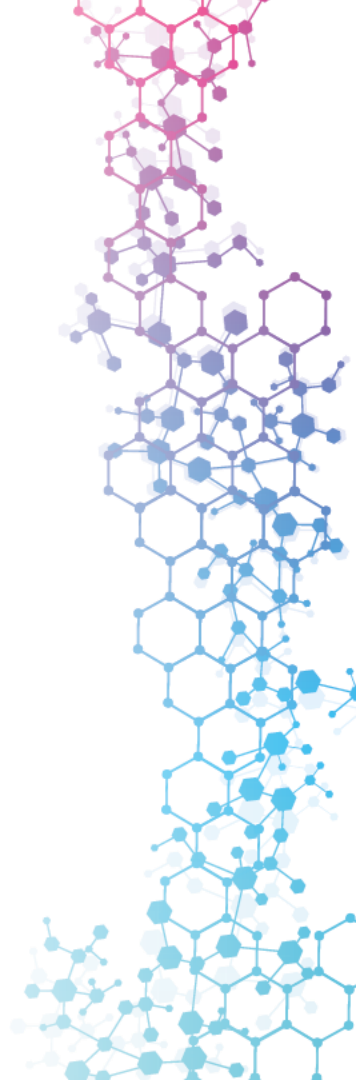
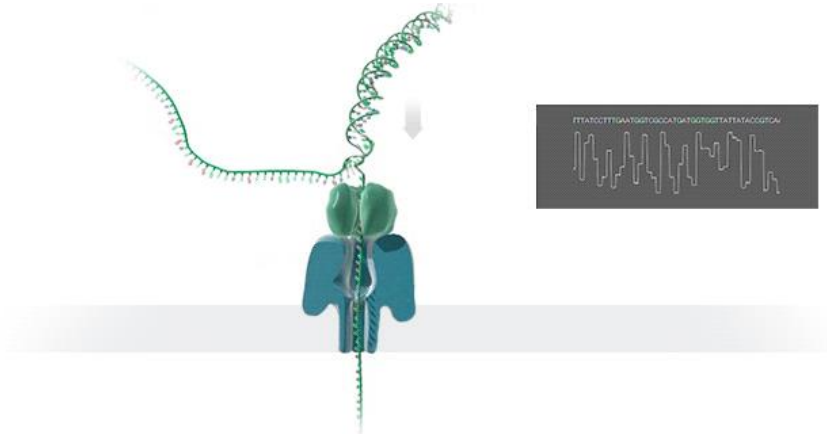
Long read sequencing

Real time sequencing

Native nucleic acid sequencing



# Principe du séquençage par Nanopore





# Les séquenceurs Nanopore

MK1C  
5 000 euros



MK1B  
1 000 euros



GridION  
50 000 euros



PromethION (P24 – P48)  
200 000 – 280 000 euros



Flongle



PromethION P2  
10 000 euros

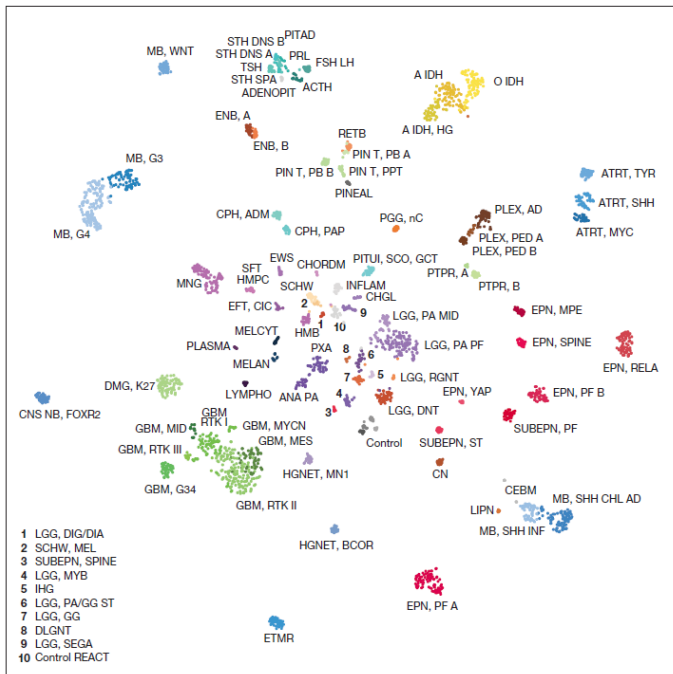


PromethION P2 solo  
50 000 euros

Quel intérêt en routine ??



# Nanopore-based CNS tumors classification



Capper et al. (Nature, 2018)

Acta Neuropathol  
 DOI 10.1007/s00401-017-1743-5

METHODS PAPER

## Same-day genomic and epigenomic diagnosis of brain tumors using real-time nanopore sequencing

Philipp Euskirchen<sup>1,2,3,6</sup> · Franck Bielle<sup>4,5</sup> · Karim Labreche<sup>4,6</sup> · Wigard P. Kloosterman<sup>7</sup> · Shal Rosenberg<sup>8</sup> · Mailya Daulau<sup>9</sup> · Charlotte Schmitt<sup>9</sup> · Julia Masliab-Planchon<sup>9</sup> · Franck Bourdeaut<sup>10</sup> · Caroline Dehais<sup>9</sup> · Yannick Marie<sup>9</sup> · Jean-Yves Delattre<sup>9,7</sup> · Ahmed Idbaih<sup>9,7</sup>

Neuropathology and Applied Neurobiology



### Robust methylation-based classification of brain tumors using nanopore sequencing

● Luis P. Kuschel, Jürgen Hench, Stephan Frank, Ivana Bratic-Hench, Elodie Girard, Maud Blanluet, Julien Masliab-Planchon, Martin Misch, Julia Onken, Marcus Czabanka, Philipp Karau, Naveed Ishaque, Elisabeth G. Hain, Frank Heppner, Ahmed Idbaih, Nikolaus Behr, Christoph Harms, David Capper, Philipp Euskirchen

## Neuro-Oncology Advances

9(1), 1–10, 2021 | <https://doi.org/10.1093/neoj/ndab149> | Advance Access date: 10 October 2021

### Intraoperative DNA methylation classification of brain tumors impacts neurosurgical strategy

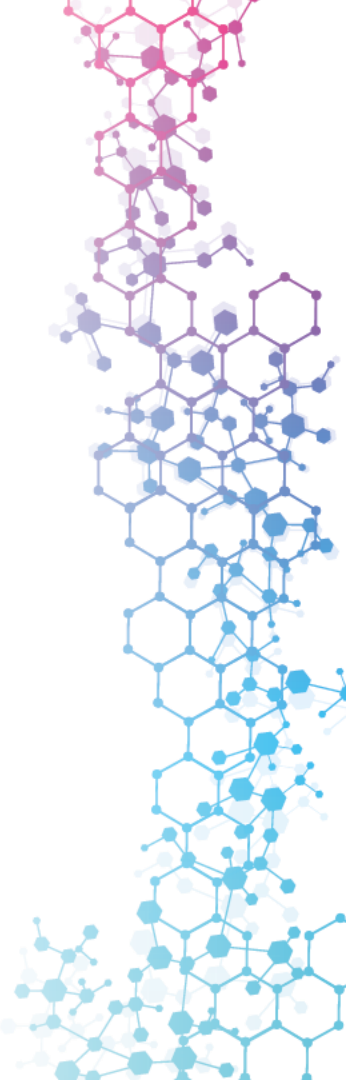
Luna Djirackor<sup>1</sup>, Skarphedinn Halldorsson<sup>2</sup>, Pitt Niehusmann, Henning Leske, David Capper, Luis P. Kuschel, Jens Pahnke, Bert J. Due-Tønnessen, Iver A. Langmoen, Cecilie J. Sandberg, Philipp Euskirchen<sup>3</sup> and Einar G. Vik-Mo<sup>4</sup>



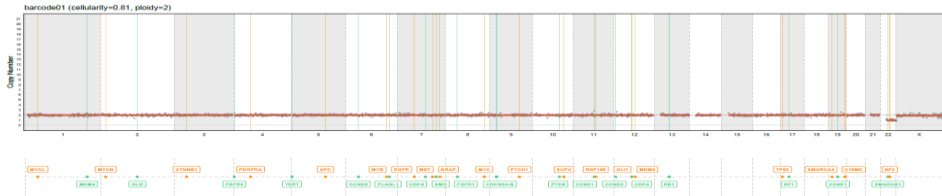
ACTA NEUROPATHOLOGICA

### Rapid-CNS<sup>2</sup>: Rapid comprehensive adaptive nanopore-sequencing of CNS tumors, a proof of concept study

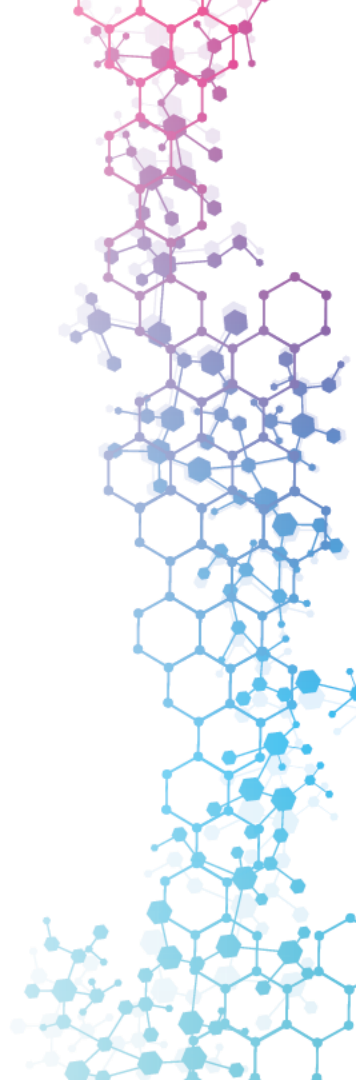
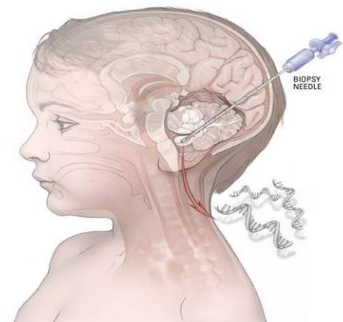
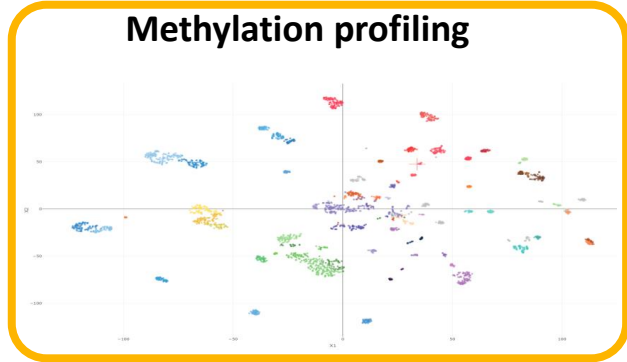
● Areeba Patel, Helin Dogan, Alexander Payne, Philipp Sievers, Natalie Schoebe, Daniel Schrimpf, Damian Stichel, Nadine Holmes, Philipp Euskirchen, Jürgen Hench, Stephan Frank, Violaine Rosenblatt-Godts, Miriam Ratliff, Nina Elmman, Andreas Unterberg, Christoph Dieterich, Christel Herold-Mende, Stefan M Pfister, Wolfgang Wick, Matthias Schliesner, Matthew Loose, Andreas von Deimling, Martin Sill, David TW Jones, ● Felix Sahm



# Whole genome copy number profiling



# Methylation profiling

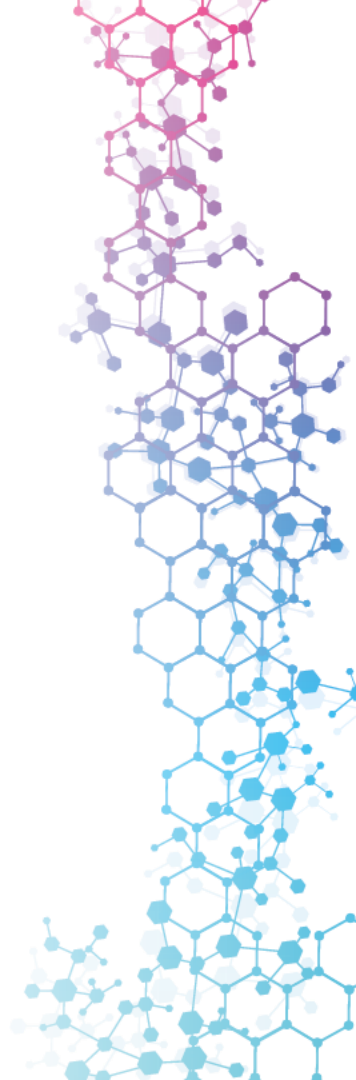
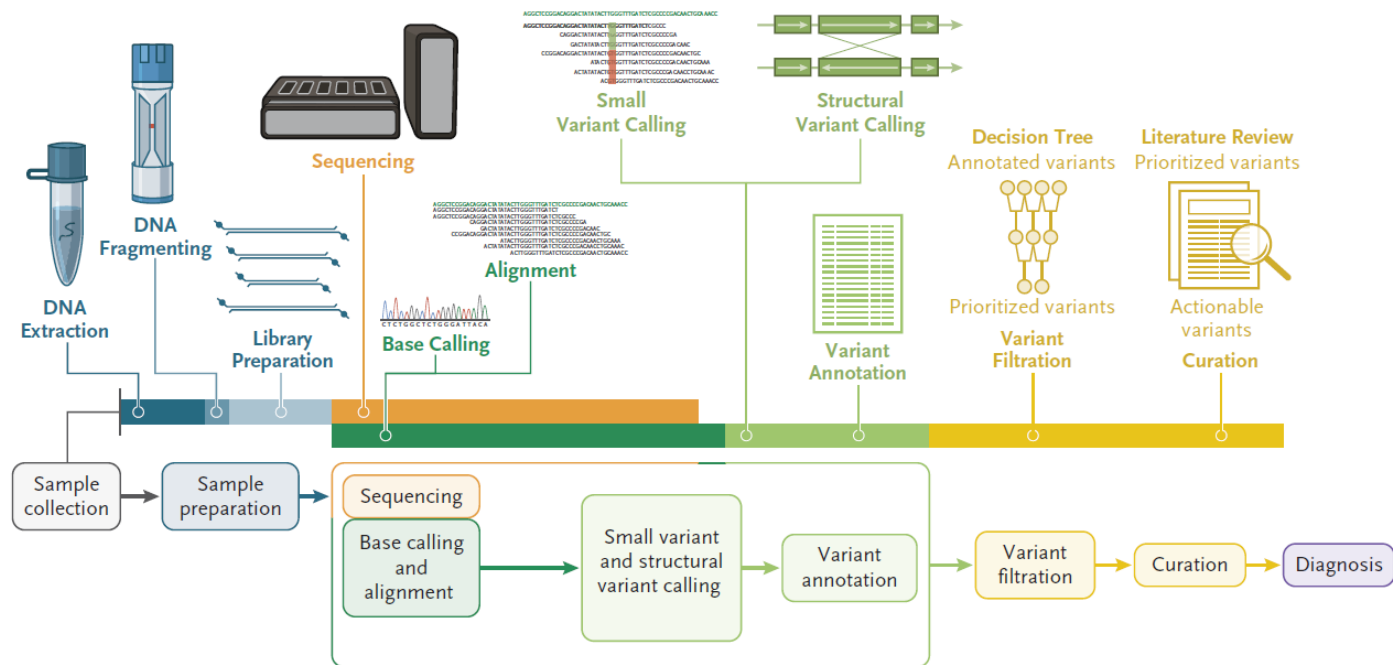


# Ultrarapid WGS

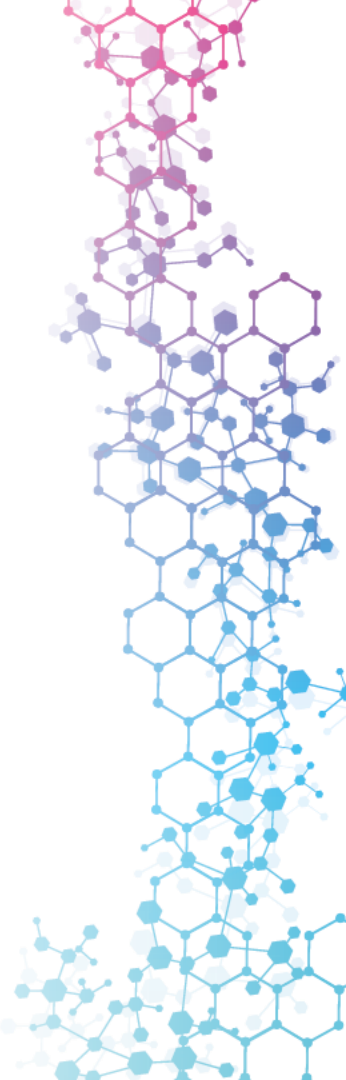
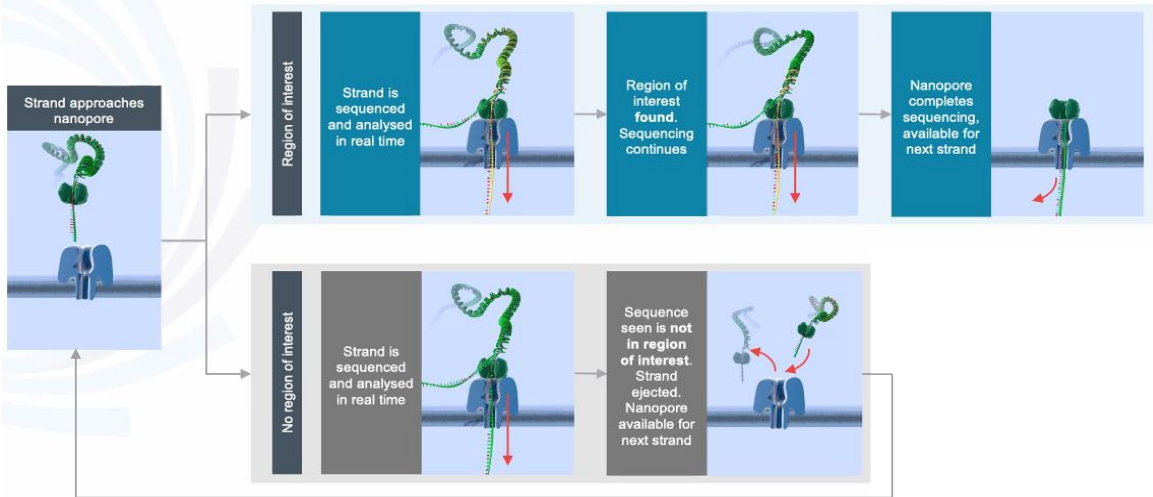
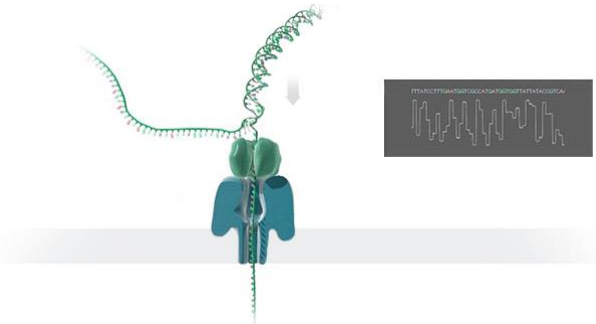
The NEW ENGLAND JOURNAL of MEDICINE

## Ultrarapid Nanopore Genome Sequencing in a Critical Care Setting

N ENGL J MED 386;7 NEJM.ORG FEBRUARY 17, 2022

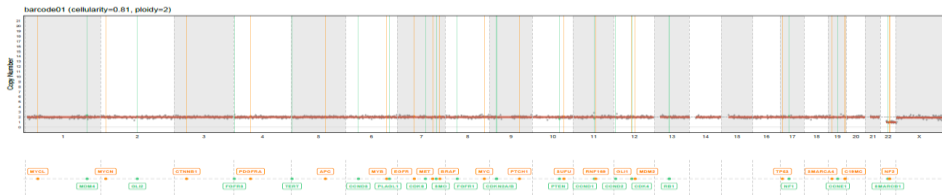


# Adaptive sequencing

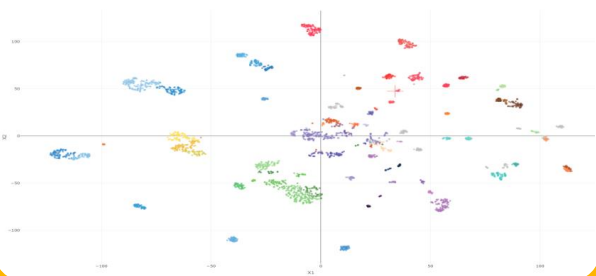




## Whole genome copy number profiling

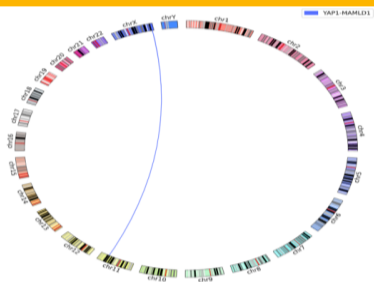


## Methylation profiling

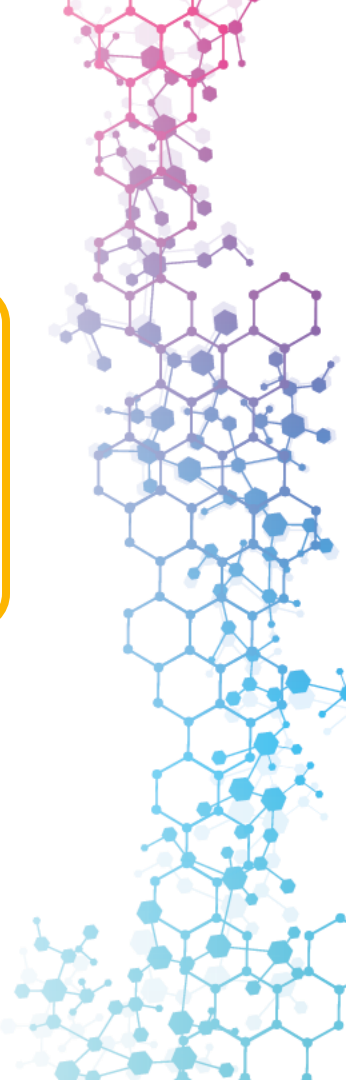
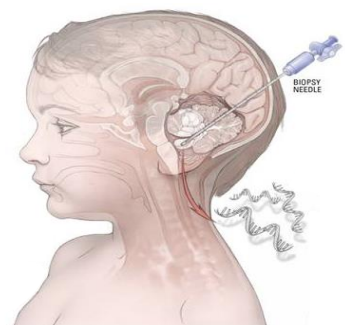


Gene	# Mut	#	Freq
PTCH1	56	51	25.2%
TERT	50	50	24.8%
DDX3X	47	43	21.3%
KMT2D	38	34	16.8%
CTNNA1	33	32	15.8%
KMT2C	31	29	14.4%
SMARCA4	27	25	12.4%
KBTBD4	27	19	9.4%
KDM6A	17	17	8.4%
TP53	18	17	8.4%

## Mutation detection

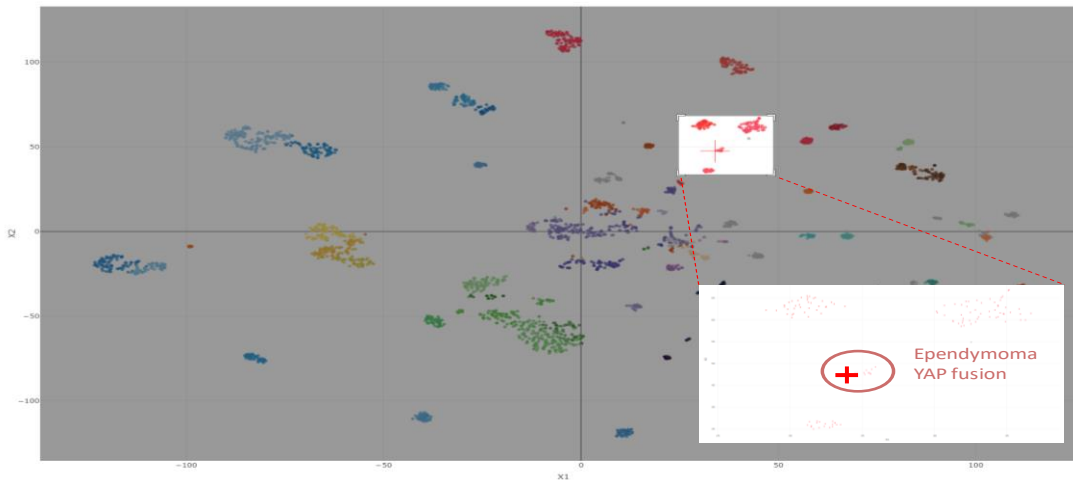


## Fusion detection

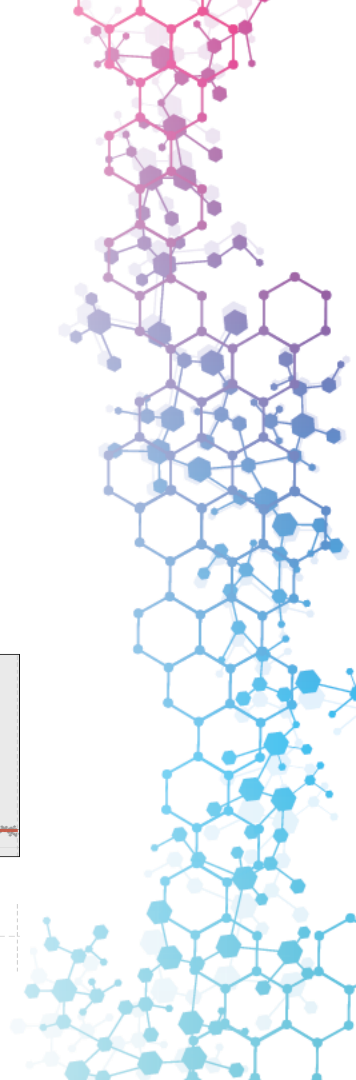
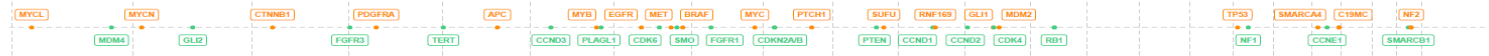
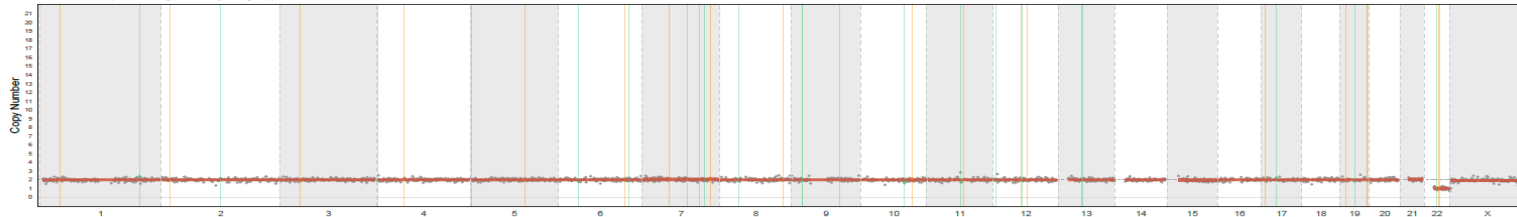


Bulky right hemispheric tumor arised at 1 year old

Histologic aspects compatible with an ependymoma



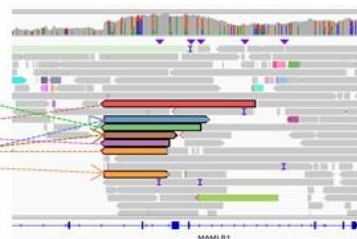
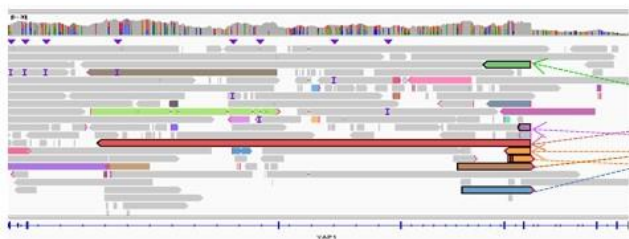
barcode01 (cellularity=0.81, ploidy=2)



**YAP1**

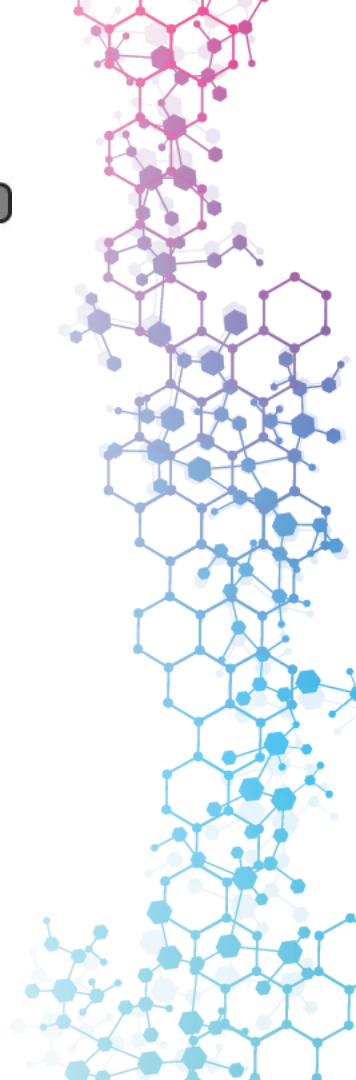
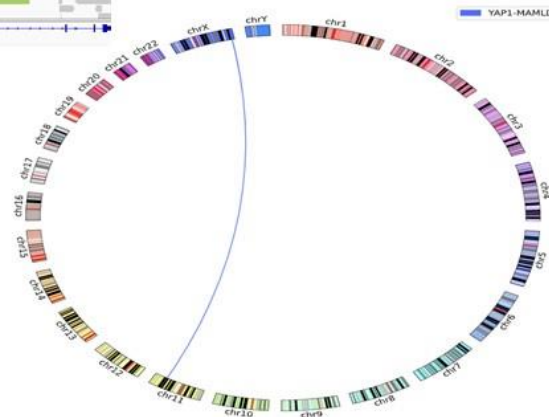


**MAMLD1**



**YAP1 fused  
ependymoma**

**YAP1-MAMLD1 fusion protein**





### Flongle

100 euros  
200 ng ADN  
10 000 CpG  
48h  
Méthylation uniquement



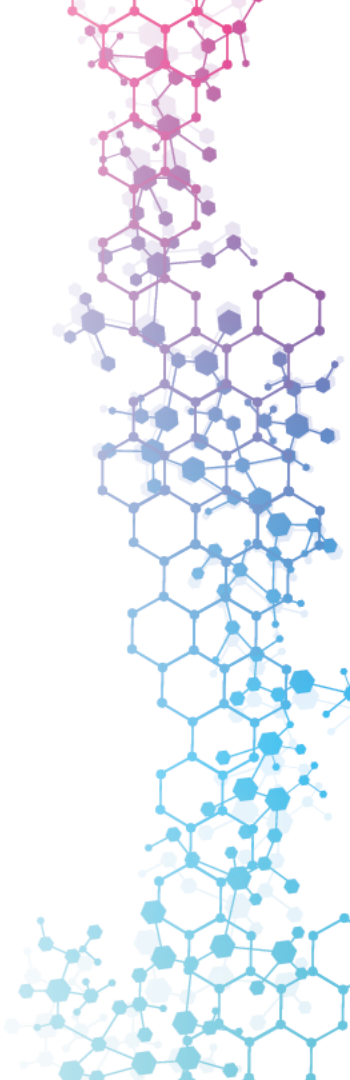
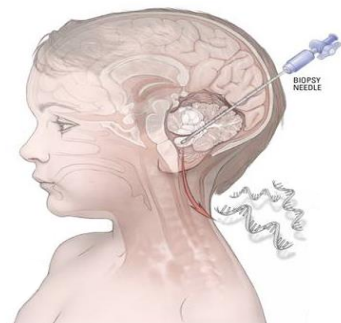
### Nanopore basique

200 euros  
400 ng ADN  
40 000 CpG  
2 semaines  
Méthylation + copy number



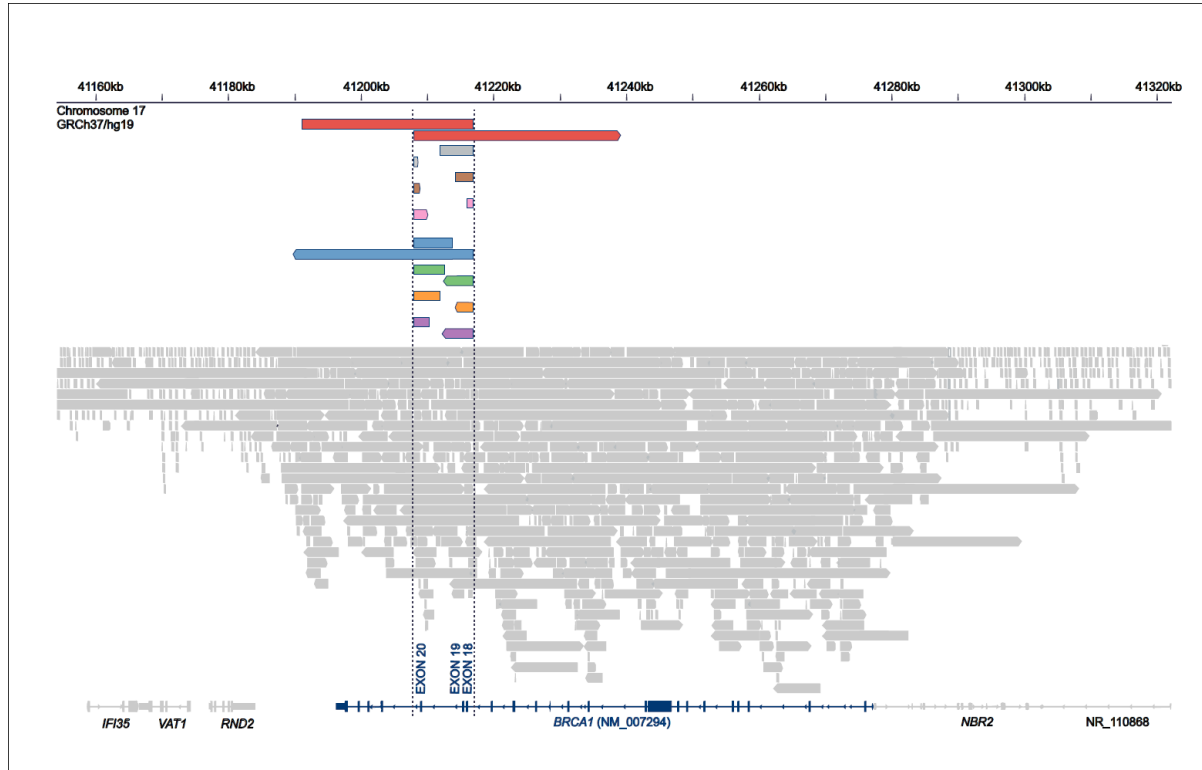
### Nanopore + adaptive

900 euros  
2  $\mu$ g ADN  
100 000 CpG  
2 semaines  
Méthylation + copy number  
+ Fusion + Mutation

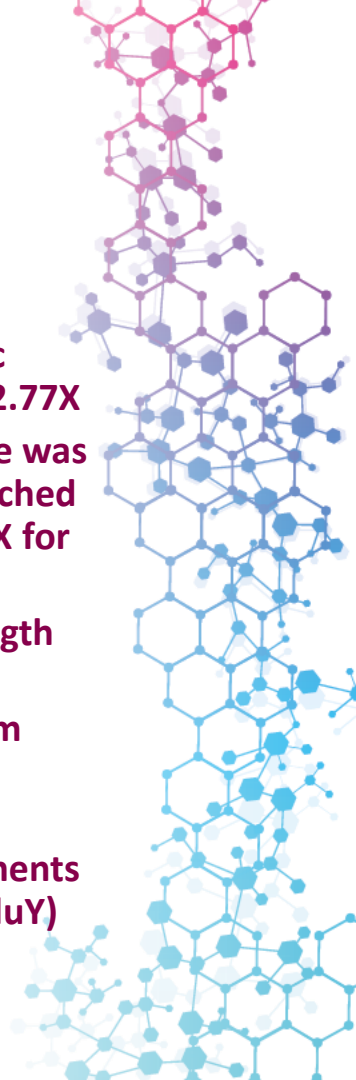


# Duplication intragénique en tandem de *BRCA1*

- 44-year-old woman with a triple negative (TN) carcinoma of the breast
- Agilent custom SureSelect QXT identify a germline duplication of *BRCA1* exons 18 to 20 (confirmed by MLPA, MRC Holland probe mix P002-D1)

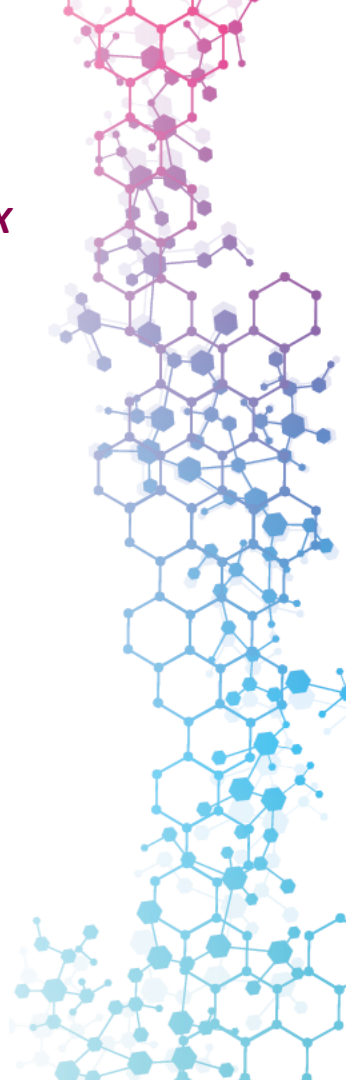
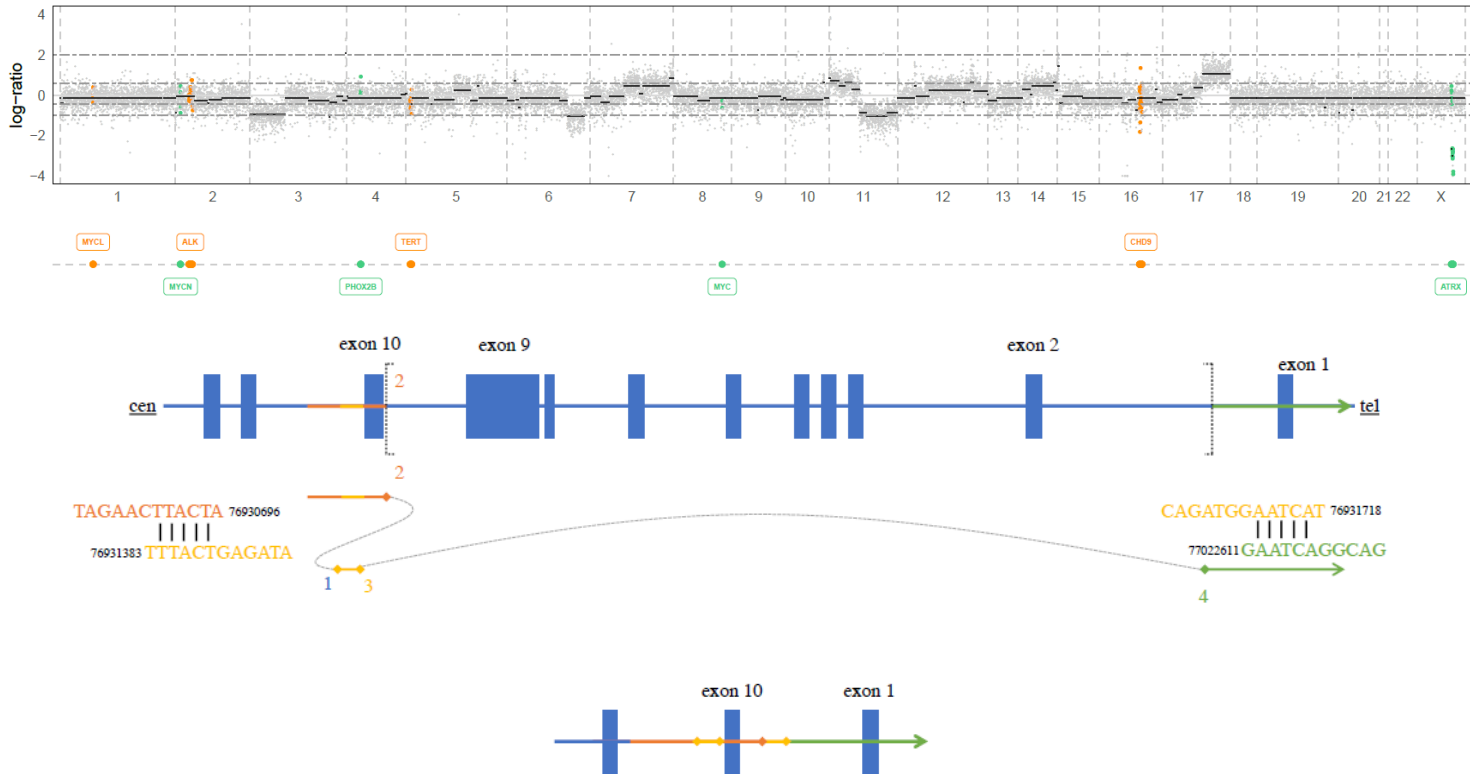


- Mean genomic coverage was 2.77X
- Mean coverage was 22.55X on enriched regions (24.63X for *BRCA1*)
- Mean read length was 3.7 Kb
- 8.67 Kb tandem duplication
- Localize in Alu repetitive elements in intron 17 (AluY) and intron 20 (AluJb)



# Caractérisation de variants structuraux

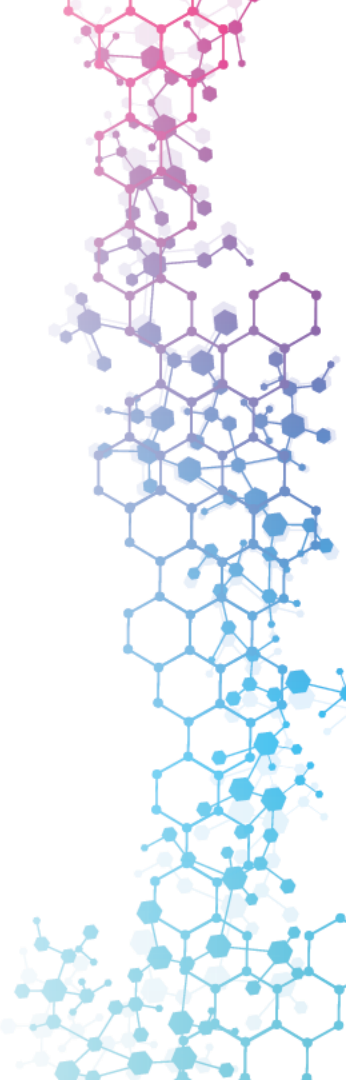
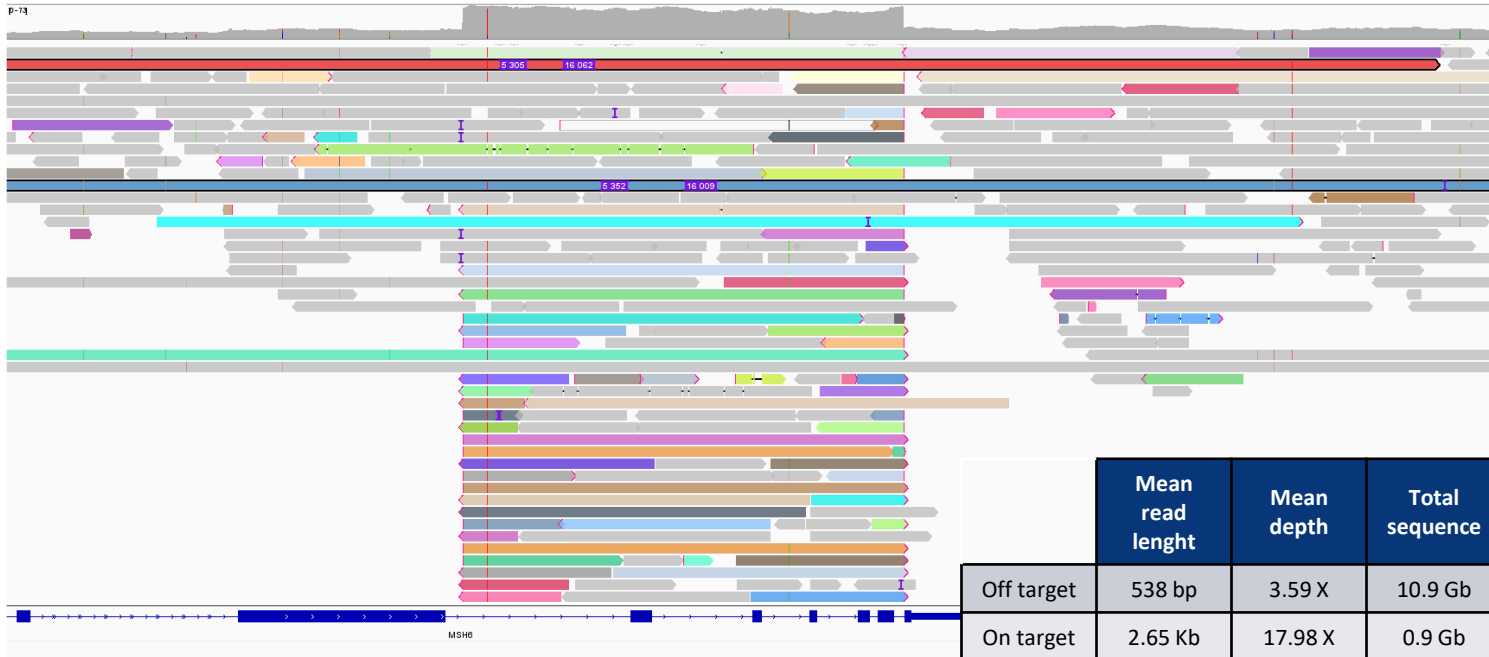
- 2-year-old neuroblastoma
- Custom short read sequencing panel identify a homozygous deletion of exon 2-9 of *ATRX*





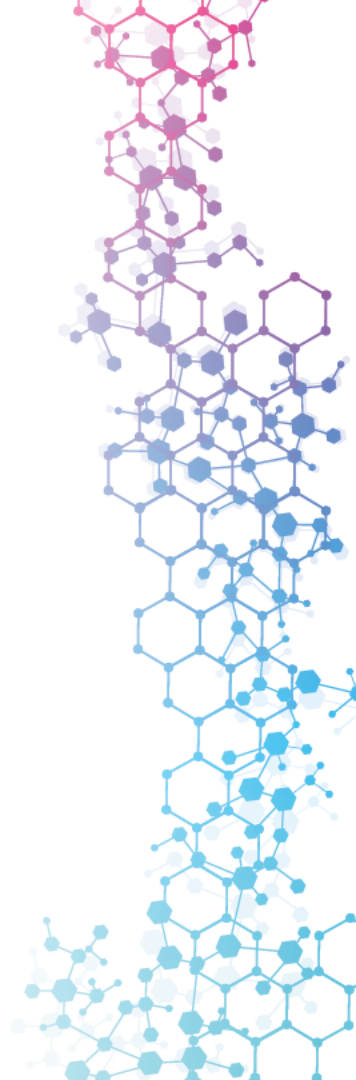
# Pentaplication intragénique de *MSH6*

- Lynch syndrome
- gain of 5 copies of exons 2-6 of *MSH6*



# Autre utilisation du séquençage par Nanopore en routine

- **Analyse de méthylation ponctuelle**
  - *BRCA1, RAD51C, MLH1*
  - *MGMT*
  - etc...
- **Phasing**
  - Maladie génétique à transmission autosomique récessive
  - Analyse tumoral de gènes suppresseurs de tumeurs
- **Intégration de génome viral chez l'homme**
  - HPV
- **Analyse d'ARN et impact sur l'épissage**
- **Longueurs des télomères**
- **Etc ...**



# Conclusion

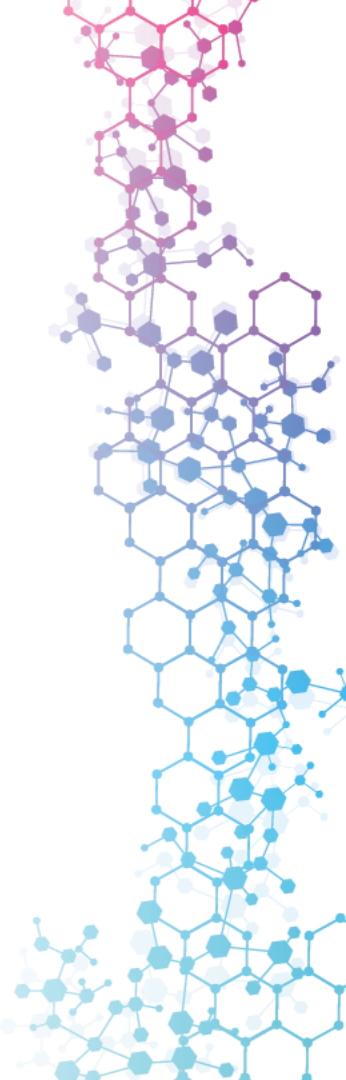
## ■ Significant limitations

- Low read accuracy
- Not diagnostic-user friendly so far

## ■ Strong advantages

- Ease of use
- Extremely fast result rendering
- Flexibility (adaptive sampling)
- Integrated analysis
- Bring information not available with other molecular technologies

In the future, Nanopore Sequencing could be implemented in medical routine diagnosis laboratories



## Oncogenetic department

- ✓ Mathilde Filser
- ✓ Abderaouf Hamza
- ✓ Christine Bourneix
- ✓ Justine Pasanisi
- ✓ Samantha Antonio
- ✓ Jennifer Wong



## Support

- ✓ SFCE
- ✓ Nvidia



## Bioinformatique clinique

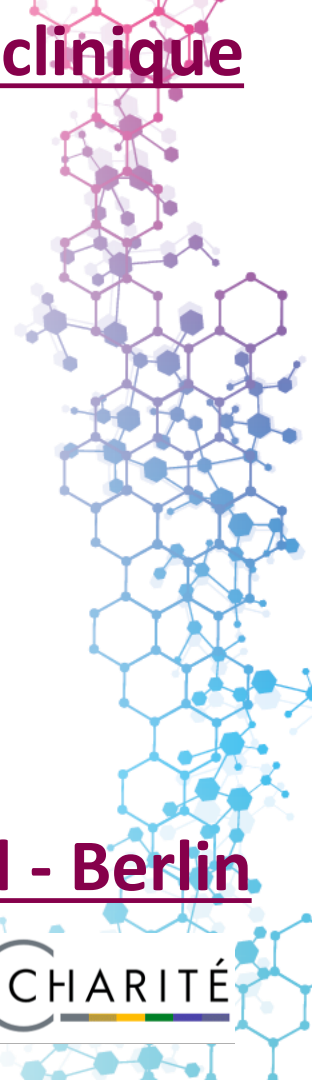
- ✓ Victor Renault
- ✓ Kevin Merchadou
- ✓ Eléonore Frouin
- ✓ Camille Benoist

## InsERM U900

- ✓ Elodie Girard
- ✓ Nicolas Servant
- ✓ Lolita Lecompte
- ✓ Natalia Rubanova

## Charité hospital - Berlin

- ✓ Luis Kuschel
- ✓ Philipp Euskirchen



MERCI DE VOTRE ATTENTION



8<sup>e</sup> ÉDITION

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