Présentation d'article: Rapide and reproducible karyotyping with Nanopore sequencing in AML patients.

Journée du Groupe Francophone de Cytogénétique Hématologique (GFCH)

Mercredi 1-02-2023

Pharm.D, spécialiste en Biologie Clinique
AHU en Génétique Médicale, Laboratoire de Cytogénétique/IPT, FPHM
M.Sc. en Cytogénétique , Faculté de Médecine de l'UdeM
M.Sc. économie de Santé (Université Paris Descartes)

blood®

Volume 140, Supplement 1, 15 November 2022, Pages 6070-6072

Poster Abstracts

613.Acute Myeloid Leukemias: Clinical and Epidemiological

Rapid and Reproducible Karyotyping with Nanopore Sequencing in AML Patients

Michael Heuser MD [1], Nouraldin Damrah MD [2*], Razif Gabdoulline PhD [2*], Courteney K. Lai PhD [3*], Eric Sträng PhD [4*], Mustafa Salim MD [5*], Gudrun Göhring MD [6*], Yvonne L. Behrens Dr. rer. nat. [5*], Ekaterina Jahn MD [7*], Martin Wichmann [2*], Jens F. Schrezenmeier [4*], Yasmine Alwie [2*], Joerg Westermann [8], Frederick Damm MD [4*], Brigitte Schlegelberger MD [9], Frank G. Rücker MD [10*], Hartmut Döhner MD [7], Arnold Ganser MD [2*], Felicitas R. Thol MD [1], Olga Blau MD [11] ...Anna Dolnik PhD [4*]

Nature Methods names ‘long-read sequencing’ as its method of 2022 and showcases Oxford Nanopore’s any read length capabilities, from short to ultra-lor

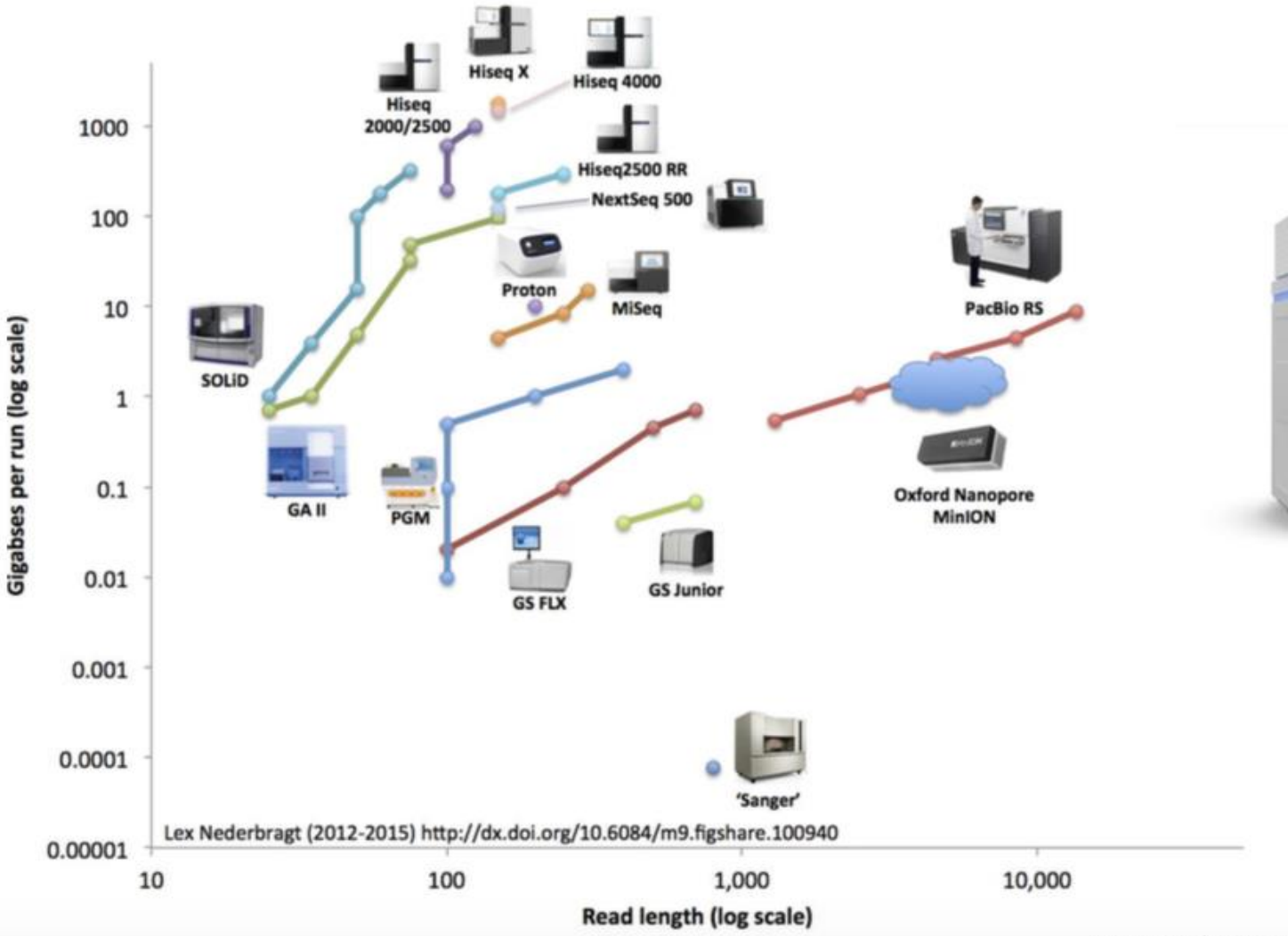
Thu 12th January 2023

Nature Methods has announced its method of the year 2022 as long-read sequencing and said it “powers a more complete reading of genomic information”. Using nanopore technology, it’s possible to sequence short to ultra-long fragments of DNA as the technology is designed to enable a user to sequence whatever DNA fragment length they pr the pore.

- The *Nature Methods* paper can be accessed in full here
- *Nature Methods*’ technology feature can be accessed in full here
- A summary of the paper can be found on our twitter here
- *Nature Methods* table of contents is here

In March 2022 a group of researchers, who make up the Telomere-to-Telomere (T2T) Consortium published the first truly complete 3.055 billion base pair sequence of a human genome and this was largely made possible by long-read sequencing.

Séquençage Troisième Génération (long read):



Novaseq
6Tb/run

Levy SE et al. *Annu Rev Genomics Hum Genet*. (2016)

Flongle

126 channels | TMO 2.8 Gb

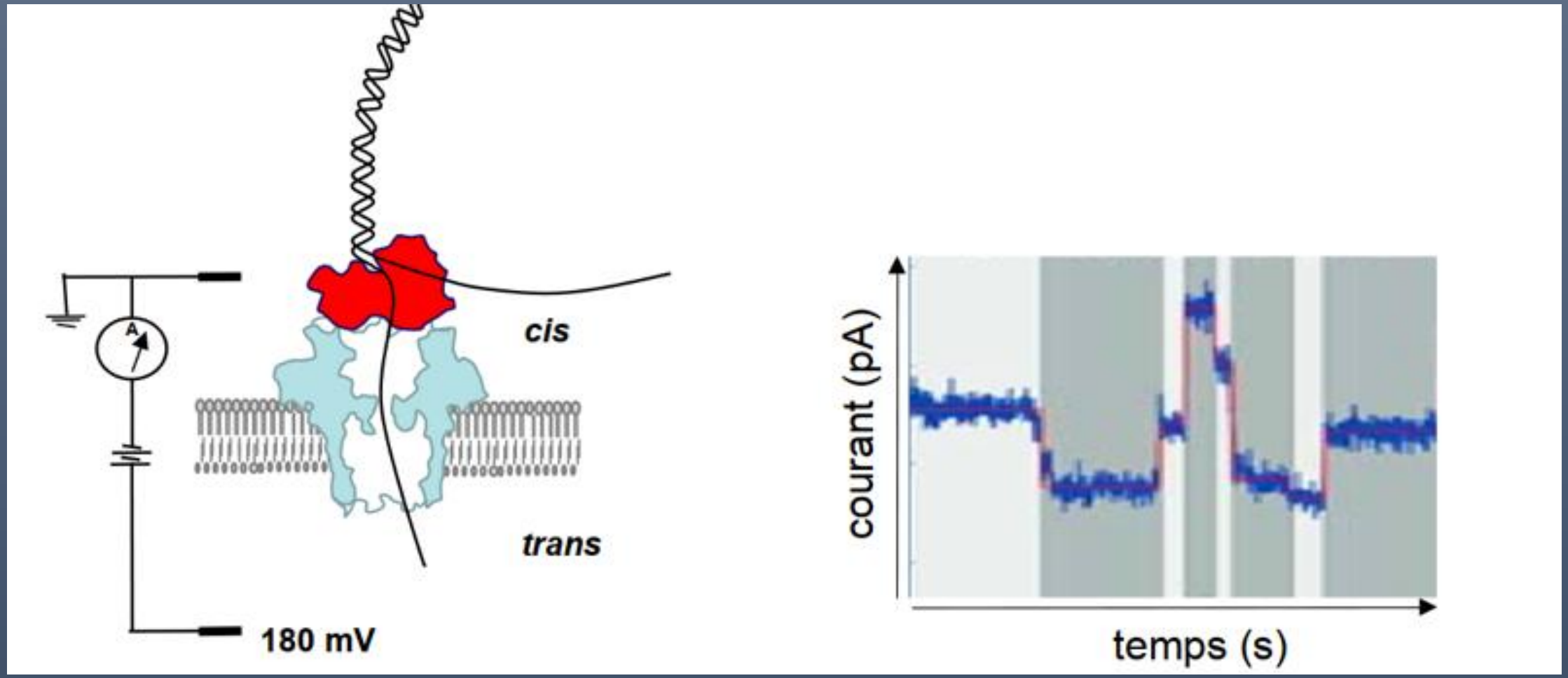
Suitable for:

- Library QC
- Plasmid, viral and bacterial sequencing
- Low-cost, disposable flow cell from $90

PromethION 24

	MinIon	GridIon	PromethIon
Nombre de pores	512	2 560	144 000
Données	10-30 Gb / 48h	50-100 Gb / 48h	7,6 – 15 Tb

From the single-use Flongle to the high-throughput PromethION, SFM is available at the scale required for users to answer their most crucial biological questions.



-Une protéine motrice (rouge) est fixée.
-Dans une cellule de flux d'Oxford Nanopore Technologies (ONT), deux compartiments (*cis* et *trans*) contenant des solutions ioniques sont séparés par une membrane qui contient des pores ménagés par des protéines.
-Un acide nucléique (noir) traverse le pore (en bleu) de façon contrôlée grâce à la présence de la protéine motrice (rouge).
-Avant de traverser le pore, l'acide nucléique est dénaturé par la protéine motrice et ainsi un seul des deux brins traverse le pore.
-Quand une molécule d'ADN ou d'ARN traverse le pore, le courant électrique est modifié ; celui-ci est enregistré en temps réel et graphiquement représenté par un *squiggle plot* (à droite).

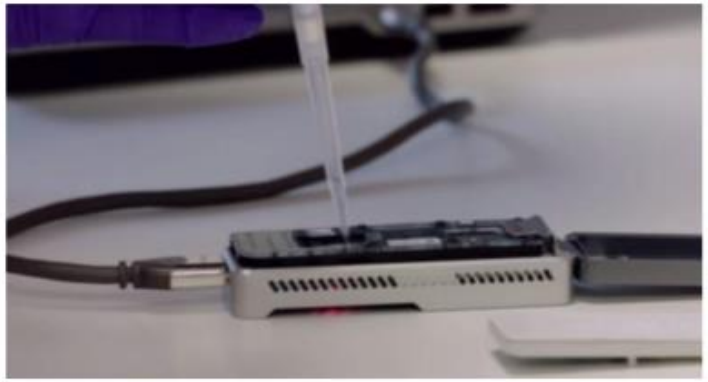
Short-read sequencing

- Alignment bias
- Low error rate
- 72 hours run
- Large device
- cDNA sequencing

Nanopore sequencing

- Reduced alignment bias
- Manageable error rate (< 2%)

  (Wan et al. Trends Genet. 2021)
- Real-time data acquisition
- Portable device: MinION
- cDNA or dRNA



- MinIon system (2012, commercialisation early access en 2014)
- 5-10 Gb par run de 48 à 72 heures (512 nanopores biologiques disponibles)
- Longueur des lectures de plusieurs dizaines de kb (jusqu'à 100 kb) : record 880 Kb

Investigations

- Structural variation
- Gene expression
- Splice variation
- Fusion transcripts
- Single cell
- SNVs and phasing
- Identification
- Assembly
- Epigenetics
- Chromatin conformation

Techniques

- Whole genome
- Targeted
- Whole transcriptome
- Metagenomics
- Short Fragment Mode

blood®

Volume 140, Supplement 1, 15 November 2022, Pages 6070-6072

Poster Abstracts

613.Acute Myeloid Leukemias: Clinical and Epidemiological

Rapid and Reproducible Karyotyping with Nanopore Sequencing in AML Patients

Michael Heuser MD [1], Nouraldin Damrah MD [2*], Razif Gabdoulline PhD [2*], Courteney K. Lai PhD [3*], Eric Sträng PhD [4*], Mustafa Salim MD [5*], Gudrun Göhring MD [6*], Yvonne L. Behrens Dr. rer. nat. [5*], Ekaterina Jahn MD [7*], Martin Wichmann [2*], Jens F. Schrezenmeier [4*], Yasmine Alwie [2*], Joerg Westermann [8], Frederick Damm MD [4*], Brigitte Schlegelberger MD [9], Frank G. Rücker MD [10*], Hartmut Döhner MD [7], Arnold Ganser MD [2*], Felicitas R. Thol MD [1], Olga Blau MD [11] ...Anna Dolnik PhD [4*]

Méthodes:

-100 LAM (+myelodysplasia-related cytogenetic abnormalities)

-Médiane d’âge (65ans)

-WGS (ONT)

-GridION sequenceur/ SQK-LSK109 kits

-50 échantillons/100: analyse d’une façon prospective

-10 échantillons par deux laboratories (reproductibilité)

-Analyse par in-house bioinformatique pipeline

-Mapping: 290 bandes chromosomiques/comparaison avec les résultats de la cytogenetique conventionnelle

-Analyse par Cytogénéticiens

-Classification en c-KT, m-KT, MRC-KT (WHO 2016), MRCA-KT (ICC 2022).

* International Consensus Classification (ICC) of myeloid neoplasms and acute leukemias

Résultats

- Couverture WG 4.4 (intervalle: 2.1-6.2)
- Résolution of ≥0.1 mega-paires bases (Mpb)
- Reproductible: 110 CNVs détectés dans les deux labos avec une longueur similaire (R^2=0.999), seulement 8 CNVs (7%) different significativement en longueur
- Une perte ou un gain chez 69 patients (≥1 chromosome)/ CNVs ou translocation retrouvés chez 72 patients avec cytogénétique conventionnelle.

Figure 1. Ideogram of the ISCN karyotypes of 100 AML patients based on (A) ONTseq results and (B) results of conventional cytogenetics.

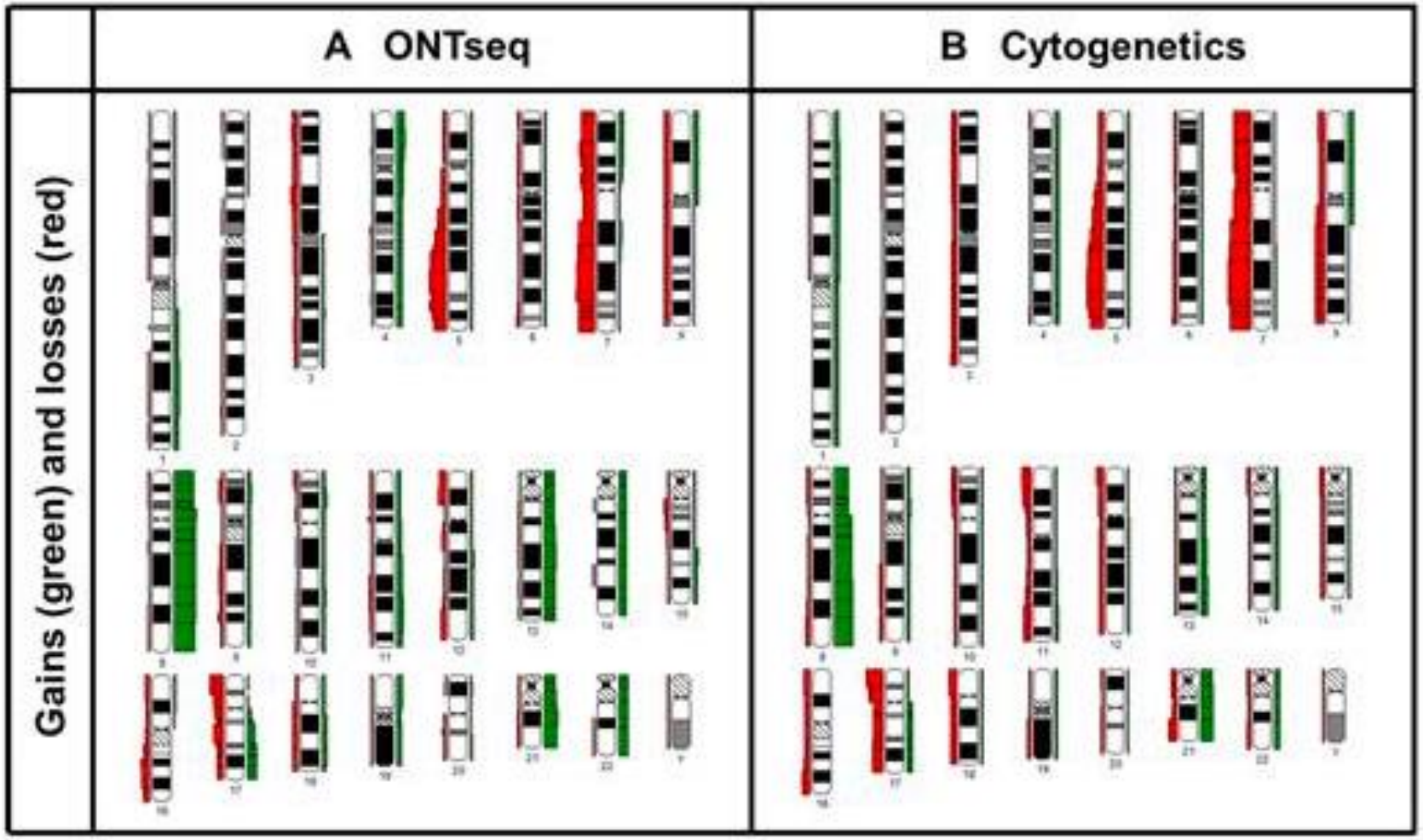


Table 1. Correlation of frequently used karyotype definitions between ONTseq and conventional cytogenetics among 100 AML patients.

ONTseq	CG	cKT N (patients)	mKT N (patients)	MRC-KT N (patients)	MRCA-KT N (patients)
Absent	Absent	49	58	40	40
Absent	Present	7	18	2	2
Present	Absent	2	5	1	1
Present	Present	42	19	57	57
P (chi-squared)		2.2E-16	9.2E-7	2.2E-16	2.2E-16

Abbreviations: ONTseq, whole genome sequencing with Oxford Nanopore Technology; CG, conventional cytogenetics; cKT, complex karyotype; mKT, monosomal karyotype; MRC-KT, karyotype defining AML with myelodysplasia-related changes according WHO 2016 classification; MRCA-KT, karyotype defining AML with myelodysplasia-related cytogenetic abnormalities according ICC 2022 classification.

La discordance la plus importante est au niveau du mKTs

➢Discordances :

- Résolution élevée de l’ONT (m-KT)

-Translocation non détectées par ONT (avec ce protocole)

➢Délai pour le rendu du résultat: 4 jours

-Préparation de la librairie: 3.45 h

-Séquencage: 24 h

-Analyse bioinformatique: 6.30 h

Third Generation Sequencing and Haematological Malignancies

ELSEVIER

Experimental and Molecular Pathology
Volume 103, Issue 1, August 2017, Pages 33-37

Mutational analysis in *BCR-ABL1* positive leukemia by deep sequencing based on nanopore MinION technology

Crescenzio F. Minervini, Cosimo Cumbo, Paola Orsini, Luisa Anelli, Antonella Zagaria, Luciana Impera, Nicoletta Coccaro, Claudia Brunetti, Angela Minervini, Paola Casieri, Giuseppina Tota, Antonella Russo Rossi, Giorgina Specchia, Francesco Albano

Minervini et al. Diagnostic Pathology (2016) 11:96
DOI 10.1186/s13000-016-0550-y

The Journal of Molecular Diagnostics, Vol. 21, No. 1, January 2019

ELSEVIER

the Journal of Molecular Diagnostics

A Nanopore Sequencing–Based Assay for Rapid Detection of Gene Fusions

William R. Jeck, Jesse Lee, Hayley Robinson, Long P. Le, A. John Iafrate, and Valentina Nardi

Diagnostic Pathology

METHODOLOGY Open Access

TP53 gene mutation analysis in chronic lymphocytic leukemia by nanopore MinION sequencing

Crescenzio Francesco Minervini, Cosimo Cumbo, Paola Orsini, Claudia Brunetti, Luisa Anelli, Antonella Zagaria, Angela Minervini, Paola Casieri, Nicoletta Coccaro, Giuseppina Tota, Luciana Impera, Annamaria Giordano, Giorgina Specchia and Francesco Albano*

Real-time molecular classification of leukemias

Mélanie Sagniez[1,2*], Shawn M. Simpson[1*], Maxime Caron[1], Marieke Rozendaal[1], Bastien Paré[1,2], Thomas Sontag[1], Sylvie Langlois[1], Alexandre Rouette[1], Vincent-Philippe Lavallée[1,3], Sonia Cellot[1,3], Daniel Sinnett[1,3], Thai Hoa Tran[1,3] and Martin A. Smith[1,2#]

[1] CHU Sainte-Justine Research Center, 3175 Chem. de la Côte-Sainte-Catherine, Montreal, H3S 2G4, Quebec, Canada.
[2] Department of Biochemistry and Molecular Medicine, Université de Montréal, 900 Edouard Montpetit Blvd, Montreal, H3T 1J4, Quebec, Canada.
[3] Department of Pediatrics, Université de Montréal, 900 Edouard Montpetit Blvd, Montreal, H3T 1J4, Quebec, Canada.

[*] Contributed equally
[#] Corresponding author: martinalexandersmith@gmail.com

Nanopore Targeted Sequencing for Rapid Gene Mutations Detection in Acute Myeloid Leukemia

Cosimo Cumbo [1,†], Crescenzio Francesco Minervini [1,†], Paola Orsini [1,†], Luisa Anelli [1], Antonella Zagaria [1], Angela Minervini [1], Nicoletta Coccaro [1], Luciana Impera [1], Giuseppina Tota [1], Elisa Parciante [1], Maria Rosa Conserva [1], Orietta Spinelli [2], Alessandro Rambaldi [2], Giorgina Specchia [1] and Francesco Albano [1,*]

1 Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, University of Bari, P.zza G. Cesare, 11 70124 Bari, Italy; cosimo.cumbo@gmail.com (C.C.); eziominervini@gmail.com (C.F.M.); paolaorsini86@gmail.com (P.O.); luisa.anelli@uniba.it (L.A.); antonellazagaria@hotmail.com (A.Z.); minervini.angela@gmail.com (A.M.); nicoletta.coccaro@uniba.it (N.C.); luciana.impera@uniba.it (L.I.); giuseppina.tota@uniba.it (G.T.); elisaparciante@libero.it (E.P.); mariarosaconserva@gmail.com (M.R.C.); specchiagiorgina@gmail.com (G.S.)

2 Hematology and Bone Marrow Transplant Unit, University of Milan, 24127 Bergamo, Italy; ospinelli@asst-pg23.it (O.S.); alessandro.rambaldi@unimi.it (A.R.)

* Correspondence: francesco.albano@uniba.it; Tel.: +39-(0)80-5478031; Fax: +39-(0)80-5508369

† These authors have contributed equally to this work.

Received: 12 November 2019; Accepted: 5 December 2019; Published: 9 December 2019

Abstract: Acute myeloid leukemia (AML) clinical settings cannot do without molecular testing to confirm or rule out predictive biomarkers for prognostic stratification, in order to initiate or withhold targeted therapy. Next generation sequencing offers the advantage of the simultaneous investigation of numerous genes, but these methods remain expensive and time consuming. In this context, we present a nanopore-based assay for rapid (24 h) sequencing of six genes (*NPM1*, *FLT3*, *CEBPA*, *TP53*, *IDH1* and *IDH2*) that are recurrently mutated in AML. The study included 22 AML patients at diagnosis; all data were compared with the results of S5 sequencing, and discordant variants were validated by Sanger sequencing. Nanopore approach showed substantial advantages in terms of speed and low cost. Furthermore, the ability to generate long reads allows a more accurate detection of longer *FLT3* internal tandem duplications and phasing double *CEBPA* mutations. In conclusion, we propose a cheap, rapid workflow that can potentially enable all basic molecular biology laboratories to perform detailed targeted gene sequencing analysis in AML patients, in order to define their prognosis and the appropriate treatment.

Acute Leukemia Classification Using Transcriptional Profiles From Low-Cost Nanopore mRNA Sequencing

Jeremy Wang [1], Nickhill Bhakta [2], Vanessa Ayer Miller [3], Mahler Revsine [4], Mark R Litzow [5], Elisabeth Paietta [6], Yuri Fedoriw [7], Kathryn G Roberts [8], Zhaohui Gu [9], Charles G Mullighan [8], Corbin D Jones [4], Thomas B Alexander [7] [10]

Affiliations + expand

PMID: 35442720 PMCID: PMC9200386 (available on 2023-04-20) DOI: 10.1200/PO.21.00326

Abstract

Purpose: Most cases of pediatric acute leukemia occur in low- and middle-income countries, where health centers lack the tools required for accurate diagnosis and disease classification. Recent research shows the robustness of using unbiased short-read RNA sequencing to classify genomic subtypes of acute leukemia. Compared with short-read sequencing, nanopore sequencing has low capital and consumable costs, making it suitable for use in locations with limited health infrastructure.

Materials and methods: We show the feasibility of nanopore mRNA sequencing on 134 cryopreserved acute leukemia specimens (26 acute myeloid leukemia [AML], 73 B-lineage acute lymphoblastic leukemia [B-ALL], 34 T-lineage acute lymphoblastic leukemia, and one acute undifferentiated leukemia). Using multiple library preparation approaches, we generated long-read transcripts for each sample. We developed a novel composite classification approach to predict acute leukemia lineage and major B-ALL and AML molecular subtypes directly from gene expression profiles.

MERCI