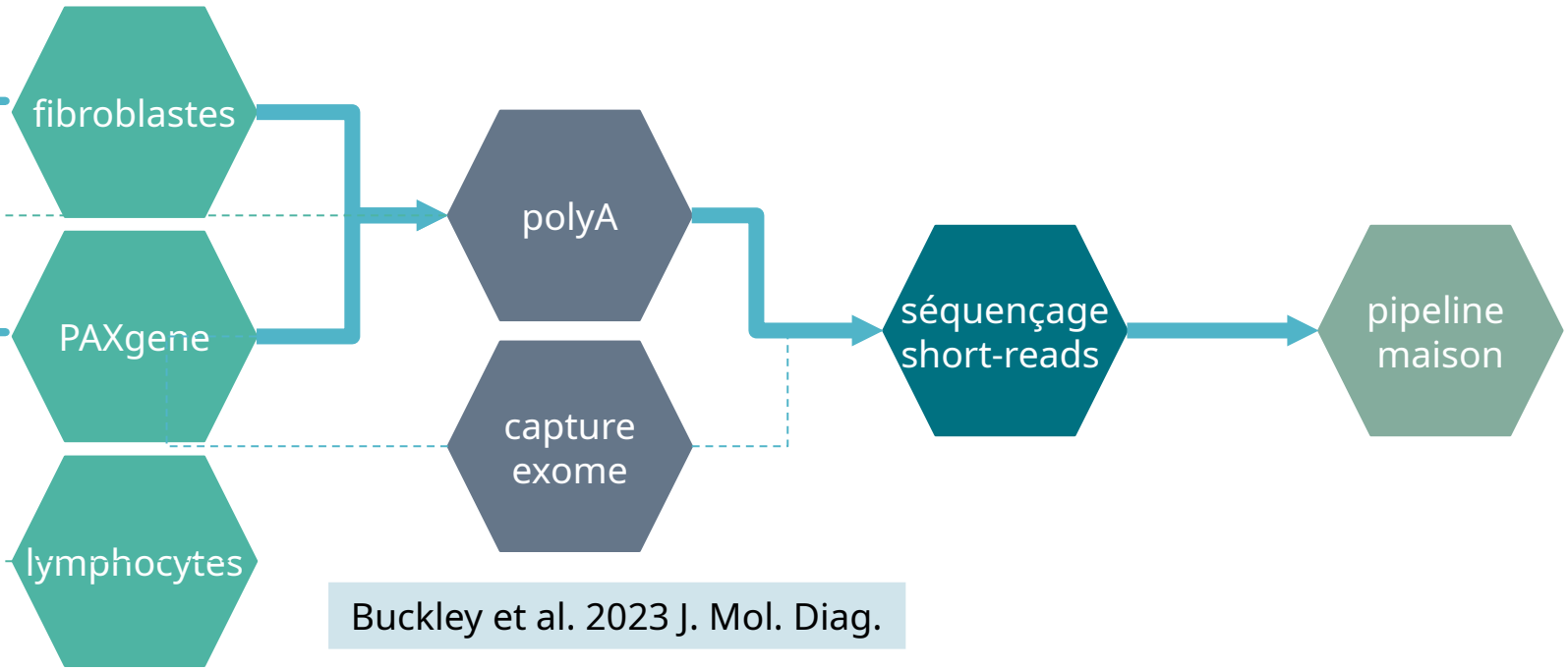
BioInfoDiag

Place du RNA-Seq dans le diagnostic génétique et l'étude des ARN non codants.

Le RNA-Seq dans le diagnostic

Etude	Pathologie	Rendement
Yepes et al. 2022 Geno. medicine	Maladies mito. (WES neg)	32/205 (16%)
Dekker et al. 2023 AJHG	Troubles du ND (WES neg)	8/67 (12%)
Frésard et al 2019 Nat Med	Hétérogène (WES neg)	6/80 (7%)
Deshwar et al. 2023 AJHG	(WGS neg)	

Hauser et al. 2020 Mol. Gen. & Met.



Buckley et al. 2023 J. Mol. Diag.

Adaptée laboratoire de diagnostic

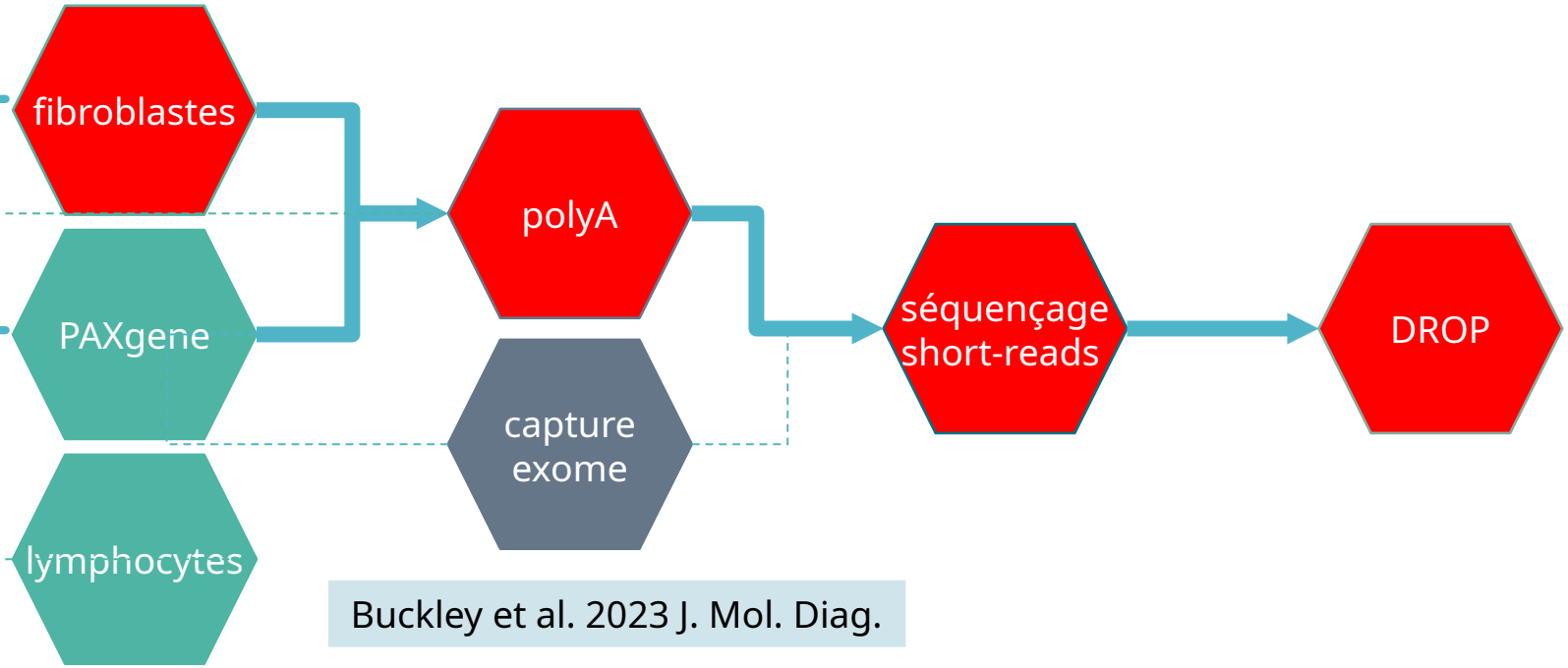
Robuste / Reproductible

Expression des gènes du ND / DI

CHU NANTES

Choix de la stratégie ?

Etude	Pathologie	Rendement
Yepes et al. 2022 Geno. medicine	Maladies mito. (WES neg)	32/205 (16%)
Dekker et al. 2023 AJHG	Troubles du ND (WES neg)	8/67 (12%)
Frésard et al 2019 Nat Med	Hétérogène (WES neg)	6/80 (7%)
Deshwar et al. 2023 AJHG		



Hauser et al. 2020 Mol. Gen. & Met.

Buckley et al. 2023 J. Mol. Diag.

Adaptée laboratoire de diagnostic

Robuste / Reproductible

Expression des gènes du ND / DI

Projet de recherche

Séquençage de 33 cas négatifs après exome en trio par WGS

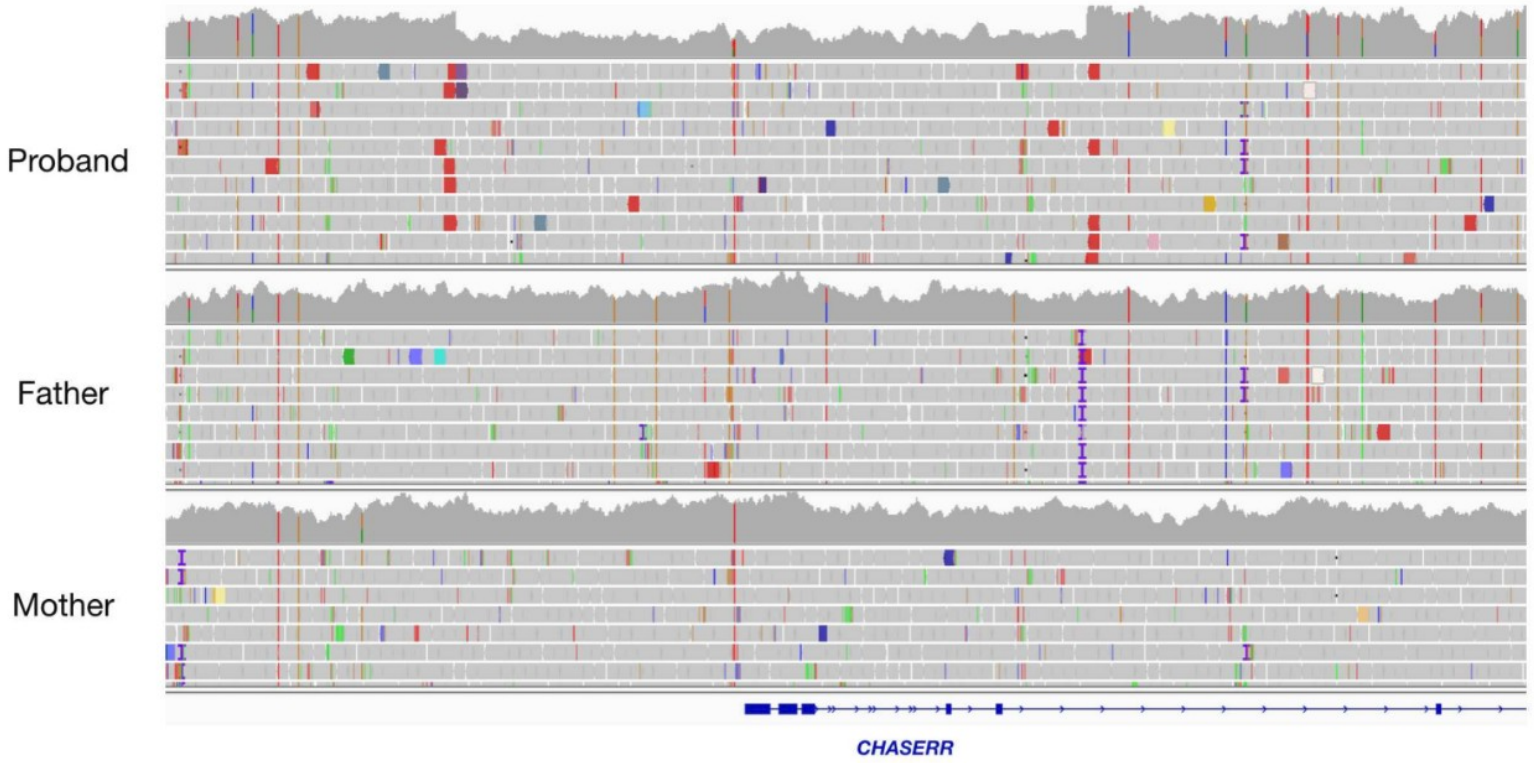
RNA-Seq sur fibroblastes de peau

- Proposé à tous les patients avec **SG non-conclusif**
- Prélèvement obtenus pour **9 patients + 1 mère**

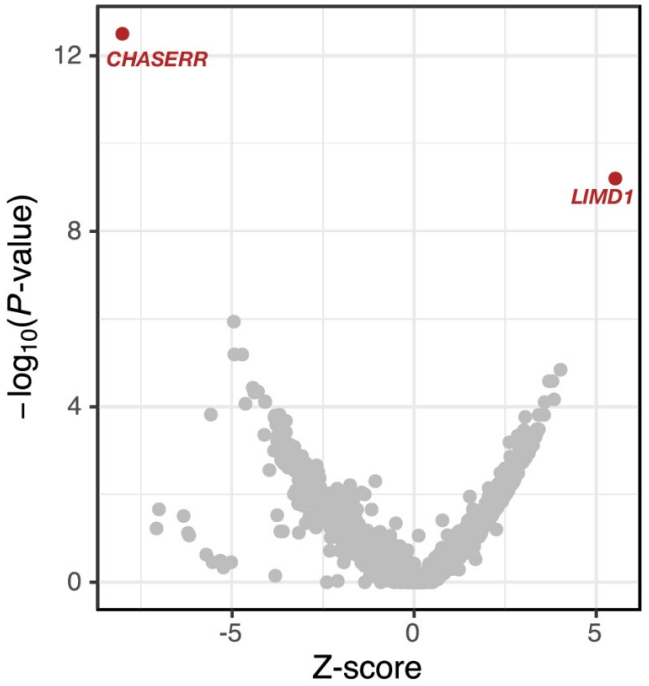
Pipeline DROP: OUTRIDER + FRASER + MAE

Délétion du lncRNA *CHASERR*

NC_000015.9:g.93422237_93430600del

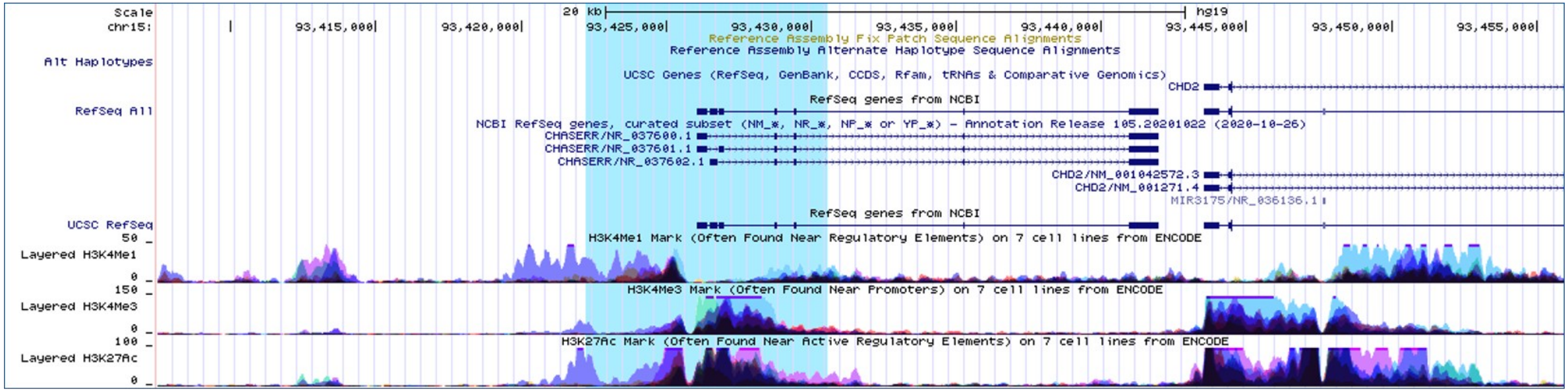


Perte d'expression confirmée en RNA-Seq



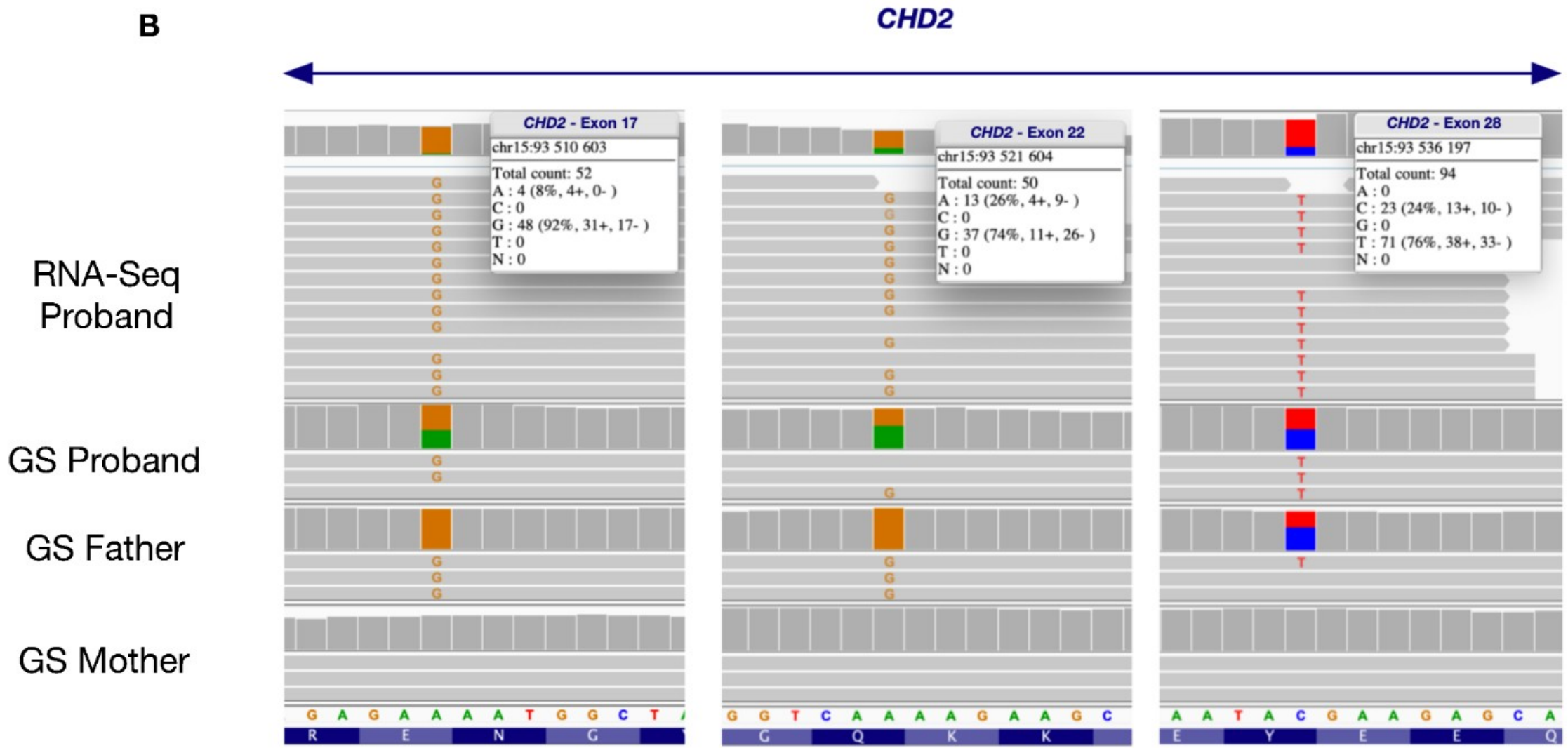
Utilisation de la matrice de compte publique fibro/poly-A

CHASERR est proche de *CHD2*



Délétion ◄ · ► ***CHD2***

Pas d’expression aberrante détectée par OUTRIDER mais <u>biais allélique</u>



lncRNA *CHASERR* : second cas

J Med Genet. 2023 Dec 21;61(1):47-56. doi: 10.1136/jmg-2023-109263.

Integrating RNA-Seq into genome sequencing workflow enhances the analysis of structural variants causing neurodevelopmental disorders

Kevin Riquin [1], Bertrand Isidor [2 3], Sandra Mercier [2 3], Mathilde Nizon [2 3], Estelle Colin [4 5], Dominique Bonneau [4 5], Laurent Pasquier [6], Sylvie Odent [6 7], Xavier Maximin Le Guillou Horn [8 9], Gwenaël Le Guyader [8], Annick Toutain [10 11], Vincent Meyer [12], Jean-François Deleuze [12], Olivier Pichon [3], Martine Doco-Fenzy [2 3], Stéphane Bézieau [2 3], Benjamin Cogné [2 3]

EMMA BROADBENT / AGE: 5

Ever since she was born, Brian Broadbent's daughter Emma has been severely delayed. Now 5, she's at the developmental age of a 5-month-old, he says. Brian and his wife, Julia, must give Emma nearly round-the-clock care to ensure her survival. She cannot feed herself, and may never walk or talk. Emma sleeps with a BiPAP machine — a portable device that pushes oxygen into a patient's airways — to help her breathe. She spent Christmas of 2019 in the hospital on a ventilator.

Shortly after their daughter's birth, the Broadbents embarked on a journey to attempt to understand what their daughter was experiencing. They spent months with a white-matter specialist analyzing Emma's brain and had her genome sequenced. They traveled to the Mayo Clinic for metabolic testing and twice to the Children's Hospital of Pennsylvania for exams. But the results from all that testing weren't very helpful. "She's at the edge of science," Brian remembers one doctor telling them.

In 2017, their search led them to the Rare Genomes Project at the Broad Institute of MIT and Harvard, and the UDN shortly afterwards. Both organizations began sequencing Emma's entire genome, as well as her RNA. And, as it turns out, both groups soon found the same thing: a mutation to the CHD2 gene. Irregularities in this gene are often associated with epilepsy, but Emma's symptoms were far worse.

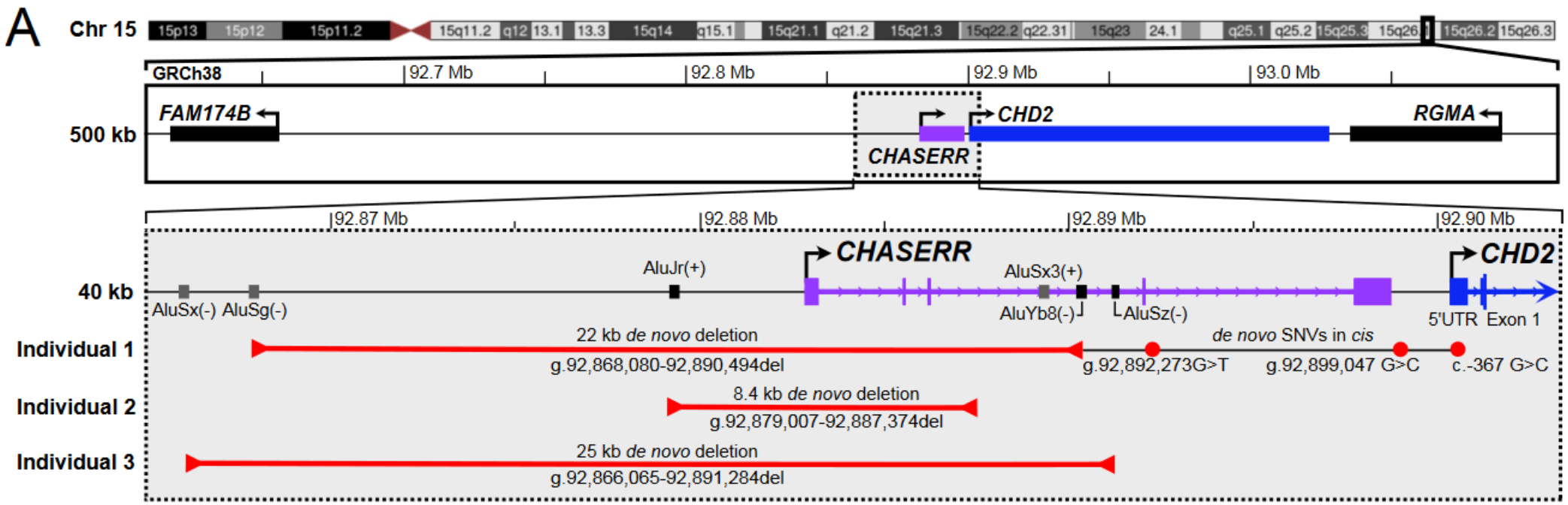
Uncovering the true root of Emma's symptoms took further digging, and a timely coincidence. It turns out Emma has another mutation on a gene near CHD2 called Chaserr. It's what's known as a long noncoding RNA, or lncRNA gene, and it affects how CHD2 is expressed. Nothing had been known about the gene until just months before, when a team of Israeli researchers published a paper on Chaserr and its role. The paper included data on mice genetically engineered to lack Chaserr, which had brain anomalies similar to Emma's.

In Emma's case, the combination of mutations appears to affect her brain's myelin, the protective sheathing that covers our nerves and brain cells, says Carlos Bacino, a clinical geneticist at the Baylor College of Medicine, a UDN site, and Emma's physician at Texas Children's Hospital. The result is what Bacino describes as a neurodegenerative disorder affecting her brain's development and function. Emma is the first patient in the world to ever be diagnosed with a condition resulting from a lncRNA mutation. There could even be a treatment for her at some point, in the form of a new kind of genetic therapy known as antisense oligonucleotides, which could alleviate some of Emma's symptoms.

Top: Emma (right) relaxes at home with her father, Brian, mother, Julia, and older sister, Claire. Bottom: Emma and Claire play dress-up.

It's bittersweet news for Brian — his daughter is truly at the forefront of modern-day medicine, and that means the chance for a cure is small. But Emma is also offering scientists potentially groundbreaking knowledge. Perhaps the next child born with a lncRNA defect will have the hope of treatment. "She's kind of like a gift to science," Brian says. "It does bring a lot of comfort."

3 cas identifiés avec une del *CHASERR*



3 cas identifiés avec une del *CHASERR*

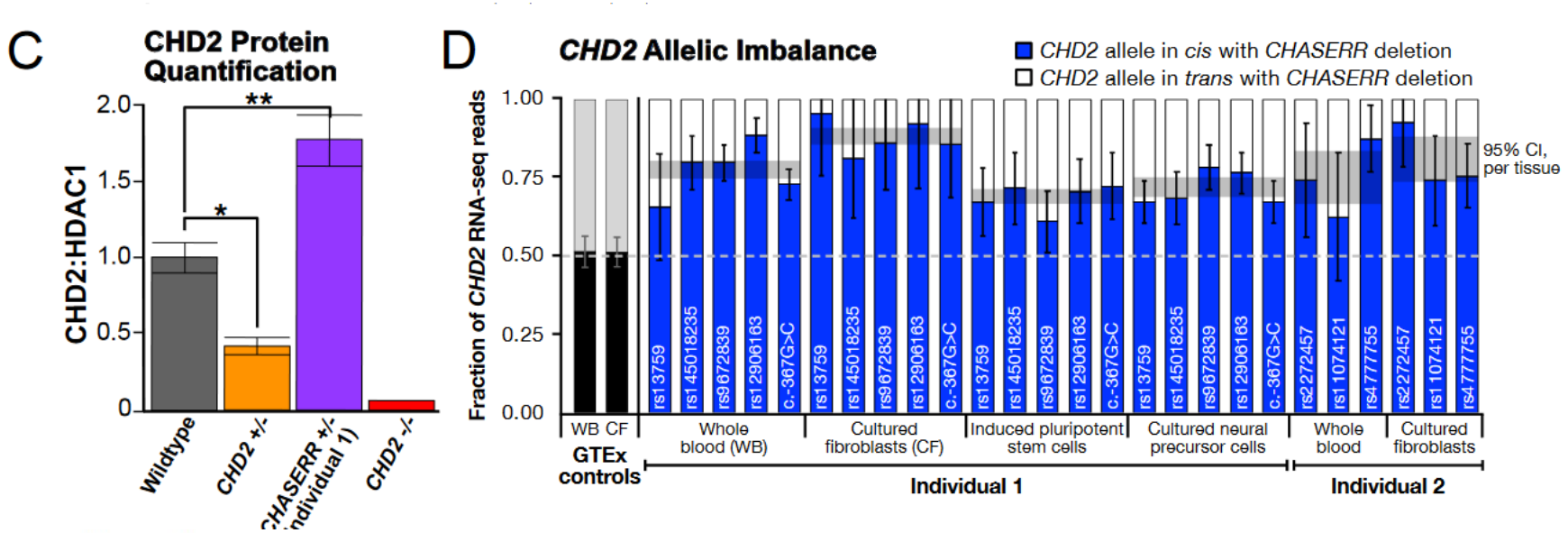
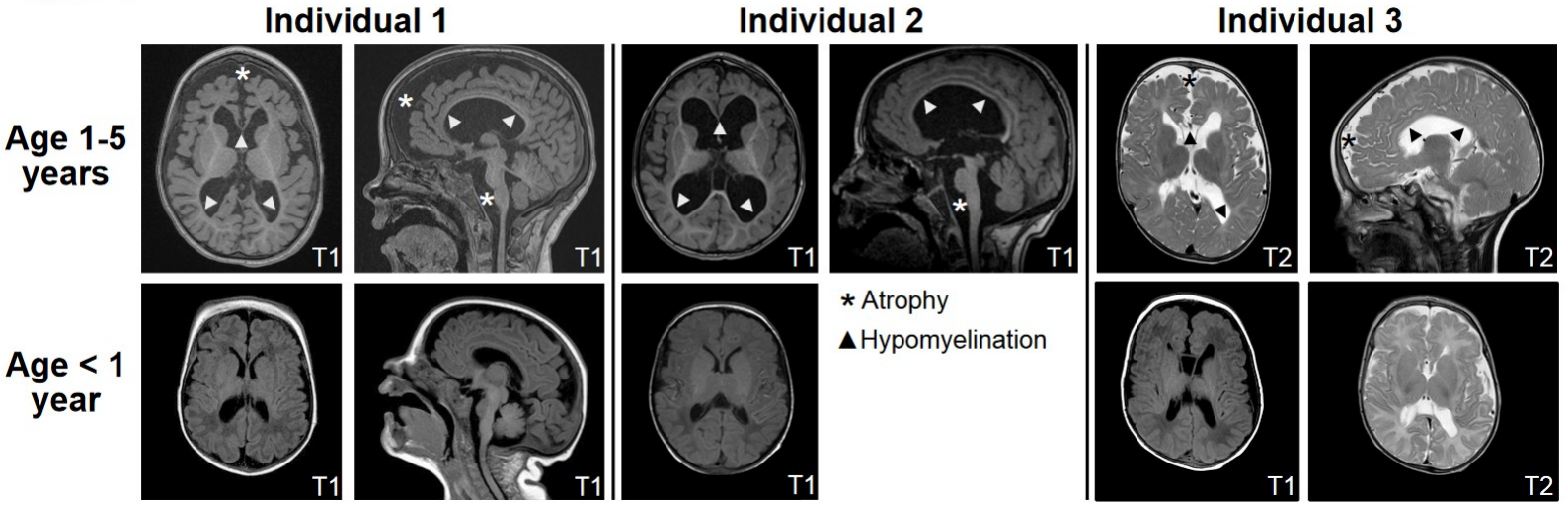
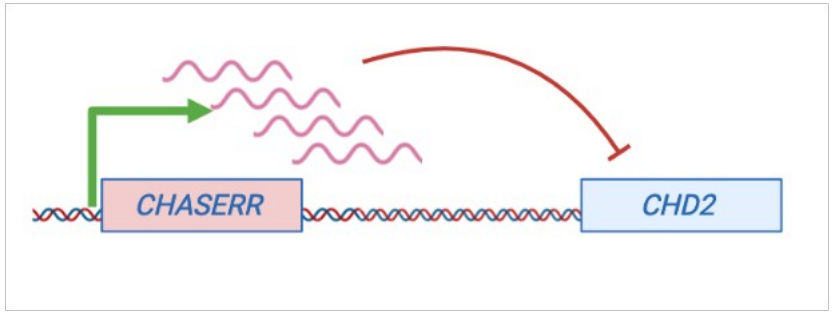
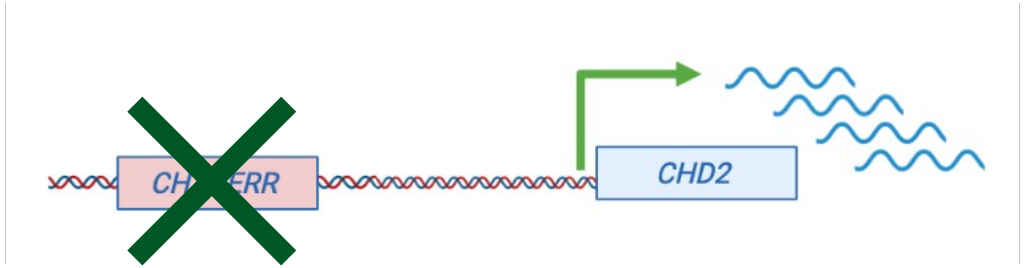


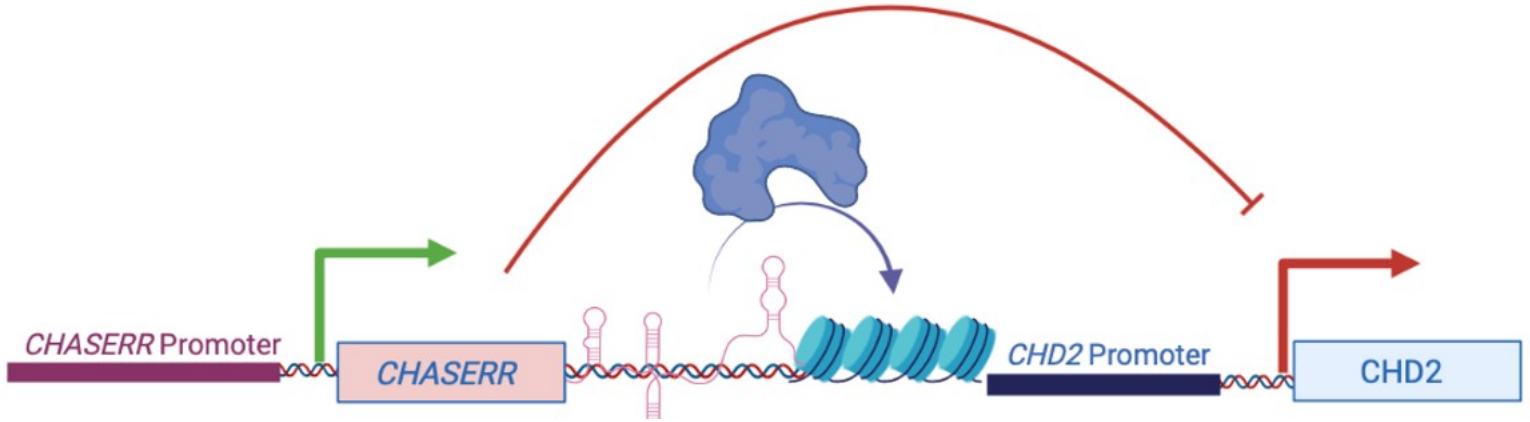
Figure 1



Un mécanisme de régulation transcriptionnelle







Un mécanisme de régulation transcriptionnelle

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE | BRIEF REPORT

Neurodevelopmental Disorder Caused by Deletion of *CHASERR*, a lncRNA Gene

Authors: Vijay S. Ganesh, M.D., Ph.D., Kevin Riquin, Ph.D., Nicolas Chatron, M.D., Ph.D., Esther Yoon, B.S., Kay-Marie Lamar, Ph.D., Miriam C. Aziz, B.S., Pauline Monin, M.D., Melanie C. O'Leary, M.Sc., Julia K. Goodrich, Ph.D., Kiran V. Garimella, D.Phil., Eleina England, M.S., Ben Weisburd, B.S., François Aguet, Ph.D., Carlos A. Bacino, M.D., David R. Murdock, M.D., Hongzheng Dai, Ph.D., Jill A. Rosenfeld, M.Sc., Lisa T. Emrick, M.D., Shamika Ketkar, Ph.D., Yael Sarusi, M.Sc., Damien Sanlaville, M.D., Ph.D., Saima Kayani, M.D., Brian Broadbent, M.B.A., Alisée Pengam, M.D., Bertrand Isidor, M.D., Ph.D., Stéphane Bezieau, Pharm.D., Ph.D., Benjamin Cogné, Pharm.D., Ph.D., Daniel G. MacArthur, Ph.D., Igor Ulitsky, Ph.D., Gemma L. Carvill, Ph.D., and Anne O'Donnell-Luria, M.D., Ph.D. -23 Author Info & Affiliations

Published October 23, 2024 | N Engl J Med 2024;391:1511-1518 | DOI: 10.1056/NEJMoa2400718

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Bilan de cette étude de recherche

- Le RNA-Seq est utile, notamment pour caractériser l'impact des SV
- Le RNA-Seq poly-A sur fibroblastes fonctionne bien y compris en utilisant une matrice externe
- On est très motivés pour un transfert de la technique vers le diagnostic…
- Mais…
- <u>Pour en faire une activité diagnostique il faut</u>:
  - Avoir une technique transférable d'un point de vue techniqu
  - Si possible automatisable
  - Pouvoir récupérer des centaines de prélèvements par

Le RNA-Seq dans le diagnostic

Etude	Pathologie	Rendement
Yepes et al. 2022 Geno. medicine	Maladies mito. (WES neg)	32/205 (16%)
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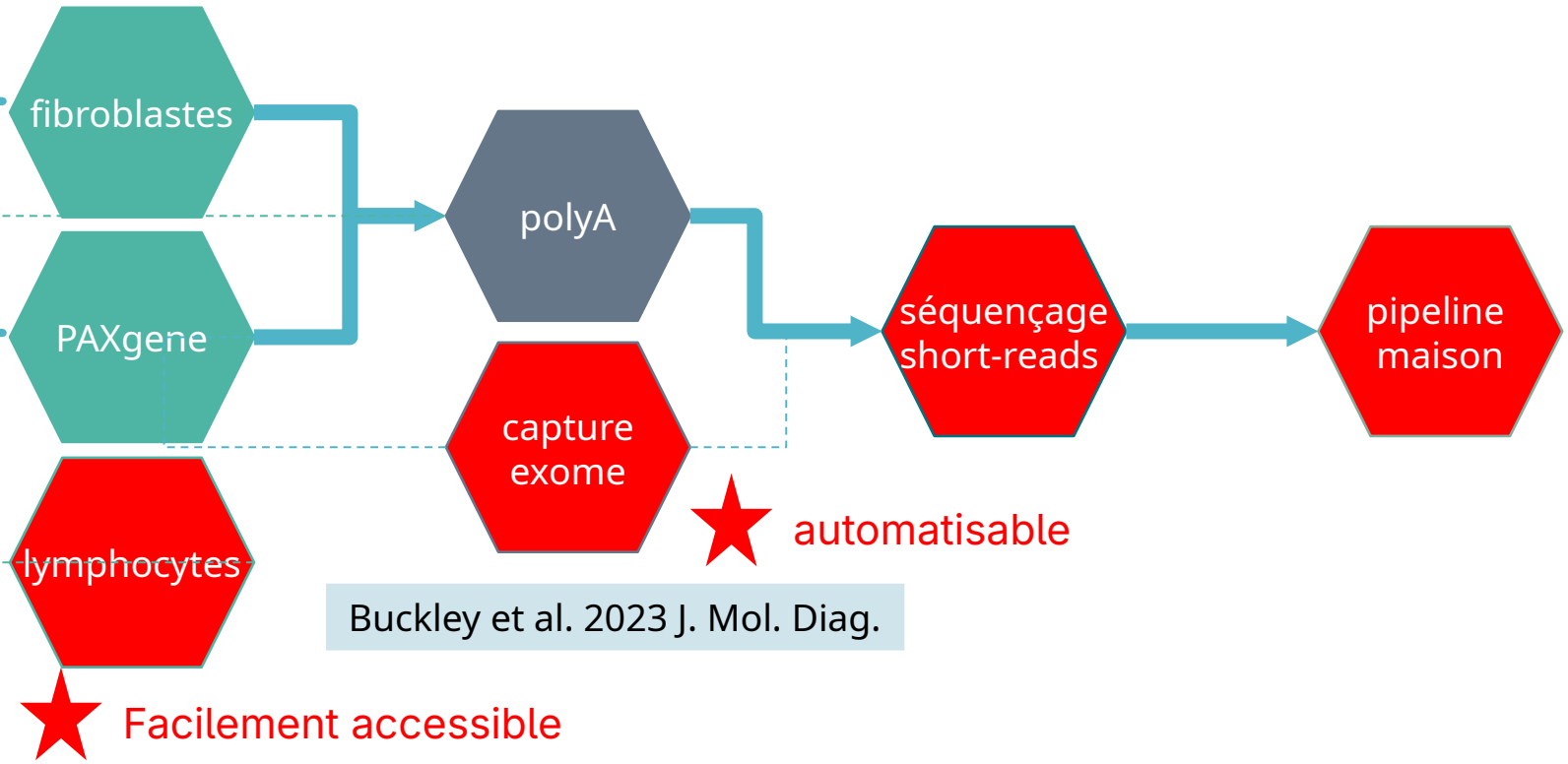
Frésard et al 2019 Nat Med	Hétérogène (WES neg)	6/80 (7%)
Hauser et al. 2020 Mol. Gen. & Met. AJHG	(WGS neg)	0/39

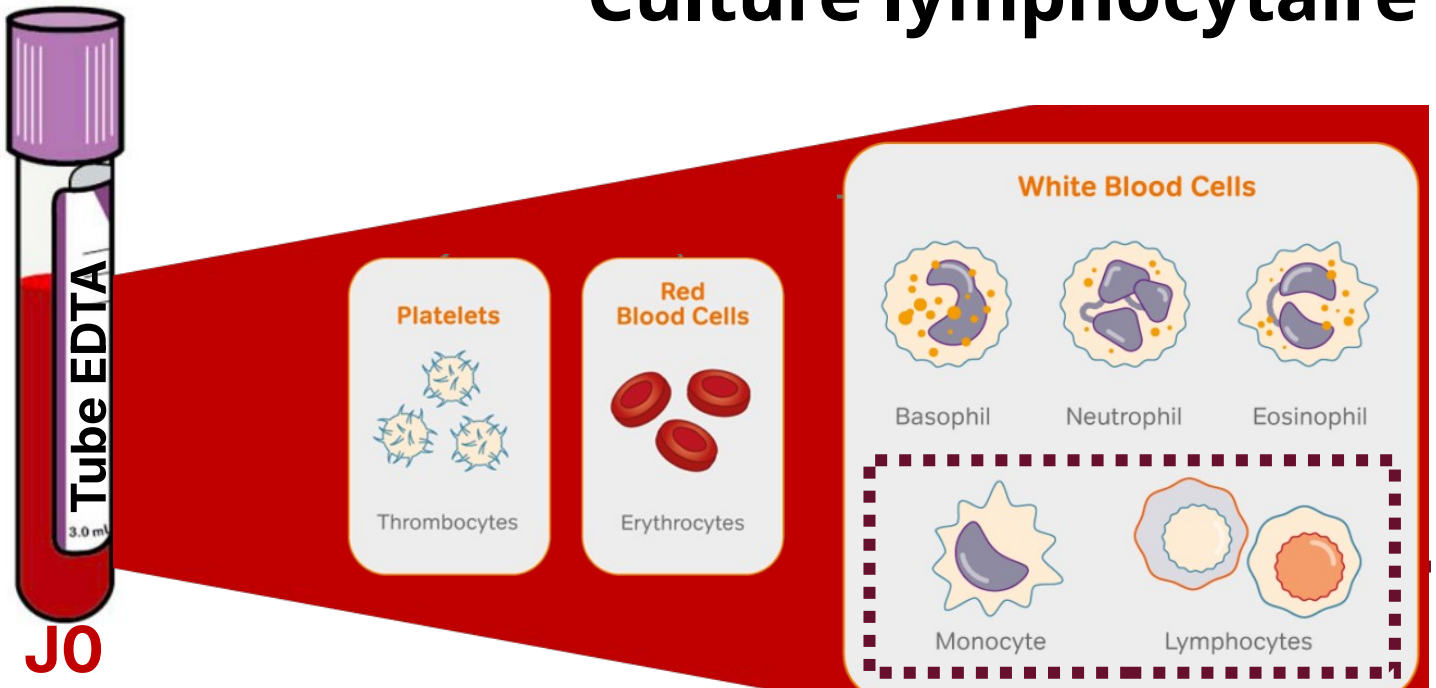


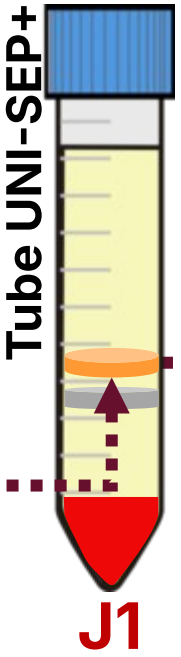
Adaptée laboratoire de diagnostic

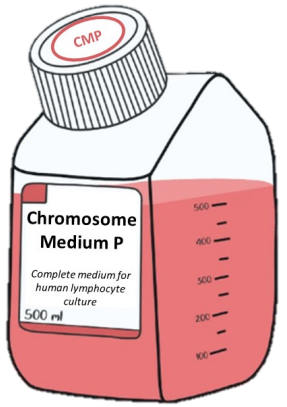
Robuste / Reproductible

Expression des gènes du ND / DI

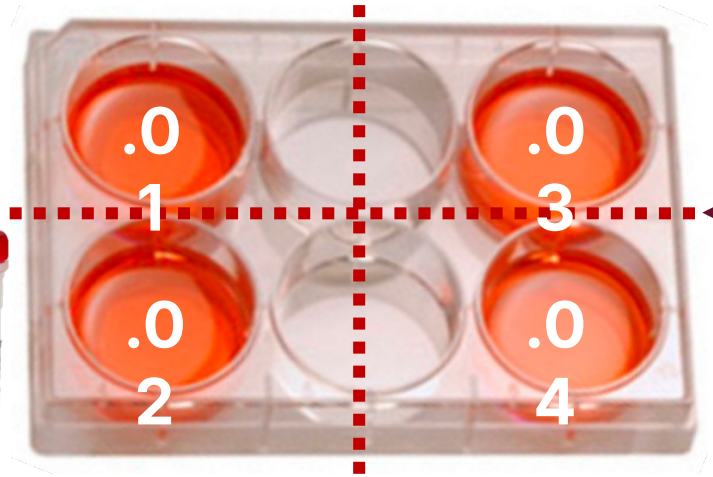
Culture lymphocytaire court terme







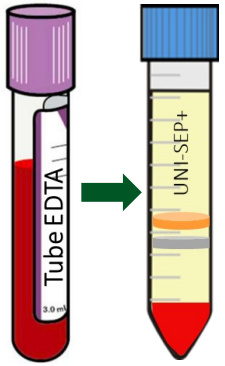
J1
Culture 48-72h (96h)

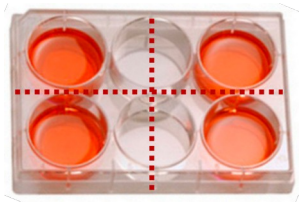


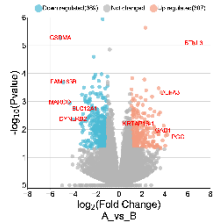
Sans puromycine

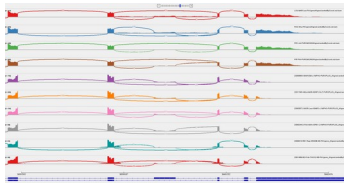
J3 Avec puromycin (NMD inhibition)

Protocole









PBMCs → culture extraction RNA → librairies Exome H. V8 sur automate → séquençage short-reads → pipeline labo*

Facilité de pvt T°c ambiante	**Inhibition NMD** +/- puromycine	Magnis Agilent SureSelect XT-HS2	Illumina NS550 High-output 2x75	QC / BAM (VCF) Outrider Fraser
0 – 48 h (72 h)	48 – 72 h	8 éch. par run 8-12h par run	16 éch. par run 18h par run	

J1 → J4 → J5 → J6 → J8

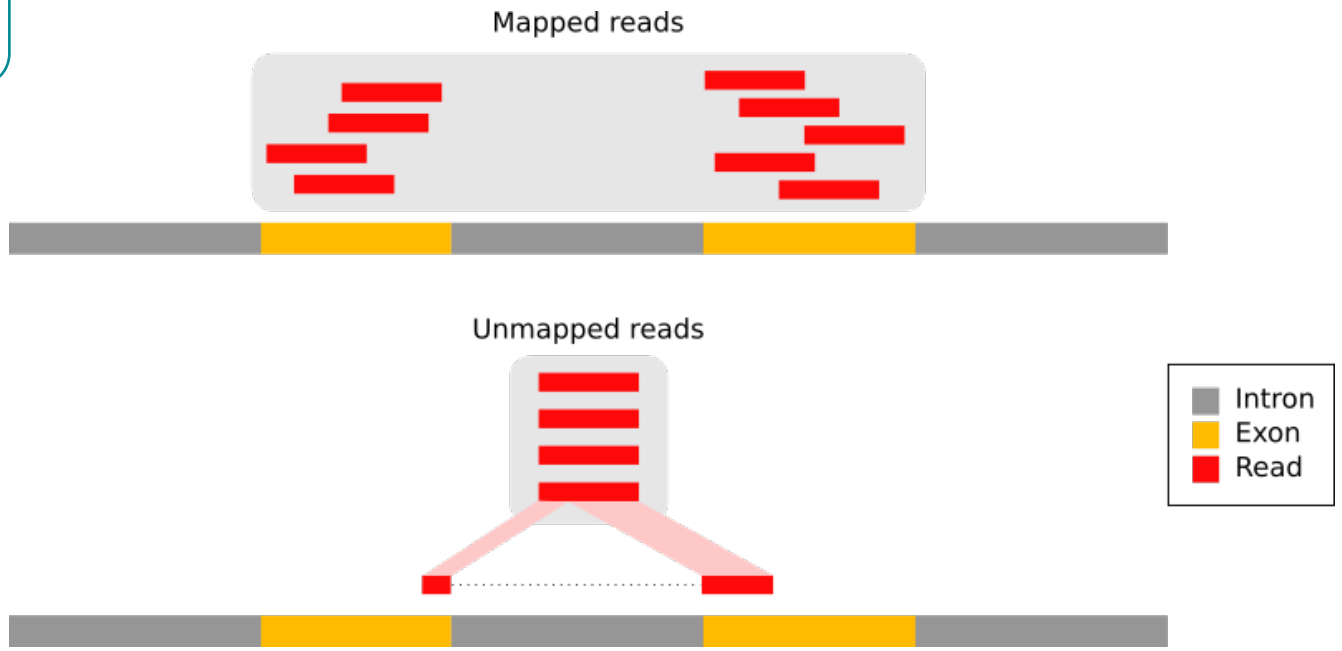
Pipeline

Qualité

- MultiQC
- fastp

Alignement

- STAR
  - → Hg38



Pipeline

Qualité

- MultiQC
- fastp

Alignement

- STAR → Hg38

Comptage

- HTSeq-count
- Kallisto

Each column is a sample

Each row is a gene

NE ID	KD.2	KD.3	OE.1	OE.2	OE.3	IR.1	IR.2	IR.3
-SBSRNA4	57	41	64	55	38	45	31	39
BG	71	40	100	81	41	77	58	40
BG-AS1	256	177	220	189	107	213	172	126
CF	0	1	1	0	0	0	0	0
A2LD1	146	81	138	125	52	91	80	50
A2M	10	9	2	5	2	9	8	4
A2ML1	3	2	6	5	2	2	1	0
A2MP1	0	0	2	1	3	0	2	1
A4GALT	56	37	107	118	65	49	52	37
A4GNT	0	0	0	0	1	0	0	0
AA06	0	0	0	0	0	0	0	0
AAA1	0	0	1	0	0	0	0	0
AAAS	2288	1363	1753	1727	835	1672	1389	1121
AACS	1586	923	951	967	484	938	771	635
AACSP1	1	1	3	0	1	1	1	3
AADAC	0	0	0	0	0	0	0	0
AADACL2	0	0	0	0	0	0	0	0
AADACL3	0	0	0	0	0	0	0	0
AADACL4	0	0	1	1	0	0	0	0
AADAT	856	539	593	576	359	567	521	416
AAGAB	4648	2550	2648	2356	1481	3265	2790	2118
AAK1	2310	1384	1869	1602	980	1675	1614	1108
AAMP	5198	3081	3179	3137	1721	4061	3304	2623
AANAT	7	7	12	12	4	6	2	7
AARS	5570	3323	4782	4580	2473	3953	3339	2666

CHU NANTES

Pipeline

Qualité

- MultiQC
- fastp

Alignement

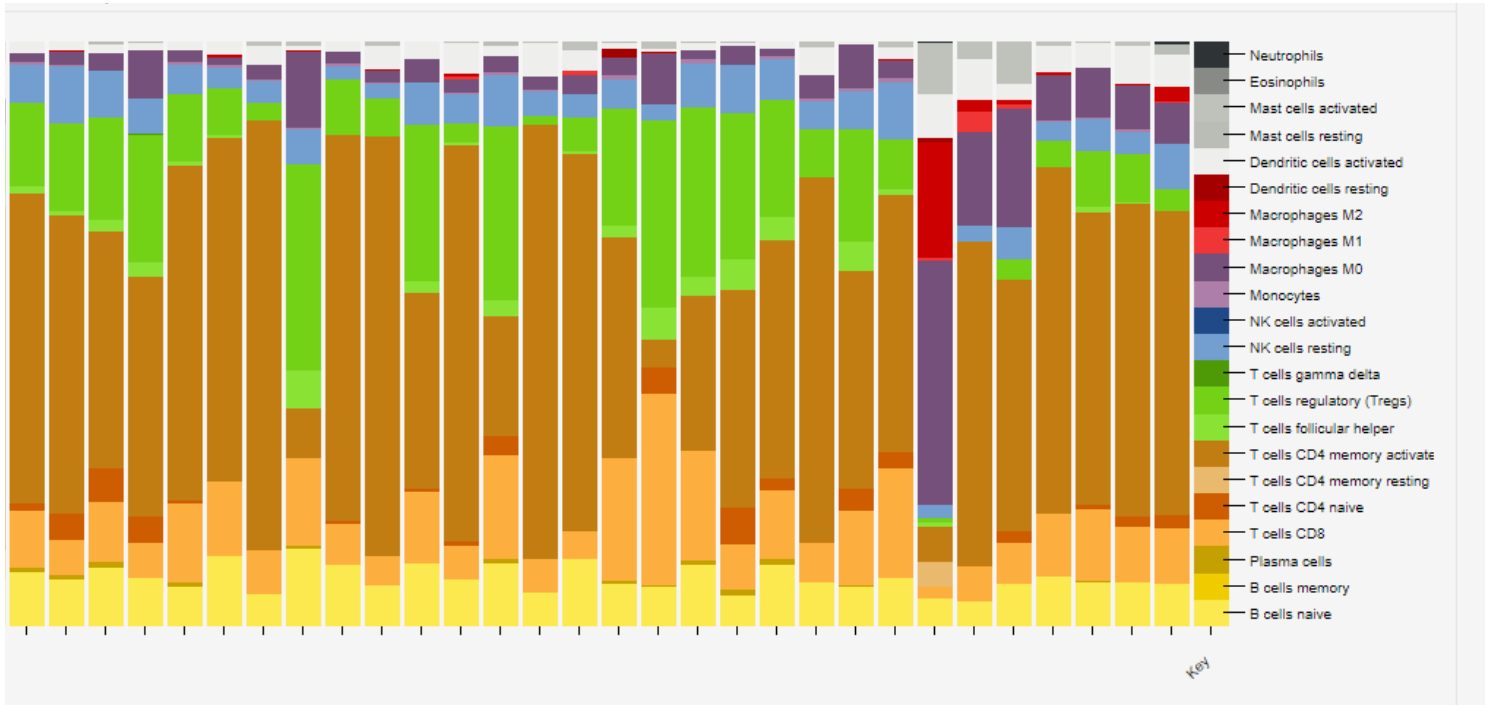
- STAR → Hg38

Comptage

- HTSeq-count
- Kallisto

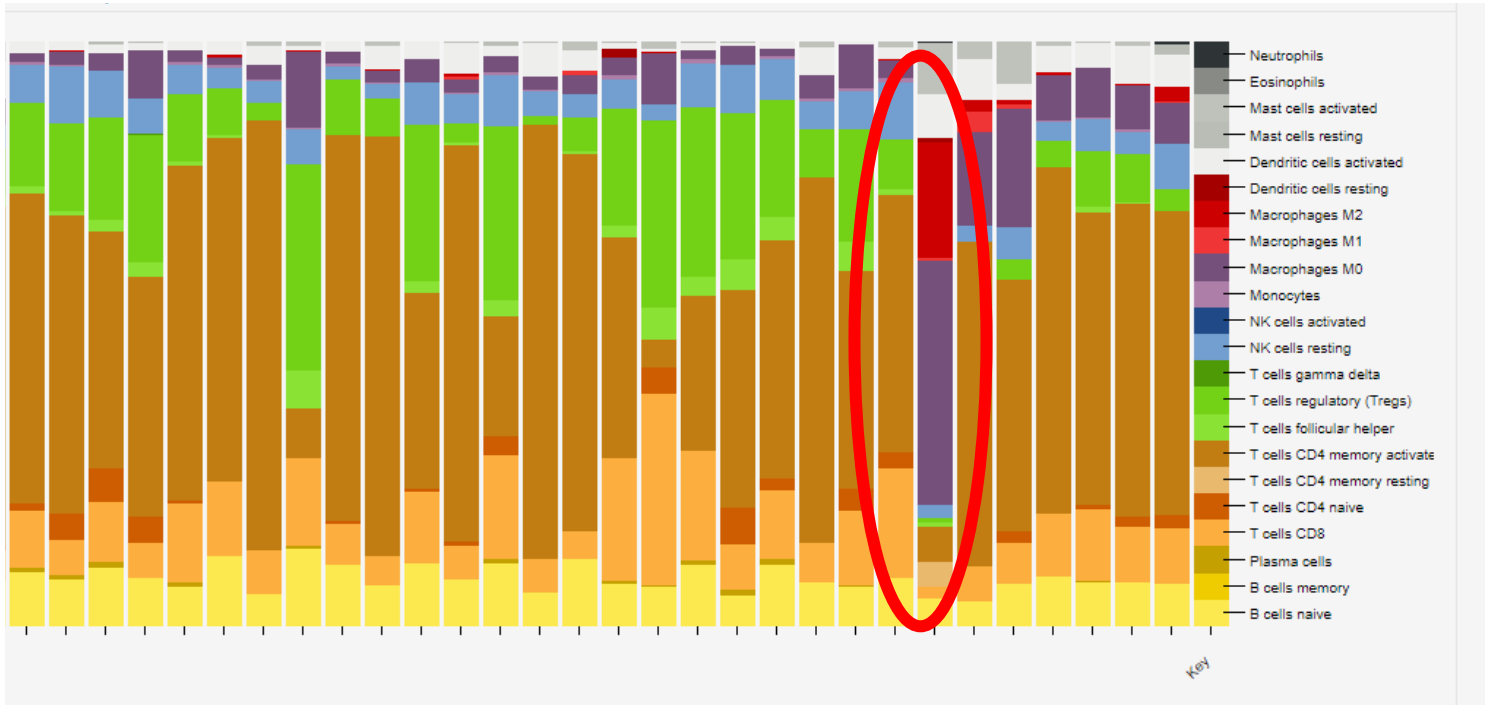
Profil type cellulaire

- Cibersortx



Pipeline

Qualité	Alignement	Comptage	**Profil type cellulaire**
MultiQC fastp	STAR → Hg38	HTSeq-count Kallisto	Cibersortx

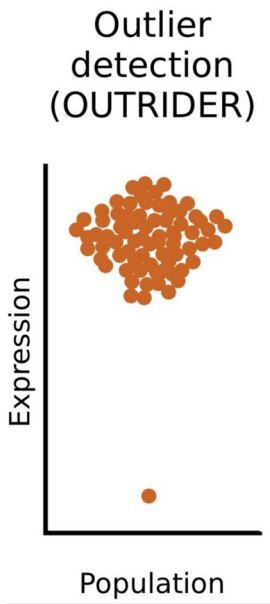


CHU NANTES

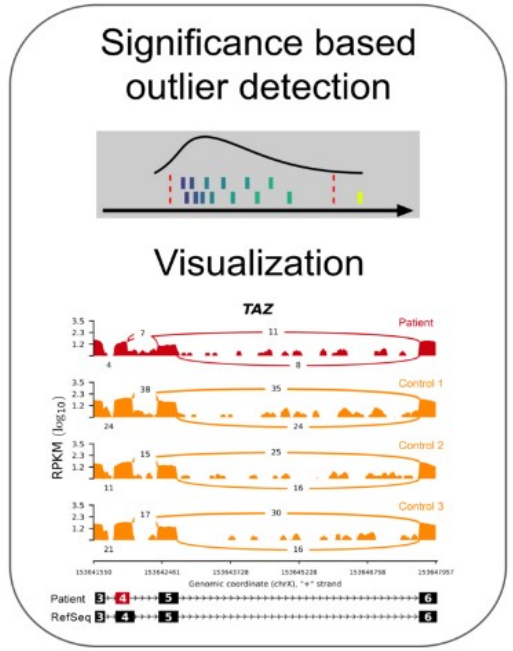
Pipeline

Qualité	Alignement	Comptage	Profil type cellulaire	Détection évènements aberrants
MultiQC fastp	STAR → Hg38	HTSeq-count Kallisto	Cibersortx	OUTRIDER FRASER2

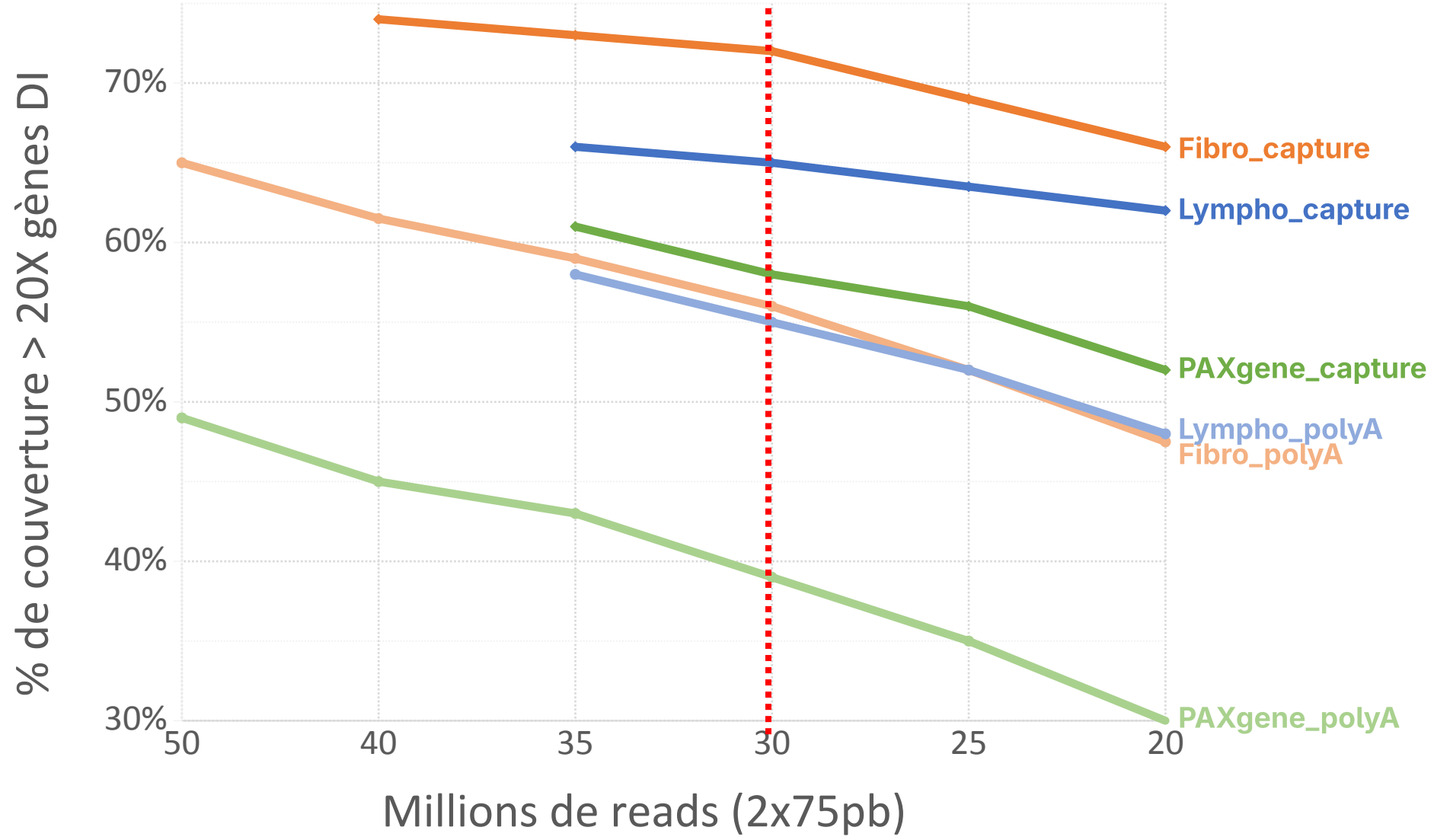
OUTRIDER



FRASER2

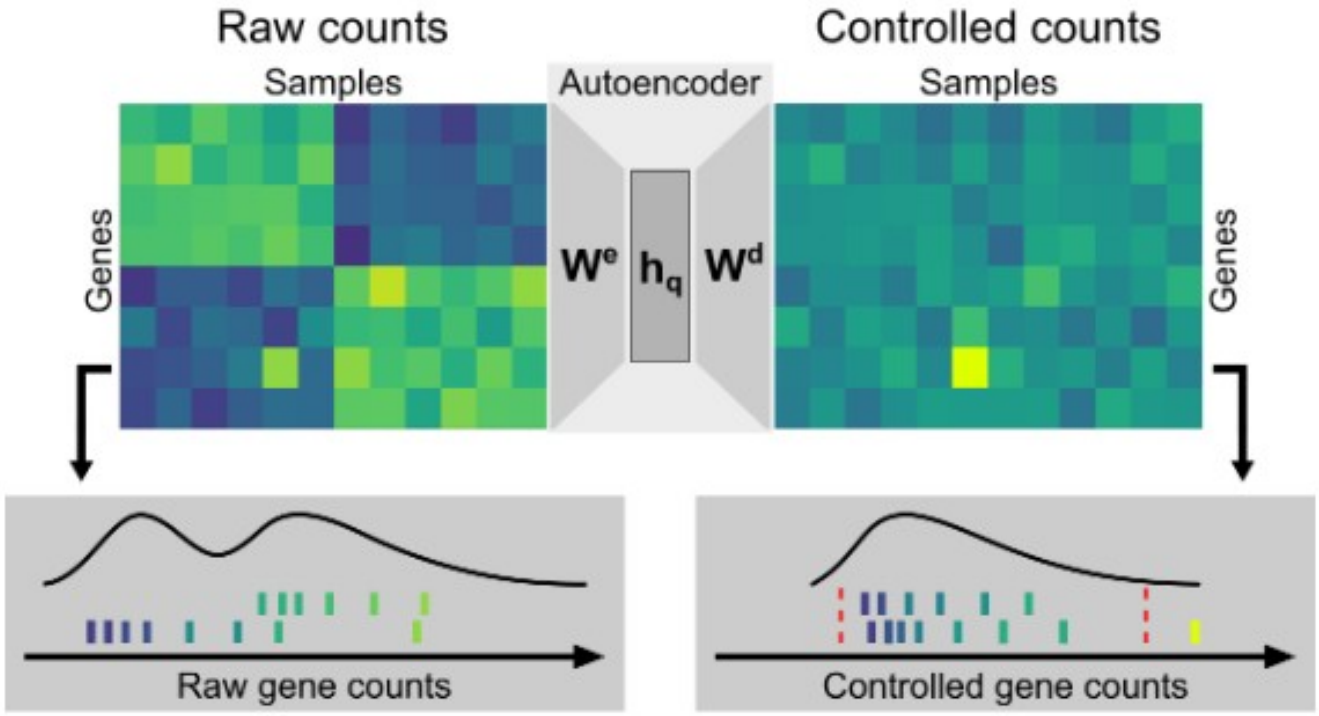


Couverture des gènes d'intérêt

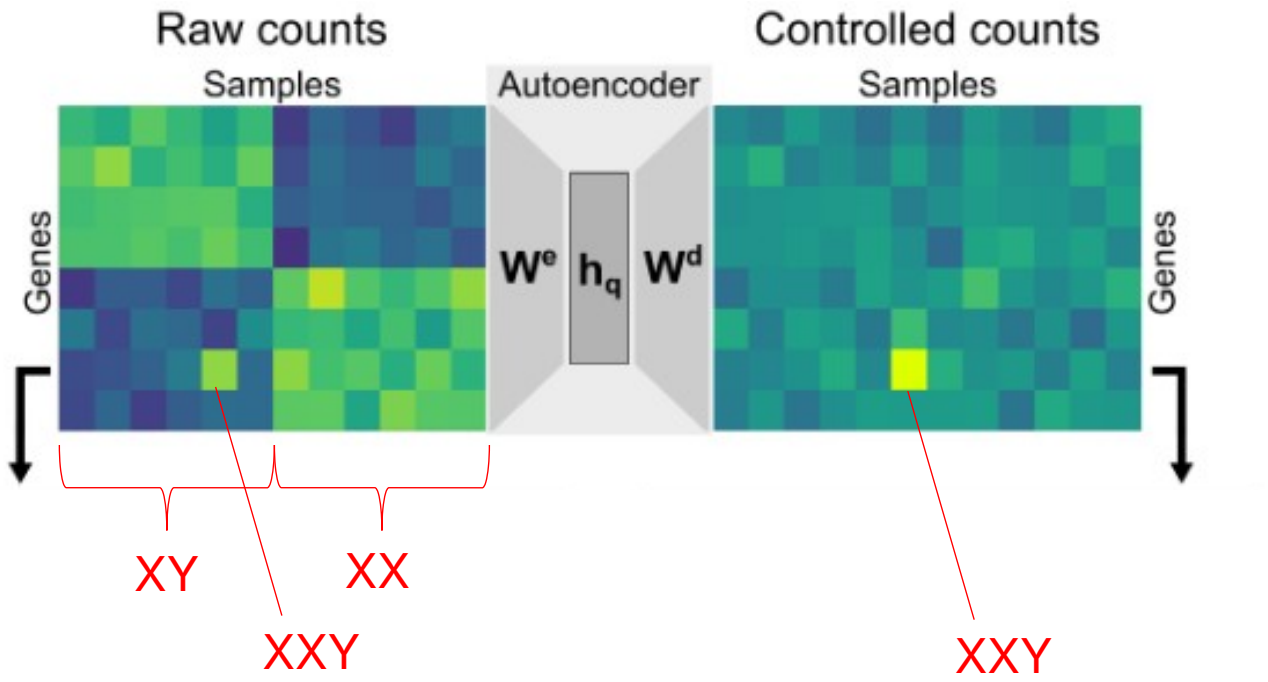


Mais est-ce que c’est quantitatif ?

- Le sang n’est pas adapté pour du quantitat
- La capture par des sondes biaise la représentation des gè
- Ça ne représente pas le transcriptome des cellules neuronales

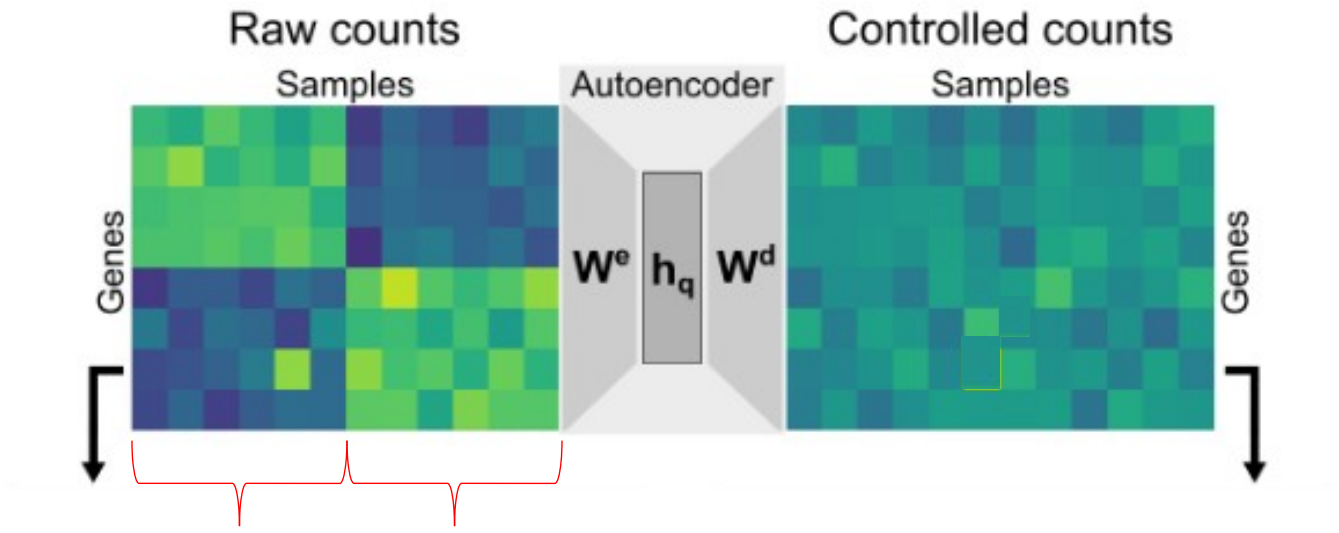


Exemple XXY


Raw counts
Samples
Autoencoder
Controlled counts
Samples
Genes
W^e
h_q
W^d
Genes
XY
XX
XXY
XXY

Exemple réponse IFN

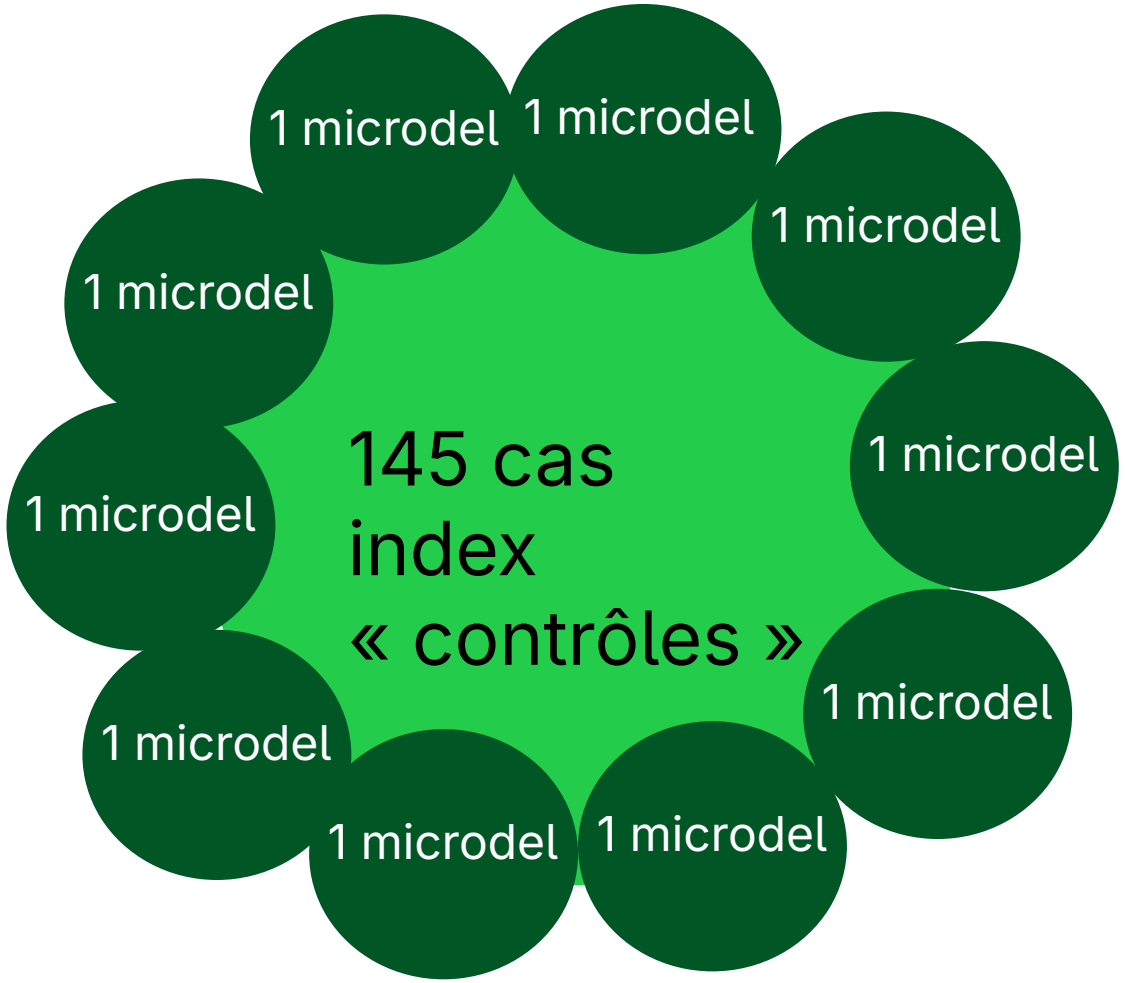
pValue	padjust	zScore	l2fc	rawcounts	meanRawcounts	normcounts	meanCorrected	theta	aberrant	AberrantBySample	AberrantByGene	padj_rank	chromosome	start	gene_name
5,42E-05	1	-4,66	-1.91	6126	733.55	127,86	480,97	14,45	False	0	0	17012.0	1	78649796	IFI44
0,00085007	1	-4,16	-3.17	7862	746.07	41,21	370,24	4,7	False	0	0	17012.0	1	78619902	IFI44L



Abs réponse IFN

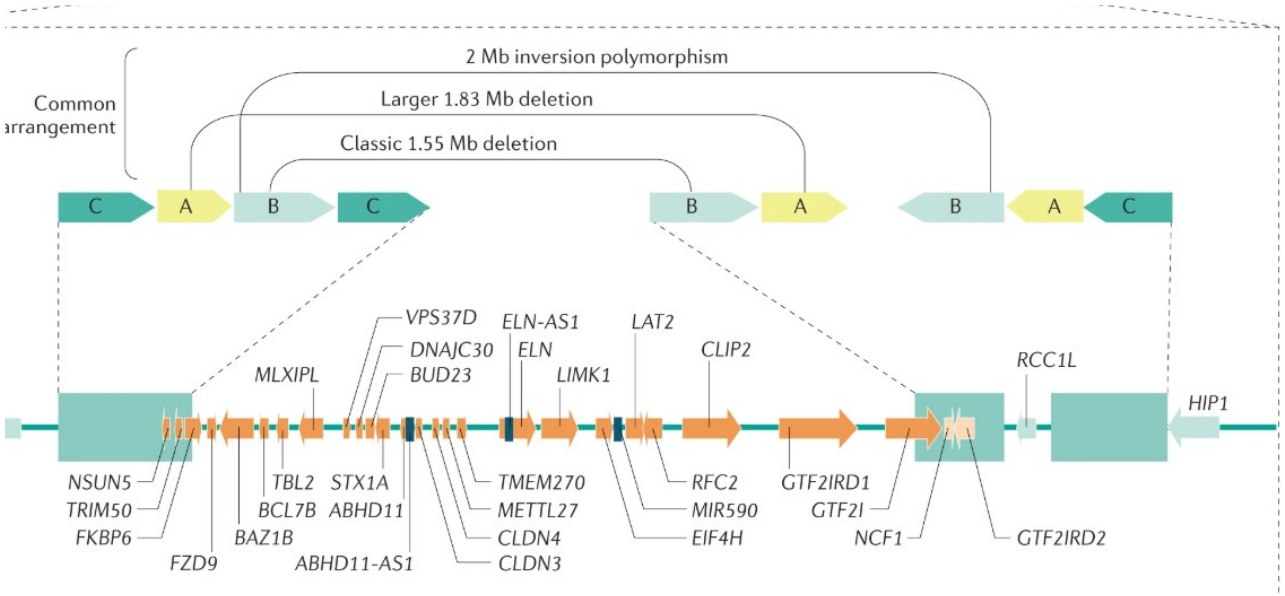
Réponse IFN

Evaluation de la sensibilité de la quantification

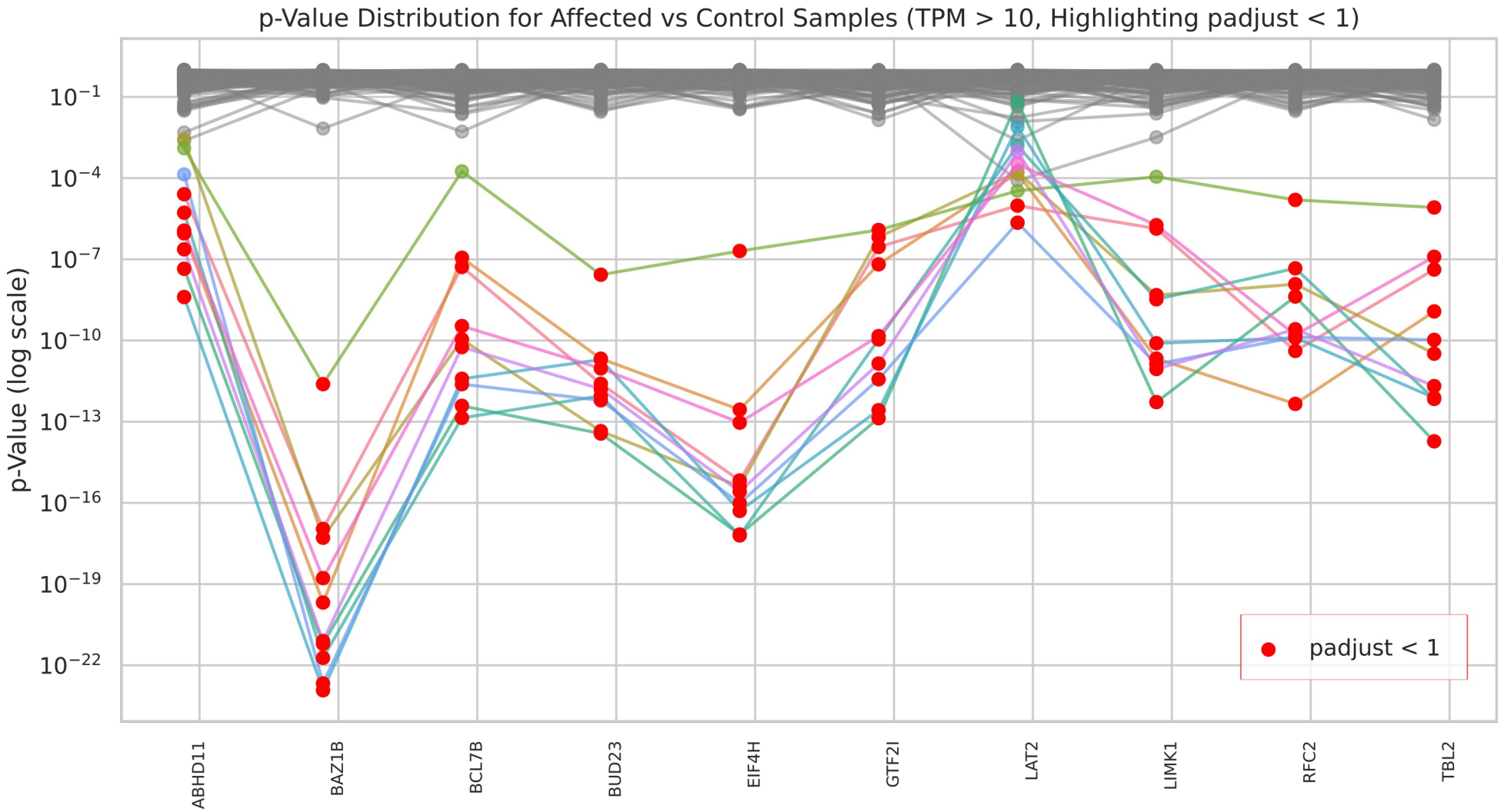


Syndrome de Williams

23 gènes – 10 gènes > 10TPM



Quantitatif – sensibilité 85% / spécificité 100%



Exemple de GTF2I

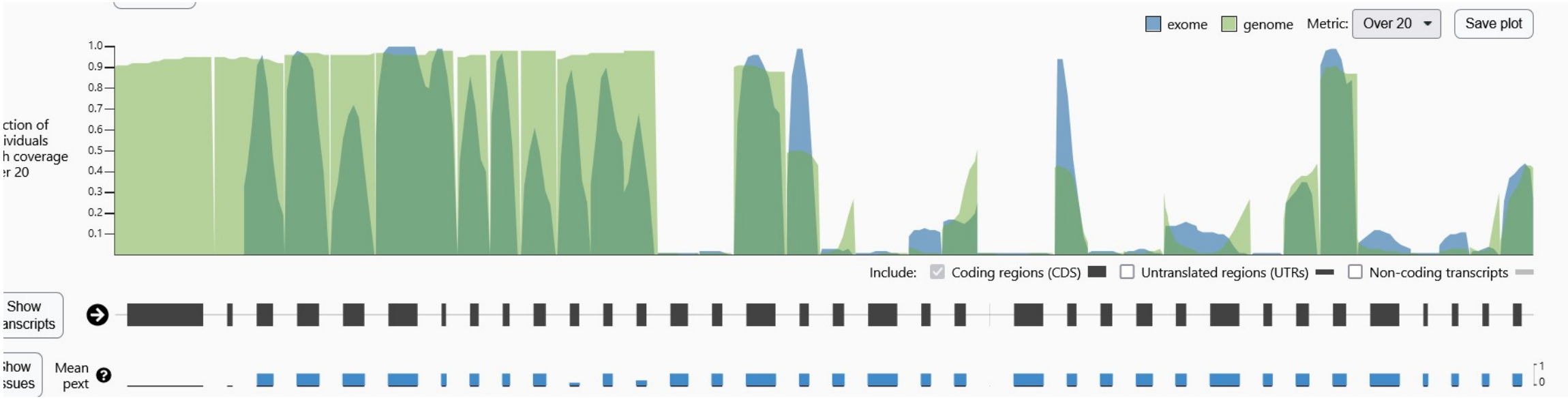
GTF2I general transcription factor IIi

Dataset gnomAD v4.1.0 gnomAD SVs v

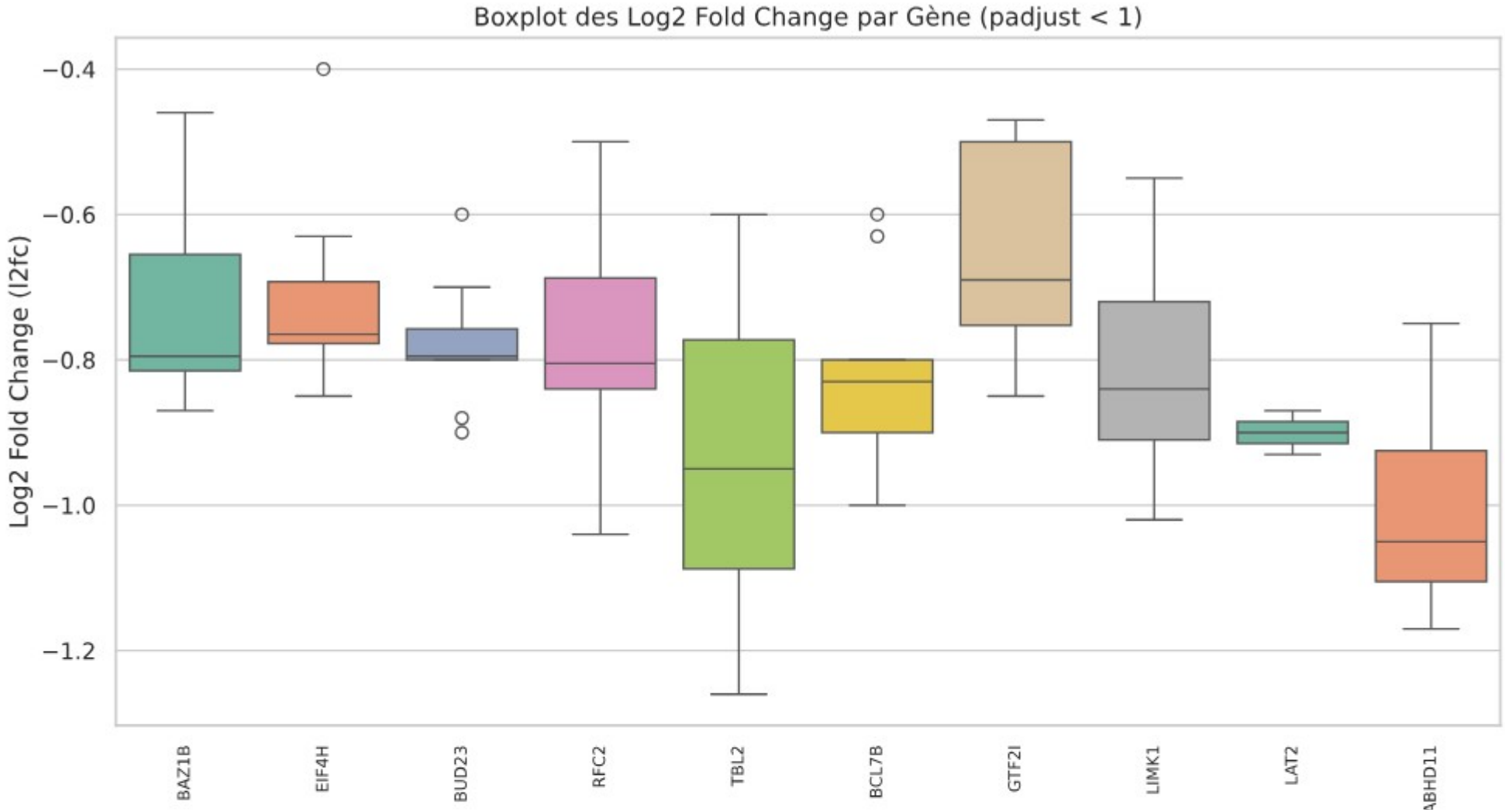
Genome build GRCh38 / hg38
Ensembl gene ID ENSG00000263001.8
MANE Select transcript ENST00000573035.6 / NM_032999.4
Ensembl canonical transcript ENST00000573035.6
Other transcripts ENST00000462915.1, ENST00000484840.5, and 21 more
Region 7:74650231-74760692
External resources Ensembl, UCSC Browser, and more

Constraint | Variant co-occurrence

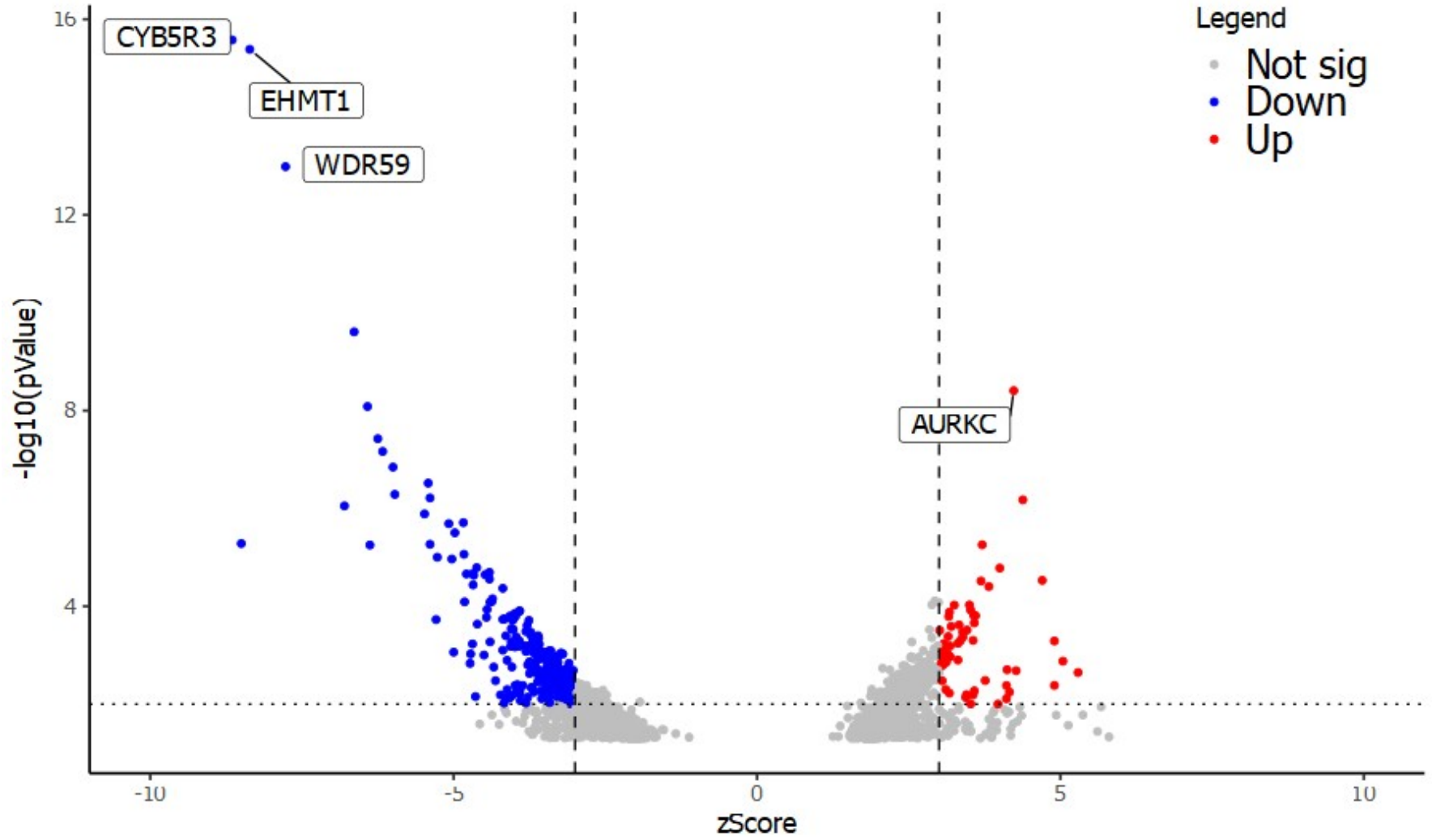
Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	83.9	65	Z = 1.13 o/e = 0.77 (0.63 - 0.95)
Missense	235.3	75	Z = 3.82 o/e = 0.32 (0.26 - 0.39)
pLoF	24.3	1	pLI = 1 o/e = 0.04 (0.01 - 0.2)



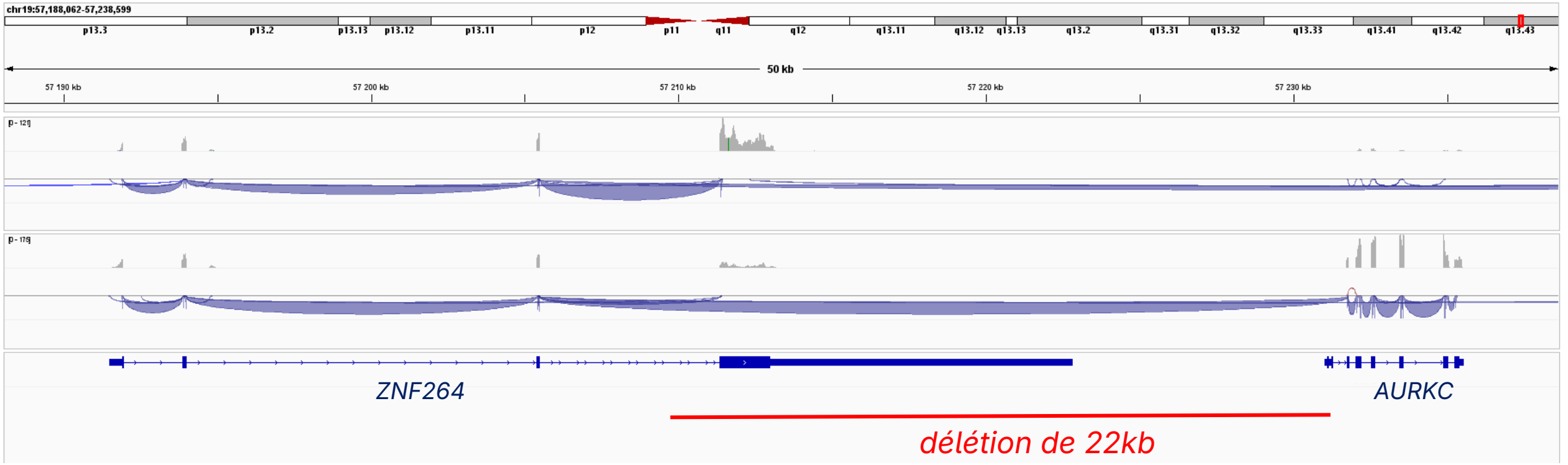
Estimation du fold change



Diagnostics par anomalie d’expression

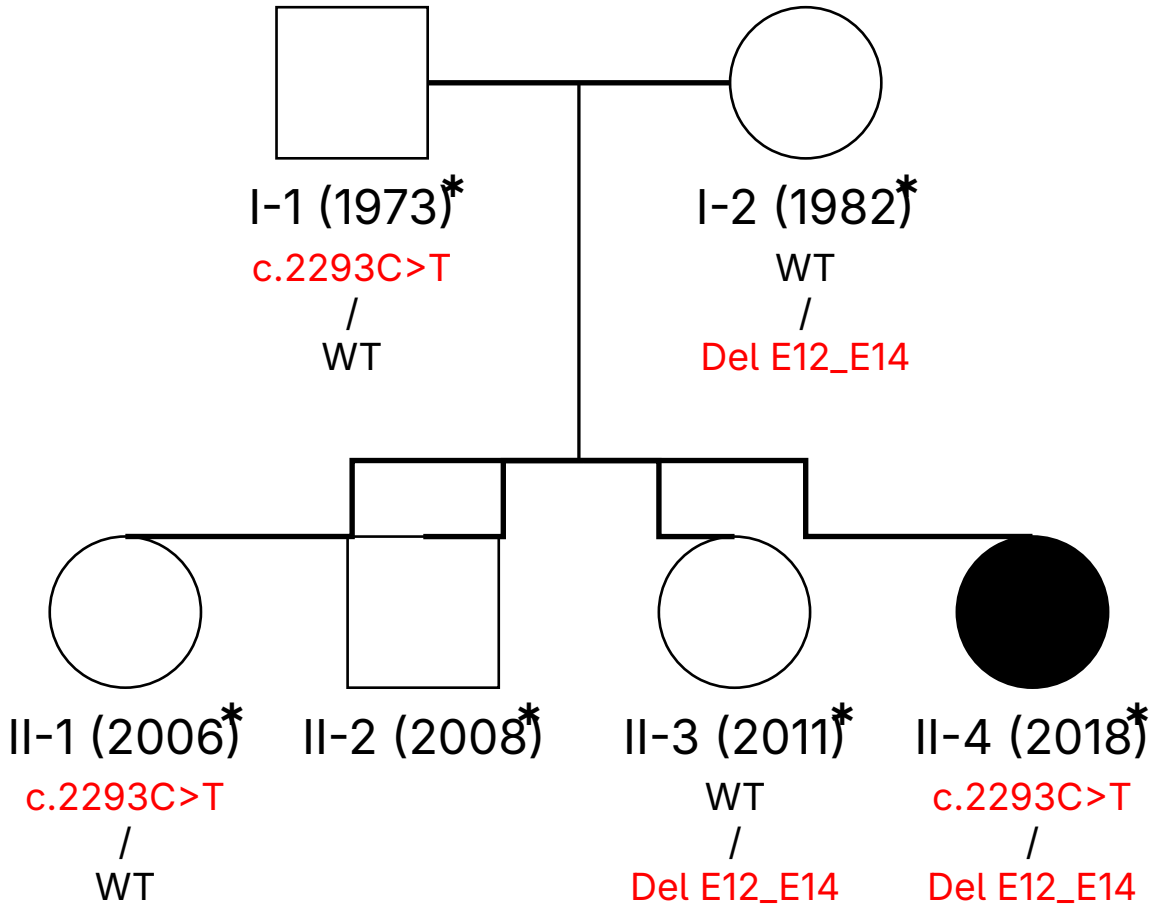


Exemple : surexpression *AURKC*

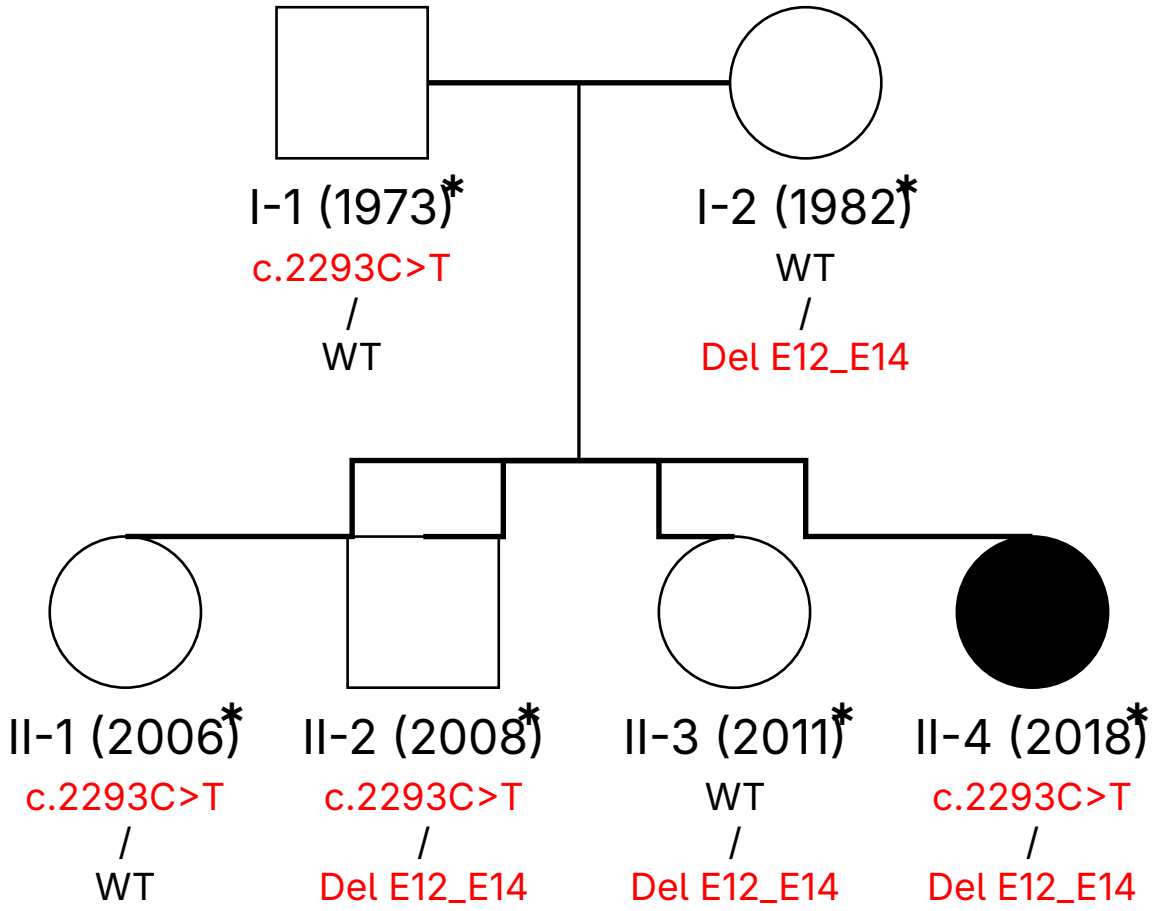


- Détection d'un transcrit de fusion qui ségrège dans une large famille avec un syndrome polymalformatif. *AURKC* normalement exprimé uniquement lors de la spermatogénèse

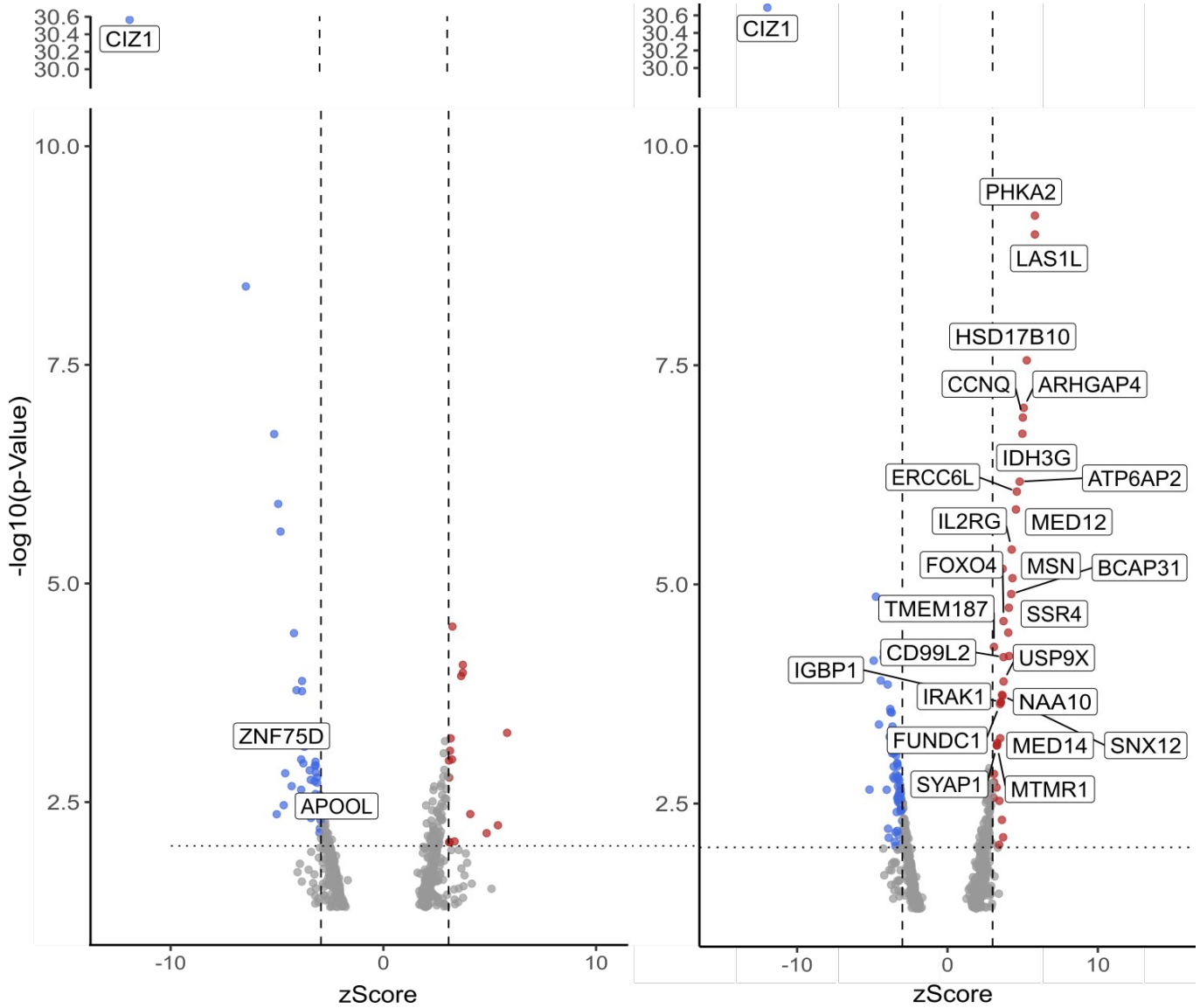
Est-on prêts pour les signatures ?



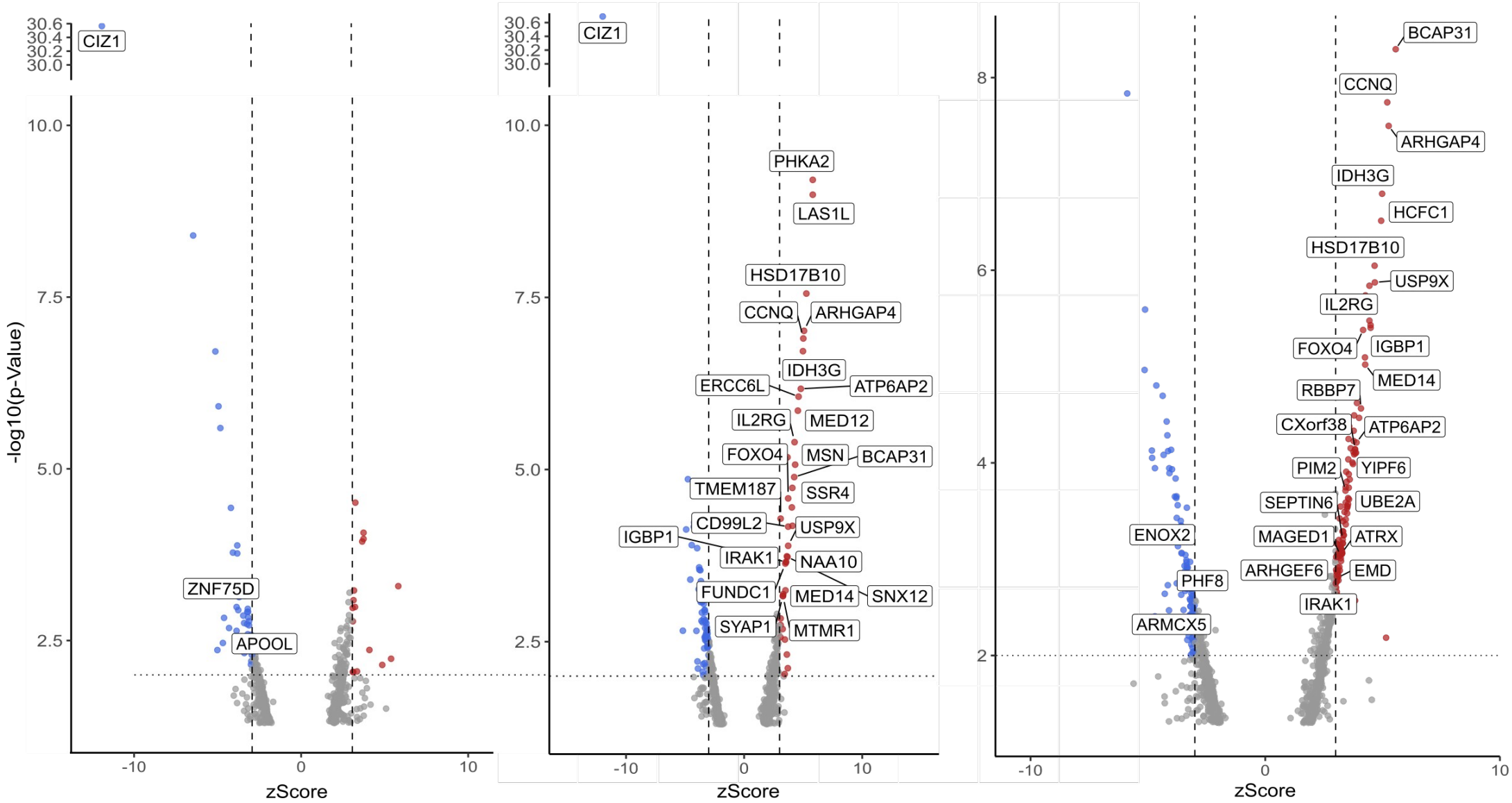
Le KO *CIZ1* est-il pathogène ?



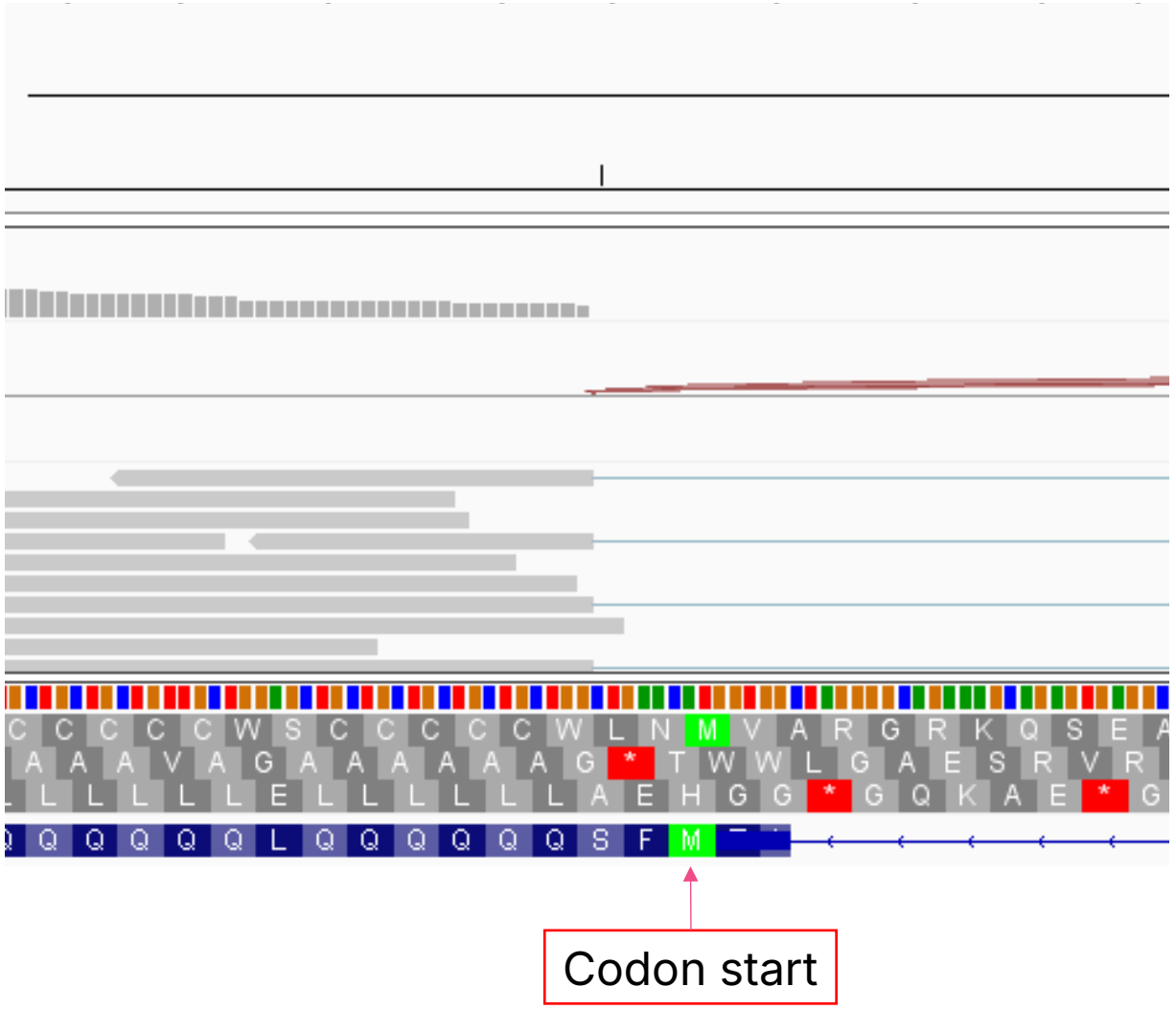
CIZ1 volcano plots

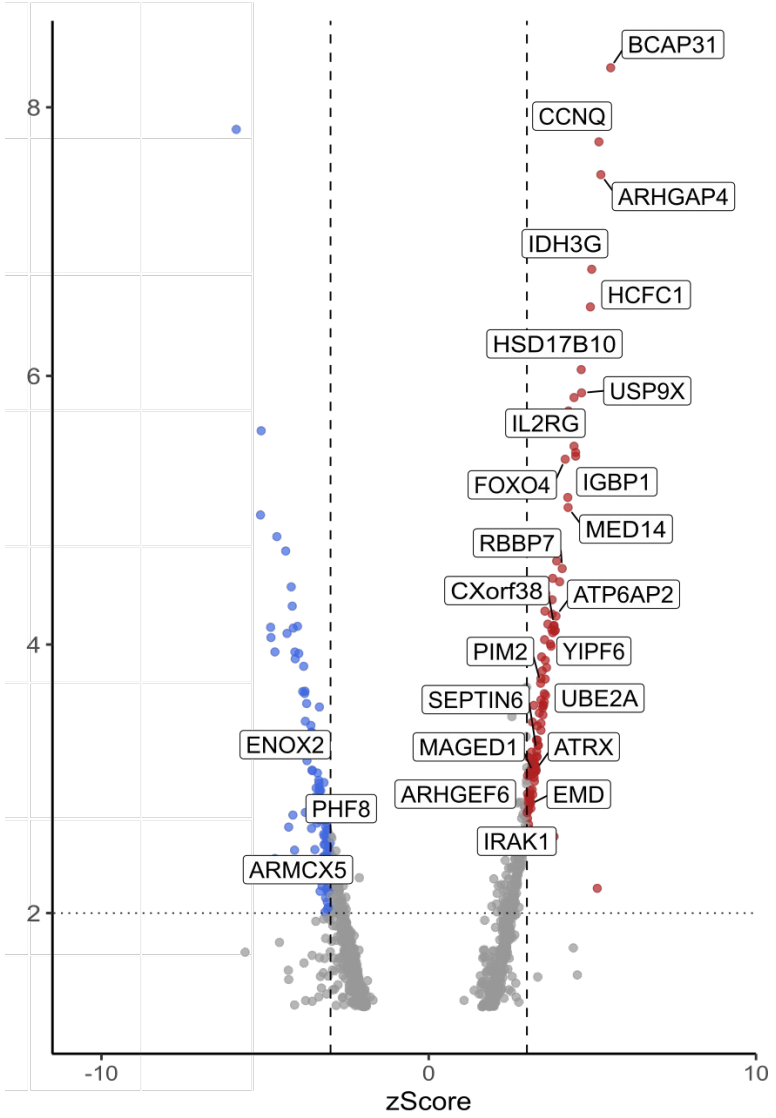


Signature commune

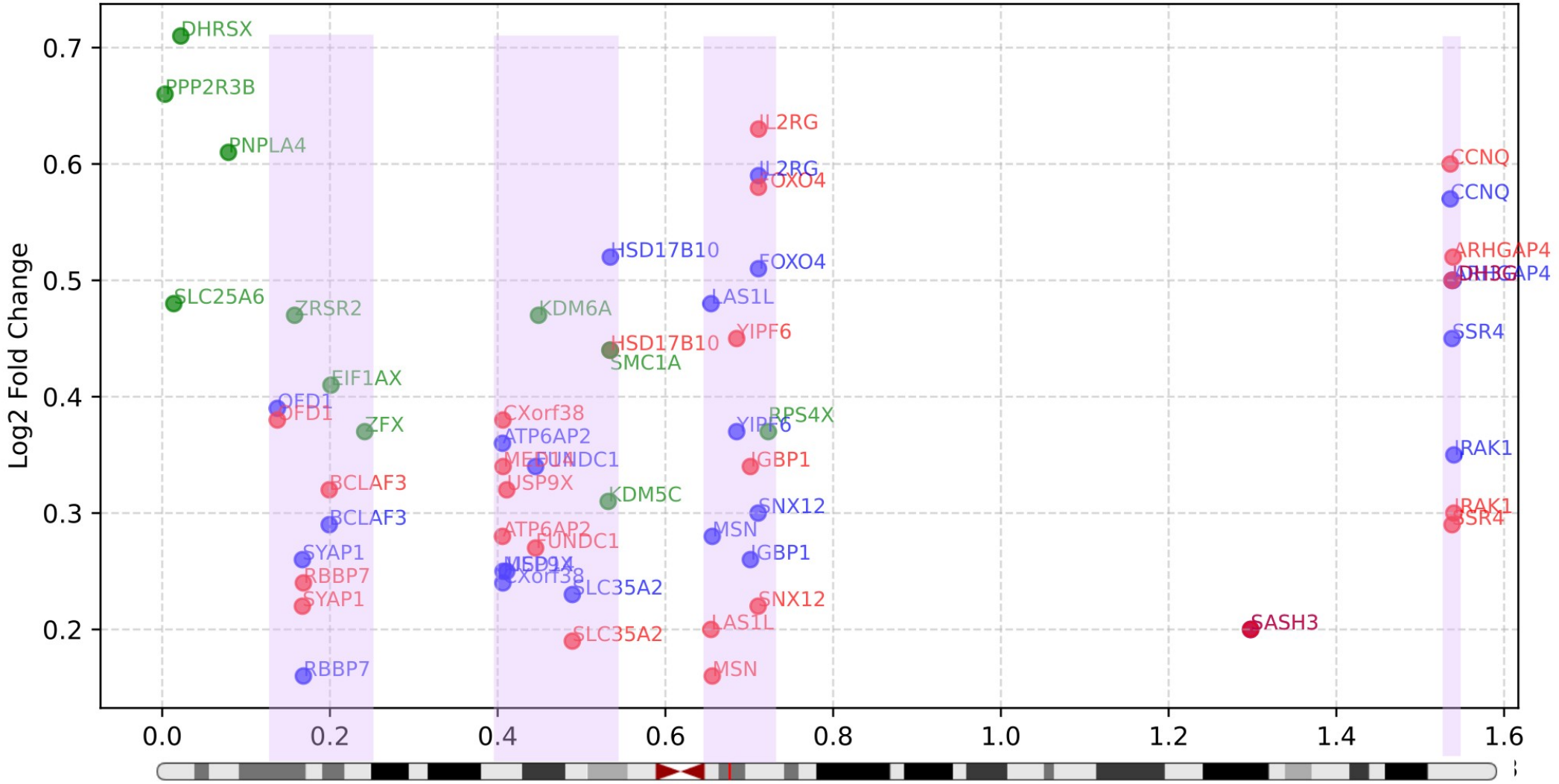


La signature permet de classer le variant





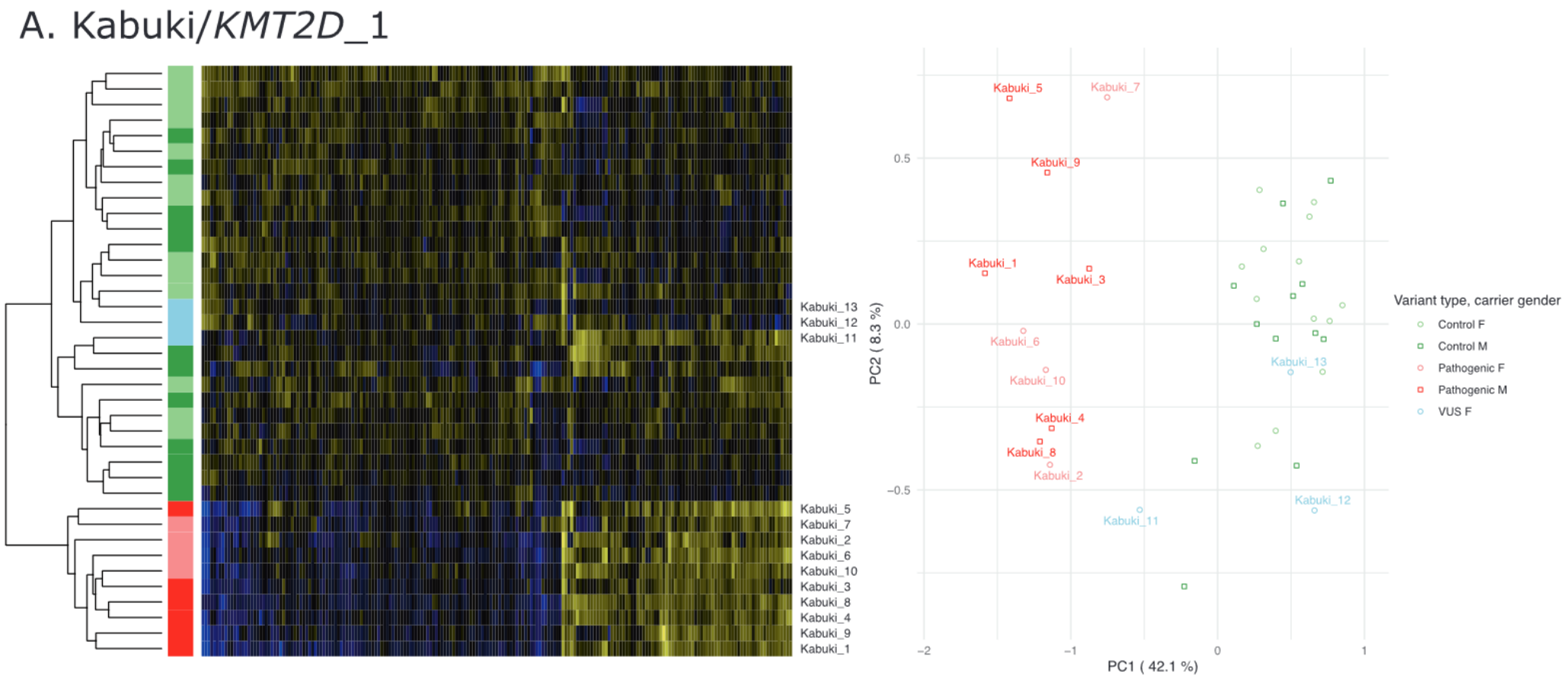
Localisation chromosomique des outliers



Signatures sur l'épissage ?

Exemple de la méthylation

T. Husson et al.

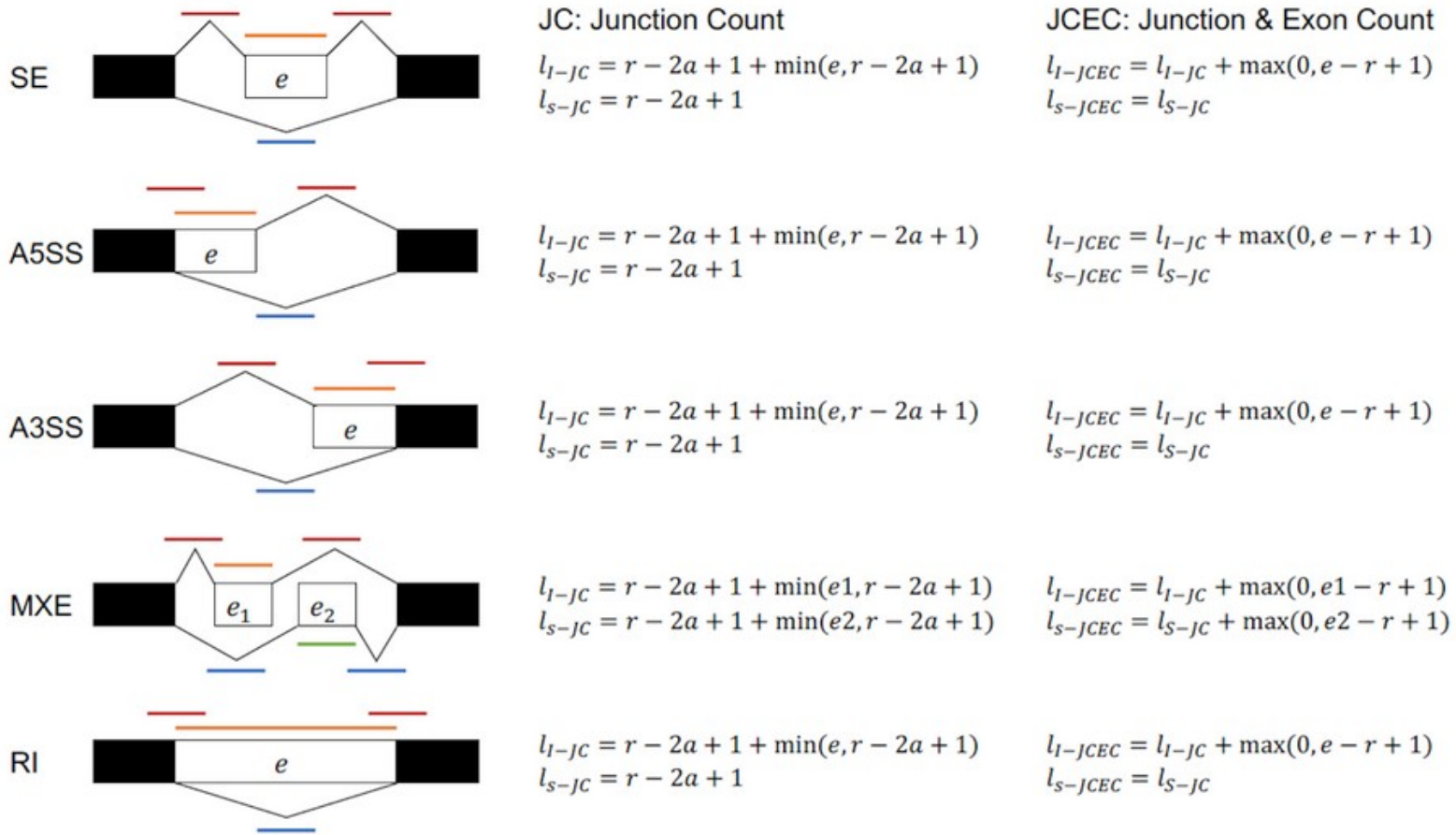


Protocol | Published: 23 February 2024

rMATS-turbo: an efficient and flexible computational tool for alternative splicing analysis of large-scale RNA-seq data

Yuanyuan Wang, Zhijie Xie, Eric Kutschera, Jenea I. Adams, Kathryn E. Kadash-Edmondson & Yi Xing

Nature Protocols **19**, 1083–1104 (2024) | Cite this article



RNU4-2

nature medicine

Brief Communication https://doi.org/10.1038/s41591-024-03085-5

Mutations in the U4 snRNA gene *RNU4-2* cause one of the most prevalent monogenic neurodevelopmental disorders

Received: 11 April 2024

Accepted: 23 May 2024

Daniel Greene[1,2], Chantal Thys[3], Ian R. Berry[4,5], Joanna Jarvis[6], Els Ortibus[7,8], Andrew D. Mumford[5,9], Kathleen Freson[3] & Ernest Turro[1,2,10,11]

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Article | Open access | Published: 11 July 2024

De novo variants in the *RNU4-2* snRNA cause a frequent neurodevelopmental syndrome

Yuyang Chen, Ruebena Dawes, Hyung Chul Kim, Alicia Ljungdahl, Sarah L. Stenton, Susan Walker, Jenny Lord, Gabrielle Lemire, Alexandra C. Martin-Geary, Vijay S. Ganesh, Jialan Ma, Jamie M. Ellingford, Erwan Delage, Elston N. D'Souza, Shan Dong, David R. Adams, Kirsten Allan, Madhura Bakshi, Erin E. Baldwin, Seth I. Berger, Jonathan A. Bernstein, Ishita Bhatnagar, Ed Blair, Natasha J. Brown, ... Nicola Whiffin

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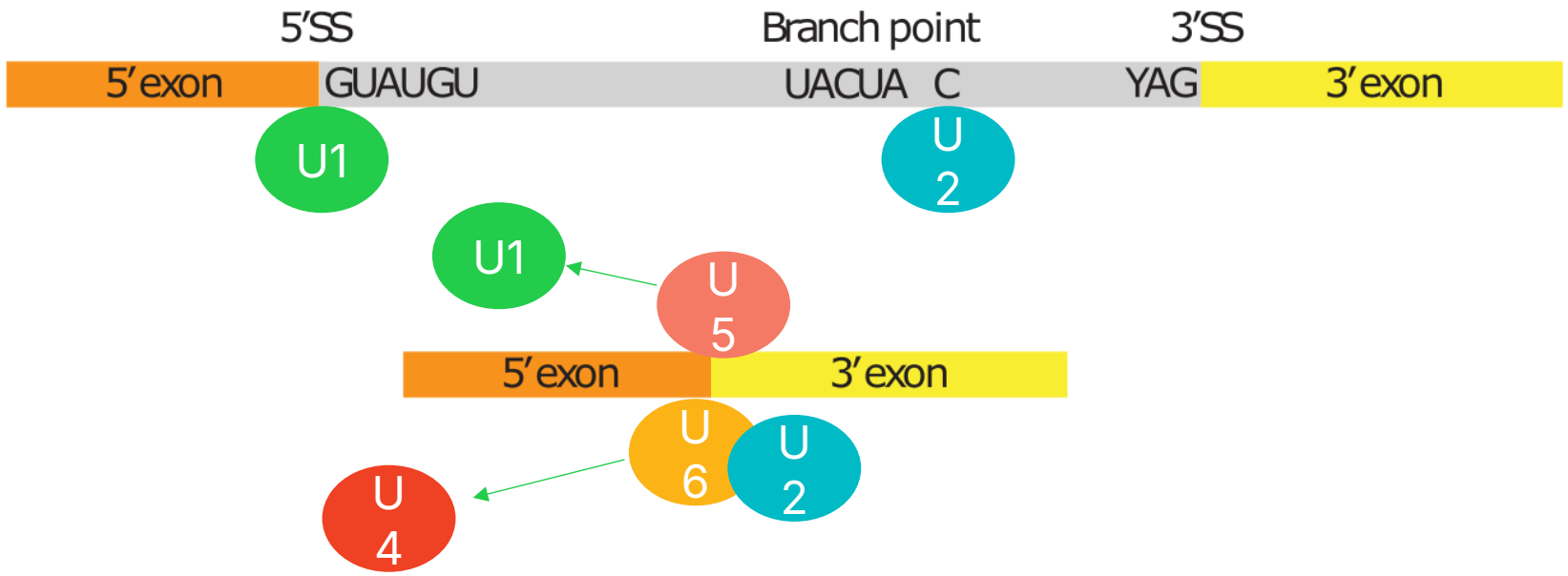
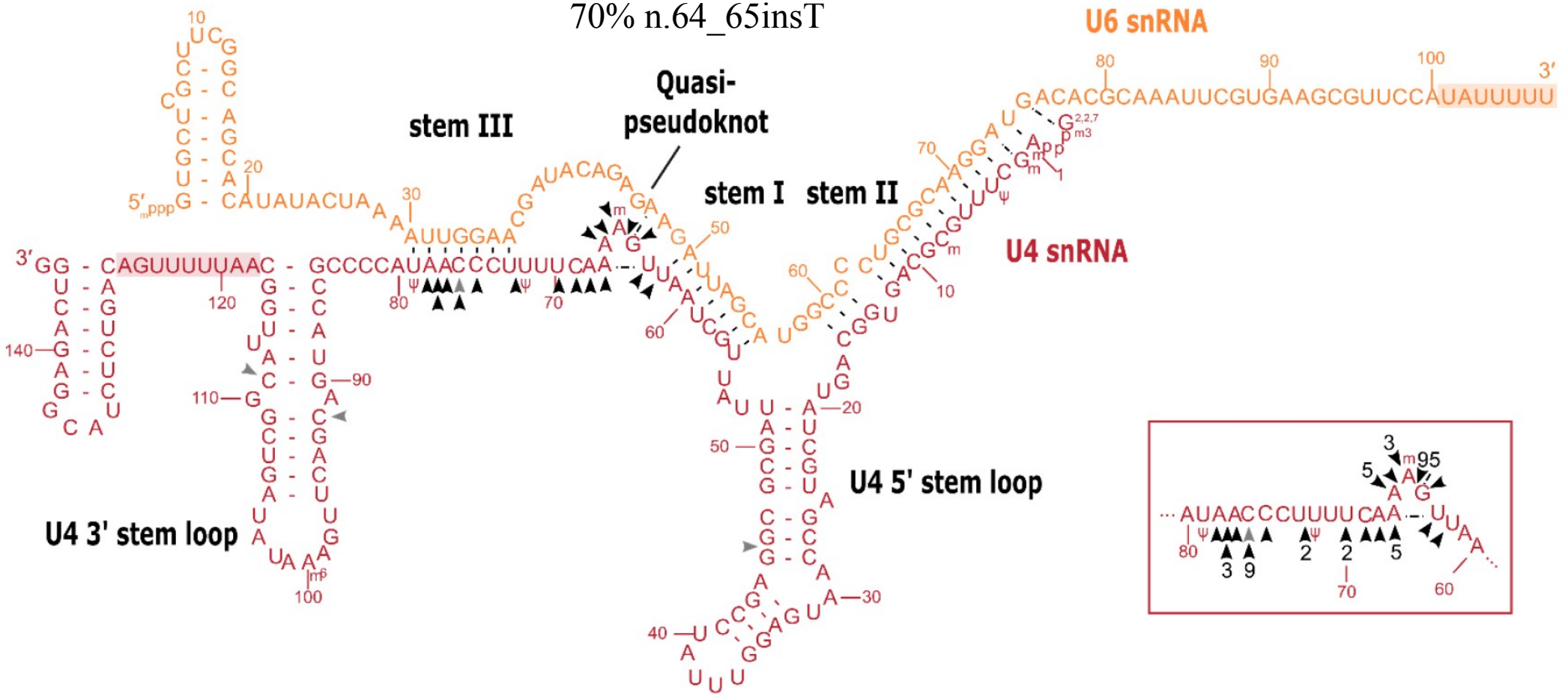
Nature **632**, 832–840 (2024) | Cite this article



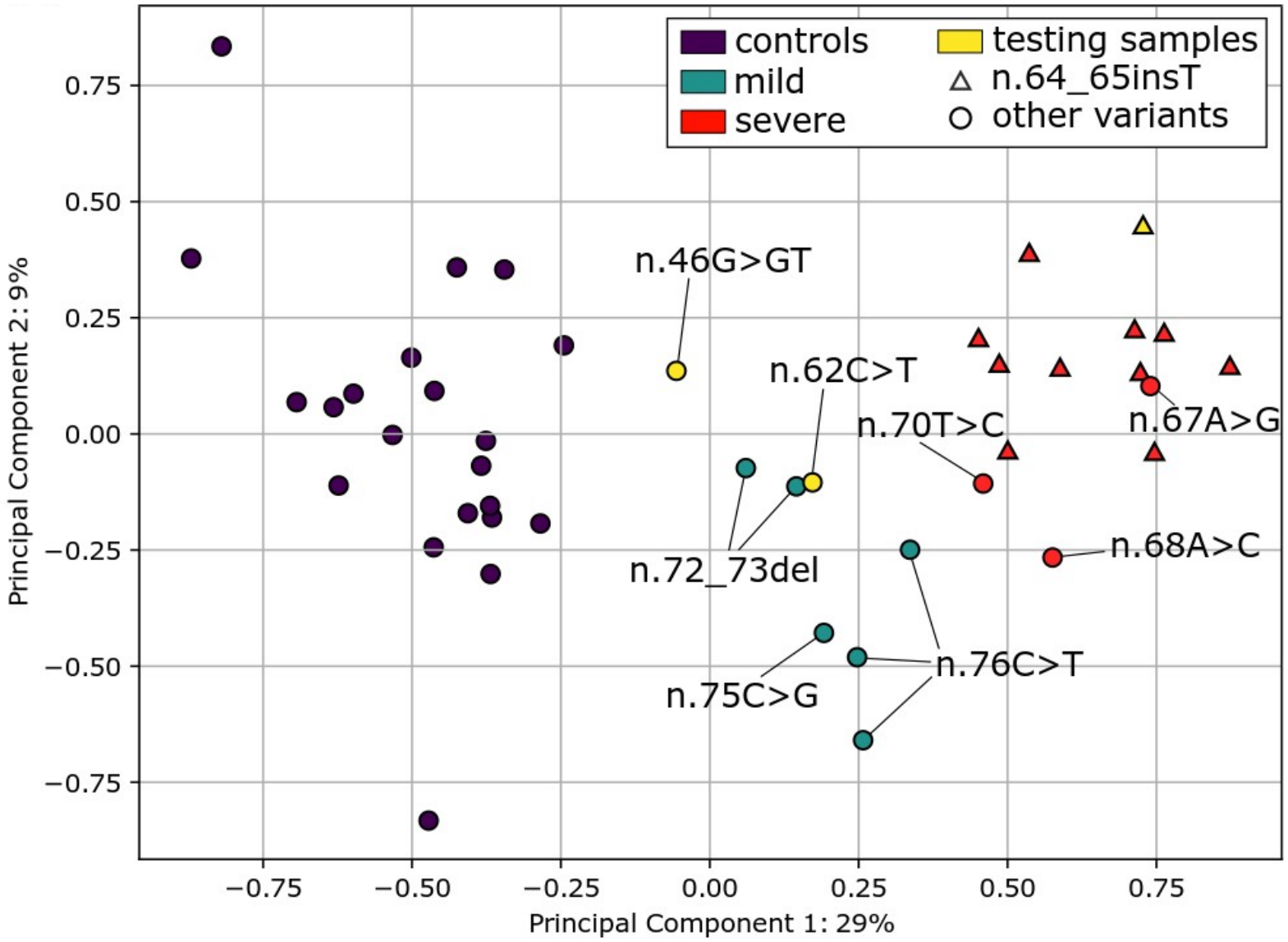
RNU4-2

70% n.64_65insT

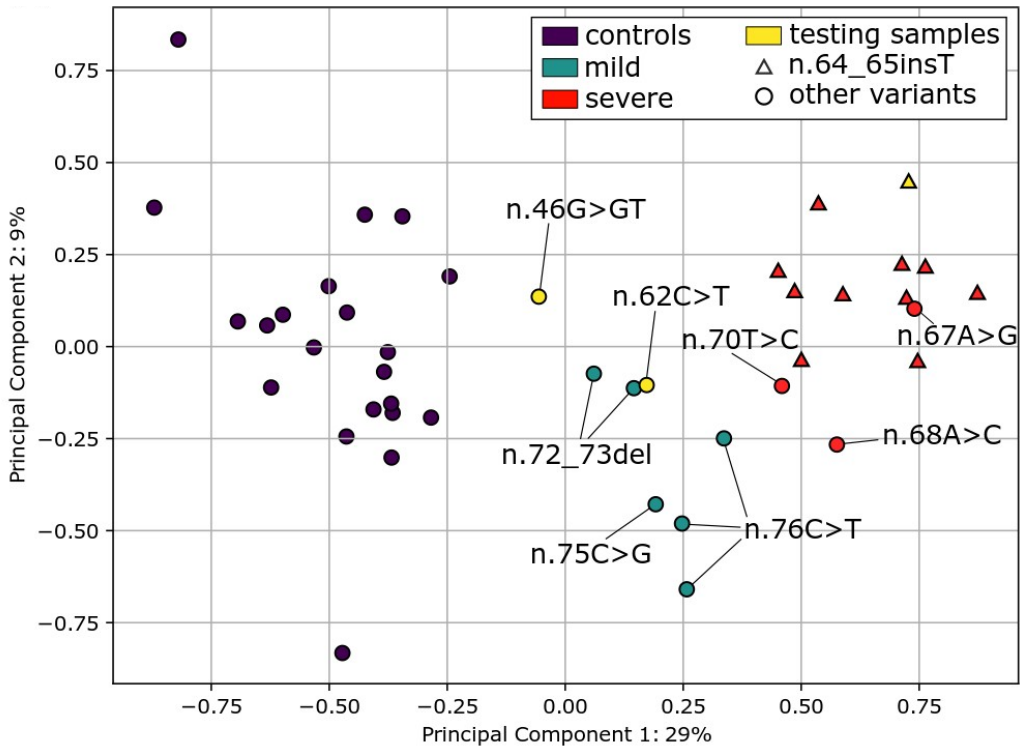


medRxiv https://www.medrxiv.org/content/10.1101/2024.10.07.24314689v1

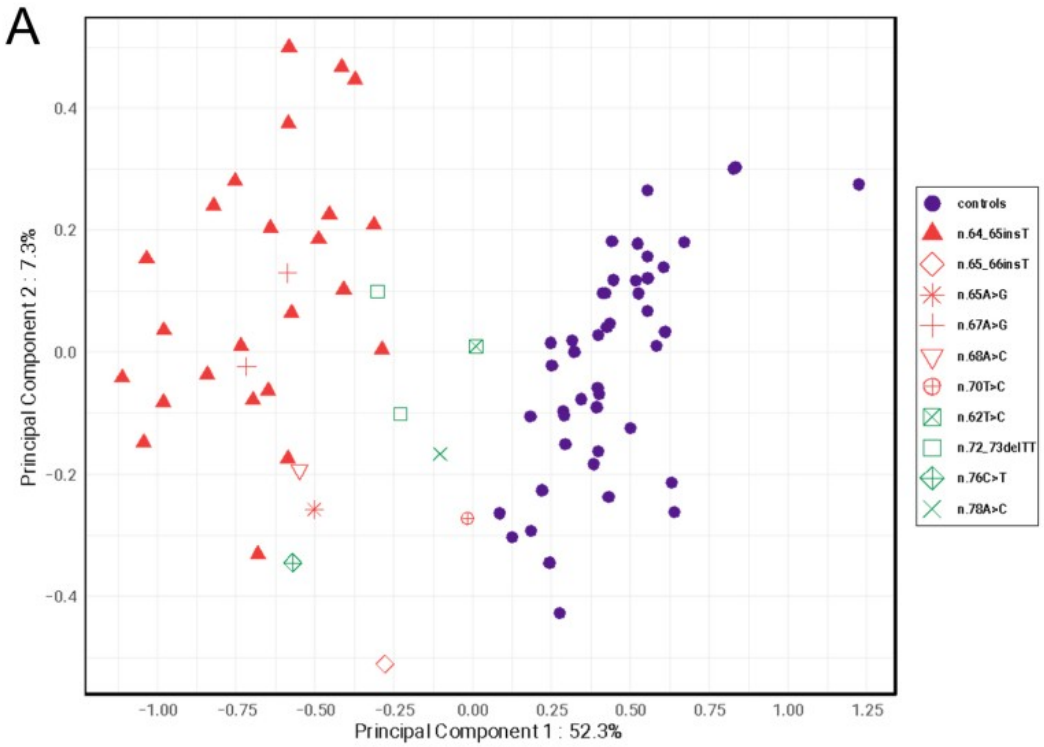
Signature sur les sites 5’ alternatifs (n=111)



Une signature utilisable en diagnostic



Signature épissage

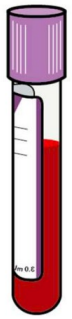


Signature méthylation

Dominant variants in major spliceosome U4 and U5 small nuclear RNA genes cause neurodevelopmental disorders through splicing disruption

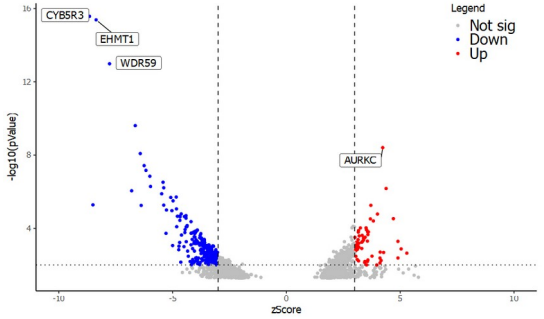
Caroline Nava, Benjamin Cogne, Amandine Santini, Elsa Leitão, François Lecoquierre, Yuyang Chen, Sarah L. Stenton, Thomas Besnard, Solveig Heide, Sarah Baer, Abhilasha Jakhar, Sonja Neuser, Boris Keren, Anne Faudet, Sylvie Forlani, Marie Faoucher, Kevin Uguen, Konrad Platzer, Alexandra Afenjar, Jean-Luc Alessandri, Stephanie Andres, Chloé Angelini, Bernard Aral, Benoit Arveiler, Tania Attie-Bitach,

Conclusion: évolution du diagnostic des maladies rares

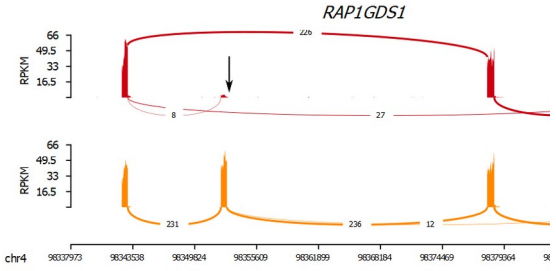


→ 1ère intention: ADN → génome

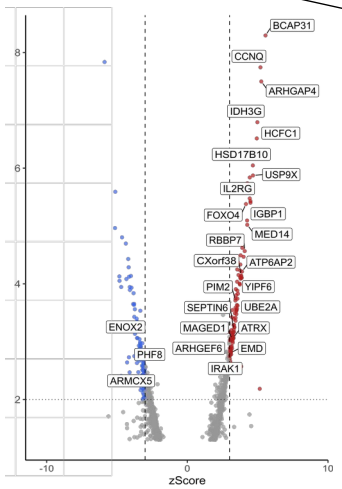
→ 2ème intention: ARN



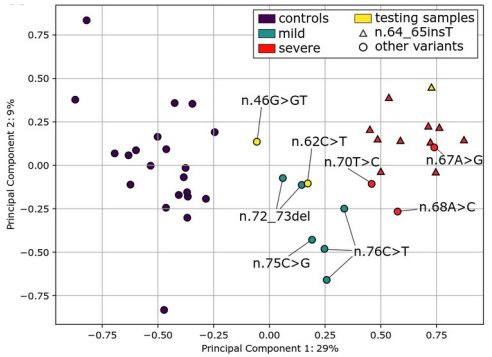
Anomalies d'expression 'primaire'



Anomalies d'épissage 'primaire'



Signature expression



Signature épissage

Remerciements

Laura Do Souto Ferreira
Thomas Besnard
Patricia Talarmain
Delphine Quinquis
Jérome Buscail
Gaëlle Landeau
Eva Trochu
Wallid Deb
Stéphane Bézieau

Bertrand Isidor
Sandra Mercier
Mathilde Nizon
Marie Vincent
Solène Conrad

Pierre Blanc
Alban Lermine
Pierre Marijon

CHU NANTES

Merci pour votre attention !

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