

# Les évolutions en génomique dans le domaine pré et post natal

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# Une demande client : Faire évoluer notre exome

RNU : Genes codants pour de petits ARN nucleaire >> epissage ARNm

**RNU4-2** = gène majeur de déficience intellectuelle (DI) = **0,4% TND**  
PPA ≈ 1

**RNU2-2P** = DI + épilepsie  
(Très rares signes en prénatal)  
PPA ≈ 0.97

**RNU5B-1**: DI, syndrome malformatif  
(**RNU5A-1**: candidat pour DI, syndrome malformatif)  
**0,05% TND**

**RNU4-2, RNU6** également impliqués dans rétinite pigmentaire

PPA (*posterior probability of association*) is a Bayesian quantity. It represents the probability, given the data and your prior, that a genetic locus or variant is truly associated with the trait of interest. A PPA of 0.97 means that, after your analysis, there's a 97 % chance the association is real under your model.

nature genetics

Nava et al, 2025



Article

<https://doi.org/10.1038/s41588-025-02184-4>

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

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**Mutations in the U2 snRNA gene *RNU2-2P* cause a severe neurodevelopmental disorder with prominent epilepsy**

Daniel Greene, Koenraad De Wispelaere, Jon Lees, Andrea Katrinecz, Sonia Pascoal, Emma Hales, Marta Codina-Solà, Irene Valenzuela, Eduardo F. Tizzano, Giles Atton, Deirdre Donnelly, Nicola Foulds, Joanna Jarvis, Shane McKee, Michael O'Donoghue, Mohanish Suri, Pradeep Vasudevan, Kathy Stirrups, Natasha P. Morgan, Kathleen Freson, Andrew D. Mumford, Ernest Turro

doi: <https://doi.org/10.1101/2024.09.03.24312863>

[Spectre clinique des variants pathogènes et probablement pathogènes de RNU4 et RNU5/ French RNUs | Health Data Hub](#)

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**De novo and inherited dominant variants in U4 and U6 snRNAs cause retinitis pigmentosa**

# Cible RNU4-2 : Gène majeur de déficience intellectuelle

## Indications en postnatal : DI +/- microcéphalie +/- épilepsie

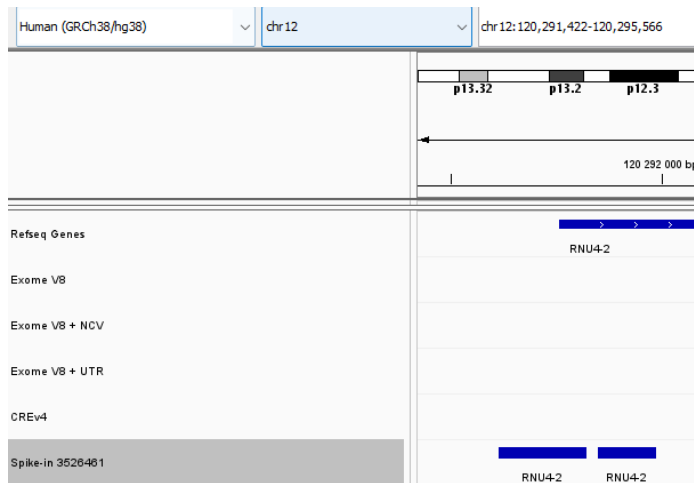
Sévérité clinique dépendante de la localisation du variant

Variant récurrent n.64\_65insT: DI sévère, microcéphalie, épilepsie, hypotonie, retard de croissance

## Indications en prénatal : agénésie/dysgénésie calleuse, microcéphalie/ventriculomégalie

## Non présent dans les différents exomes commerciaux

RNU4-2



### Design Information

Design Name: RNU\_addon\_v20250109  
Design ID: 3526461  
Species: H. sapiens (UCSC hg38, GRCh38, December 2013)

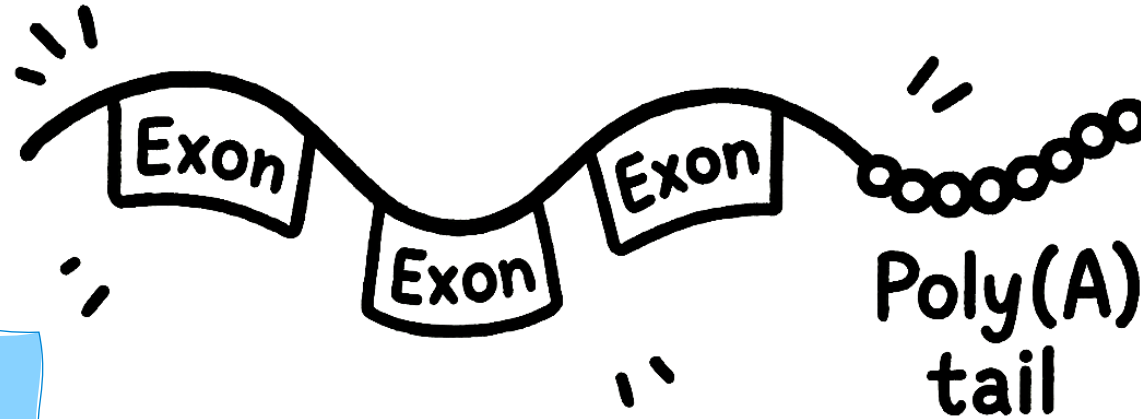
Probe Summary  
Total Probes: 95  
Total Probes Size: 1.900 kbp  
Recommended Minimum Sequencing per Sample: 380.000 kbp  
Pricing: Tier 1 (Probe Region Size = 1 - 500 kbp; up to 60.5K probes)

Ajout d'un groupe de sondes

Exome V8 "Plus" disponible UNIHA

# RNAseq : application et usage

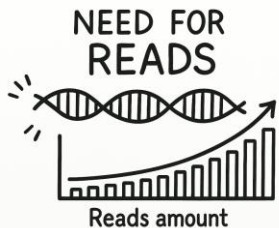
## RNA



Capture Exome V8 ARN :  
Couverture exon +++  
Pas de rRNA, moins de globine, TPM augmenté  
Pas de couverture autre qu'exon

RNA après Ribodepletion:  
Le plus complet,  
(hors miRNA)

mRNA : PolyA  
Enrichissement tout mRNA donc élimination rRNA/ LincRNA et couverture exhaustive !  
Mais saturation de certains mRNA



# Comparison between Poly A, Direct to Capture, and RiboMinus using XTHS2 RNA kit

PolyA (mRNA) prep

Direct to capture prep

RiboMinus prep

POOL NAME/DESCRIPTION				NORMALIZATION 20M		
Sample Name	rRNA Rate	Mean /Base Coverage	Strand Specificity	% on Target	UMI Metrics Dupes	Est Lib Size UMI
PA-MXT 15575-1	0.1%	124.4	91.9%	NA	40.6%	2.6E+06
PA-MXT 15575-2	0.3%	124.5	91.5%	NA	41.4%	2.6E+06
PA-MXT 15507-1	0.2%	56.7	91.0%	NA	44.7%	9.0E+05
PA-MXT 15507-2	0.1%	51.2	90.8%	NA	49.0%	7.3E+05
PA-NGI 15550-1	0.6%	177.5	95.3%	NA	39.3%	4.0E+06
PA-NGI 15550-2	0.3%	142.5	94.8%	NA	44.0%	2.7E+06
PA-SCv4-A-1	1.1%	546.9	96.7%	NA	10.4%	3.9E+07
PA-SCv4-A-2	2.2%	549.6	96.7%	NA	11.8%	3.4E+07
DTC-MXT 15575-1	0.4%	265.9	96.8%	69.8%	17.8%	2.2E+07
DTC-MXT 15575-2	0.7%	400.3	97.2%	77.7%	17.1%	2.3E+07
DTC-MXT 15507-1	1.3%	378.0	97.8%	75.0%	34.7%	9.6E+06
DTC-MXT 15507-2	1.2%	375.1	97.9%	75.0%	35.2%	9.4E+06
DTC-NGI 15550-1	0.5%	439.6	98.3%	77.8%	16.3%	2.5E+07
DTC-NGI 15550-2	0.4%	443.9	98.4%	77.8%	14.2%	2.5E+07
DTC-SCv4-A-1	0.3%	558.4	98.8%	75.5%	8.4%	5.5E+07
DTC-SCv4-A-2	0.4%	563.3	98.7%	75.3%	8.6%	5.3E+07
Ribo-MXT 15575-1	13.0%	43.6	79.6%	NA	36.2%	5.8E+06
Ribo-MXT 15575-2	12.9%	44.5	79.4%	NA	35.7%	5.9E+06
Ribo-MXT 15507-1	19.8%	32.6	85.4%	NA	39.6%	4.5E+06
Ribo-MXT 15507-2	19.2%	32.6	85.1%	NA	38.9%	4.6E+06
Ribo-NGI 15550-1	11.5%	47.09	79.3%	NA	34.9%	6.2E+06
Ribo-NGI 15550-2	13.5%	47.49	79.2%	NA	34.3%	6.2E+06
Ribo-SCv4-A-1	11.0%	157.25	82.2%	NA	27.1%	8.5E+06
Ribo-SCv4-A-2	11.4%	150.14	82.4%	NA	27.3%	8.4E+06

Conditions:  
 SCV4 >> ref RNA  
 Other >> lower quality RNA DV200 30-60%

All exon 20 Millions de reads

# SURESELECT « MAX » NEW LIBRARY PREP Innovation

➤ **Couverture, uniformité et complexité supérieures à XTHS2**

- Complexité de bibliothèque plus élevée en pré- et post-capture (ADN et ARN)

➤ **Couverture plus uniforme au niveau du génome entier (avant capture)**

➤ **Rendements post-capture 2 à 5x plus élevés**

➤ **PRE and POST capture pooling prévu et validé**

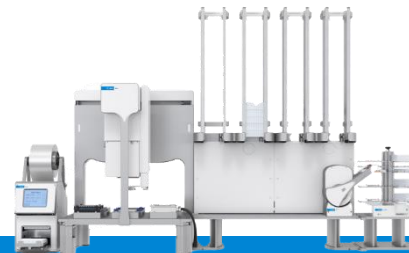
➤ Hybridation overnight ou courte

➤ Adaptateurs ILM/MGI

➤ UMI ou sans UMI



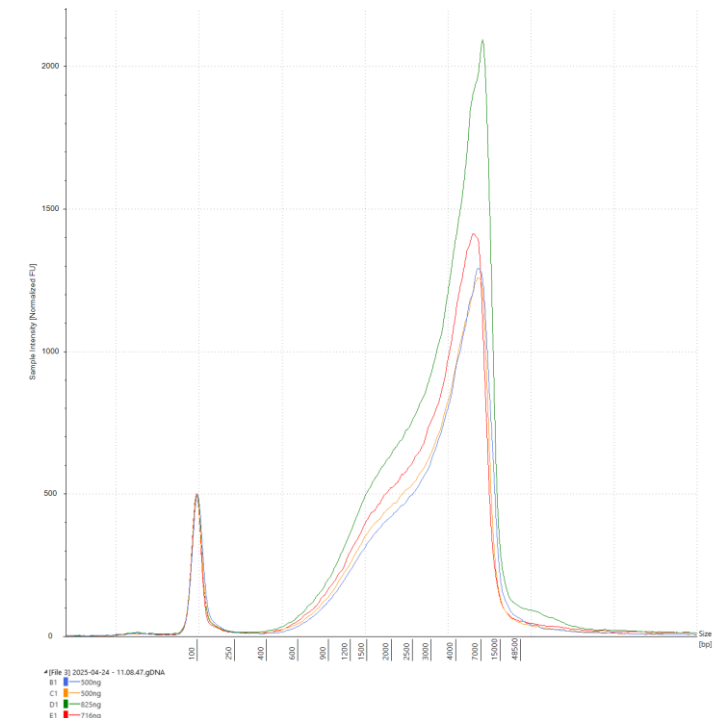
**AUTOMATISE SUR BRAVO**



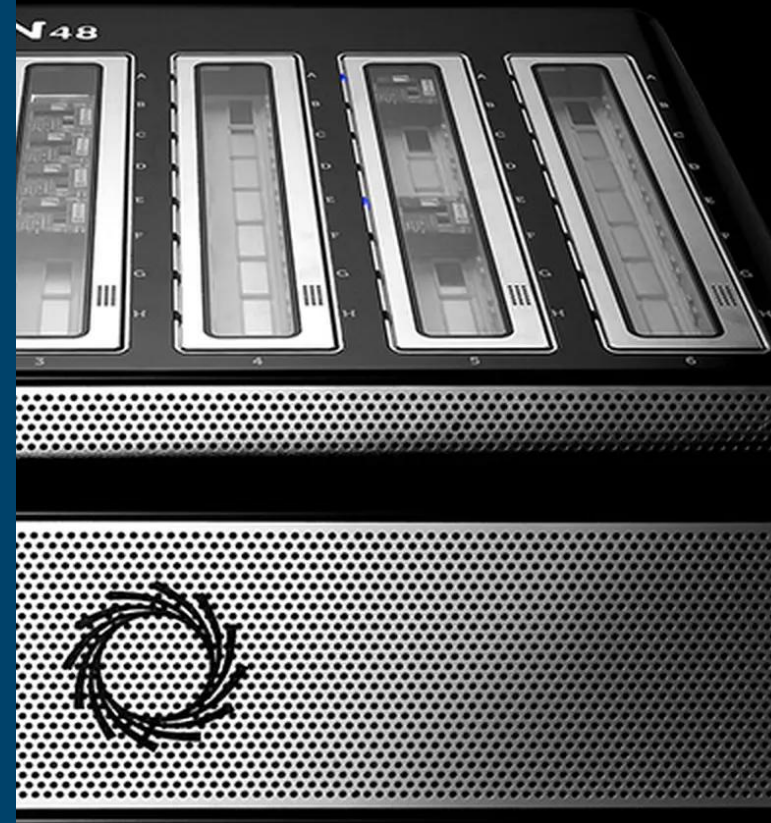
➤ **Quelques modifications limitées du protocole permettent d'obtenir des bibliothèques capturées pour du long reads**

➤ **Range 3-10Kb**

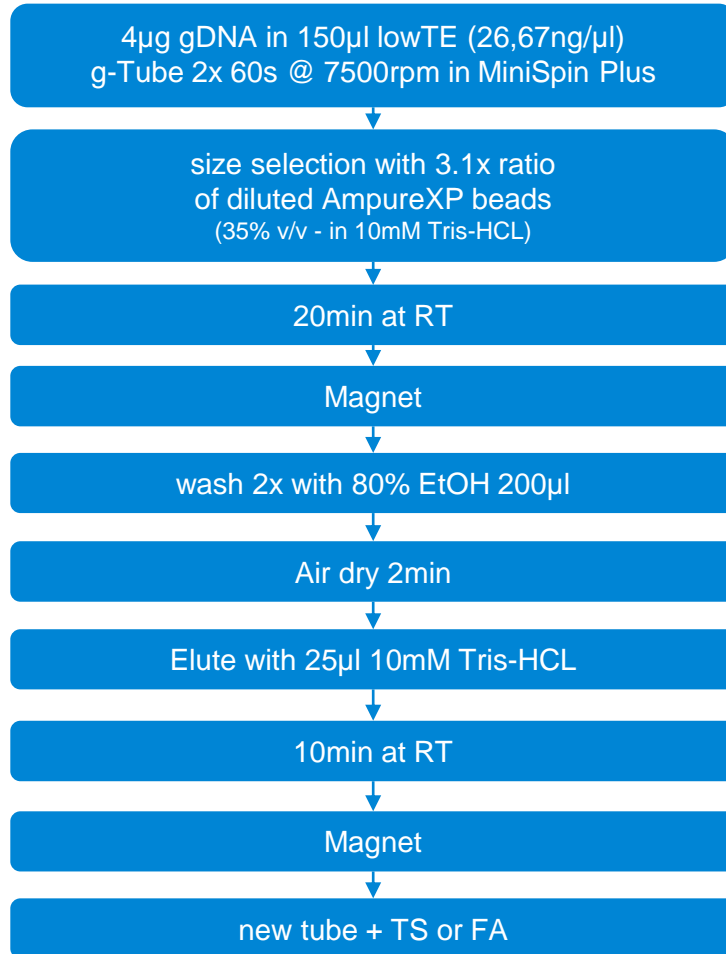
➤ **ONT ou PACBIO**



# Magnis, et pourquoi pas pour du long read ?

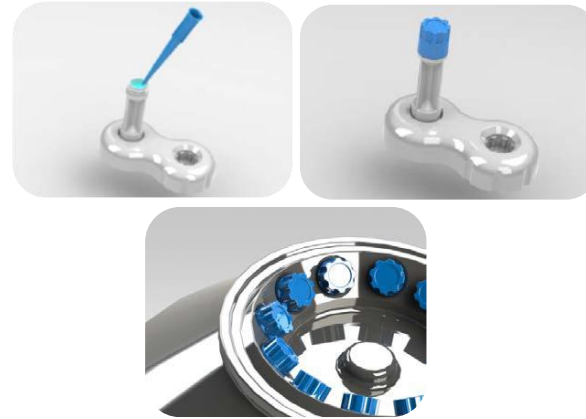


# V8 + XTHS2 + Magnis



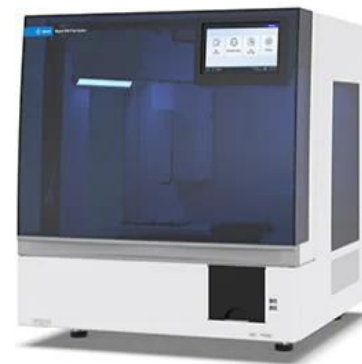
1

Shearing



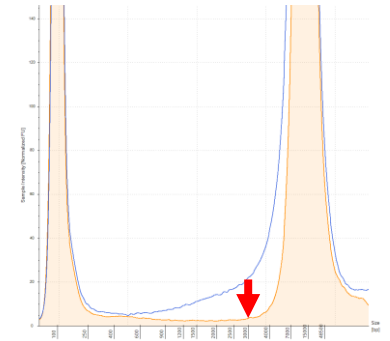
3

Magnis proto modifié



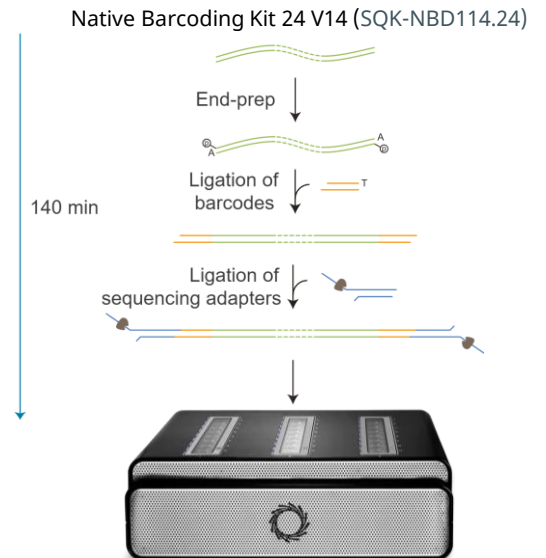
2

Selection taille



4

ONT index/adaptor  
Ligation



# Exemple Exome V8 short read (en bas) versus Long reads

