TAGC — Implementation of whole genome longreads sequencing to decode somatic genotypes in T-ALL

30 October 2024

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— Thèse co-dirigée par Pr. Vahid Asnafi (PU-PH Necker) et Dr. Salvatore Spicuglia (DR1 Inserm)

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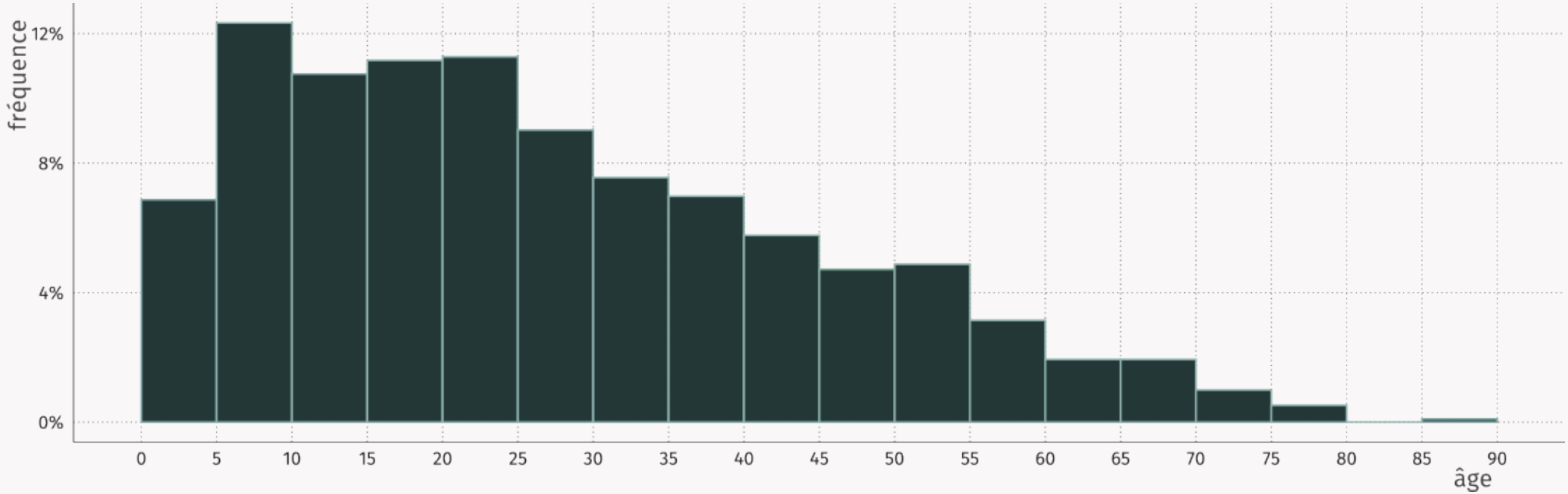
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

❓ Main Hypothesis

Tumor proliferations harbor within their genome intergenic somatic mutations that disrupts the expression of oncogenes.

👨 Model — T-ALL

Acute lymphoblastic leukemia is a rare disease (≃ 120/yr in Fr) resulting from a tumoral process driven by the clonal **proliferation of immature T lymphocytes**.



Internal unpublished data

👨‍⚕️ Model — T-ALL

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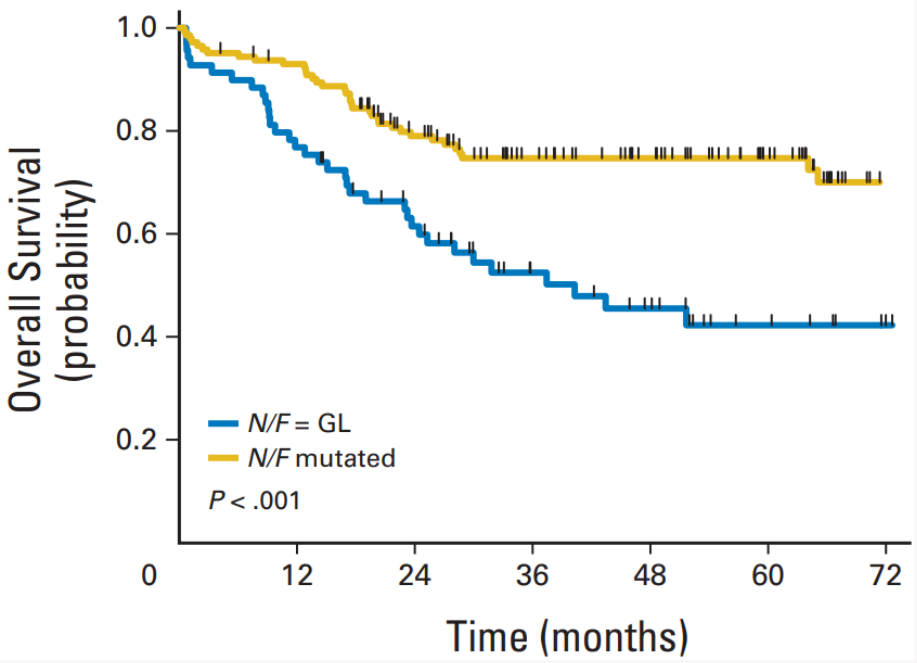
- Adults OS 3 years: 67% (GRAALL-2005)



Trinquand A et al. J Clin Oncol. 2013;31(34):4333-4342

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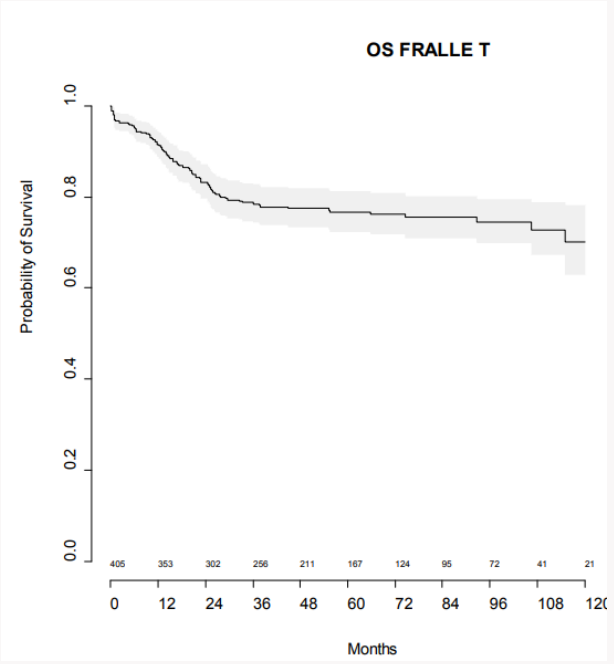
- Youths OS 5 years: 77% (FRALLE)



Petit A et al. Blood. 2018;131(3):289-300.

👨‍⚕️ Model — T-ALL

Refractory cases to standard chemotherapy as well as **relapses** (UKALL12: adults at 5 years 42%) have a poor prognosis.

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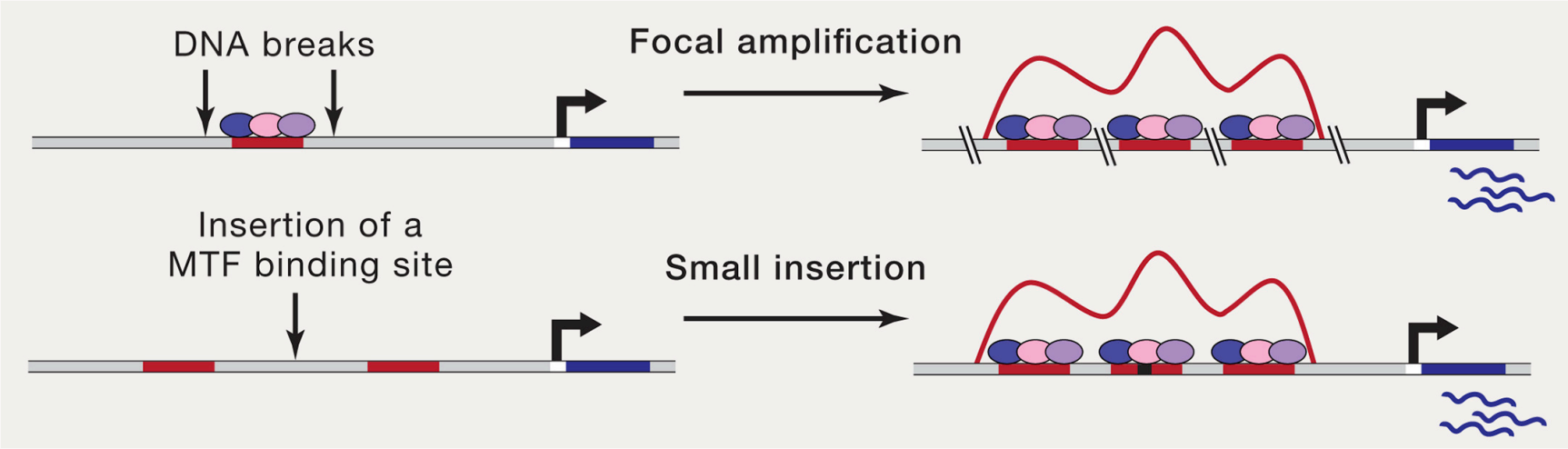
The tumor phenotype emerges from alterations in their genotype.

⇨ A better description of the genotype will lead to a better understanding of oncogenic mechanisms and to the discovery of more **effective therapies tailored to specific alterations**.

Intergenic alterations

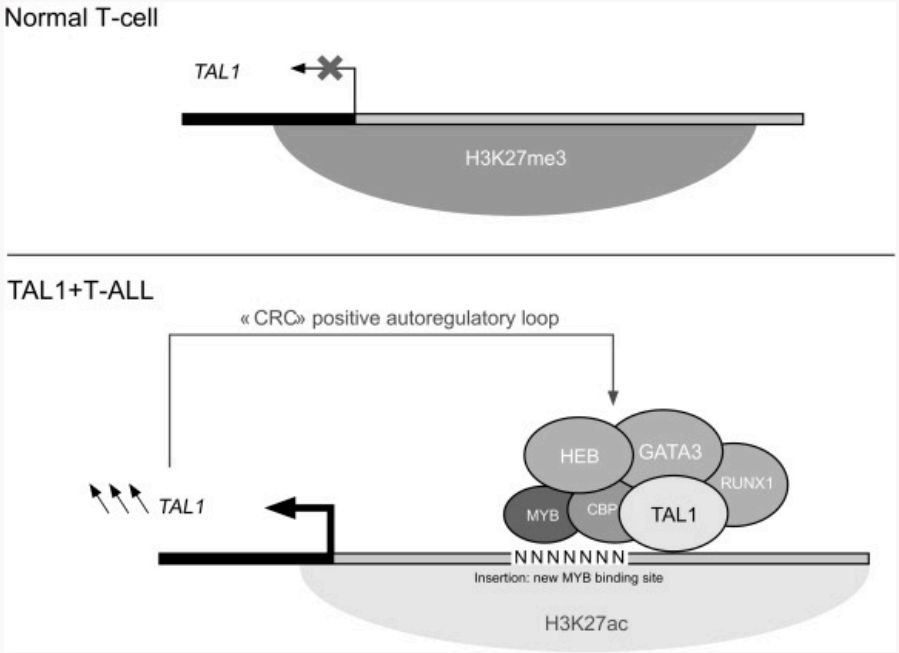
Somatic intergenic alterations are responsible of the deregulation of oncogenes.



Bradner JE, et al. Cancer. Cell. 2017 Feb9 ;168(4):629-643

Preliminary results

- Our laboratory has shown that a somatic insertion upstream of *TAL1* leads to the formation of a **neo-enhancer** and thus leads to the overexpression of *TAL1*.



Smith, C et al. "TAL1 activation in T-cell acute lymphoblastic leukemia: a novel oncogenic 3′ neo-enhancer." Haematologica vol. 108,5 1259-1271. 1 May. 2023

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- **Implement** a method for sequencing the **whole tumoral genome**.
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⇨ Implementation of the **Oxford Nanopore sequencing method** and integrate multi-omics data.

Infrastucture

Computer A for processing and analyzing the signal generated by the sequencer (MAD).

- 2x Intel Xeon Platinum 8380 CPU 2.30GHz
- 160 cores
- 503 GB RAM
- 4 nVidia A100 GPU

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Computer B for data archiving and sharing.

- 52 To fo HD in raidz1 mode (redundancy)
- LTO: magnetic tape drive (raw data archiving)

🧪 Wet lab

- Intermediate difficulty level.
- 3 half-days of work.

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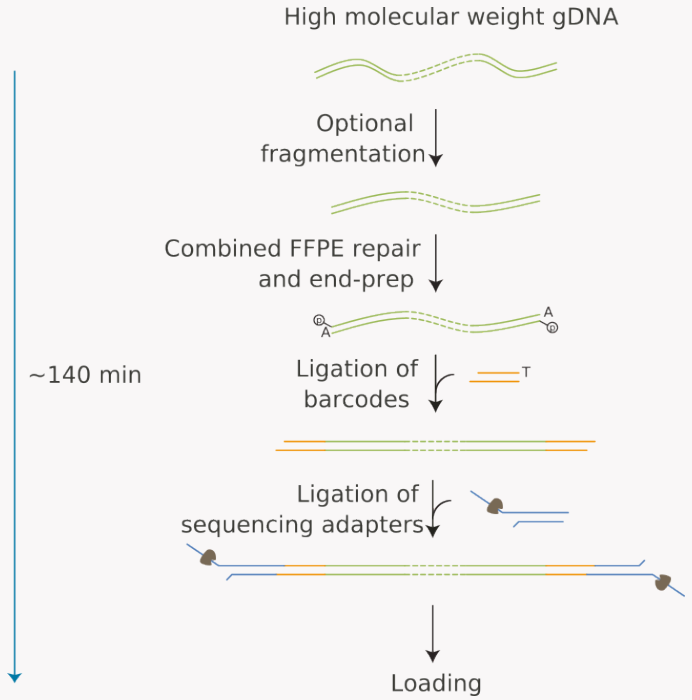
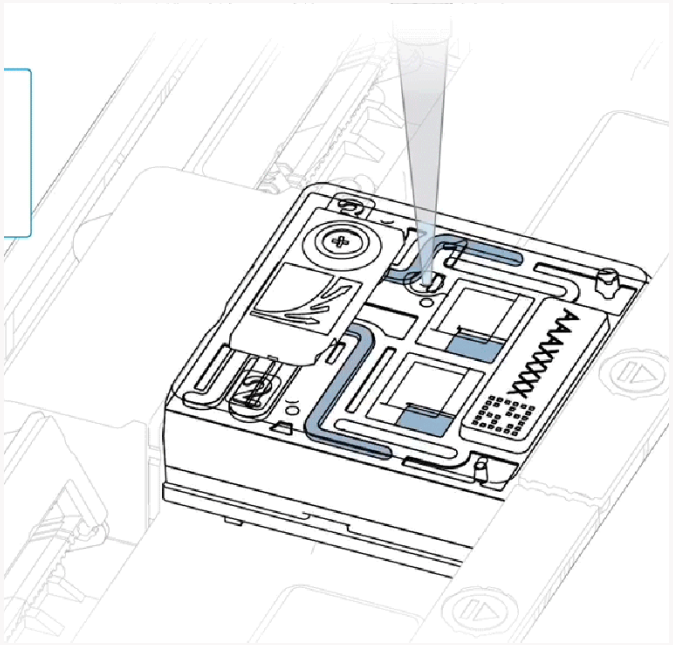
1. Genomic DNA **shearing** Covaris g-TUBE (3µg, 8,000rpm, 1min)

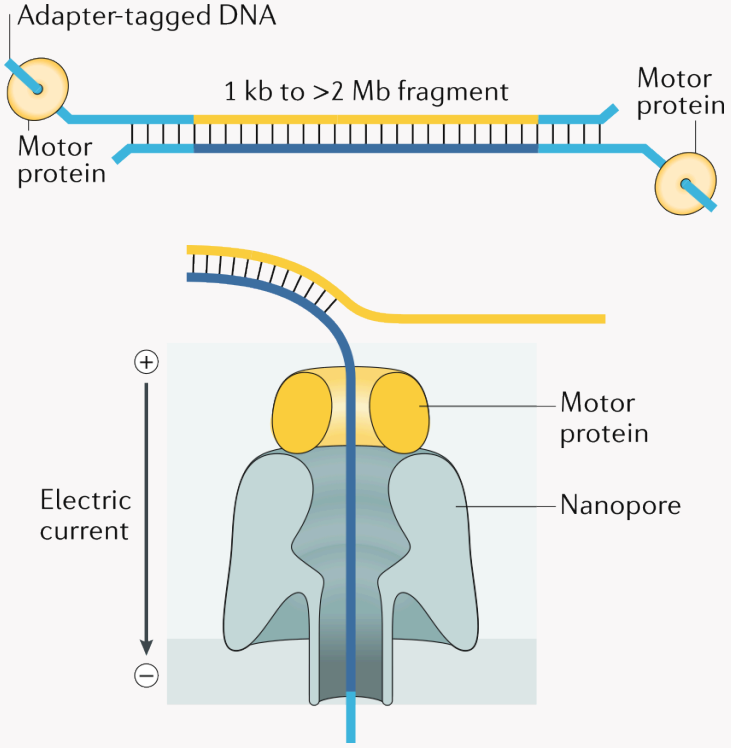
2. Preparation of the sequence library

DNA **repair** and end-prep ⇨ **barcode** ligation ⇨ **adapter** ligation



3. Flowcells **loading** and **sequencing run**

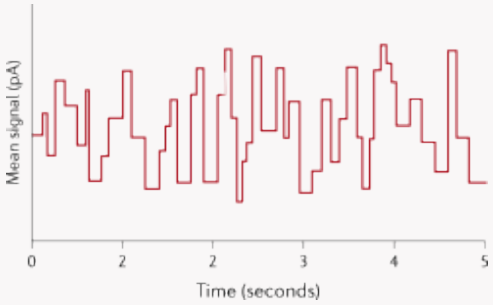




Bioinformatic Pipeline

1. **Base calling** and **Alignement** on hs1 (T2T).

Latest version of Dorado 0.8.1 with latest AI model of 5mC 5hmC modified basecalling (v5.0).



→

```
059b2db8-416c-4fdf-b7eb-3cdd3390a3bd 0 chr1 2 1
1075S5M1D8M1D36M1D4M1D116M1D111M1I485M3I595M...69M1I12M1I16M1D48M1641S * 0 0
AAGGTTAAAACCAAGACTCGCTGTGC 8;88;;<<<:::98...87877676645654456798997 qs:i:22 du:f:14.
              137 ns:i:70685 ts:i:904 mx:i:2 ch:i:1307 st:Z:2023-12-14T18:30:59.187+00:00
rn:i:36917 fn:Z:PAS34492_pass_barcode
              09_2e65ae3a_a7dfa7d7_4228.pod5 sm:f:-764.172 sd:f:0.00798761 sv:Z:pa dx:i:0
RG:Z:a7dfa7d727c0e04ecb6e9c5c3dac8080fe7cffaa_dna_r10.4.1_e8.2_400bps_sup@v4.3.0 MN:i:5392
MM:Z:C+h.,1,0,1,0,0,4,5,117,36,0,93,99,56,0,65...; ML:B:C,152,3,5,3,3,1,...; NM:i:1
              71 ms:i:4485 AS:i:4438 nn:i:0 de:f:0.0502045 tp:A:P cm:i:11 s1:i:82 s2:i:148
MD:Z:5^A8^C36^C4^A116^C...; rl:i
              :3576 SA:Z:chr8,146252402,-,1604M1D3788S,36,10;
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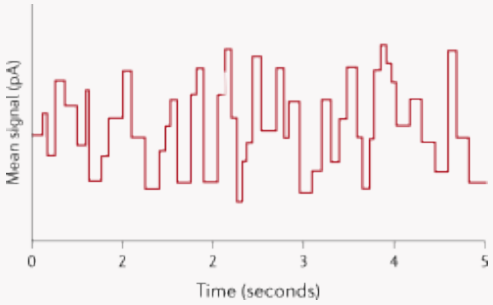

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059b2db8-416c-4fdf-b7eb-3cdd3390a3bd 0 chr1 2 1
1075S5M1D8M1D36M1D4M1D116M1D111M1I485M3I595M...69M1I12M1I16M1D48M1641S * 0 0
AAGGTTAAAACCAAGACTCGCTGTGC 8;88;;<<<:::98...87877676645654456798997 qs:i:22 du:f:14.
                137 ns:i:70685 ts:i:904 mx:i:2 ch:i:1307 st:Z:2023-12-14T18:30:59.187+00:00
rn:i:36917 fn:Z:PAS34492_pass_barcode
                09_2e65ae3a_a7dfa7d7_4228.pod5 sm:f:-764.172 sd:f:0.00798761 sv:Z:pa dx:i:0
RG:Z:a7dfa7d727c0e04ecb6e9c5c3dac8080fe7cffaa_dna_r10.4.1_e8.2_400bps_sup@v4.3.0 MN:i:5392
MM:Z:C+h.,1,0,1,0,0,4,5,117,36,0,93,99,56,0,65...; ML:B:C,152,3,5,3,3,1,...; NM:i:1
                71 ms:i:4485 AS:i:4438 nn:i:0 de:f:0.0502045 tp:A:P cm:i:11 s1:i:82 s2:i:148
MD:Z:5^A8^C36^C4^A116^C...; rl:i
                :3576 SA:Z:chr8,146252402,-,1604M1D3788S,36,10;
```
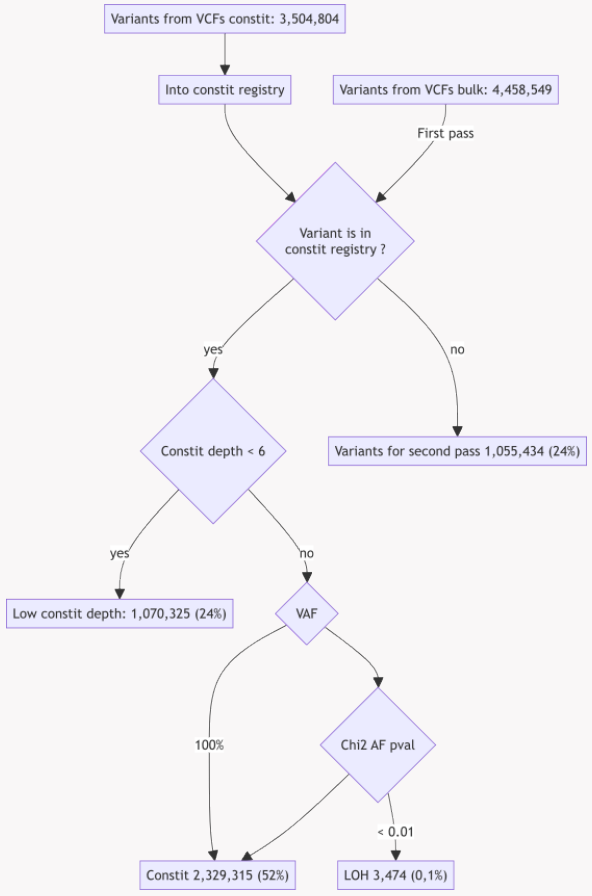

2. **Variant calling**
   - DeepVariant (Google v1.6.1) on constit and tumoral BAMs.
   - ClairS (HK-UBAL v0.1.7) takes both constit and tumoral BAMs.
   - ~~Sniffles (Fritz Sedlazeck v2.2) on constit and tumoral BAMs.~~
   - NanoMonSV (Yuichi Shiraishi v0.7.2) takes both constit and tumoral BAMs.
   - Exogene (Z. Stephens v15) viral integration.

Bioinformatic Pipeline — Aggregation

1. VCF filter



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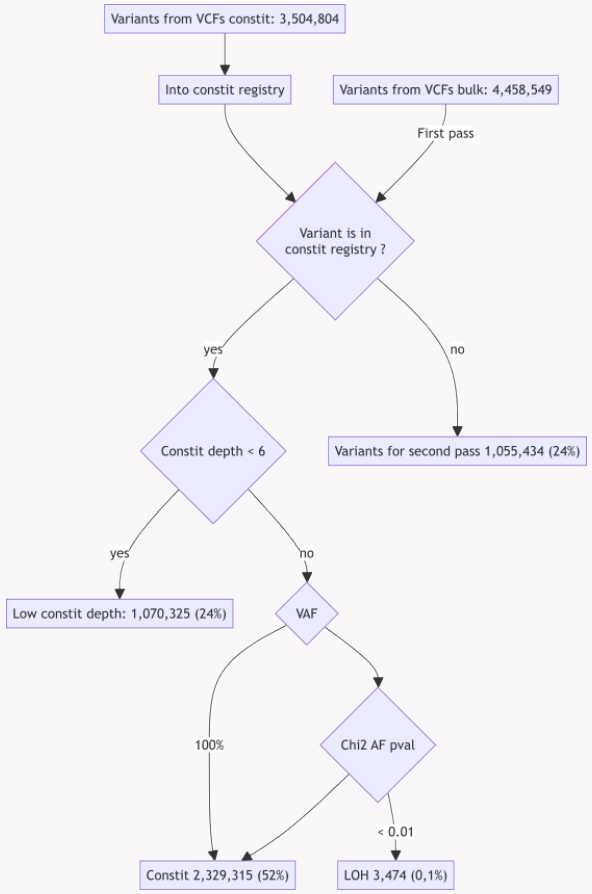
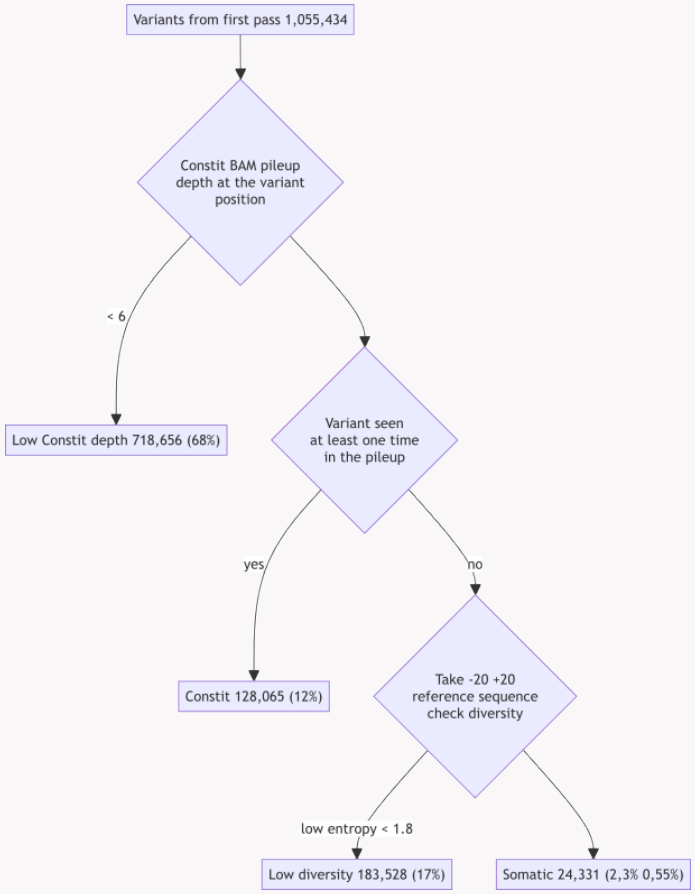
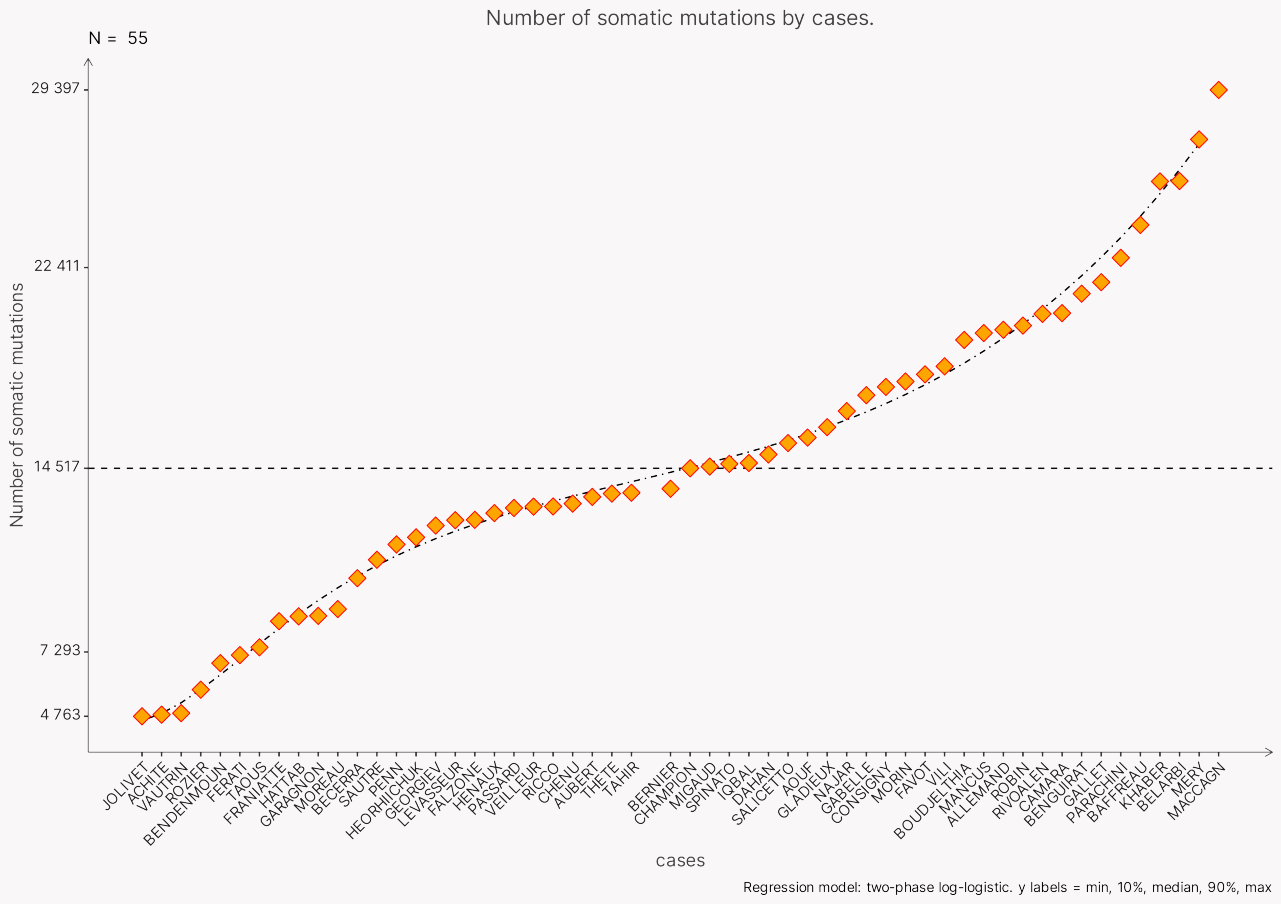


2. BAM filter



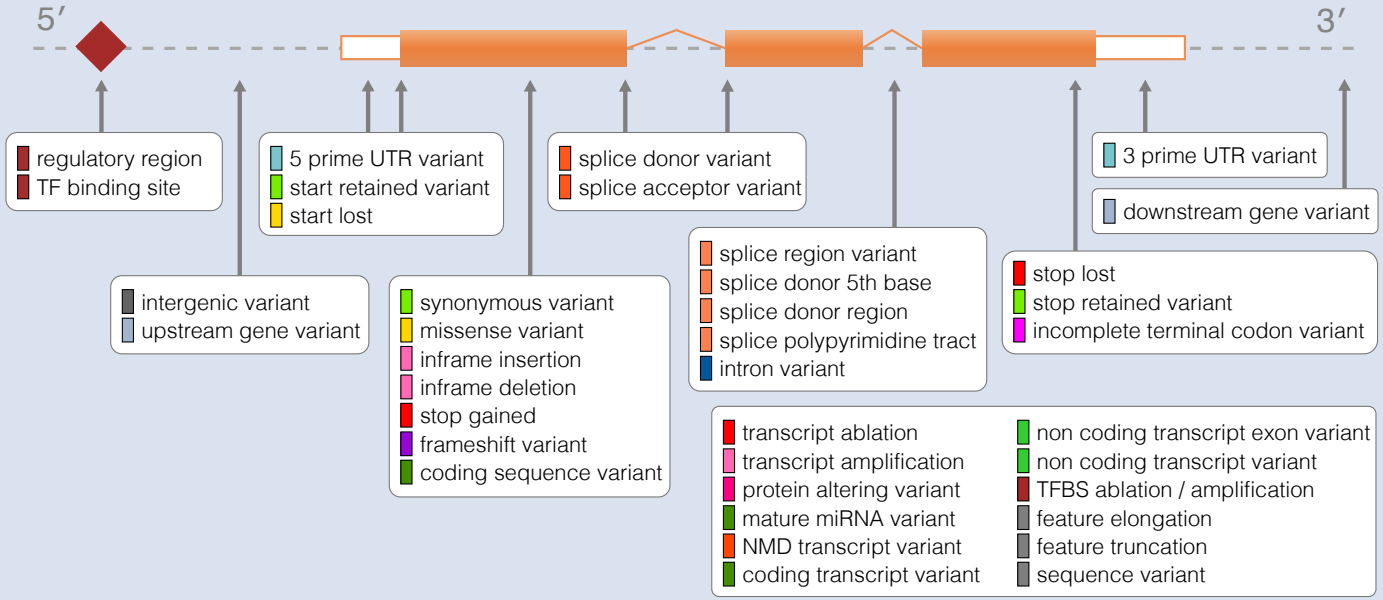
Results — Number of somatic alterations



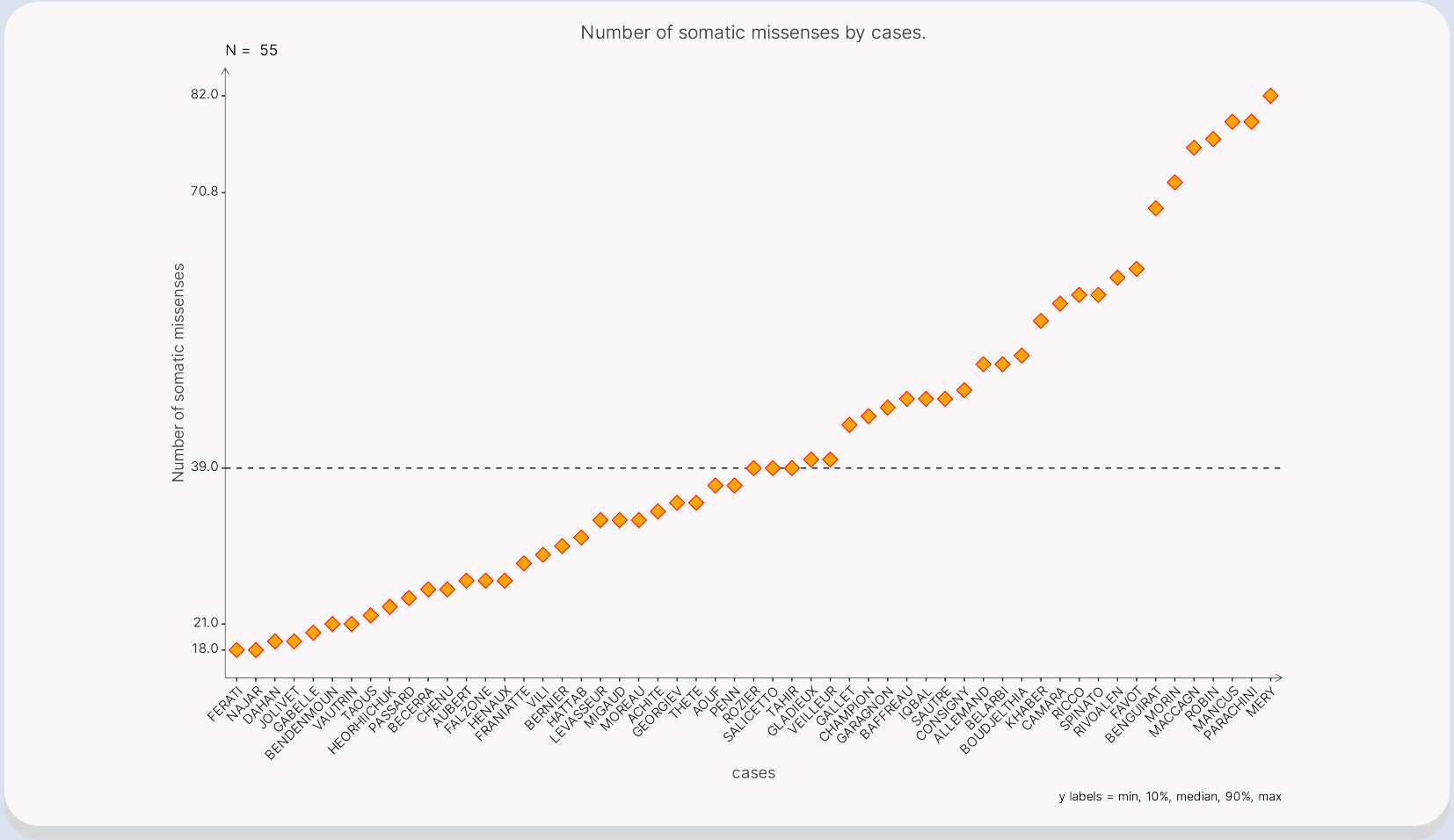
Bioinformatic Pipeline — Annotation

3. Annotations

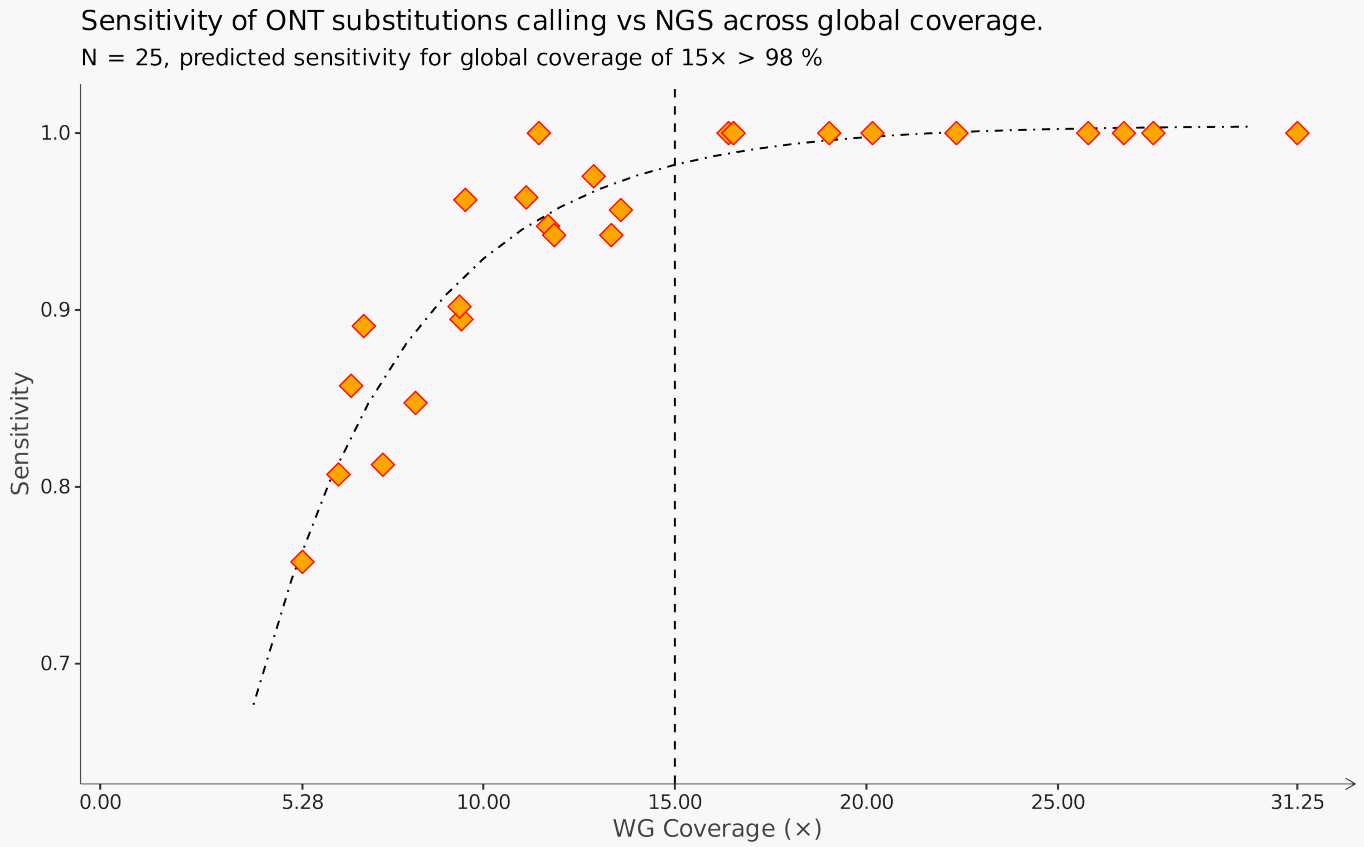
- VEP for variant consequence prediction (ensembl v112)
- Cosmic DB (latest)
- dbSNP
- NCBI genomic regions (latest)



Results — Number of somatic missense alterations



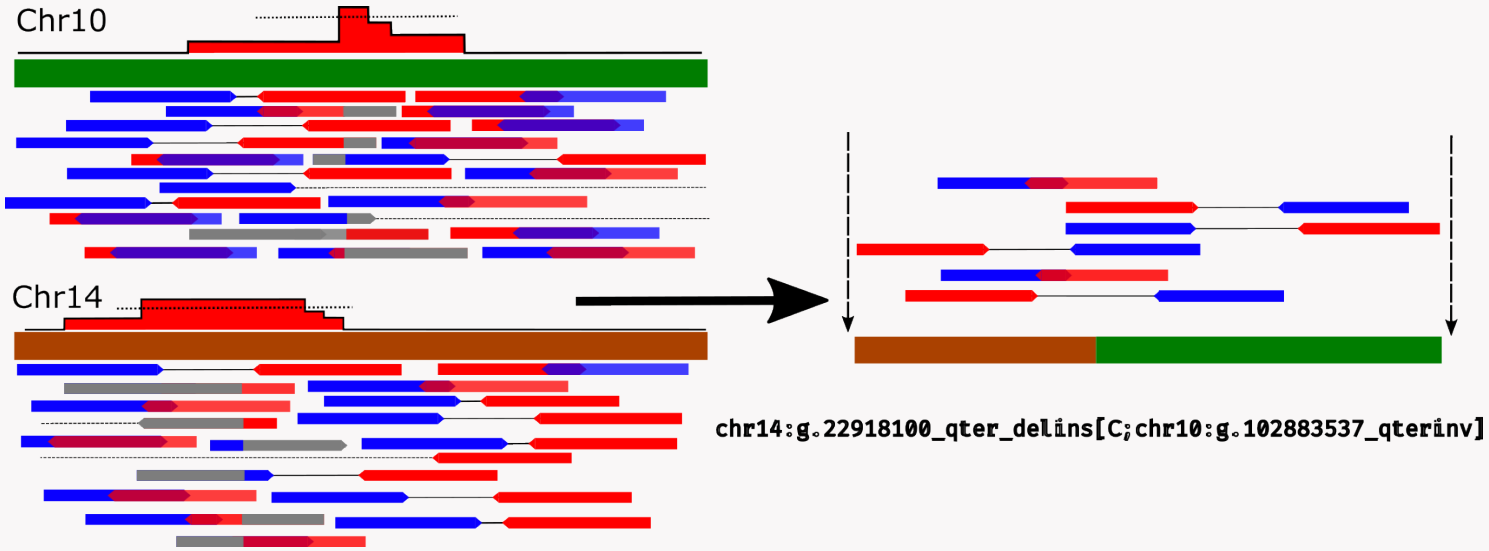
Performances — Substitution calling



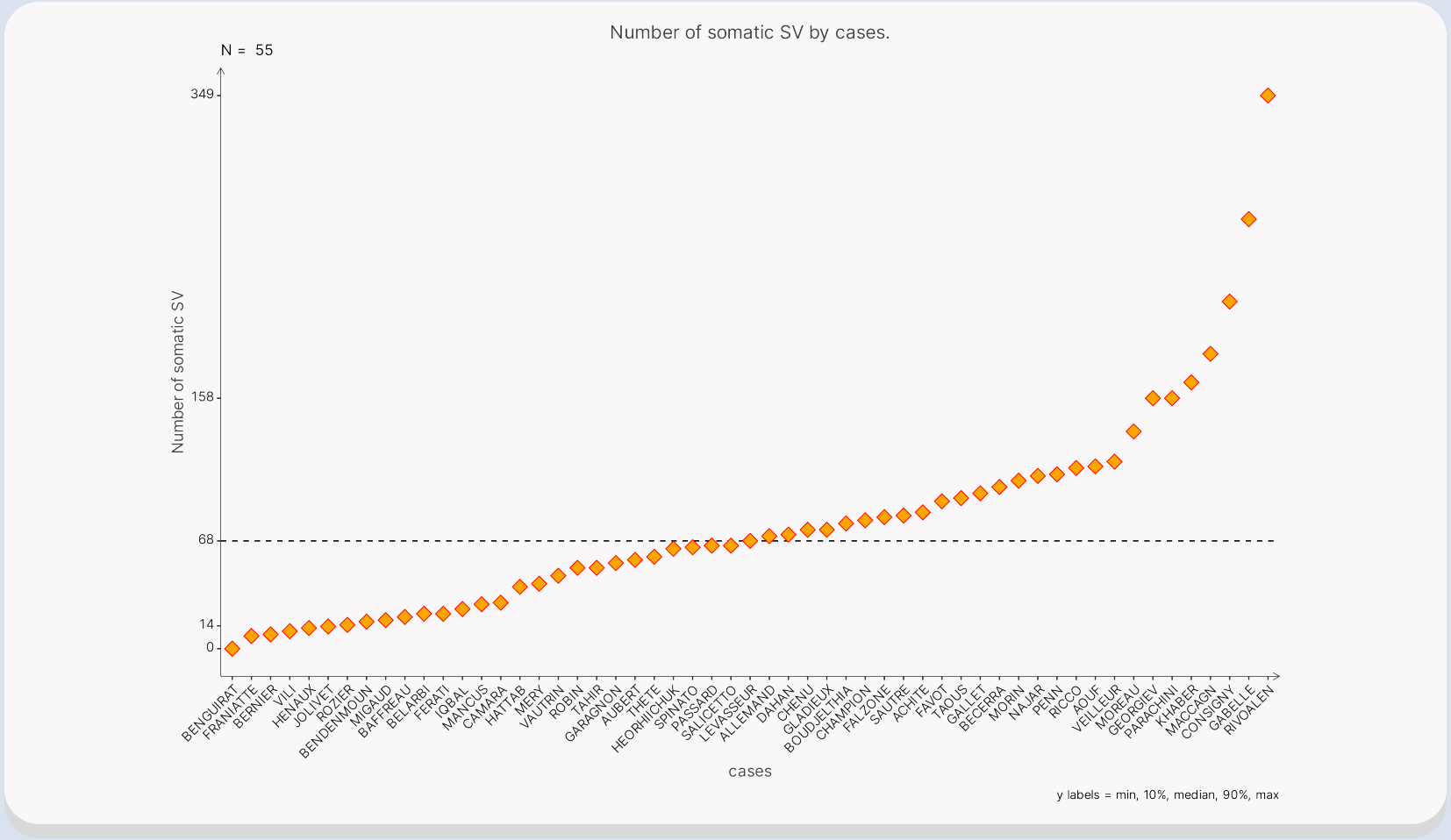
Bioinformatic Pipeline — *de novo*

Implementation of *de novo assemblage* for LRS:
Inspired by **SV-finder** (local de novo assembly)

- **Scan** alignements and select locally misaligned reads (outliers detection).
- **Assemble** them together (wtdbg2 v2.5 and spades v4.0).
- **Describe** the resulting consensus sequence (Blast, minimap2).



Results — Number of somatic SV



🔬 Visualization and interpretation of results

Development of a web service (HTMX + Bun) for **sharing, visualization, and interpretation of the results**.

DEMO (C.... ex.: *PHF6* et *AEBP2*)

Reporting

After the manual interpretation of the results and the redaction of a conclusion.

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The system generates a detailed PDF report that seamlessly integrates:

1. Detailed quality metrics (with graphics generation)
2. Interpreted genetic mutations (Pathogenic, ...)
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Example...

👥 Cohorts

Initially, to investigate our hypothesis, we decided to sequence T-ALLs harboring deregulation of the expression of known frequent oncogenes in T-ALL:

- ***TAL1*** (cis-deregulated),
- ***HOXA9*** (RT-MLPA neg),
- ***TLX1*** deregulated without genetic explanation (FISH neg and NGS panel neg).

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We also decided to sequence a cohort of pediatric T-ALLs and an adult one with deregulation of *TLX3* (Pediac/Manon project). As well as a cohort of T-ALLs **< 3 years**.

👥 Bilan des flowcells

Bilan WGS Nanopore par projet

	Fait WGS Nanopore	
projet	patients	echantillions
<3ans	20	40
	20 D+M	
TLX3 ped	13	24
	11 D+M; 2 D	
TLX3 ad	13	25
	12 D+M; 1 D	
TAL1	14	20
	6 D+M; 8 D	
TLX1	17	28
	11 D+M; 6 D	
HOXA	17	34
	17 D+M	
LBL-T	9	16
	7 D+M; 2 D	
ZFP36L2	1	2
	1 D+M	
TOTAL	104	189
TOTAL*	98	179
	81 D+M; 17 D	

* 6 patients comuns entre les projets

	Reste a faire	
projet	patients	echantillions
<3ans	0	0
TLX3 ped	1	2
	1 D+M	
TLX3 ad	0	0
TAL1	3	5
	2 D+M ; 1 D	
TLX1	0	0
HOXA	8	16
	8 D+M	
LBL-T	8	16
	8 D+M	
ZFP36L2	1	2
	1 D+M	
TOTAL	21	41

commandes FC WGS :

1- Commande en Mai de 40 pack de FC (soit 160 FC)

a. Réception de 5 pack (20 FC) en Juin – reste 0

b. Réception de 4 pack (16 FC) en septembre - reste 16

2- Réception prévu en décembre : 13 pack (52 FC) – reste 52

3- Réception prévu en Mars 2025 : 17 pack (68 FC) – reste 68

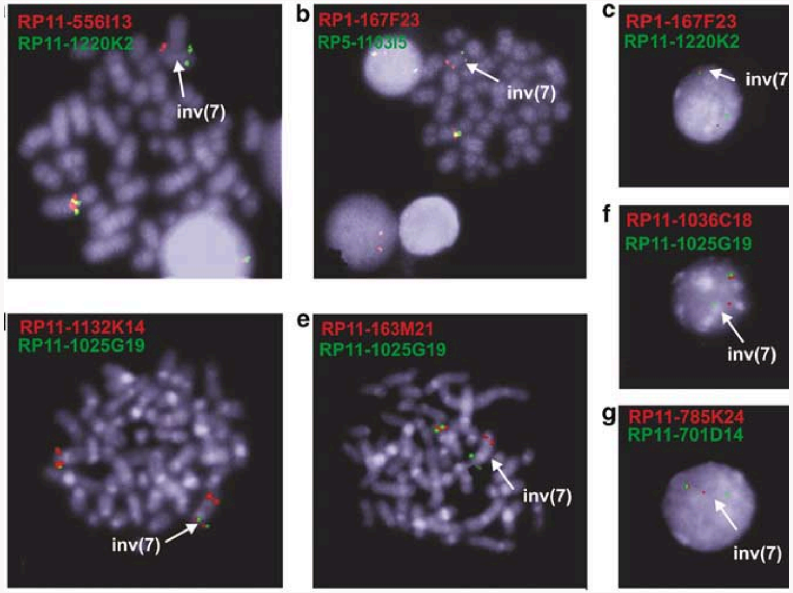
👥 Cohort *HOXA* N = 14

id	X	mécanisme	interpretation	info
ALLEMAND	11	Multiples mutations de *MLLT3* + hotspot CSF3R	❓	
BELARBI	13	Altération de GATA3 + KMT2C	❓	
BERNIER	15	DPP10::SET	✅	
BOUDJELTHIA	13	KMT2E ter	❓	JAK/STAT
GARAGNON	13	TRB::HOXA inv7	✅	JAK/STAT
HATTAB	11	AFDN::KMT2A	✅	
KENNOUCHE	*en cours*			
LAVIDALE	*en cours*			
MANCUSO	10	TNRC18::KMT2A	✅	
MERY	16	TRB::HOXA inv7	✅	JAK/STAT
MICHELAS	*en cours*			
MIGAUD	26	mutation EN1 (homeobox)	❓	
MORIN	22	TRB::HOXA inv7	✅	JAK/STAT
SAUTRE	28	AFDN::KMT2A	✅	

Interesting results: *HOXA9*

ME

- **Inv(7)(p15q34)** TRB/HOXA10
- `chr7:27,287,813_delins[ATGGGGGGGG_chr7:144,128,326inv]`
- `chr7:27,313,165_delins[GATGG_chr7:144,161,537inv]`



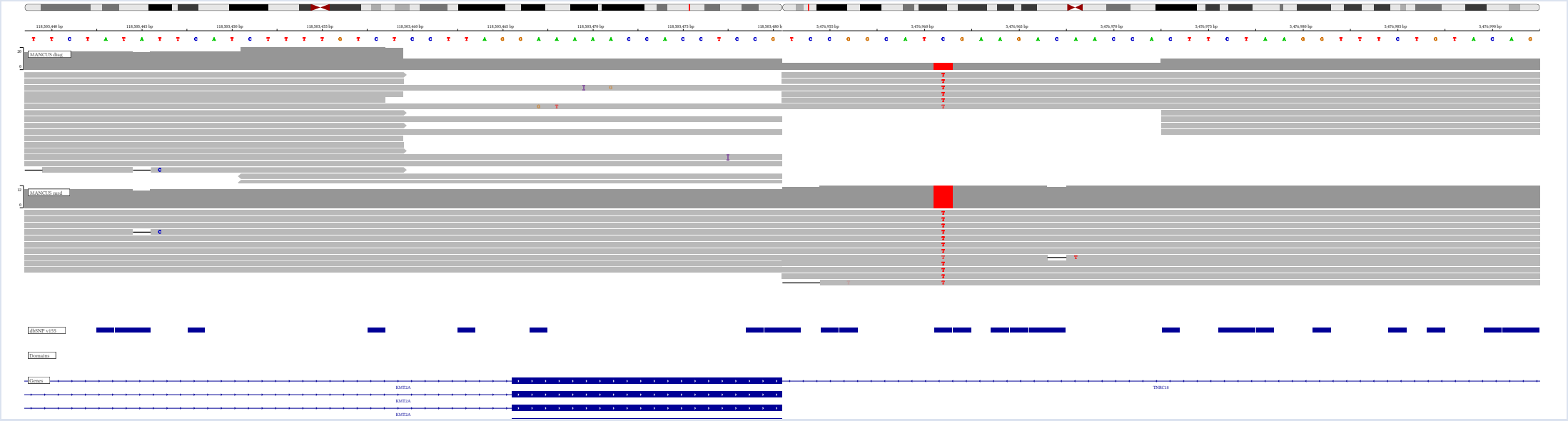
Résultats FISH break apart.

Speleman, F et al. "A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias." Leukemia vol. 19,3 (2005): 358-66.

Interesting results: *HOXA9*

MA

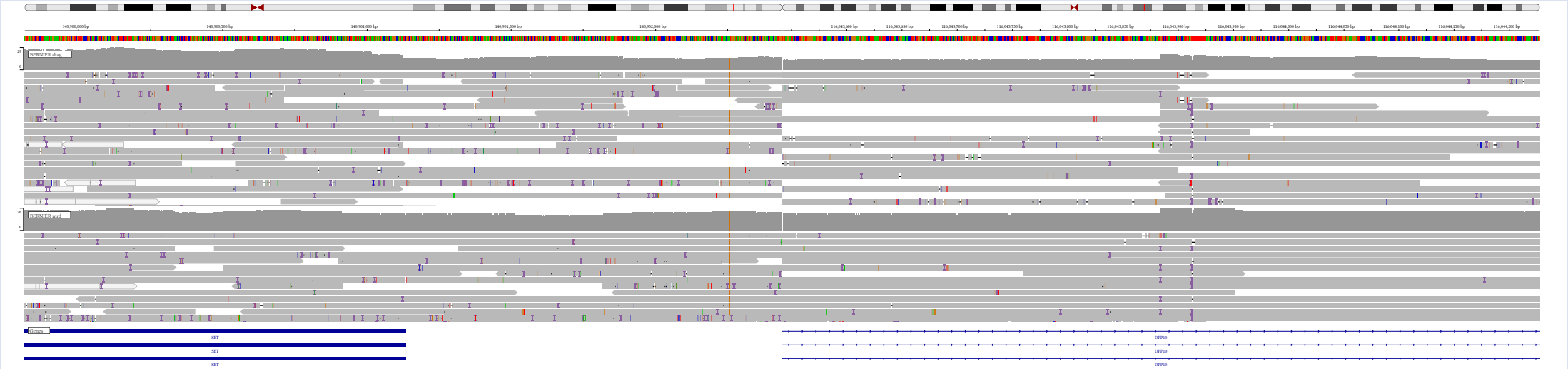
- t(7;11)(p22;q23) likely ***KMT2A::TNRC18***
- `chr11:118,503,451(KMT2A)_delins[CCCC_chr7:5476973(TNRC18)]`
- Known fusion transcript (2 pediatric cases / 759 LAL. Meyer, C et al. Leukemia 2009)
- RT-MLPA probes w/o TNRC18



Interesting results: *HOXA9*

BE

- Translocation t(2;9)(q14.1;q34.11) likely fusion transcript ***SET::DPP10***
- `chr9:140,901,114(SET)_delins[GAACATAAAGAAAAAAA_chr2:116,043,899(DPP10)]`
- **New fusion transcript in T-ALL** (only described one time in CRC: Xia, Li C et al. “Identification of large rearrangements in cancer genomes with barcode linked reads.” Nucleic acids research vol. 46,4 2018)



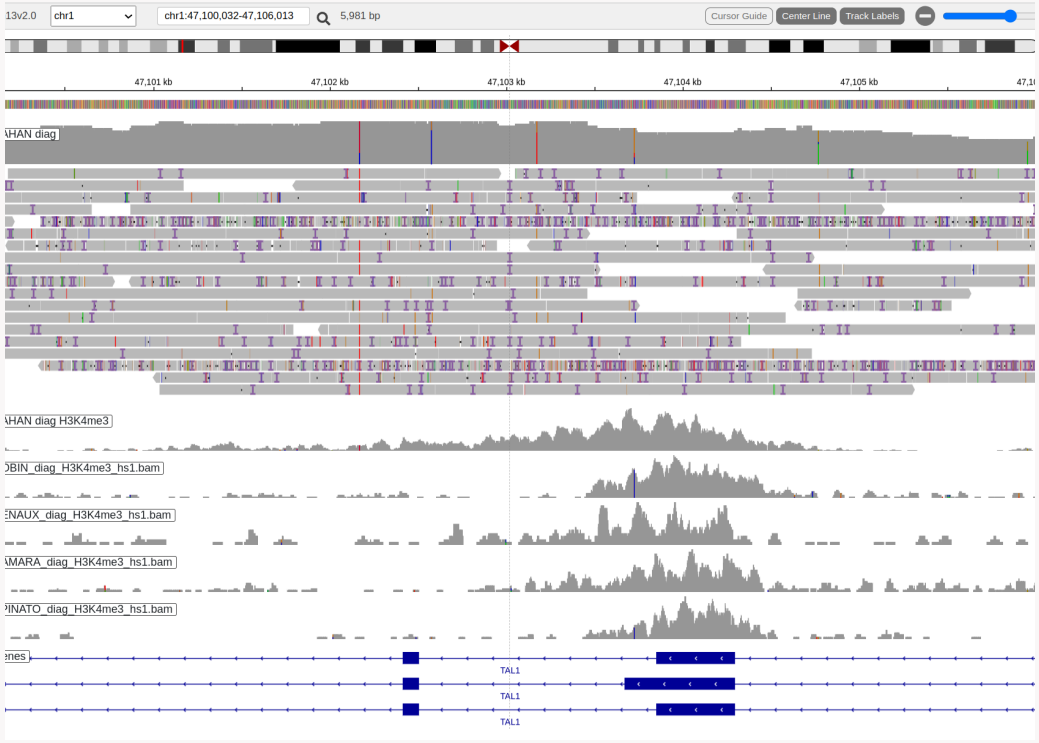
👥 Cohort *TAL1* N = 8

id	X	mécanisme	interpretation
ACHITE	12	t(1;17) *TAL1::RPTOR*	✅
BECERRA	12	*TCR::LMO2*	❓
CAMARA	20	insertion dans *MYB*	❓
COLLE	7		
DAHAN	13	duplication intronique *TAL1* (ex2-3)	❓
DOUYERE	22 pas mrd	(*BCL11B::TLX3*)	❓
FALLE	*en cours*		
GILLOUX	15 pas mrd	pas d'altération locus *TAL1*	❓
HENAUX	12	ins 9 nt ex3-4	❓
JEANSELME	*en cours*		
ROBIN	14	*LMO2::RAG1* (del 11q)	❓
SCHOLZ	16		

Interesting results: *TAL1*

DAH

- Mono-allelic surexpression of TAL1.
- Somatic tandem duplication between exon 2 and 3 of TAL1.
- Also observed in ChIP-seq data showing broad H3K4me3 coverage.



👥 Cohort *TLX1* N = 12

id	X	mécanisme	interpretation
AUBERT	17	del 10q (10mb) *TLX1*	✅
CHAMPION	32	inv10	✅
LEVASSEUR	20	inv10 (avec 5' UTR ralongé)	✅
PARACHINI	17	t(10;14)	✅
PASSARD	18	chromotripsis du 10	✅
PAYSAN	*en cours*		
RIVOALEN	19	inv10	✅
SALICETTO	12 pas mrd	inv10	✅

Interesting results: TLX1

LEV

- Surexpression of TLX1.
-
-

Interesting results: TLX1

LEV

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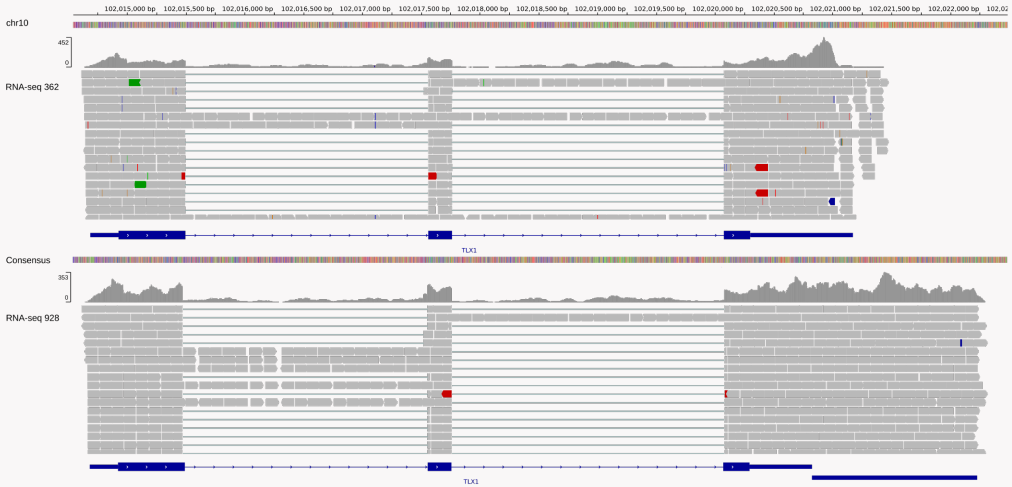
- Inv10
-

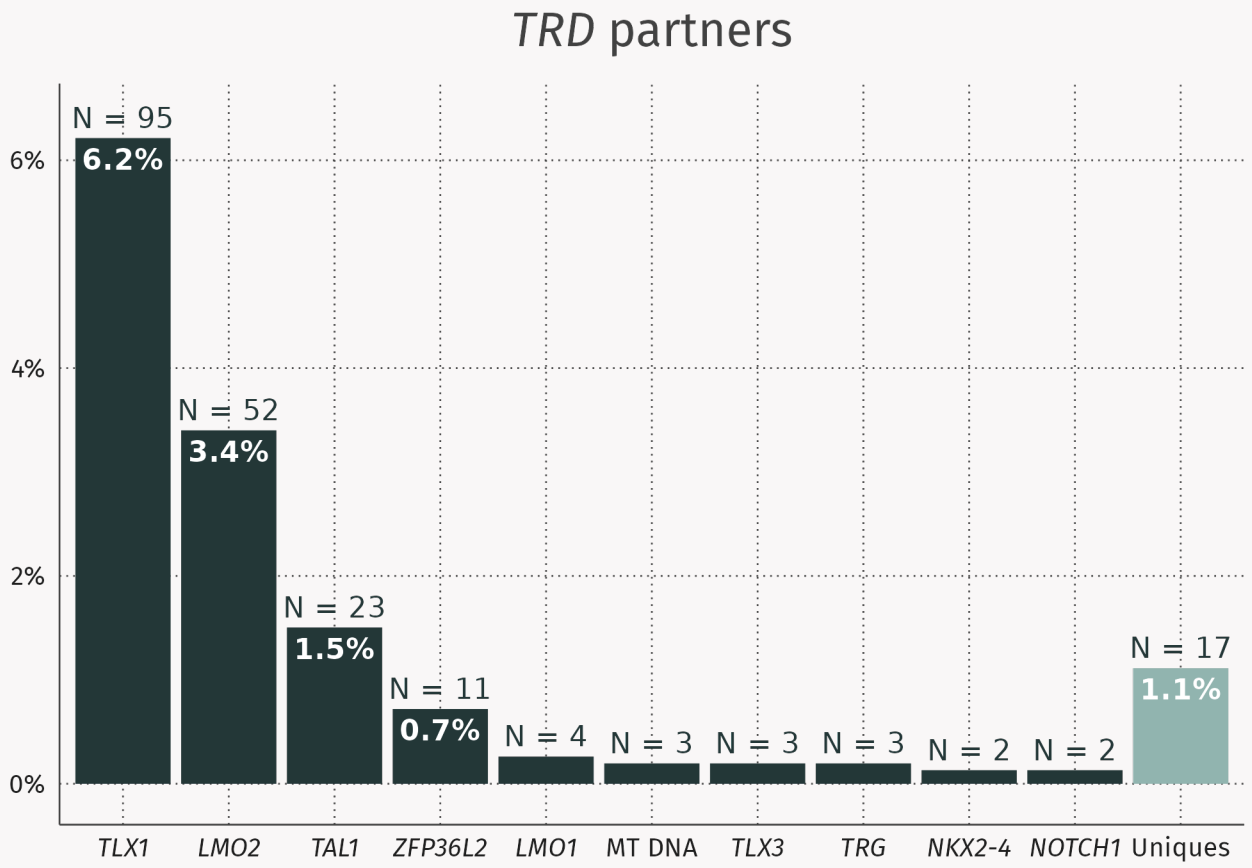
Interesting results: TLX1

LEV

- Surexpression of TLX1.
- Inv10
- Modification of 3′ UTR.



Discussion


TRD partners
6%
4%
2%
0%
N = 95
6.2%
N = 52
3.4%
N = 23
1.5%
N = 11
0.7%
N = 4
N = 3
N = 3
N = 3
N = 2
N = 2
N = 17
1.1%
TLX1
LMO2
TAL1
ZFP36L2
LMO1
MT DNA
TLX3
TRG
NKX2-4
NOTCH1
Uniques

🏁 Conclusions

What is **done**

- ✅ The implementation of a robust and more informative whole-genome sequencing method.
- ✅ The development of a pipeline for detecting somatic alterations (SNV, SV, viral insertion) as well as a simple way to visualize and interpret the results.
- ✅ Compare missense/indels variant calling with NGS panel.
- ✅ The integration, if available, of RNAseq / ChIPseq.
- ✅ Develop a de novo assembly pipeline (better accuracy and visualization of SV).
- ✅ Automate reporting.

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TODO

- ❌ Functional experiments ?
- ❌ Complete sequencing of cases (> 200).
- ❌ Aggregate results by cohorts and tag recurrency (at gene and mutation levels).

Remerciements

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