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

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07 October 2024

Dr. Thomas Steimlé (MD)

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

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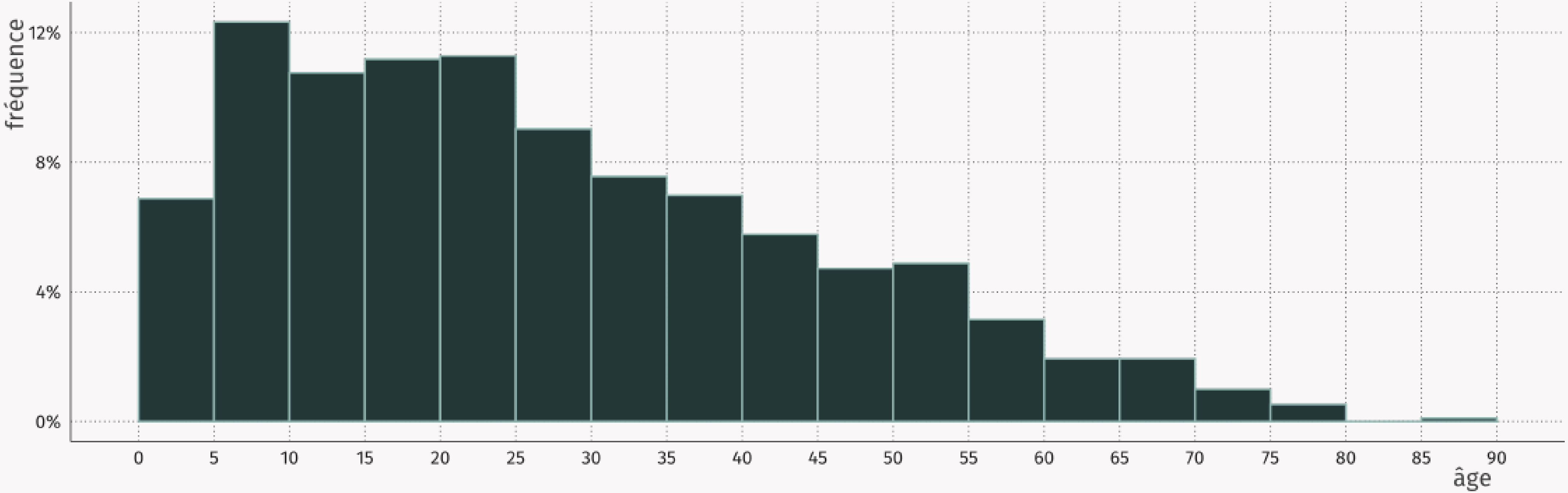
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

❓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 ($\simeq$ 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Internal unpublished data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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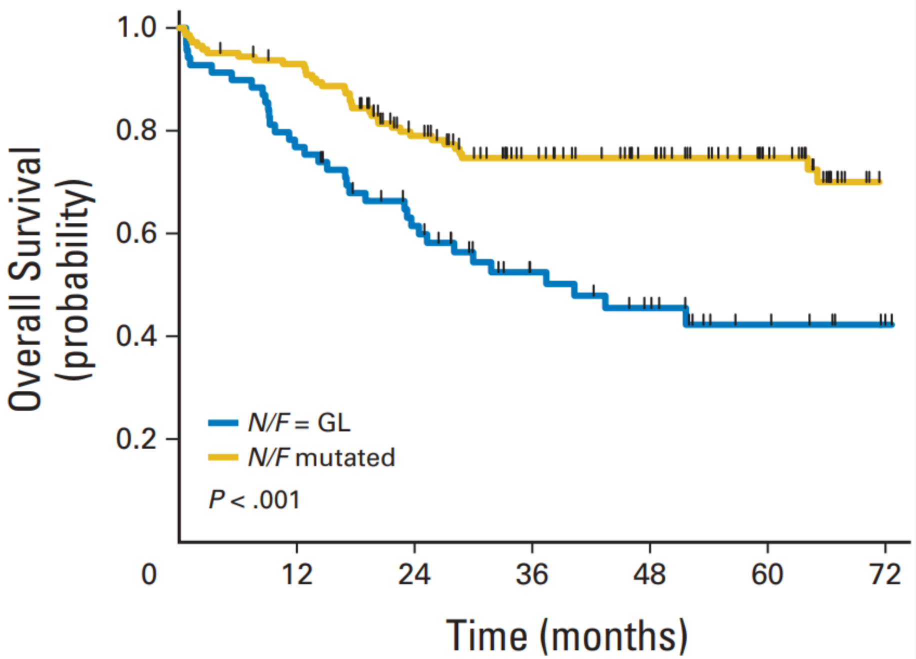
- Adults OS 3 years: 67% (GRAALL-2005)

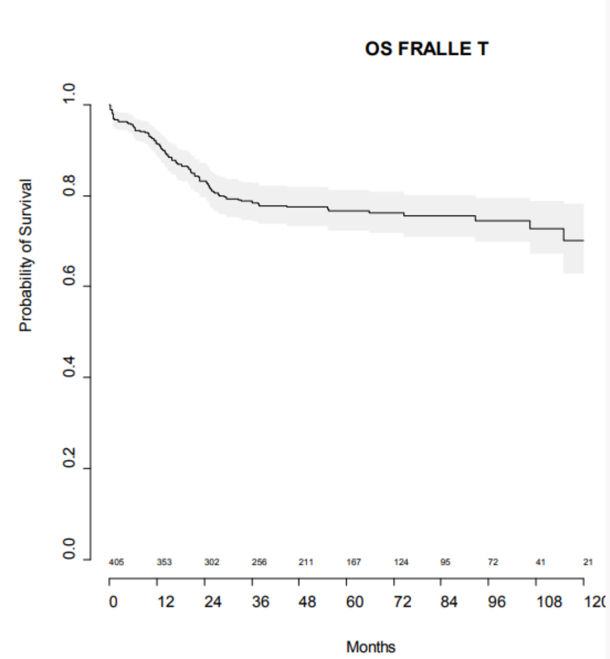


Trinquand A et al. J Clin Oncol. 2013;31(34):4333-4342

👨‍⚕️ Model — T-ALL

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

- Adults OS 3 years: 67% (GRAALL-2005)
- 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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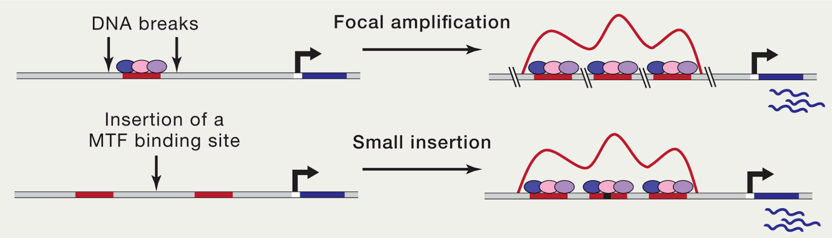
We need new treatments to address these cases !

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

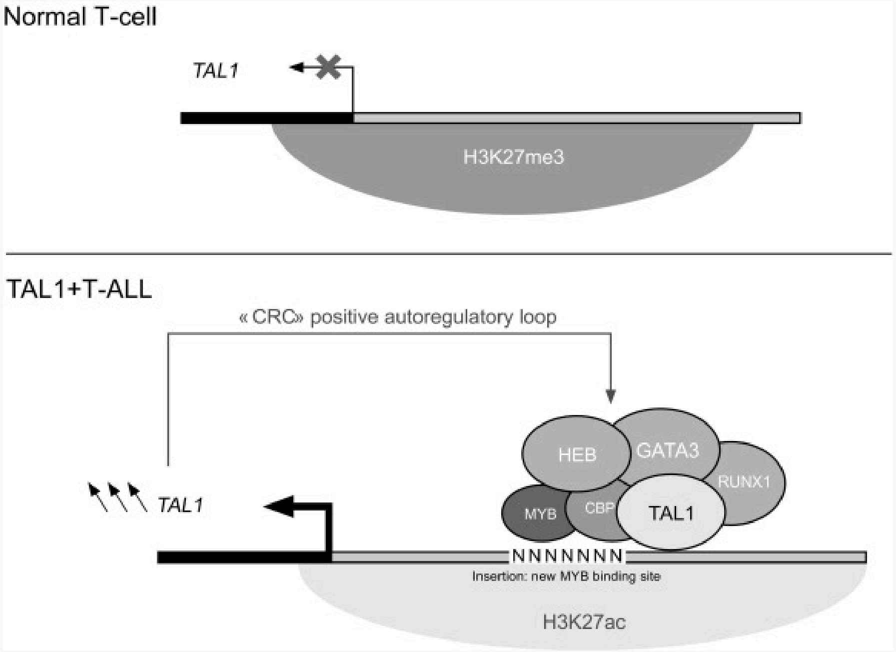
Somatic intergenic alterations are responsible of the deregulation of oncogenes.



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

🎯 Goals

- Implement a method for sequencing the whole tumoral genome.
- Detect structural variations (SV) and SNV with good sensitivity/specificity.
- Describe a set of somatic intergenic alterations likely responsible for the deregulation of known oncogenes.
- Discover similar alterations deregulating genes not known to be oncogenes.

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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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- 4 nVidia A100 GPU

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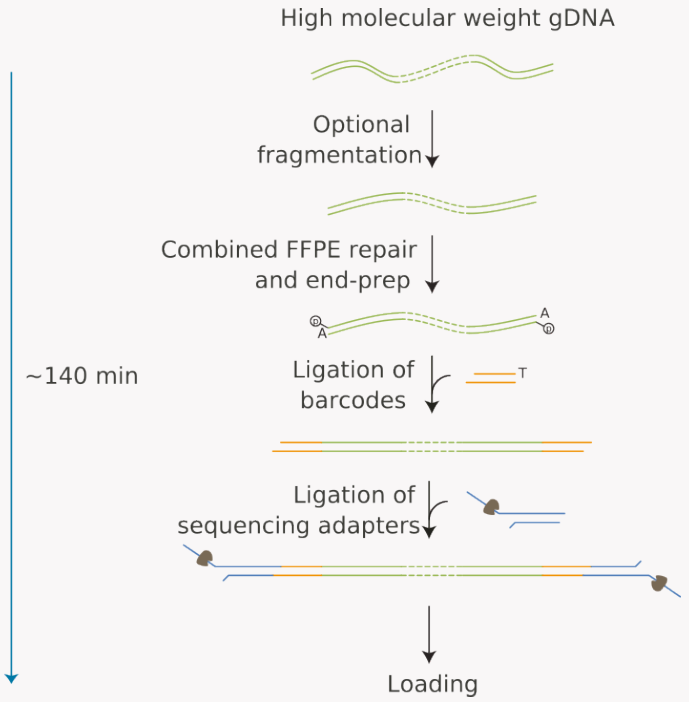
1. Genomic DNA shearing Covaris g-TUBE (3µg, 8,000rpm, 1min)

🧪 Wet lab

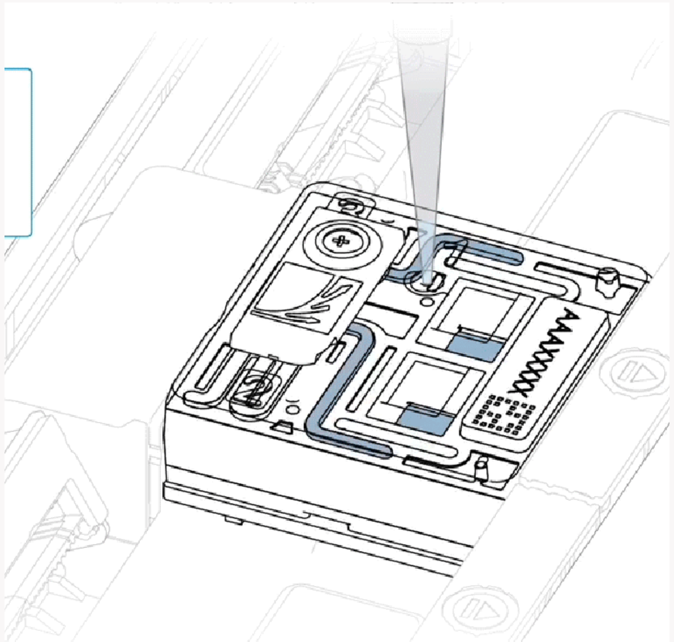
2. Preparation of the sequence library

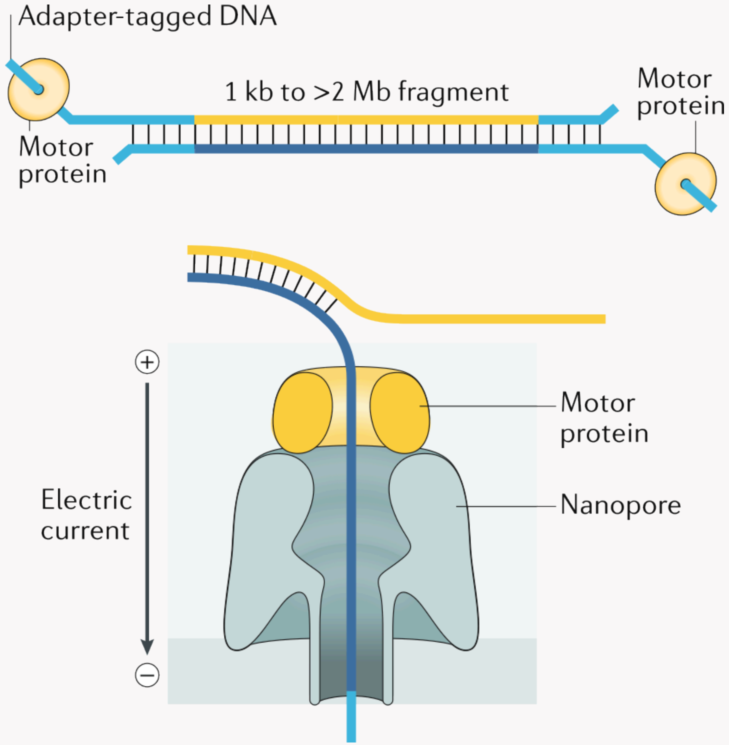
DNA repair and end-prep ⇨ barcode ligation ⇨ adapter ligation



Wet lab

3. Flowcells loading and sequencing run

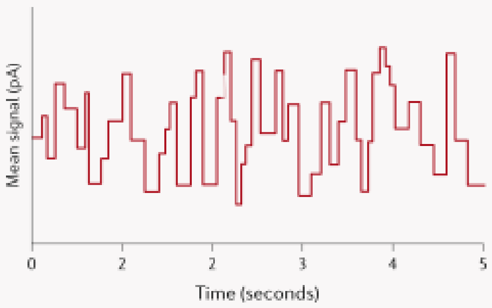




💾 Bioinformatic Pipeline

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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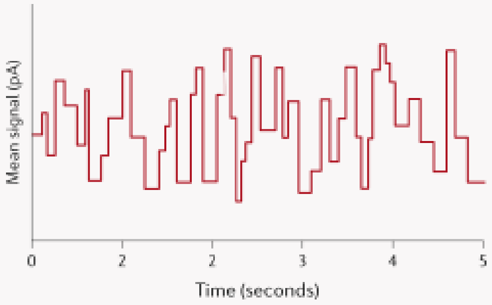

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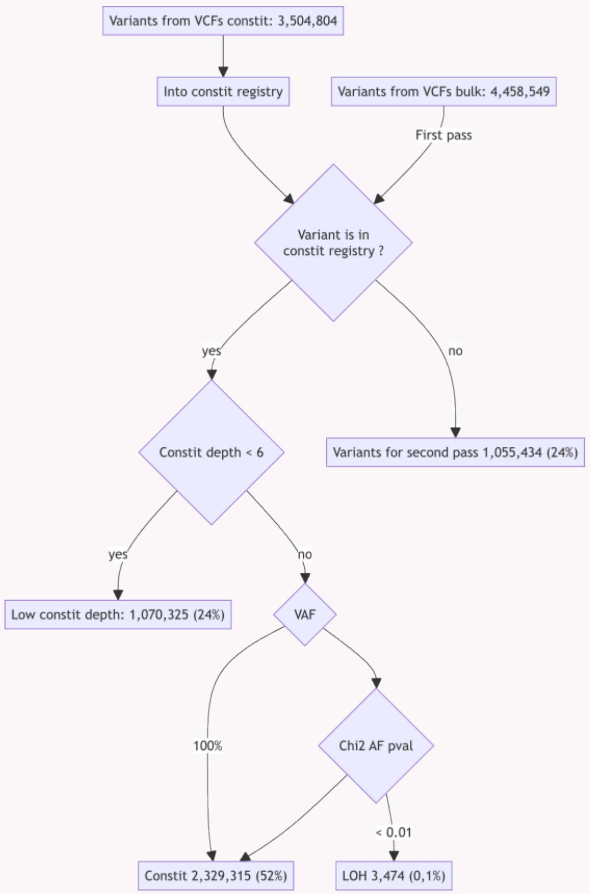
1075S5M1D8M1D36M1D4M1D116M1D111M1I485M3I595M...69M1I12M1I16M1D48M1641S * 0 0
AAGGTTAAAACCAAGACTCGCTGTGC 8;88;;<<<:::98...87877676645654456798997 qs:i:22 du:f:14.
               137 ns:i:70685 ts:i:904 mx:i:2 ch:i:1307 st:Z:2023-12-14T18:30:59.187+00:00
rn:i:36917 fn:Z:PAS34492_pass_barcode
               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



Variants from VCFs constit: 3,504,804

Into constit registry

Variants from VCFs bulk: 4,458,549

First pass

Variant is in constit registry ?

yes

no

Constit depth < 6

Variants for second pass 1,055,434 (24%)

yes

no

Low constit depth: 1,070,325 (24%)

VAF

100%

Chi2 AF pval

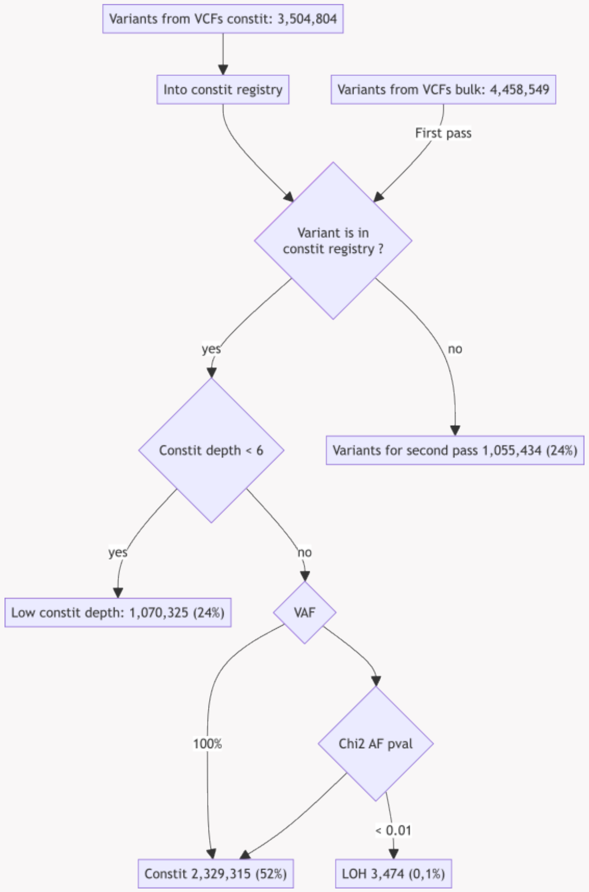
< 0.01

Constit 2,329,315 (52%)

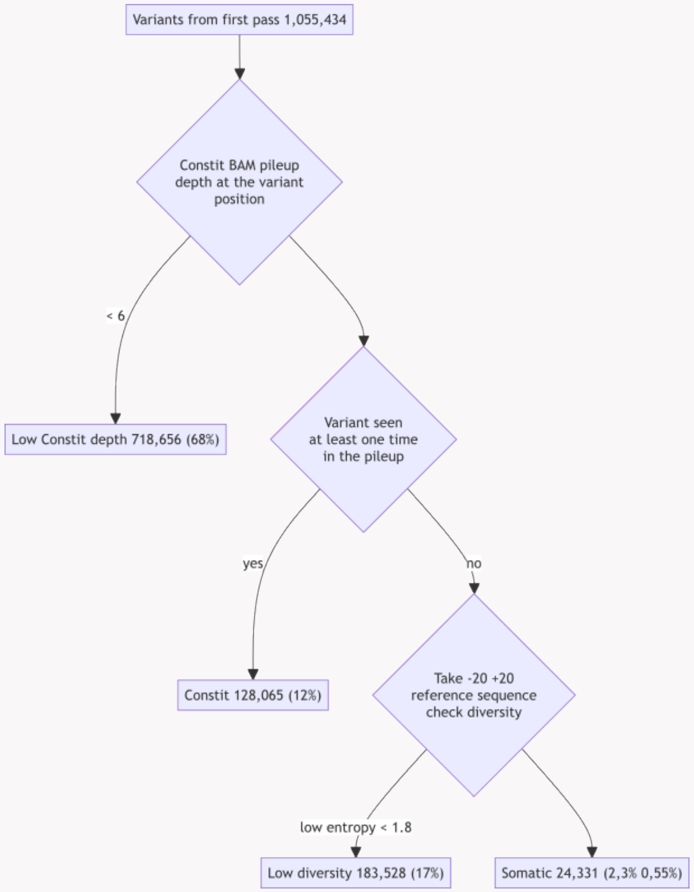
LOH 3,474 (0,1%)

💾 Bioinformatic Pipeline – Aggregation

1. VCF filter



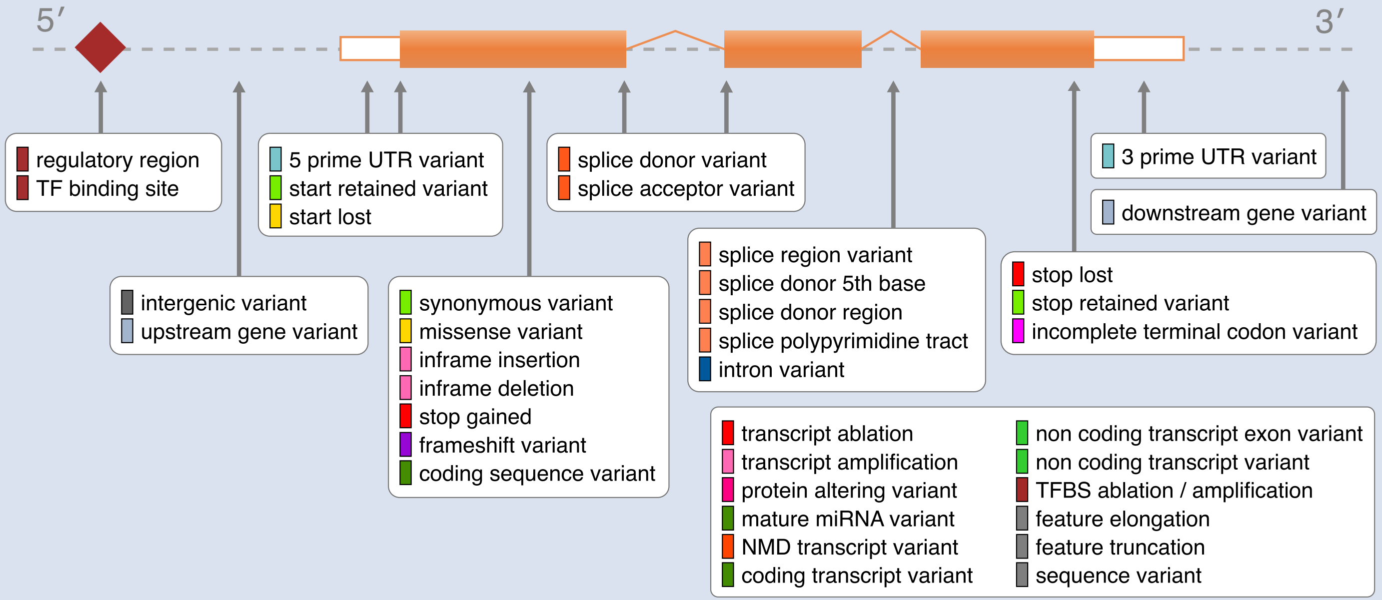
2. BAM filter



💾 Bioinformatic Pipeline – Annotation

3. Annotations

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



🟥 regulatory region
🟥 TF binding site

🟦 5 prime UTR variant
🟩 start retained variant
🟨 start lost

🟧 splice donor variant
🟧 splice acceptor variant

🟦 3 prime UTR variant

⬛ downstream gene variant

⬛ intergenic variant
⬛ upstream gene variant

🟩 synonymous variant
🟨 missense variant
🟪 inframe insertion
🟪 inframe deletion
🟥 stop gained
🟪 frameshift variant
🟩 coding sequence variant

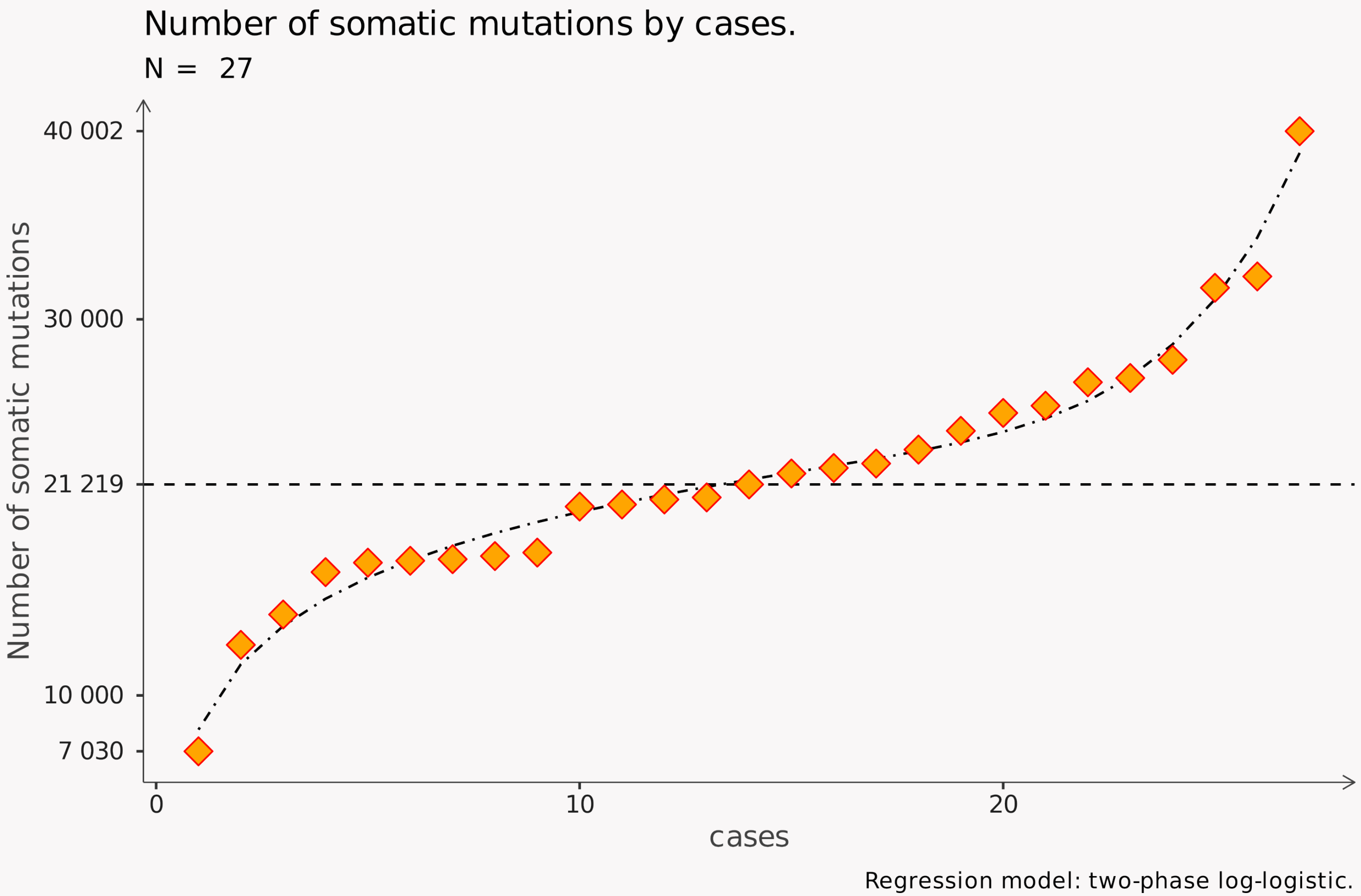
🟧 splice region variant
🟧 splice donor 5th base
🟧 splice donor region
🟧 splice polypyrimidine tract
🟦 intron variant

🟥 stop lost
🟩 stop retained variant
🟪 incomplete terminal codon variant

🟥 transcript ablation
🟪 transcript amplification
🟪 protein altering variant
🟩 mature miRNA variant
🟧 NMD transcript variant
🟩 coding transcript variant

🟩 non coding transcript exon variant
🟩 non coding transcript variant
🟥 TFBS ablation / amplification
⬛ feature elongation
⬛ feature truncation
⬛ sequence variant

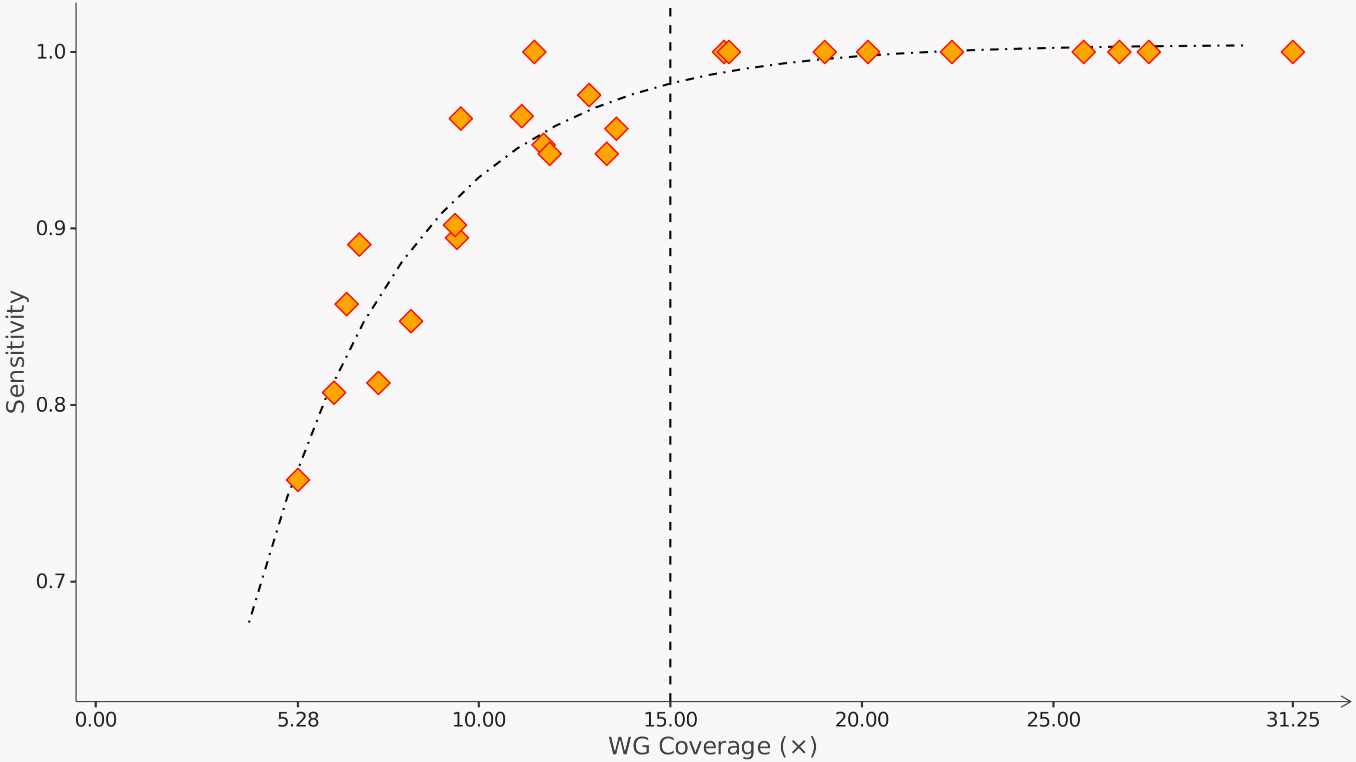
Results – Number of somatic alterations



Performances – Substitution calling

Sensitivity of ONT substitutions calling vs NGS across global coverage.

N = 25, predicted sensitivity for global coverage of 15× > 98 %

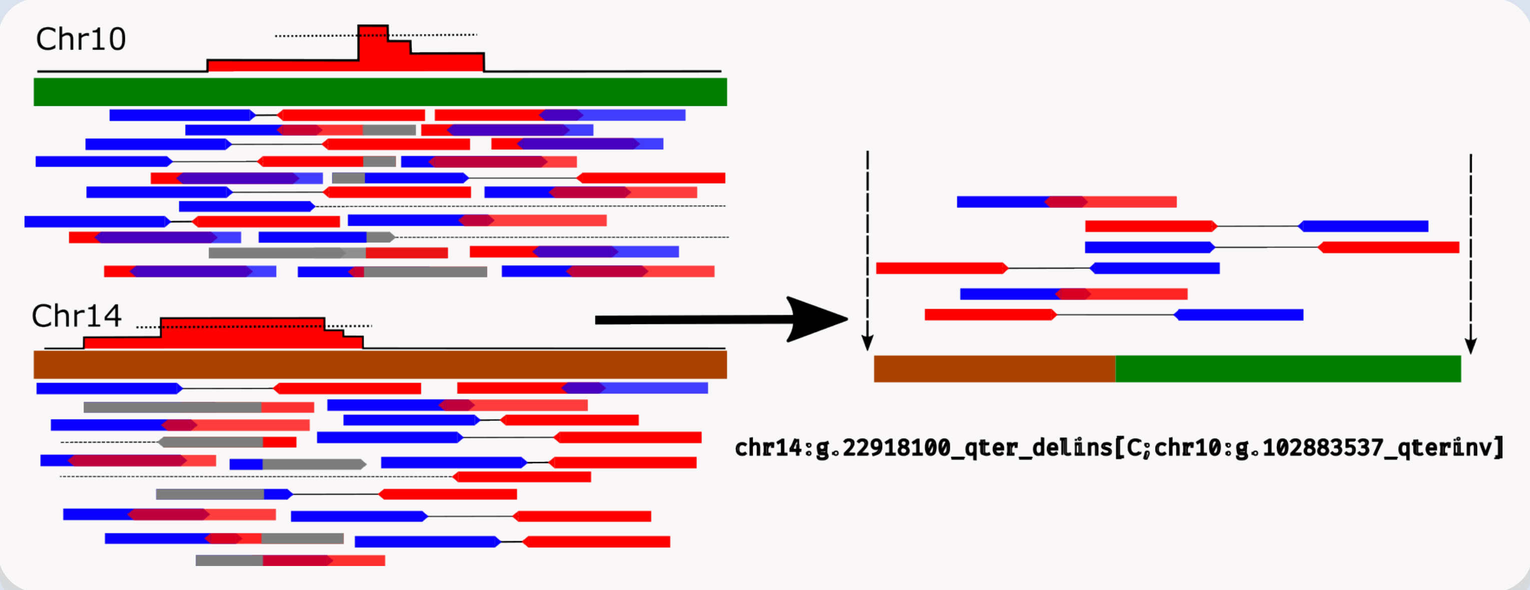


Calling on both NGS and ONT: DeepVariant (v1.6.1), coverage: cramino (v0.9.0), regression model: asymptotic.
342 930 genomic positions for each case were analysed. Specificity is always > 99 %.

Bioinformatic Pipeline – de novo

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

- 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).



🔬 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 interpretation of the results and the redaction of a conclusion.

The system generates a detailed PDF report that seamlessly integrates:

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

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 ,
- HOXA9 and
- TLX1 deregulated without genetic explanation.

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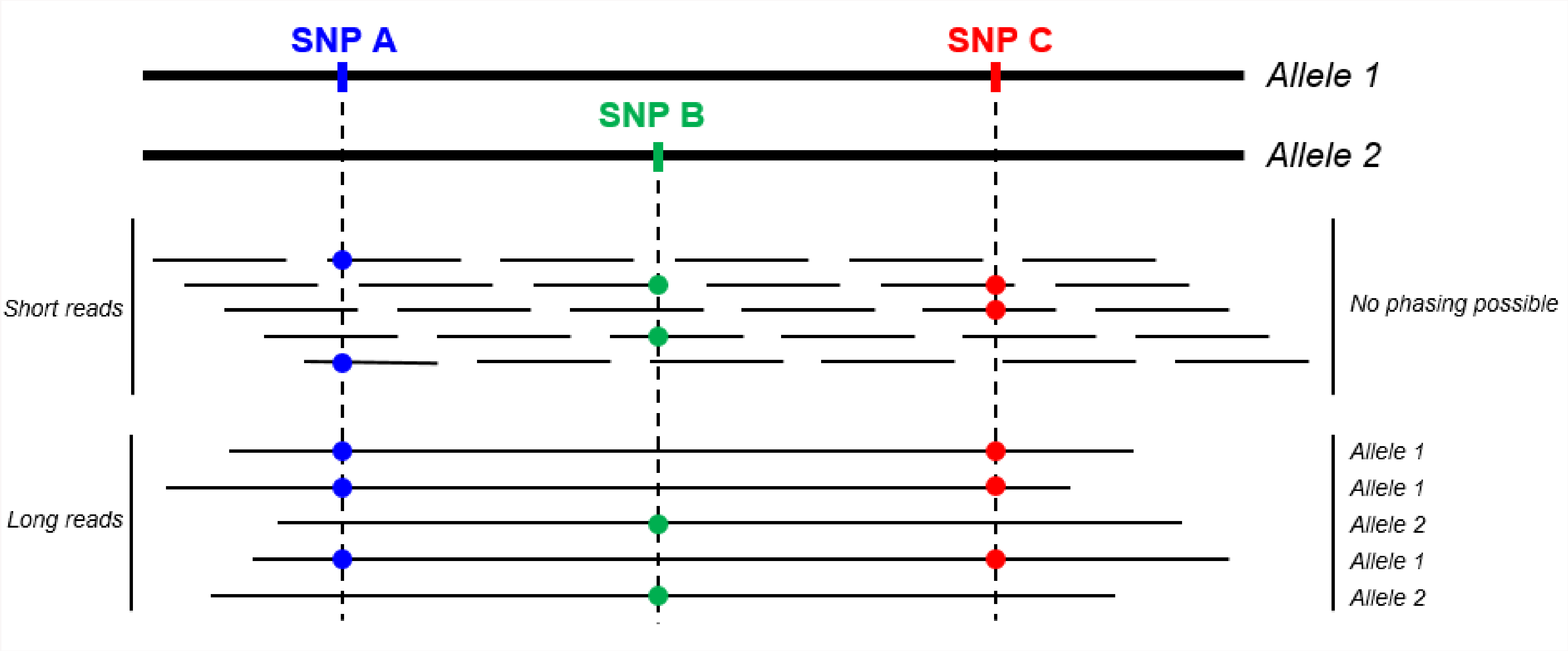
- TAL1 ,
- HOXA9 and
- TLX1 deregulated without genetic explanation.

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.

Performances – Phasing



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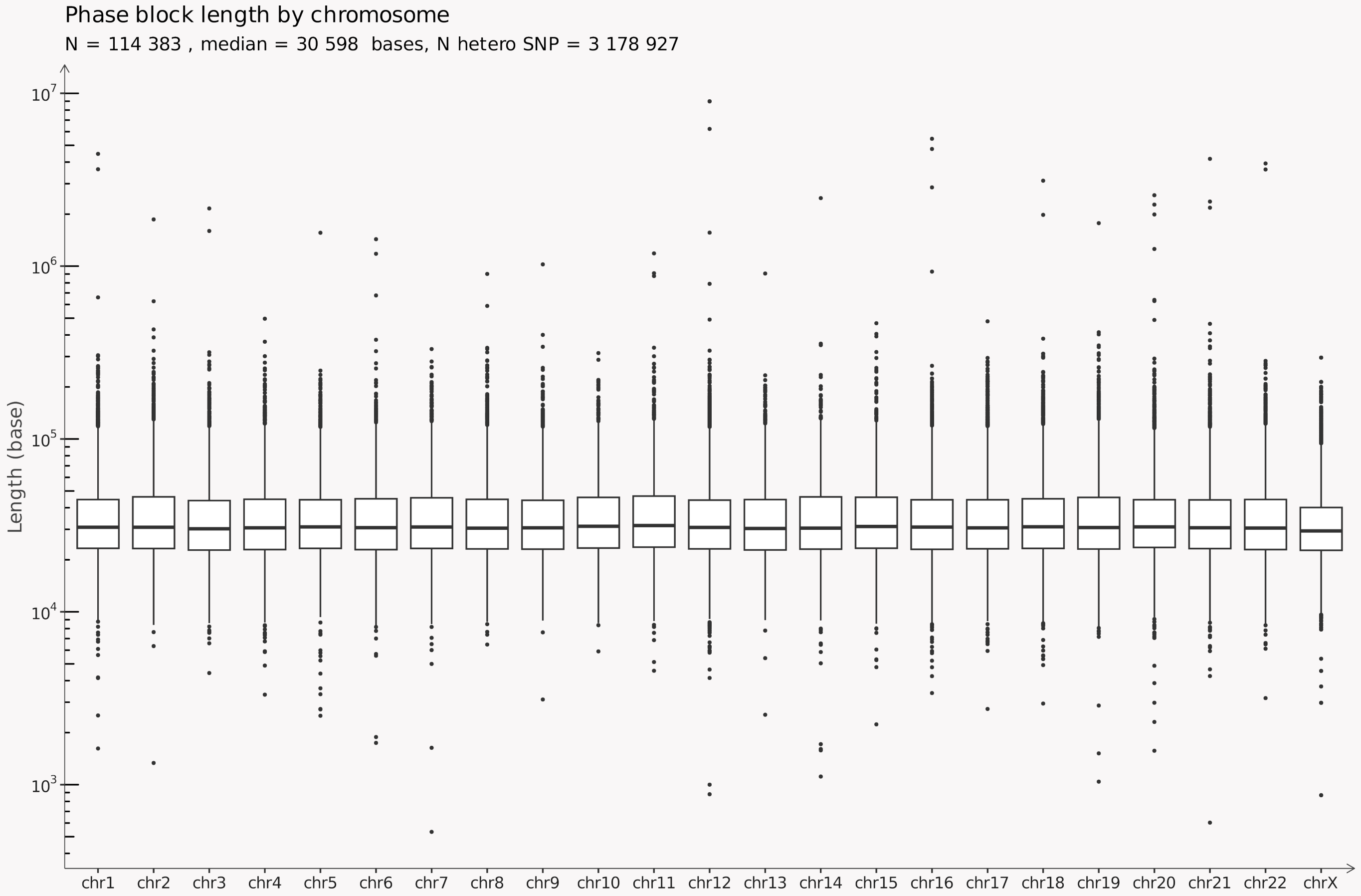
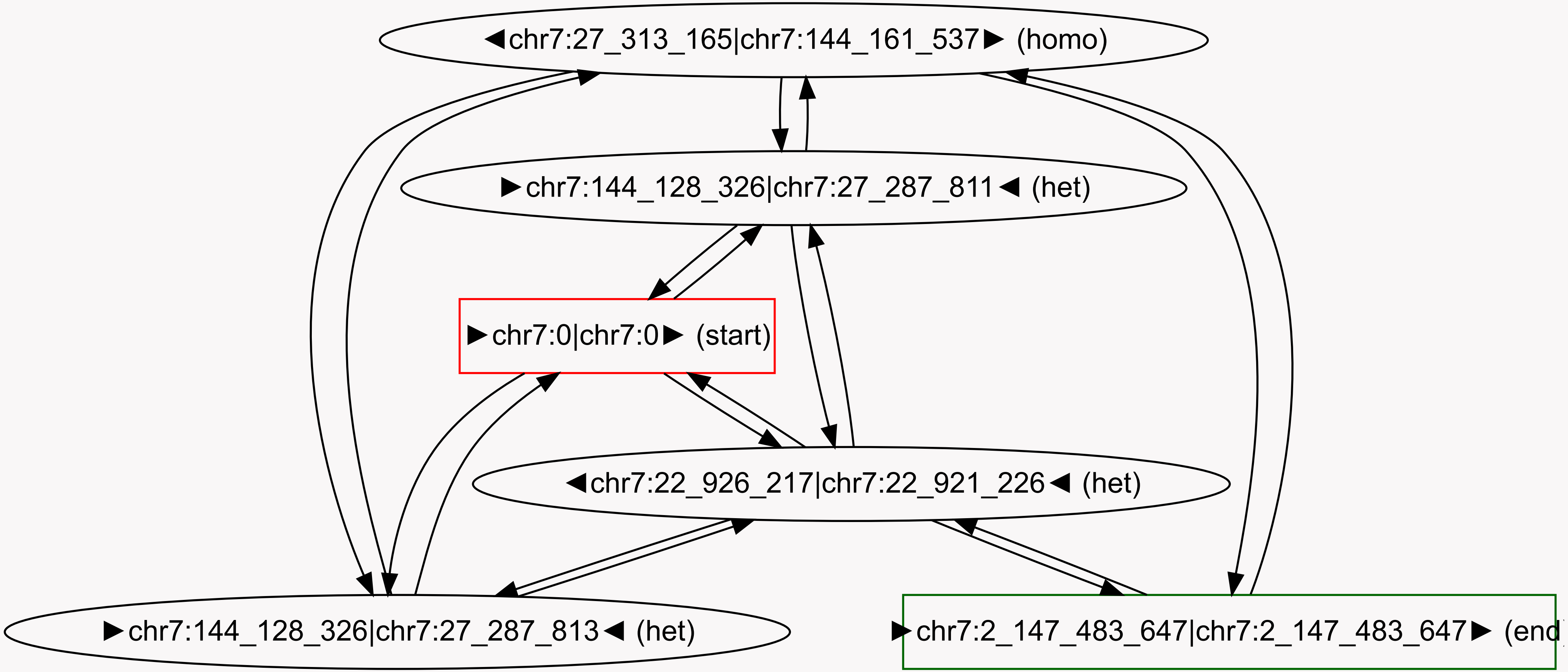
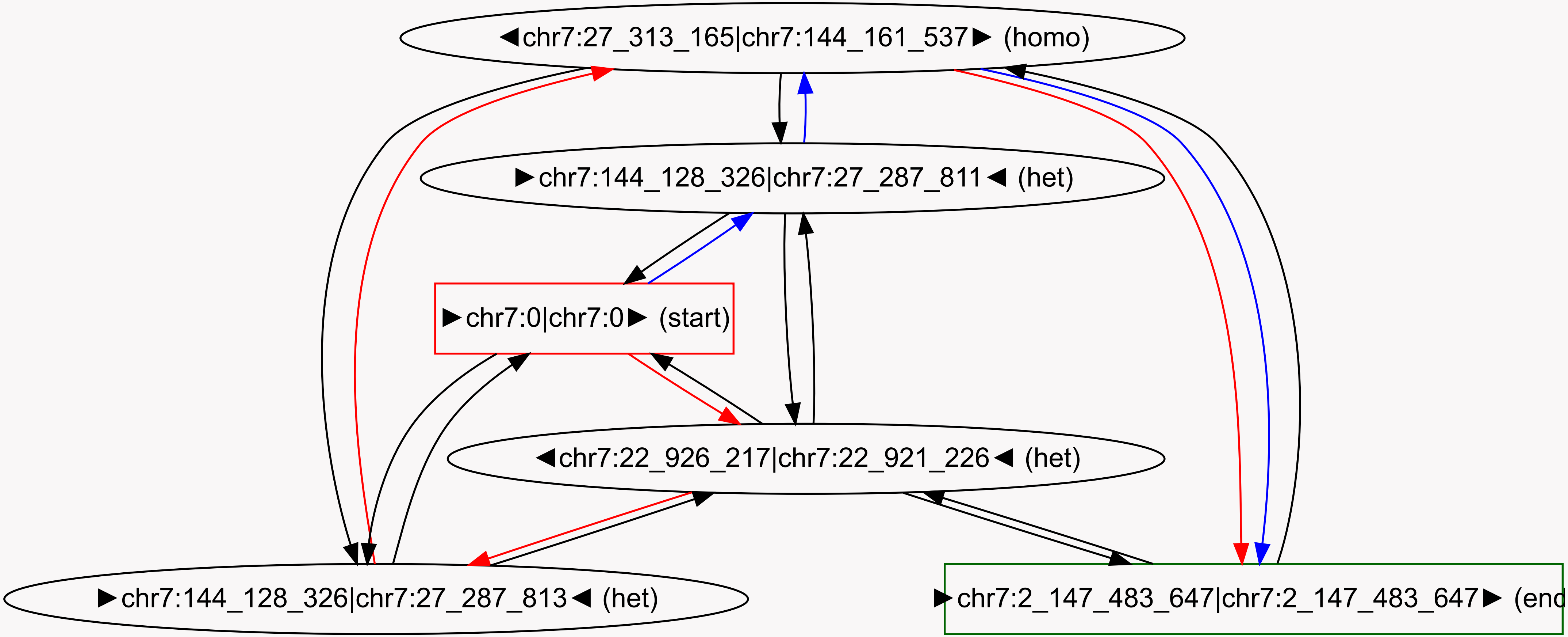


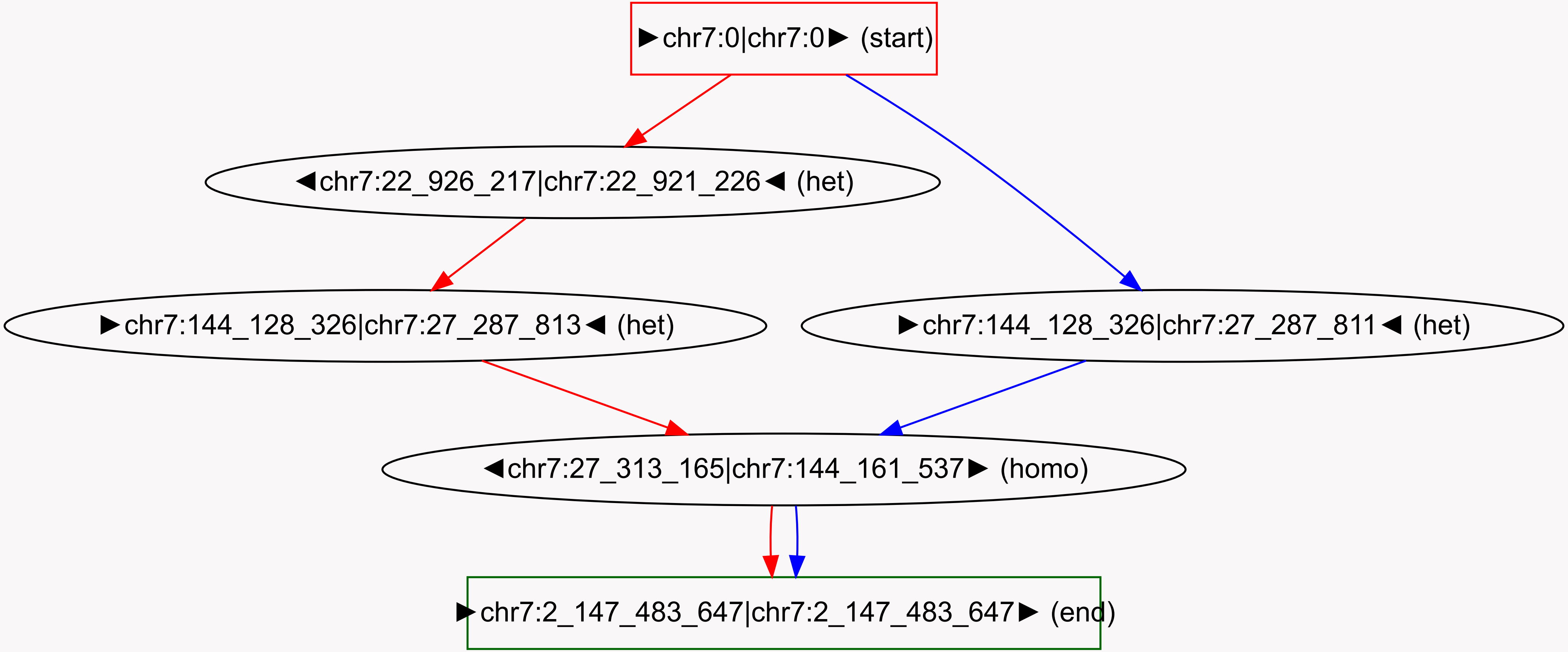
🤖 SV calling – inv7 case – bp chromosome order



🤖 SV calling – inv7 case – paths visiting max bp



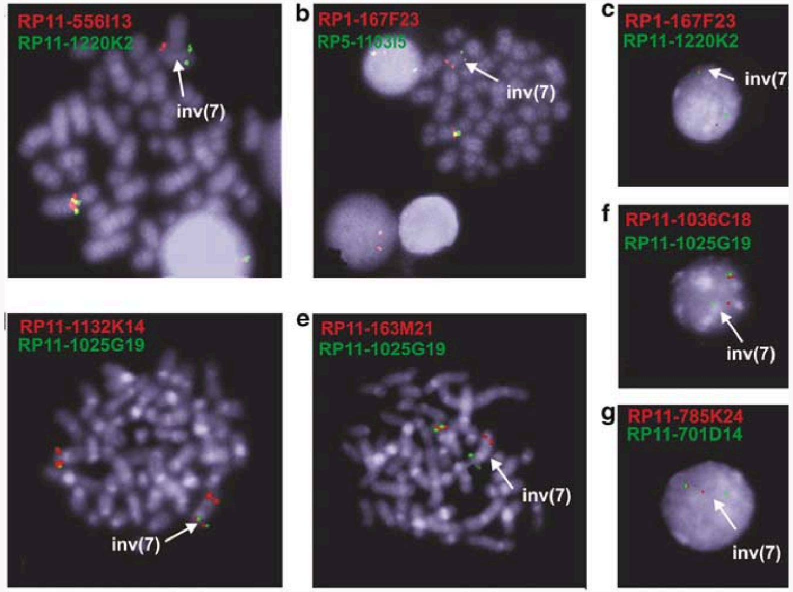
🤖 SV calling – inv7 case – simplification



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]`



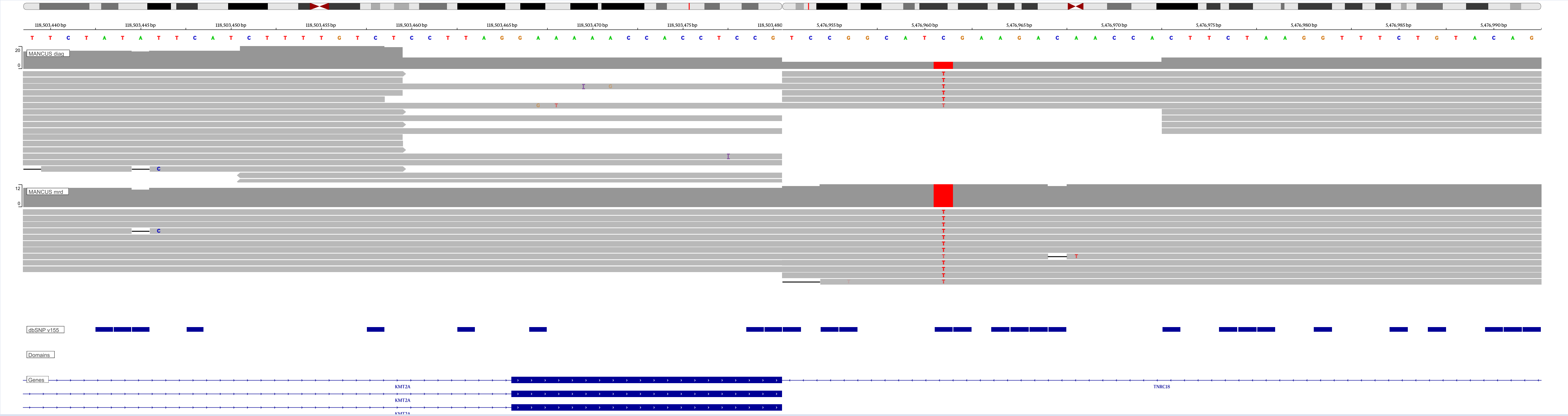
Résultats FISH break apart.

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

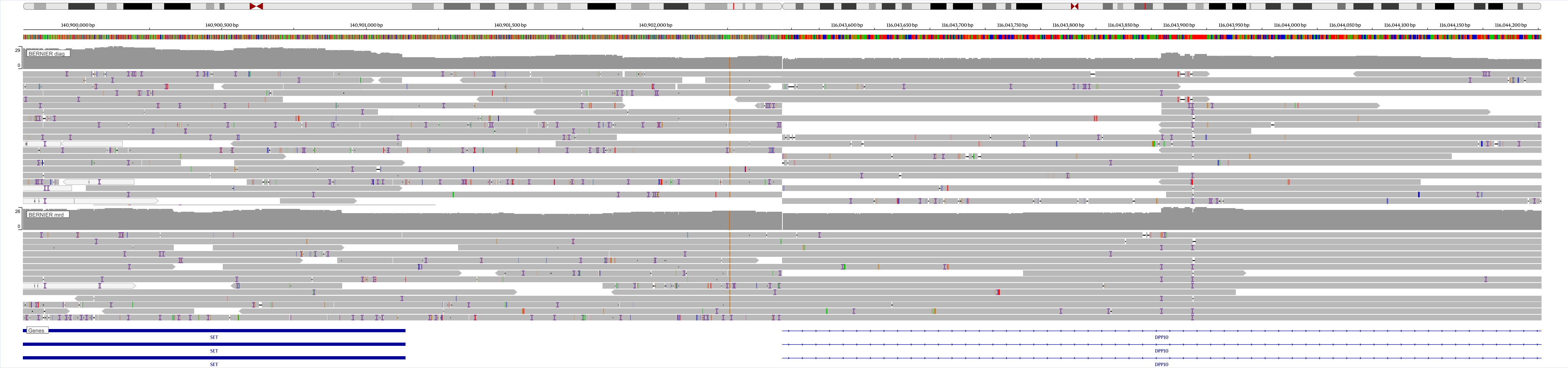
- t(7;11)(p22;q23) likely **KMT2A::TNRC18**
- `chr11:118,503,451(KMT2A)_delins[CCCC_chr7:5476973(TNRC18)]`
- Known fusion transcript (2 pediatric cases / 759 LAL. Meyer, C et al. Leukemia 2009)
- RT-MLPA probes w/o TNRC18



Interesting results: HOXA9

BE

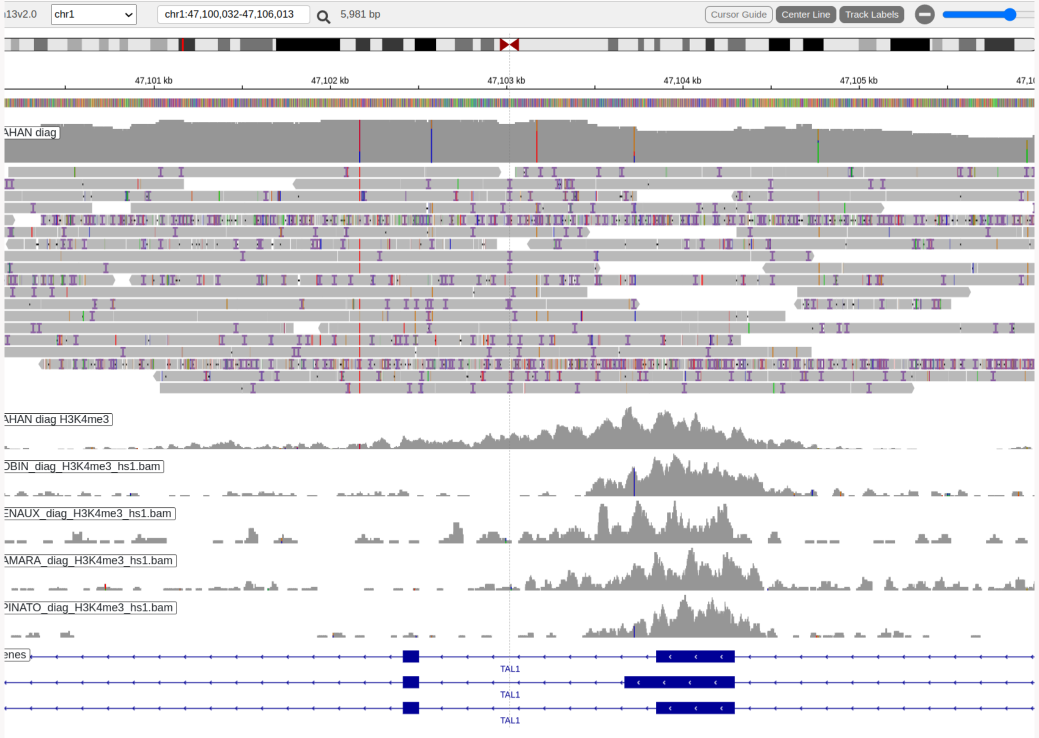
- Translocation t(2;9)(q14.1;q34.11) likely fusion transcript SET::DPP10
- `chr9:140,901,114(SET)_delins[GAACATAAAGAAAAAAA_chr2:116,043,899(DPP10)]`
- New fusion transcript in T-ALL (only described one time in CRC: Xia, Li C et al. “Identification of large rearrangements in cancer genomes with barcode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



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.



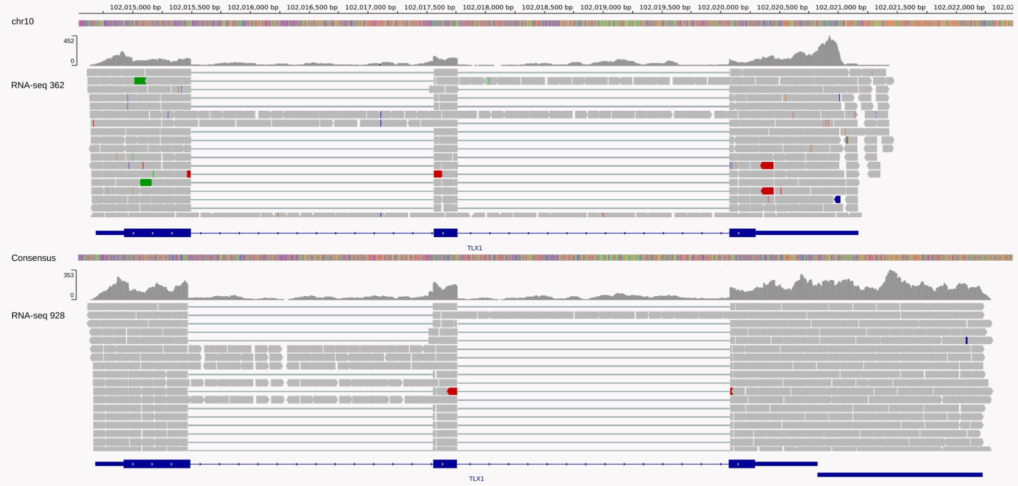
Interesting results: TAL1

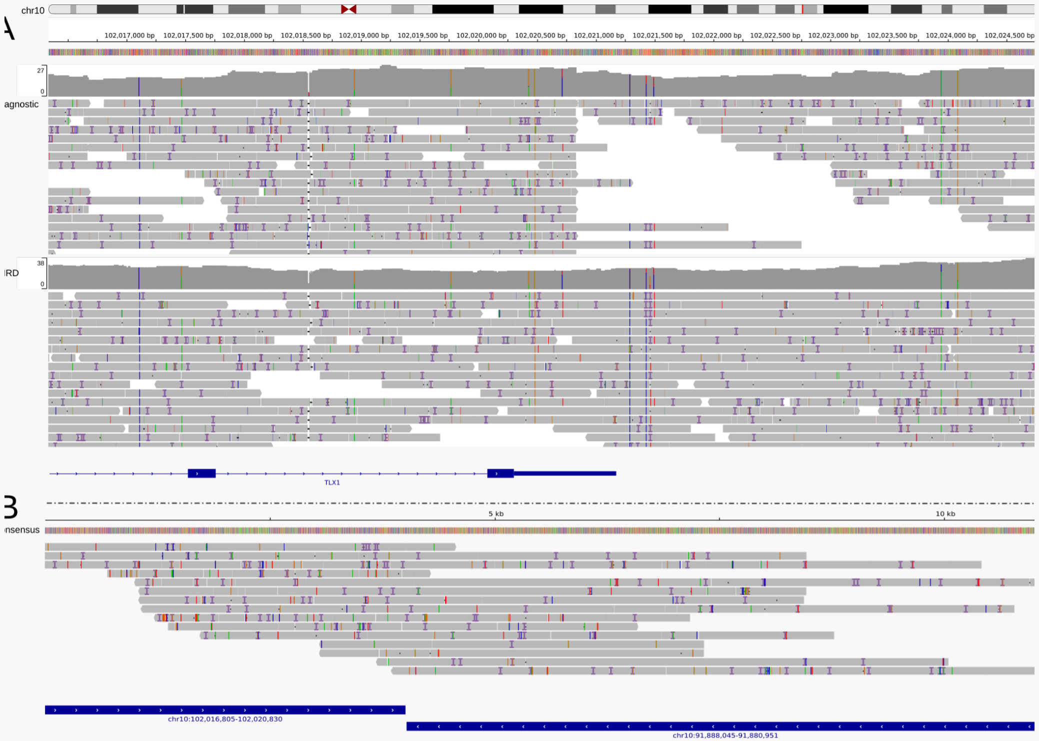
show CAMA MYB insertion on line...

Interesting results: TLX1

LEV

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





🏁 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).
- ❌ Develop methylation analysis and compare to RRBS.

💐 Remerciements

The Necker team

Pr. Vahid Asnafi

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