RNAseq :

Analyse des réarrangements, fusions, variants

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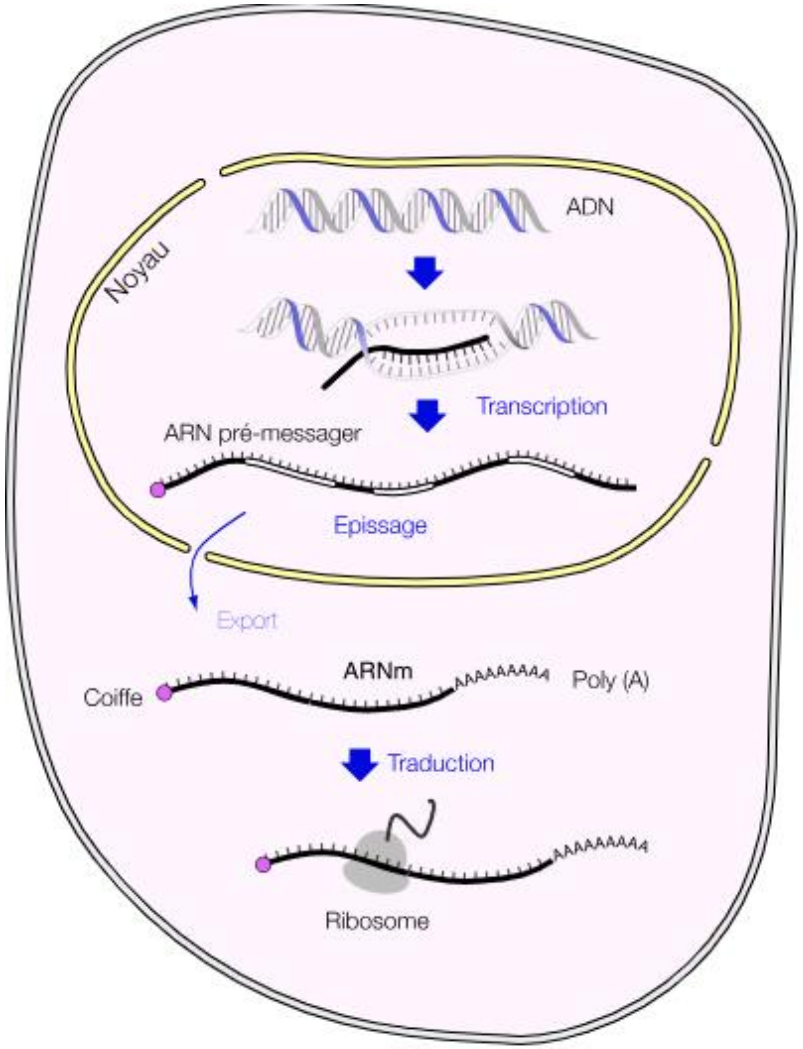
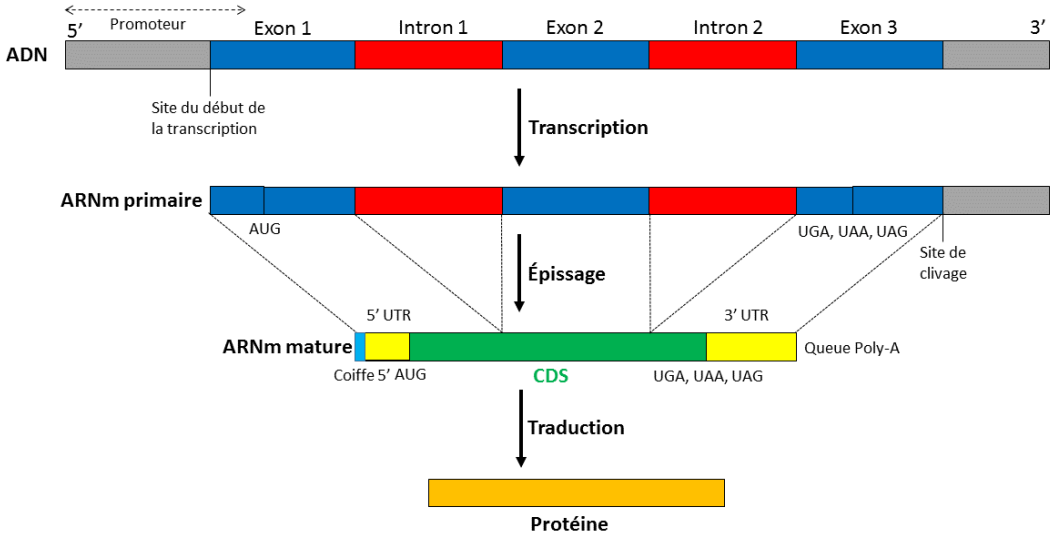
L'ARNm





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NGS

ThemoFisher

→ H+

Illumina

→ SBS-CRT cyclic reversible termination

MGI

→ DNBSEQ cyclic reversible termination

PacBio

→ Émission lumineuse

ZMW (zero-mode waveguide)
SMRT (single molecule real time technology)

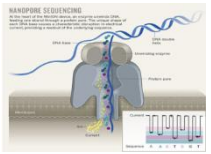
Nanopore

Nanopore MinION

Nanopore GridION X5

Nanopore PromethION P48

→ A



Mesure de l'intensité d'un courant électrique

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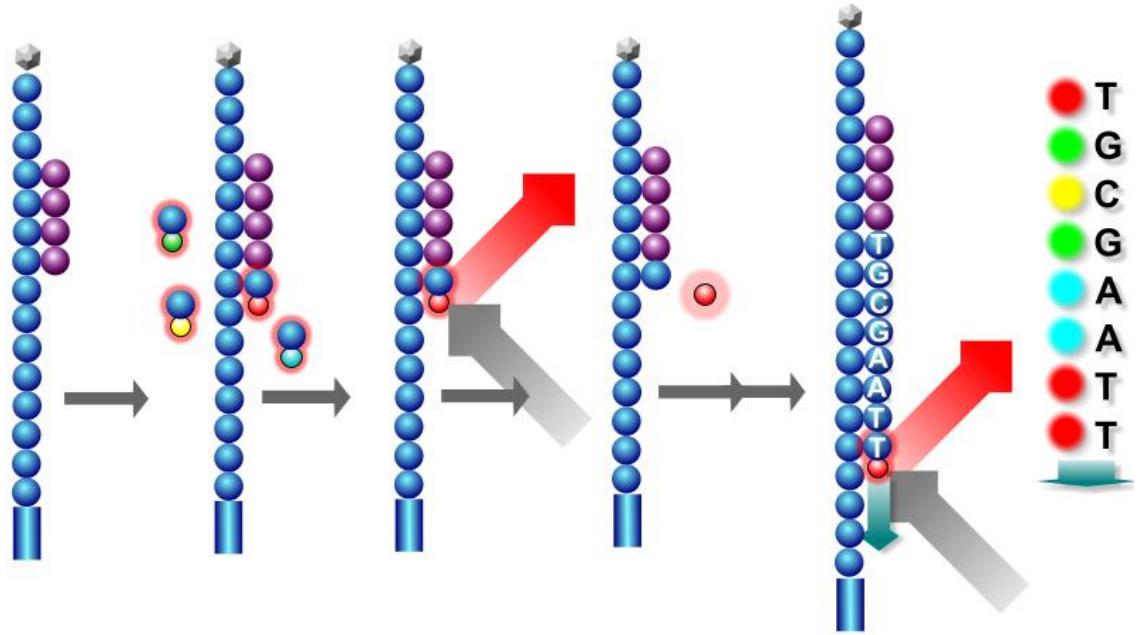
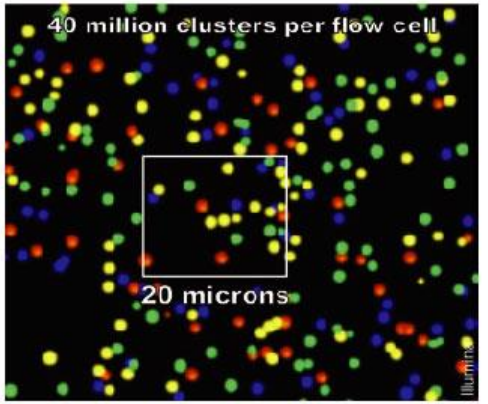
NGS Illumina

SEQUENCING WITH REVERSIBLE TERMINATORS

ddNTP ⇔ dNTP

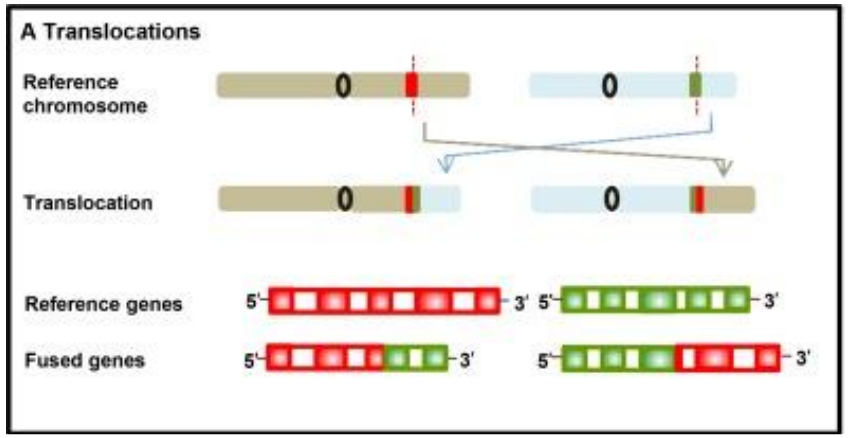




Objectifs

- Recherche des fusions/réarrangements
- Analyse des variants (substitution, délétion, insertion)
- Analyse de l'expression génique (hyper-expression, sous-expression)
- Intérêts : tumeurs solides (CBNPC, cholangiocarcinomes, sarcomes, vessie, glioblastome), constitutionnel (analyses fonctionnelles) et oncohématologie (LAM, LMC, Syndrome hyperéosinophilie, Lymphomes)

Fusions

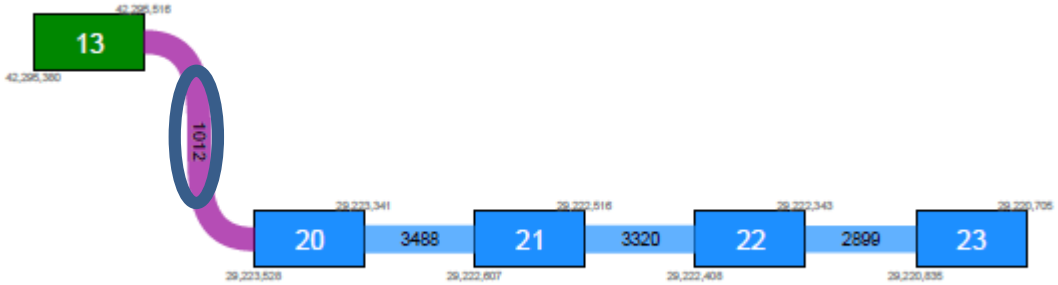

A Translocations
Reference chromosome
Translocation
Reference genes
Fused genes
5'
3'

Data Analysis using CLC Genomics Workbench

Fusion reports :

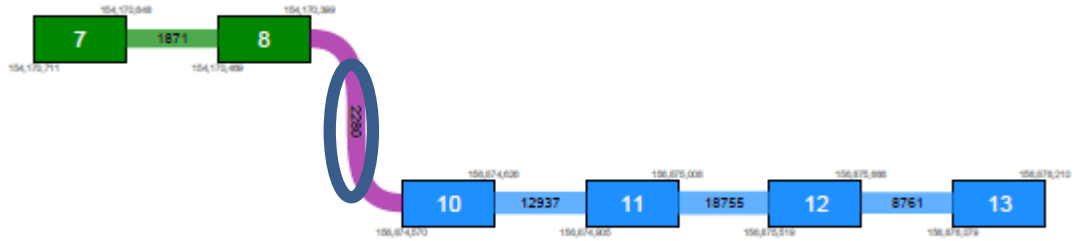
3.2 EML4-ALK

usion name	EML4-ALK
gene	EML4
chromosome	2
gene	ALK
chromosome	2
Reported transcript 3'	NM_004304.4
Translocation name	EML4{NM_019063.4}:r.1_1763_ALK{NM_004304.4}:r.4125_6265
P-value	0.00
Z-score	1,001.00
Fusion crossing reads	1,012
5' read coverage	1,012
3' read coverage	1,012



3.1 TPM3-NTRK1

Fusion name	TPM3-NTRK1
5' gene	TPM3
5' chromosome	1
3' gene	NTRK1
3' chromosome	1
Reported transcript 5'	NM_152263.3
Reported transcript 3'	NM_002529.3
Translocation name	TPM3{NM_152263.3}:r.1_892_NTRK1{NM_002529.3}:r.1252_2655
P-value	0.00
Z-score	1,486.68
Fusion crossing reads	2,280
5' read coverage	2,280
3' read coverage	2,340



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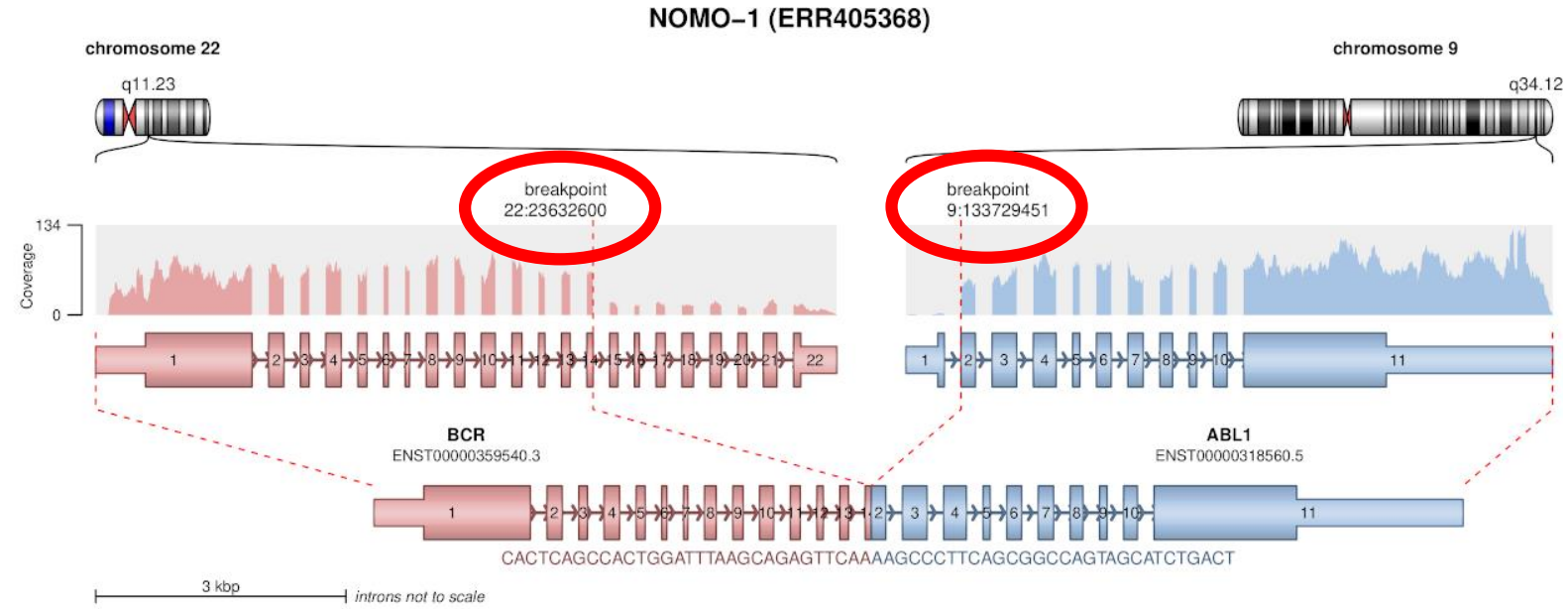
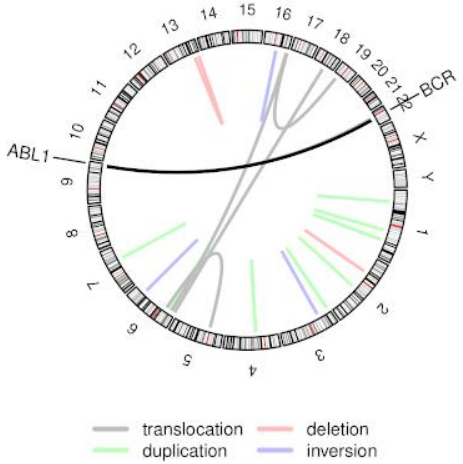
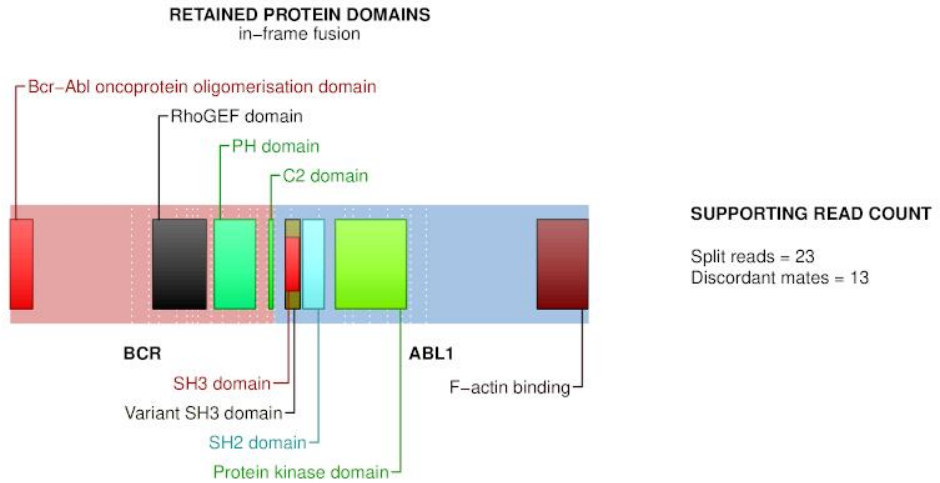
LMC

- Hémopathie, Syndrome Myéloprolifératif SMP
- Cellules atteintes : granulocytes (myélémie)
- Translocation entre le chromosome 9 et 22 -> **Chromosome Philadelphie**
- **Examens à réaliser : Myelogramme -> Caryotype -> Biologie moléculaire**
- Traitements : ITK (imatinib, nilotinib, ponatinib…)

LMC : Fusion BCR::ABL







SUPPORTING READ COUNT

Split reads = 23
Discordant mates = 13

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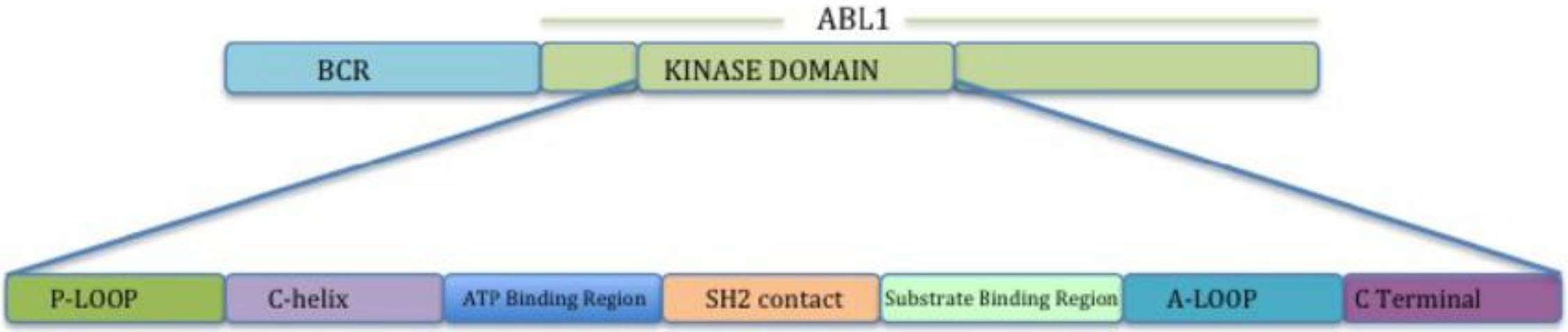

(Drug contact site) (Drug contact site)

K245R	Y257H	F281K	E292G	T315I	E334E	I360T	T392T	P439L
G249S	Y264C	E286E	I293T	F317L	V335V	D363D	H396P	L451L
G250R	L266P	A288T	L298P	T319A	N336D	A366A	I403T	R460R
Q252stop	E275E	V289A	V304I	Y326Y	A337A	R367stop	W405C	Q477stop
Y253Y			R307Q	L327L	Y342Y	K378E	A407A	N479S
G254E					A344T	A380A	L411P	A487V
					C345stop	L384P		
					E352G/K	T389T		
					Y353Y			

Conclusion

- RNAseq = Séquençage NGS à partir d'ARN
- Intérêts RNAseq = Diagnostic, thérapeutique, pronostic
- Objectifs = Recherche de fusions +++, variants, expression
- Fusion = Translocation, inversion, délétion, insertion, duplication
- LMC = Chromosome Philadelphie, fusion entre le gène BCR sur le chromosome 22 et le gène ABL sur le chromosome 9

MERCI POUR VOTRE ATTENTION

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