Alternative splicing analysis in plasmacytoid dendritic cells

Master 1 - Bio-informatic

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Introduction

Context :

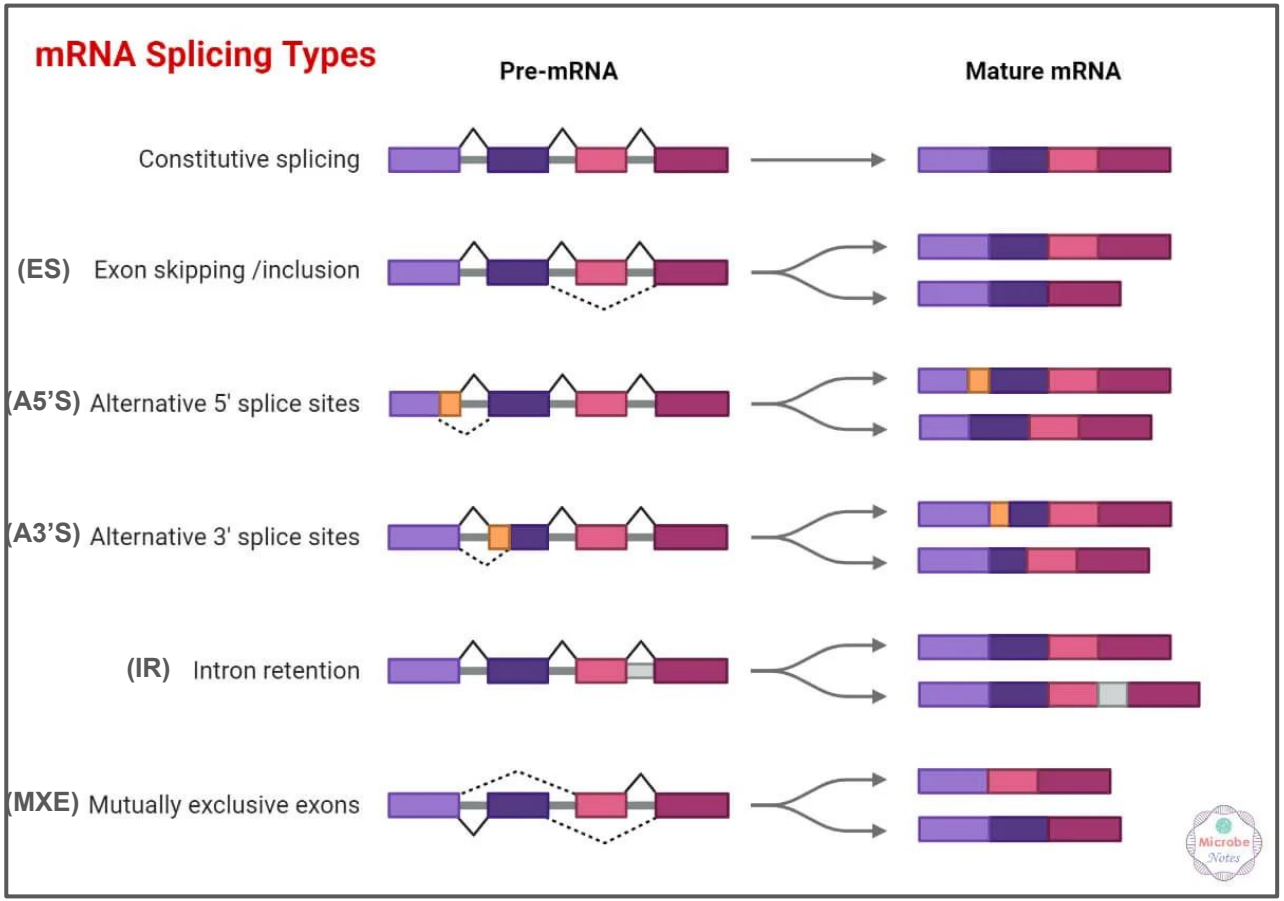
1. Alternative Splicing (AS)
    - Definition

NIH → “Alternative splicing is a cellular process in which exons from the same gene are joined in different combinations, leading to different, but related, mRNA transcripts. These mRNAs can be translated to produce different proteins with distinct structures and functions, all from a single gene. “

Introduction

Context :

1. Alternative Splicing
    - Types



Introduction

Context :

1. Alternative Splicing
    - In immune cells, what we know…

- [1] STING gene→ Exon skipping ⇒ **Viral DNA sensing**
- [2] IFNAR2 gene → Exon skipping ⇒ **IFN sensing & Immunomodulatory effects**
- [3] IRF7 gene → ES, IR, Alternative splicing sites ⇒ **IFN pathway modulation**
- …

Introduction

Context :

1. Alternative Splicing
    - In plasmacytoid Dendritic Cells (pDC)

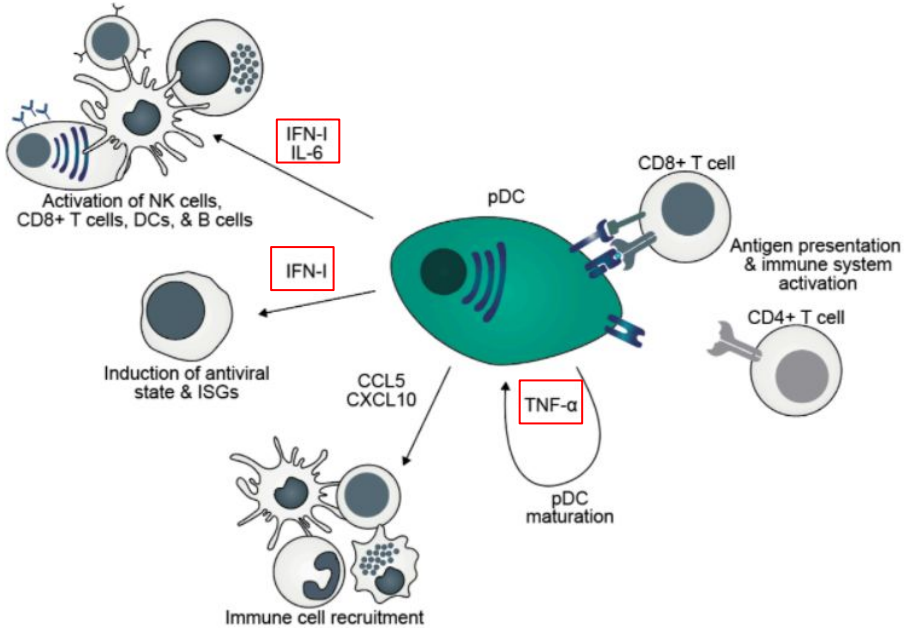
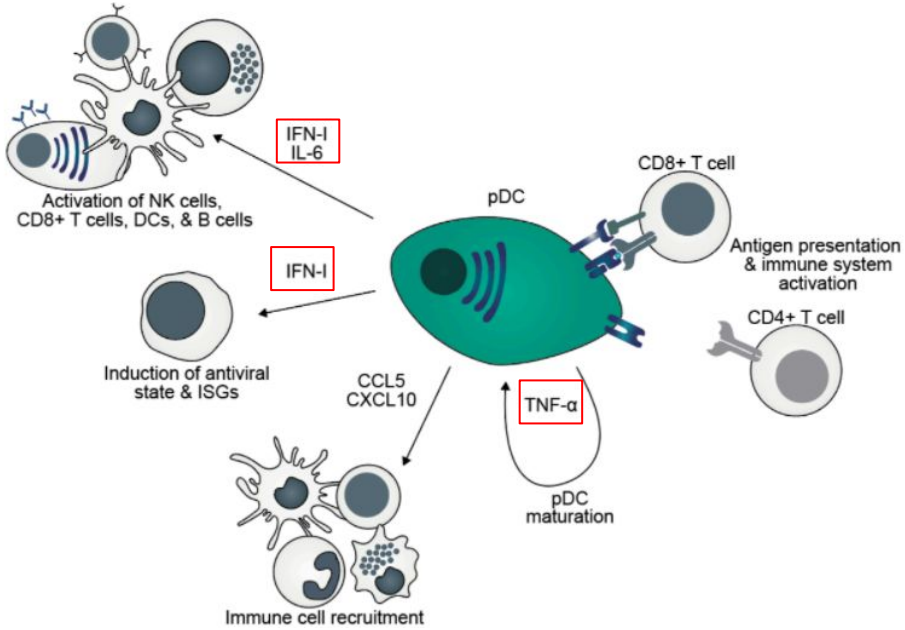


Image Link

Introduction

Context :

1. Alternative Splicing
    - In plasmacytoid Dendritic Cells (pDC)



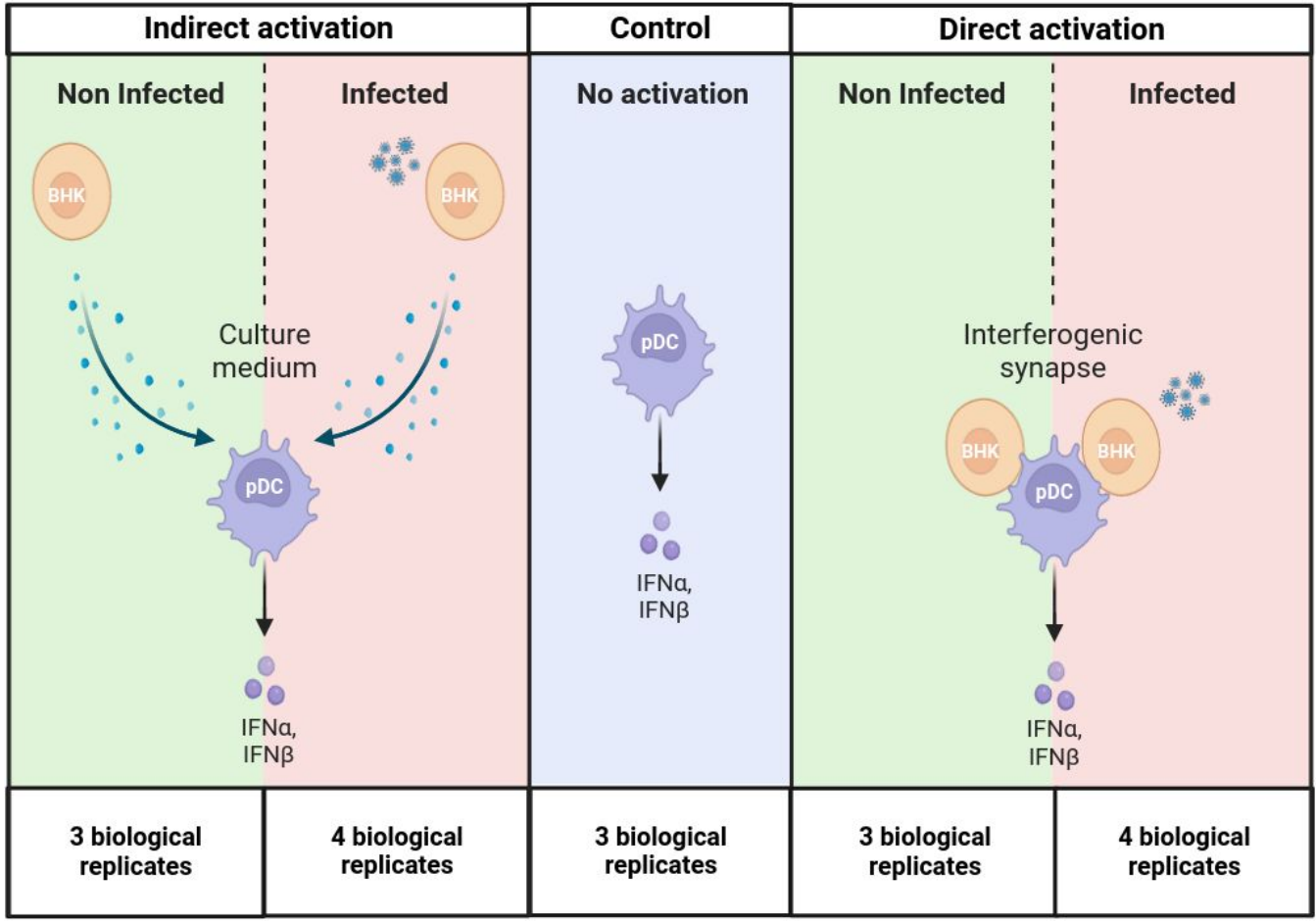
- AS in type 1 IFN as other immune cells ?
- Very little knowledge about AS in these cells and implications…

Image Link

Introduction

RNAseq data description :

- 2x150pb Paired-end Illumina seq (NovaSeq)
- Sequencing depth ~ 20-100 M reads
- BHK = Baby Hamster Kidney cells



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Introduction

Aims of this project :

- Are the quality and depth of the dataset sufficient to detect signals of differential alternative splicing between conditions?

- If so, is there alternative splicing remodelling between conditions? And which transcripts are concerned?

Preliminary analysis

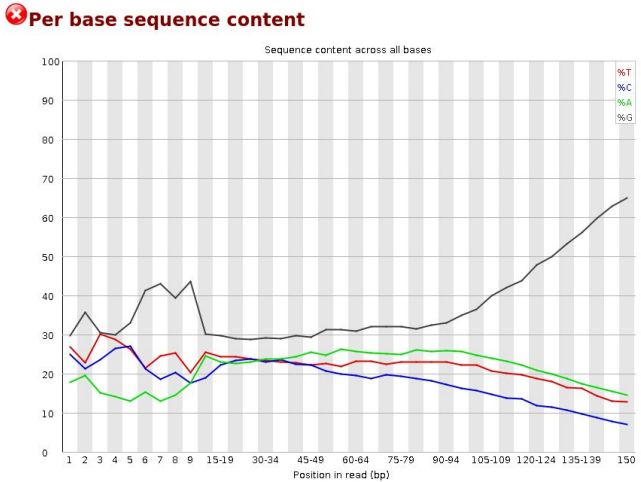
- Gives an idea of the state of the dataset

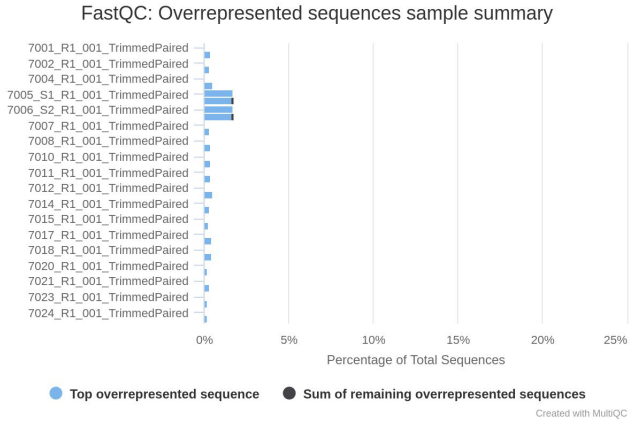
- Helps in identifying problems from sequencing and earlier data manipulation

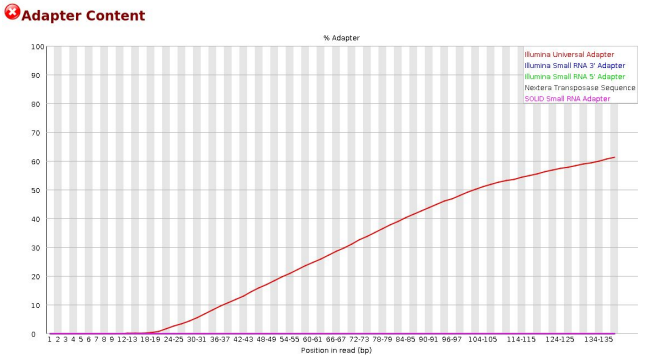
Preliminary analysis

FastQC

- Summarizes a variety of stats such as quality scores and GC content
- Ran on raw data



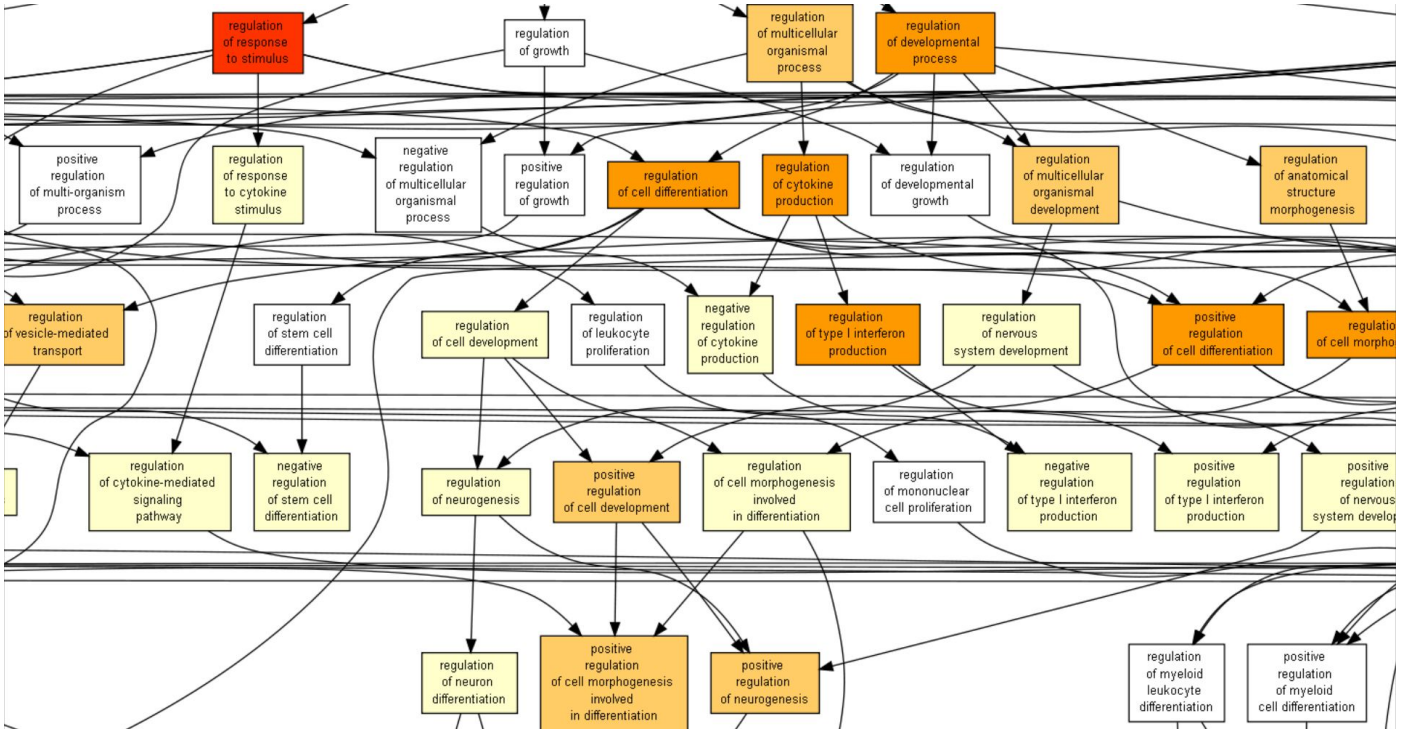




Preliminary analysis

GO diagram

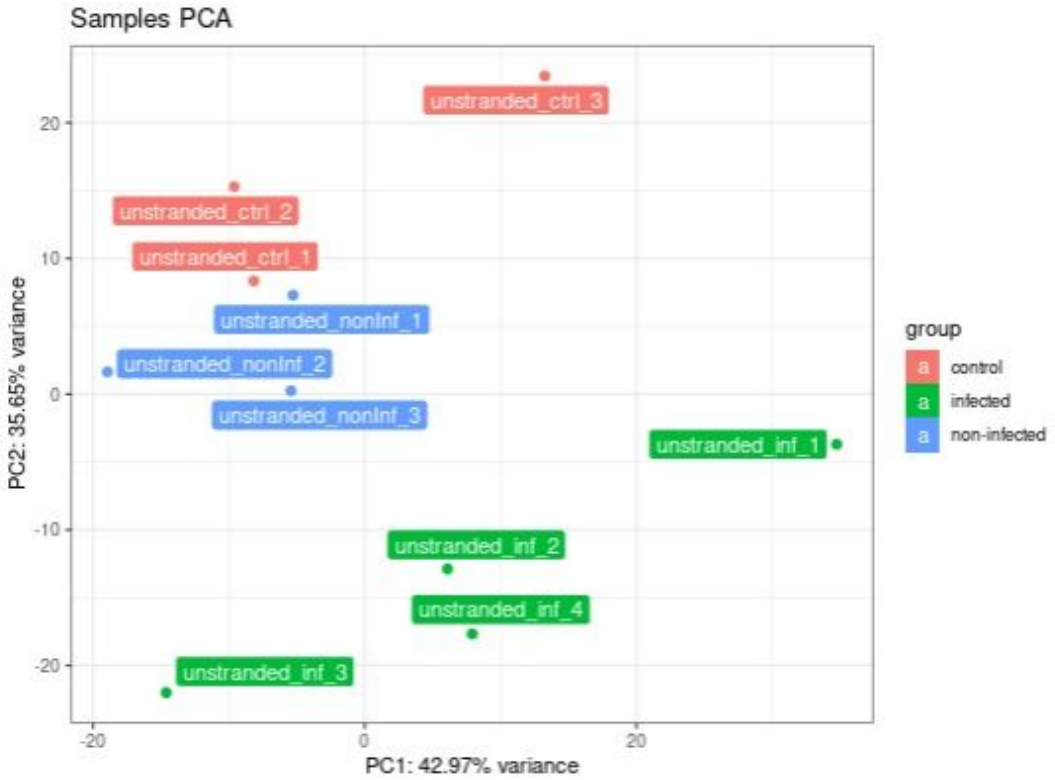
- Summarizes molecular function, cellular components and biological processes



Preliminary analysis

PCA diagram

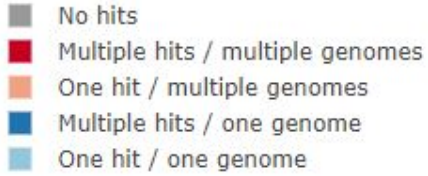
- Helps in identifying signals from the dataset
- PCA on transcript counts

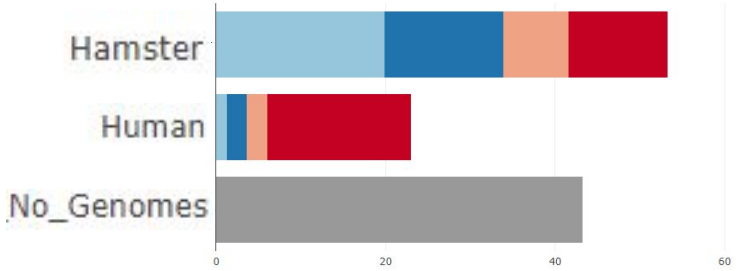


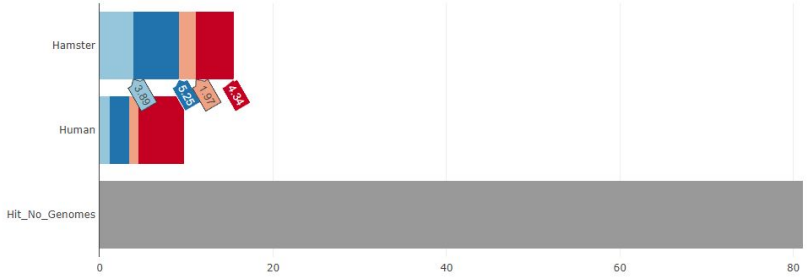
Preliminary analysis

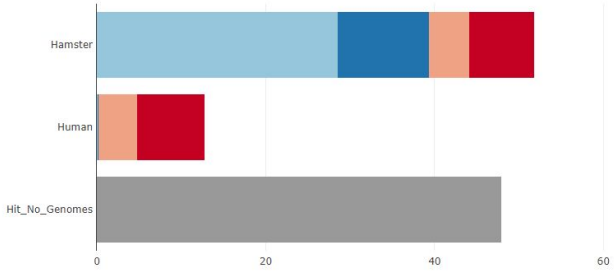
FastQscreen

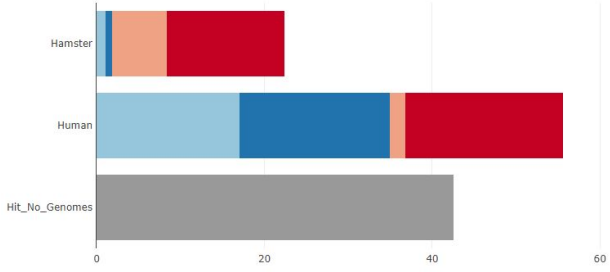
- Determines from where the sequences in our data originates
- Helps in detecting contamination
- Samples 7015, 7018, 7020 and 7024



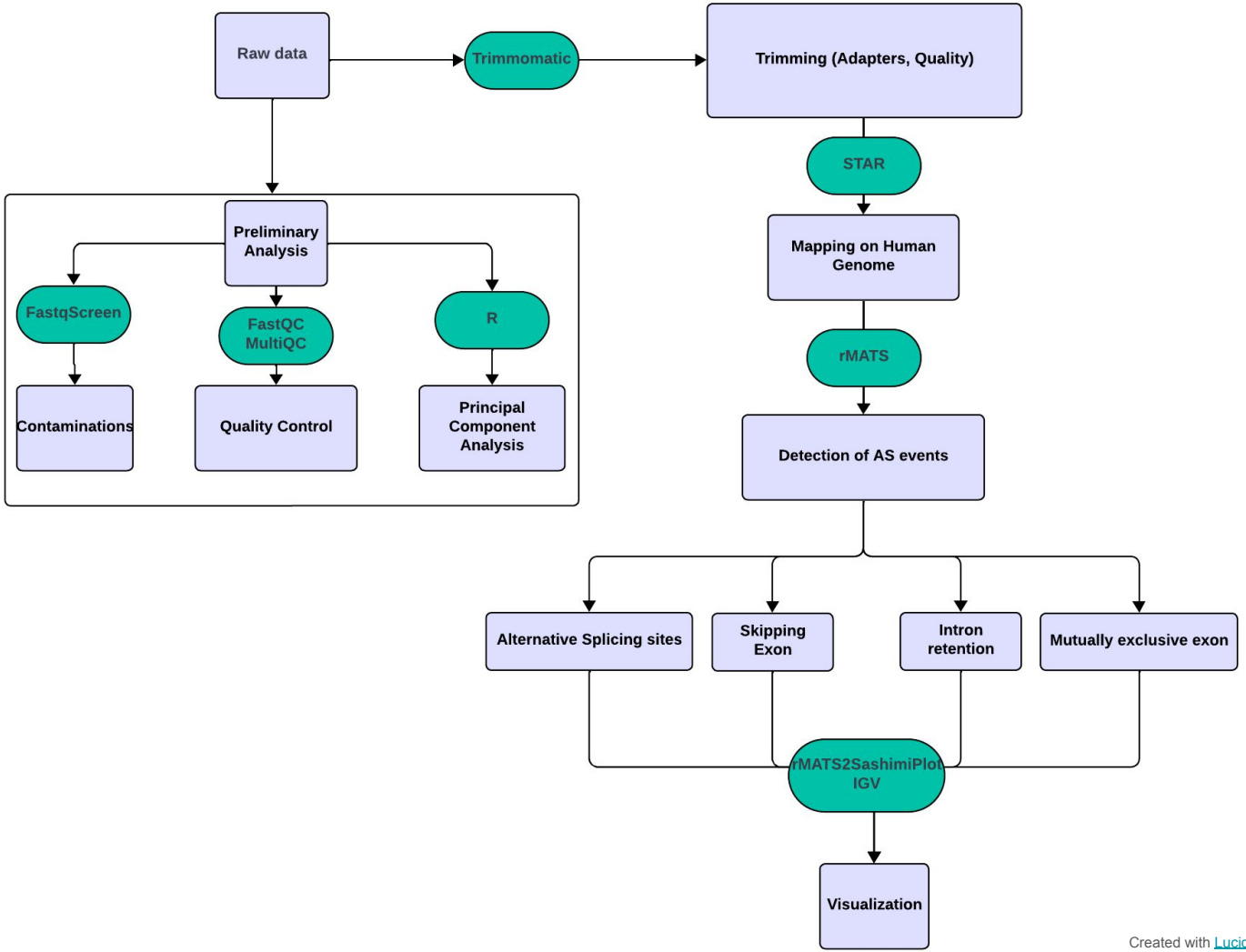








Pipeline



Created with LucidChart

Results: Mapping - STAR

	pDC (activation indirecte)	pDC + BHK (activation directe)
Reference genome	hg38	hg38
Alignment scores	homogeneous (~55%)	highly fluctuating (10% to 50%)

How to sort pdc + BHK reads ?

Concatenated index

Analysis with RMATS : Procedure

rmats_ output_List → Inclusion difference level > 0,5 ; FDR < 0,05 → pre_List

Infected - Control : pre_List_A

Not_Infected - Control : pre_List_B

→ Final_List = pre_List_A \ pre_List_B

What about pDC with direct contact of BHK ?

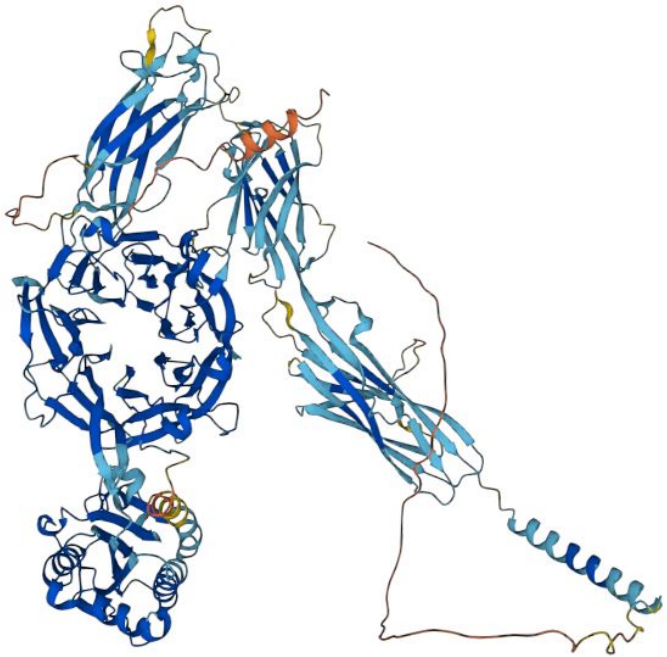
mapping scores + Not_Infected sample's quality analysis → pre_List only

Alternative splicing events: Results

	SE	MXE	RI	A5SS	A3SS
Genes with alternative splicing due to infection without direct activation	"SMG1" "HNRNPD" "LPIN1" "HNRNPA3" "IRF7"	"LDHA" "ITGAL"	"HGS" "ANAPC5" "YJU2B" "ARHGAP4" "FN1" "COPA" "CARS2"		“CNBP”

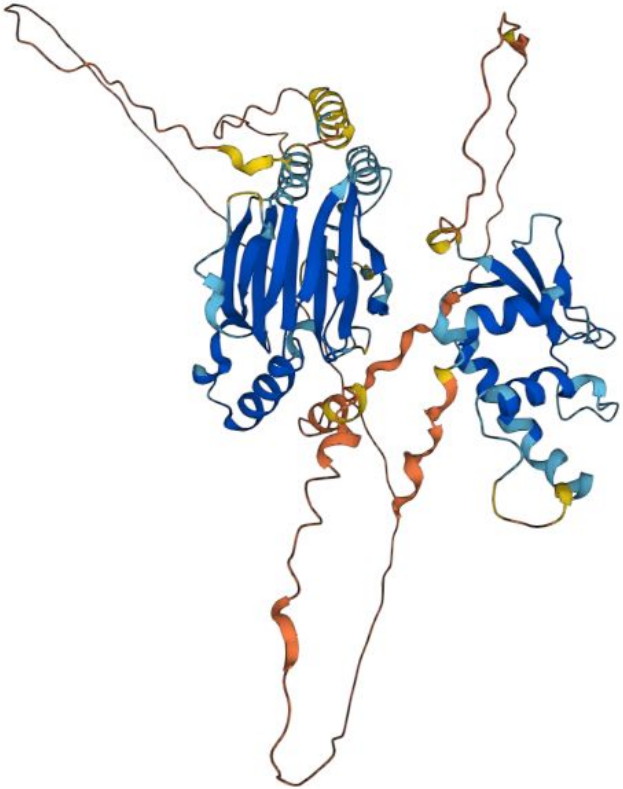
	SE	MXE	RI	A5SS	A3SS
Genes with alternative splicing and direct activation to BHK cells	"HNRNPA3" "TPM2"		“MYH9" "CIRBP" "GOLGA3" "ANAPC5" "MTCH1" "EWSR1" "ANKRD11" "CTSC"		

Functional analysis: ITGAL



- Integrin: involved in synaptogenesis
- essential role in immune processes:
  - leukocytes - endothelial cells interaction
  - clearance by cytotoxic T cells, granulocytes and monocytes.
  - phagocytosis of apoptotic neutrophils by macrophages
- involvement in lymphopoiesis

Functional analysis: IRF7



- transcriptional regulator
- involved in type I interferon (IFN)-dependent immune responses
- critical role in innate immune defense against DNA and RNA viruses

Conclusion

Aims of this project :

- Are the quality and depth of the dataset sufficient to detect signals of differential alternative splicing between conditions?
  - ⇒ We can detect a signal, but the list of differentially spliced gene is very short
  - ⇒ A greater quality and depth sequencing should be better to extend the list and our trust in results

- If so, is there alternative splicing remodelling between conditions? And which transcripts are concerned?
  - ⇒ With provided dataset, interesting genes have been found, like IRF7 and ITGAL

Remerciements

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Basile SUGRANES

Hélène BADOUIN

References

- The UniProt Consortium, UniProt: the Universal Protein Knowledgebase in 2023, Nucleic Acids Res. 51:D523–D531 (2023)
- https://www.genome.gov/genetics-glossary/Alternative-Splicing
- Li, Xiaobo et al. “An alternatively spliced STING isoform localizes in the cytoplasmic membrane and directly senses extracellular cGAMP.” *The Journal of clinical investigation* vol. 132,3 (2022): e144339. doi:10.1172/JCI144339
- Hurtado-Guerrero, Isaac et al. “Antiviral, Immunomodulatory and Antiproliferative Activities of Recombinant Soluble IFNAR2 without IFN-ß Mediation.” *Journal of clinical medicine* vol. 9,4 959. 31 Mar. 2020, doi:10.3390/jcm9040959
- Xu, Xiaofang et al. “Alternative mRNA Processing of Innate Response Pathways in Respiratory Syncytial Virus (RSV) Infection.” *Viruses* vol. 13,2 218. 31 Jan. 2021, doi:10.3390/v13020218
- https://pereiralab.com/publication/using-direct-cell-reprogramming-to-uncover-plasmacytoid-dendritic-cell-specification-programs-and-function/