

## Introduction - TU Delft

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## A FAMILIAR FACE



### Investigating the differential instructive roles of WT1's isoforms

#### Introduction: The Wilms' tumour 1 gene and its isoforms

The Wilms' tumour 1 (WT1) gene was given its name because of its crucial role in the development of the eponymous paediatric kidney tumour, where the gene was originally identied. The human gene is located on chromosome 11 and consists of 10 exons, spanning about 50 kilobases (kb) of genomic sequence.

Mutations in the gene were identifed not only in a considerable portion of Wilms' tumours, but also in diffrent congenital syndromes, characterised by severe kidney disease, gonadal dysgenesis, heart and diaphragm problemsThe +/+ isoform includes both the 17 aa (amino acids), deriving from the usage of exon 5, and the KTS between the thirds and fourth zinc fingers, generated by the use of the alternative splice donor site in exon 9; the +/- variant encodes for the 17 aa, but lacks the KTS; the -/+ isoform includes only the KTS residues, while in the -/- variant both the 17aa and the KTS are excluded.

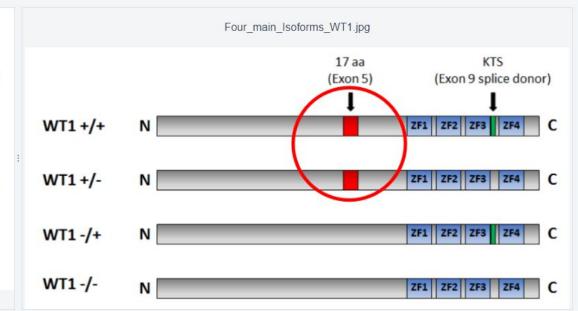
#### **Objectives**

The functions of WT1 and its isoforms have been so far addressed in vivo using KO models, which have provided pivotal insights on the role of WT1 in development and disease. Nonetheless, they did not allow the investigation of the instructive role of the transcription factor. In order to address in vivo which processes WT1 is able to induce, mice models for the upregulation of WT1 will be needed.

Aiming to address the instructive role of WT1 and to dissect the differences between its variants, I wanted to create cellular systems for the inducible expression of single isoforms of the transcription factor. I derived two epithelial cell models in which is possible to induce WT1 isoforms expression and I started characterizing the effects of the induction by gene expression analysis and cellular assays.

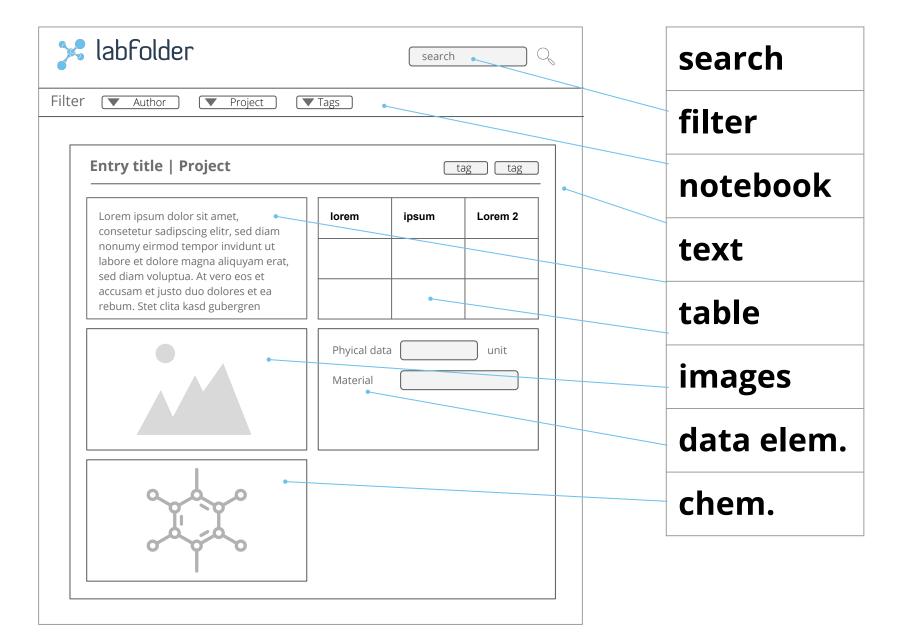
As WT1 exhibits specific functions depending on tissue and cellular context, using these cell lines disclosed interesting outcomes following WT1 induction, but came along with multiple limitations. Therefore, my final goal was to derive embryonic stem cells (ES) to generate mouse models, in which the expression of single variants could have been temporally(and spatially) controlled.

In this thesis I will first explain the cloning process to generate plasmids for the inducible expression of WT1 single isoforms, second I will describe the stable and inducible epithelial cell models and ES cells derived with these plasmids. Last, I will discuss the results obtained from the induction of single WT1 isoforms in the differentiated cell lines.



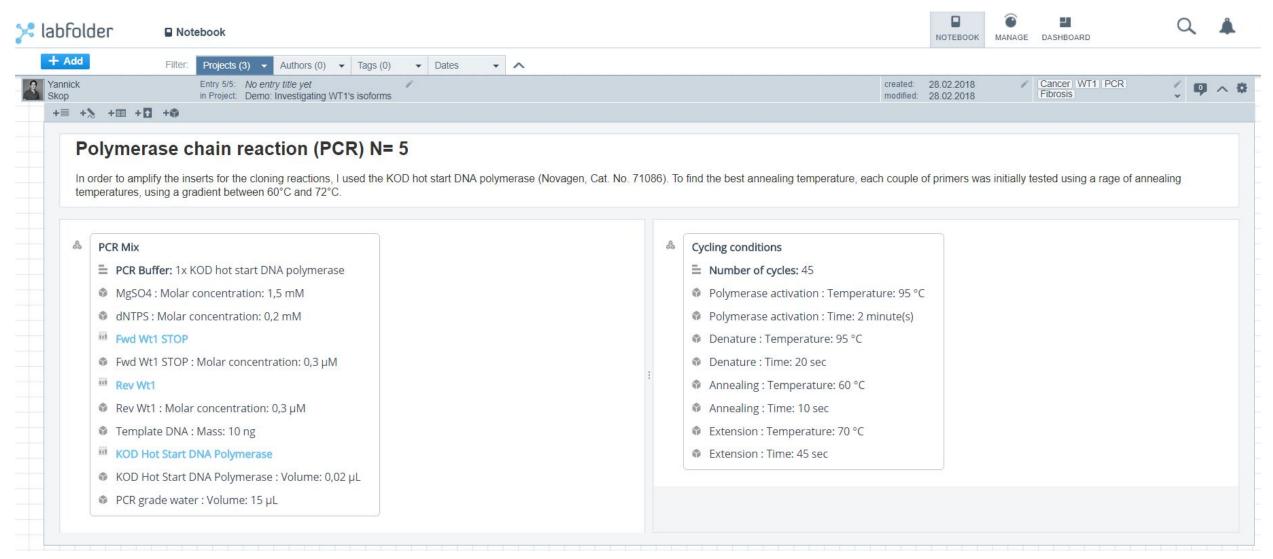


## PRODUCT: CORE FUNCTIONALITIES



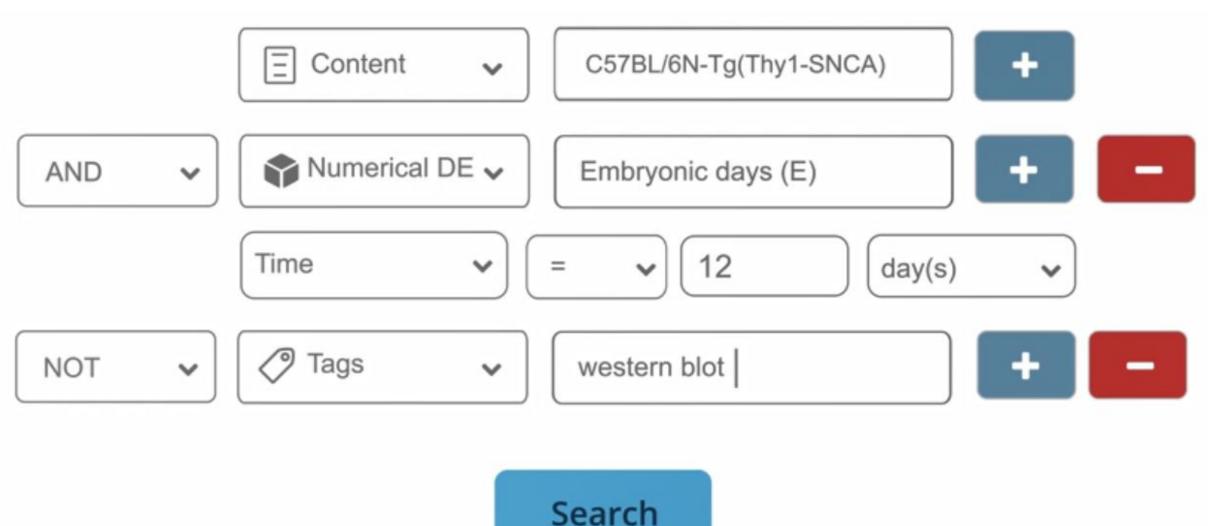


## PROTOCOL TEMPLATES





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## Academia & Healthcare





















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## THANK YOU FOR YOUR ATTENTION!



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