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## Search for ncRNA targets in *Clostridioides difficile* by crossreferencing MAPS data and RNA-RNA interaction predictions

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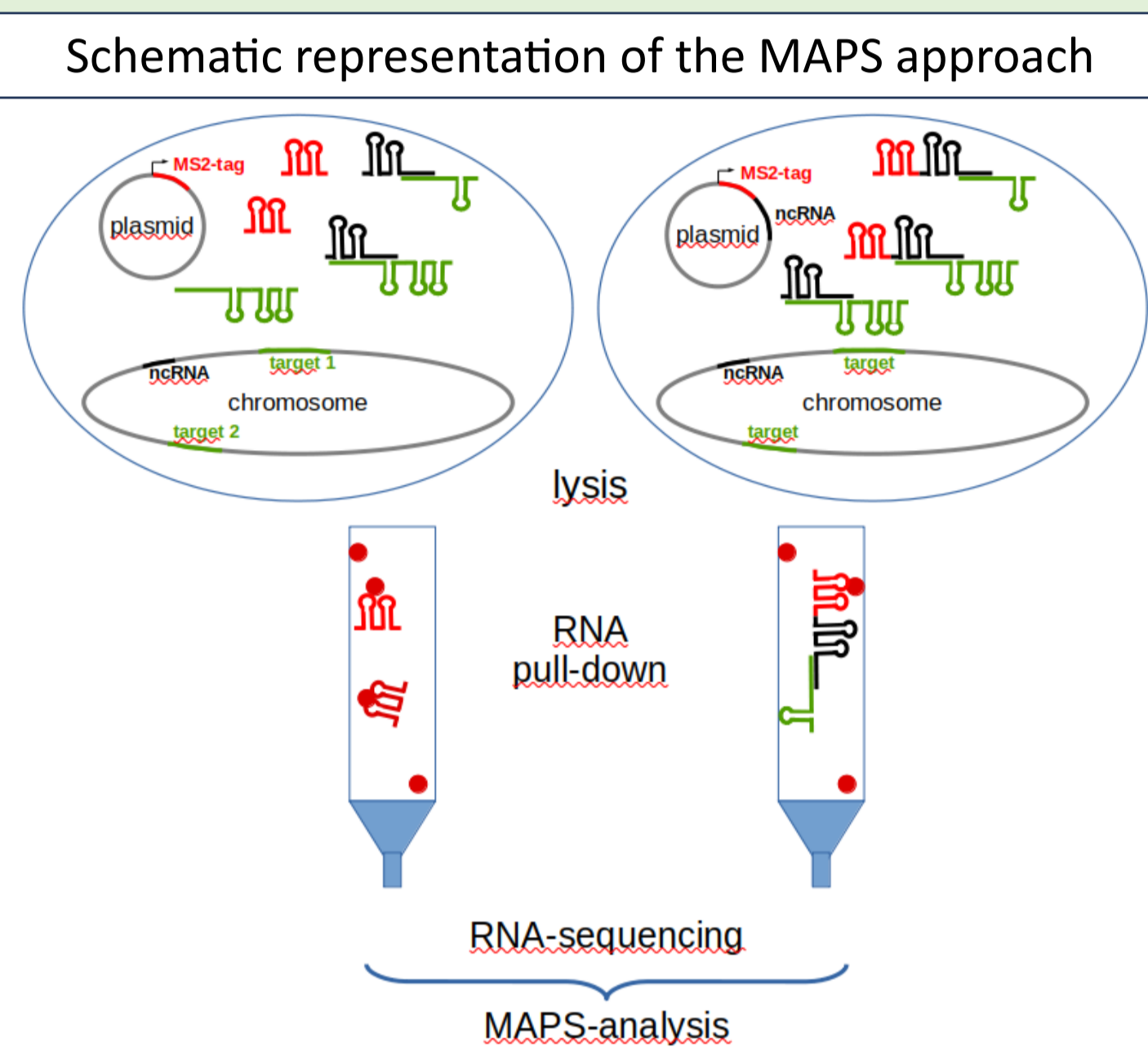


## Introduction

*Clostridioides difficile* (CD) is a Gram positive, anaerobic, spore-forming bacterium. It is known to be the major cause of **nosocomial infections** associated with antibiotic therapy. Despite being classified as an urgent Antibiotic Resistance Threat by the Centre for Disease Control and Prevention, its pathogenesis control is **poorly understood**. Some of these **ncRNAs** regulate their targets by associating with their target **messenger RNA** to form an RNA duplex leading to modulation of mRNA translation and/or stability.

To understand the mechanism of action of ncRNA candidates selected in a previous work [1], we **combine experimental (MAPS) and *in silico* (intaRNA)** approaches to identify their target genes. 2 **Snakemake** pipelines [2] have been developed to analyse these data.

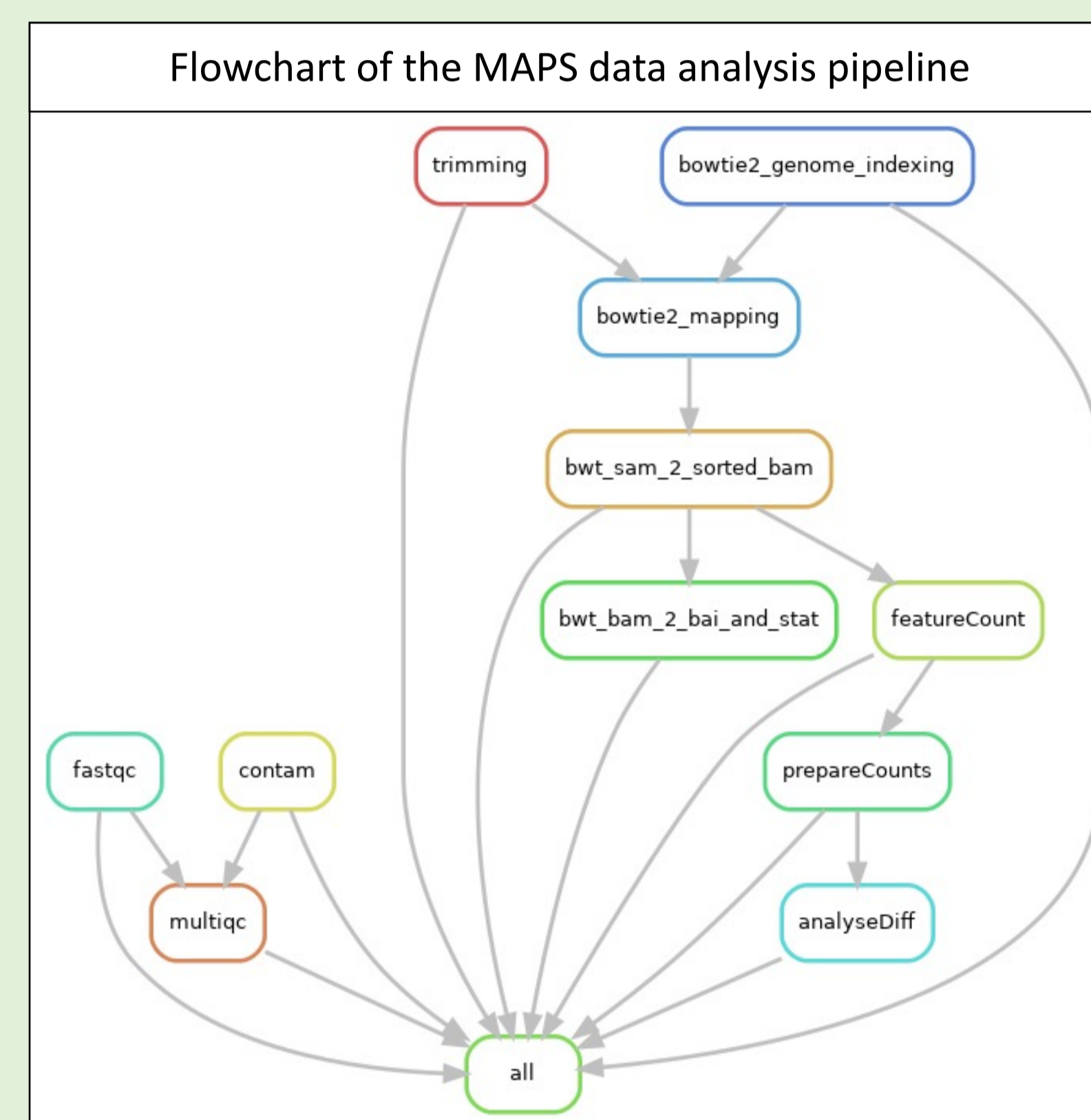
## MAPS



The MAPS (MS2-Affinity Purification Coupled With RNA Sequencing) approach consists in tagging a ncRNA with a MS2 tag and letting interact the ncRNA with its targets. The complexes are then purified using the MS2 tag and the RNAs are sequenced [3].

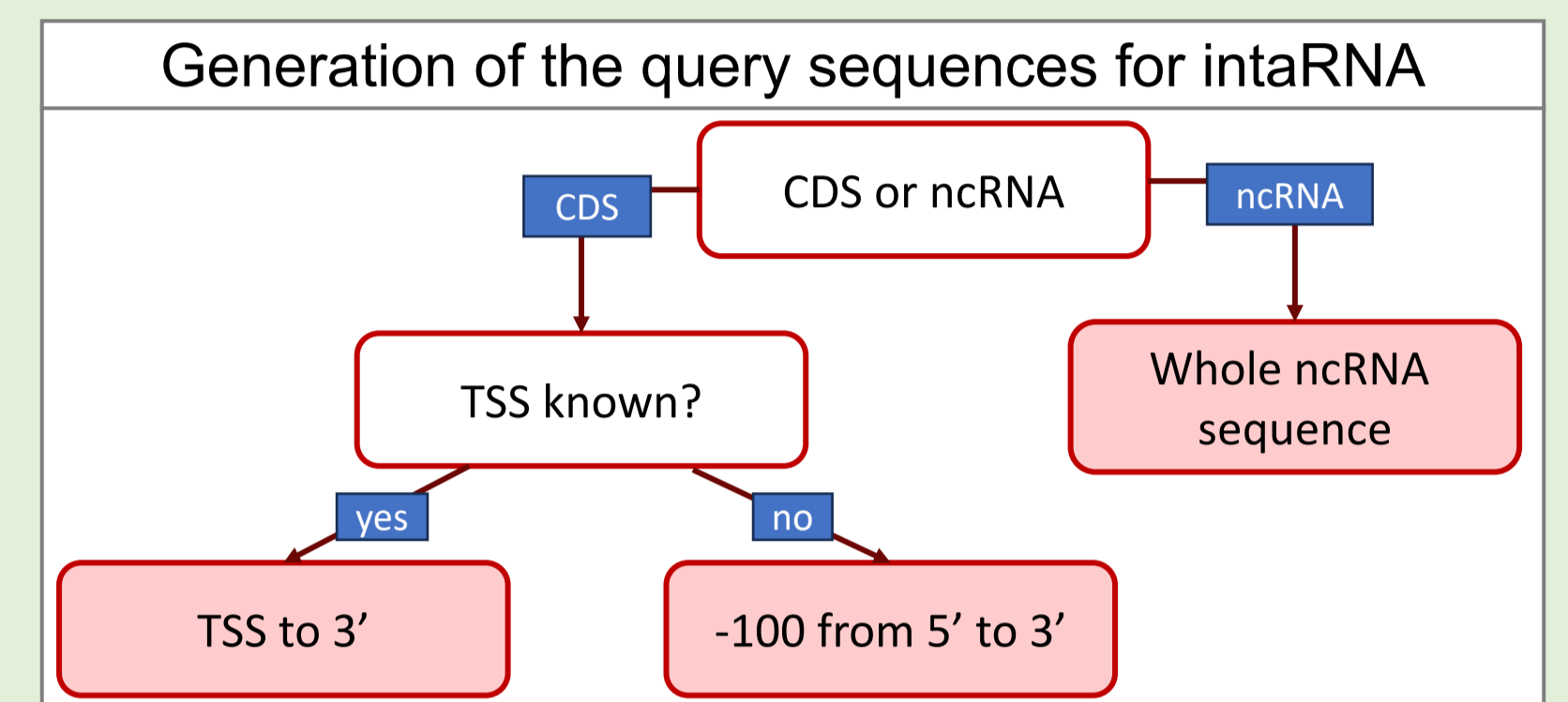
**Differential analysis** of the captured complexes compared to a control condition (untagged ncRNA and MS2 tag alone) **gives the targets of the ncRNA of interest**.

We developed a Snakemake pipeline to manage the MAPS data (quality control, detection of contamination, trimming, mapping, counting).



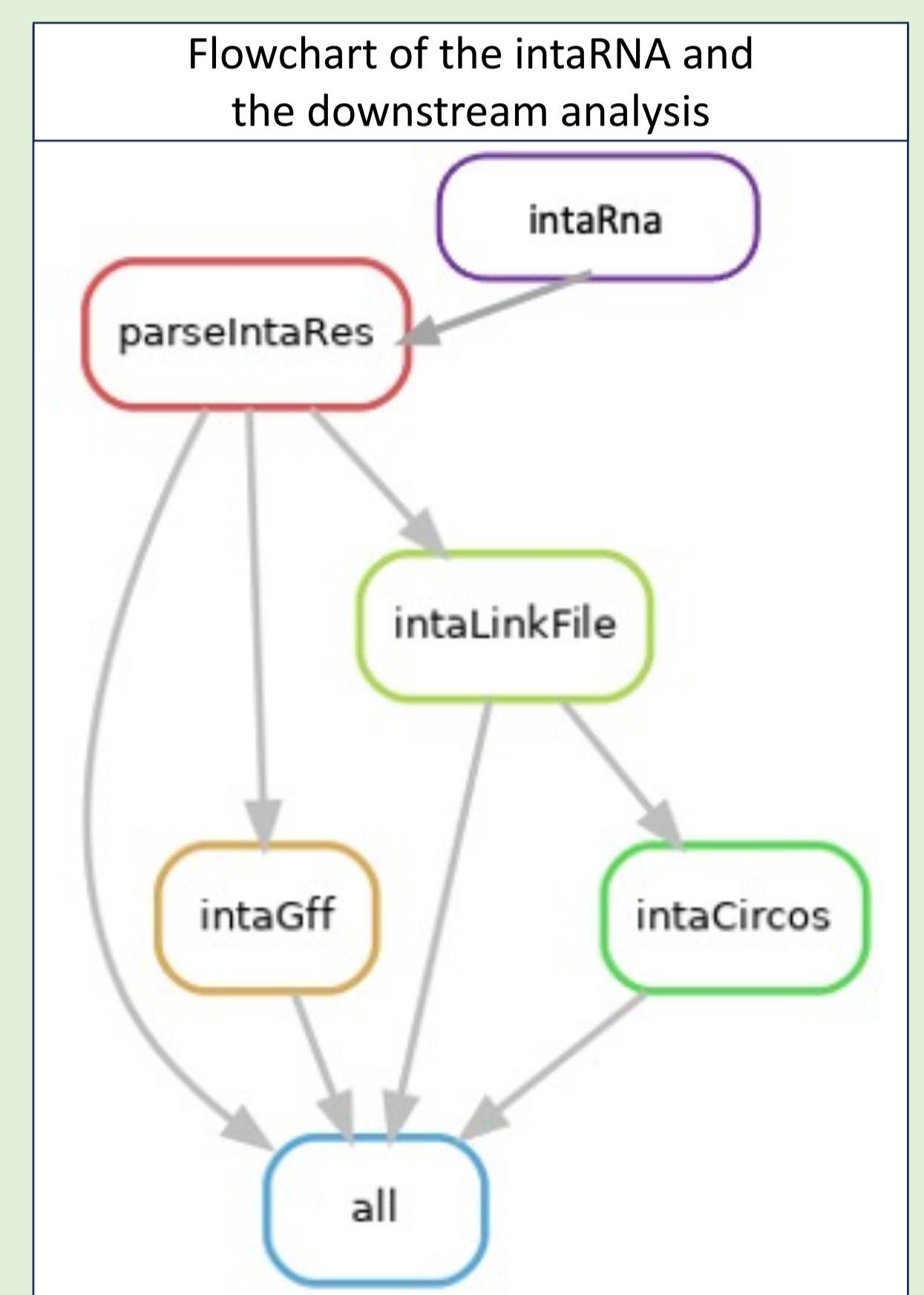
## intaRNA

We used the intaRNA bioinformatic tool [4] to **predict the interactions** between candidate **ncRNAs** and all **mRNAs / ncRNAs** of CD (if TSS known).

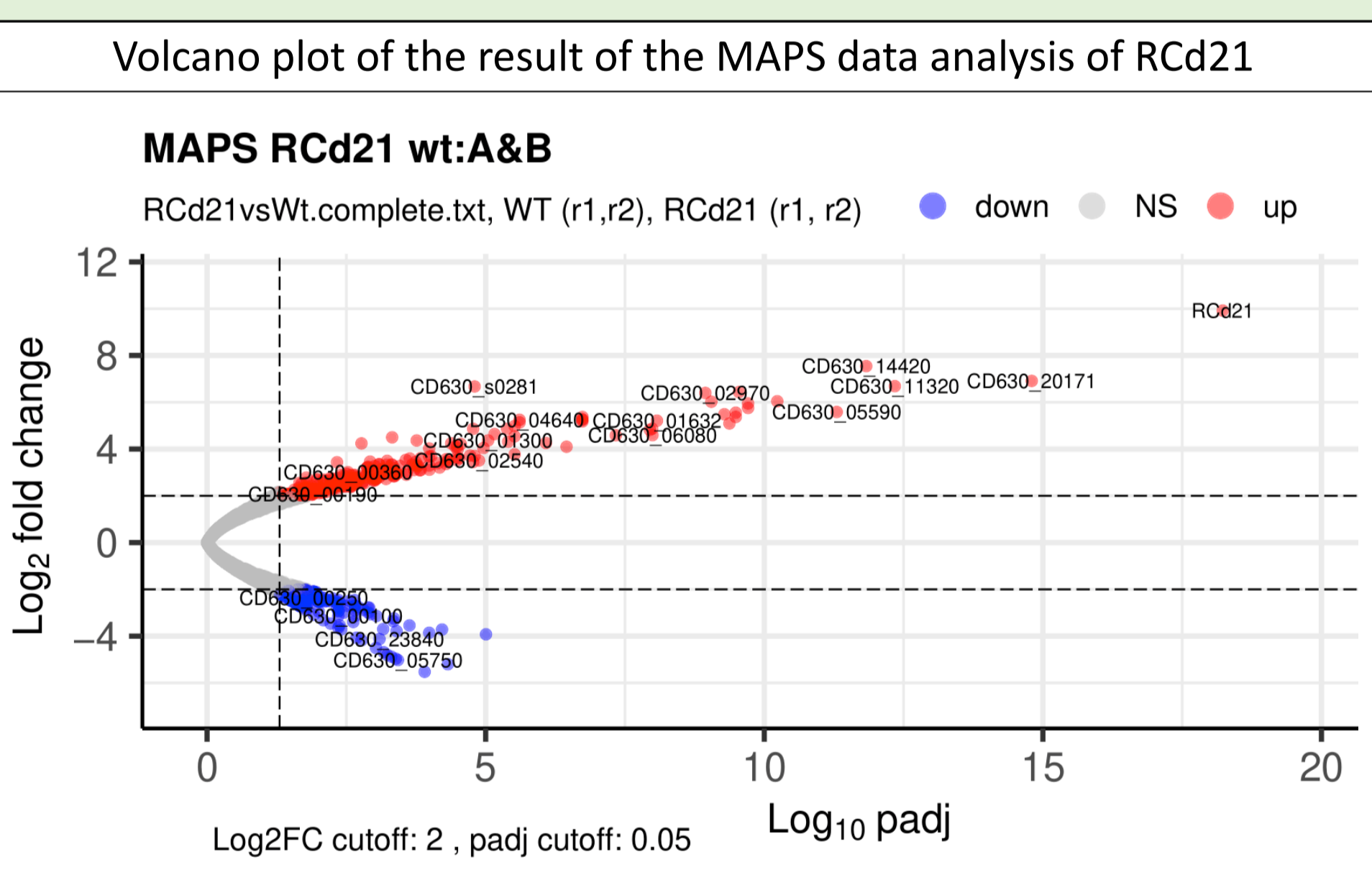


```
Example of an intaRNA output
CD630_07160 39 71
5'-GAA...AAAA UA-- UG AAU AUUG...GCU-3'
      |   |   |   |   |   |   |   |   |   |   |   |   |
      GGGGA GGAUAUGGA AAAA GUAACA
      ||||| ++++++ ||||| ++++++
      CCCCC UCUUAUACU UUUU UAAUUGU
3'-AAU...AAAA UUCA UA -- GAAA...UAU-5'
      |   |   |   |   |   |   |   |   |   |   |   |   |
      171 140
CD630_S01002
interaction seq1 = 39..71
interaction seq2 = 140..171
interaction energy = -17.88 kcal/mol
```

We developed a Snakemake pipeline that can handle the execution of intaRNA, the filtering of the results (keeping the targets with the lowest interaction energy) and the generation of the circos plot for all the ncRNAs of interest.



## Combining the two analyses: example of RCd21

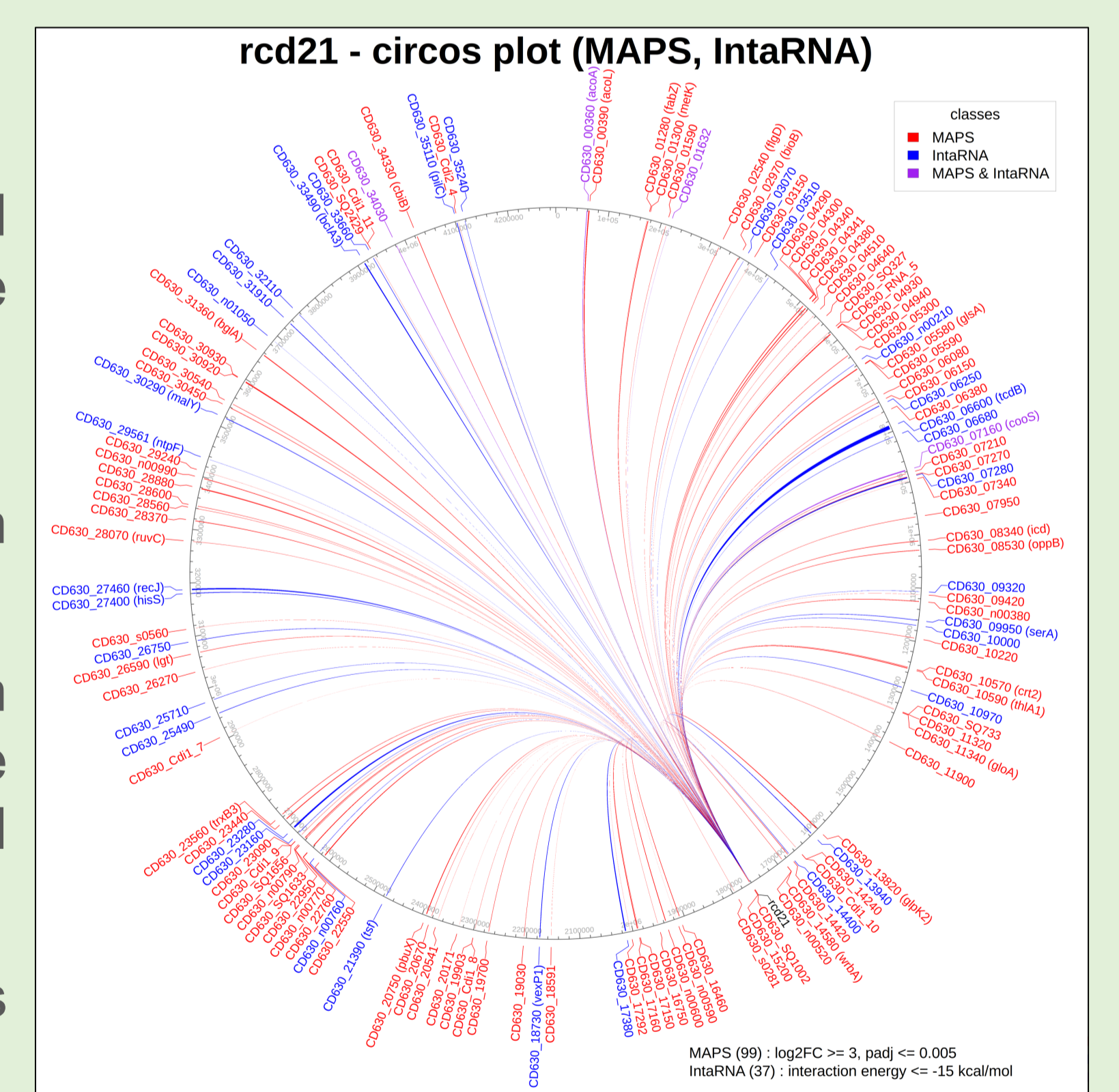


Due to the MAPS purification, only the “up” or targeted RNAs (red dots) of the volcano plots (log2FC vs. log10 of padj) are significant.

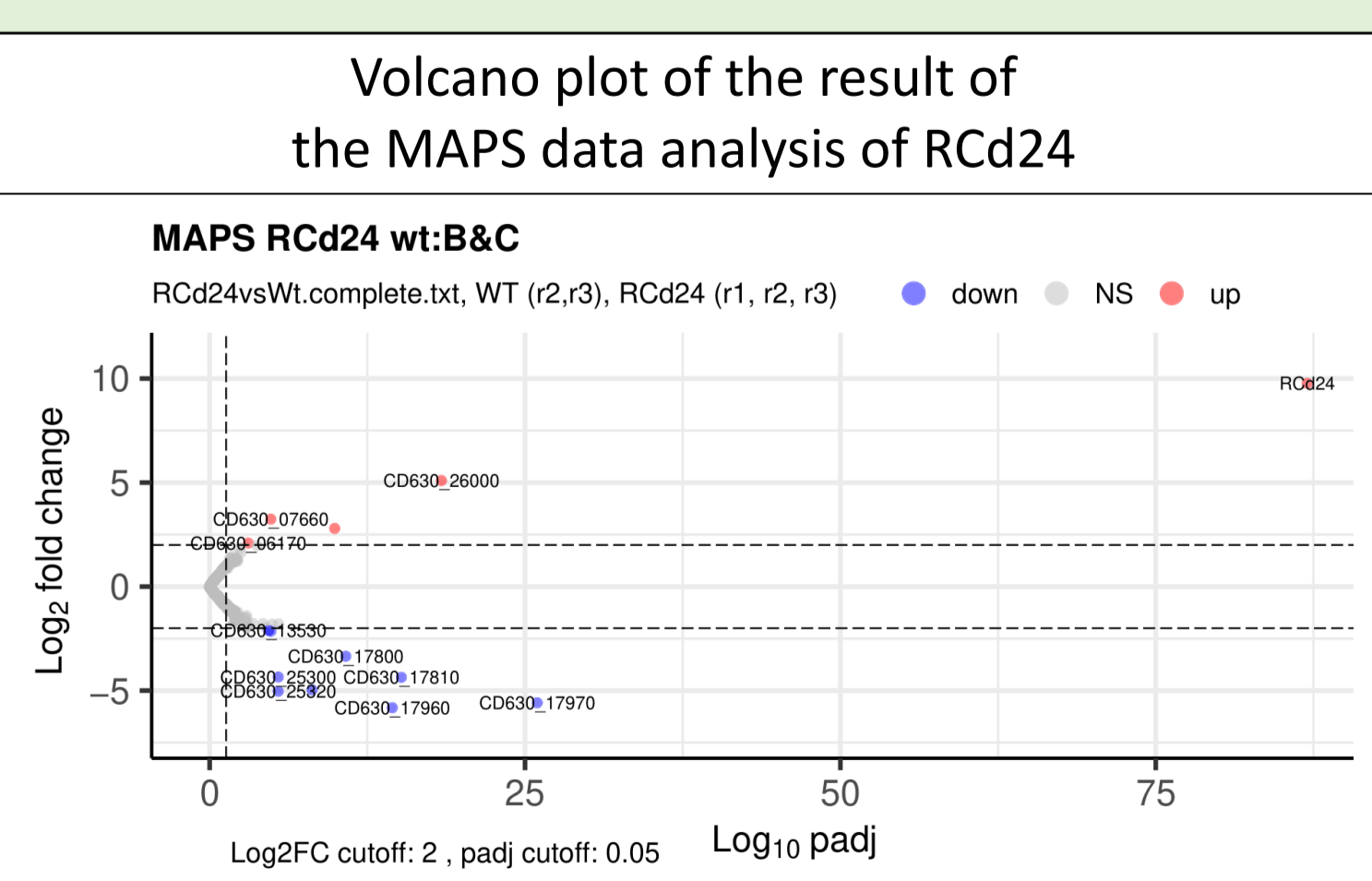
For RCd21, 257 candidates have passed the thresholds. RCd21 appears to be the best because it has been over-expressed in the sample.

In the circos plot, each line connects RCd21 to a target based on the results of the two approaches. The number of displayable target is limited but it is an efficient way to visually identify and locate target candidates.

The 4 genes in purple are the ones qualified by the two methods with customized thresholds for visualisation.



## example of RCd24



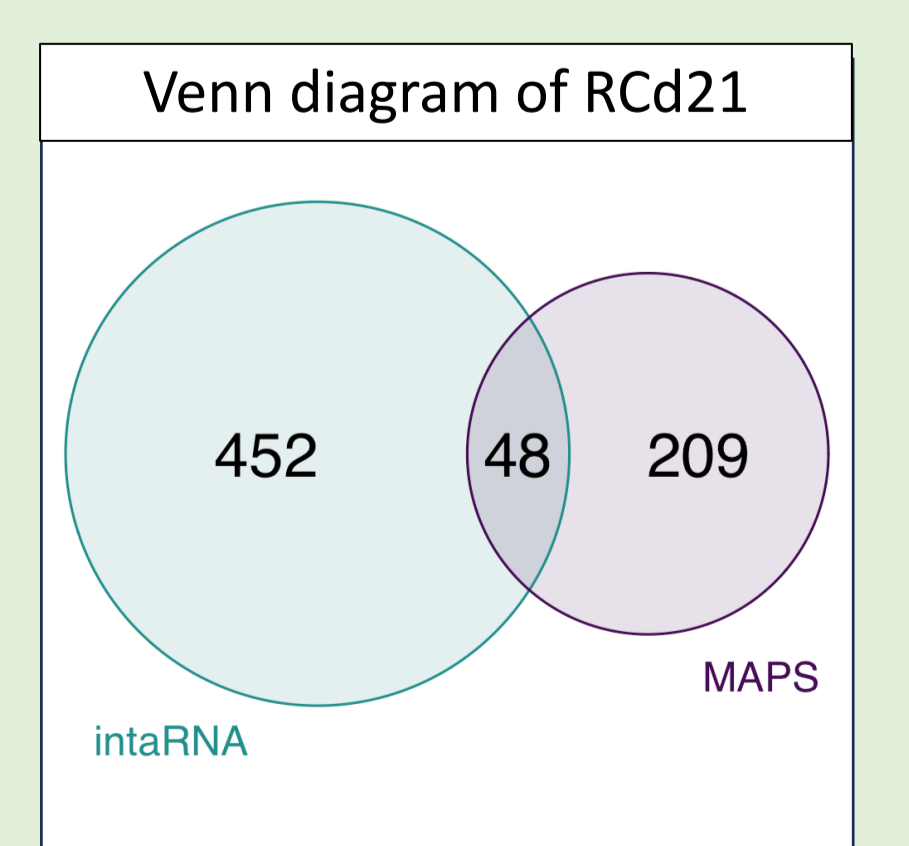
For RCd24, only a few genes have passed the thresholds with low log2FC. In this case, when combined with intaRNA result (215 targets), the benefit remains limited.

## Conclusion and perspectives

By combining experimental and *in silico* approaches, we successfully established a list of promising targets for the selected ncRNA candidates as discussed in [5].

The reliability of the predicted targets could be increased by adding other analyses detecting ncRNA-target interactions such as RIL-seq. Additionally, it is essential to find a way to attribute a weight to each approach to classify them and manage false positives.

GitHub: [https://github.com/jueda99/MAPS\\_intaRNA.git](https://github.com/jueda99/MAPS_intaRNA.git)



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