Detecting transcriptional regulation despite incomplete annotation

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Biological question

☞ What are the transcriptome differences between two biological conditions?

- molecular markers of a specific condition ?

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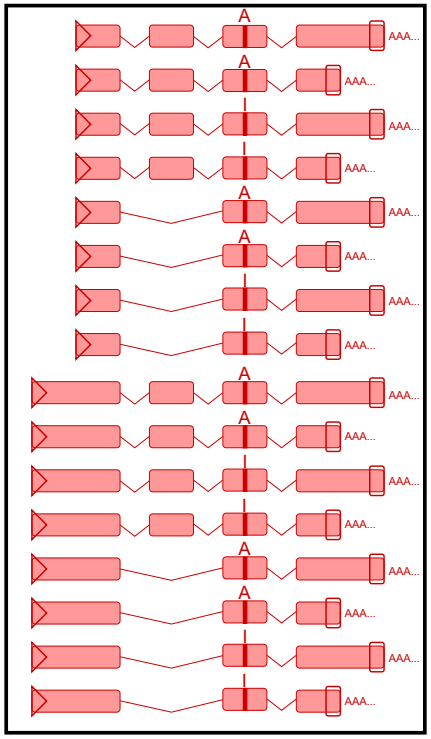
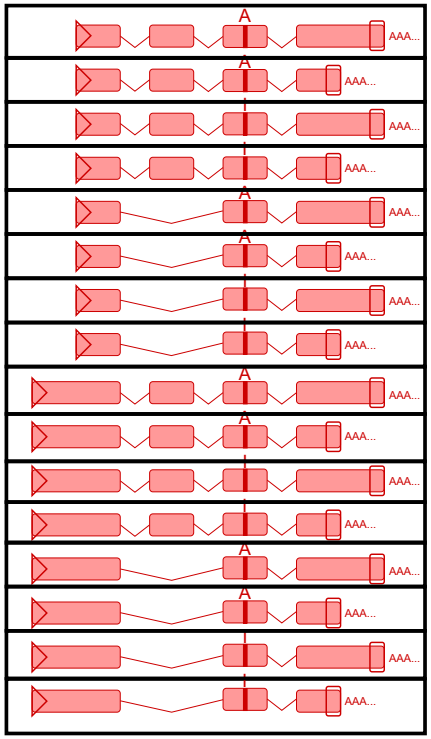
RNA-Seq experiment

- numerous analysis strategies (gene/events/isoforms)

Transcriptome analysis: two extreme views

Gene-level and isoform-level analysis



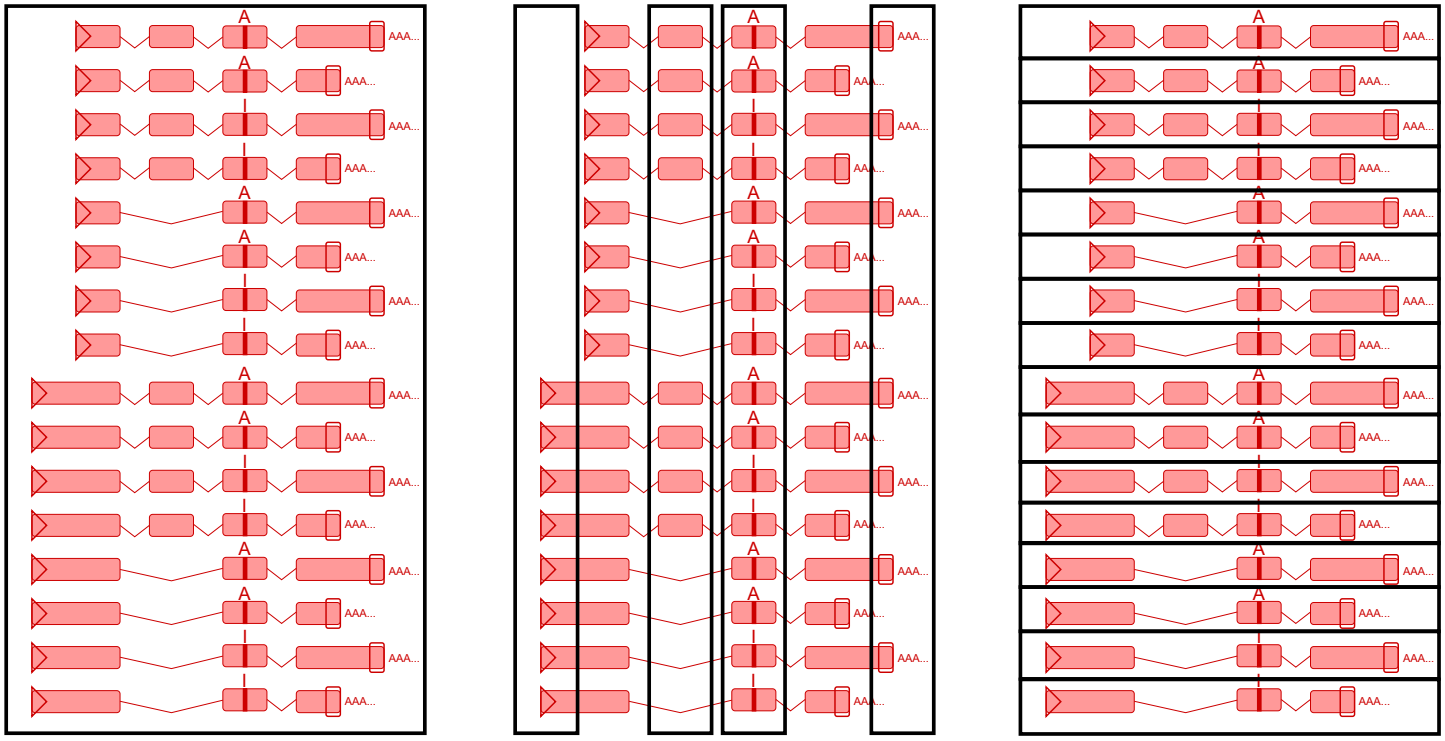


gene-level: miss post-transcriptional regulations [Rhinn et al., 2012]

isoform-level: technically, statistically and biologically more challenging [Soneson et al., 2019, Karlebach et al., 2022]

Transcriptome analysis: the correct scale

Event-level analysis



- should detect post-transcriptional regulations
- [events] <<< [isoforms] $\sim 2^{[\text{events}]}$

Biological question

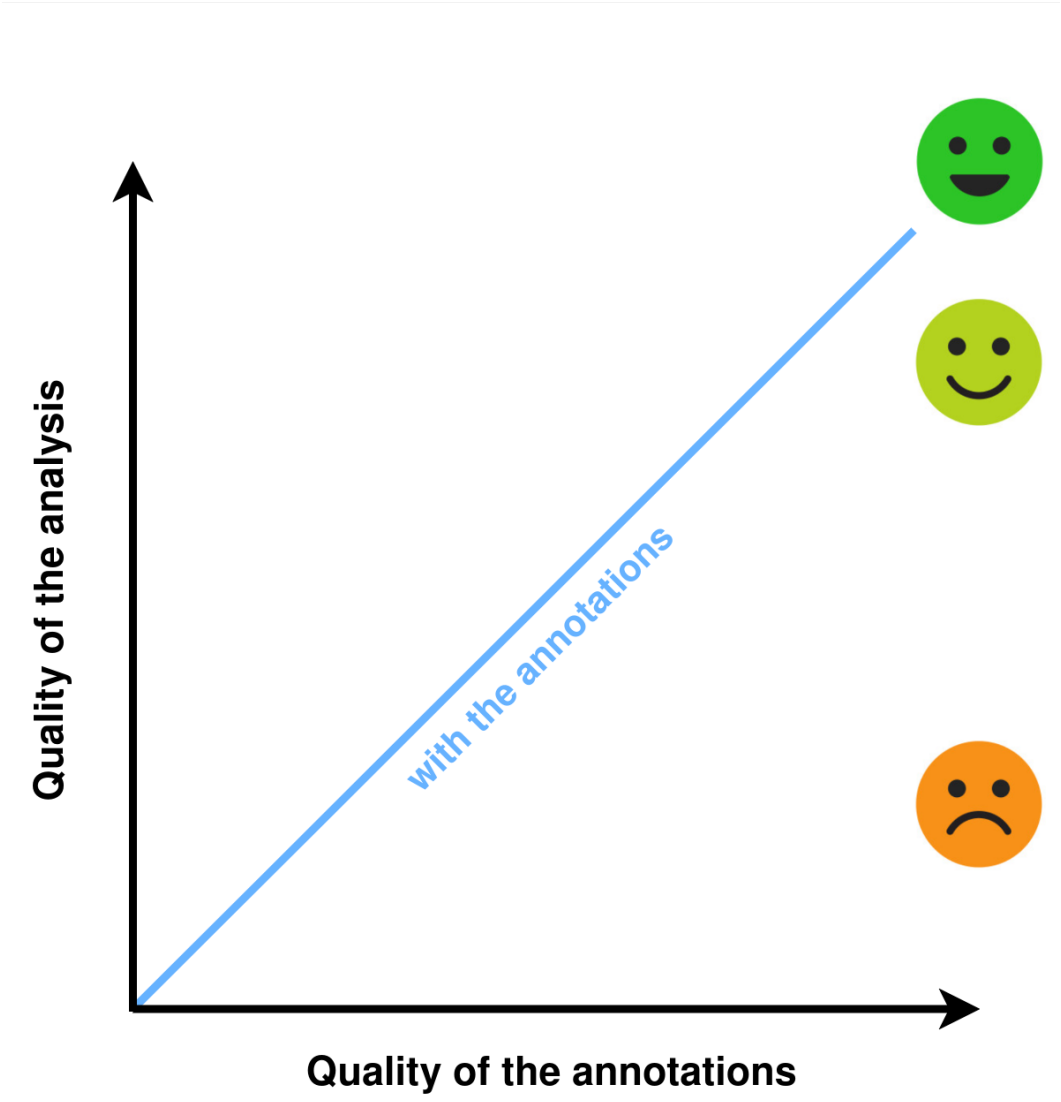
☞ What are the transcriptome differences between two biological conditions?

- molecular markers of a specific condition ?

RNA-Seq experiment

- numerous analysis strategies (gene/events/isoforms)
- highly dependent on annotations quality (incomplete)

Better Annotations, Better Analyses



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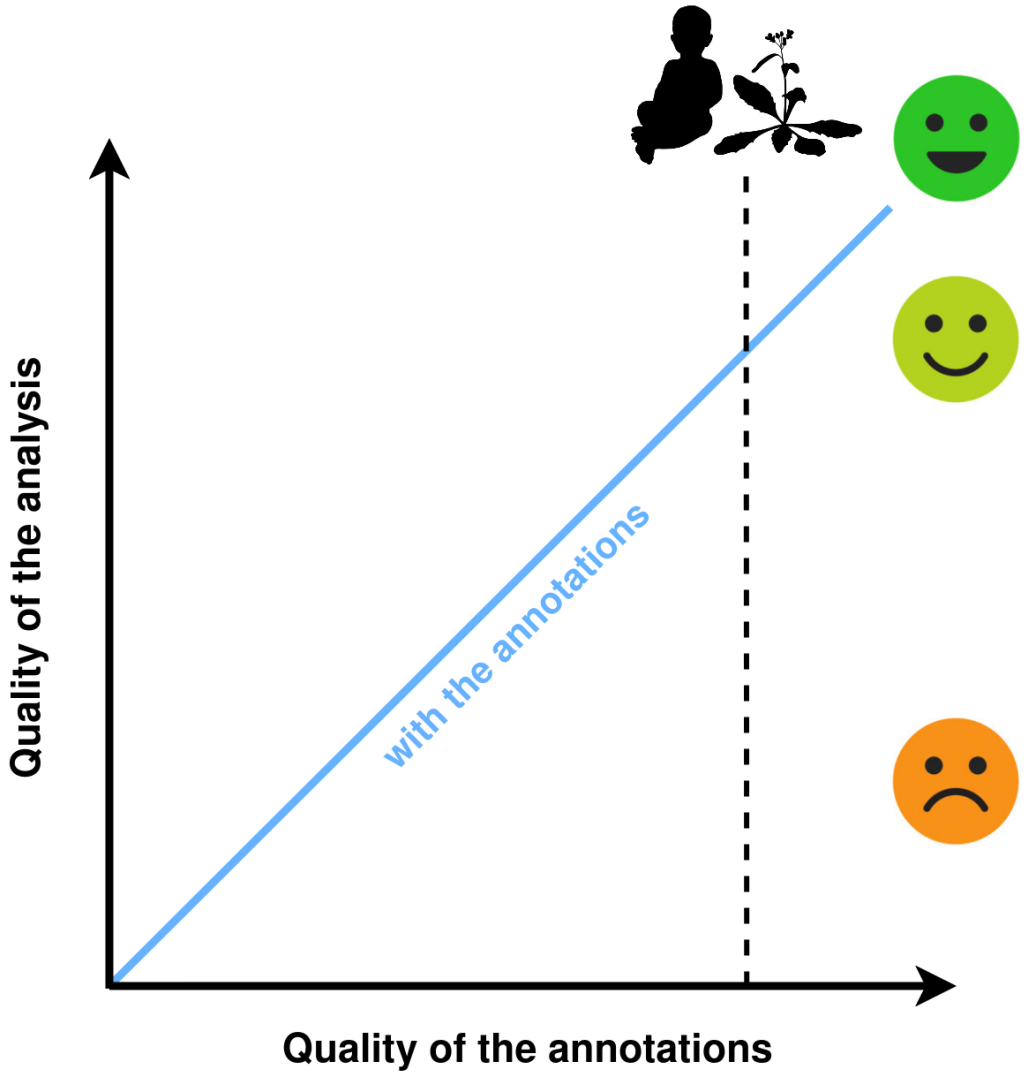

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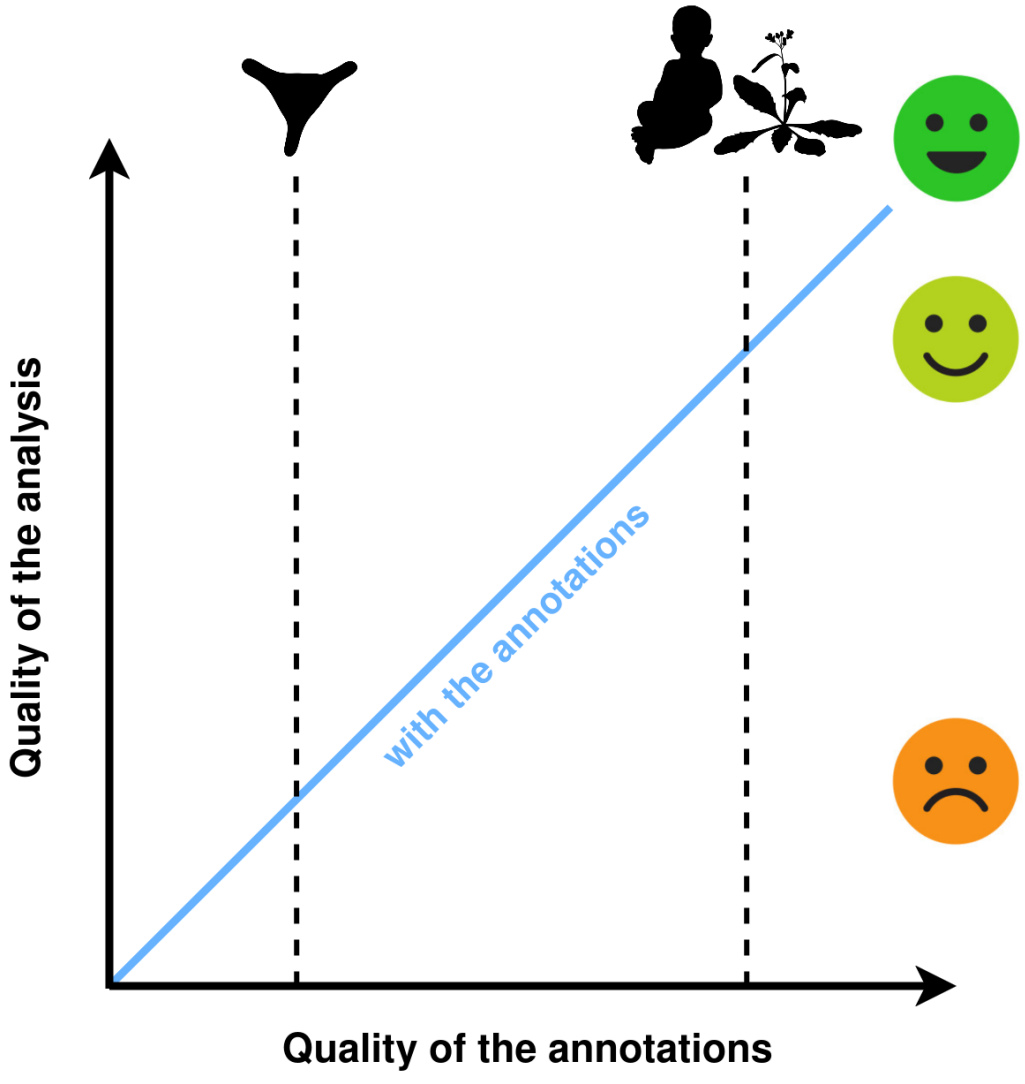

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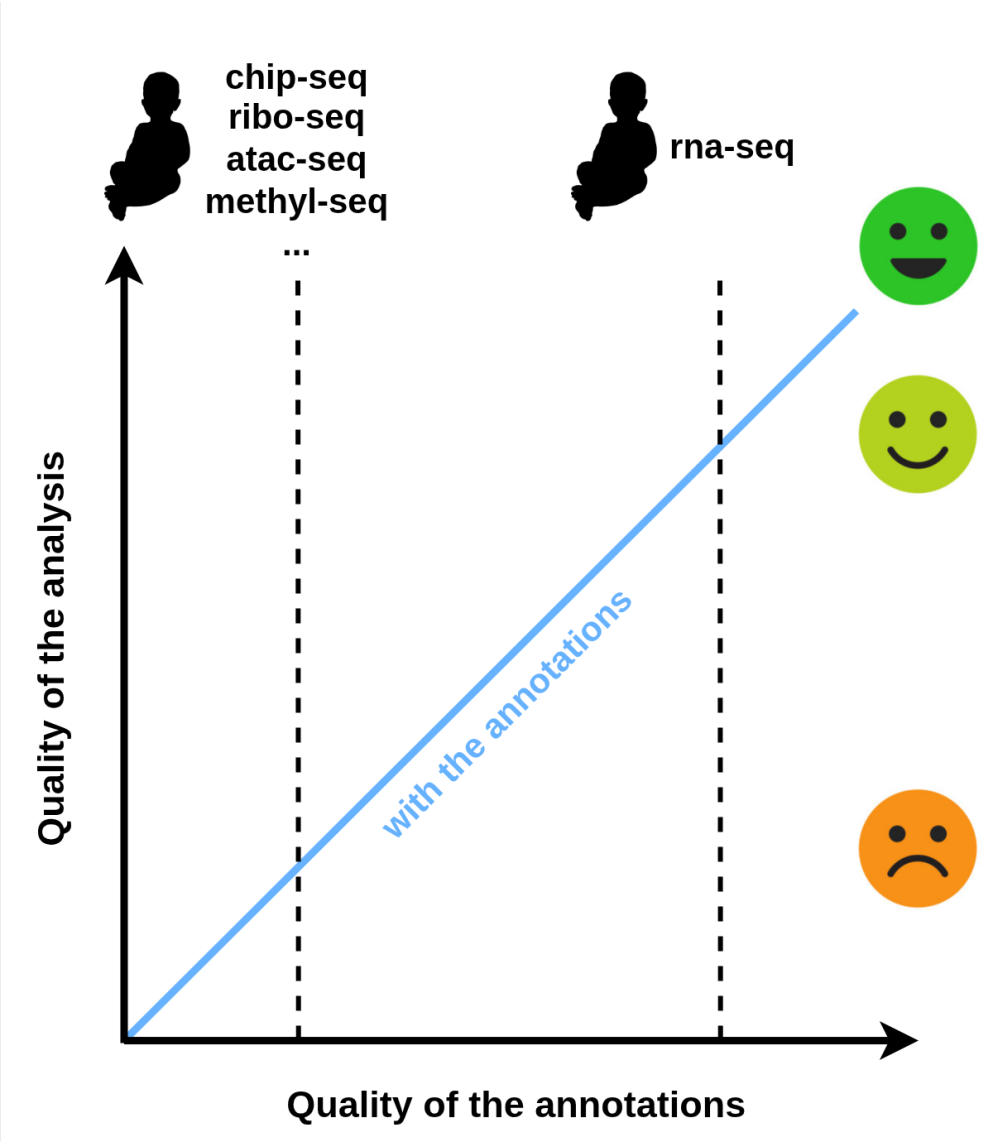
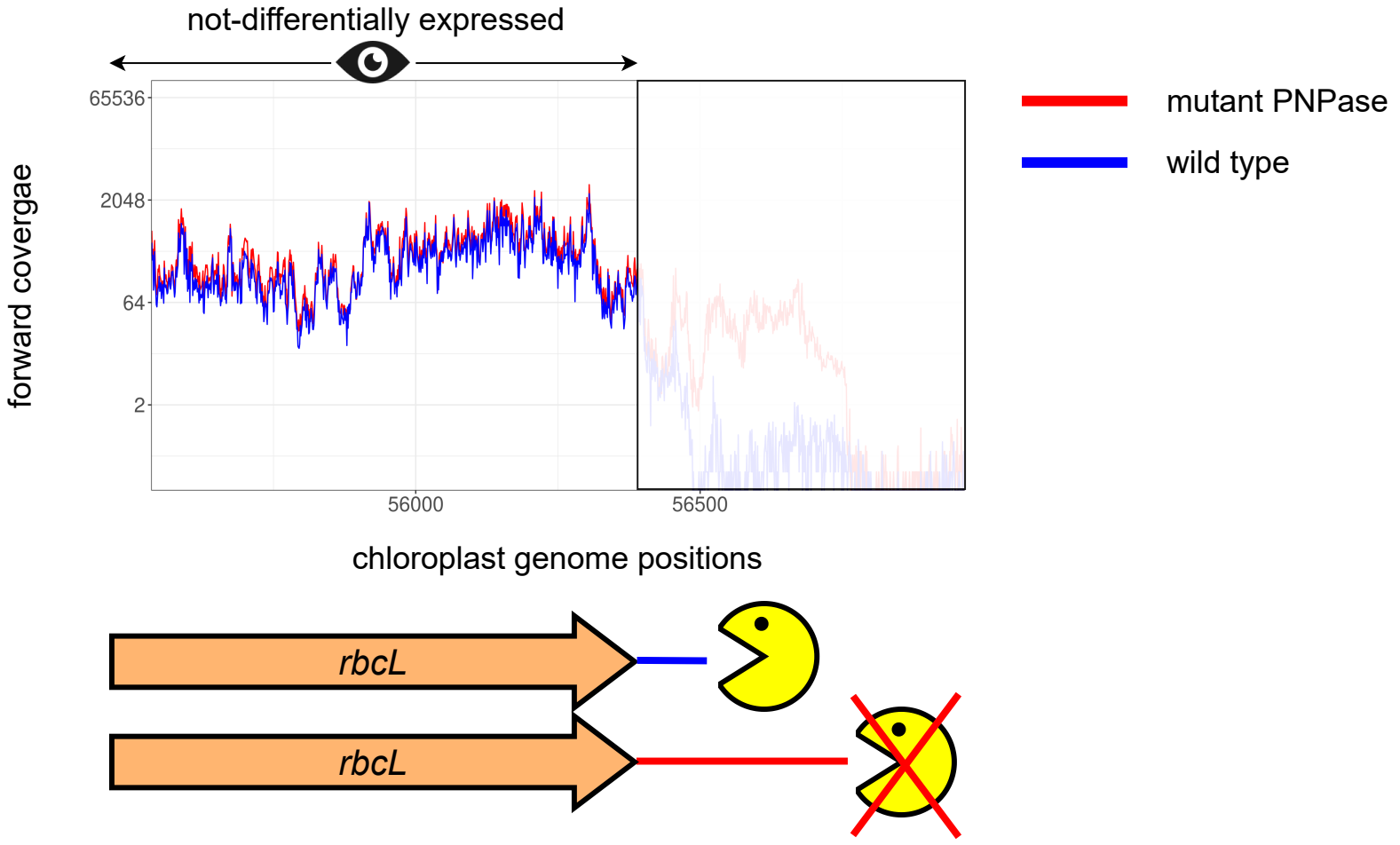


Differential expression analysis within annotations

The annotation of the *rbcL* gene does not capture the 3' extension observed in the *Arabidopsis thaliana* mutant deficient in PNPase activity



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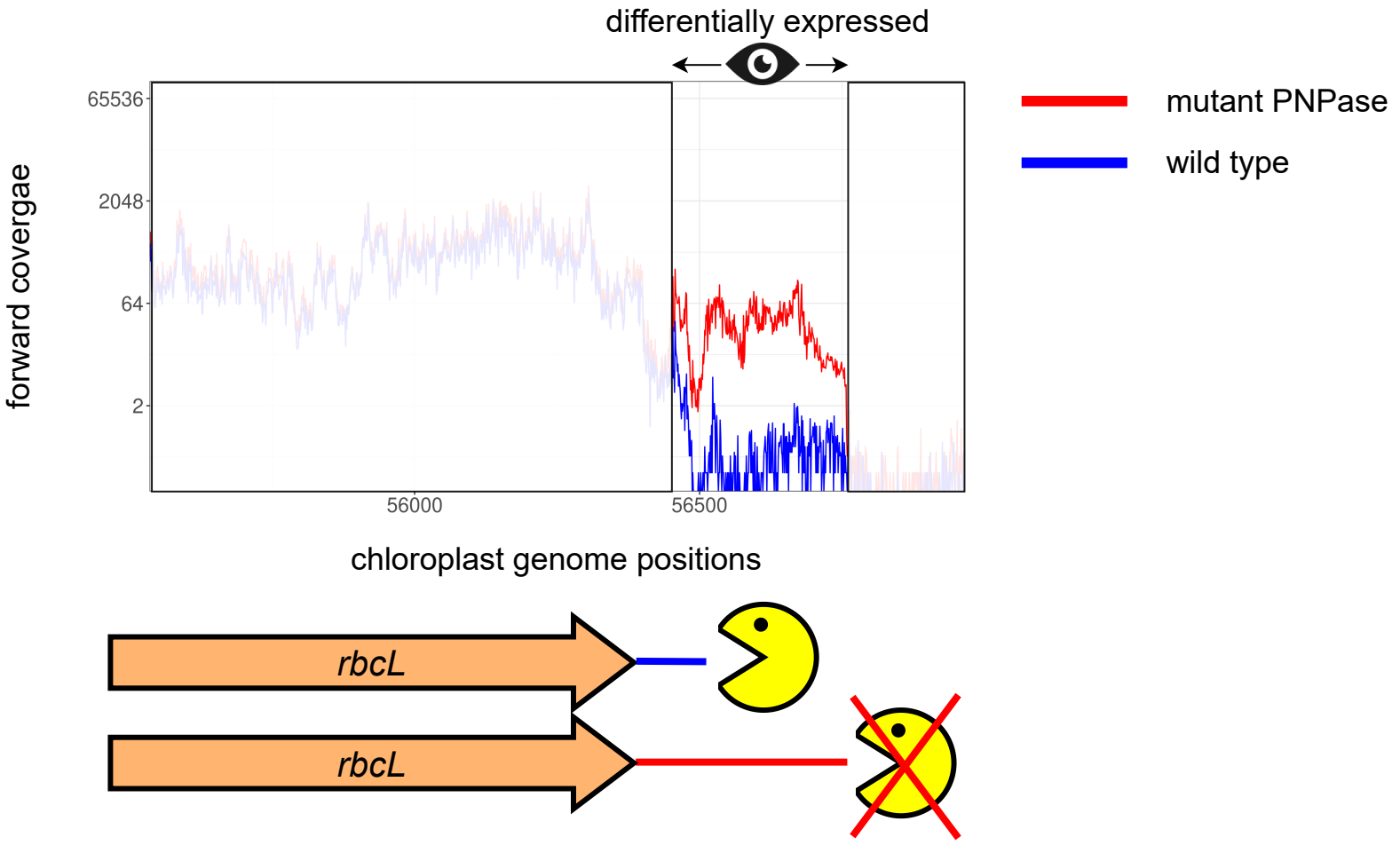
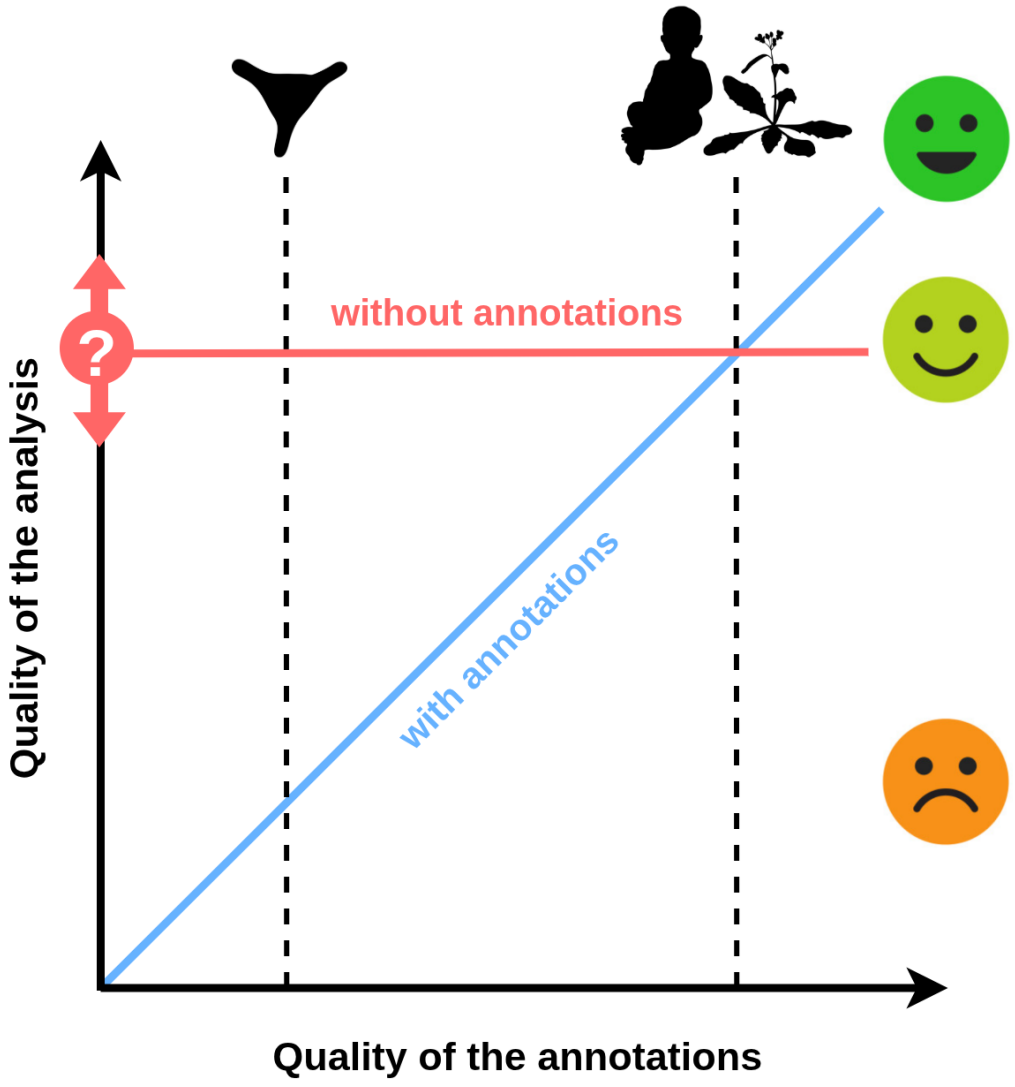
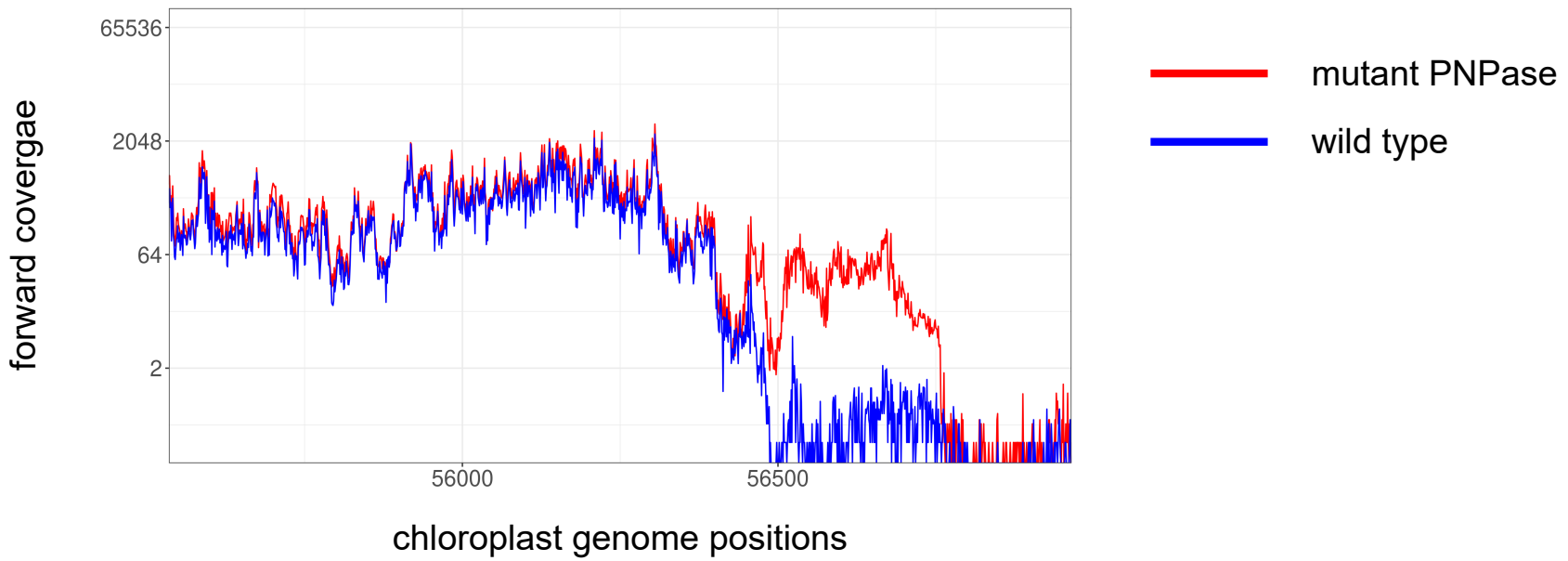


How well can we perform without annotations?



Summarize the signal into regions that are easier to interpret



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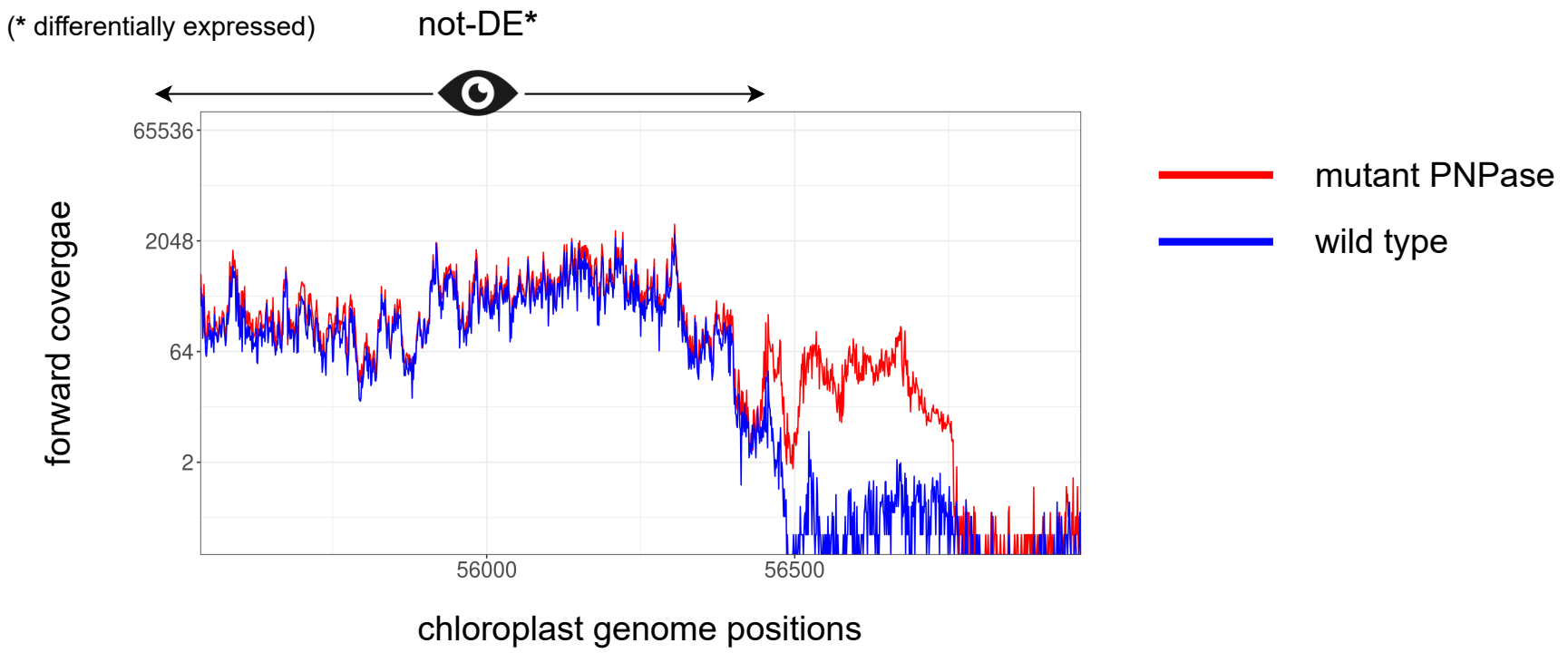

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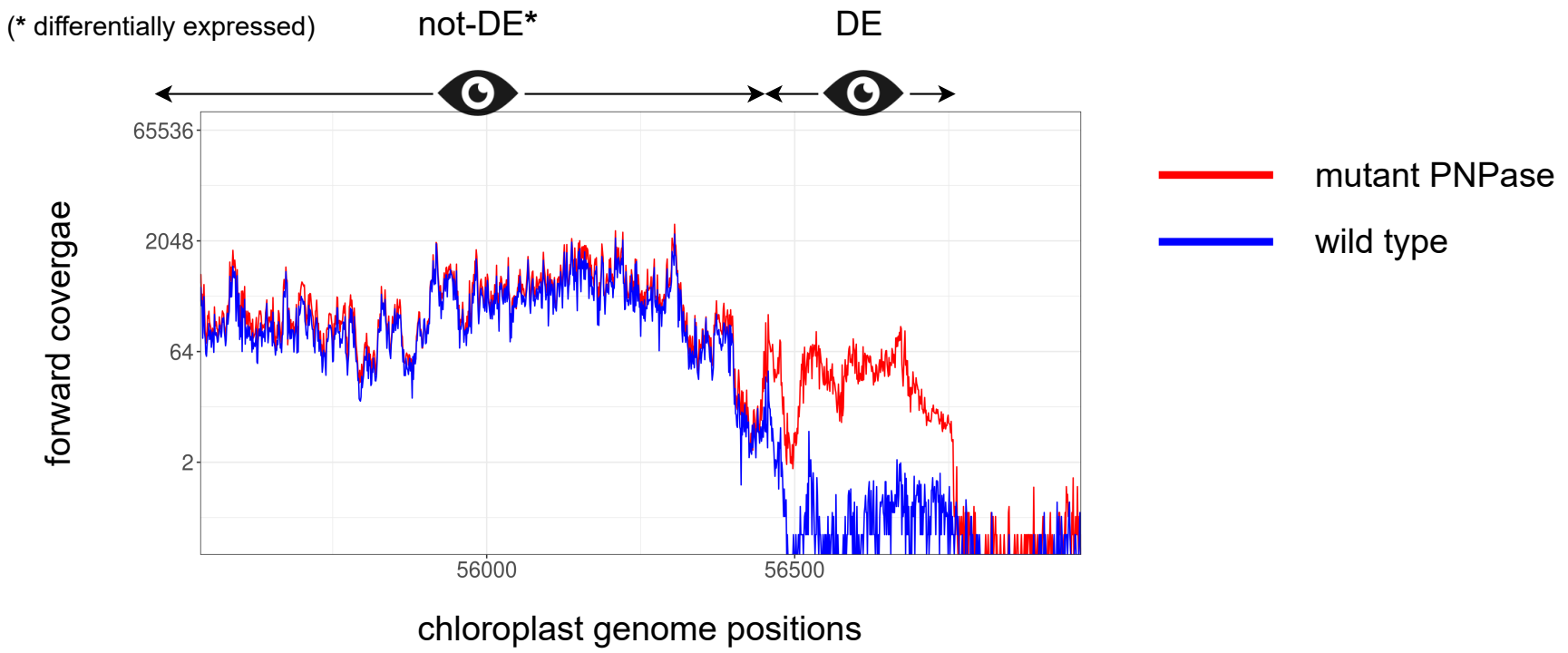

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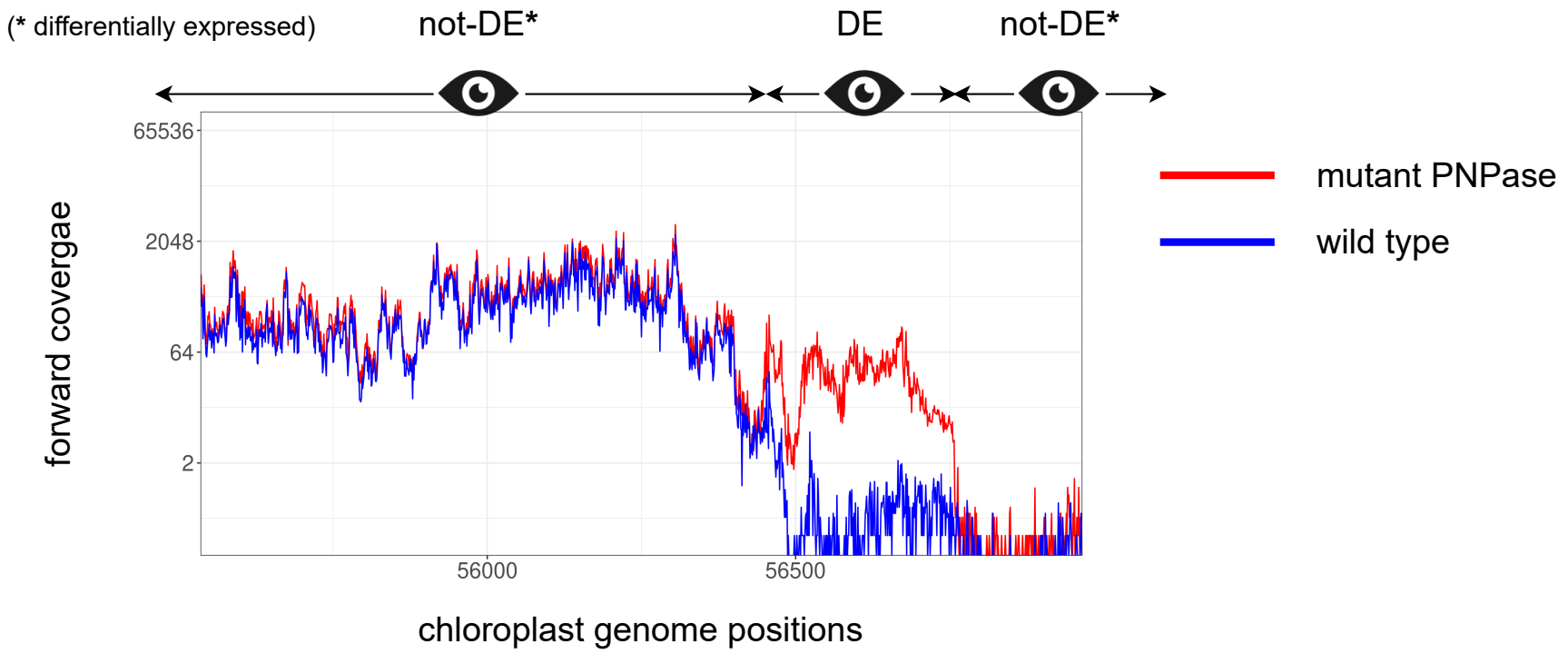

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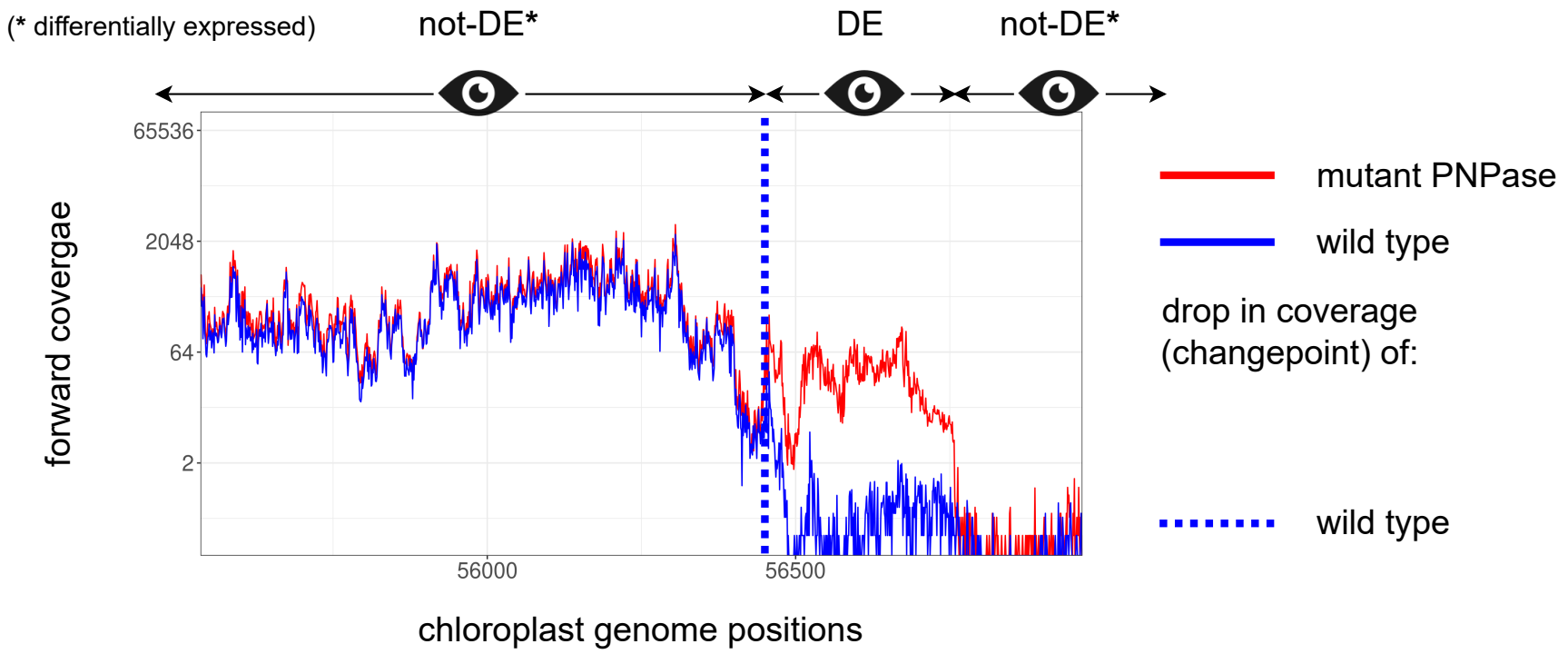

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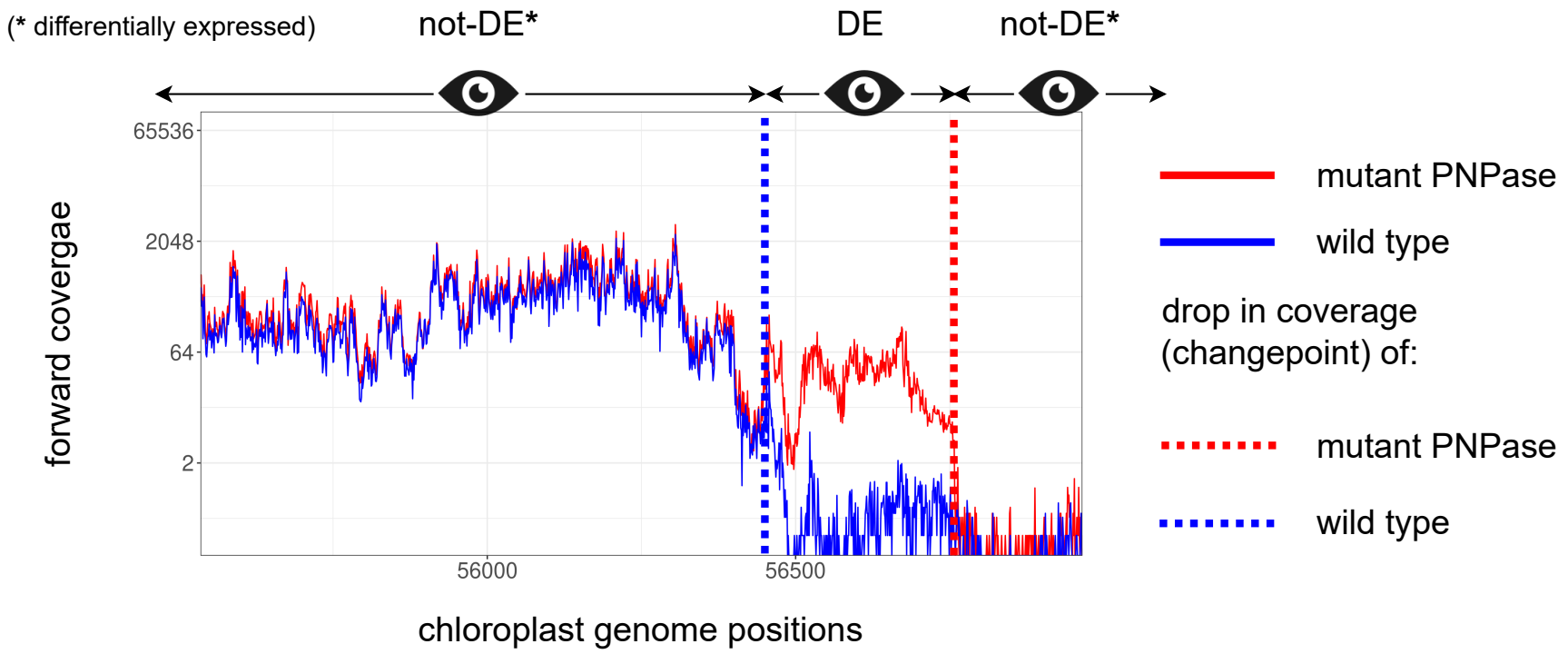

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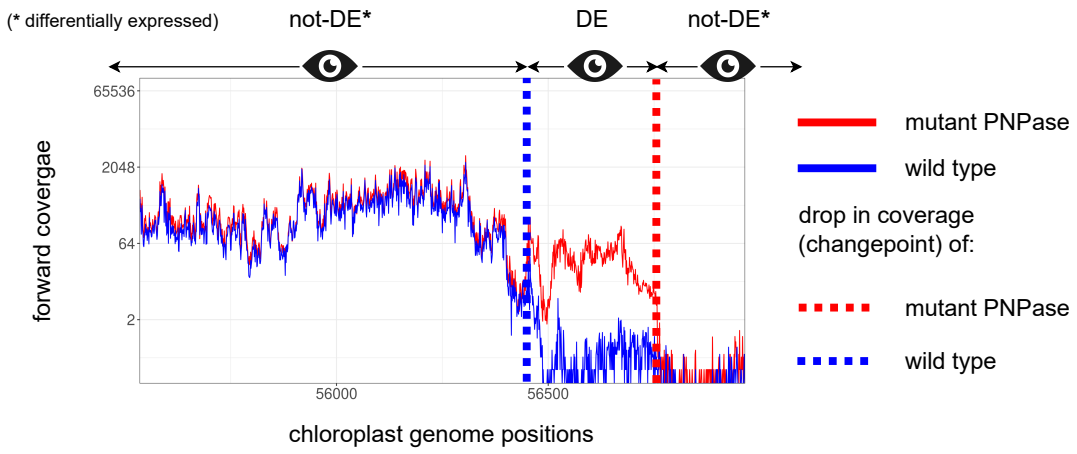
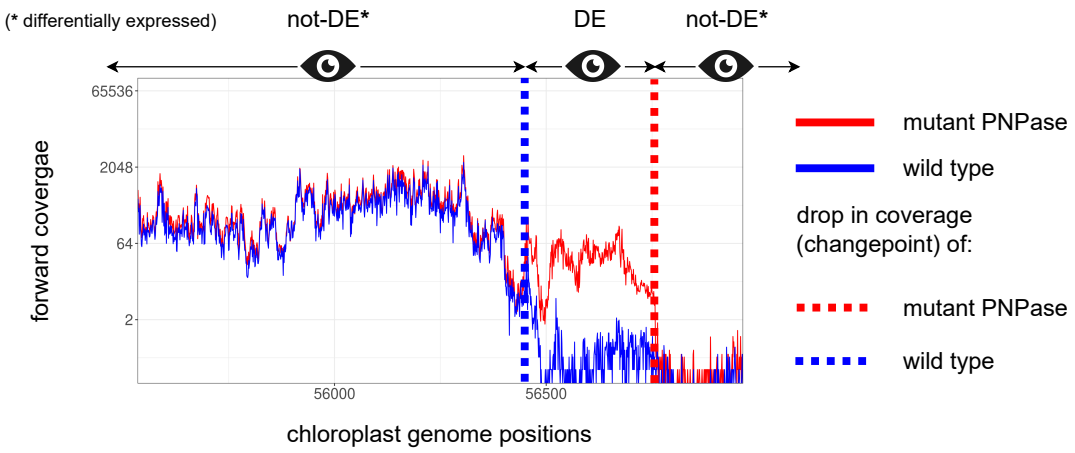


The multiple changepoint detection problem [Fearnhead and Rigaill 2019; Truong et al., 2020]

1. estimate the number of changepoints
2. estimate the positions of changepoints

Summarize the signal into regions that are easier to interpret



The multiple changepoint detection problem [Fearnhead and Rigaill 2019; Truong et al., 2020]

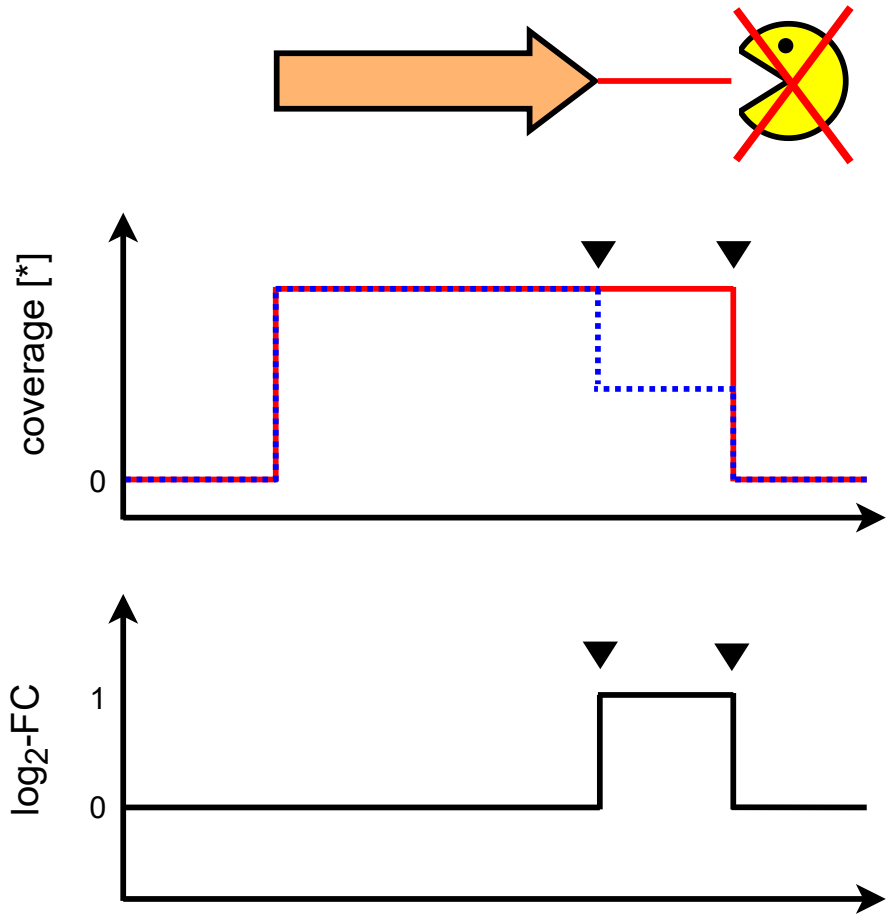
1. estimate the number of differentially expressed regions
2. estimate the positions of differentially expressed regions

What signal should we segment ?

Per-base $\log_2$-FC *"difference of coverages on the log scale"*

1) scales with transcription differences between two conditions

[*Collado-Torres et al., 2017; Mirauta et al., 2014]



changepoint :

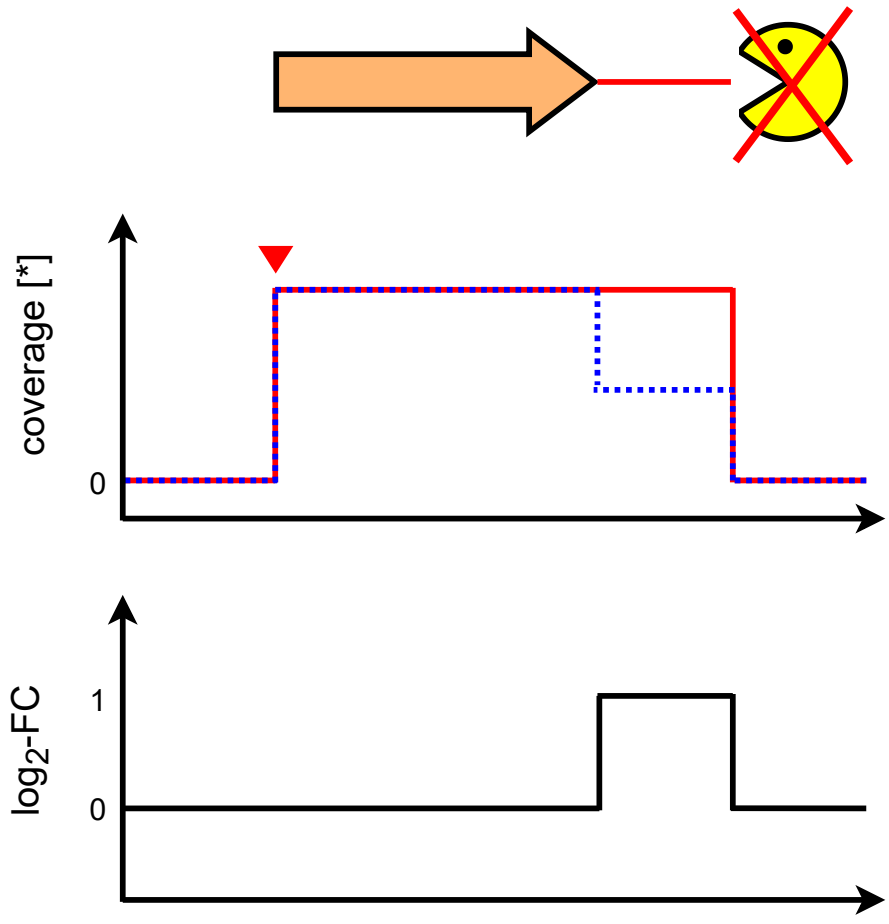
▼ matches transcription differences

What signal should we segment ?

Per-base $\log_2$-FC *"difference of coverages on the log scale"*

2) robust to biological/technical variations in the coverage

[*Collado-Torres et al., 2017; Mirauta et al., 2014]



changepoint :

▼ does not match transcription differences

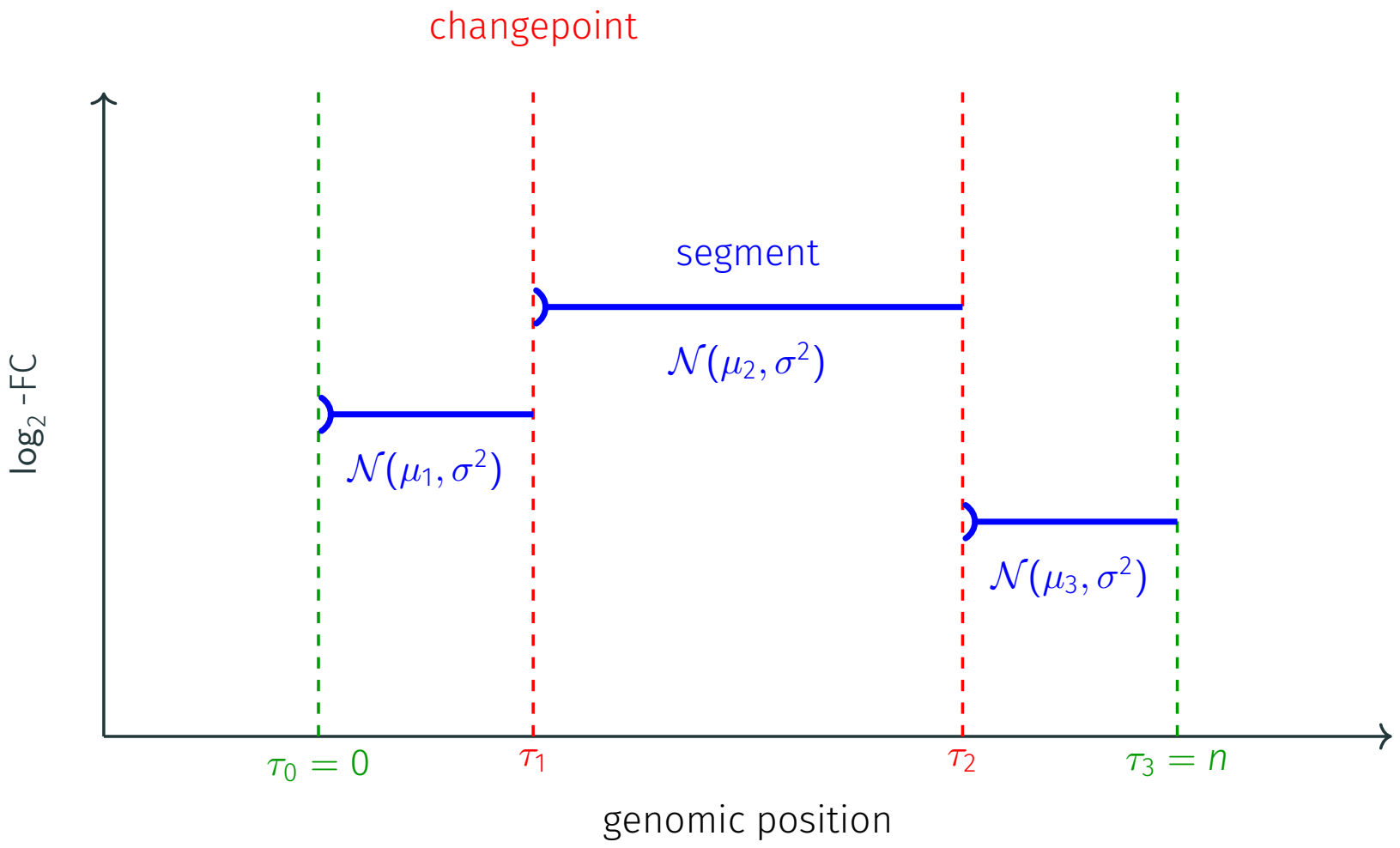
Which methodology should we use?

Changepoint in the mean of a Gaussian signal with FPOP

model $\log_2 \text{-FC}_i \sim \mathcal{N}(\mu_{(\tau_{d-1},\tau_d]}, \sigma^2)$ *i.i.d* (1)

Which methodology should we use?

The homoscedastic piecewise constant Gaussian model (graphically)



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estimate τ minimize a penalized least-squares criterion

[Yao and Au 1989]

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Changepoint in the mean of a Gaussian signal with FPOP

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estimate τ minimize a penalized least-squares criterion

[Yao and Au 1989]

algorithms dynamic programming

[Jackson et al., 2003; Maidstone et al., 2017 (FPOP, log-linear)]

- FPOP : $\sim$ 1 sec on bacterial genome (8Mb)

Which methodology should we use?

Changepoint in the mean of a Gaussian signal with FPOP

$$\text{model} \quad \log_2\text{-FC}_i \sim \mathcal{N}(\mu_{(\tau_{d-1},\tau_d]}, \sigma^2) \;\; i.i.d \qquad (1)$$

More complex models were considered, but (1) is sufficient !

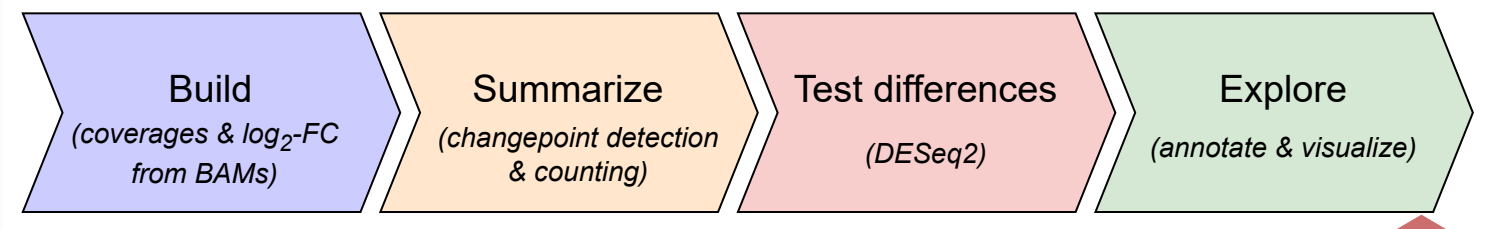
- finds all experimentally validated RNA regulations [1]

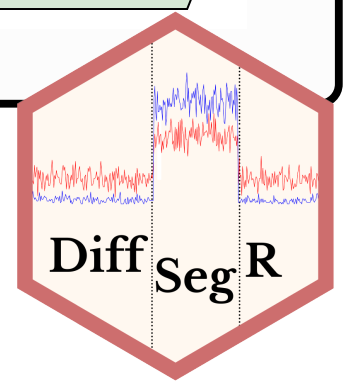
  (threshold heuristics : 13/16 at best [Tran et al., 2016; Collado-Torres et al., 2017; Zytnicki et al., 2021])
- controls the FP on blank experiments

[1] 5' & 3' extension; antisenses, introns and protected RNAs accumulation, (up/down)-regulated exons [Hotto et al., 2011, Germain et al., 2011, Ruwe et al., 2016]

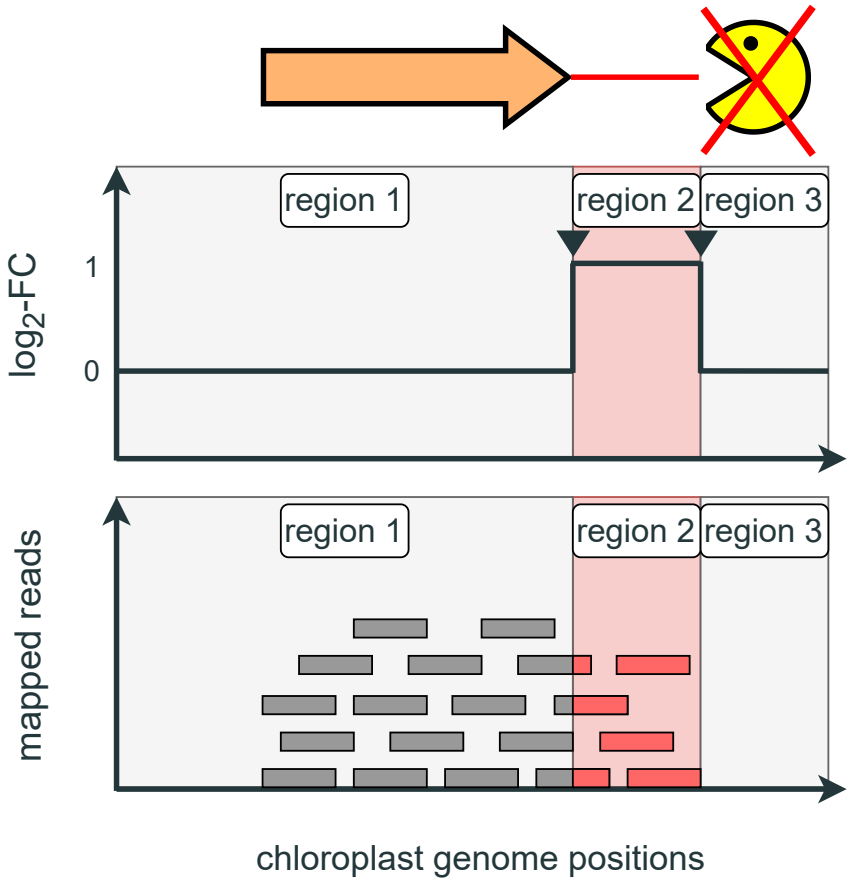
DiffSegR [Liehrmann et al., 2023]

DiffSegR segments the per-base $\log_2$-FC with FPOP to automates the discovery of differentially expressed regions along the genome.





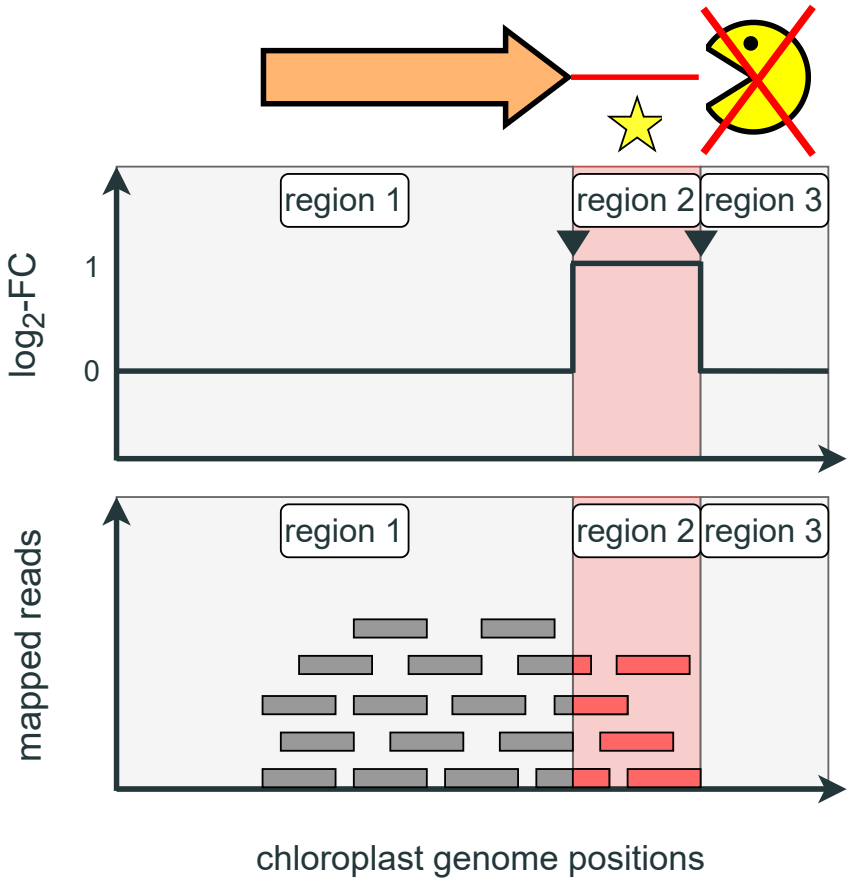
Step 2/4: Counting overlapping reads



region \ sample	mutant 1	mutant 2	wild type 1	wild type 2
region 1	100	104	106	98
region 2	100	104	53	49
region 3	0	0	0	0

counting

Step 3/4: Differential expression analysis



sample / region	mutant 1	mutant 2	wild type 1	wild type 2	adjusted p-value
region 1	100	104	106	98	1
region 2	100	104	53	49	1e-4
region 3	0	0	0	0	1

counting

- $\widehat{\log_2 -\text{FC}} =_? 0$ with **DESeq2** (*NB* generalized linear model) [Love et al., 2014]

Step 4/4: Detection of fine regulations with IGV

mutant PNPase

wild type

up-regulated (DESeq2)

not-differentially expressed (DESeq2)

Step 4/4: Detection of fine regulations with IGV

1 up-regulated exon

2 3' extension

3 5' extension

4 intron accumulation

[Walter et al., 2002; Germain et al., 2011; Hotto et al., 2011; Castandet et al., 2013]

Evaluating TPR on *A. thaliana* nuclear genome [preliminary]

- Peersim dataset :
  - ambient T° vs elevated T°
  - 20 biological replicates per condition

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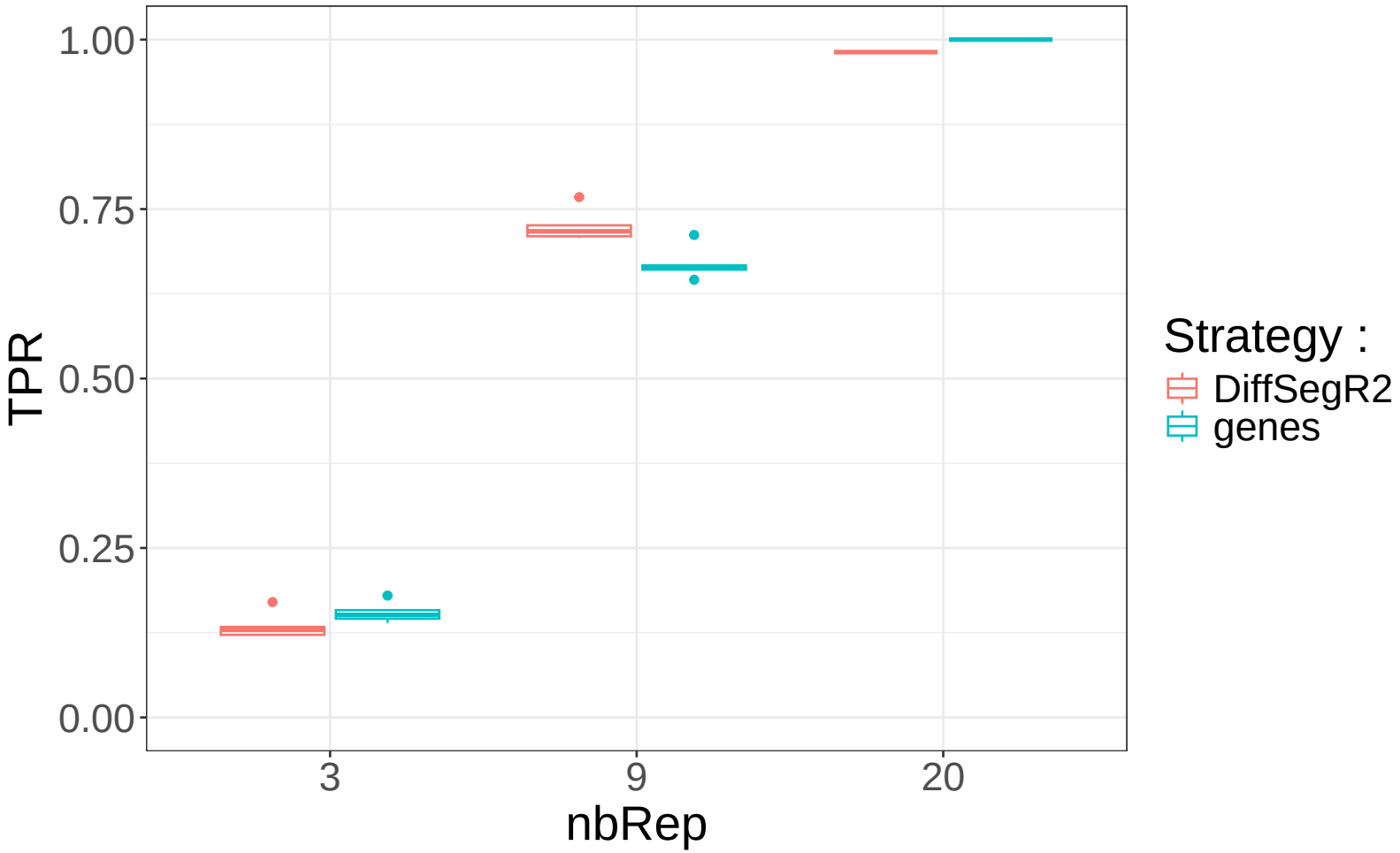
- Peersim dataset :
  - ambient T° vs elevated T°
  - 20 biological replicates per condition
- Power experiment :
  - differential gene expression analysis, 20 vs 20 [gold-standard]
  - Resampling : 3 vs 3 - 9 vs 9
    - Ex : {rep 1, rep 9, rep 17} vs {rep 1, rep 9, rep 17}
  - chromosome 1 (30M bp)

DiffSegR2 performs on par with gene annotations [preliminary]

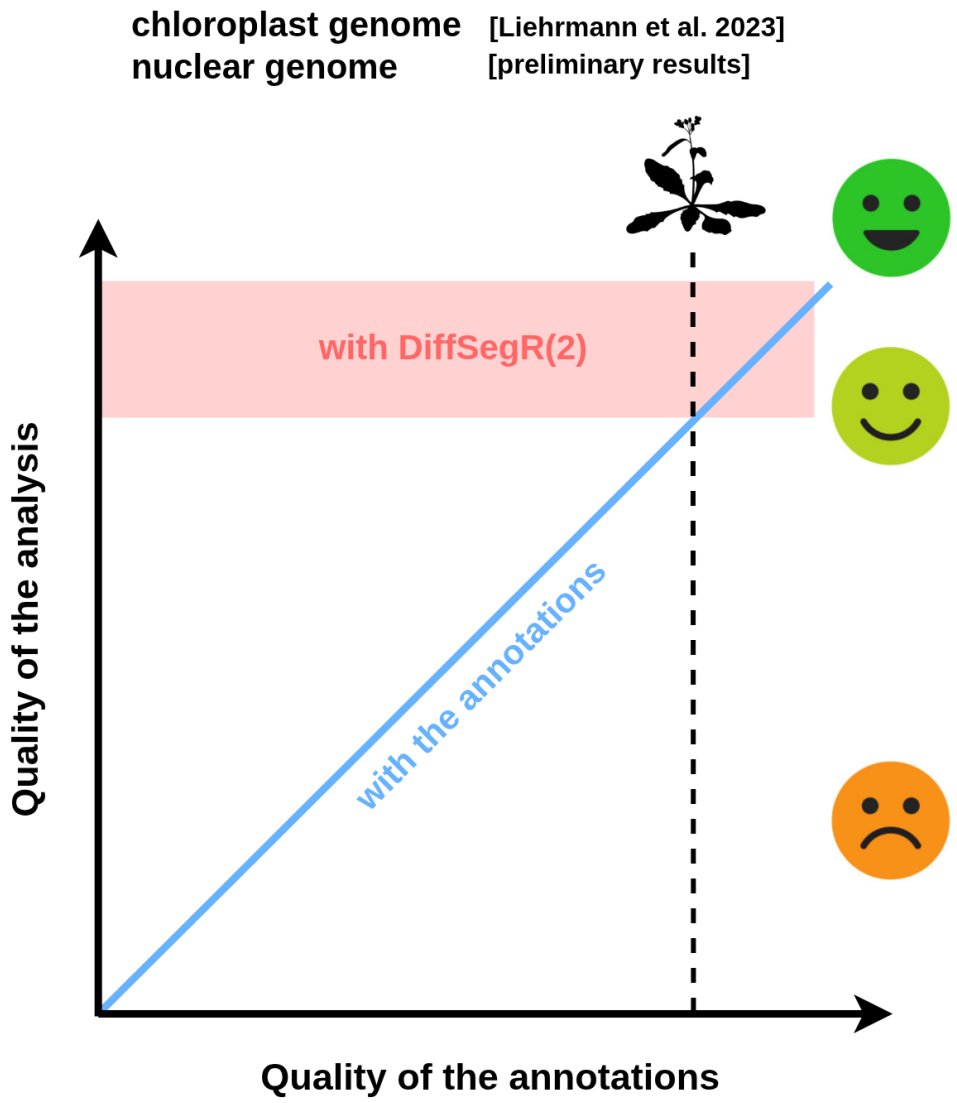


Up to 16% of segments fall outside the gold-standard

[preliminary]

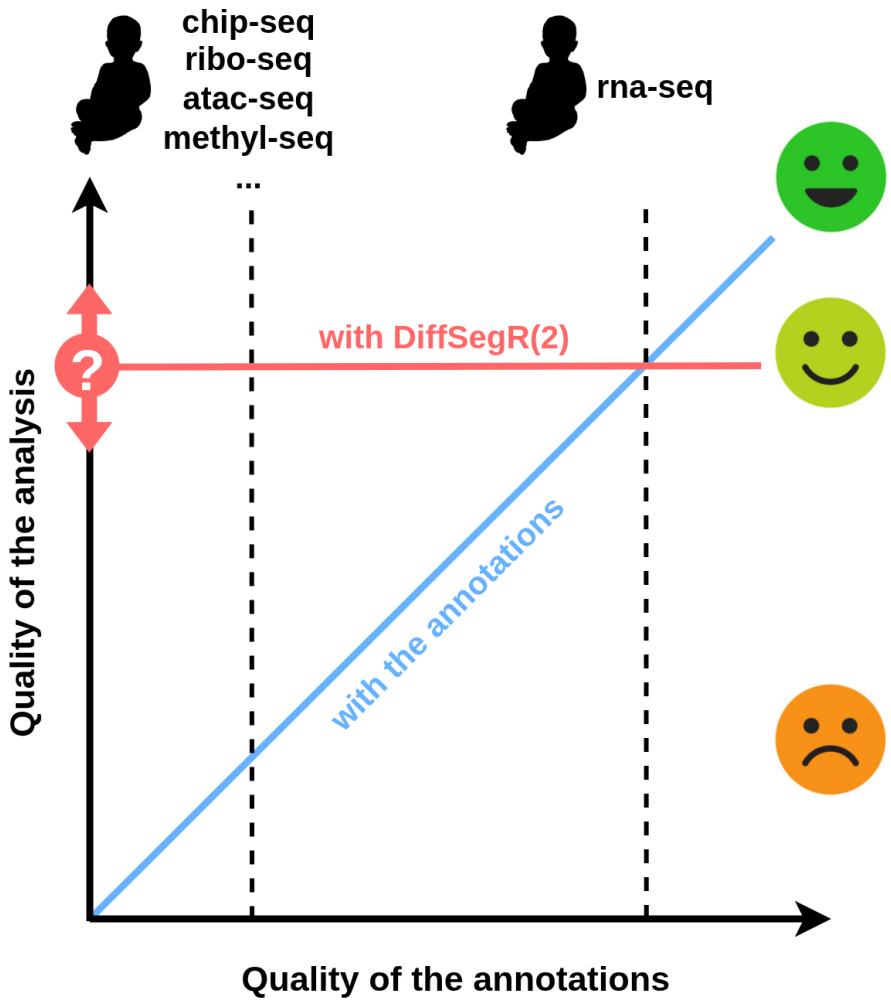
- 9% fall within genes not found in the gold-standard
- 5% fall within antisense of genes
- 2% fall within intergenic regions ($\sim$ 200 segments)

Take home message



Perspectives

- DiffSegR can analyze other experiments (RIP-Seq, [Tran et al., 2023])



Acknowledgements

Collaborators

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- Véronique Brunaud

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- Véronique Brunaud
- Sophie Lemoine
- Erwan Corre