



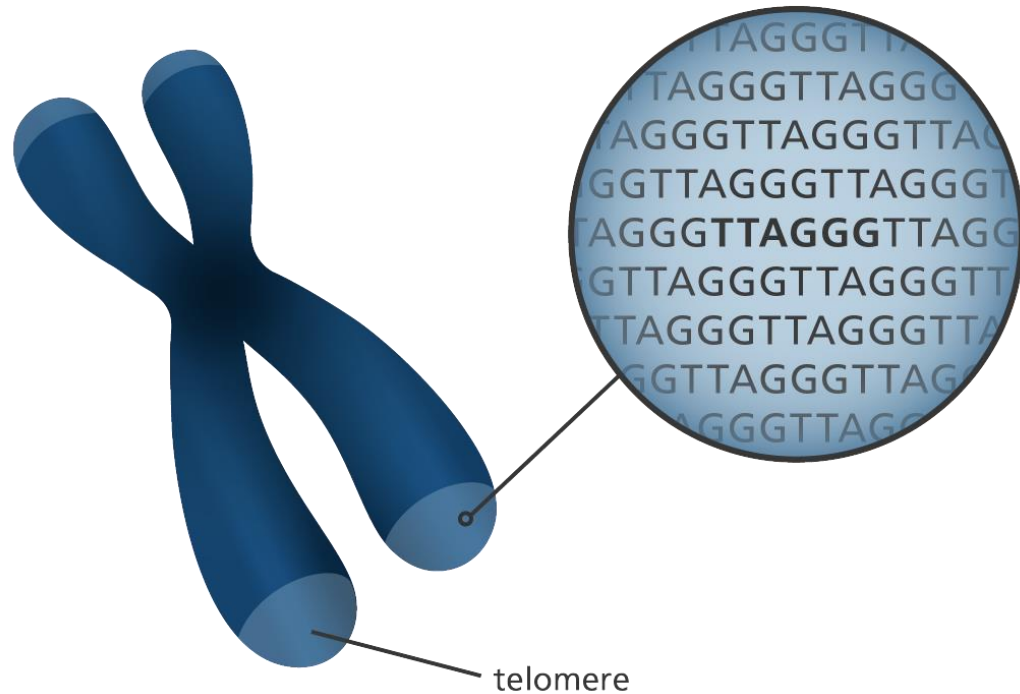
*Fantastic
telomeres*
(and where to find them)

- what telomeres, telomerases and TRs are
- how we can predict TR genes *in silico*
- how we can verified our results *in vitro/in vivo*



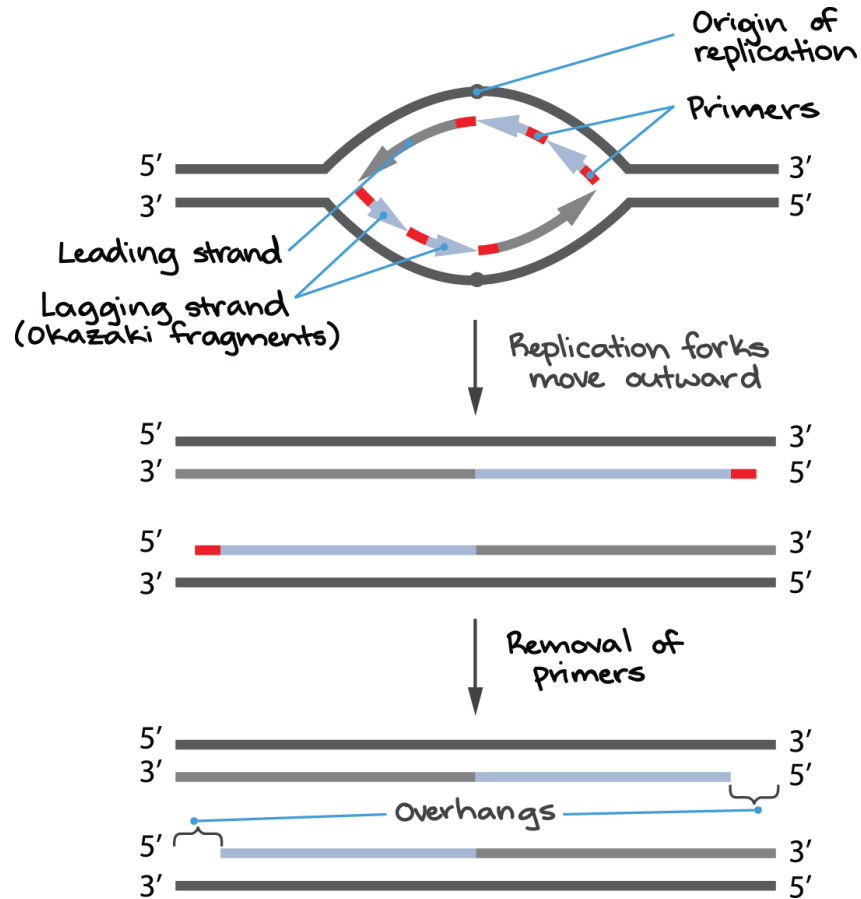
Concerning telomeres ...

Chromosome



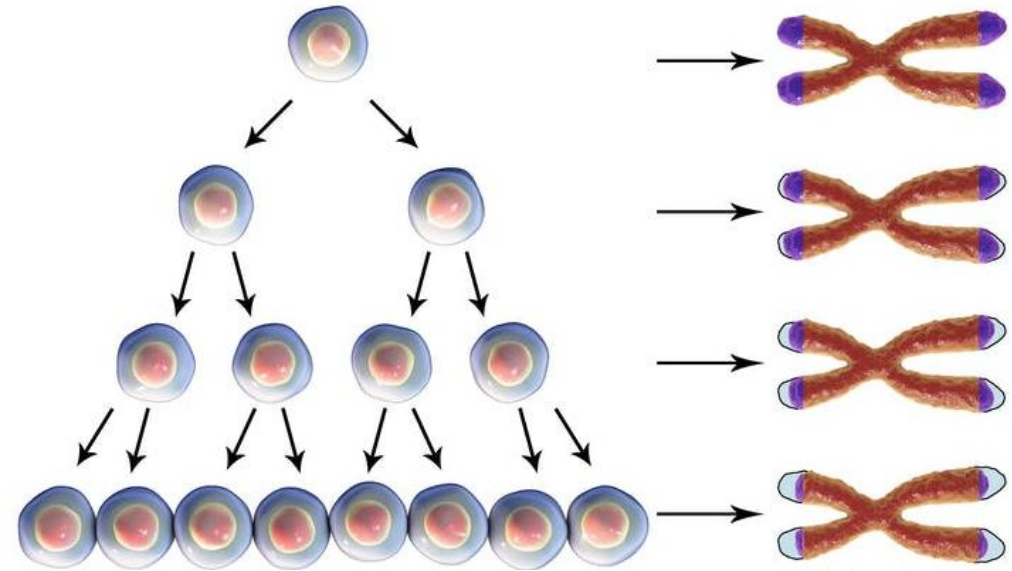
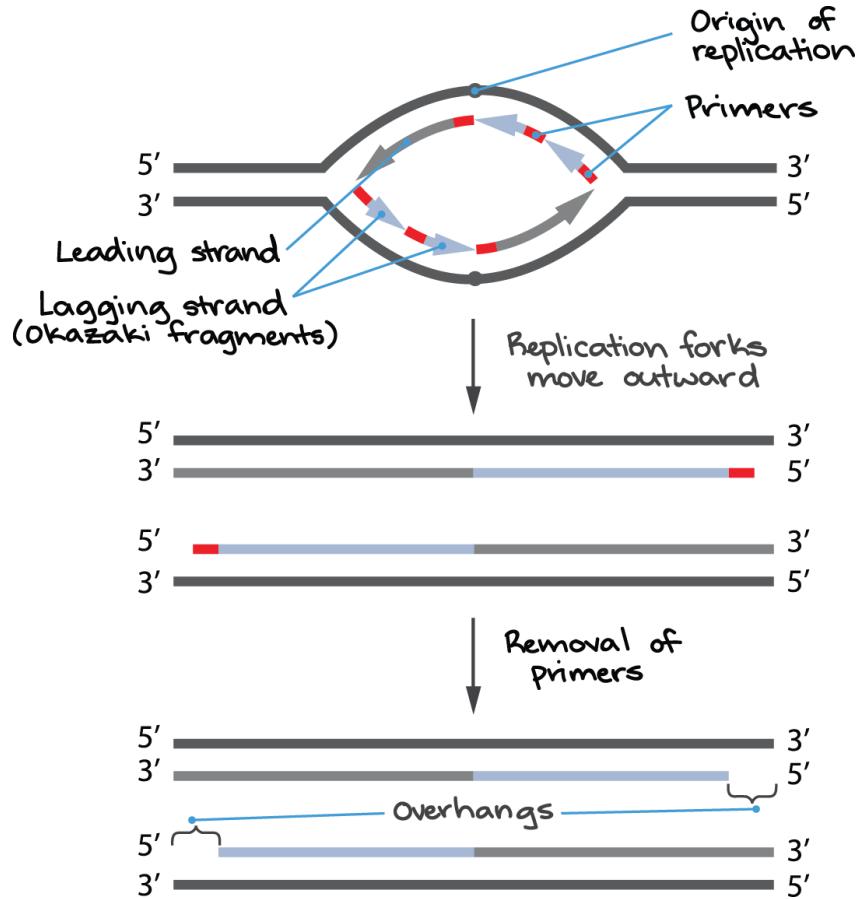
- nucleoprotein complexes at the ends of chromosomes
- length: 300 bp in yeasts, 5 – 15 kb in humans
- short tandem sequence

The function of telomeres



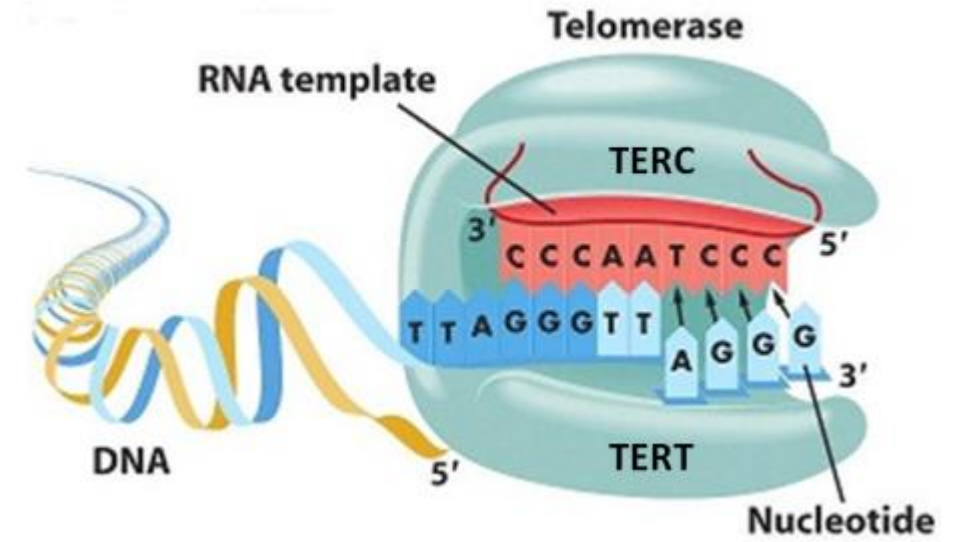
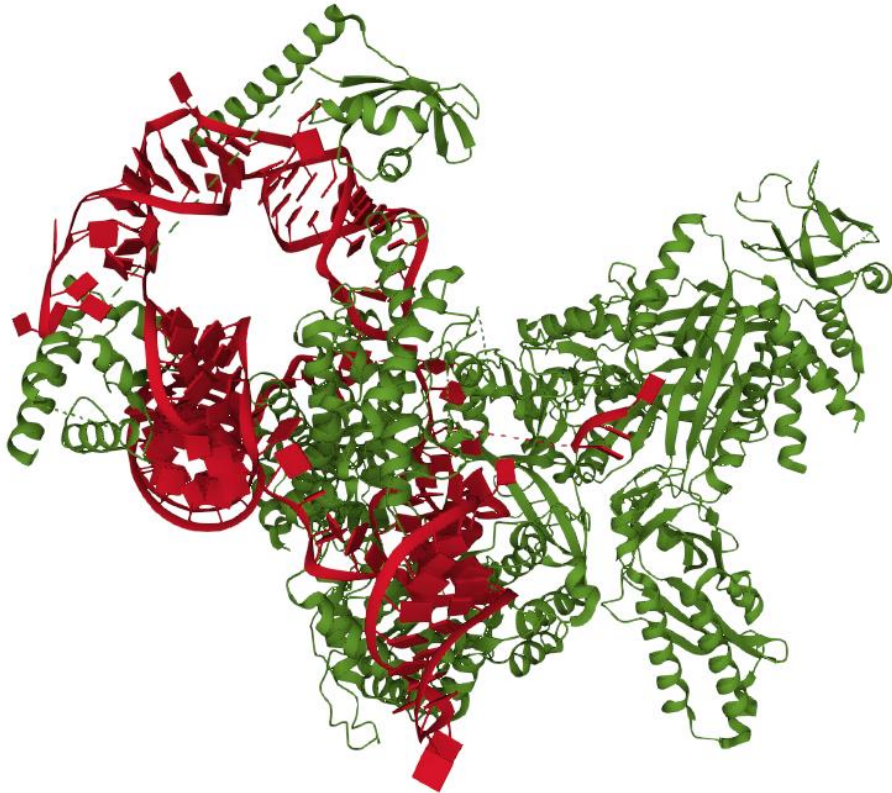
Telomeres solve end-replication problem

The function of telomeres



Telomeres solve end-replication problem and prevent the loss of genes.

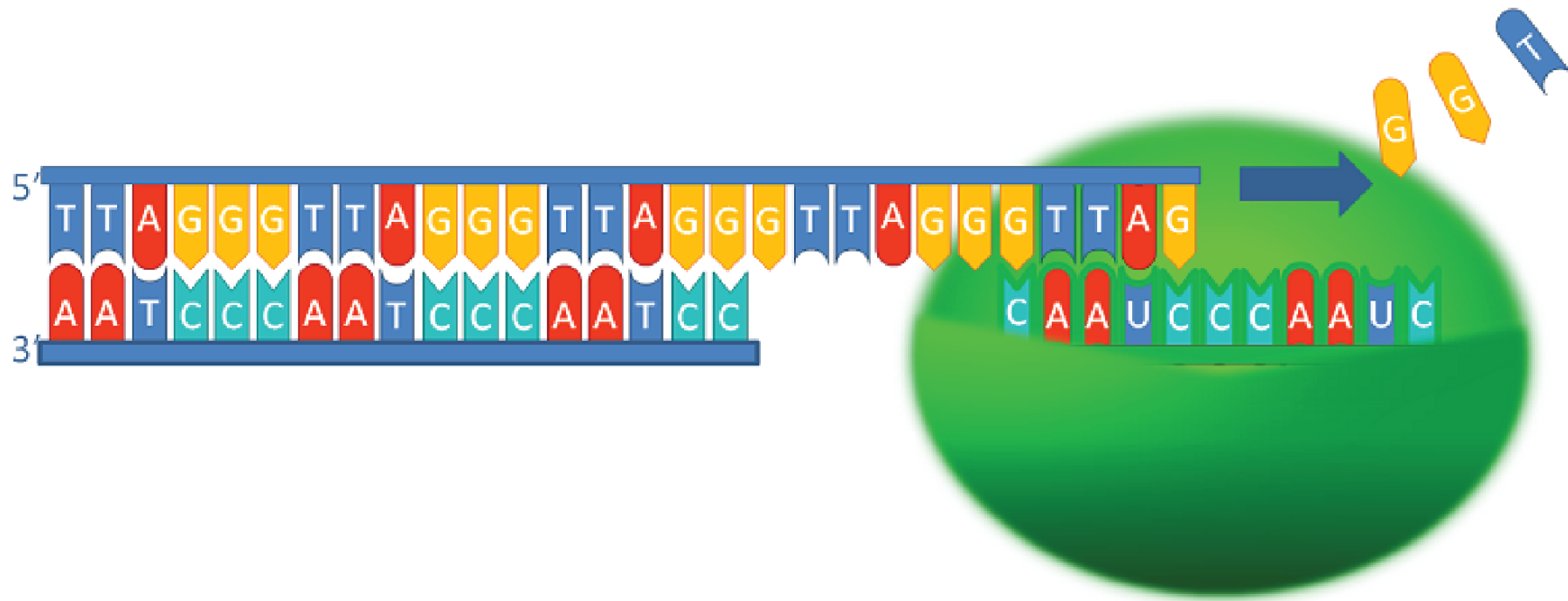
Telomerase



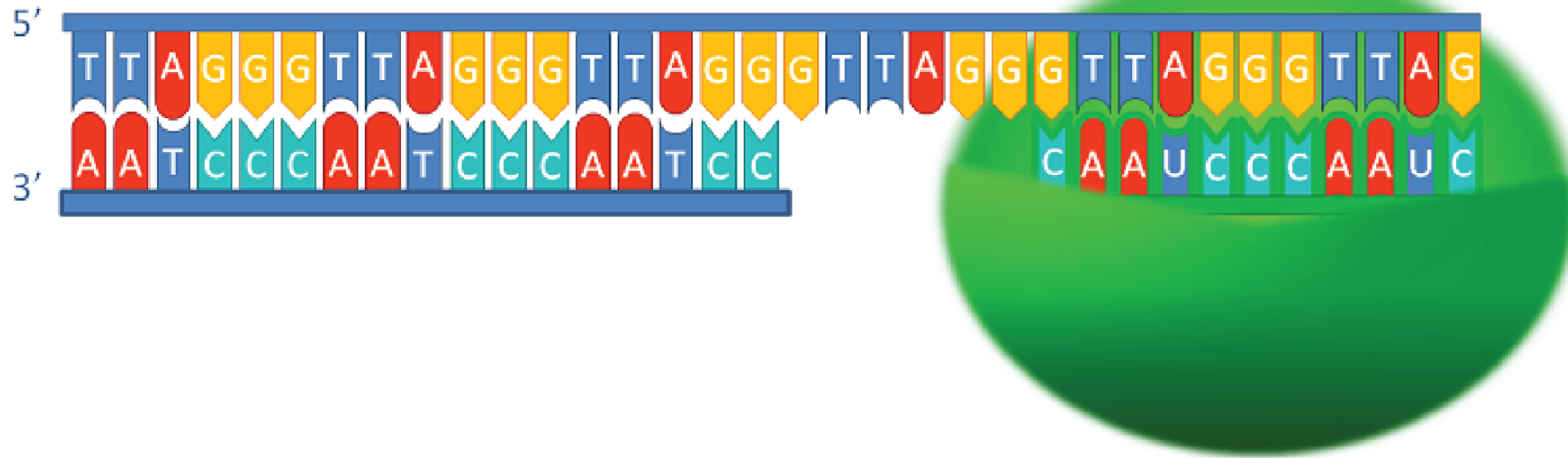
Telomerase



Telomerase



Telomerase



Telomerase





Telomere sequence

CTCGGTTATGGG

Allium cepa

TTTTTTAGGG

Cestrum elegans

TTTAGGG

Arabidopsis thaliana

TTAGGG

Homo sapiens

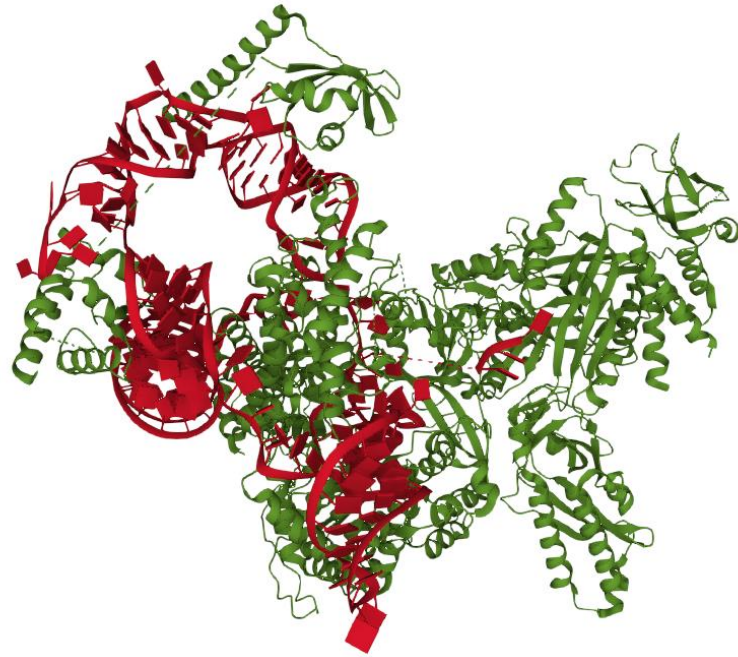
Asparagus officinalis

?

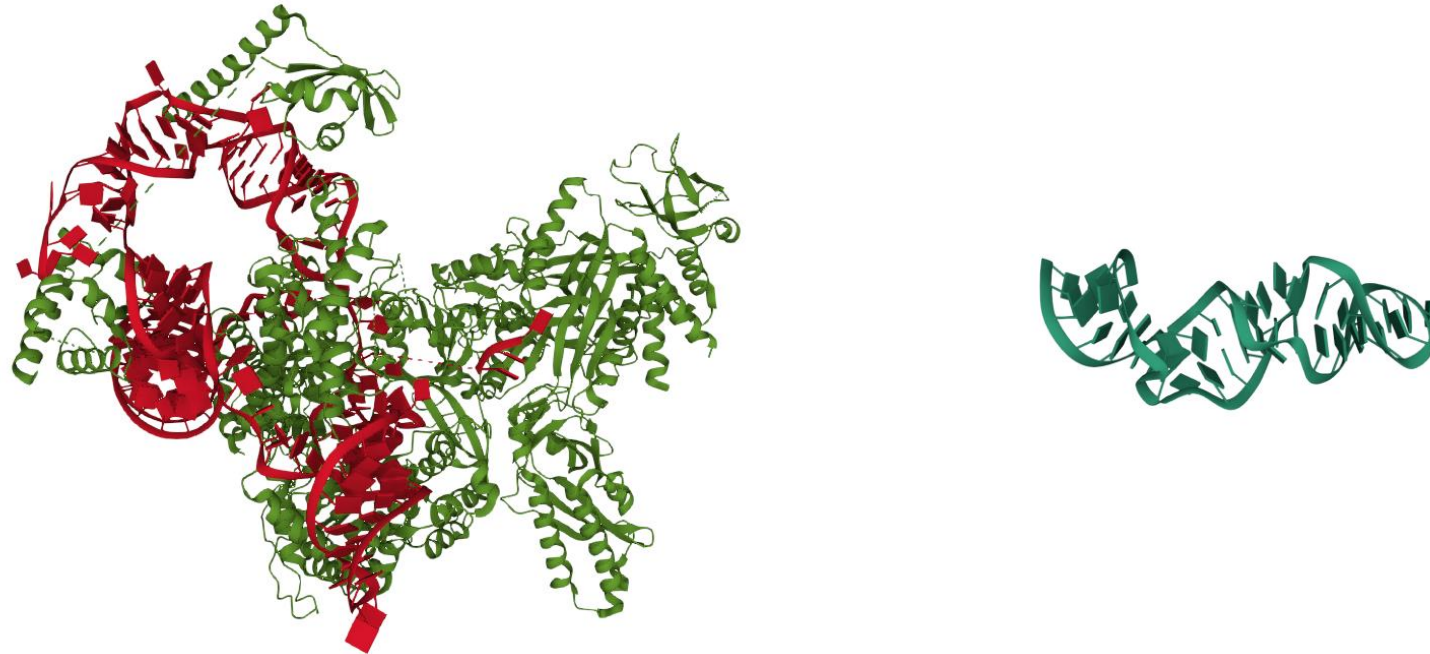
Fagopyrum esculentum

Changes in telomere sequences correspond to plant phylogeny.

Telomerase RNA subunit (TR)

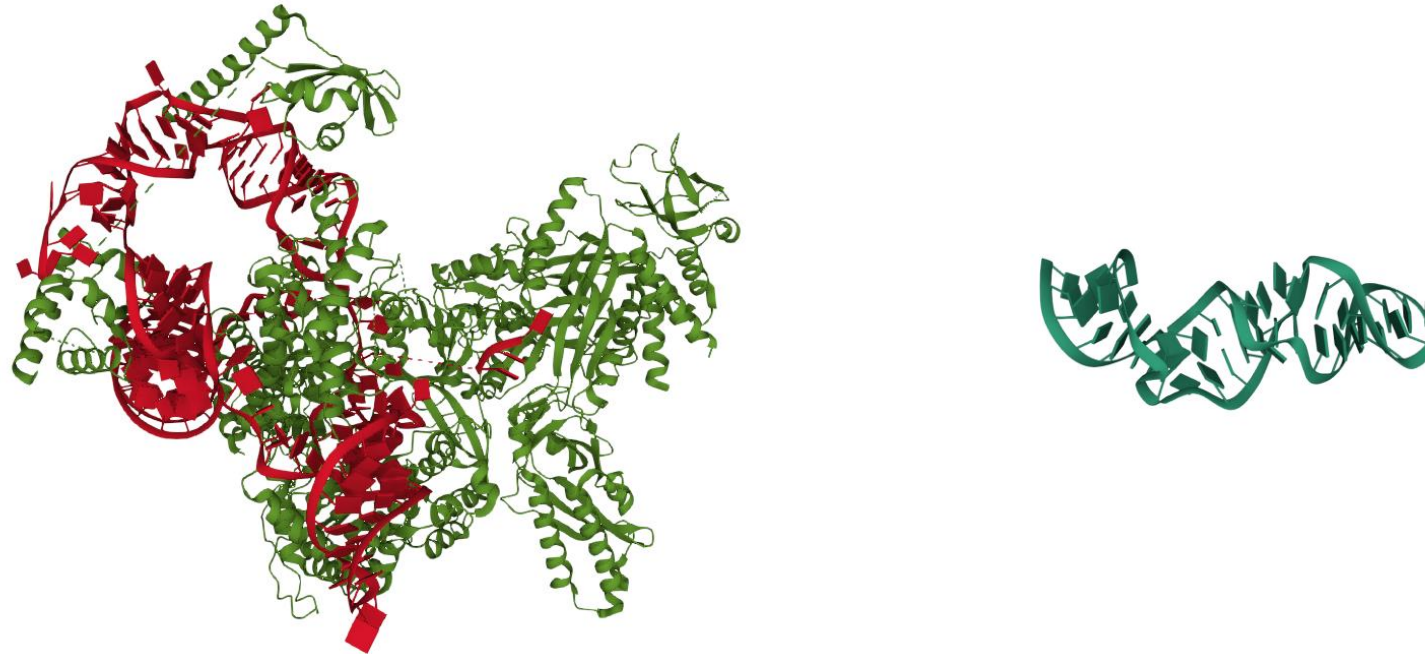


Telomerase RNA subunit (TR)



What could have caused change in telomere sequence?

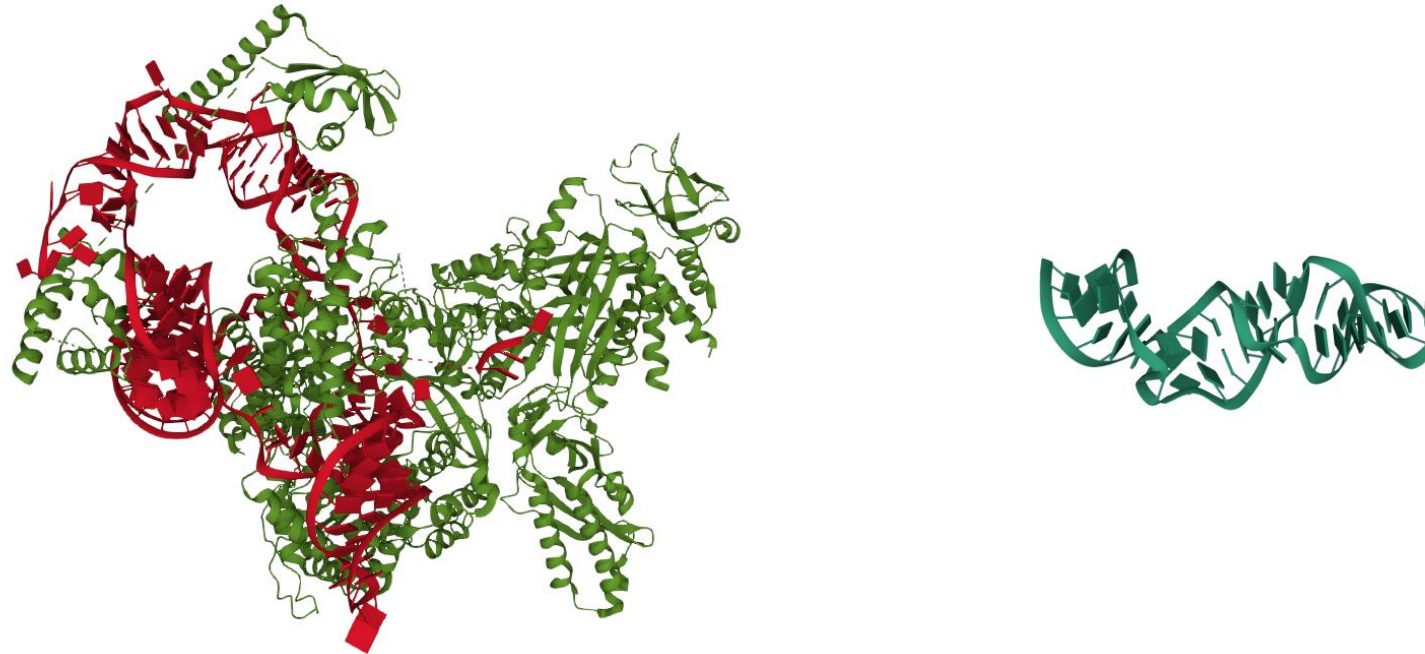
Telomerase RNA subunit (TR)



What could have caused change in telomere sequence?

- change in telomerase RNA subunit

Telomerase RNA subunit (TR)



What could have caused change in telomere sequence?

- change in telomerase RNA subunit
- different RNA took over the telomerase RNA subunit function



Main purpose of the research, methods

main purpose: understand evolutionary changes of telomeres and TRs in plants

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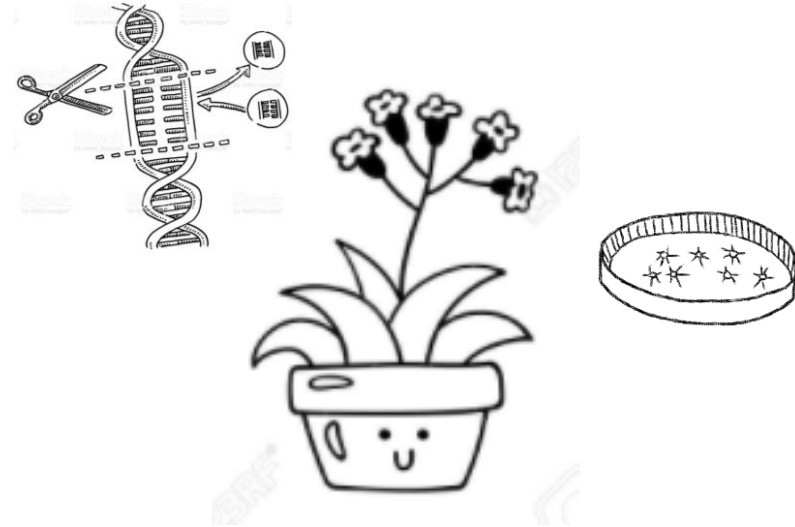
identification of TRs *in silico*

Main purpose of the research, methods

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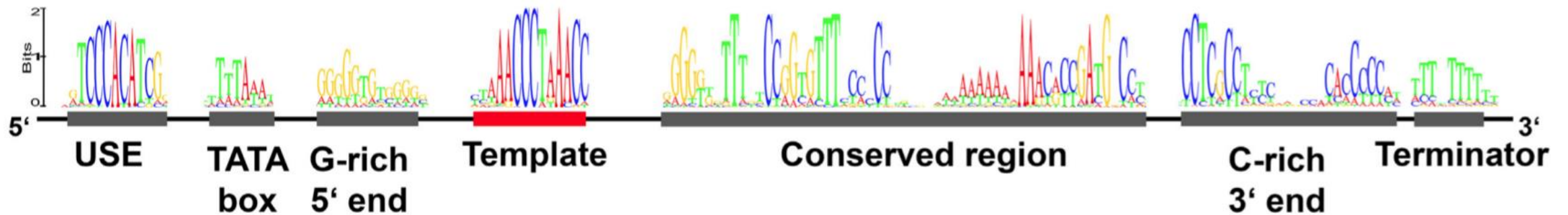
identification of TRs *in silico*



in vitro/in vivo validation

Identification of TR *in silico*

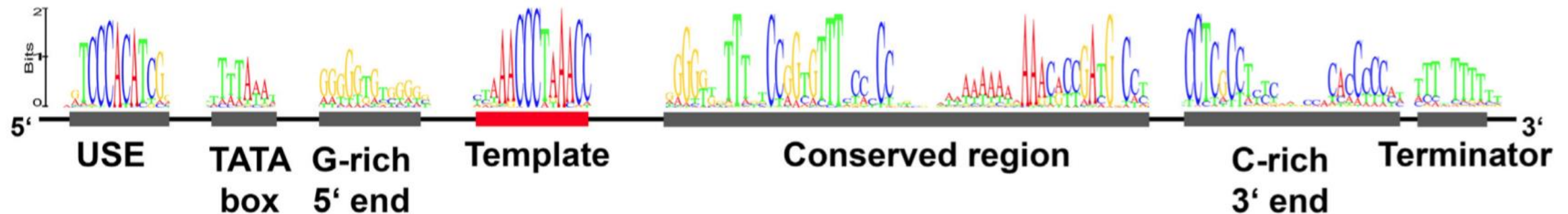
Characterization of TRs is complicated:



Identification of TR *in silico*

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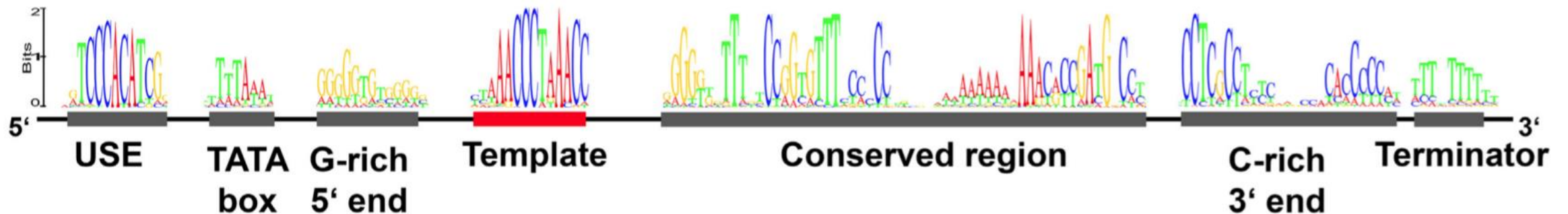
- extreme divergence in size, nucleotide sequence, and pathways of biogenesis among organisms



Identification of TR *in silico*

Characterization of TRs is complicated:

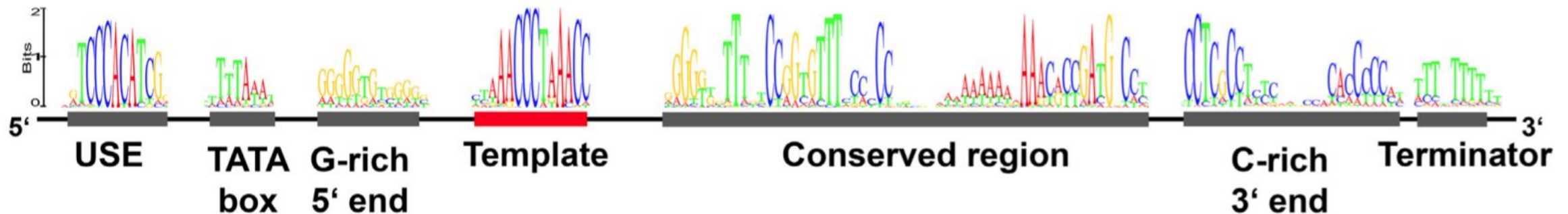
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- conservation only at the level of secondary structure



Identification of TR *in silico*

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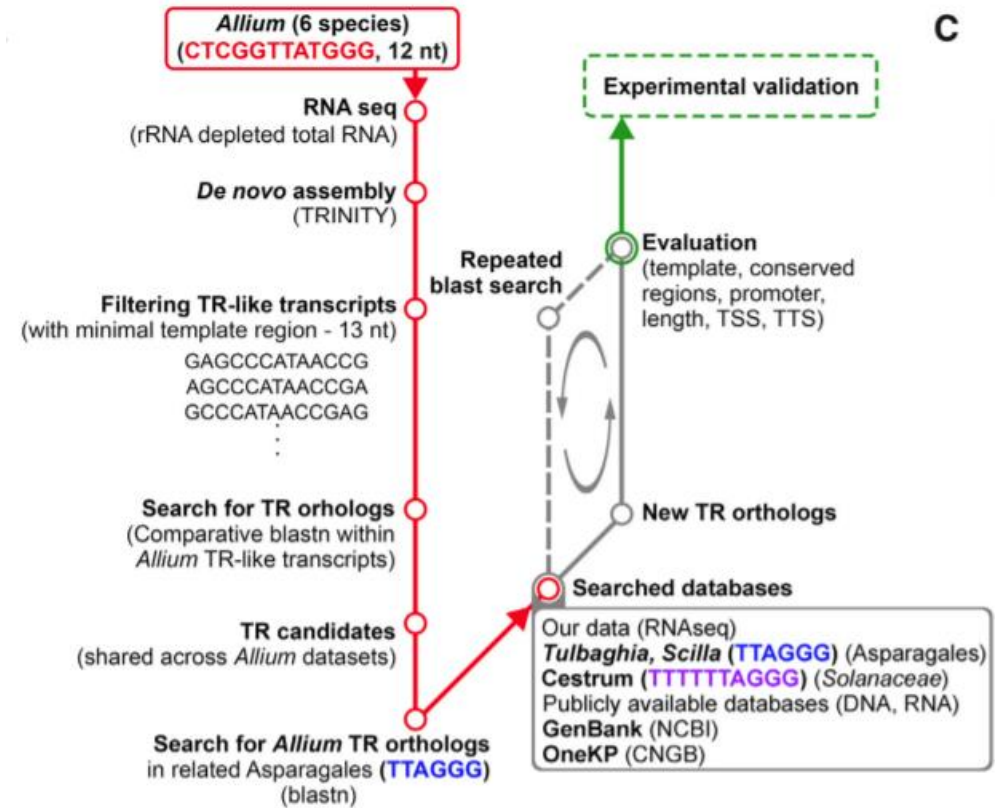
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The only predictable TR domain is the **template region**.

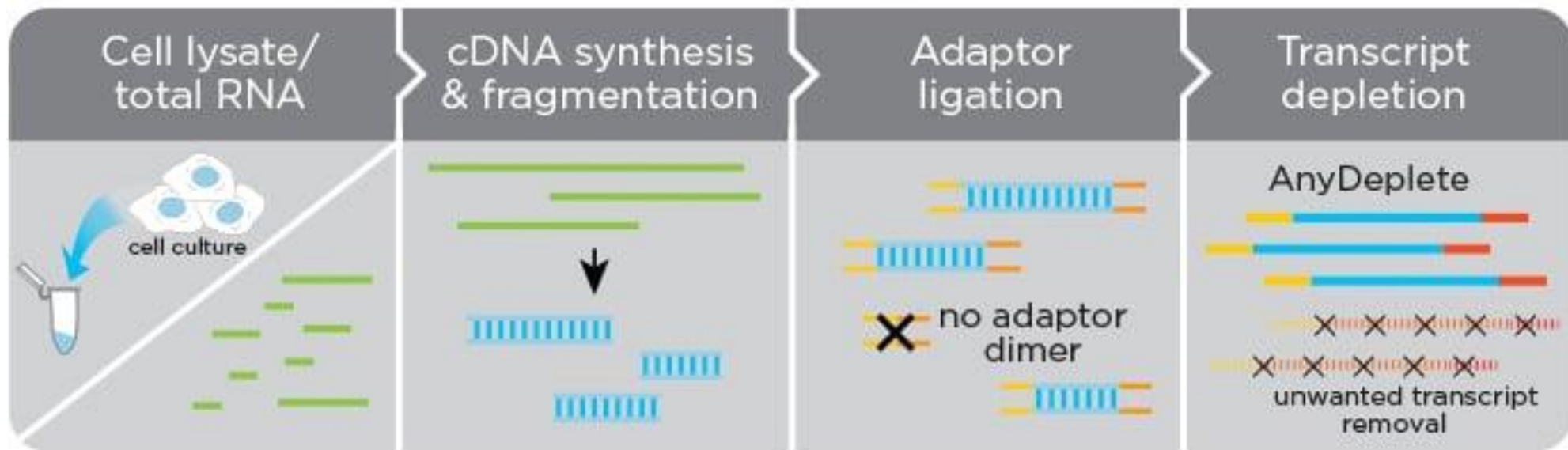
Identification of TR *in silico*

- RNA-seq library preparation and sequencing
- transcriptome *de novo* assembly
- filtering for TR candidates
- identification of TR orthologs



Identification of TR *in silico*

RNA-seq library preparation and sequencing



Identification of TR *in silico*

transcriptome *de novo* assembly



Identification of TR *in silico*

transcriptome *de novo* assembly



Inchworm assembles the read data set into linear contigs.

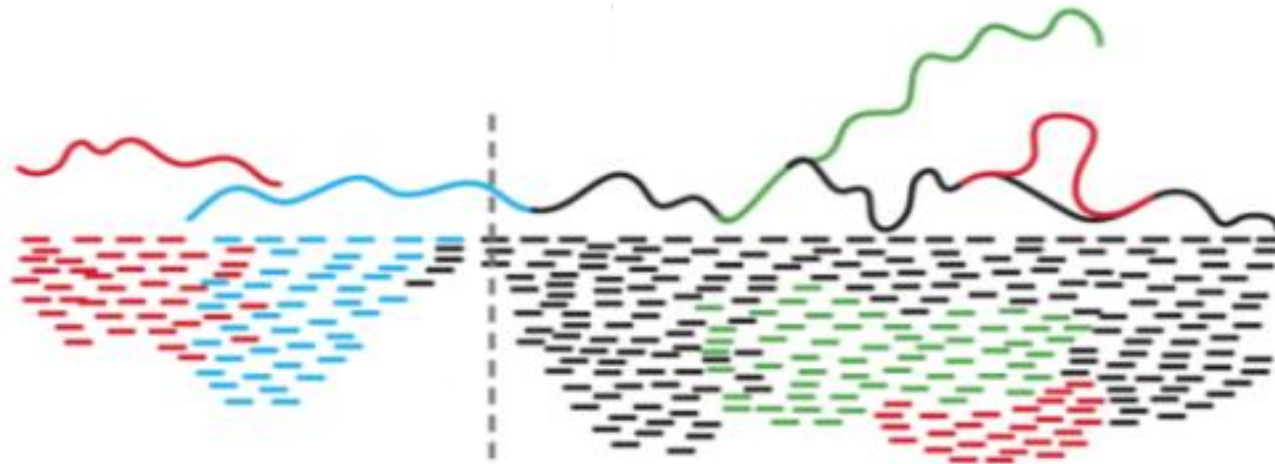


Identification of TR *in silico*

transcriptome *de novo* assembly



Chrysalis clusters contigs into components, assign reads to components and split overlapping transcripts.

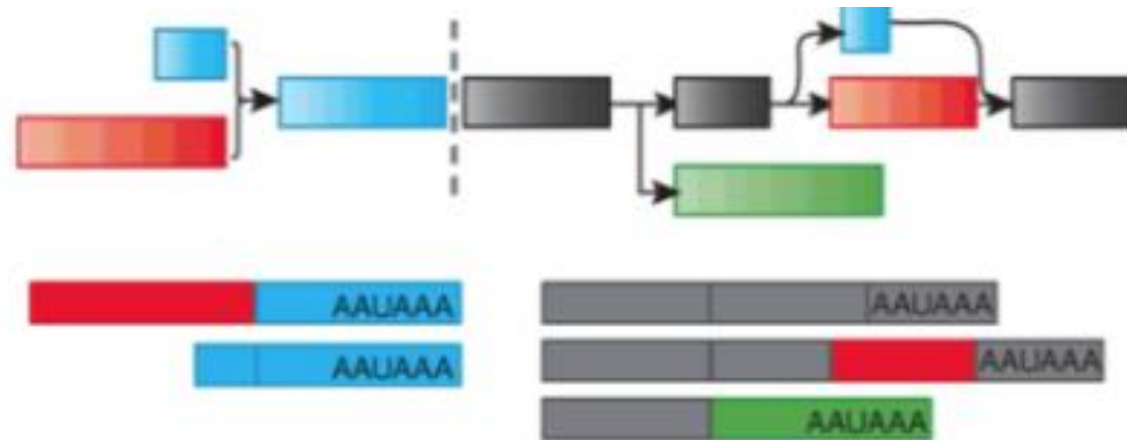


Identification of TR *in silico*

transcriptome *de novo* assembly



Butterfly enumerate transcript isoforms using reads.



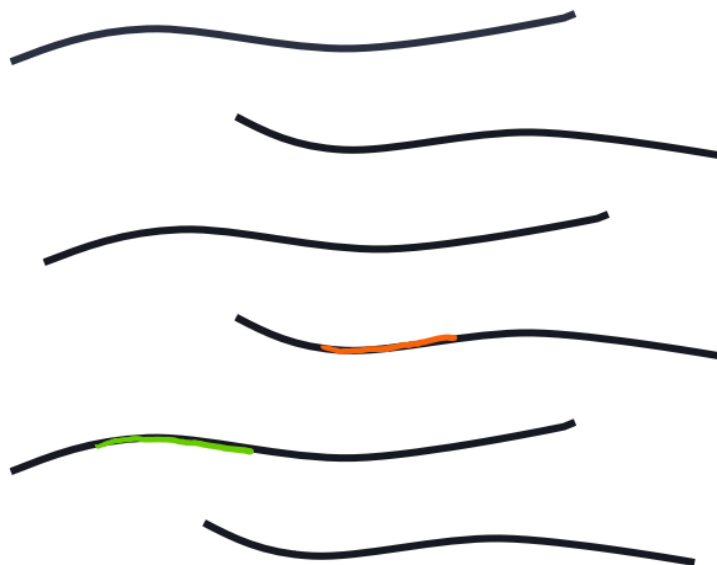
Identification of TR *in silico*

filtering for TR candidates

CCCTAAC
CCTAACC
CTAACCC
TAACCCCT
AACCCCTA
ACCCCTAA



transcripts assembled by Trinity



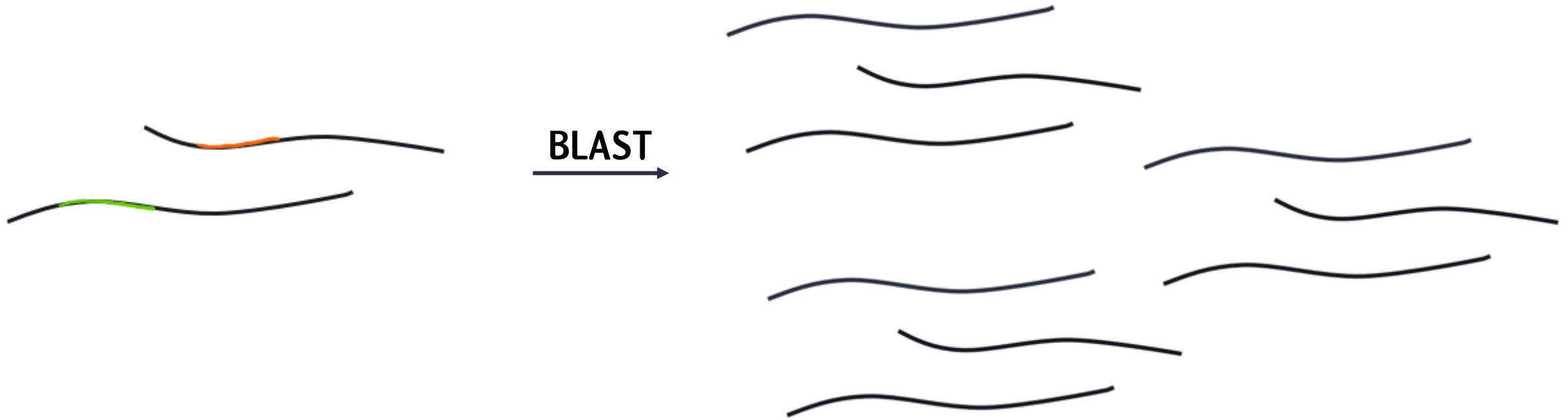
BLAST



Identification of TR *in silico*

identification of TR orthologs

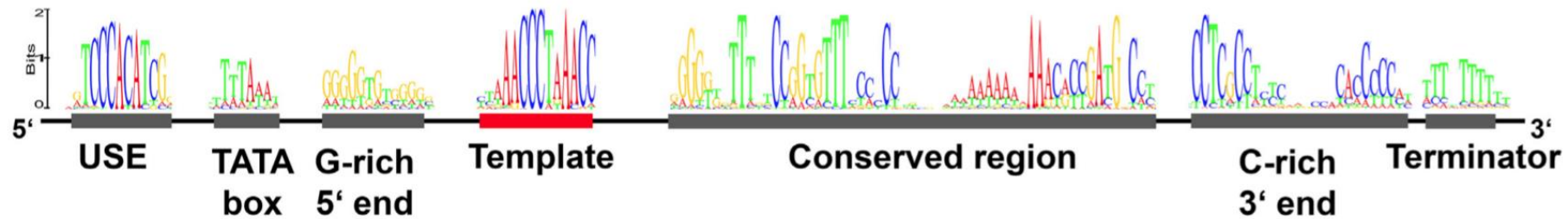
transcript datasets from different species



Identification of TR *in silico*

Results

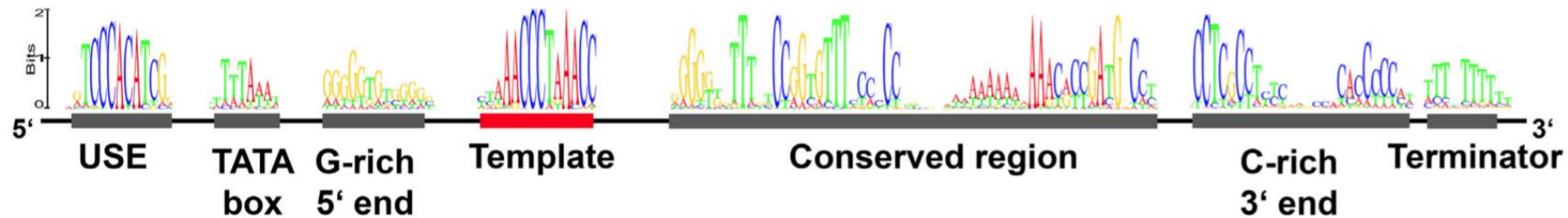
- TR gene in plants is highly conserved in contrary to the very divergent TR genes found in animals or yeast



Identification of TR *in silico*

Results

- TR gene in plants is highly conserved in contrary to the very divergent TR genes found in animals or yeast

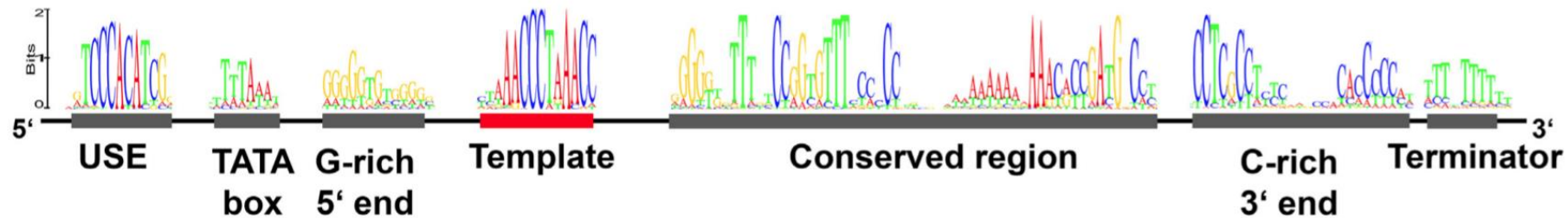


- plants with unusual telomere repeats have TR orthologs with the corresponding template region sequences

Identification of TR *in silico*

Results

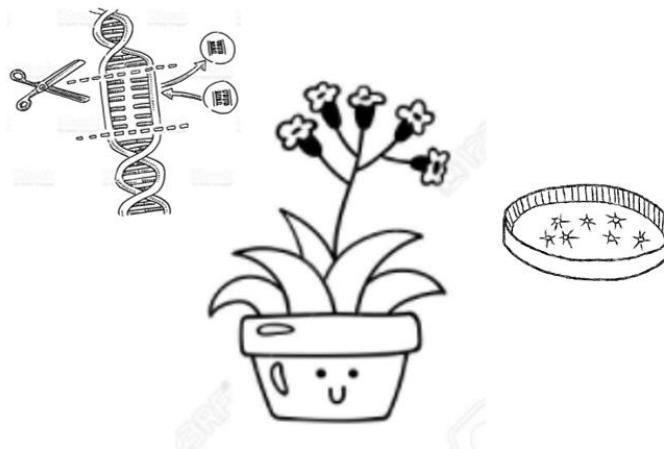
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- plants with unusual telomere repeats have TR orthologs with the corresponding template region sequences
- identification of new TR gene in *Arabidopsis thaliana*

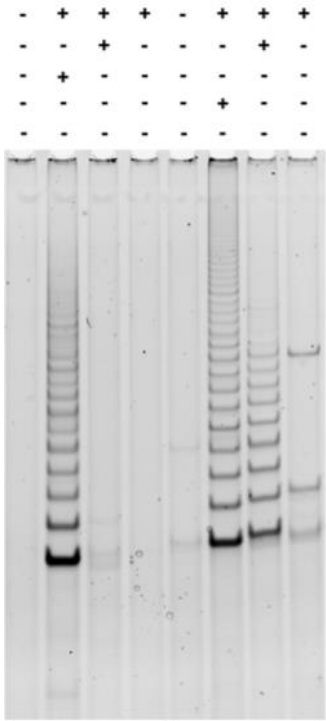


in vitro/in vivo validation



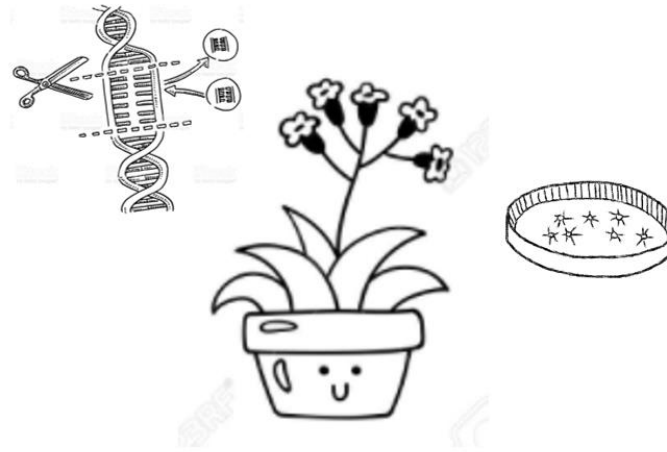
in vitro/in vivo validation

Nicotiana

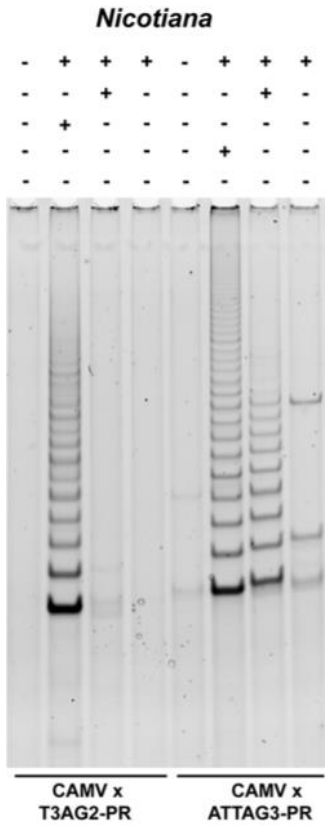


CAMV x T3AG2-PR CAMV x ATTAG3-PR

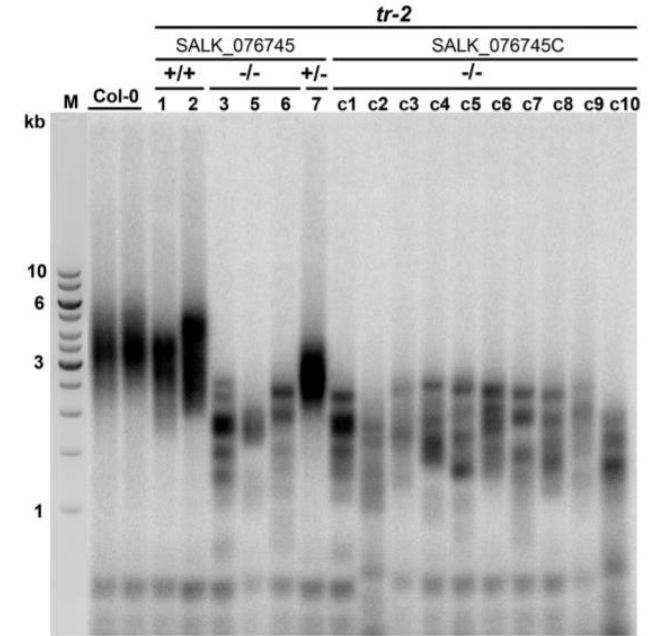
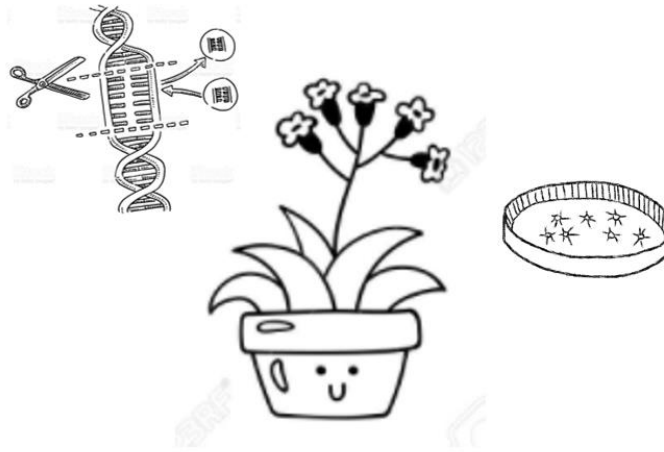
TRAP assay



in vitro/in vivo validation

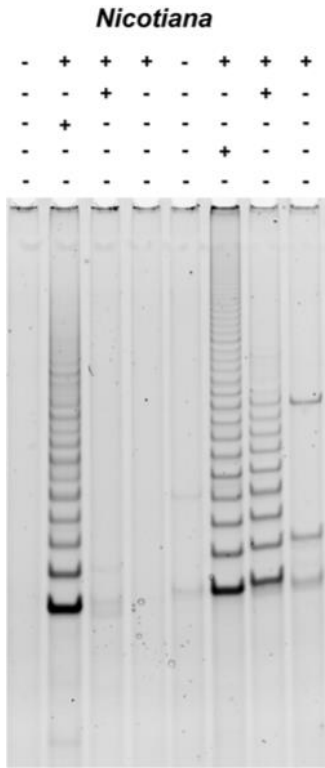


TRAP assay



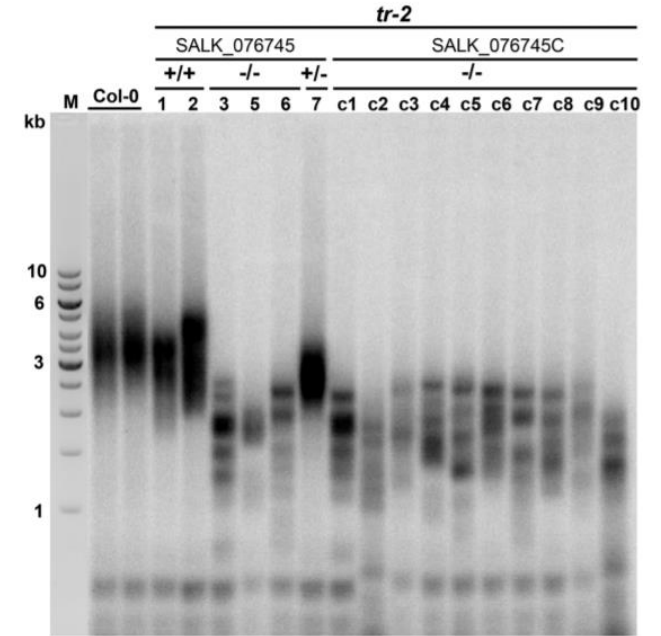
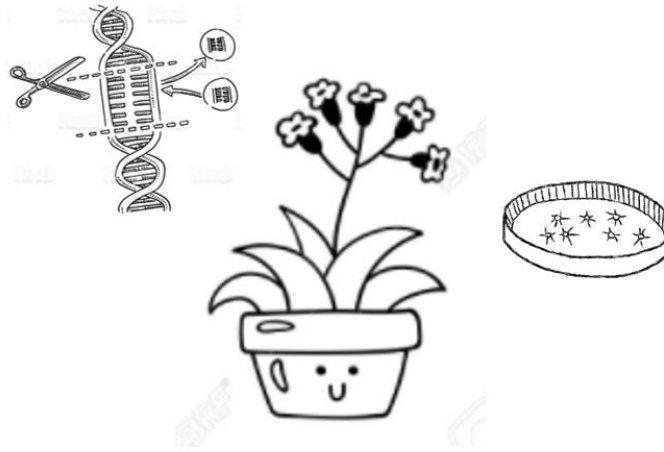
TRF analysis

in vitro/in vivo validation

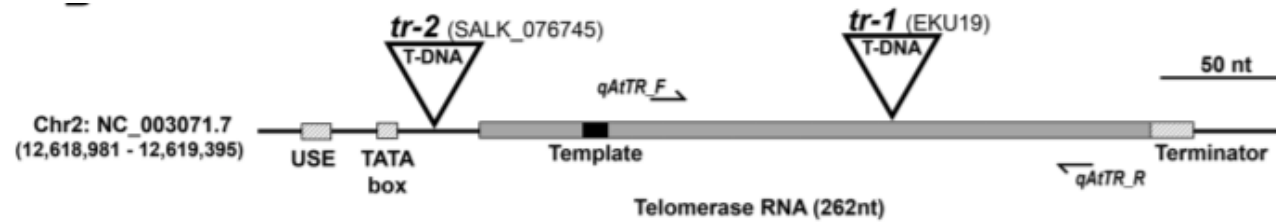


CAMV x T3AG2-PR CAMV x ATTAG3-PR

TRAP assay



TRF analysis



knockout mutations

Why is this research important?

Using this bioinformatical approach, we can predict telomere sequence in virtually any land plant species.





Thank you

References

Fajkus, P., Peška, V., Závodník, M., Fojtová, M., Fulnečková, J., Dobias, Š., Kilar, A., Dvořáčková, M., Zachová, D., Nečasová, I., Sims, J., Sýkorová, E., & Fajkus, J. (2019). Telomerase RNAs in land plants. *Nucleic acids research*, 47(18), 9842–9856.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., ... Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature biotechnology*, 29(7), 644–652.

