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CRBM

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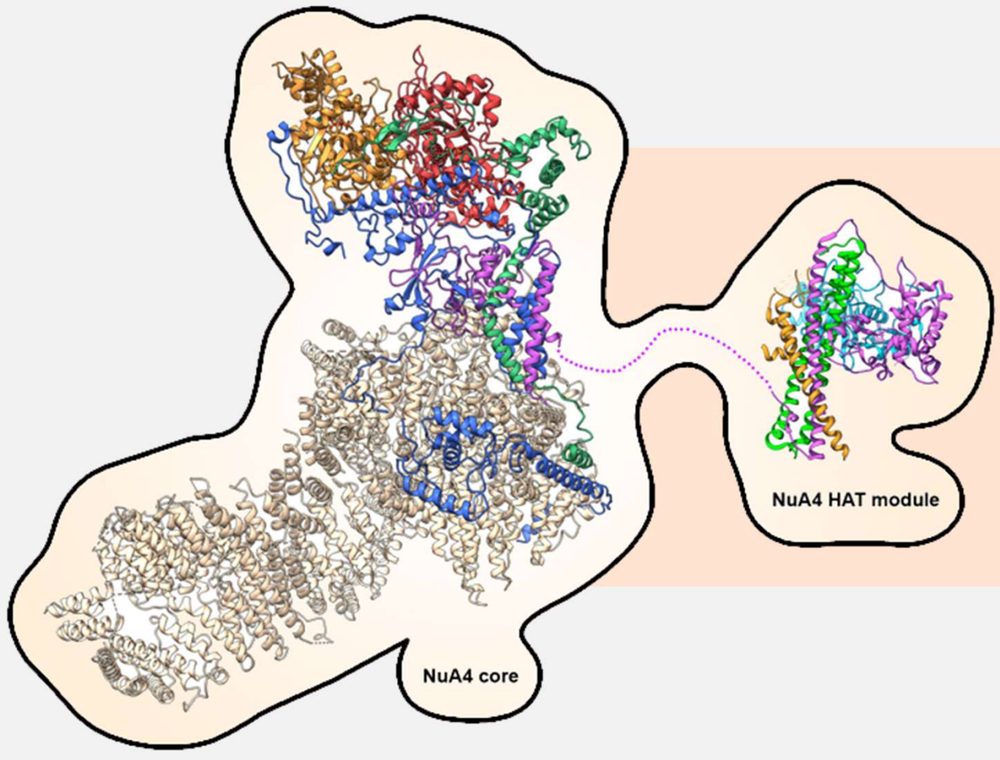

Functional Relevance of Structural Flexibility in the NuA4 Coactivator Complex

Conor McKeon **Helmlinger Group, CRBM** **Spring 2022**

The Transcription Initiation Machinery

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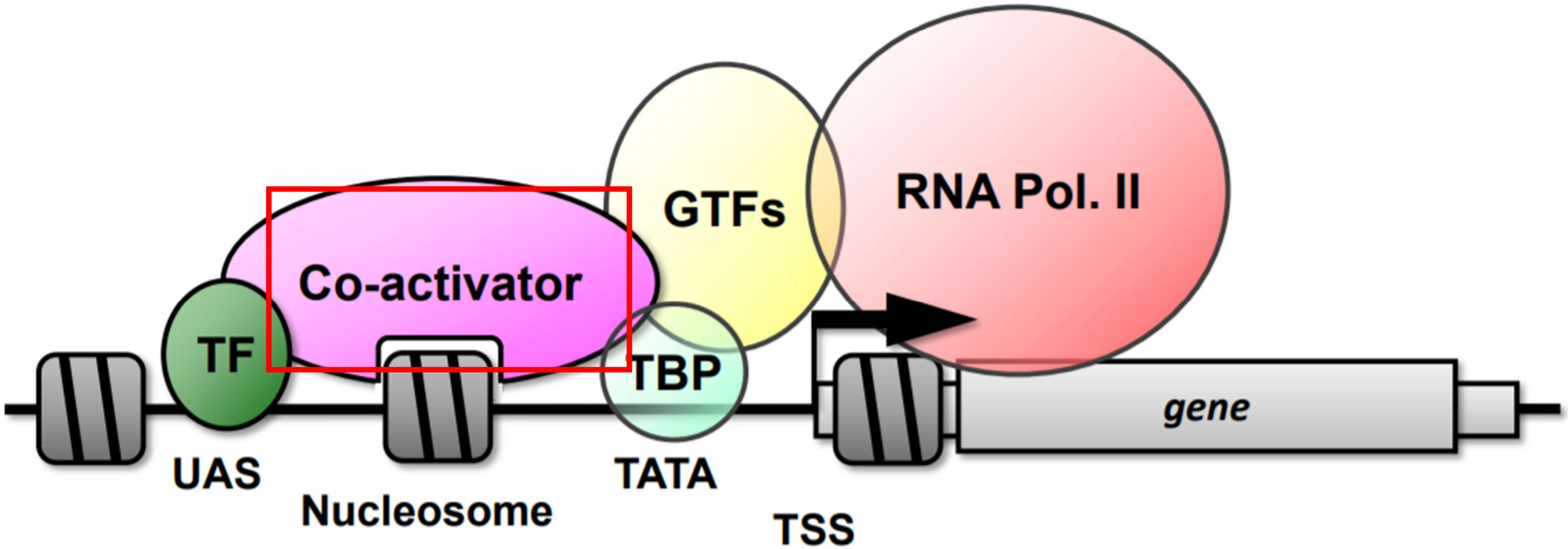

Flexibility of the HAT Module

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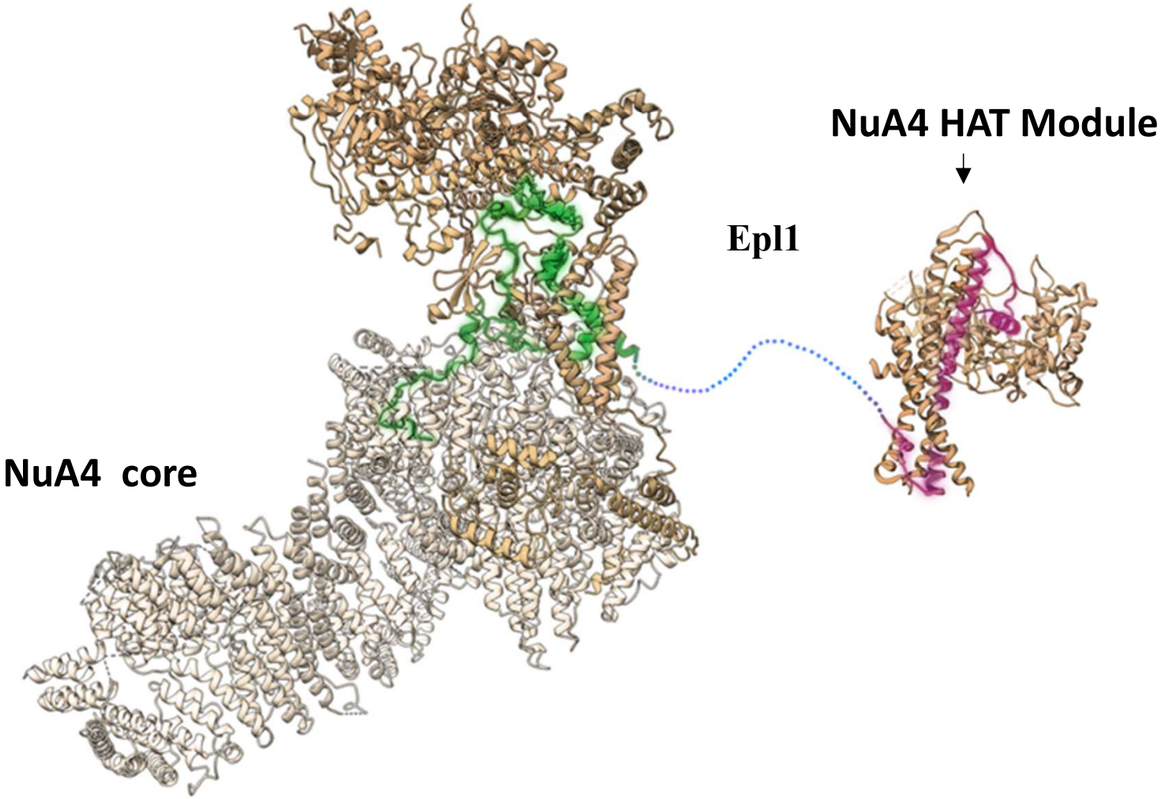
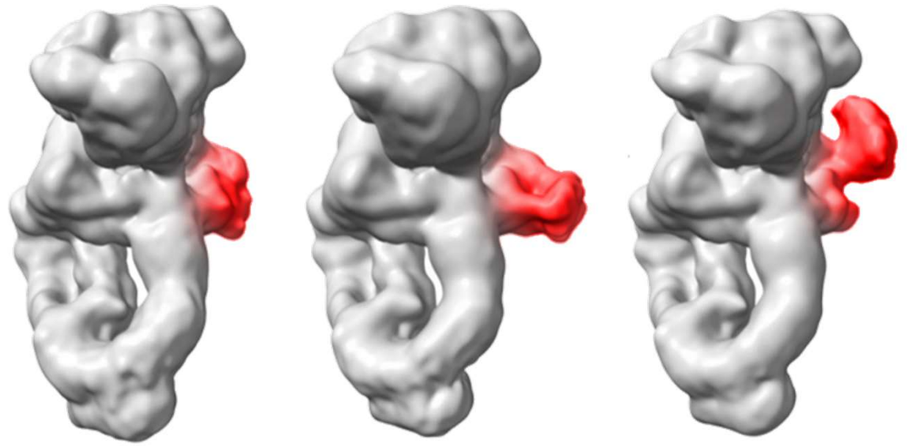


Loss of HAT Module Mobility

∴ Increasing resolution in Cryo-EM



Unpublished Cryo-EM structures - Collaborators of Host-Laboratory

NuA4 mediates long-range acetylation

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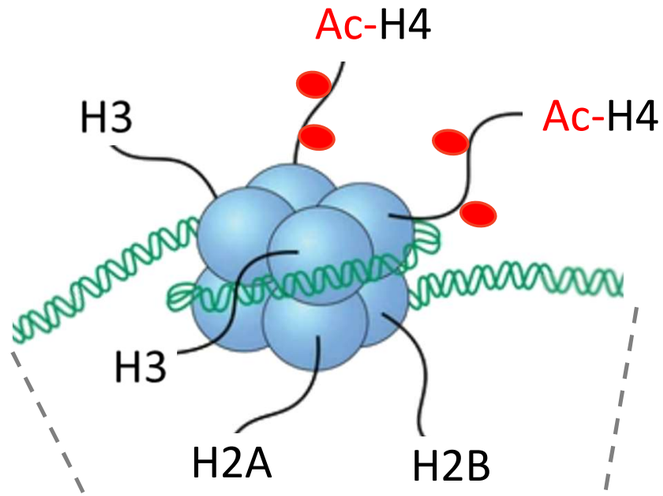
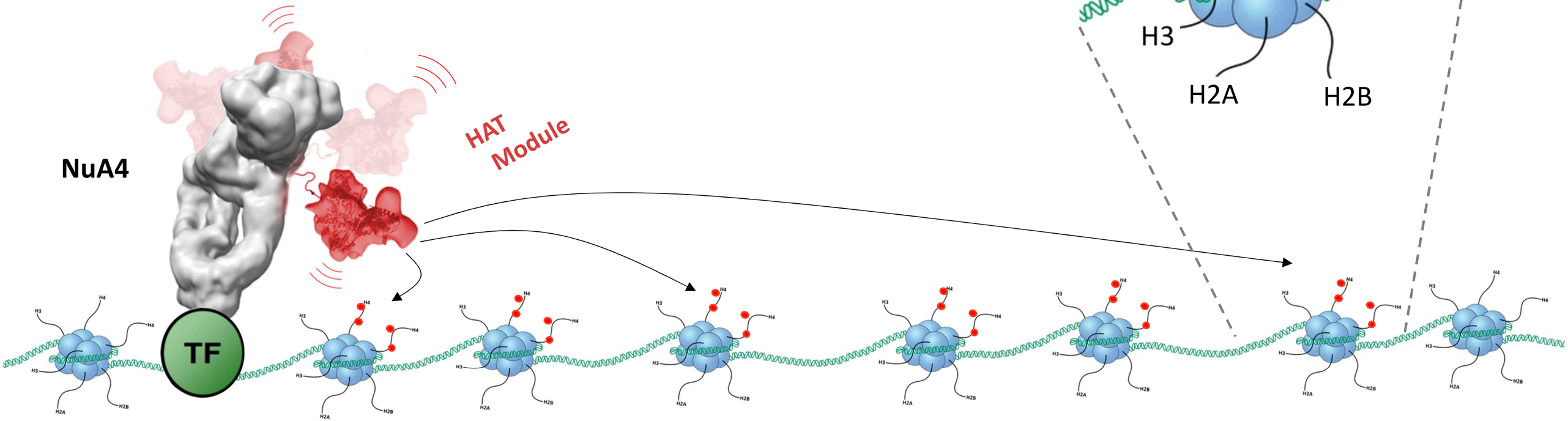
Is the Epl1 linker flexibility responsible for this long-range activity in yeast?





Reid et al. (2000) Vignali et al. (2000)

FLEx conditional mutation system

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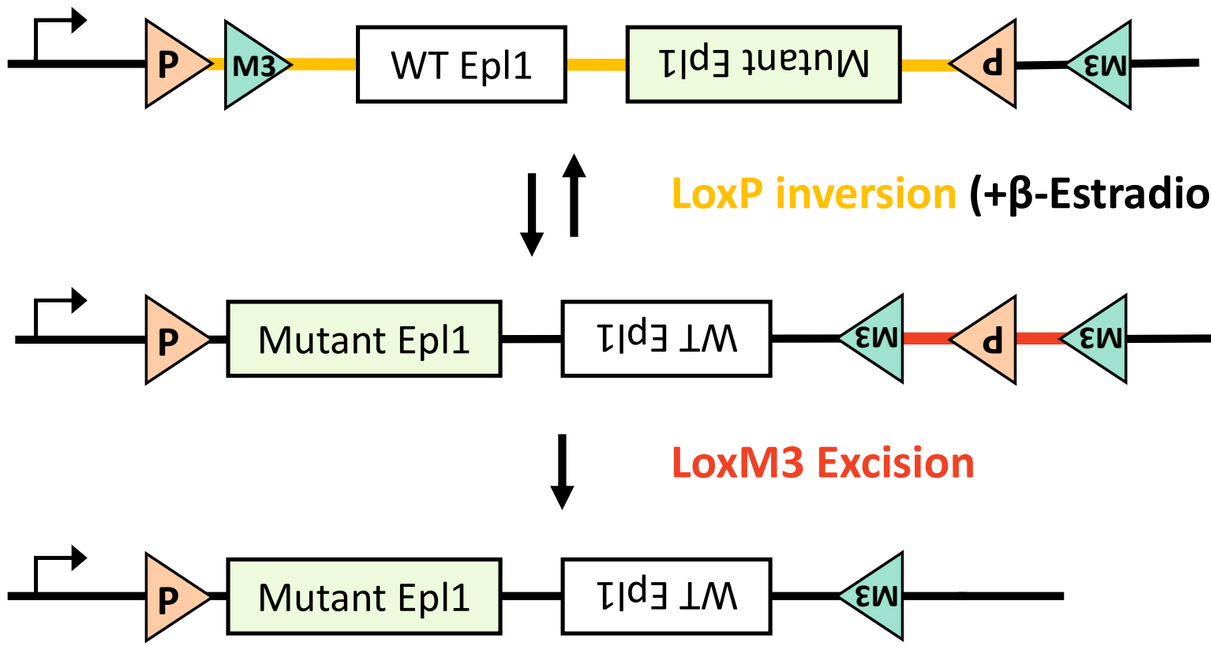
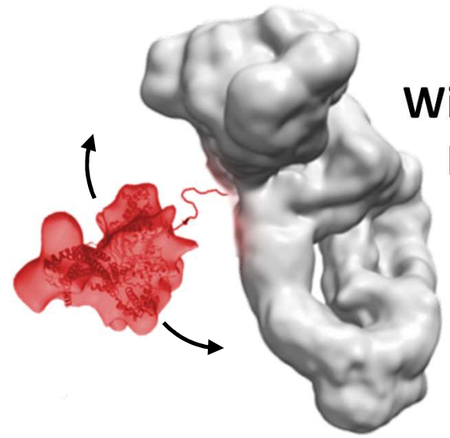
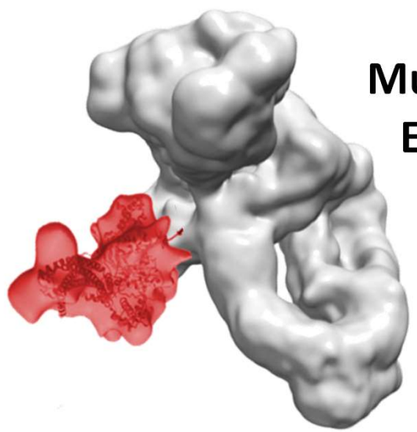
Flip-Excision (FLEx) system to induce conditional mutations upon addition of β-Estradiol





Wild-Type Epl1 Expressed Before Induction



Mutant Epl1 Expressed After induction

Epl1 mutants

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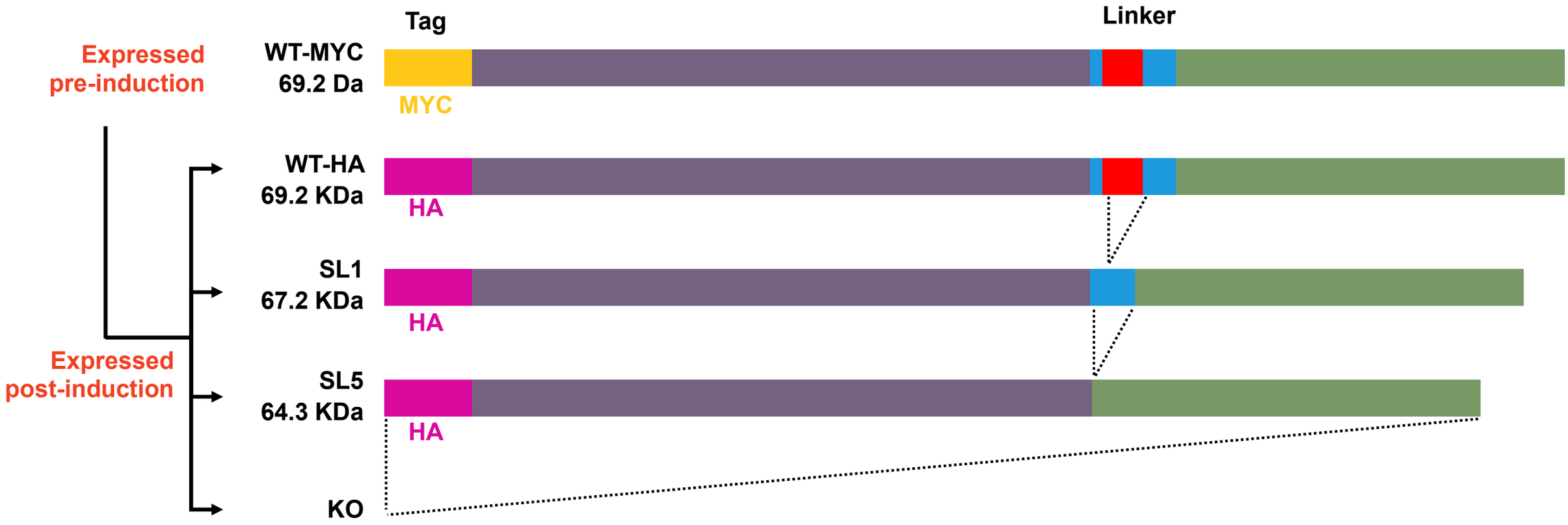

Overview

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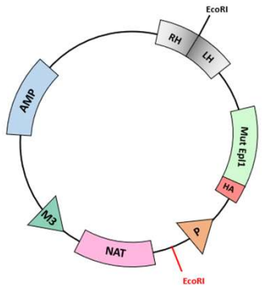
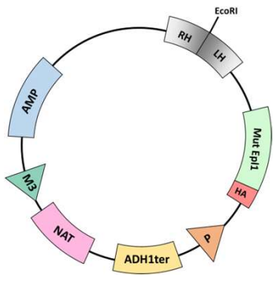
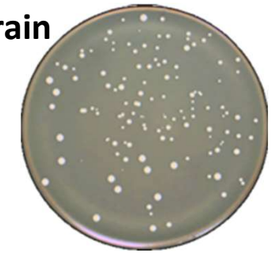


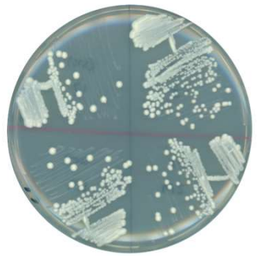
Edit plasmids



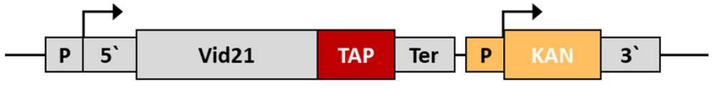
Transform plasmids in un-tagged yeast strain



Screen clones for functional FLEx system by Western Blot



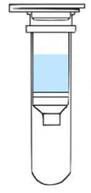
- Phenotypic analysis
- Candidate gene-expression analysis
- Genome-wide NuA4 ChIP-seq & Nascent RNA-seq



Transform and isolate Vid21-TAP background yeast strain

Transform plasmids in tagged yeast strain

TAP-purify NuA4



Validate Catalysis

Designing FLEx system - Plasmid editing

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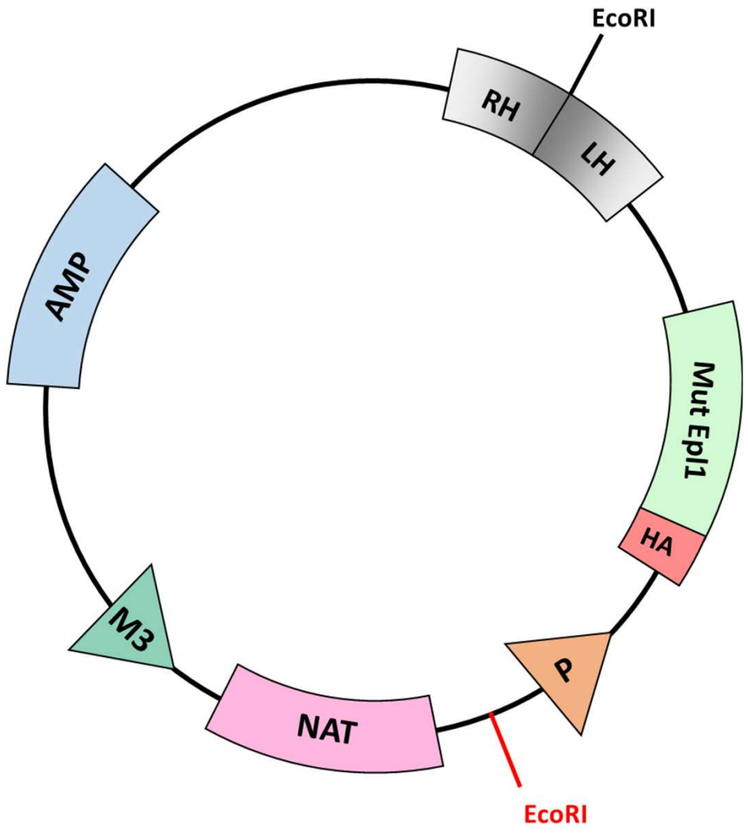
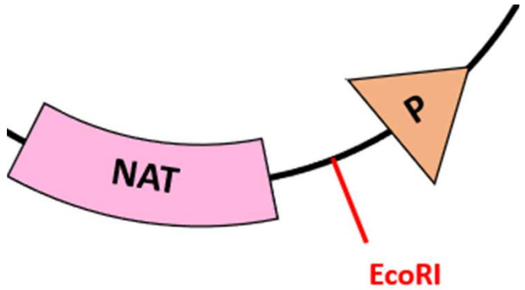
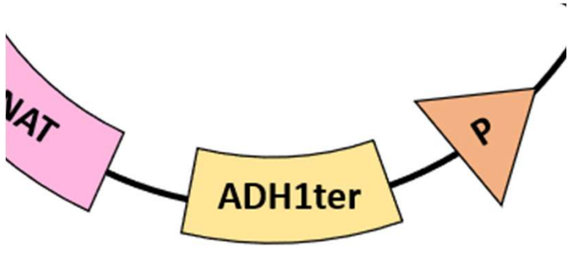
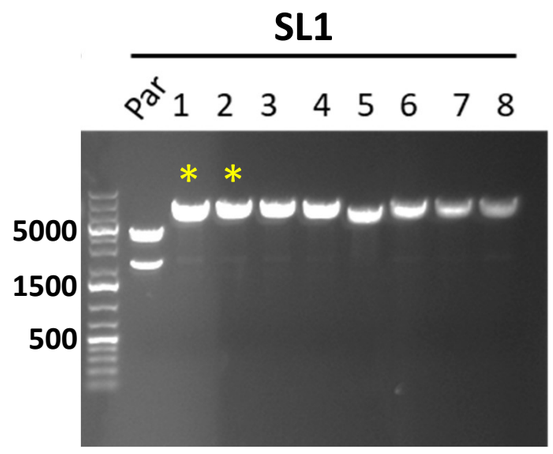




Gibson Assembly





EcoR1 digestion

* Successful insertion confirmed by sequencing.

TAP tagging Vid21

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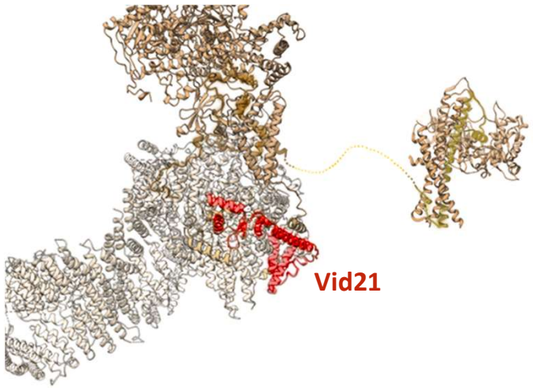

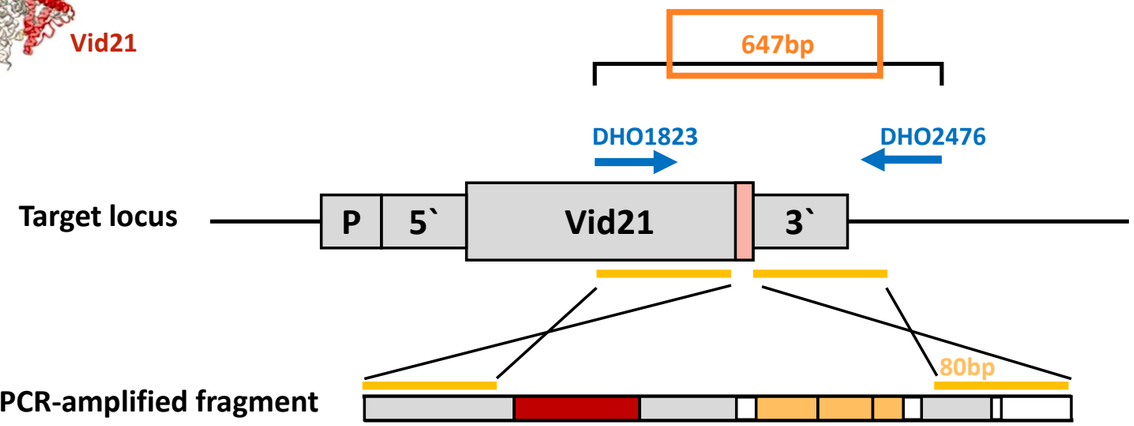

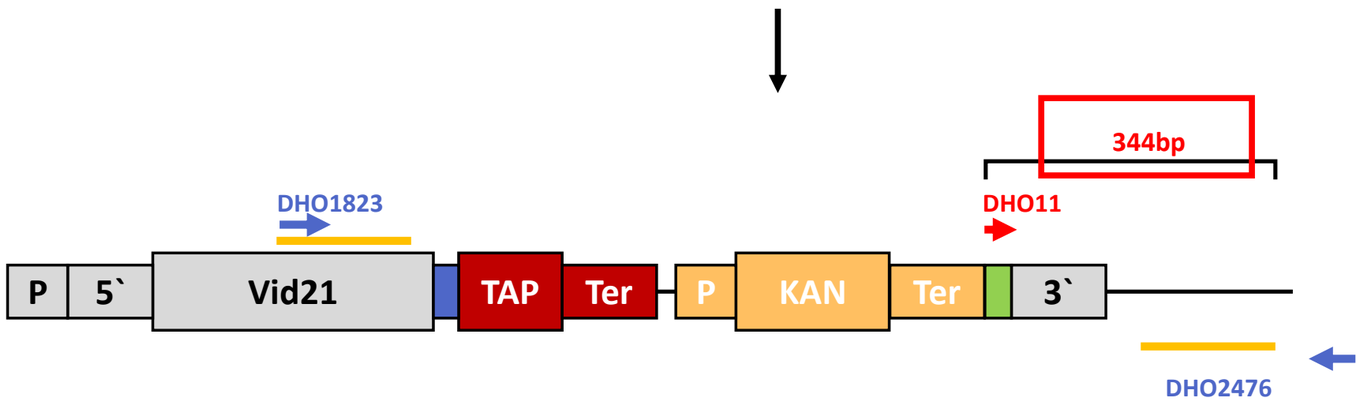

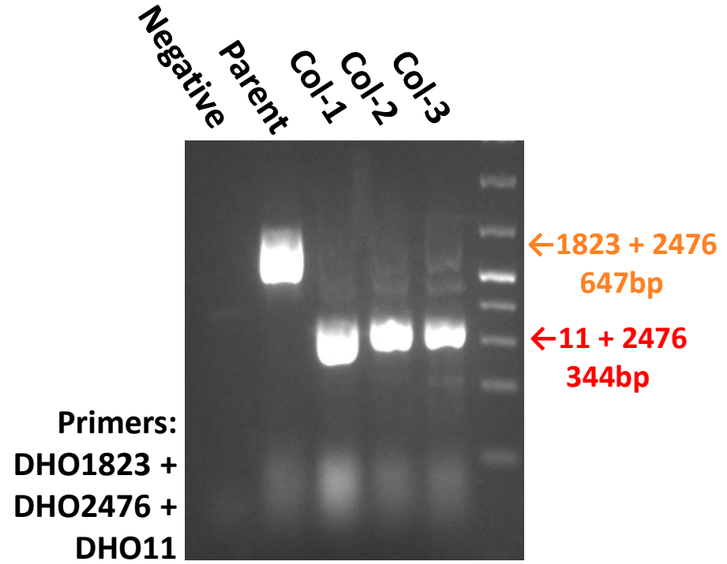

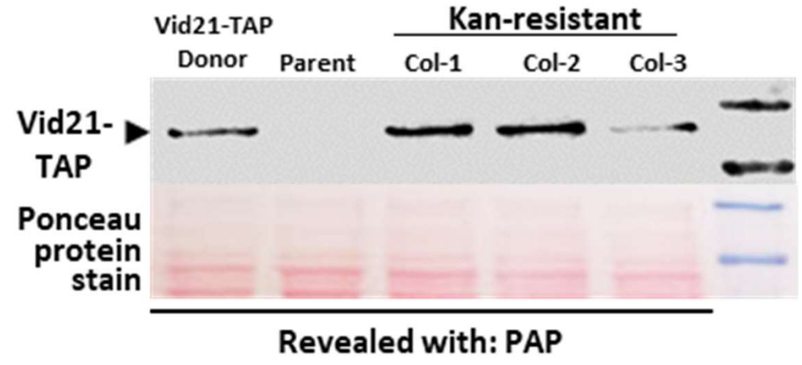

Validation of FLEx system by Western Blot

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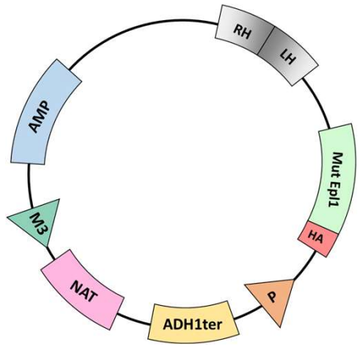
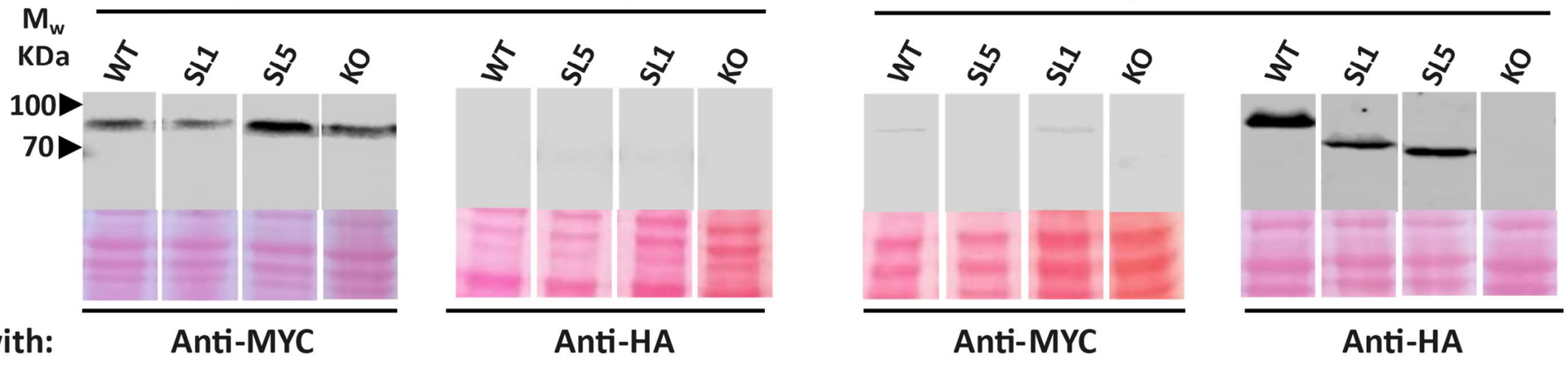


Transform plasmids in tagged yeast strain

Screen NAT-resistant colonies for functional FLEx system by Western Blot



Phenotypic Analysis

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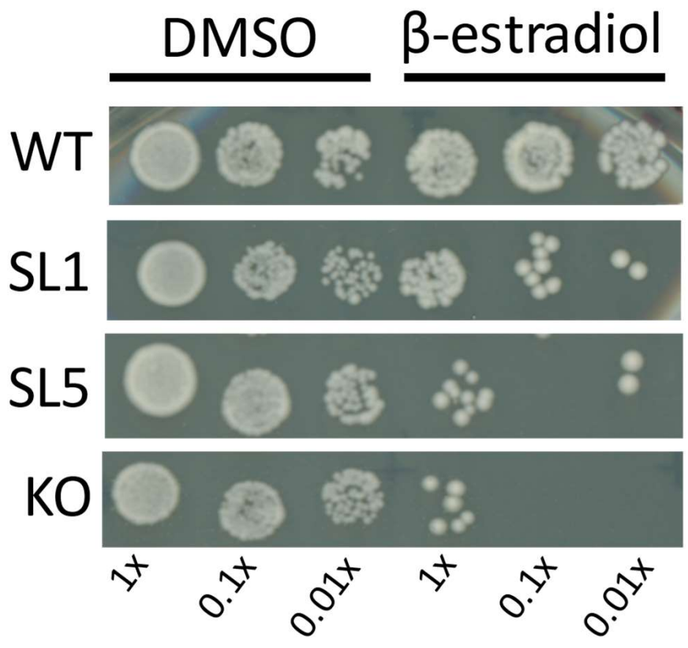
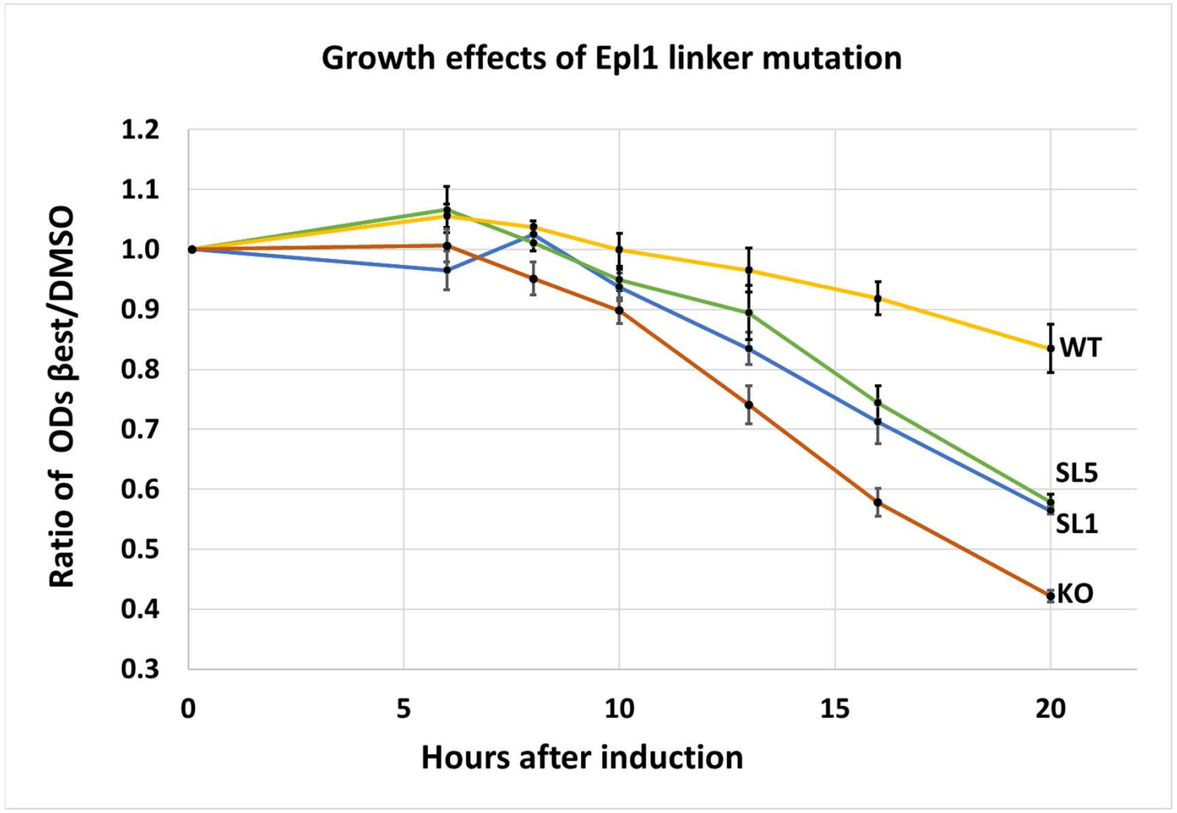


20 hours post-induction



- **n = 5**
- **Error bars = Standard error of the mean**

Conclusion

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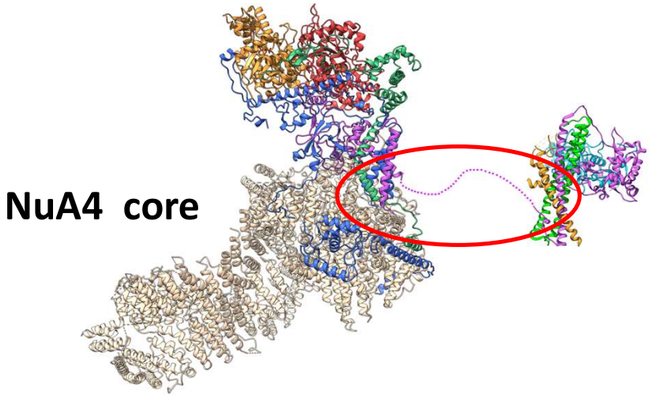
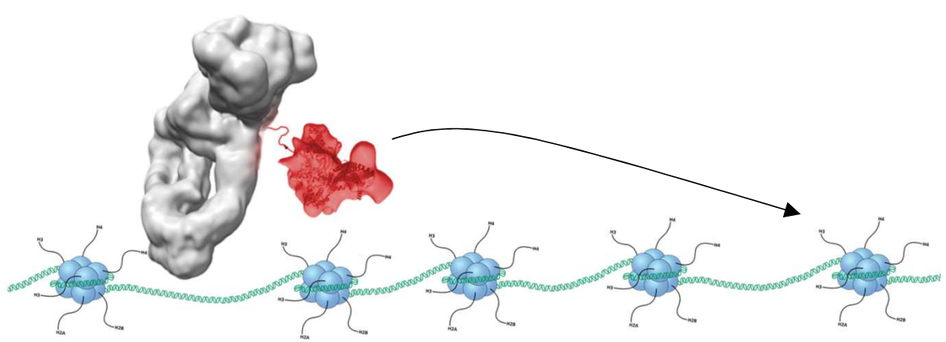
Using the First FLEx system to be established in yeast , we have learned that flexibility in the Epl1 linker is essential





Evidence that unstructured regions can be have a very significant biological role

Identify the transcription regulatory role of this essential long-range activity

Thank you for Your Attention

Facilities

MGX

Funding

anr agence nationale de la recherche
AU SERVICE DE LA SCIENCE

Contributors

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Affiliations

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Annex - 4tU-seq

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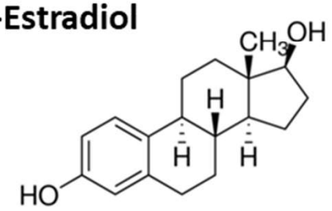
β-Estradiol

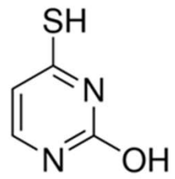


epl1-DCt-FLEx

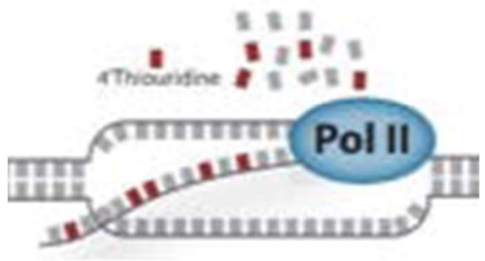
Add β-Estradiol to induce conditional mutation

1-6 hours

4-thiouracil (4tU)



Add 4tU

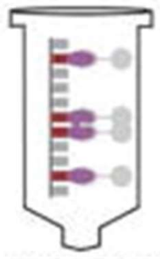


4sU incorporates into newly transcribed RNA

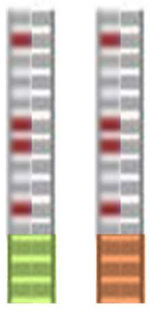
5 minutes

Halt Metabolism and Extract RNA

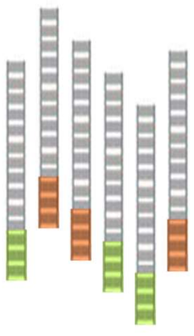
Biotinylate Labeled RNA



Isolate labeled RNA on Streptavidin column which binds biotin



Produce cDNA And add barcodes



Amplify, Pool and Sequence

Annex – FLEx strategy

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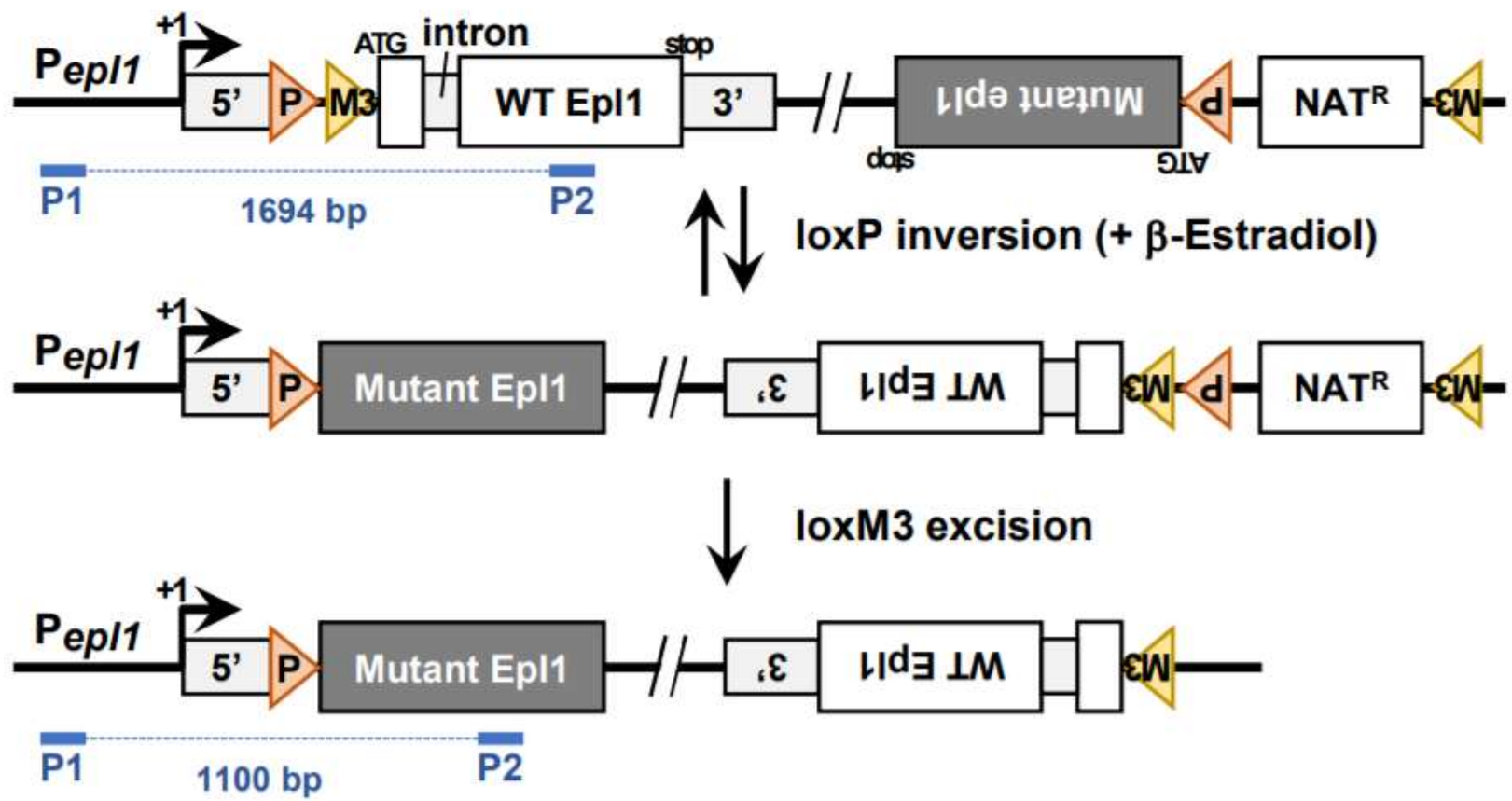
FLEx strategy:



Annex – FLEx validation

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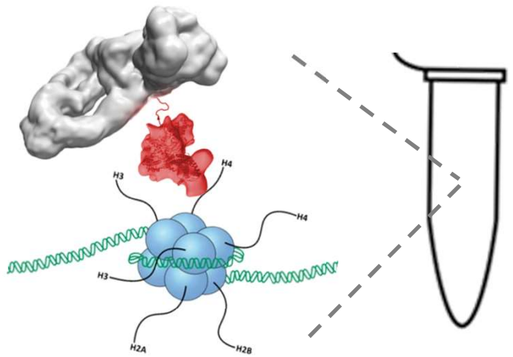
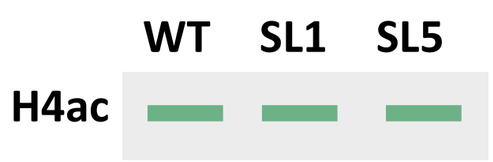
FLEx validation - HAT Activity





- **Equal levels of catalytic activity *in vitro***

Annex – NuA4 and acetyl-H4 ChIPseq

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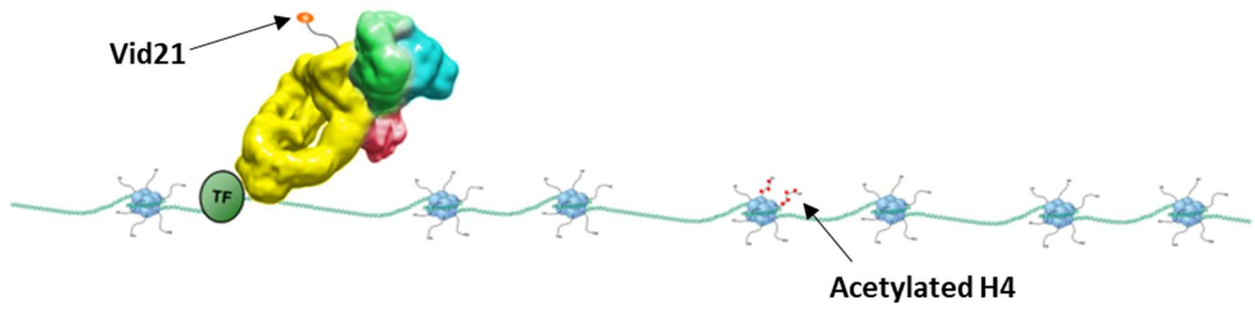
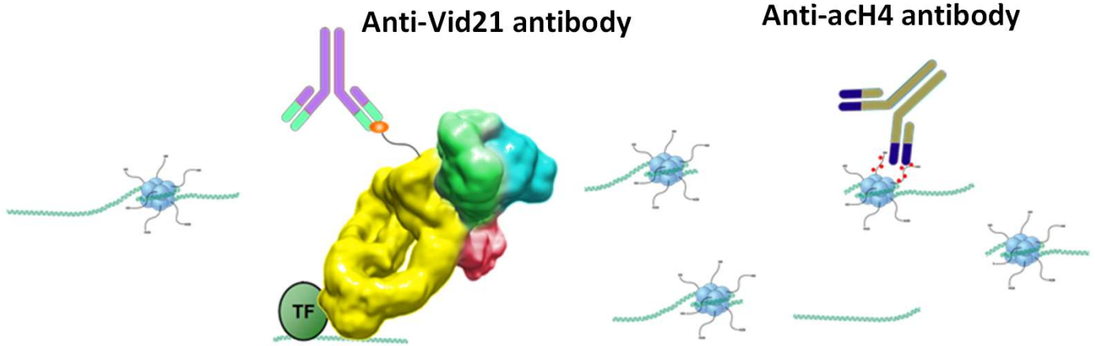
- Crosslink proteins and DNA





- Sonicate into 200-500 bp fragments
- Add antibody for target protein

- Isolate antibody-protein-DNA complex
- dissociate DNA,
- isolate DNA for barcoding, amplification and sequencing