

Contribution of promoter architecture to Pol II initiation by scanning in *Saccharomyces cerevisiae*

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Introduction:

- Eukaryotic protein-coding genes are transcribed by RNA polymerase II (Pol II), which is highly conserved in structure.
- As the first step of transcription, initiation determines where and how efficiently transcription initiates and therefore is a key component of gene expression.
- Pol II initiation in yeast proceeds by a proposed promoter scanning mechanism. (Panel 1)
- To understand why any individual transcription start site (TSS) is used, we are determining how scanning is affected by or interacts with different promoter "architectures". (Panel 1 & 2)

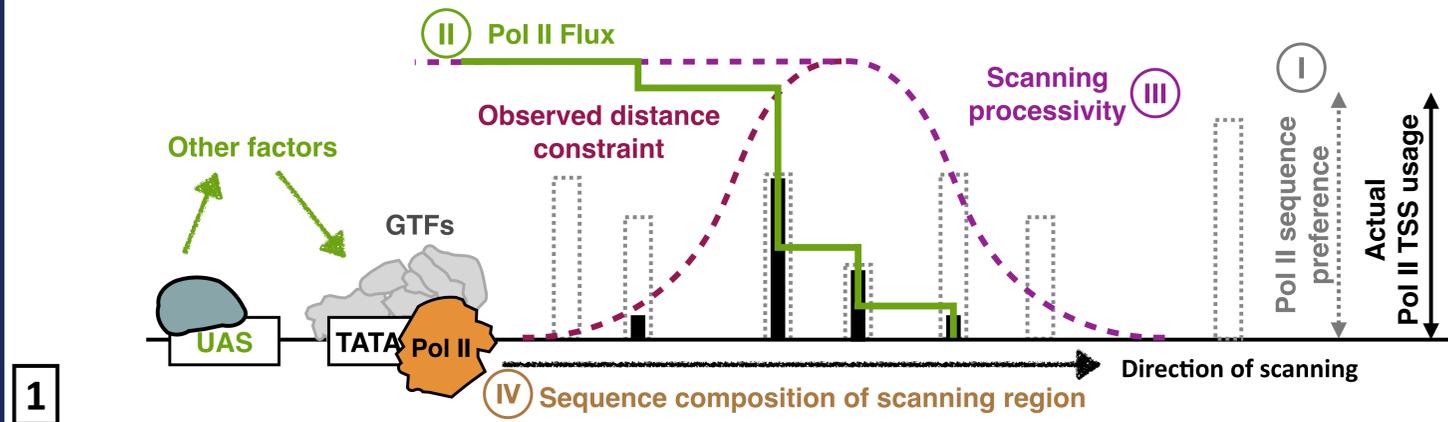
Method: Designed promoter variant libraries where promoter attributes are systematically varied in a controlled fashion (Panel 2)

Main Results:

- Pol II shows distinct sequence preference at positions around TSS. (Panel 5)
- Pol II mutants change overall efficiency for $A_8Y_1R_{+1}$ and $B_8Y_1R_{+1}$ motifs but at different levels. (Panel 6)

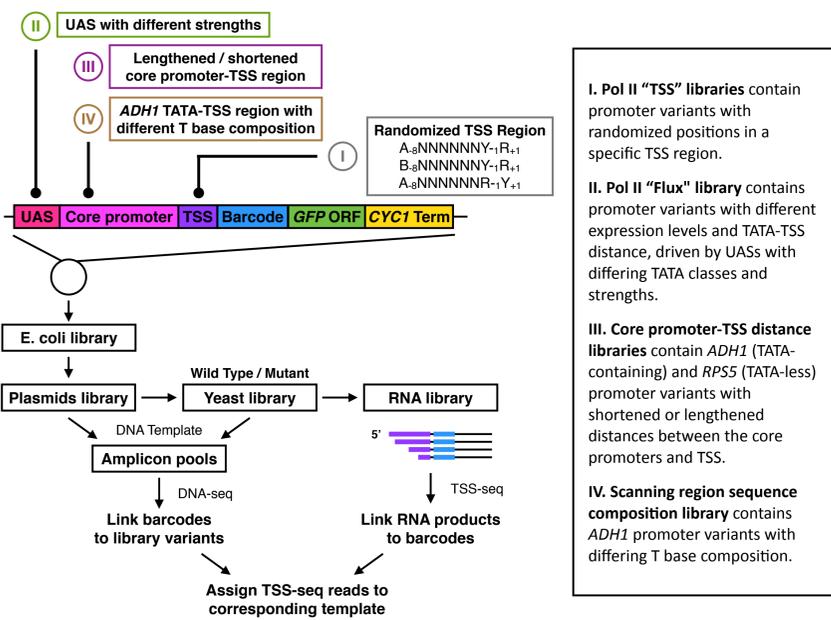
Goal: By combining data from these libraries, our aim is to quantitatively model and predict Pol II initiation distributions for any particular promoter.

Pol II initiation proceeds by promoter scanning in yeast and is controlled by promoter architectural features



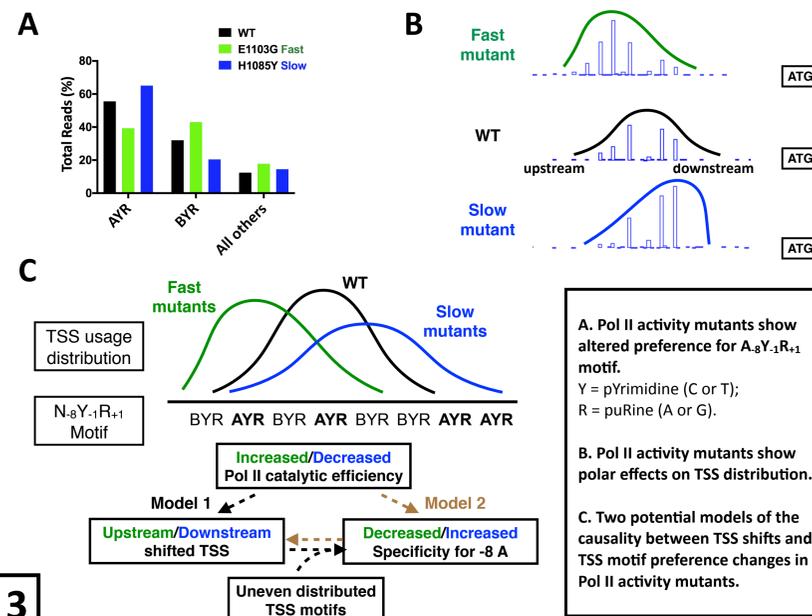
Promoter scanning model: Pol II pre-initiation complex (PIC), comprising Pol II and initiation factors, assembles upstream of the initiation region and then scans downstream to select appropriate transcription start sites (TSSs) to initiate transcription.

Systematic analysis of promoter architectural effects on Pol II initiation

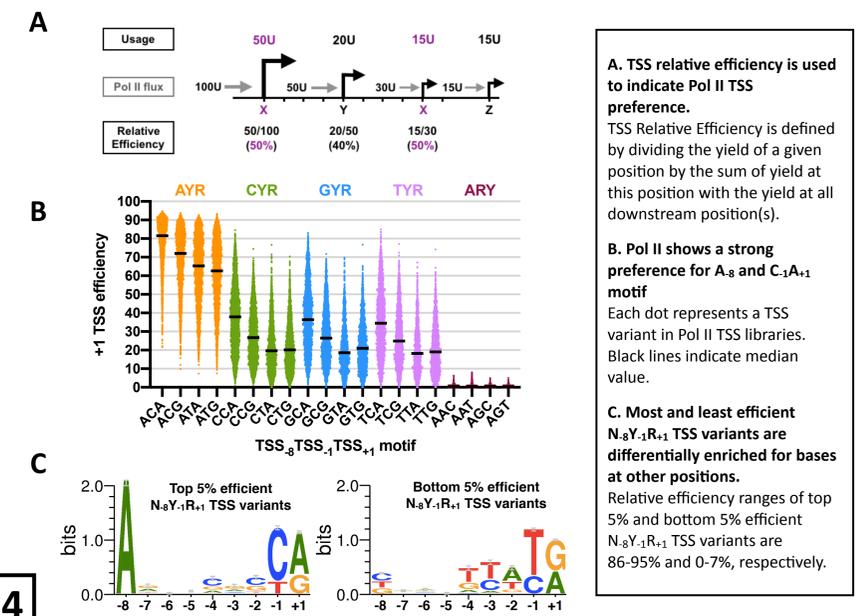


- Pol II "TSS" libraries contain promoter variants with randomized positions in a specific TSS region.
- Pol II "Flux" library contains promoter variants with different expression levels and TATA-TSS distance, driven by UASs with differing TATA classes and strengths.
- Core promoter-TSS distance libraries contain *ADH1* (TATA-containing) and *RPS5* (TATA-less) promoter variants with shortened or lengthened distances between the core promoters and TSS.
- Scanning region sequence composition library contains *ADH1* promoter variants with differing T base composition.

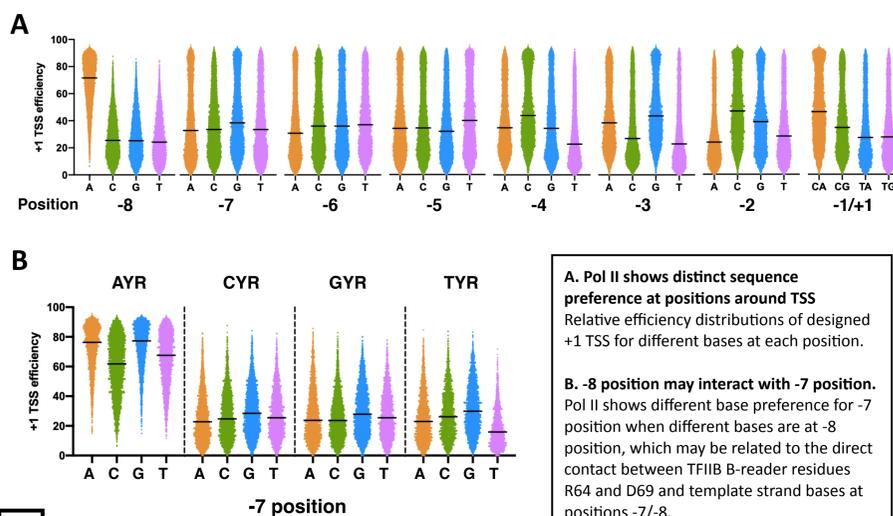
The causality between Pol II TSS motif preference changes and TSS distribution shifts is uncertain



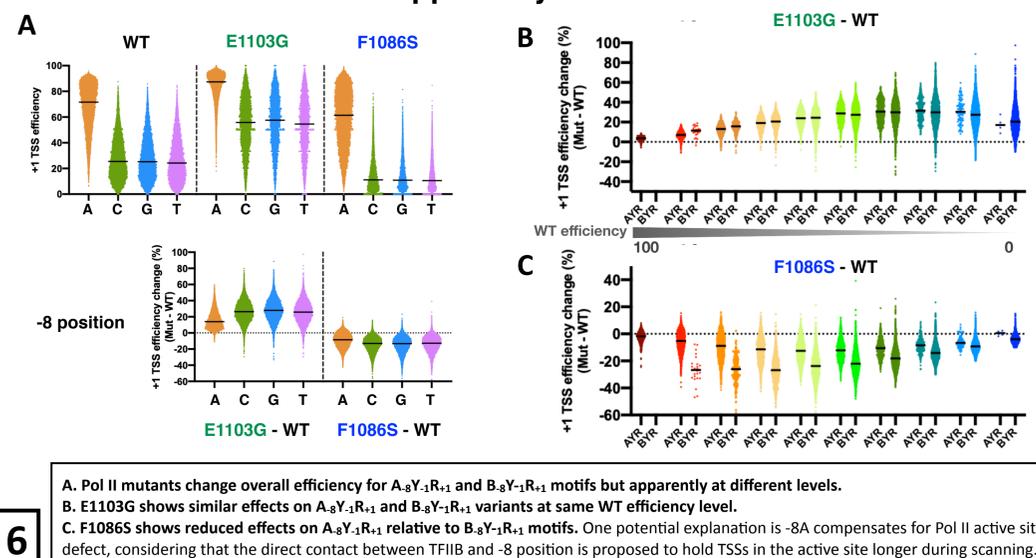
As expected, Pol II shows a strong preference for A_8 and C_1A_{+1} motif



Pol II shows distinct sequence preference at positions around TSS



Pol II mutants change overall efficiency for $A_8Y_1R_{+1}$ and $B_8Y_1R_{+1}$ motifs but apparently at different levels



References

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