

# Discovering functional enhancers with nascent RNA profiling

Erin Wissink

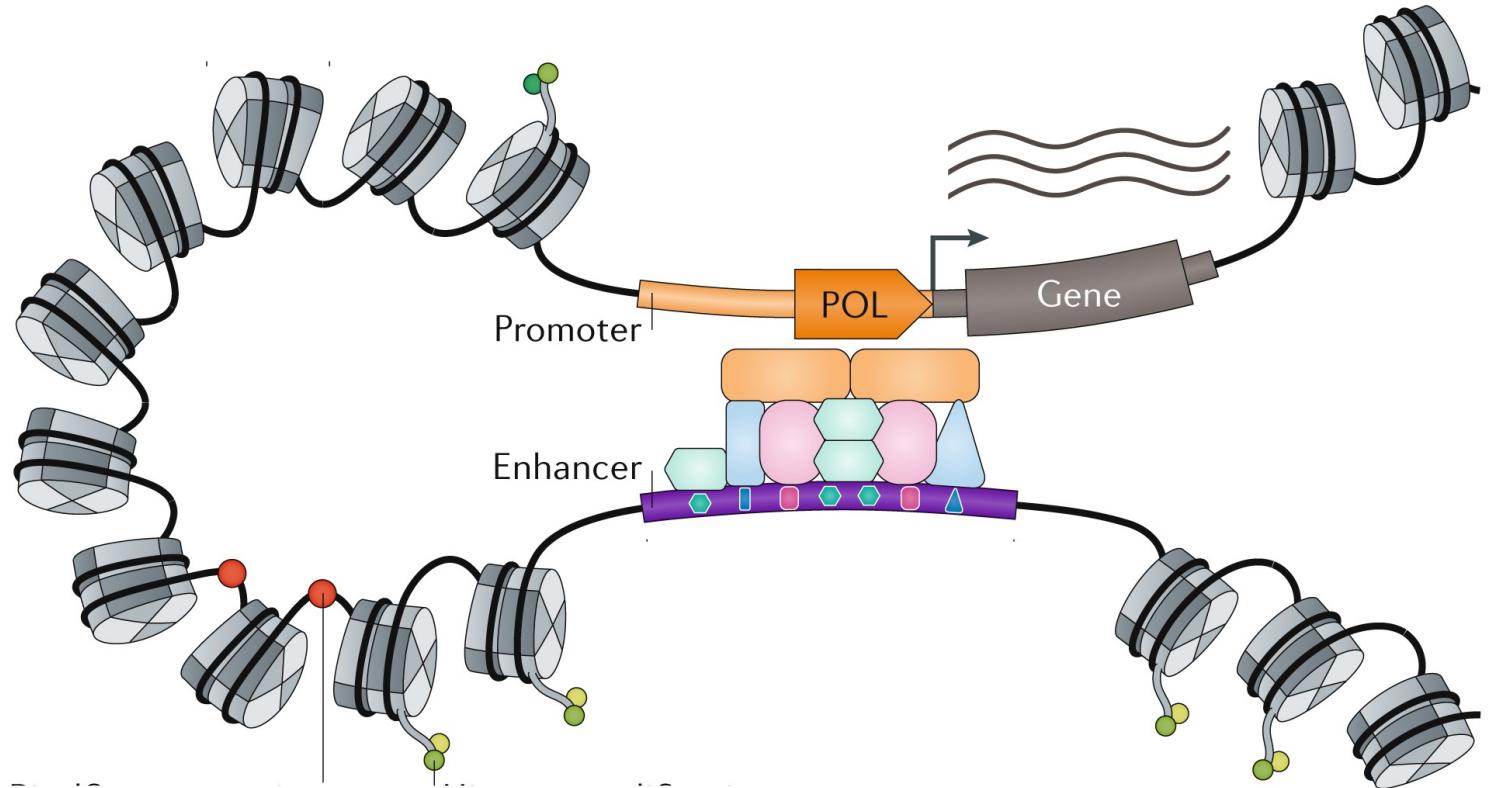
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Cornell University

September 17, 2021

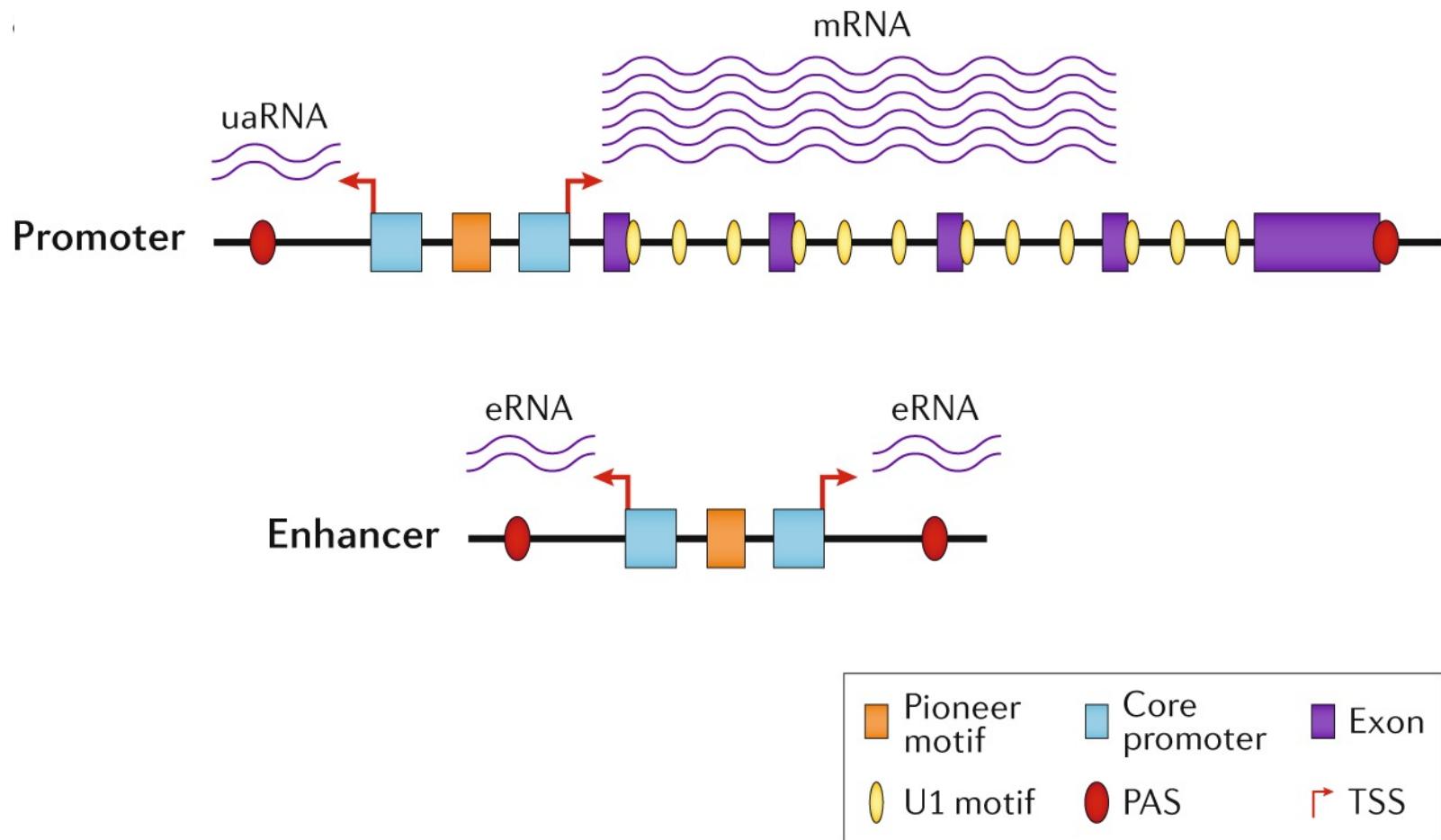
# Transcription is encoded at promoters and enhancers

- Transcription factor (TF) binding sites specify when and where RNAs are synthesized
- Clusters of binding sites present at gene promoters and distal enhancers
- TFs increase chromatin accessibility, recruit histone modifiers, and enable looping



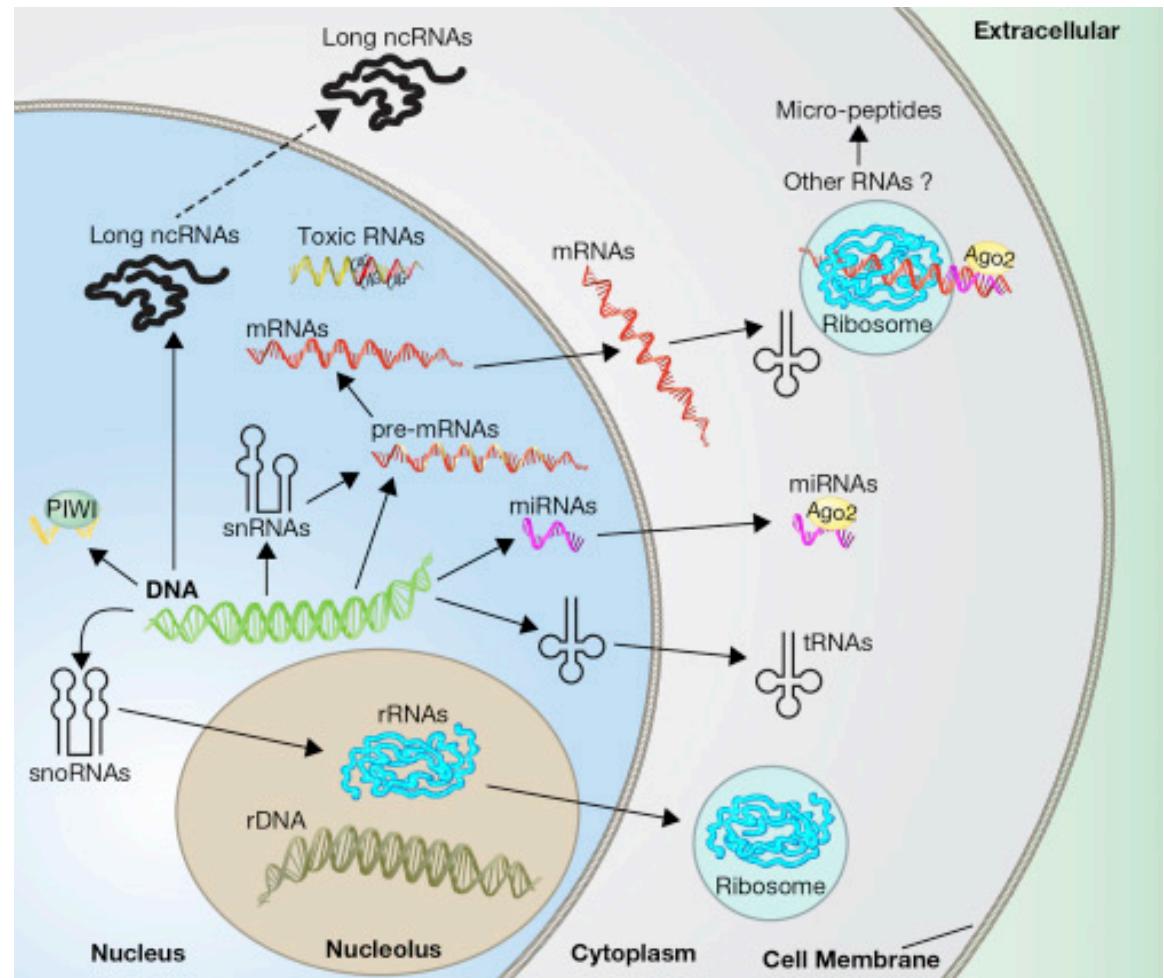
# Promoters and enhancers exhibit bidirectional transcription

- Promoters generate:
  - Stable mRNA (capped and polyadenylated)
  - Unstable upstream antisense RNA (capped, not always polyadenylated)
- Enhancers generate:
  - Two unstable RNAs
  - Capped
  - Not always polyadenylated



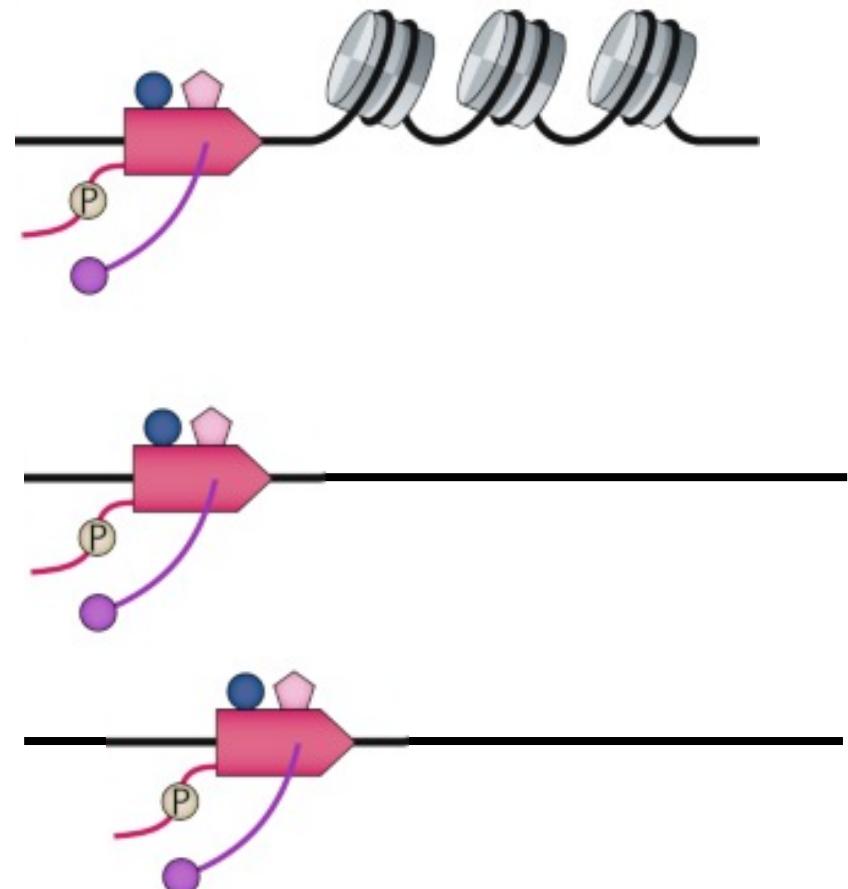
# Enhancers RNAs are needles in a haystack

- >90% of RNA in a cell is ribosomal
- eRNAs make up < 1% RNA molecules and are unstable
- Need to make the haystack smaller! How to capture only RNAs that are actively being synthesized?



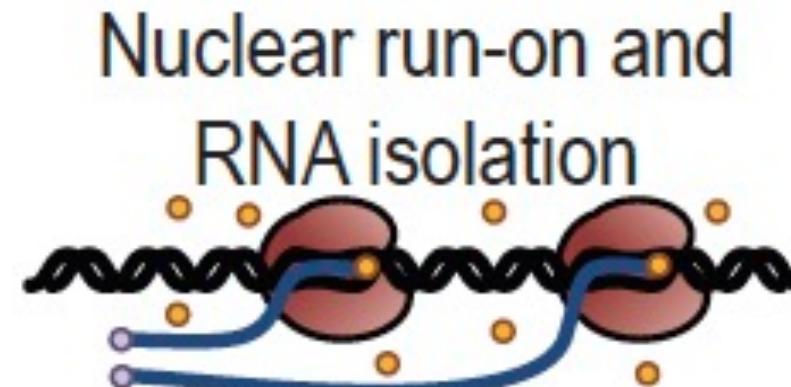
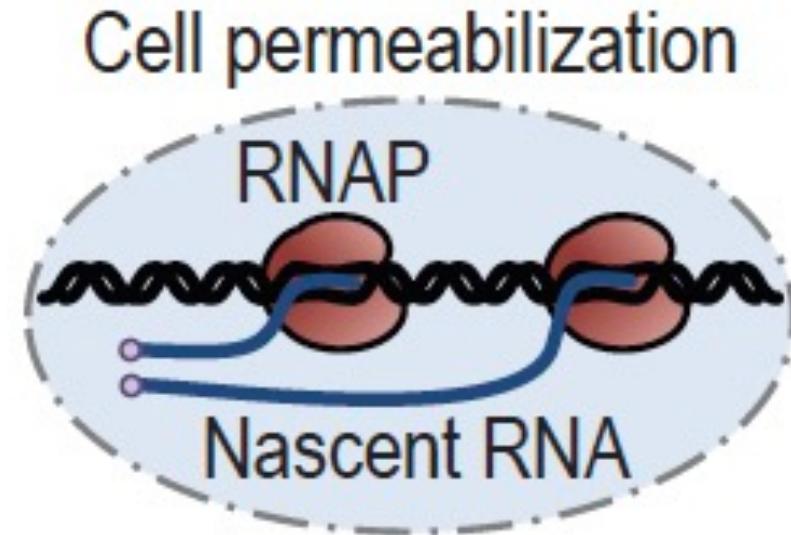
# Principles of run-on assays

- How do we find out where Pol II is transcribing at a given moment?
- Transcription can be stopped by:
  - Placing cells on ice
  - Removing nucleotides
- Pol II transcription bubble is very, very stable
- Strong detergents will remove all barriers to transcription but NOT transcribing Pol II
- Transcription can be restarted *in vitro*
- Labeled nucleotides can be detected



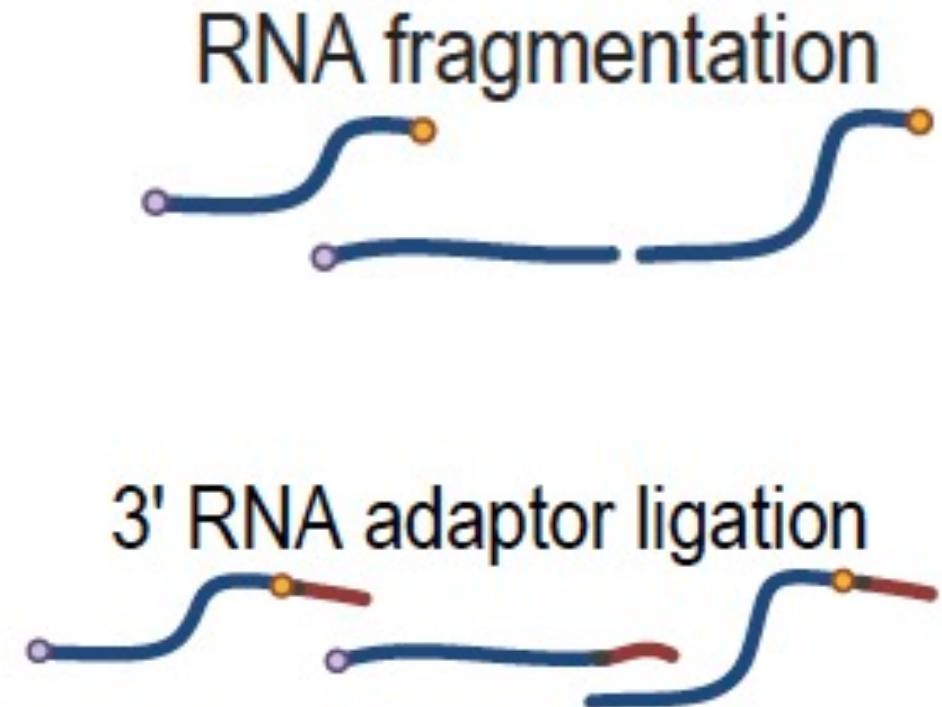
# How to sequence RNAs being actively transcribed

1. Wash out endogenous nucleotides to freeze Pol II in place
  - can flash-freeze cells after permeabilization and store at -80°C until ready to proceed
2. Perform nuclear run-on with biotinylated nucleotides
  - Bulky biotin stops Pol II from continuing to transcribe – base pair resolution
  - Can lower cost by only using biotin-UTP and biotin-CTP



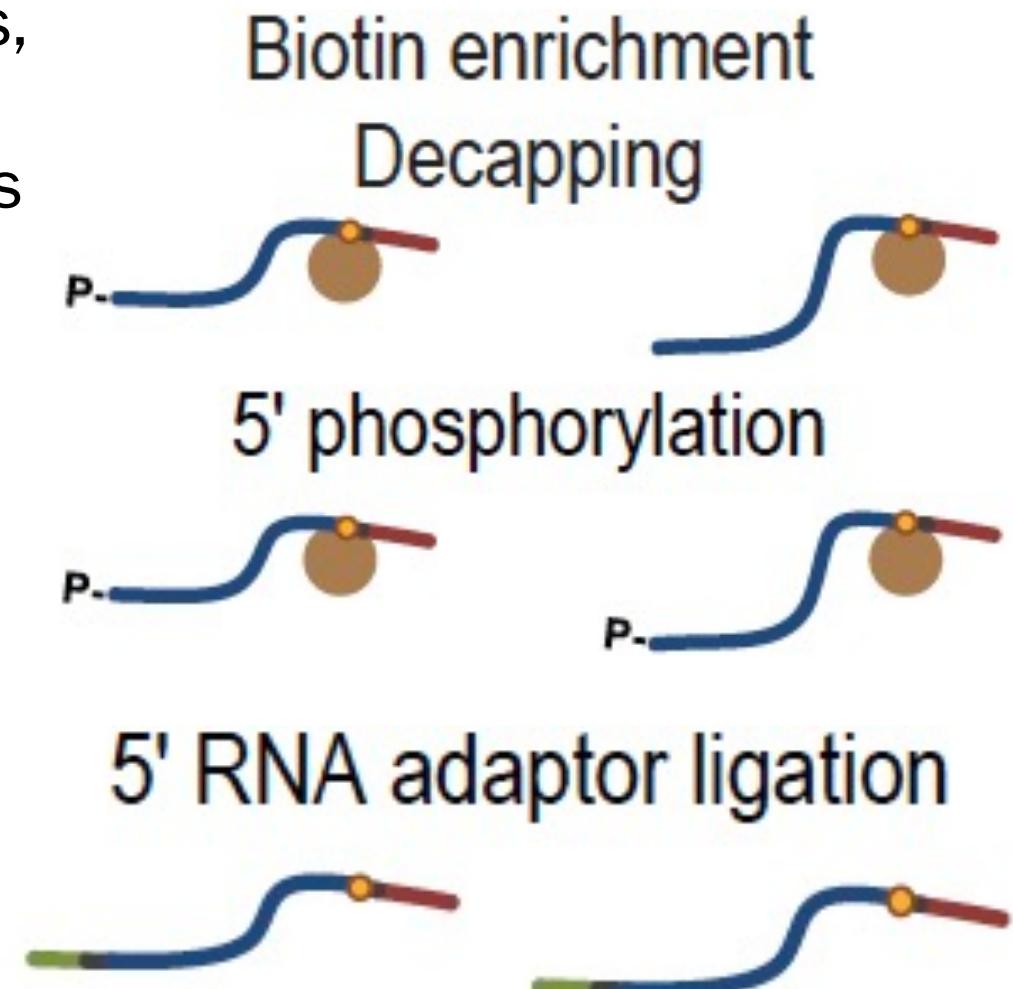
# How to sequence RNAs being actively transcribed

3. Use NaOH to hydrolyze RNAs to ~100 nt
4. Ligate sequencing adapter to 3' end
  - Adapter has sequences for binding molecule to Illumina flow cell
  - Ligation is not specific for nascent RNA
  - Only attaches to 3' end (not 5') of RNA because T4 RNA ligase requires a 3' hydroxyl and 5' phosphate. The adapter is phosphorylated on 5' end. RNA with cap or that has been fragmented does not create a ligation substrate

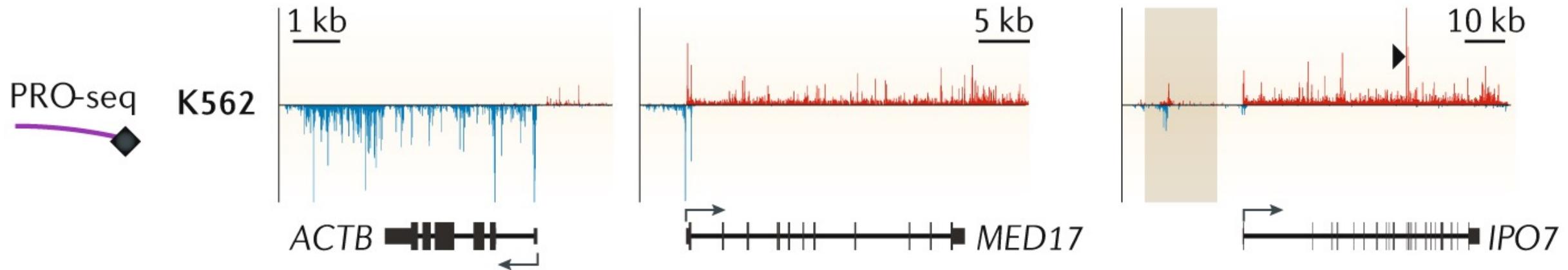


# How to sequence RNAs being actively transcribed

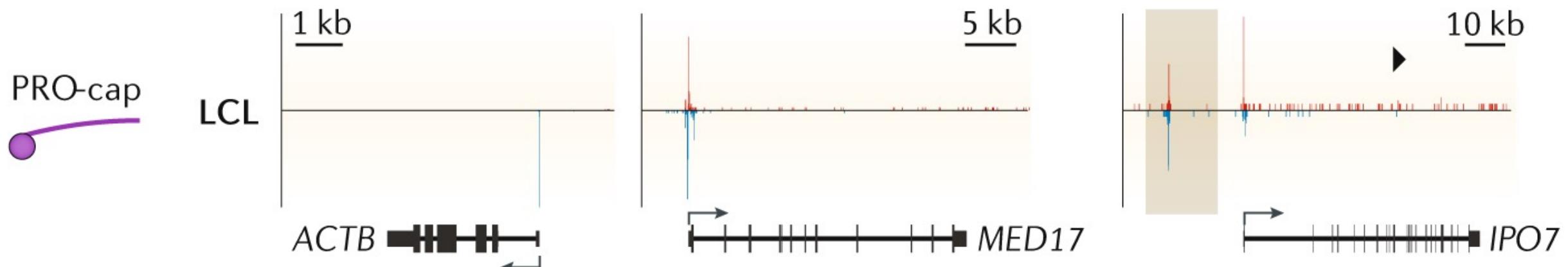
5. Bind RNA to streptavidin magnetic beads, and wash away non-nascent RNA
  - Following steps are performed on beads
6. Do end repair to 5' to allow for adapter ligation
  - Remove RNA cap
  - Add phosphate
7. Ligate adapter to 5' end
8. Isolate RNA from beads
9. Perform RT and PCR



# Example data

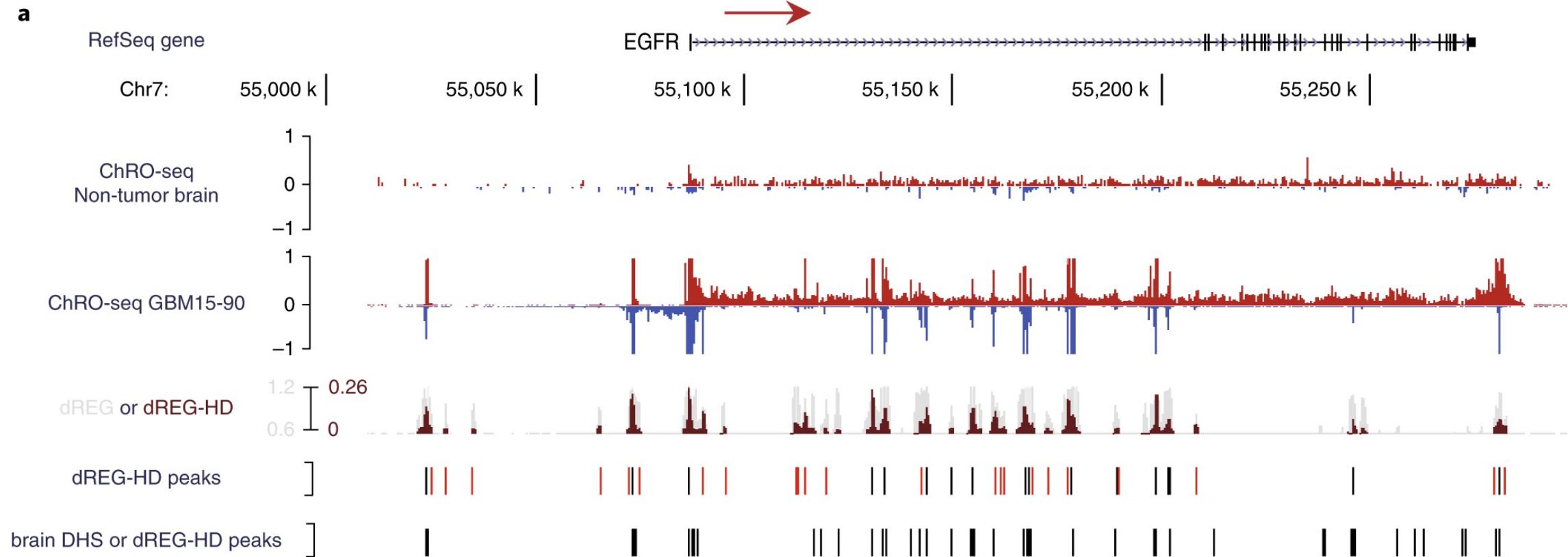


Can alter protocol to capture the 5' end of nascent RNAs, allowing TSS mapping



# Can perform ChRO-seq on tissues and banked material

a



- Found genes and enhancers specific to different glioblastoma subtypes

# Protocols

## Base-pair-resolution genome-wide mapping of active RNA polymerases using precision nuclear run-on (PRO-seq)

Dig Bijay Mahat, Hojoong Kwak, Gregory T Booth, Iris H Jonkers, Charles G Danko, Ravi K Patel, Colin T Waters, Katie Munson, Leighton J Core  & John T Lis 

*Nature Protocols* 11, 1455–1476 (2016) | [Cite this article](#)

### A rapid, sensitive, scalable method for Precision Run-On sequencing (PRO-seq)

 Julius Judd, Luke A. Wojenski, Lauren M. Wainman,  Nathaniel D. Tippens, Edward J. Rice, Alexis Dziubek, Geno J. Villafano,  Erin M. Wissink, Philip Versluis, Lina Bagepalli, Sagar R. Shah, Dig B. Mahat, Jacob M. Tome, Charles G. Danko,  John T. Lis, Leighton J. Core

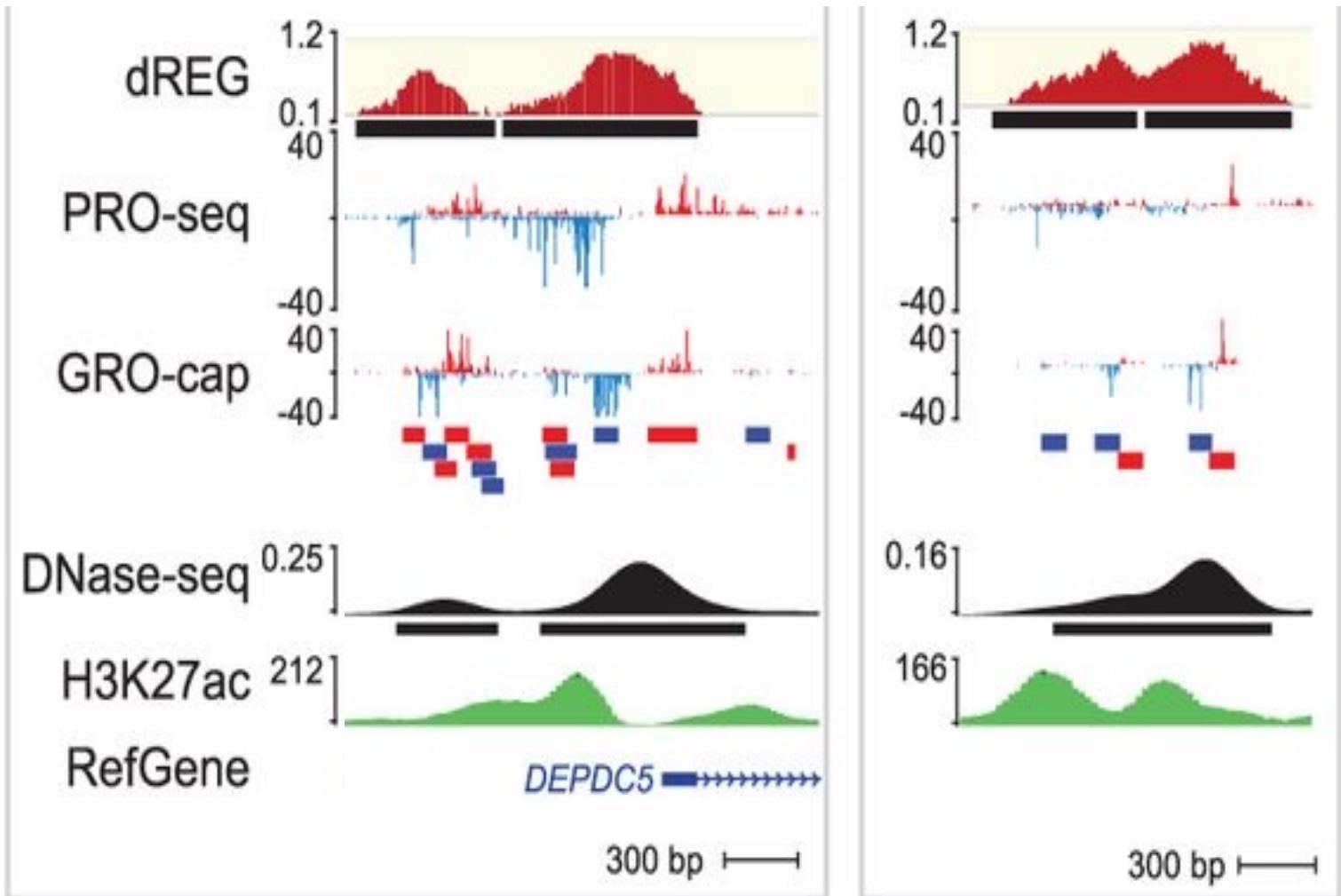
**doi:** <https://doi.org/10.1101/2020.05.18.102277>

This article is a preprint and has not been certified by peer review [what does this mean?].

<https://www.protocols.io/view/a-rapid-sensitive-scalable-method-for-precision-ru-57dg9i6>

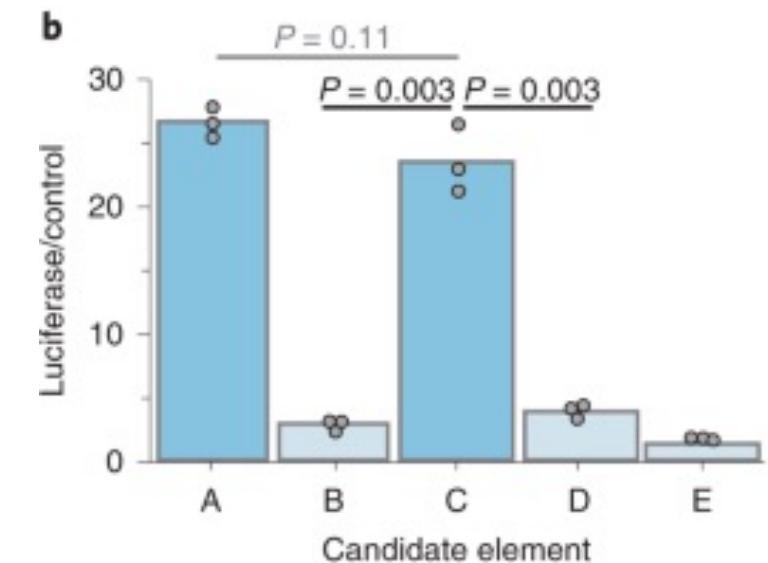
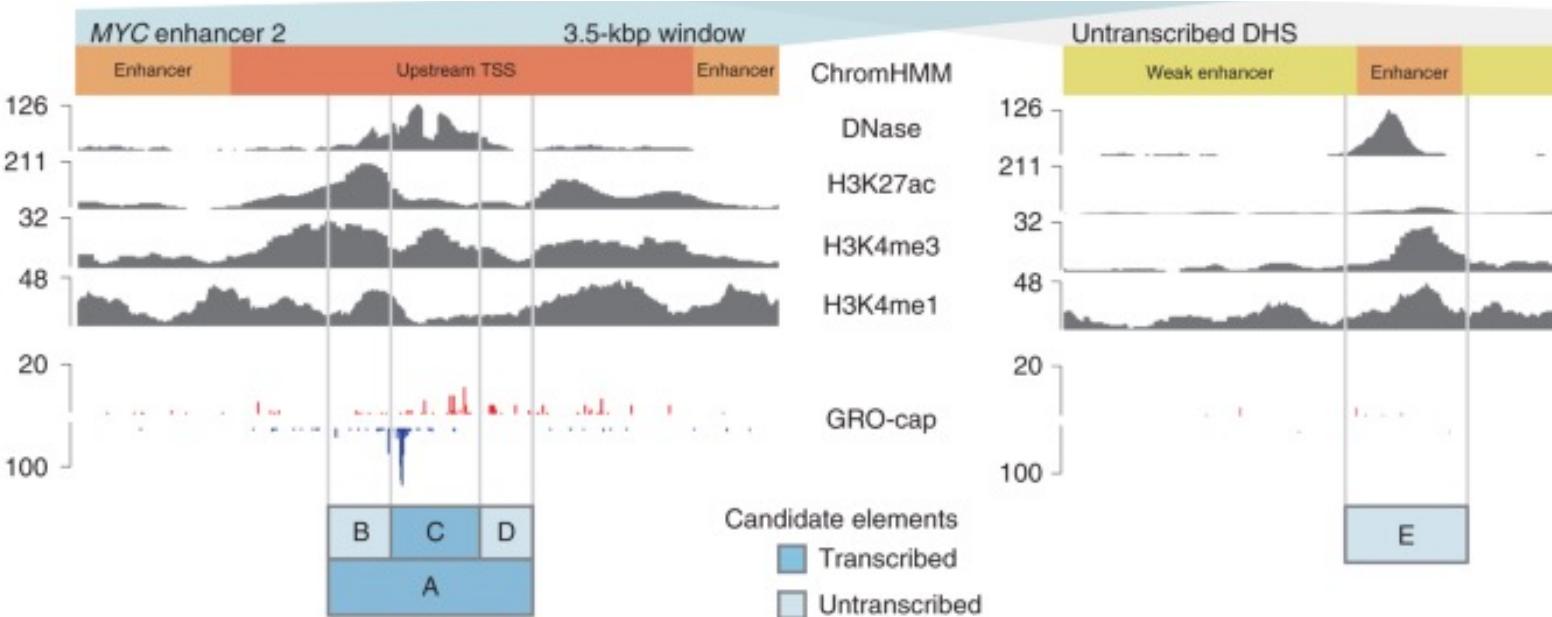
# Finding enhancers from PRO-seq data

- Input PRO-seq data at  
<https://django.dreg.scigap.org/>
- Receive regulatory regions as output, aka sites of bidirectional transcription



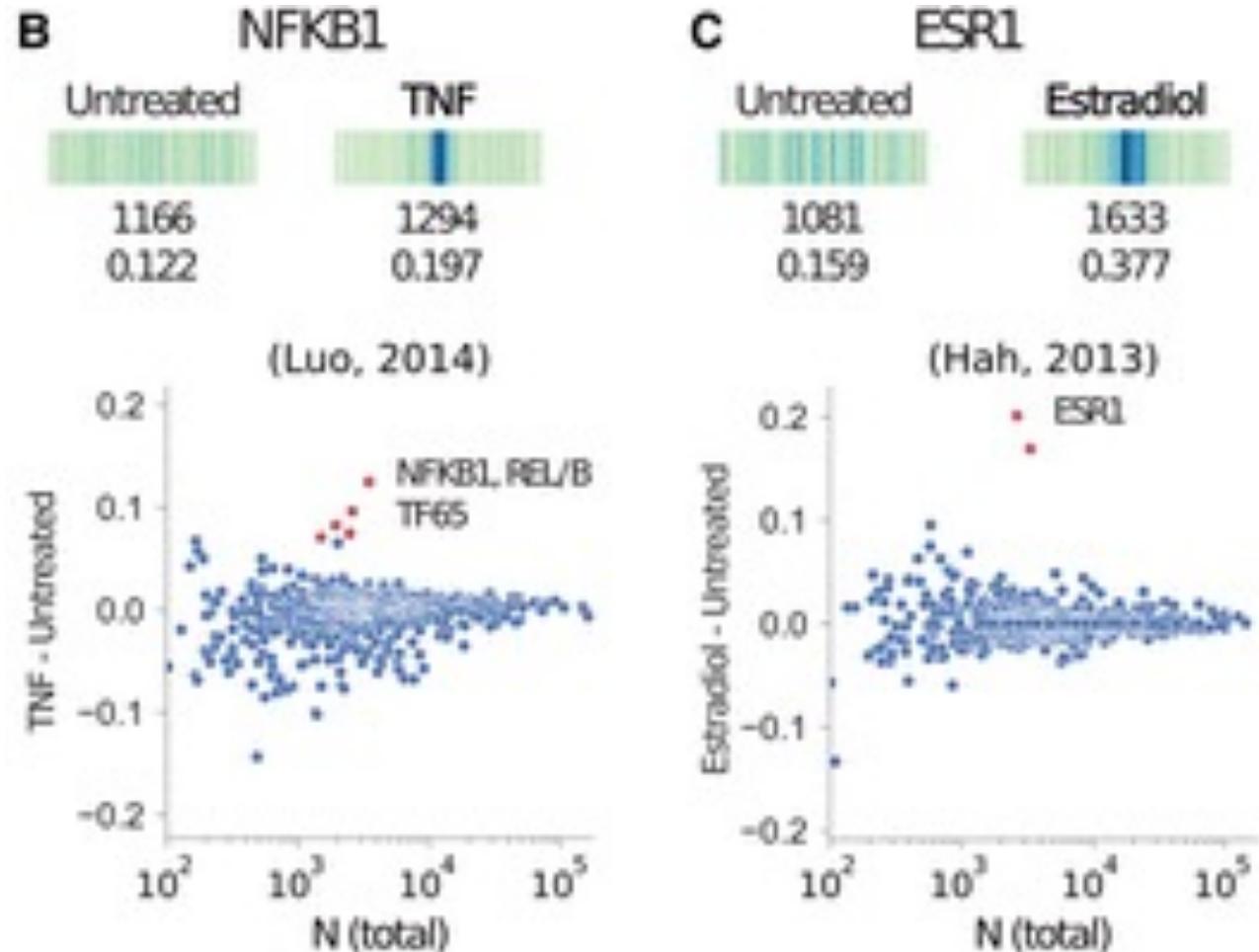
# Active enhancers produce RNAs

- Used a high-throughput reporter assay (eSTARR-seq) to measure enhancer activity
- Accessible chromatin that is transcribed has more activity than untranscribed accessible chromatin

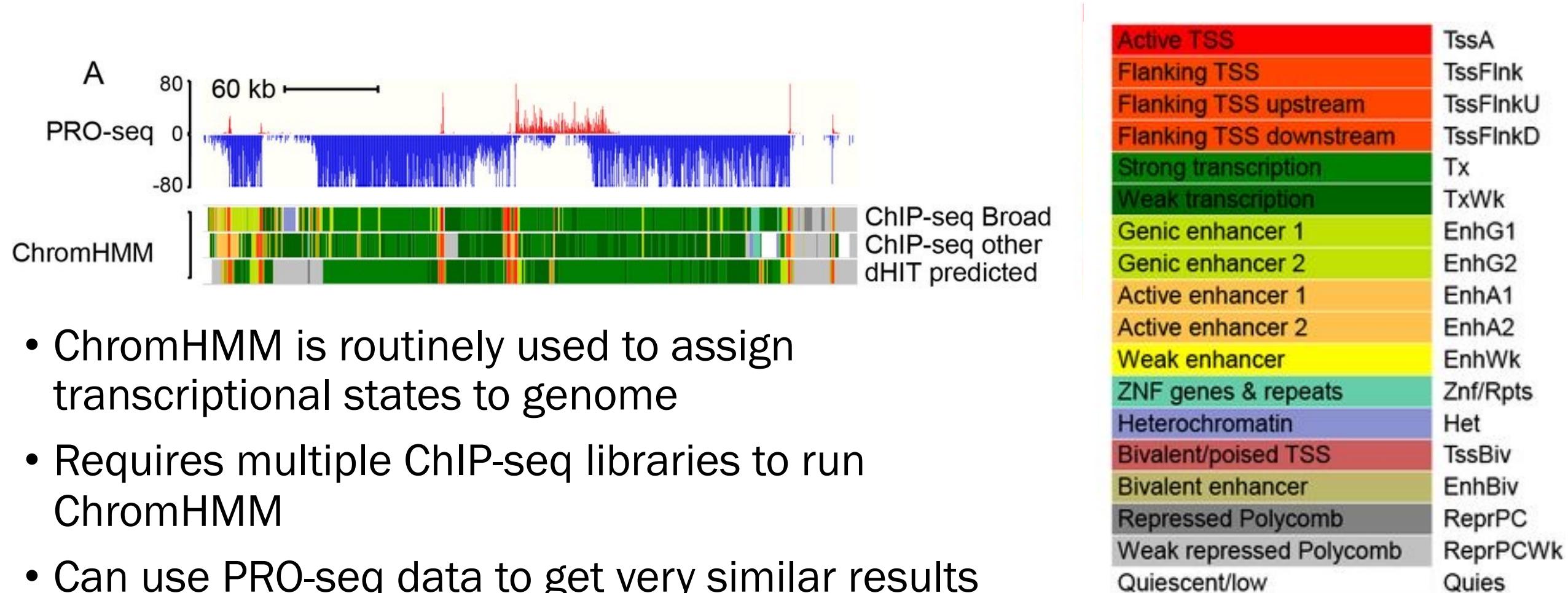


# Finding TFs that are active in different conditions

- Found eRNAs by looking for bidirectional transcripts
- Identified the TF motifs present between transcripts
- Found differential motif usage between conditions

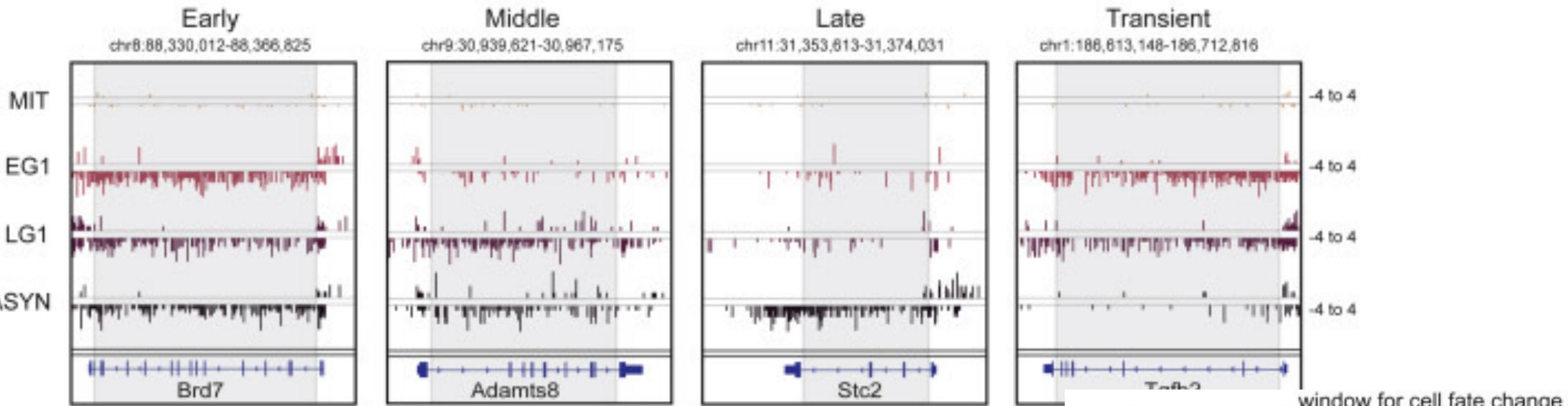


# Run-on data predicts ChromHMM states without needing to do ChIP-seq

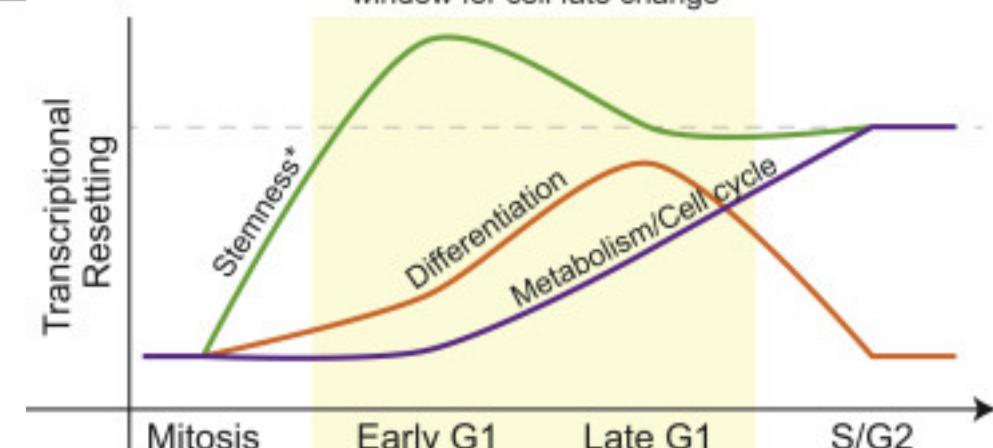


# Studying cell cycle effects on transcription

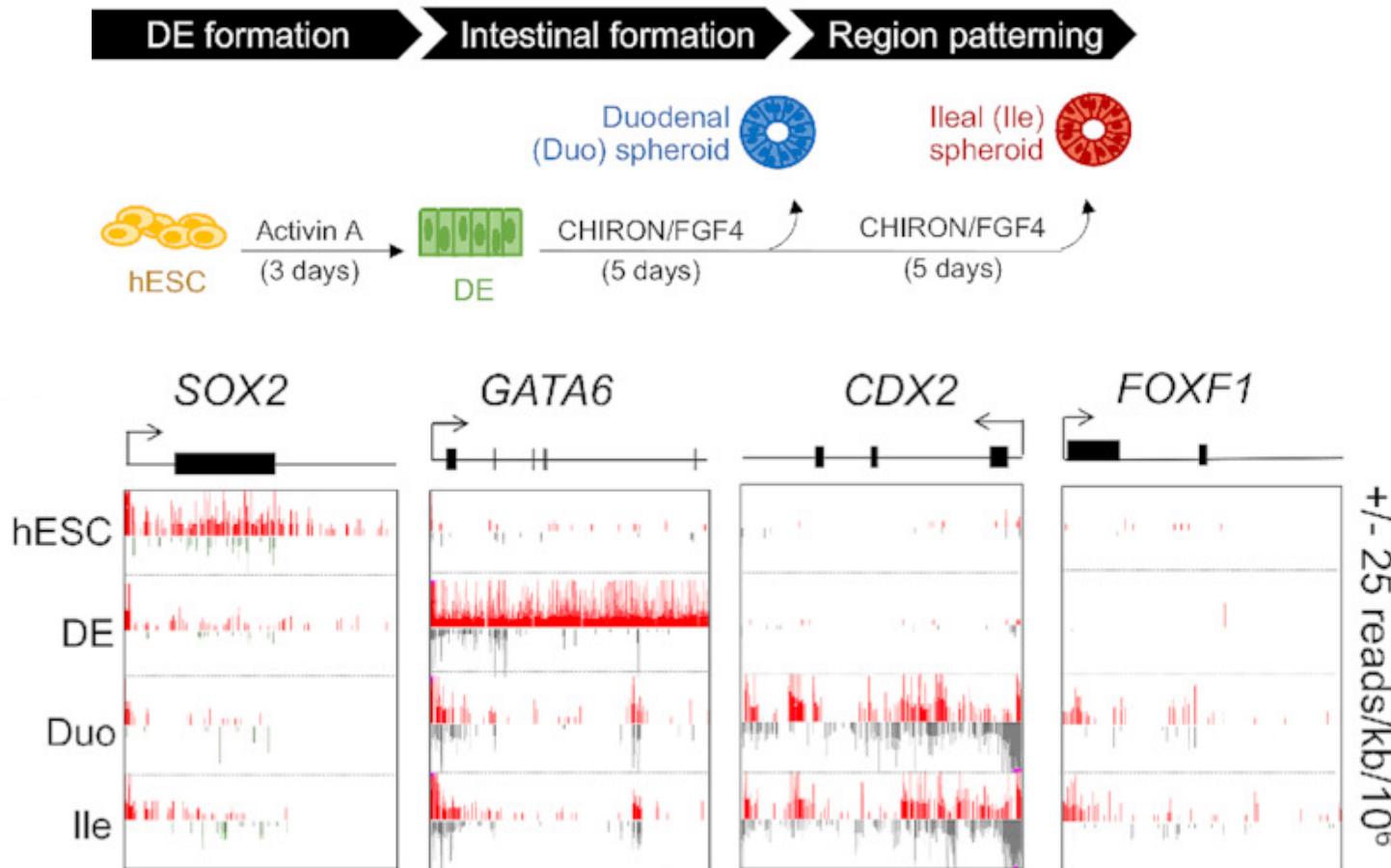
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- Measured nascent transcription after cell cycle arrest in mESCs
- Defined parts of the genome that turned on early vs late



# Discovering developmental changes in gene expression

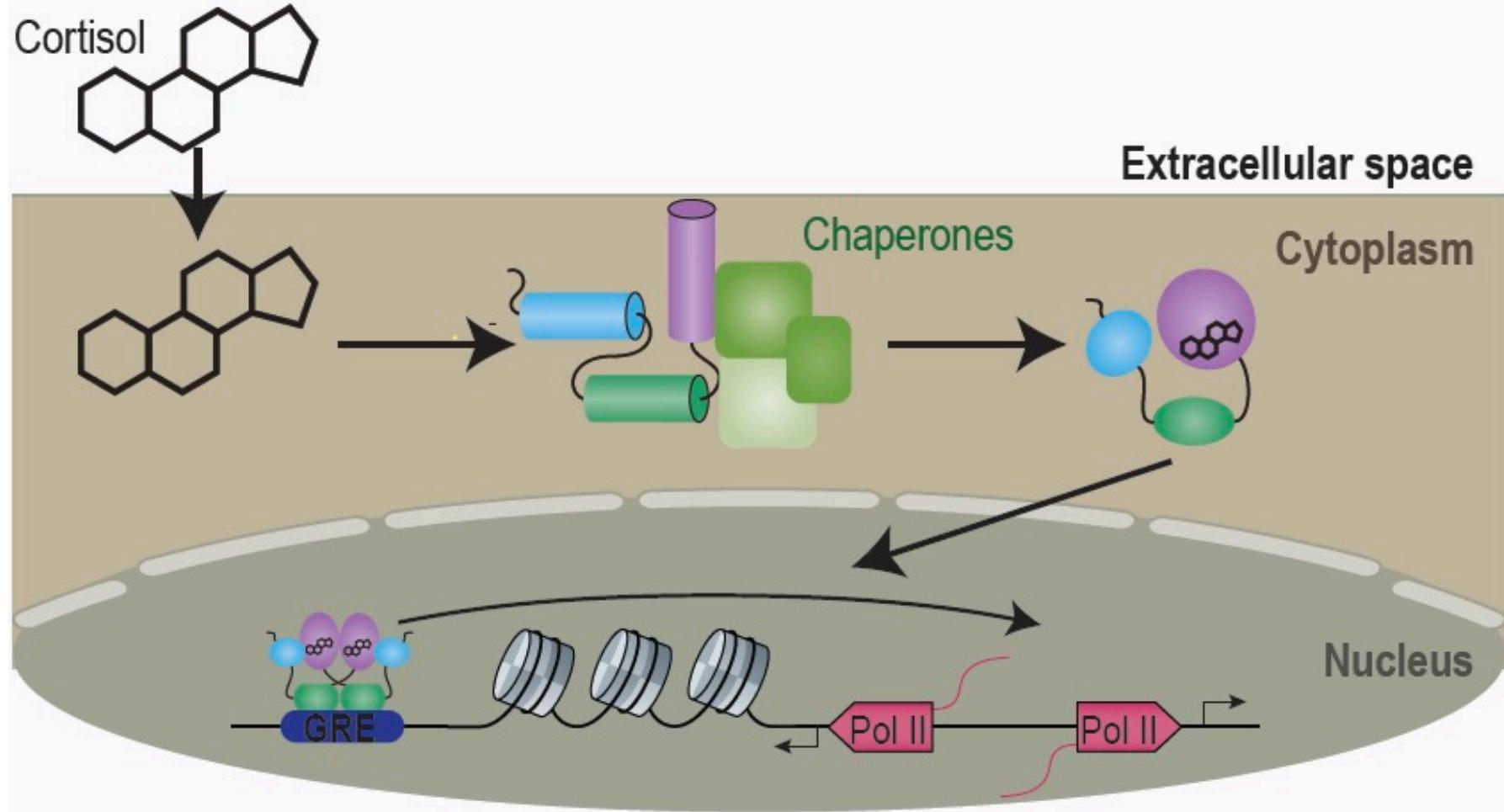


- Measured nascent transcription in differentiation model for gut
- Easier to see which genes are shut off in nascent RNA – no stable, mature RNAs interfere
- Found unexpected influence of HOX genes and important TFs at each stage

DE=definitive endoderm

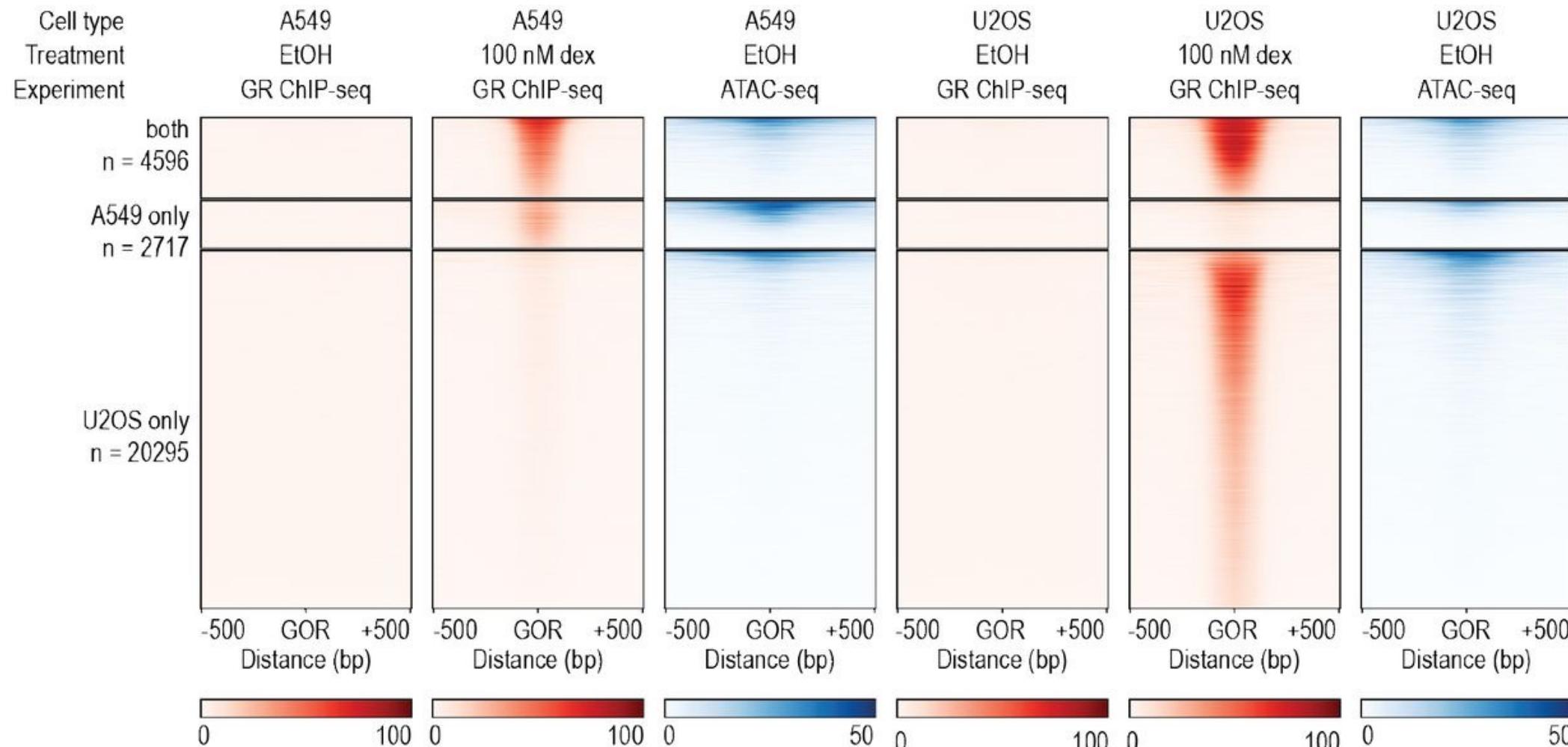
Hung, et al. NAR (2021).

# Assessing roles of different transcription factors in gene regulation

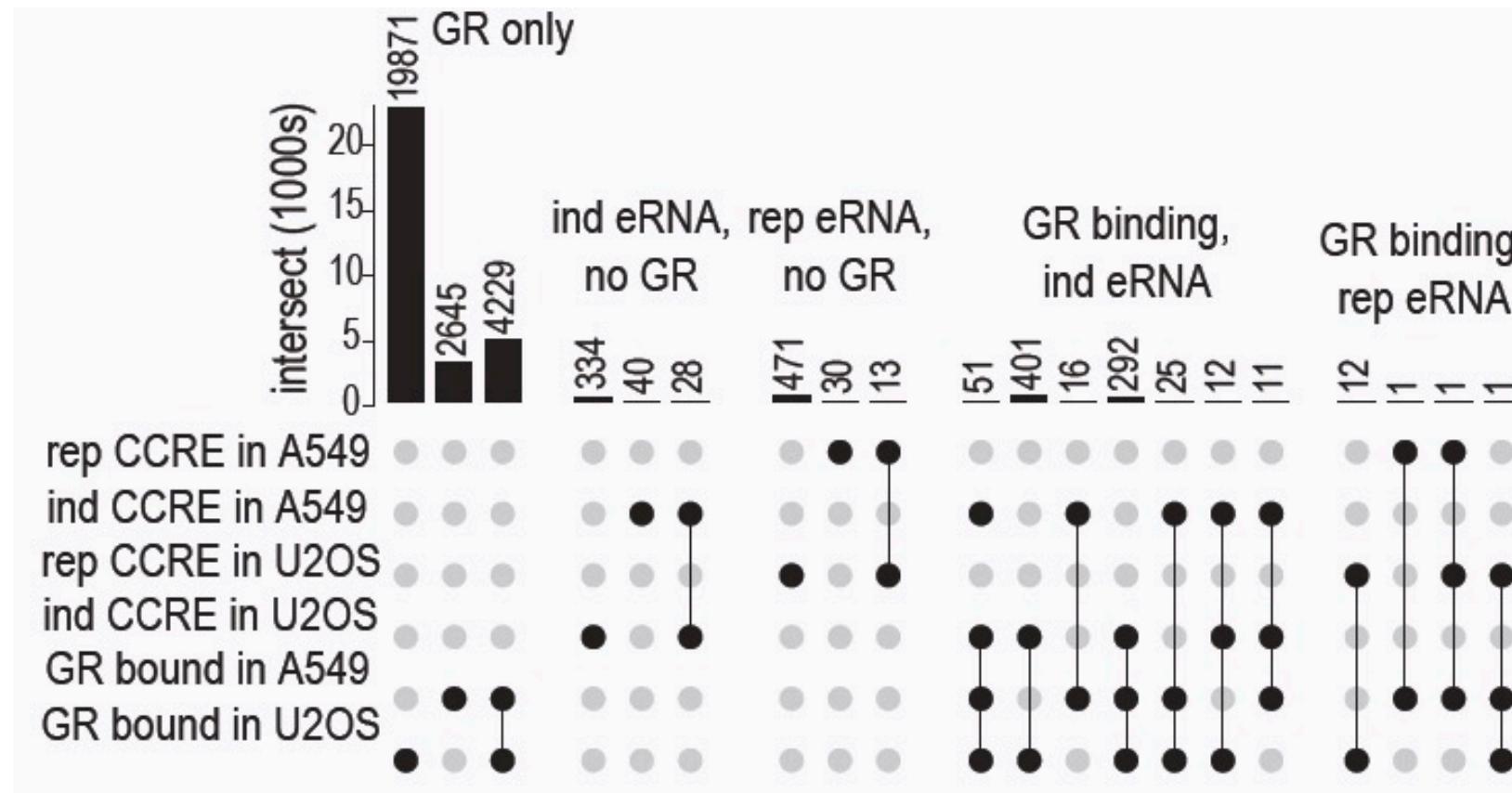


- Assayed changes in nascent transcription and GR protein binding after glucocorticoid (dexamethasone) treatment

# Glucocorticoid receptor binds to thousands of sites in the genome

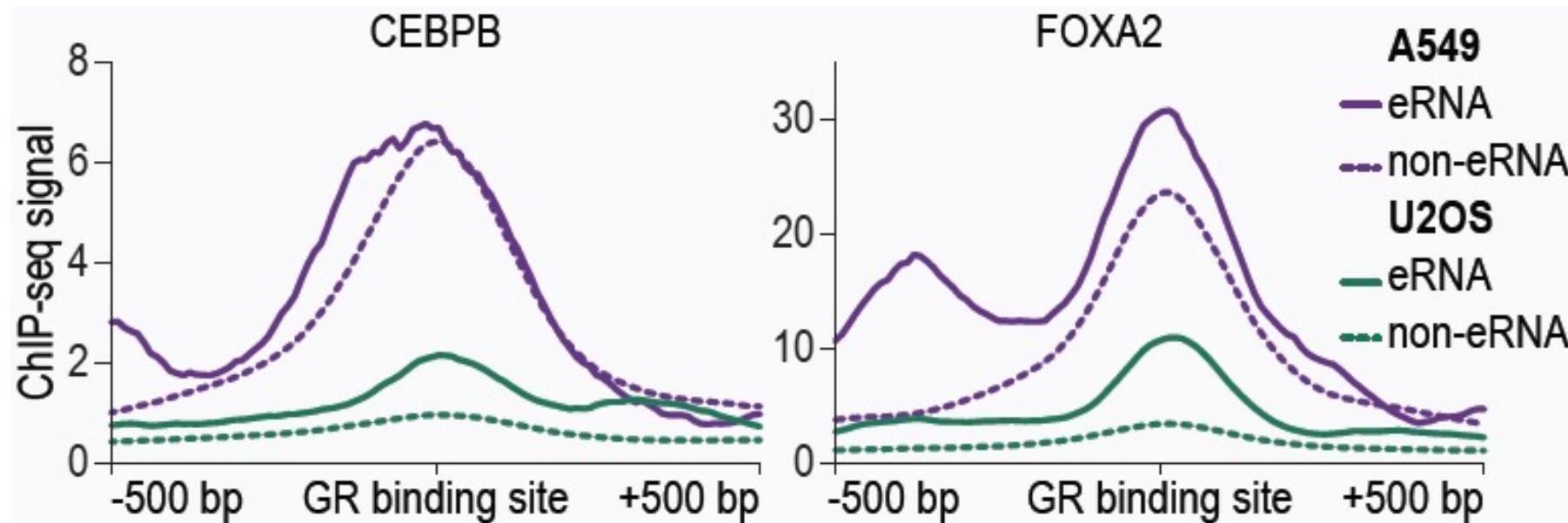


# Far fewer eRNAs have differential transcription after dex treatment



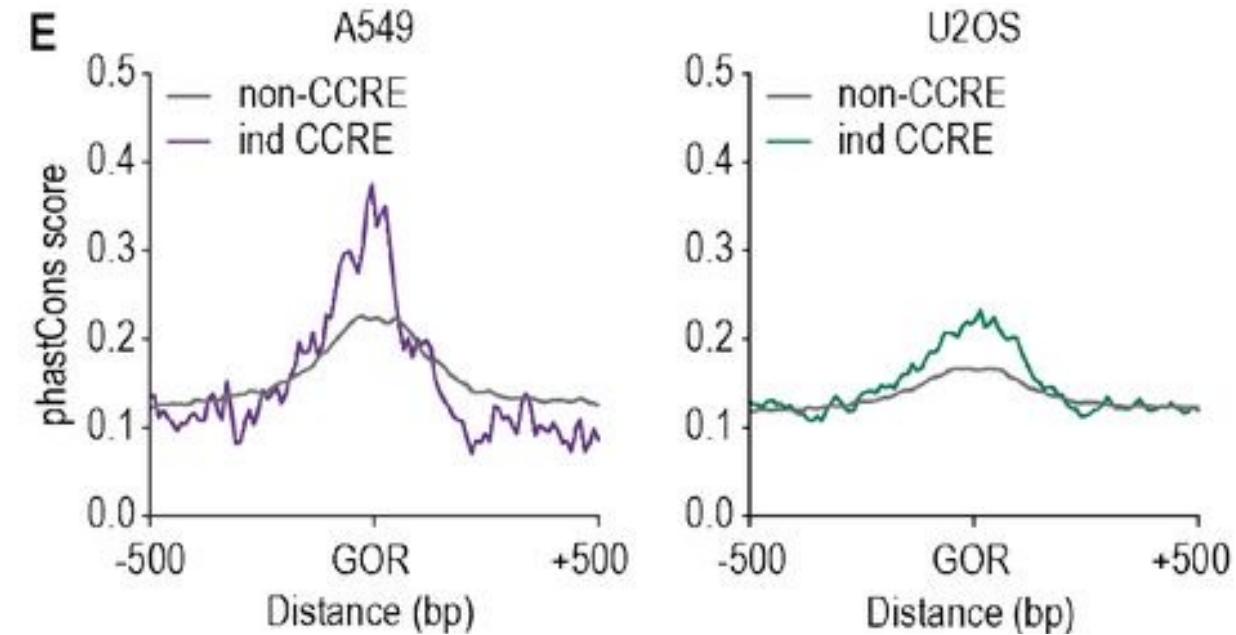
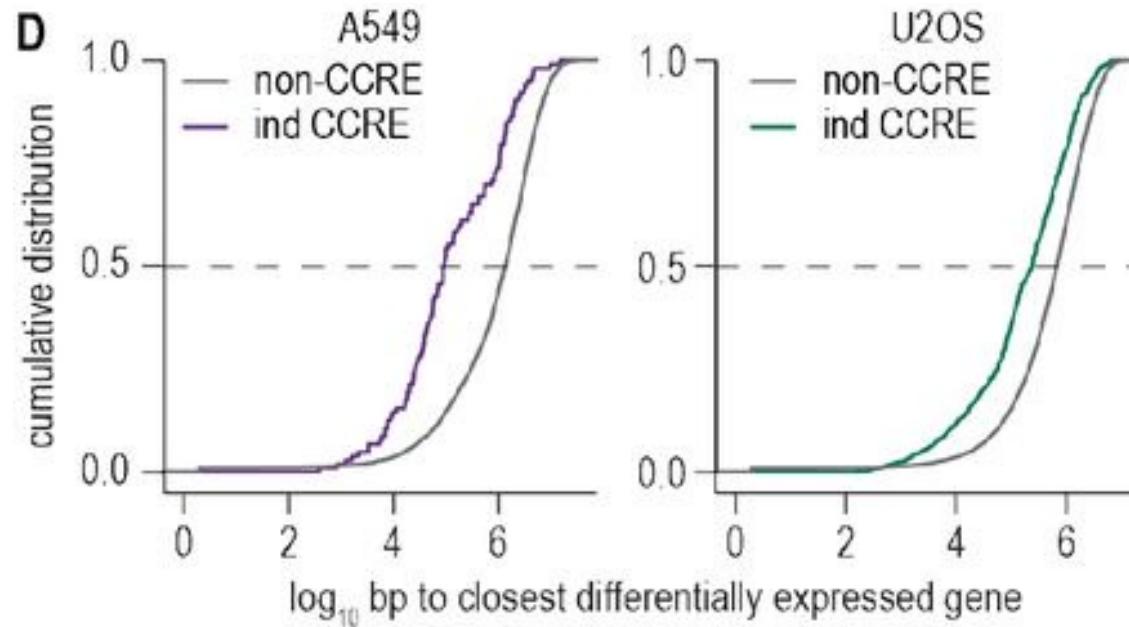
- Almost all induced eRNAs are bound by GR
- <5% of GR bound sites overlap eRNAs

# GR binding overlaps pioneer factors, regardless of eRNA transcription



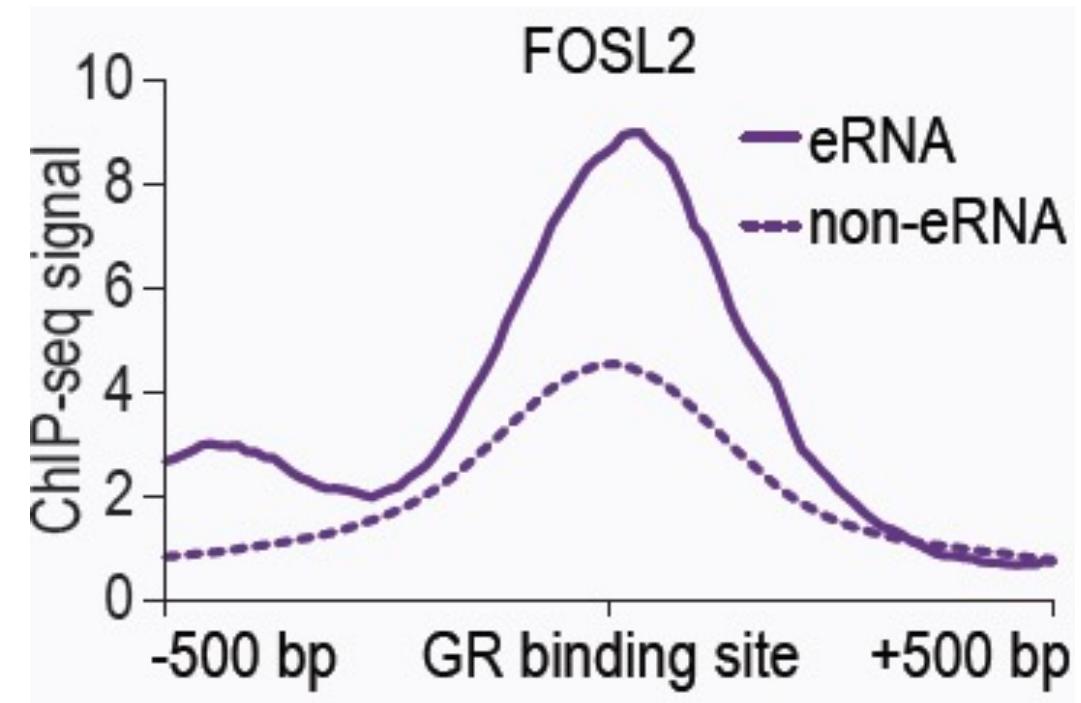
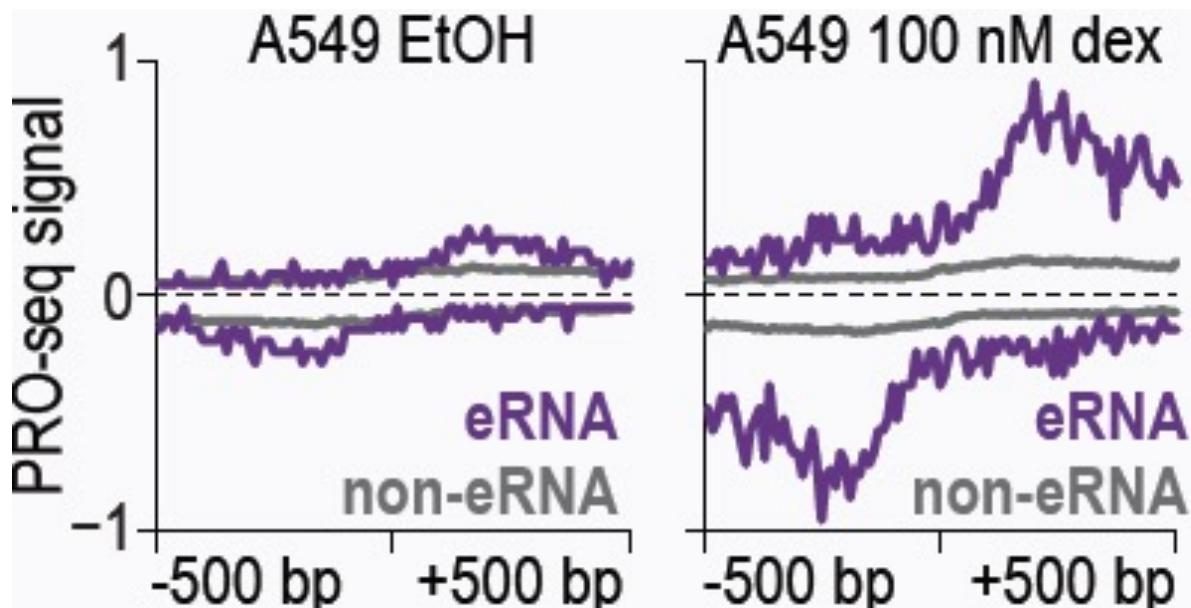
- CEBP and FOXA are reported to increase chromatin accessibility
- They are specifically expressed in A549 cells (not U2OS)

# Dex-induced eRNAs are near dex-induced genes and conserved

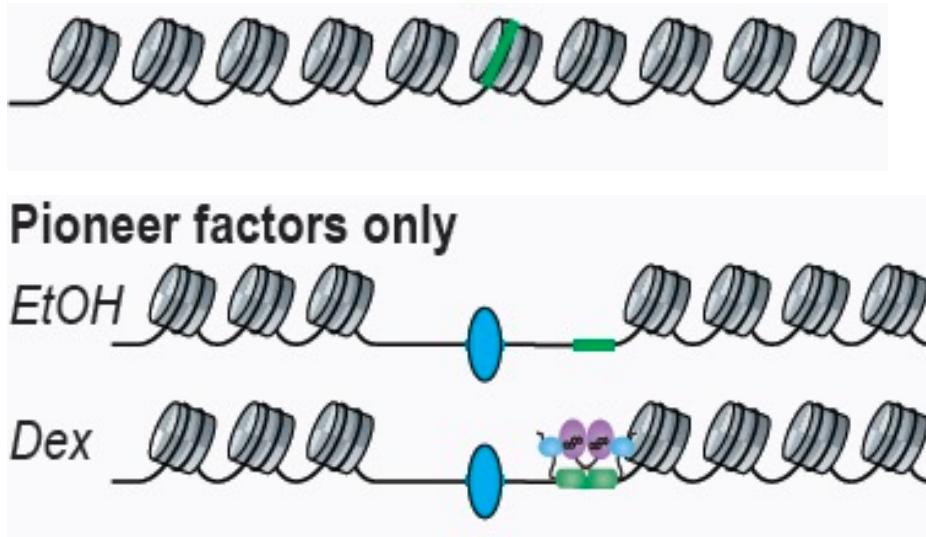


- GR binding sites that overlap eRNAs have features expected of enhancers

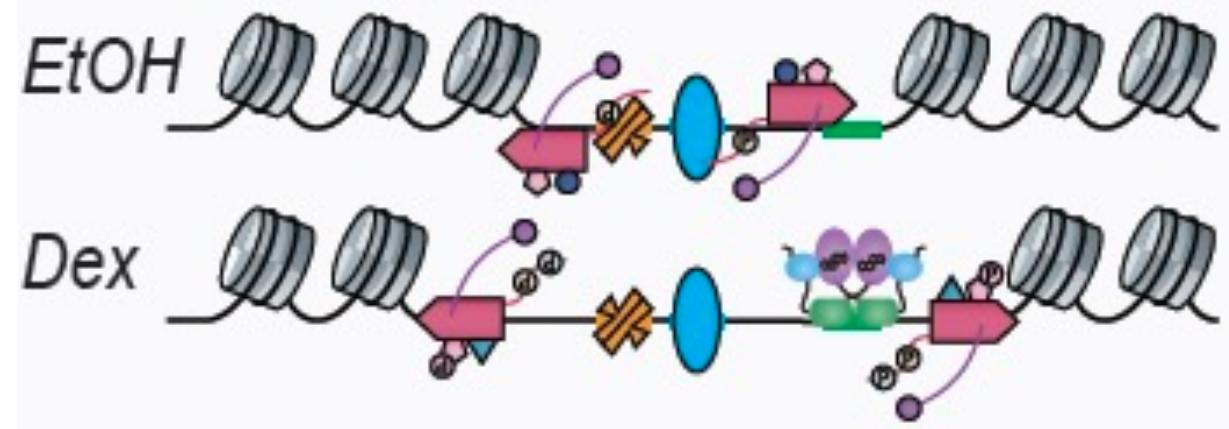
# Dex-induced eRNAs are lowly transcribed and bound by AP-1 in controls cells



# Model: different TFs play different roles in GR response



## Pioneer factors and AP-1



# Acknowledgements

John Lis and Lab



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