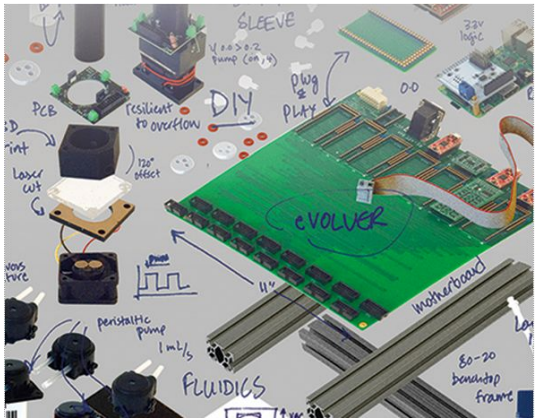
Measuring Kinetics and Dynamics of SynZiFTER 2.0 via PROseq

Nicholas White



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Background

Lab: Khalil Lab at BU

Project PI: Dr. Ahmad Khalil, Liz Tchantouridze

Synthetic Reconstitution of Complex Cellular Behavior

- “Can we build biological systems that recapitulate complex cellular functions like those seen in nature? Answering this question is the central goal of our research.”
- Develops tools of synthetic biology that allow construction of regulatory circuits inside living cells.
- Continuous evolution technologies that are automated and scalable, and applying these to generate biomolecules with radically altered or new functions

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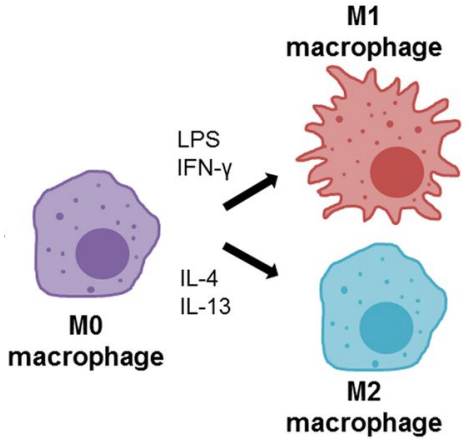
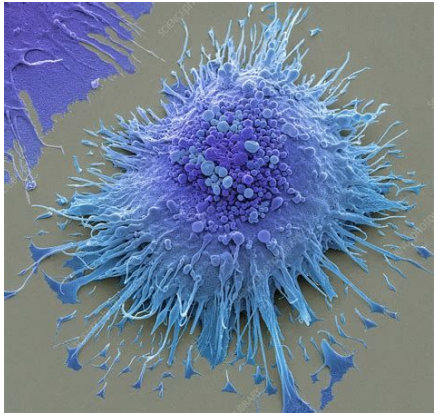
Macrophage Engineering

- Macrophages are immune cells, polarize to 2 subtypes
  - M1 are pro-inflammation and show anti-tumor activity
  - M2 treat anti-inflammatory diseases
- Cancer hijacks, and there are issues with current treatments of antibodies, AAV gene therapy, peptides and small molecule
- 3 tools developed for inducible polarization:
  - engineered STAT effector proteins that can drive macrophage polarization to M1 or M2 state
  - **synZiFTRs 2.0**, an optimized human gene regulation toolkit built upon our previous work, enabling drug-inducible and enhanced transgene expression with minimal basal activity
  - monocyte-targeting nanoparticles for cell-specific delivery of synZiFTR induction agents.





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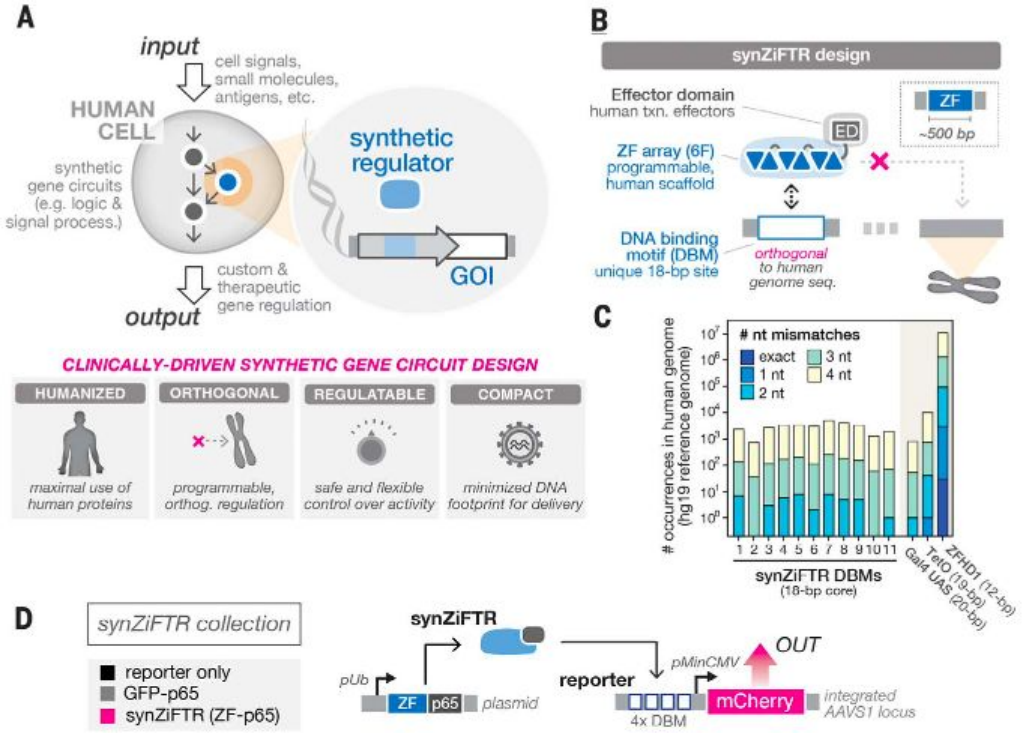
On / Off switch: Synthetic Zinc Fingers

- On/off switch enables more effective cell therapies
- CAR-M is like CAR-T, but aimed at solid tumors and still pre-clinical
- Necessary to have certain features
  - No “leakiness”
  - Low toxicity
  - Strong activation
  - Easily tunable



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Key Actors

- TAM = Tumor associated Macrophage
- STAT =
- TAD = Transactivation Domain
- TND = TFIIS N-terminal domain
- TIM = TND interacting domain
- p65 transactivation domain
- TIM2 subdomain binds to PPP1R10/(PNUTS)
    - PNUTS as a versatile transcriptional rheostat whose effects are highly context-dependent
    - TFIIS N-terminal domains (TNDs) and natively unstructured TND-interacting motifs (TIMs)
- Tox4

Key Actors

- TAM: Tumor associated Macrophage
- STAT: signal transducer and activator of transcription
- TAD: Transactivation Domain
- TND: TFIIS N-terminal domain
- TIM: TND interacting domain (unstructured region)
- p65 transactivation domain
- PPP1R10/(PNUTS) = versatile transcriptional rheostat whose effects are highly context-dependent
- IWS1: Elongation factor interacts with RNAP2

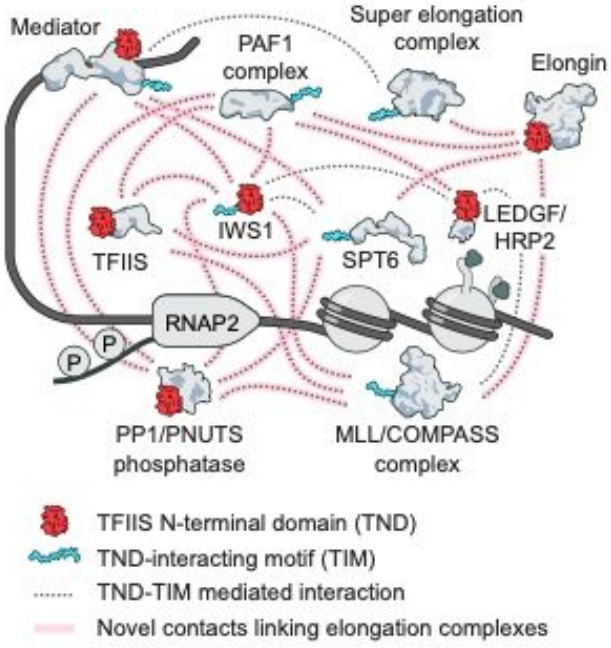

Fig. 6. Schematic of the TND-TIM module–driven interaction network. All TND-TIM interactions are represented by gray lines, and previously unknown TND-TIM interaction interfaces in the elongation machinery are highlighted in pink.

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Key Actors

- IWS1 TIMs -> (p65 + 2x TIMs) SynZFTR 2.0
- Better than traditional architecture, low basal exp and not toxic like viral
- Success comes from kinetic synergy between complementary activation mechanisms.
- conventional TADs like p65, NFZ and VPR primarily interact with initiation-focused complexes
- TIMs uniquely engages the elongation machinery through its interaction with PPP1R10 (PNUTS).
- This division of labor allows simultaneous enhancement of distinct transcriptional processes - initiation/remodeling by classical TADs and elongation efficiency by TIMs - resulting in multiplicative rather than merely additive effects on transcriptional output.
- The generalizability of this synergy is evidenced by TIMs' ability to enhance both p65 and NFZ, suggesting broad applicability to other non-elongation- focused TADs.

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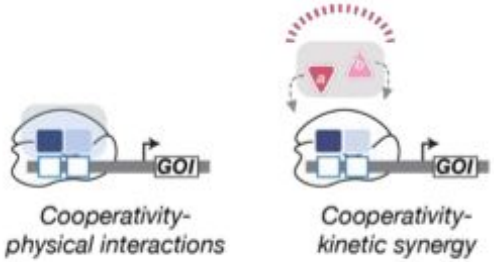
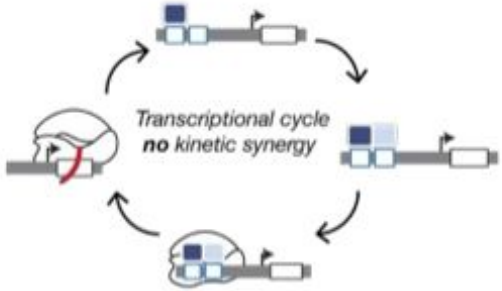
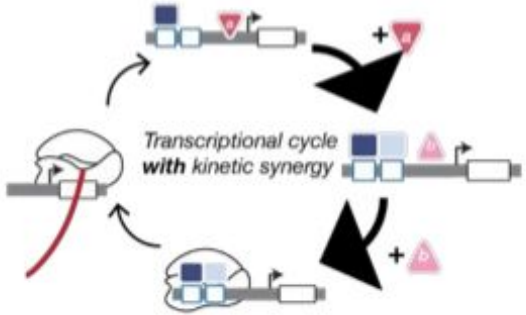
synZiFTR2.0: Is Kinetic Synergy Possible?

- Physical cooperativity for transcription factors breaks the circuit in annoying ways, need a better strategy
- Kinetic synergy is a less explored form of cooperativity that has only been tested in quantitative models
- Can two TFs can synergistically promote transcription by stimulating distinct steps in transcriptional cycle that are rate-limiting
- Targeted screen identified the **IWS1** TIMs domain as a p65 synergist
- Short sequence so you can stack multiples
- Relay race metaphor

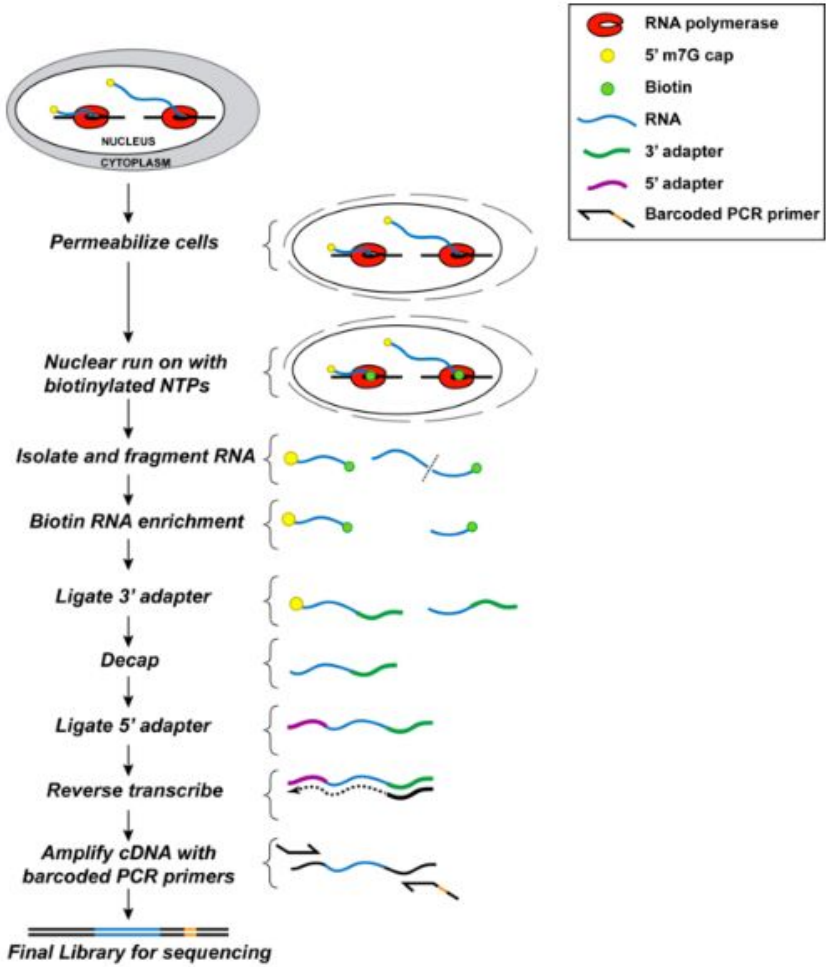






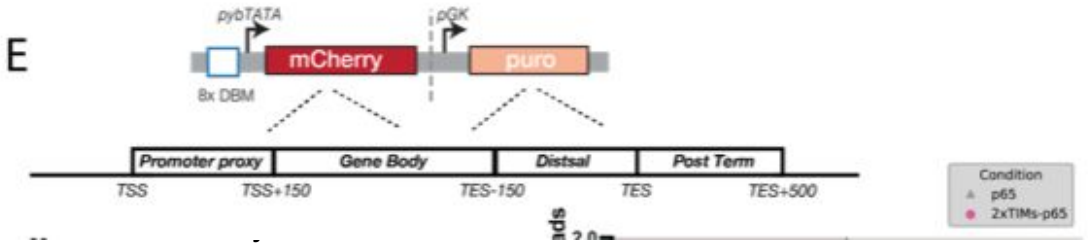
Precision Run On Sequencing

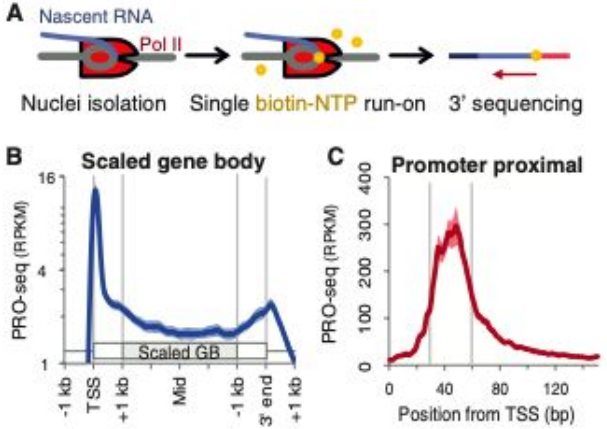
- Cells are permeabilized, followed by a nuclear run-on assay
- Engaged RNA polymerase complexes incorporate a biotinylated nucleotide into the nascent RNA chain
- Labeled RNAs are enriched using streptavidin beads and sequencing of the final libraries reveals RNA 3’ ends at single nucleotide resolution.



Precision Run On Sequencing

- PRO-Seq illuminates process of elongation: RNAP2 activities such as pausing, pause release, RNA synthesis and termination are rate-related and time-sensitive
- PRO-Seq is particularly suitable to gain insights in TIMs' effects on not only stimulating transcriptional activity, but also mediating the kinetics of different stages of elongation
- Quantifying the average counts of 3' end reads in different regions on the gene body, such as promoter proximal, gene body, distal and post termination
- Normalize the average counts of 3' end reads in each region with that of the gene body.
- TIMs should be able to facilitate efficient elongation by increasing RNAP2 activity level by modulating RNAP2 dynamics such as post-termination clearance

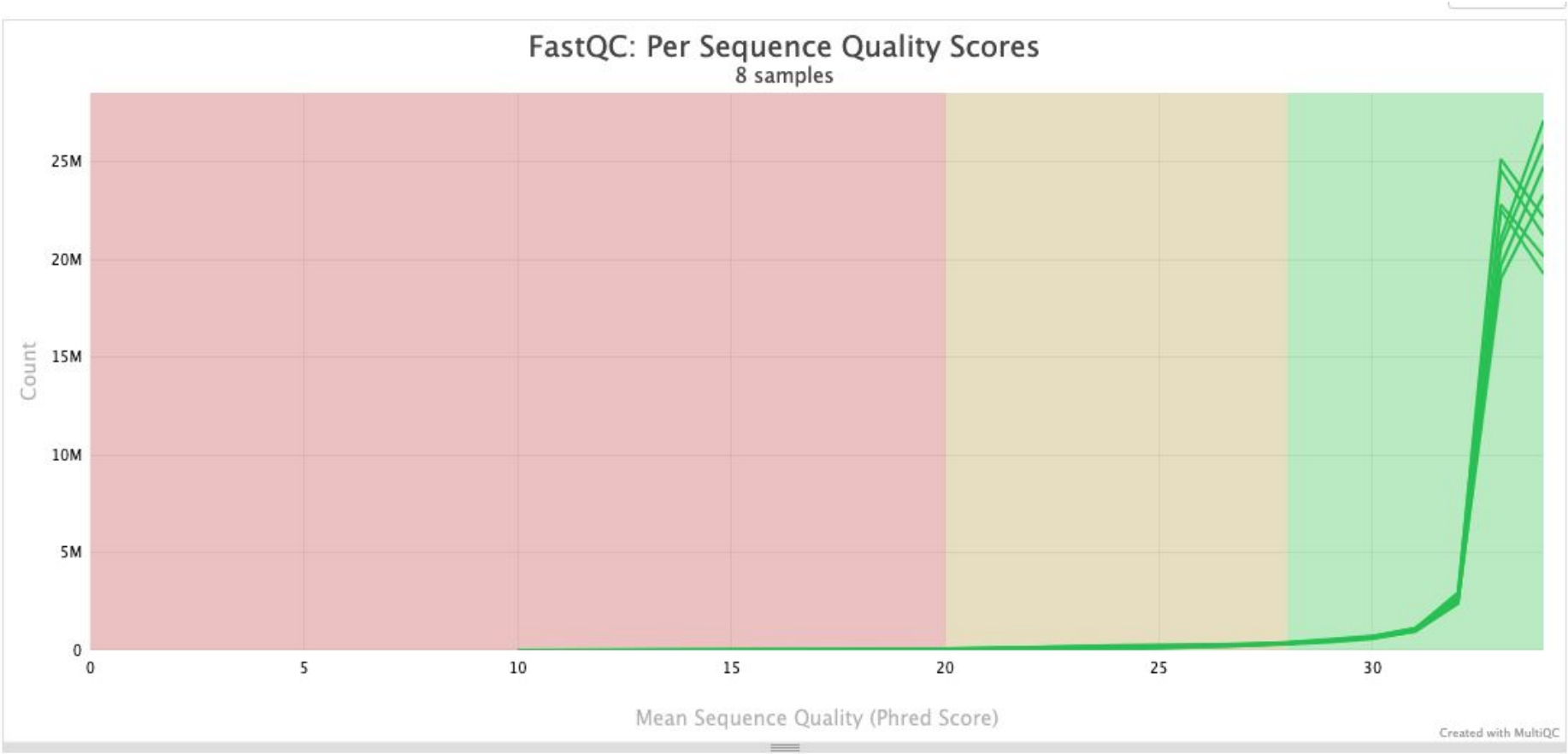




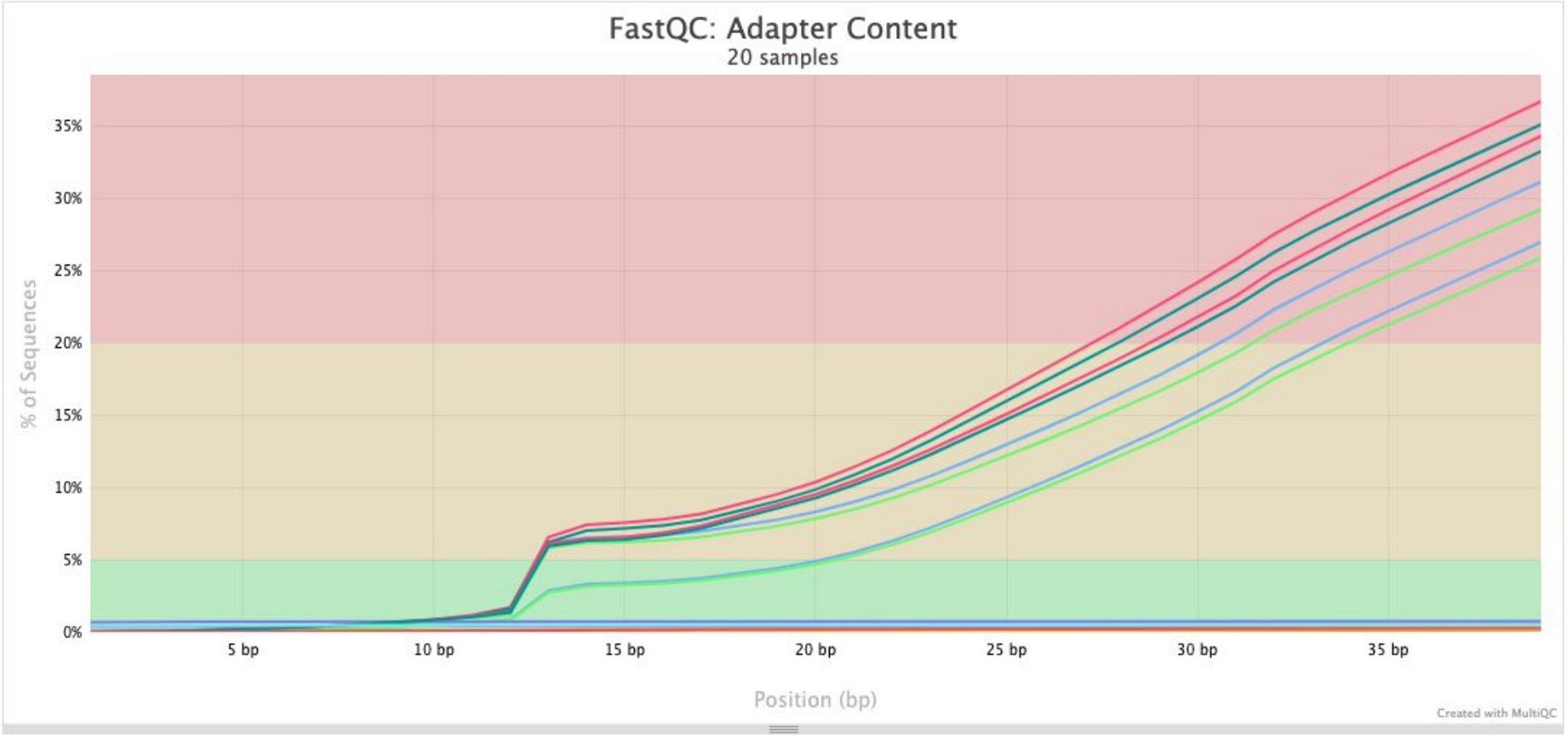
Project Plan

1. Create PROseq pipeline with snakemake
2. Identify 20% reads not human (mystery reads identification)
3. Adaptor sequences cleanup
4. Check differences in expression between samples
5. Align to the plasmid and make bedfiles
6. Differences on the reporter expression
7. Proseq for elongation, dynamics (RNAP2 pause and release), is there more pausing?
8. Calculate density in nucleotide windows

Initial Results



Initial Results



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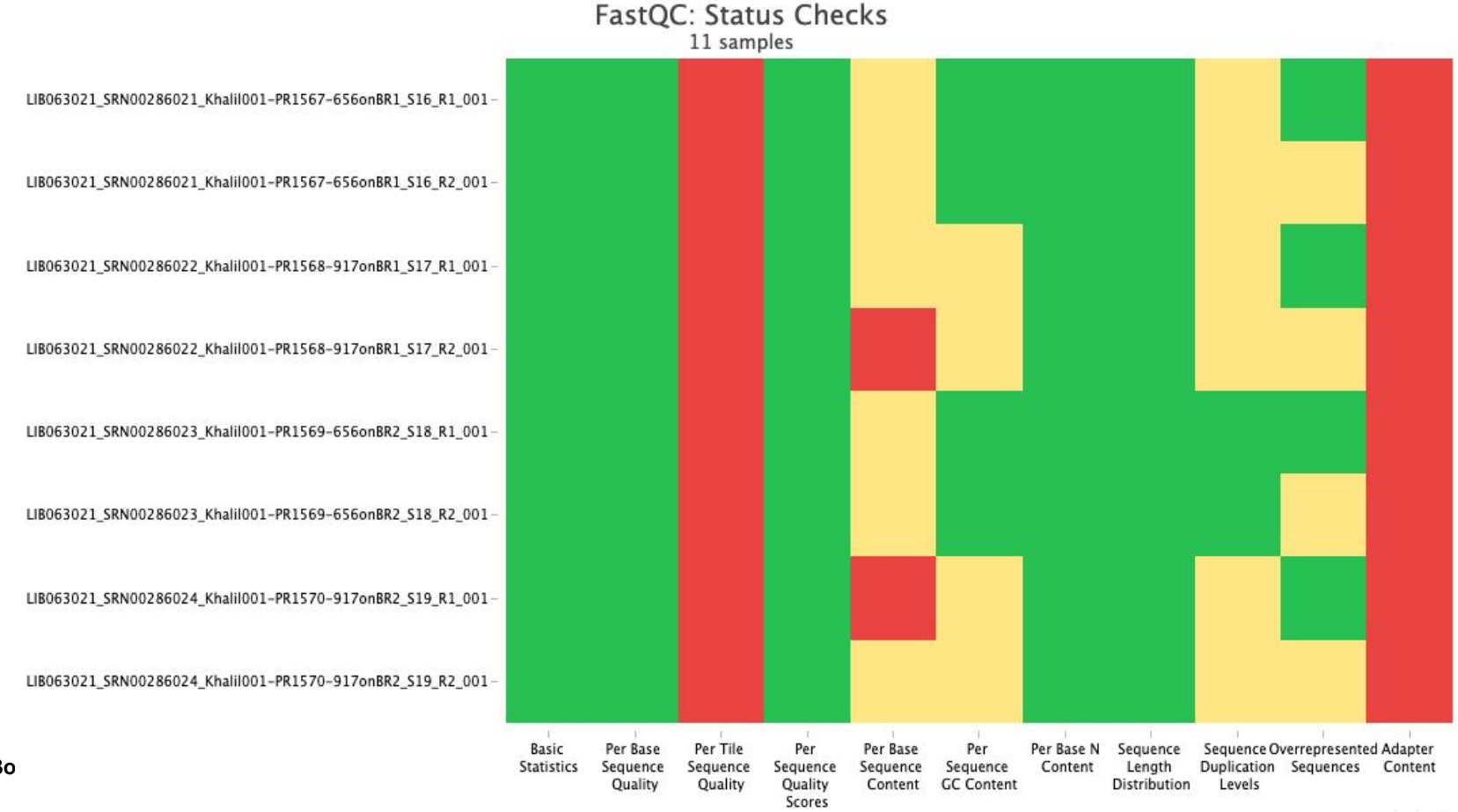

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THANK YOU

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4. A ubiquitous disordered protein interaction module orchestrates transcription elongation
5. Co-transcriptional splicing regulates 30 end cleavage during mammalian erythropoiesis
6. Precise Maps of RNA Polymerase Reveal How Promoters Direct Initiation and Pausing

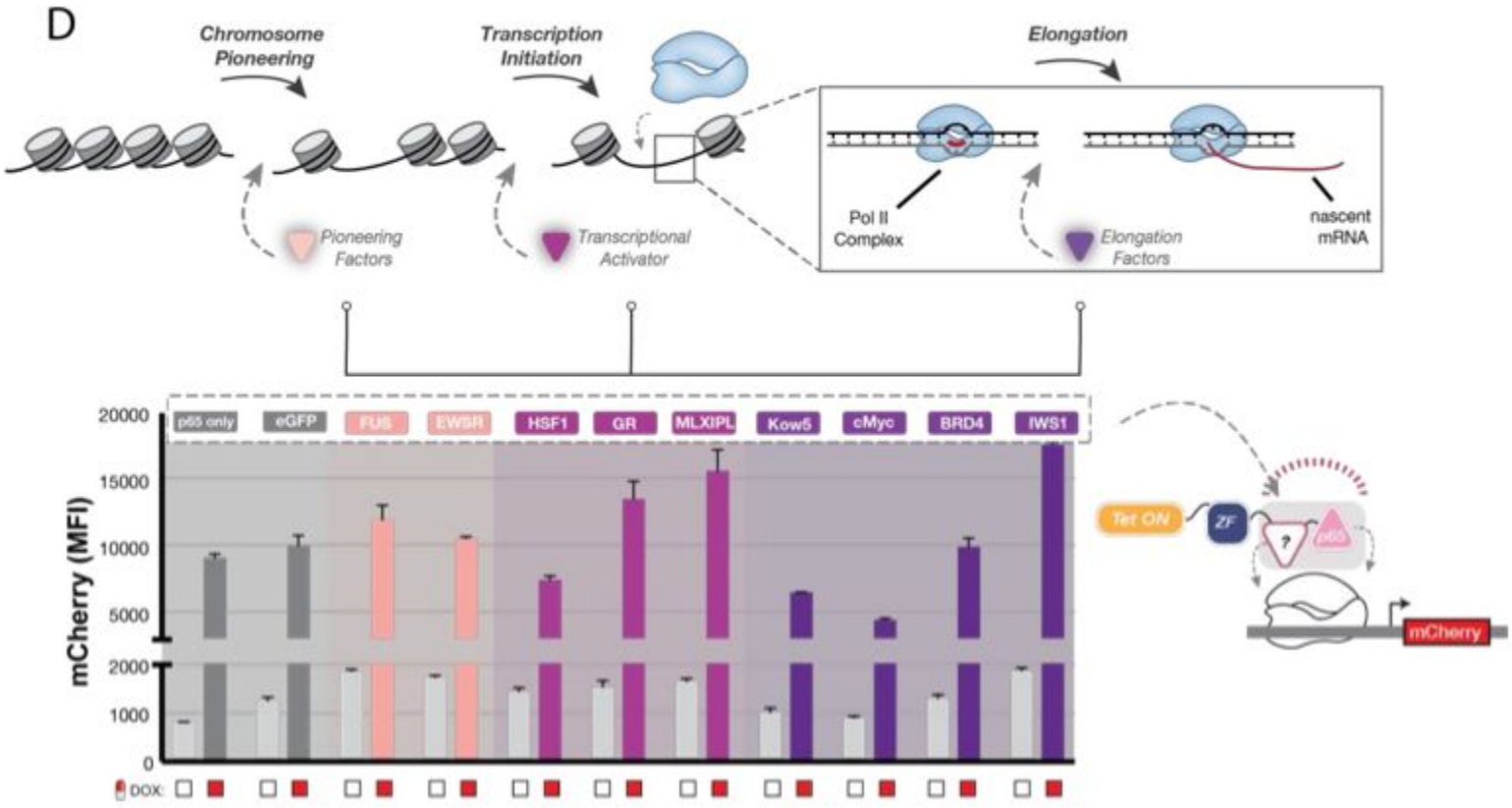
Background

Methods/Plan 1-2

Results

- shitty 20% reads not human (mystery reads identification)
- adaptor sequences cleanup
- Differences in expression between samples (shouldn't be but good to check)
- recap by aligning to the plasmid and make bedfiles
- Differences on the reporter expression (interesting and useful)
  - Do a statistical analysis on them
- Proseq for elongation, dynamics (RNA pol pause and release), more pausing? Calc density in nucleotide windows? papers
- Clean, align, improve align, mystery reads
- BDC 4:30 June 25th

Background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Chromosome Pioneering
Transcription Initiation
Elongation
Pioneering Factors
Transcriptional Activator
Pol II Complex
Elongation Factors
nascent mRNA
mCherry (MFI)
20000
15000
10000
5000
2000
1000
0
p65 only
eGFP
FUS
EWSR
HSF1
GR
MLXIPL
Kow5
cMyc
BRD4
IWS1
DOX:
Tet ON
ZF
?
p65
mCherry