BROAD INSTITUTE

Mapping and exploring the functions of N^6-methyladenosine in mRNA

Kayla Lee
Summer Research Program in Genomics 2012

RNA and Cell Regulation

- The roles of RNA within a cell include the regulation of genes and the synthesis of proteins

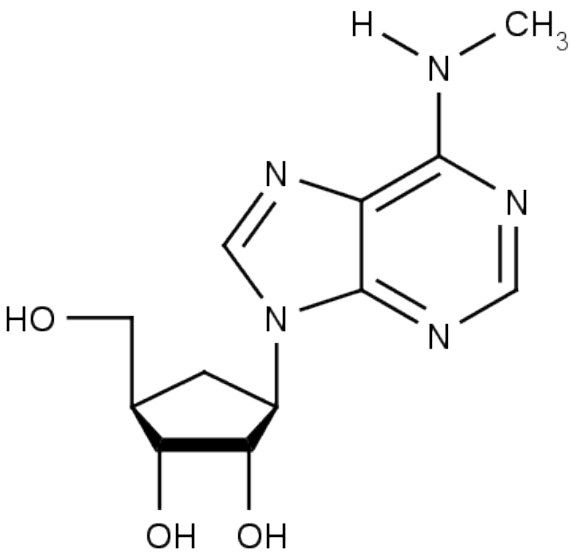
- Post transcriptional modifications:
  - 5' capping
  - 3' polyadenylation
  - RNA splicing
  - Base modifications

- Limited amounts of hypotheses and analytical methods leave many of these modifications uncharacterized

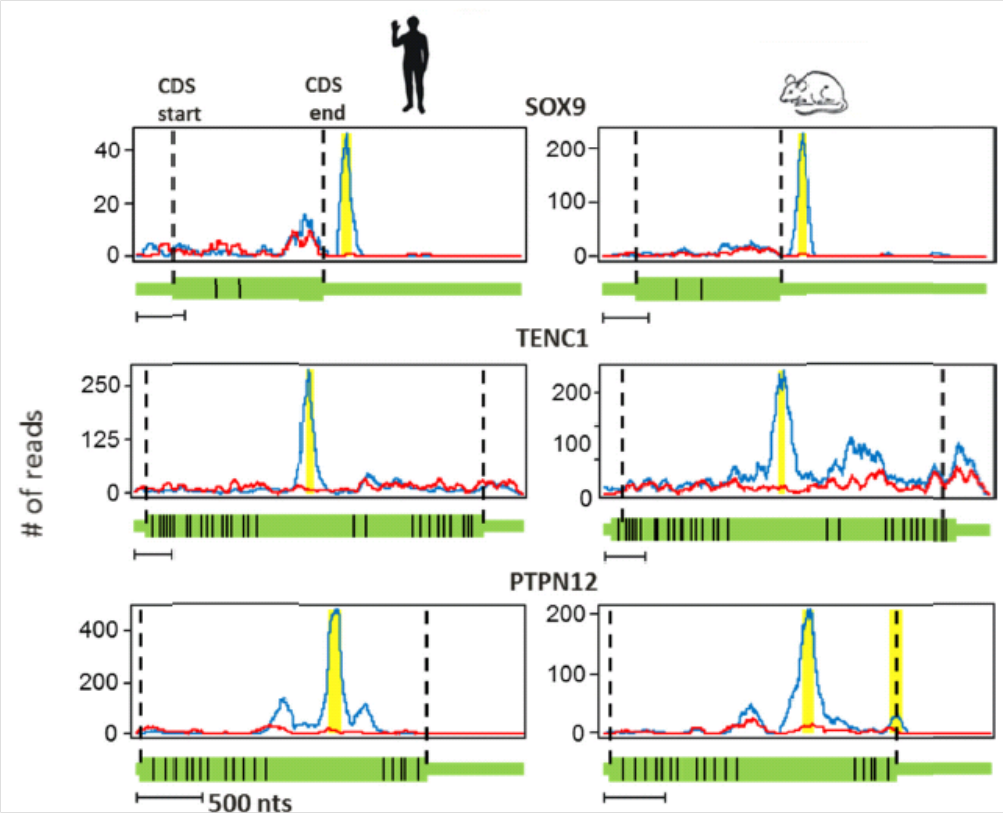
N^6-methyladenosine (m^6A)

- Most common, internal base modification on eukaryotic messenger RNA (mRNA)

- Occurs on almost 50% of expressed transcripts within the consensus motif *RR**A**CH*
  - where *R*=purine, *A*= m^6A, and *H*=A, C, or U



m^{6}A is highly conserved



Highly conserved between mouse and human genomes and strongly enriched in long exons and near stop codons

Dominissini, Moshitch-Moshkovitz, Schwartz *et al.* (2012) Nat.

Phenotypic observations suggest regulatory role

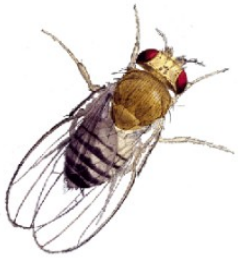
- Catalyzed by a multi-component conserved enzyme
  - Only known subunit: methyltransferase like 3 (METTL3)

- Silencing of METTL3 leads to:
  - Apoptosis in *Homo sapiens*
  - Impaired gametogenesis in *S. cerevisiae* and *D. melanogaster*

How can we elucidate the functions of m^{6}A?

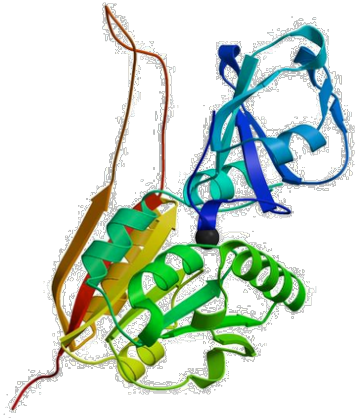
Objectives

Map m^{6}A in selected model systems



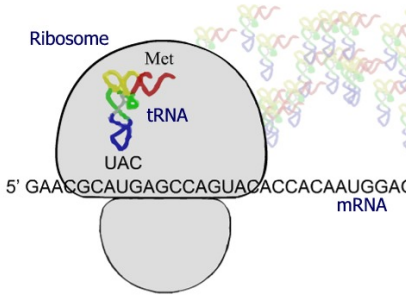
Understand genome wide trends

Pull down associated proteins



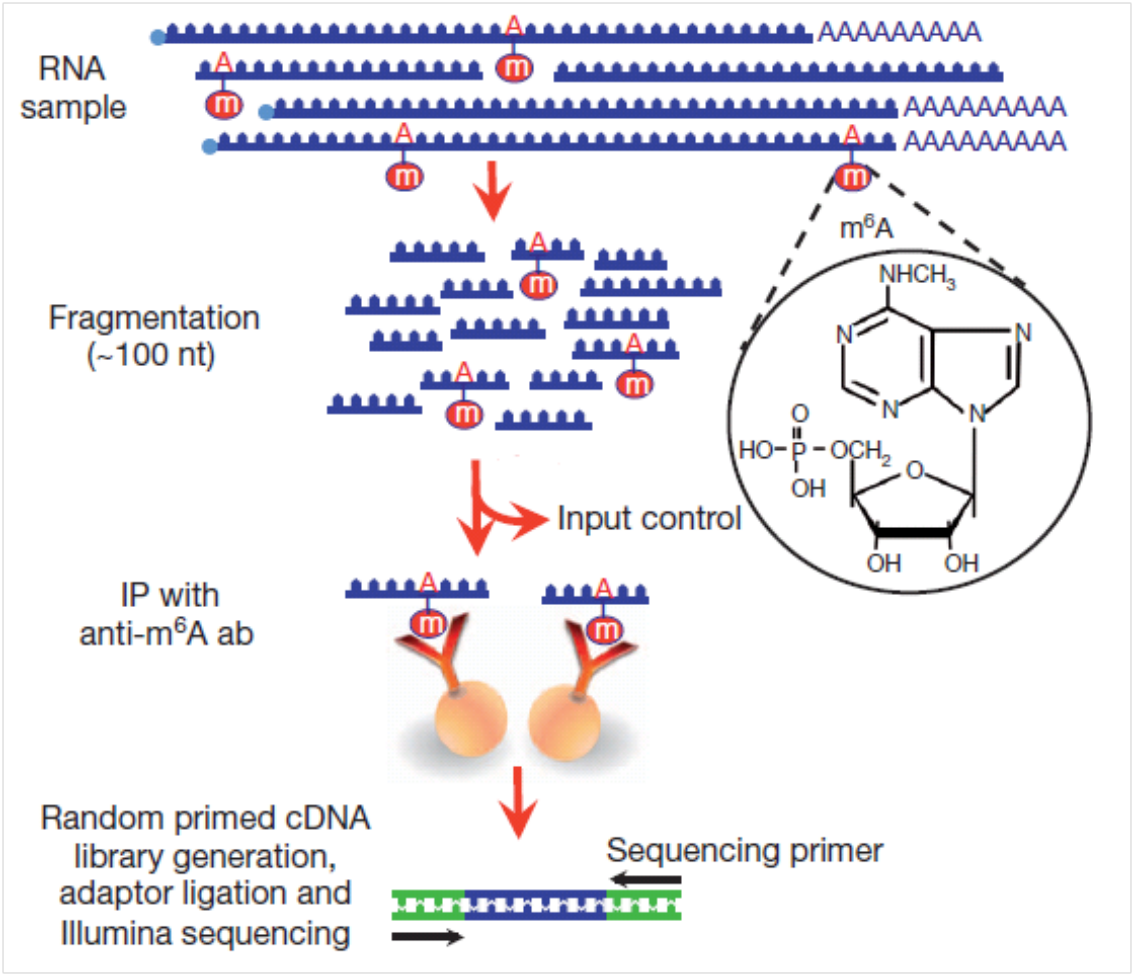
Identify unknown components recognizing this modification

Understand m^{6}A in the life cycle of RNA



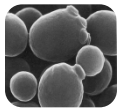
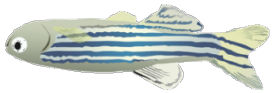
How does methylation affect cellular processing?

Mapping of m^{6}A



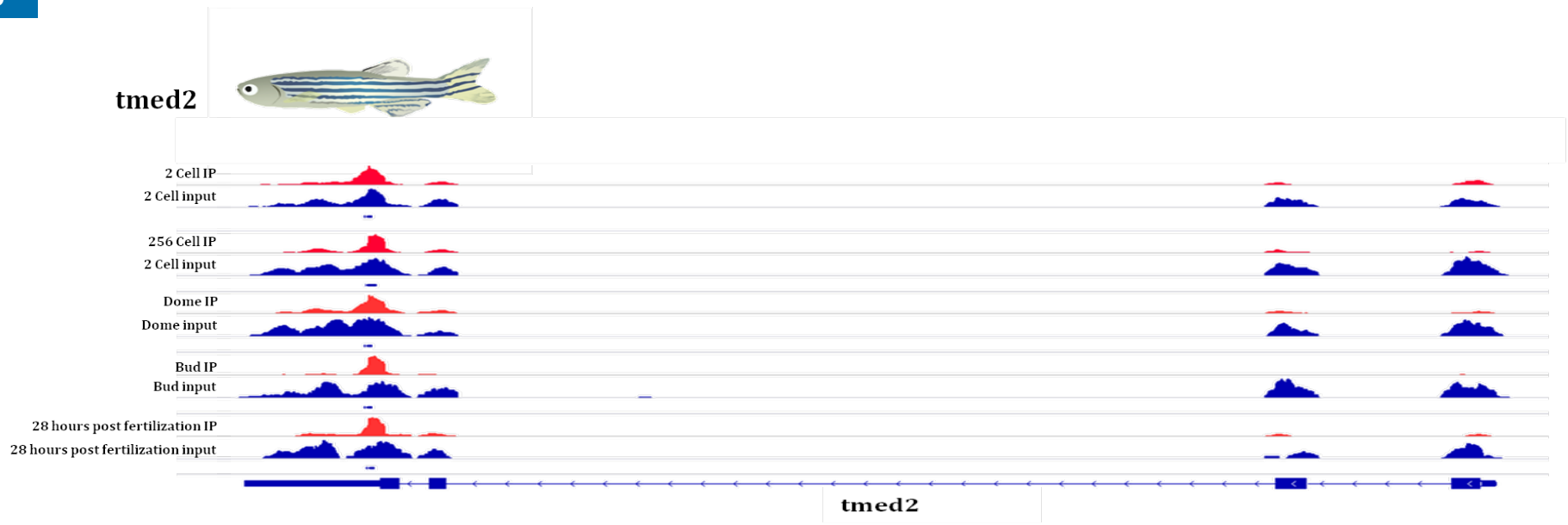
Dominissini, Moshitch-Moshkovitz, Schwartz *et al.* (2012) Nat.

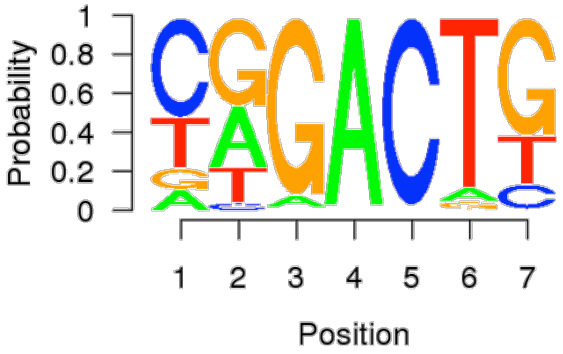
Intriguing m^{6}A observations in model systems

Selected organisms	Samples	Purpose
Yeast *S. cerevisiae* 	*ime4Δ*/*ime4Δ* and wild type	IME4 required for induction of meiosis; increased methylation during sporulation
Fruit fly *D. melanogaster* 	Ovary and body tissues	IME4 homolog expressed in ovaries and testes; *ime4Δ* has fused-egg chambers
Zebrafish *D. rerio* 	Developmental time points	Decrease in METTL3 throughout embryonic development

Locate trends on genome-wide m^{6}A maps

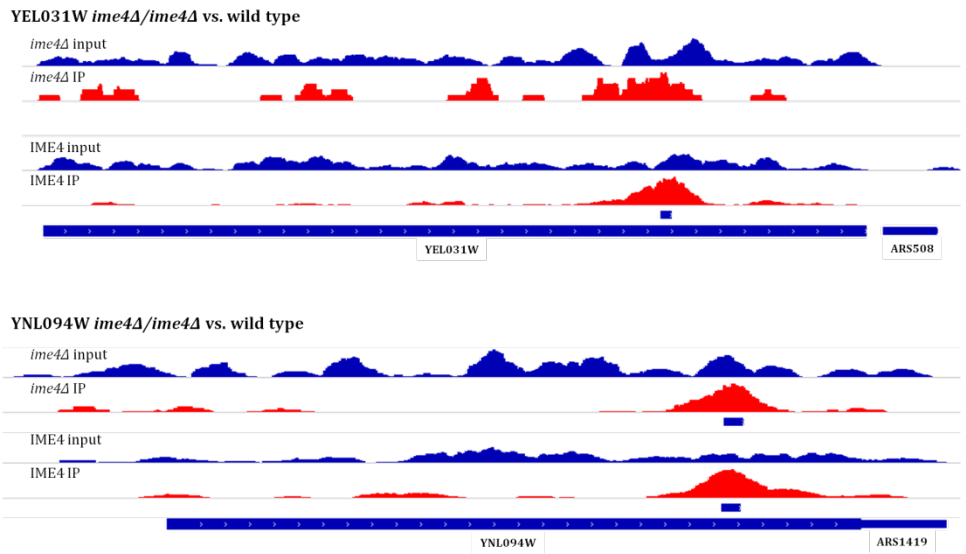
Zebrafish m^{6}A enrichment show similar conservation to human and mouse genomes



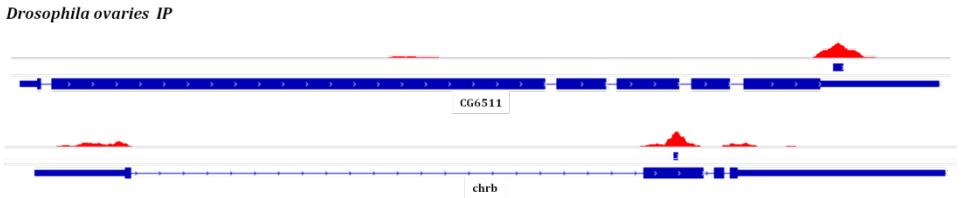


D. melanogastor and *S. cerevisiae* SK1 show signs of enrichment

- *Drosophila* and *S. cerevisiae* show distinctive enrichment peaks throughout the genome
- Does not follow the consensus motif
- Data is currently being replicated



S. cerevisiae SK1

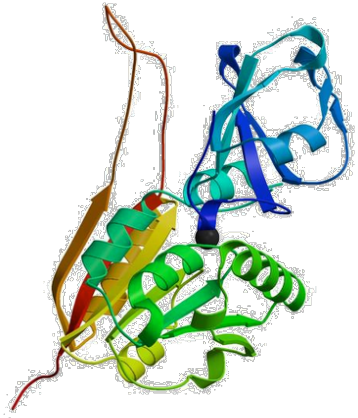


Objectives

Map m^{6}A in selected model systems

Understand genome wide trends

Pull down associated proteins



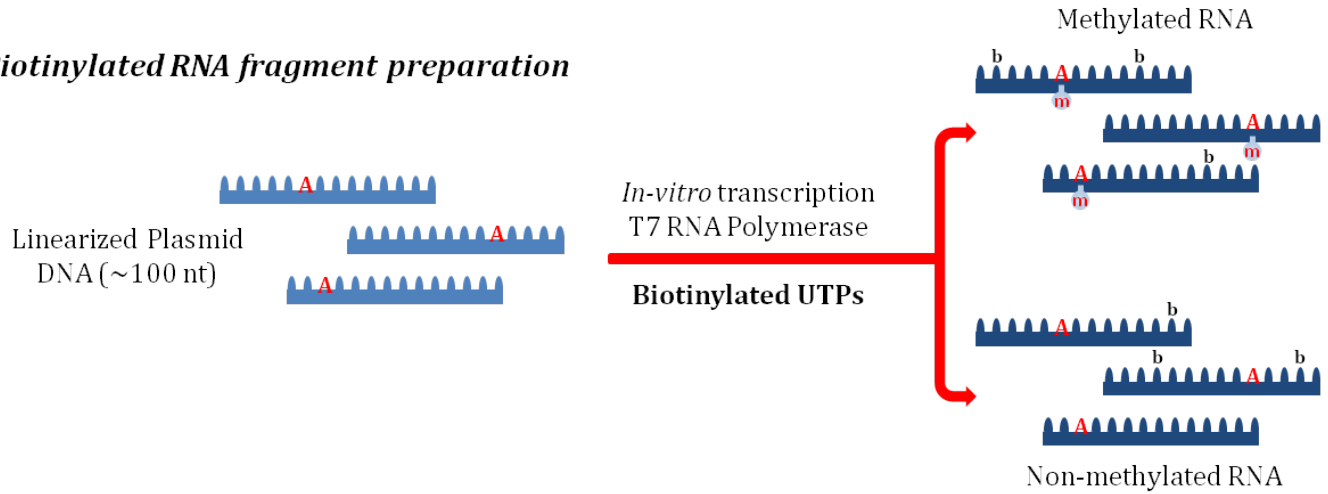
Identify unknown components recognizing this modification

Understand m^{6}A in the life cycle of RNA

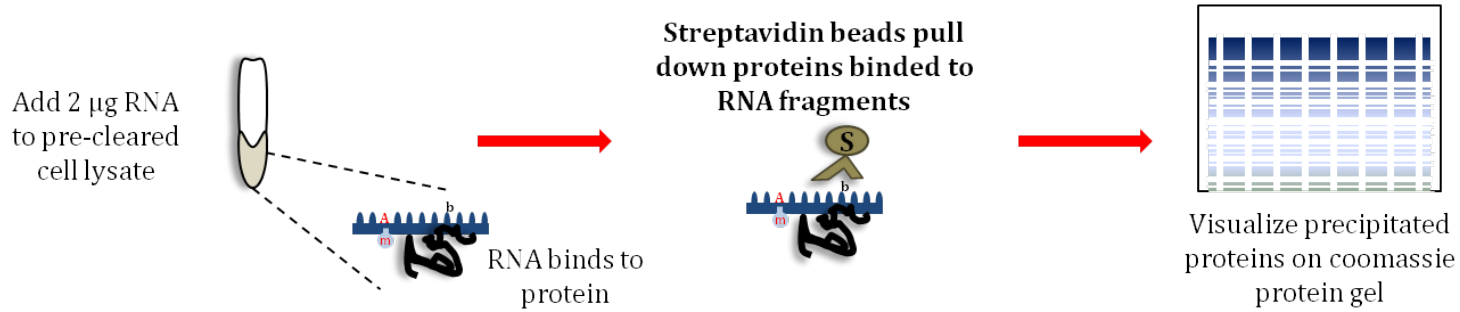
How does methylation affect cellular processing?

Pulling down associated proteins

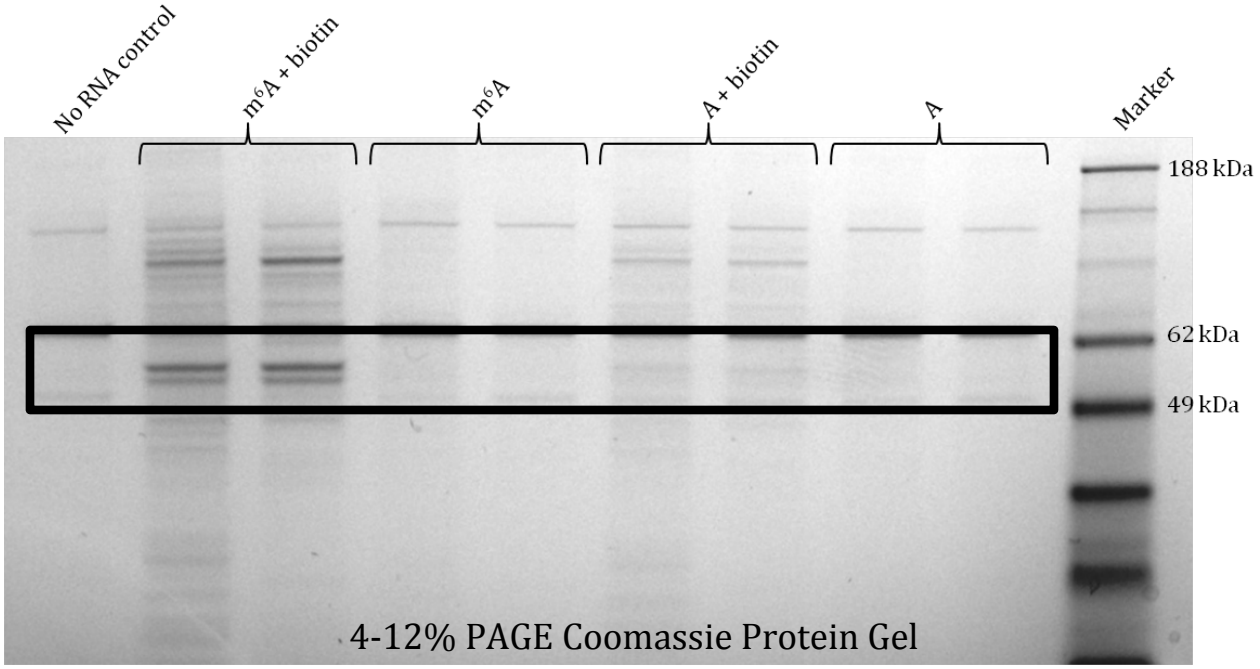
I. Biotinylated RNA fragment preparation



II. Protein pull down



Biotin-methylated RNA show unique protein bands



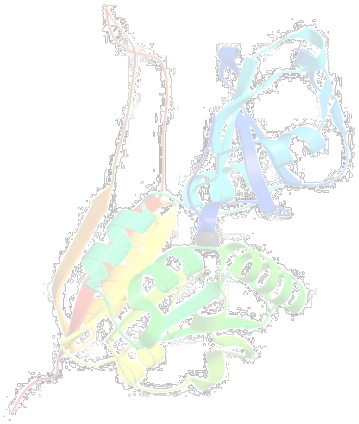
- Two distinct protein bands are observed in m^6A + biotin lanes
  - Estimated size: ~49 - 62 kDa

Objectives

Map m^{6}A in selected model systems

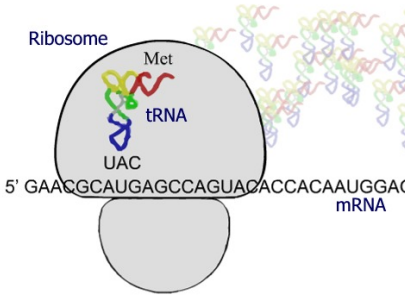
Understand genome wide trends

Pull down associated proteins



Identify unknown components recognizing this modification

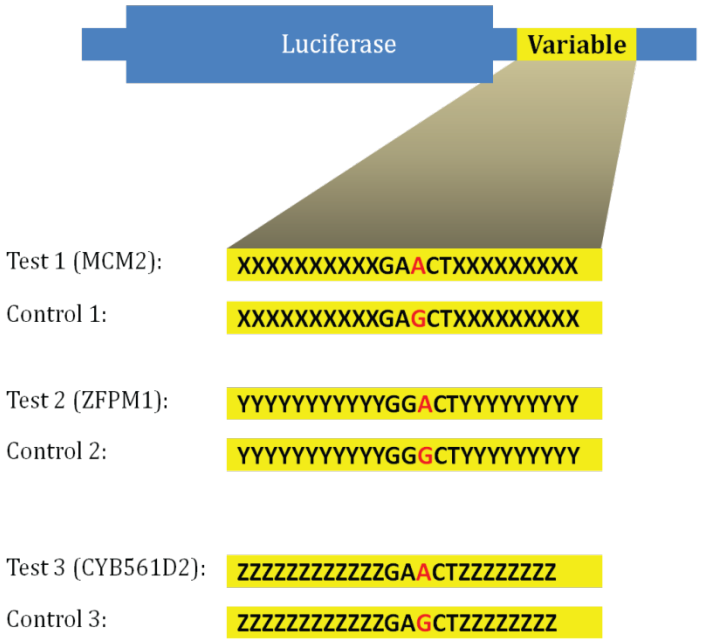
Understand m^{6}A in the life cycle of RNA

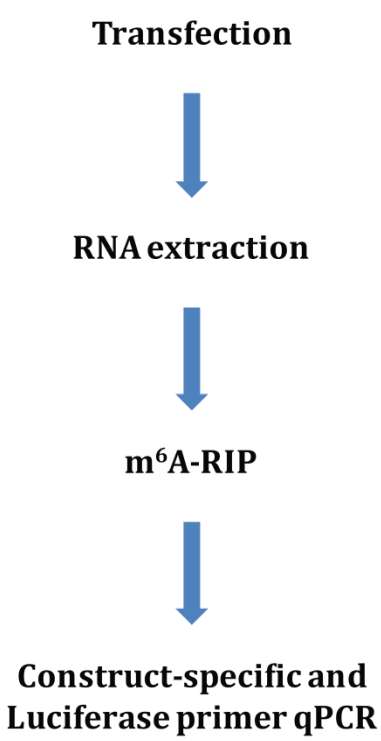


How does methylation affect cellular processing?

Constructs designed to eliminate consensus sites followed by qPCR

Construct design with endogenous methylation site:





Conclusions/Future Directions

- *S. cerevisiae* show distinct peaks of enrichment with a strong tendency to occur at the 3’ end of genes
  - Replicate m^6A-RIP and continue computational analysis of mapped organisms
- Zebrafish data provides a developmental model of methylation enrichment that shows similar conservation to human and mouse genomes
- Protein mass spectrometry of potential candidates as identified by pull down
- Ensure the constructs and their mutant strains design a system that can selectively methylate

Acknowledgments

Regev and Lander Groups

Aviv Regev
Eric Lander
Schragi Schwartz
Ivo Wortman
Dima Ter-Ovanesyan
Dawn Thompson

Diversity Initiative

Bruce Birren
Eboney Smith
Francie Latour
Brandon Ogbunu

Summer Research Program in Genomics

Broad Institute

BROAD INSTITUTE