Integrate RNA-seq and eCLIP-seq for mining regulation functions of RNA binding proteins

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Alternative Post-transcriptional Regulation in simple form

Alternative splicing, initialization, and termination from computational perspective
Motivating example using SRSF1

SRSF1 binding to cassette exon promotes inclusion.

Differentially used exon.
Alternative Post-transcriptional Regulations

- **Skipped exon**
- **Retained intron**
- **Alternative 1st exon**
- **Alternative transcription promoter**
- **Alternative 3’ SS**
- **Alternative 5’ SS**
- **Alternative last exon**
- **Alternative Polyadenylation**

**Abbreviations:**
- AFE: Alternative 1st exon
- A5SS: Alternative 5’ SS
- A3SS: Alternative 3’ SS
- APA: Alternative Polyadenylation
- RI: Retained intron
- SE: Skipped exon
Look at those events systematically? Use the SURF.

We developed the Statistical Utilities for RBP Functions (SURF) to learn RBP regulatory functions.
The intuition behind the SURF

- Hypothesis testing
The SURF pipeline

WT RNA-seq

KD RNA-seq

AR event

eCLIP-seq

REUC = \frac{K_1}{K_0}

WT 5/19

KD 2/13

Differential Regulation

Phenotype

Regulation effect

inclusion

exclusion

SURF Dataset

Extract Features

Signal / Count
PUM2 regulating functions from the SURF

- Promotes exclusion of APA, binding to the body or upstream
- Promotes inclusion of SE, binding to exonic regions
PUM2 leads to exclusion of APA site

• One example out of the 715 APA events
SURF is scalable, so we can study many RBPs.

A systematical analysis to the ENCODE consortium.
ENCODE is generating lots of these data

105 RBPs (K562 cell)

WT RNA-seq (Control)

KD RNA-seq

eCLIP-seq
Profiling 68 RBPs in alternative regulations

- RBPs typically have impacts on multiple AR events
- Individual RBP is involved in different types of AR events
Inferenced regulatory functions of 68 RBPs

Sanity checks

HNRNPC Regulating SE
How about RBP relationships?

An example of CPSF6 in regulating APA
CPSF6 has dual functions in regulating APA

CPSF6 Regulating APA

- inclusion supported by 1301 APA events
- exclusion supported by 580 APA events
Pairwise screening indicates an association between CPSF6 and PUM2.

Association Testing

Numerical validation

log10(p value)

Factors

CPSF6

RBP 1

RBP 2

RBP n

Panel

w/ PUM2

w/o PUM2

Average log2 (w/ PUM2 - w/o PUM2) vs. normalized eCLIP signal cutoffs
The inclusion function of CPSF6 associates with PUM2

- PUM2 is enriched in APA where CPSF6 promotes inclusion
- Supported by 693 APA events
Summary

The SURF
• Utilizes abundant AR events to study RBP.
• Inferences RBP’s regulatory functions.
• Screens for potential RBP interactions.

Thank you!

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Open to questions.