Profiling Nascent Transcription using Bru-seq

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University of Michigan
OUTLINE
1. Description of Bru-seq technologies
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2. Dynamics of transcriptional regulation
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3. RNA processing
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4. Mapping active enhancers
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Bru-seq technology platform

1) Label nascent RNA* in cells with bromouridine
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2) Isolate Bru-RNA from total RNA using magnetic beads conjugated to anti-BrdU antibodies
Bru-seq technology platform

1) Label nascent RNA* in cells with bromouridine

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3) Prepare cDNA libraries from the isolated Bru-RNA and perform deep sequencing
Bru-seq technology platform

1) Label nascent RNA* in cells with bromouridine

2) Isolate Bru-RNA from total RNA using magnetic beads conjugated to anti-BrdU antibodies

3) Prepare cDNA libraries from the isolated Bru-RNA and perform deep sequencing

4) Analyze the data using the Bru-seq analysis pipeline and perform bioinformatics
Bru-seq technologies
Bru-seq technologies

Bru-seq
Rates of RNA synthesis
**Bru-seq technologies**

**Bru-seq**
Rates of RNA synthesis

**BruChase-seq**
RNA processing
RNA stability
Bru-seq technologies

**Bru-seq**
Rates of RNA synthesis

**BruChase-seq**
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RNA stability

**BruUV-seq**
Mapping active enhancers
Rates of transcription elongation

Bru-seq
Rates of RNA synthesis

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RNA processing
RNA stability

BruUV-seq
Mapping active enhancers

BruDRB-seq
Rates of transcription elongation

Figure 1. Transcription elongation rates measured genome-wide using BruDRB-seq. (10 min) Appearance of a nascent transcription wave at the 5' end of genes during a 10-min recovery after DRB removal (10-min Bru labeling). (0 min) Following a 60-min DRB treatment of the cultured cells with DRB to arrest RNAPII at promoter-proximal sites, the drug was washed out, expected for nascent RNA, the signal was fairly evenly distributed throughout the first 50 kb of these genes. Following a 60-min recovery period. Cells were lysed in TRIzol, and total RNA is labeled with bromouridine (Bru), isolated with anti-BrdU antibodies conjugated to magnetic beads. The captured Bru-labeled RNA was then reverse-transcribed, and the resulting cDNA library was subjected to deep sequencing using anti-BrdU antibodies, and subjected to deep sequencing. By measuring the length in the diploid human fibroblast cell line HF1 are represented by median normalized expression (an aggregate view). As genes in HF1 cells ordered by elongation rate for a 10-min recovery following DRB removal are shown. Examples of transcriptional recovery in individual genes after 0-, 10-, and 20-min recovery after DRB removal in HF1 cells are shown in (A) Aggregate view of nascent RNA reads through the first 50 kb of the transcription start site (TSS), (B) Aggregate view of BruDRB-seq (10-min recovery) showing the upstream region of TSS having a low signal (A), advancing wave (B), and region downstream from the advancing wave with low signal (C). (D) A hidden Markov model was developed to identify advancing waves and enhancers. Normalized signals are presented by median normalized expression (an aggregate view). As genes in HF1 cells ordered by elongation rate for a 10-min recovery following DRB removal are shown. Examples of transcriptional recovery in individual genes after 0-, 10-, and 20-min recovery after DRB removal in HF1 cells are shown in (A) Aggregate view of nascent RNA reads through the first 50 kb of the transcription start site (TSS), (B) Aggregate view of BruDRB-seq (10-min recovery) showing the upstream region of TSS having a low signal (A), advancing wave (B), and region downstream from the advancing wave with low signal (C).
OUTLINE

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transcriptional regulation of the acute serum response
transcriptional regulation of the acute serum response

serum starve → serum stimulate

48 h

Bru-seq
transcriptional regulation of the acute serum response

serum starve

48 h

serum stimulate

Bru-seq

1. 30 min Bru starved
   serum collect

2. 0-30 min
   serum collect

3. 30 min
   30-60 min
   serum collect

4. 60 min
   60-90 min
   serum collect

5. 90 min
   90-120 min
   serum collect
TPM1

starved

0-30 min

30-60 min

60-90 min

90-120 min

actin cytoskeleton

log2 fold change

TPM1

time in serum (min)

starved

30

60

90

120

4

3

2

1

0

-1

-2

-3

-4

-5

100.00 MB

chr15

102.531 Mb

chr15 coordinate (Mb)

TPM1

−150

−50

50

150

−150

−50

50

150

−150

−50

50

150

−150

−50

50

150
**TPM1**

chr15

<table>
<thead>
<tr>
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<th>time in serum (min)</th>
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<tbody>
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<td>0-30 min</td>
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<tr>
<td>TPM1</td>
<td>[chart showing expression levels over time]</td>
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</tbody>
</table>

Log2 fold change vs. time in serum (min):

- [chart showing log2 fold change for TPM1]

**FERMT2**

chr14

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<td>[chart showing expression levels over time]</td>
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Log2 fold change vs. time in serum (min):

- [chart showing log2 fold change for FERMT2]

Actin cytoskeleton vs. scaffolding protein:

- [chart comparing actin cytoskeleton and scaffolding protein expression]
### TPM1
**chr15**
102.531 Mb

**log2 fold change**

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<td>90-120</td>
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</tbody>
</table>

#### Actin Cytoskeleton

- **log2 fold change**
  - 0-30 min
  - 30-60 min
  - 60-90 min
  - 90-120 min

#### Scaffold Protein

- **log2 fold change**
  - 0-30 min
  - 30-60 min
  - 60-90 min
  - 90-120 min

### FERMT2
**chr14**
107.35 Mb

**log2 fold change**

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<td>90-120</td>
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#### Scaffolding Protein

- **log2 fold change**
  - 0-30 min
  - 30-60 min
  - 60-90 min
  - 90-120 min

### APCDD1
**chr18**
78.077 Mb

**log2 fold change**

<table>
<thead>
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<th>Time (min)</th>
<th>Starved</th>
<th>0-30</th>
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<th>60-90</th>
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<td>90-120</td>
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</table>

#### Inhibitor of WNT

- **log2 fold change**
  - 0-30 min
  - 30-60 min
  - 60-90 min
  - 90-120 min
L0g2-fold

starve 30 min 60 min 90 min 120 min

GADD45B ID3 EGR2 SIK1 ID1 DUSP2 ACTC1 FOXD1 GREM1

"sustained induction"
a. Heatmap showing gene expression levels over time.

b. Graph showing sustained induction over time with gene names indicated.

c. Graph showing induction reset over time with gene names indicated.

d. Graph showing delayed induction over time with gene names indicated.

Gene names include: GADD45B, ID3, EGR2, SIK1, ID1, DUSP2, ACTC1, FOXD1, GREM1, USP36, ZNF548, MTHFD1L, KDM3A, JAG1, STX12, HSPA2, GZF1, NFIL3, DOT1L, BMPR2, HECTD2, BNC1, ADAM19, KIF21A, OLFM2, CD9, EML1.

Key:
- Sustained induction
- Induction reset
- Delayed induction
A study on gene expression changes during starvation. The graphs show the log2 fold changes of various genes over time (30, 60, 90, 120 min).

- **a** shows sustained induction of genes like GADD45B, ID3, EGR2, SIK1, ID1, DUSP2, ACTC1, FOXD1, GREM1.
- **b** indicates that the expression of genes such as USP36, ZNF548, MTHFD1L, KDM3A, JAG1, STX12, HSPA2, GZF1, and NFIL3 is reset after the initial induction.
- **c** demonstrates delayed induction of DOT1L, BMPR2, HECTD2, BNC1, ADAM19, KIF21A, OLFM2, CD9, and EML1.
- **d** shows reversal of expression for genes like MSC, CXXC5, MTSS1, DLX2, HMOX1, PLXND1, CHST3, PODXL, and SEMA7A.
- **e** illustrates sustained repression of ZNF416, YAE1D1, C1QBP, HARS2, TMEM11, SYPL2, MRPL44, TMPPE, and SAP30L.
- **f** reveals reversion of expression for genes such as NCKAP5L, HBP1, GAB1, LINC00265, CDC42EP2, ATP6V1A, SYT1, GLCCI1, and FAM129A.
- **g** shows delayed repression of FOS, ATF3, PER1, EGR1, ING1, FHOD3, BTG2, KIF18A, and KLF4.

**Additional Information:**

- The graphs are color-coded to represent log2 fold changes.
- The x-axis represents time points (0-30 min, 30-60 min, 60-90 min, 90-120 min).
- The y-axis represents log2 fold changes.

**References:**

Biology Open, 5:837-847, 2016
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co-transcriptional splicing is inefficient
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Bru-seq

YBX1

30 min Bru labeling

BruChase-seq

6-hour chase
co-transcriptional splicing is inefficient
co-transcriptional splicing is inefficient
co-transcriptional splicing is inefficient

\[
\text{Splicing Index} = \frac{a}{a + b + c}
\]

Splicing index following 30 min Bru-labeling period (>15,000 introns)
post-transcriptional removal of introns
post-transcriptional removal of introns

CRKL

Layered H3K4Me3
Layered H3K4Me1
Layered H3K27Ac

CRKL

Scale
chr22:
21,280,000
21,290,000
21,300,000
21,310,000
10 kb
hg19
post-transcriptional removal of introns
post-transcriptional removal of introns

![Graph showing RPKM levels over time for CRKL gene](image)

**CRKL**

- **0h**
- **0.5h**
- **1h**
- **2h**

**Scale**

chr22: 21,280,000 | 21,290,000 | 21,300,000 | 21,310,000
post-transcriptional removal of introns

CRKL

0h

0.5h

1h

2h

4h

Layered H3K4Me3

Layered H3K4Me1

Layered H3K27Ac

DNase Clusters

SINE

LINE

LTR

DNA

Simple

Low Complexity

Satellite

RNA

Other

Unknown

10 kb

hg19

21,280,000

21,290,000

21,300,000

21,310,000

chr22:
post-transcriptional removal of introns

CRKL

Layered H3K4Me3
Layered H3K4Me1
Layered H3K27Ac

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SINE
LINE
LTR
DNA
Simple
Low Complexity
Satellite
RNA
Other
Unknown

Scale

10 kb

hg19
MODELS
MODELS

1 Post-transcriptional splicing
MODELS

1. Post-transcriptional splicing

2. All splicing is “co-transcriptional” with quality control and turnover of aberrantly spliced transcripts by the RNA exosome.
RNA exosome
RNA exosome

**TRAMP complex**
- MTR4
- TRF4
- ZCCHC7

**NEXT complex**
- MTR4
- RBM7
- ZCCHC8

- rRNA 5'-ETS

- eRNA

- PROMPT RNA

- aberrantly spliced mRNAs

**SKI complex**
- SKI2
- SKI3
- SKI8

- mRNA

**nucleus**

**cytoplasm**

- RNA degradation

- Central channel
RNA exosome

TRAMP complex
MTR4
TRF4
ZCCHC7
rRNA 5′-ETS

NEXT complex
MTR4
RBM7
ZCCHC8
eRNA
PROMPT RNA
aberrantly spliced mRNAs

RNA degradation

nucleus
cytoplasm

SKI complex
SKI2
SKI3
SKI8
mRNA

RNA degradation
RNA exosome

TRAMP complex
MTR4
TRF4
ZCCHC7

NEXT complex
MTR4
RBM7
ZCCHC8

eRNA

PROMPT RNA

aberrantly spliced mRNAs

rRNA 5’-ETS

5’ RNA

Rrp6

Dis3

Central channel

nucleus

inhibitor
Nouri Neamati

RNA degradation

mRNA

cytoplasm

SKI complex
SKI2
SKI3
SKI8

PROMPT RNA

5’ RNA

SKI complex

RNA degradation

Nouri Neamati
RNA exosome degrades unspliced transcripts

K562 cells

Splicing Index

30 min  6 h  30 min  6 h  30 min  6 h
control  RNA exosome inhib  splicing inhib
RNA exosome degrades unspliced transcripts
RNA exosome degrades unspliced transcripts
RNA exosome degrades unspliced transcripts

K562 cells

Splicing Index

0.0

0.2

0.4

0.6

0.8

1.0

30 min 6 h control

30 min 6 h RNA exosome inhib

30 min 6 h splicing inhib
Splicing-coupled transcription
Splicing-coupled transcription

spliceosome

RNAPII
Splicing-coupled transcription
Splicing-coupled transcription

Spliceosome

RNAPII

fully spliced

Splicing-uncoupled transcription
Splicing-coupled transcription

Spliceosome

RNAPII

fully spliced

Splicing-uncoupled transcription
Splicing-coupled transcription

Splicing-uncoupled transcription
Splicing-coupled transcription

Spliceosome

![RNA Pol II](RNA Pol II)

fully spliced

Splicing-uncoupled transcription

RNAPII

fully un-spliced

RNA degradation

RNA exosome
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**RNA exosome**

**nucleus**

**cytoplasm**

**TRAMP complex**
- MTR4
- TRF4
- ZCCHC7

**NEXT complex**
- MTR4
- RBM7
- ZCCHC8

**SKI complex**
- SKI2
- SKI3
- SKI8

- TRAMP complex: MTR4, TRF4, ZCCHC7
- NEXT complex: MTR4, RBM7, ZCCHC8
- SKI complex: SKI2, SKI3, SKI8

**rRNA 5'-ETS**

**PROMPT RNA**
- aborted transcripts
- aberrantly spliced mRNAs

**eRNA**

**Central channel**

**RNA degradation**

**5' RNA**

**5' RNA**
UV light suppresses the RNA exosome

RNA degradation

3'-5' exonuclease

UV light suppresses the RNA exosome

p38MAPK

MK2

RBM7

RNAP II blocked

exosome unable to access 3'-end of RNA

5' RNA

3'-5' exonuclease

RNA exosome

Central channel

RNA degradation
UV light suppresses the RNA exosome

HCT116 cells with auxin-inducible degradation of DIS3

Davidson et al, Cell Report 2019
UV light suppresses the RNA exosome

HCT116 cells with auxin-inducible degradation of DIS3

Davidson et al, Cell Report 2019
UV light suppresses the RNA exosome

HCT116 cells with auxin-inducible degradation of DIS3

Davidson et al, Cell Report 2019
Using **BruUV-seq** to identify TNF-inducible enhancers
Using **BruUV-seq** to identify TNF-inducible enhancers
Using BruUV-seq to identify TNF-inducible enhancers

**Chart 1:**
- **Y-axis:** RPKM
- **X-axis:** Chromosome 4
- **Legend:**
  - **BrU-seq control**
  - **TNF**
- **Markers:**
  - 100 kb
  - 103,400,000
  - 103,500,000
  - NFKB1
  - H3K4Me3
  - H3K4Me1
  - H3K27Ac

**Chart 2:**
- **Y-axis:** RPKM
- **X-axis:** Chromosome 4
- **Legend:**
  - **BrU-seq control**
  - **TNF**
  - **IL8**
- **Markers:**
  - 20 kb
  - 74,600,000
- **Annotations:**
  - CXCL8
  - H3K4Me3
  - H3K4Me1
  - H3K27Ac

*Scientific Reports, 5:17978, 2015*
**Bru-seq technologies**

**Bru-seq**
Rates of RNA synthesis

**BruChase-seq**
RNA processing
RNA stability

**BruUV-seq**
Mapping active enhancers

**BruDRB-seq**
Rates of transcription elongation