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# SELECT-based quantification of site-specific m$^6$A modification

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**Protocol status:** Working
**We use this protocol and it's working**

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## Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

## Abstract

This protocol describes SELECT (single-base elongation- and ligation-based qPCR amplification) assays for site-specific quantification of m6A RNA modification using total RNA, followed by qPCR-based detection of SELECT products.

## Materials

- **40 nM Up primer****
- **40 nM Down primer****
- **5 µM dNTPs** (NEB, N0447S)
- **1x CutSmart buffer** (NEB, B7204S)
- **0.01 U Bst 2.0 DNA polymerase** (NEB, M0537S)
- **0.5 U SplintR ligase** (NEB, M0375S)
- **10 nmol ATP** (NEB, P0756S)
- **SYBR Green Master Mix** (Applied Biosystems, A25742)

## Before start

- Use RNase-free tubes, tips, and reagents throughout.
- Prepare all primer working solutions in nuclease-free water.
- Thaw enzymes on ice and keep on ice until use.
- All centrifugation steps are performed briefly to collect liquid unless otherwise specified.

## Annealing

1 Prepare the annealing reaction for each sample by mixing:
500 ng total RNA
40 nM Up primer
40 nM Down primer
5 µM dNTPs (NEB, N0447S)
1x CutSmart buffer (NEB, B7204S)
Adjust volume to 17 µL with nuclease-free water.

2 Perform primer annealing using the following temperature program:
90°C for 1 min
80°C for 1 min
70°C for 1 min
60°C for 1 min
50°C for 1 min
40°C for 6 min

## Elongation and ligation

3 Prepare anenzyme mixture containing:
0.01 U Bst 2.0 DNA polymerase (NEB, M0537S)
0.5 U SplintR ligase (NEB, M0375S)
10 nmol ATP (NEB, P0756S)

4 Add 3 µL enzyme mixture to each annealed reaction to reach a final volume of 20 µL.

5 Incubate reactions using the following program:
40°C for 20 min
80°C for 20 min
Hold at 4°C

## qPCR detection

6 Dilute SELECT reaction products five-fold with nuclease-free water.

7 Use 2 µL diluted product as template for qPCR amplification using:
SELECT common primers
SYBR Green Master Mix (Applied Biosystems, A25742)

8 Perform qPCR according to the manufacturer’s instructions.

## Data interpretation

9 The abundance of SELECT qPCR products reflects the relative methylation level at the interrogated adenosine site.

10 All SELECT assays are performed with at least three independent biological replicates.

## Protocol references

Xiao Y, Wang Y, Tang Q, Wei L, Zhang X, Jia G. An Elongation- and Ligation-Based qPCR Amplification Method for the Radiolabeling-Free Detection of Locus-Specific $N^6$ -Methyladenosine Modification. Angew Chem Int Ed Engl. 2018 Dec 3;57(49):15995-16000. doi: 10.1002/anie.201807942. Epub 2018 Nov 8. PMID: 30345651.

## Acknowledgements

**Notes**
- Primer sequences used for SELECT assays are provided in *Supplementary Table 8_*.
- Annealing temperature steps are critical for specificity and should not be shortened.
- Relative comparisons should be performed using matched input RNA amounts across samples.