



Oct 24, 2020

## Improve

Hung Yu Chen<sup>1</sup>, Huan Jui Chang<sup>1</sup><sup>1</sup>Chung Shan Medical University

1

Works for me

This protocol is published without a DOI.

Chung Shan Medical University



Iris Chen

### PROTOCOL CITATION

Hung Yu Chen, Huan Jui Chang 2020. Improve . **protocols.io**  
<https://protocols.io/view/improve-bnw2mfge>

### LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

### CREATED

Oct 24, 2020

### LAST MODIFIED

Oct 24, 2020

### PROTOCOL INTEGER ID

43706

### In vitro protein synthesis

- 1 Add the reagents(on ice) for synthesis of amilCP into 15 wells of the 384-well plate. Pipette well before adding reagents into the wells.

#### banana amilCP

Order	Location	Reagent	Amount
1	Upper-left	DNase/ RNase free water	till 5 µL
2	Upper-left	Solution A	2 µL
3	Lower-right	Solution B	1.5 µL
4	Lower-right	RNase inhibitor	0.2 µL
5	Lower-left	banana amilCP	50 ng
		Total	5 µL

#### banana trigger + banana amilCP

Order	Location	Reagent	Amount
1	Upper-left	DNase/ RNase free water	till 5 µL
2	Upper-left	Solution A	2 µL
3	Lower-right	Solution B	1.5 µL
4	Lower-right	RNase inhibitor	0.2 µL
5	Lower-left	banana amilCP	50 ng
6	Lower-left	banana trigger	50 ng
		Total	5 µL

#### T7 Strong Promoter + amilCP

Order	Location	Reagent	Amount
1	Upper-left	DNase/ RNase free water	till 5µL
2	Upper-left	Solution A	2 µL
3	Lower-right	Solution B	1.5 µL
4	Lower-right	RNase inhibitor	0.2 µL
5	Lower-left	T7 Strong Promoter amilCP	50 ng
		Total	10 µL



Do triple repeat

- 2 Seal the plate with microseal (on ice)
- 3 Centrifuge (4000rpm, 0.01 (1 min), 4°C)

#### Setup plate reader

- 4 Temperature: Setpoint 37°C  
Shake: Orbital for 1:00 (1 min)  
Start Kinetic [ Run 6:00:00 (6 hour), Internal 0:05:00 (5 min)]  
Read: (A) 588 nm  
End Kinetic

#### Incubation

- 5 Put the plate into plate reader.  
Incubate the mixture and measure the fluorescence in plate reader follow Setup 1 for 06:00:00

#### Measuring

- 6 Measure the fluorescence excitation and emission intensity of the parts.