cell-free system

Materials：

• Nuclease-free, barrier tips and pipets capable of pipetting 0 – 100 µL
• Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
• Nuclease-free, molecular biology-grade water
• Incubator, thermo block or water bath
• Table-top microcentrifuge
• Vortex mixer
• Fluorescence plate reader (e.g. Tecan Genios)

Procedure：

The following steps describe setting up myTXTL reactions with the positive control plasmid P70a(2)-deGFP that is supplied with the myTXTL Sigma 70 Master Mix Kit.

1. Preheat incubator (or thermo block or water bath) to 29 °C.
2. Completely thaw the myTXTL Sigma 70 Master Mix and the positive control plasmid on ice. Keep reagents on ice till use.

Note: To minimize freezing and thawing cycles, only thaw the number of reagent tubes required to set up the desired number of myTXTL reactions.

3. Directly before use, vortex the myTXTL Sigma 70 Master Mix for 2-3 seconds and briefly spin down. If any precipitate is visible hereafter, gently resuspend master mix solution about 10 times to ensure homogeneity. Avoid formation of bubbles and foam.
4. Setting up a myTXTL reaction. The recommended total volume of a myTXTL reaction is 12µL.

a.Make stocks which are 10× of desired final reaction concentrations.
b.Assemble a reaction (either prepackaged or multi-component),completing with water to 80% volume

5. Vortex gently to mix and split the reaction into each tube evenly.
6. Add 1.2 µL of respective plasmid encoding sigma factors under P70a promoter to each tube. Add 1.2 µL of the corresponding deGFP plasmid to each tube to complete to 12 µL. To the background tube, add water to 12 µL. Gently mix all the tubes.
7. Pipette 5 µL from each reaction into two wells in the well plate.
8. Seal the wells with caps, place the well plate into the plate reader, and begin measurement.
9. When kinetics are done, use the GFP calibration (488/535)to calibrate the raw data.
10. Subtract the background reaction from all other reactions for all time points.
11. Compare the slope of the deGFP kinetics between each transcription cascade
12. Repeat as necessary for error bars.