Overexpression and depletion of a protein using the *nmt1* promoter

1) Overexpression of a protein using the *nmt1* promoter

Grow cells in 5 ml in appropriate medium containing thiamine in a flask O/N @30˚C

Check OD in the morning. Dilute cell in 20 ml of YES so that the culture OD reaches between 0.6 and 1.2 at 3~4pm

Wash cells with thiamin-free medium three times. (Centrifugation: 2300rpm, 4min, RT)

Dilute cells to OD=0.05 in 100 ml of thiamin-free medium.

Grow cells for 16~20h at 30˚C (five generations)

Collect and freeze cells for appropriate analysis

2) Depletion of a protein using the *nmt1* promoter

Grow cells in 50 ml in appropriate medium in a flask O/N @30˚C

Check OD. If the OD is between 0.6 and 1.2, dilute cells to OD=0.4 in 100ml medium.
(If the OD is more than 1.2, dilute cells to OD=0.3 and grow cells for another 3 hours. Then adjust OD to 0.4)

Transfer 20 ml into a 50ml tube (0h sample). Split the rest of the culture into two flasks (40 ml each). Add 80 ul of thiamine (2000 x stock) to one of two flasks, and start a timer. Grow cells @30˚C.

Freeze cells for 0h samples. **For detail, follow the protocol “Preparation of *S. pombe* protein samples for Western Blot (Urea Method)”

2, 4, and 6 hr after the addition of thiamine, transfer 16 ml into a 50ml tube and freeze cells (two samples at each time point).

Prepare SDS-PAGE samples from the frozen cells obtained above.
**Follow the protocol “Preparation of *S. pombe* protein samples for Western Blot (Urea Method)”. Need to adjust protein concentration.

SDS-PAGE and Western Blotting to examine overexpression of proteins