# TX-TL (Cell-free protein expression)

Aims of the experiment

Cell-free transcription–translation (TXTL) system is used for analyzing the level of gene expression by our homemade cell extract. It is aimed to achieve as comparative as possible with the ordinary gene expression in vivo.

Materials

- Extract produced from Dialysis Protocol
- TX-TL buffer
- Nuclease-free $H_2O$
- Expression plasmid: e.g. yPet / GFP / mTurq / Malachite Green
- 200µl micro-centrifuge tube
- Square 384-wells plate

Procedure

Always work on ice for this experiment and work quickly to avoid early expression in the extract!

1. Pre-cool the plate reader to 29°C.
2. Thaw the plasmids and TX-TL buffer on ice.
3. Prepare Master Mix for every plasmid, each with concentration of 3 nM.
4. Prepare Malachite Green (MG) with concentration of 200 µM if needed for mTurq.
    I. The Master Mix for mTurq will be changed into the following:
    II. ( # samples + 0.5 ) * ( 3.75 – "plasmid per well" – 1.5 ) = amount of water added
    III. Add 1.5µl Malachite Green into this mTurq/MG Master Mix.
    IV. Amount of mTurq remain unchanged.
5. Add 3.75µl plasmid Master Mix into the 384-wells plate.
6. Prepare 200µl micro-centrifuge tube for each extract sample.
7. Mix 22.8µl extract samples with 28.51µl TX-TL buffer (for 3 plasmids sample and 1 blank).
8. Add 11.25µl Extract-Buffer Solution into each plasmid Master Mix in the well, add one more well without plasmid Master Mix as blank.

9. Mix well by pipetting up and down.
10. Spin down the plate for 30 seconds to remove air bubbles.
11. Set up the plate reader with the following:
    - No. of cycle: 200
    - Cycle time: 180 seconds
    - Bottom optics
    - Monochromats as following:

| Fluoresecent protein/Aptamer | Excitation wavelength | Emission wavelength | Optimal gain |
|---|---|---|---|
| GFP | 485 | 520 | 1000 |
| mTurq | 440 | 480 | 1000 |
| YPet | 500 | 540 | 1000 |
| Malachite Green | 630 | 650 - 670 | |