Benchling

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Introduction

Dimerisation assays for yeast containing BiFC and NanoBiT systems

Materials

- NanoGlo® substrate and buffer
- white or black 96 well plate
- elicitor elf18,csp22
- Synergy H_1 Hybrid Reader Biotek

Procedure

Preparations of yeast cultures

1. Grow overnight cultures of yeast you want to investigate and UT yeast in appropriate medium.
2. Centrifuge the ONC, pour out supernatant and resuspend with 1XTE in such a way that they have the same OD600

BiFC mCherry assay

3. add 100µL of the resuspended cells into the wells of a black 96 well plate
4. add 1µL of 1mM elicitor to the appropriate wells.
5. wrap in aluminium foil and incubate for 30 min on a shaker at RT
6. read the fluorescence with the plate reader with excitation wavelength 587 nm and emission wavelength 610nm

   If you do a kinetic assay, measure fluorescence every 5 min for 2 hours with constant linear shaking

NanoBiT® assay

7. add 50µL of the resuspended cells into the wells of a white 96 well plate
8. Prepare the NanoGlo® reagent by pipetting n+4(50µL) into a 10mL tube. (a is the number of wells you have filled with your yeast samples. you calculate for 4 in excess to account for reagent that might get lost due to foaming of the reagent). You then add the substrate in a 1:50 ratio to the buffer.
9. add 50µL the prepared reagent to each well.
10. add 1µL of 1mM elicitor to the appropriate wells. (If you investigate yeast containing FRB/$FKBP_{12}$, then add 10µL of 10µM of Rapamycin instead.
11. measure chemiluminescence using the plate reader with an integration time of 2 seconds for each well. Measure the samples each 5 mins for 2 hours with for a kinetic assay.