酿酒酵母中经修饰后 GFP 的半衰期检测方法

实验目的

比较 TJU 改良的绿色荧光蛋白（Green fluorescence protein, GFP）与未经修饰的 GFP 半衰期变化。

Purpose

To compare the half-life of Green fluorescent protein (GFP) modified by TJU with that of unmodified GFP.

实验材料

酶标仪，超声裂菌仪，灭菌锅，液体培养基，无菌枪头，移液枪，超净台。基因组上整合有改良 GFP，且能够表达 CRISPR/Cas9 并完成切割基因组目的的酿酒酵母菌种①。基因组上整合有 GFP，且能够表达 CRISPR/Cas9 并完成切割基因组目的的酿酒酵母菌种②。

Material & Equipment

Microplate tester, ultrasonic splitting tester, sterilizing pot, liquid culture medium, Sterile head, pipette gun, ultra clean table. *Saccharomyces cerevisiae* strains ① that have modified GFP integrated on their genomes and can express CRISPR/Cas9 which can complete genome cutting purposes. *Saccharomyces cerevisiae* strains② that have GFP integrated on their genomes and can express CRISPR/Cas9 with the same function of strains①.

实验步骤

一、酶标仪检测荧光强度

在液体培养基中分别接种①与②，设置转速与温度为酿酒酵母的最适条件。

在培养时间为 0/12/24/48/72h 时，使用无菌枪头，在超净台中取 1ml 样品（严格无菌操作，避免染菌），取样后将 200μl 样品加于 96 孔板中，设定酶标仪程序的激发光，在合适的发射光波长条件下检测吸光度，记录数据。

二、SDS-PAGE

在至少 200ml 的液体培养基中分别接种①与②，设置转速与温度为酿酒酵母的最适条件。

4000rpm，4℃，离心 10min，回收沉淀。

向沉淀中加入适量缓冲液（10%甘油, 20Mm HEPES, 500mM NaCl, pH 值调整至 GFP 最适的条件,一般 7.5 即可）

超声裂菌仪裂解菌体。设置为 600w，超声 5s，间隔 5s，共计 40min。

15000rpm，4℃，离心 20min，重复 3 次。取上清液备用。

制备 SDS-Page 样品：20μl 上清液 + 5μl Loading Buffer，98℃条件下加热 15min 完成变性。

上样，150V 条件下泳动 45min。

完成电泳后使用染色液染色（考马斯亮蓝 R250 1.2g/L；甲醇：水：乙酸（5：4：1)），60℃，8min。

染色后，使用脱色液（甲醇：水：乙酸 （5：4：1 ））脱色，脱至可见到清晰条带。

Procedure

A. Detection of fluorescence intensity by microplateReader

Inoculate ① and ② respectively in liquid medium, and set the speed and temperature as the optimal conditions of *S. cerevisiae*.

At the incubation time of 0/12/24/48/72h, 1ml sample was taken in a clean bench with sterile head (strictly sterile operation, avoid bacterial contamination), and 200μ L sample was added to the 96-well plate after sampling. Excitation light was set for the microplate reader program, and absorbance was measured at appropriate emission wavelength and recorded.

B. SDS-PAGE

Inoculate ① and ② respectively in liquid medium of at least 200ml, and set the speed and temperature as the optimal conditions of *S. cerevisiae*.

The precipitate was recovered at 4000rpm, centrifuged at 4℃ for 10min.

Add an appropriate amount of buffer solution (10% glycerol, 20Mm HEPES, 500mM NaCl, adjust the pH value to the optimal conditions of GFP, generally 7.5) to the precipitation.

Ultrasonic schizotometer lyses thallus. Set to 600W, ultrasound 5s, 5s interval, a total of 40min.

Centrifuging at 15000rpm, 4℃ for 20min(Repeat 3 times). Take the supernatant and reserve.

SDS-PAGE samples were prepared with 20 μl supernatant + 5 μl Loading Buffer and heated at 98℃ for 15min to complete denaturation.

Sample loading, swim for 45min at 150V.

After electrophoresis, stain with staining solution (Coomassie bright blue R250 1.2g/L; Methanol: water: acetic acid (5:4:1)) at 60℃ for 8min.

After dyeing, decolorization solution (methanol: water: acetic acid (5:4:1)) was used to decolorize until a clear band was visible.

数据处理

使用酶标仪测定荧光强度后导出数据绘制柱状图

SDS-Page 可定性，只可半定量。在蛋白浓度足够高时，可使用显影仪检测荧光强度。

Data processing

The fluorescence intensity was measured with a microplate reader and the data were derived to draw a histogram

SDS-PAGE can be qualitative, only semi-quantitative. When the protein concentration is high enough, fluorescence intensity can be measured using a developer.

注意事项

上述条件均需要根据酿酒酵母的性质进行调整优化。

检测荧光的激发光与发射光均需要根据所使用的 GFP 实际情况进行调整

如有染胶仪，可不使用手动染色法。

Precautions

The above conditions need to be adjusted and optimized according to the properties of *S. cerevisiae*.

The excitation light and emission light for fluorescence detection should be adjusted according to the actual situation of GFP used.

Do not use manual dyeing method if there is a glue dyeing machine.