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Tag and Catcher Assembly

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igemnthuth[1]

[1]iGEM_NTHU_Taiwan

iGEM NTHU

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Protocol status: Working
We use this protocol and it's working

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Abstract

Induce the strain to secrete SpyCatcher and SpyTag proteins for in vitro conjugation.

Materials

- 1 M IPTG
- PBG
- Centrifuge
- 0.22 µm filter membrane
- Ultrasonic cell disruptor (sonicator)

Cell Disruption by Sonication

1 Resuspend cells in PBS, aiming for approximately **1–5 × 10^9 cells/mL per mL suspension** for effective lysis.

2 Place the cell suspension in an **ice bath** to prevent overheating.

3 Disrupt cells using a sonicator:

- **Power setting:** 20–40% amplitude
- **Pulse duration:** 30 seconds to 1 minute total, with cycles of **10 s on / 10 s off** to avoid overheating.
- **Total sonication time:** 3–5 minutes, adjusted according to sample volume and lysis efficiency.

Collecting Supernatant (SpyCatcher/SpyTag Proteins)

4 Centrifuge at **4500 × g, 4 °C, 5 min**.

5 Collect the supernatant and filter through a **0.22 µm membrane**.

Preparing Reaction Conditions

6 Adjust protein concentration to fall within **1–10 µM**.

7 Prepare PBS buffer for the reaction.

SpyCatcher/SpyTag Covalent Ligation

8 Mix SpyCatcher and SpyTag proteins at a **1:1 molar ratio**.

- Example: if both are at 1 µM, add 1 µL of each into a 50 µL reaction volume.

9 Incubate at room temperature for **1 hour**.

10 Stop the reaction by placing the mixture on ice.