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## Tag and Catcher Assembly

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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  $\mu$ m filter membrane
- Ultrasonic cell disruptor (sonicator)

## Cell Disruption by Sonication

- 1 Resuspend cells in PBS, aiming for approximately  **$1\text{--}5 \times 10^9$  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  **$4500 \times g$ , 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.