Protein Necklace: Harnessing the Glow of Jellyfish

Safety

Use established laboratory safety practices, including appropriate personal protective equipment (PPE), at all times. Wear gloves throughout this activity, and wear proper eye protection when using UV or LED light sources.

Materials

Freeze-Dried *E. coli* Cells
Buffer C
Centrifuge Tubes
Microcentrifuge Tubes
3-mL Transfer Pipettes
2-mL Transfer Pipette
Needle-Point Pipette
Small Applicator Sticks
UV Transilluminators
Reagents:
- Ammonium Sulfate
- Tris Solution
- Isopropyl Alcohol

Procedure

1. Resuspend the *E. coli* cells by filling the tube to the 7.5-mL mark with Buffer C. Close the cap and shake thoroughly for 1 minute so all of the cells are resuspended in the buffer.
2. Now fill the centrifuge tube to the 15-mL mark with isopropyl alcohol. Close the cap and shake vigorously for 5 minutes.
3. Allow the tube to sit for about 20 minutes. You will see the precipitate clumping together between the uppermost organic layer and the lower aqueous phase containing the green fluorescent protein (GFP).

Stopping Point (20 min or overnight)

4. After the mixture has settled, remove the upper layer (organic phase) of alcohol with a 3-mL transfer pipette.
5. Use a 3-mL transfer pipette to push through the disc of cellular debris and suck up the lower layer (aqueous phase containing GFP) only and transfer it to a new 15-mL centrifuge tube. The clump of cellular debris can be thrown away in a waste bin.
6. Now fill the tube to the 15-mL mark again with isopropyl alcohol and shake vigorously for 1 minute.
7. Allow the tube to sit for another 15 to 20 minutes. You will see the GFP clump together and collect in between the uppermost organic layer and the lower aqueous phase, which will now be clear.

Stopping Point (15 to 20 min or overnight)

8. After the mixture has settled, remove the upper layer (organic phase) of alcohol with a 3-mL transfer pipette.

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9. Carefully use the 3-mL transfer pipette to remove as much of the liquid left in the bottom layer (clear aqueous layer) of the tube, while leaving the green precipitate inside the tube.
10. Use the 2-mL transfer pipette to dispense about 6 drops of Ammonium Sulfate Tris Solution into the tube with the green precipitate.
11. Use a 3-mL transfer pipette to resuspend the disc into the buffer by continually sucking up and mixing the buffer and disc until the mixture is homogeneous. Be careful not to suck the mixture all the way up into the bulb of the transfer pipette—you'll have difficulty getting the mixture out of the pipette. You may add more buffer, drop by drop, to aid the process, but be careful not to add too much. In the end, it should take no more than 1 mL of buffer to resuspend the disc. You may still see some precipitate in the mixture when it is finished.
12. Transfer the contents of the tube into a smaller microcentrifuge tube.
13. Allow the sample to sit for 5 to 10 minutes.

Stopping Point (5 to 10 min or overnight)

14. After the mixture has settled, carefully use a small needle-point pipette to transfer the green liquid (located in the aqueous phase) into a new microcentrifuge tube, leaving behind the clumps of precipitate, if possible. The experiment is now complete.

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