Preparing crude cell extracts for cell free Tx-Tl reactions

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Abstract

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Protocol

Notes

Step 1.

This protocol is based on:

Sun, Z. Z., Hayes, C. A., Shin, J., Caschera, F., Murray, R. M., Noireaux, V. Protocols for Implementing an *Escherichia coli* Based TX-TL Cell-Free Expression System for Synthetic Biology. *J. Vis. Exp.* (79), e50762, doi:10.3791/50762 (2013).

Preparing crude cell extract over three days **requires two people** to conduct efficientlly.

Day One

Step 2.

Prepare a culture tube with 5mL of 2XYT+P and inoculate it with a glicerol stock of BL21 DE3 STAR. Grow the bacterias at 37°C with agitation.This is the **mini-culture 1**.

Day Two

Step 3.

Pour 50 mL of 2xYT + P culture medium into a 500mL Erlenmeyer flask and inoculte it with 300 µL of the overnight **mini-culture 1**.

Let it grow for 12 hrs at 37°C with agitation. This is the **mini-culture 2.**

Day three

Step 4.

Weigh an empty sterile 50 ml Falcon tube and record mass in Table 1. Chill Falcon tubes on ice; these will be subsequently used later.

Step 5.

Pour 330 mL of 2xYT + P culture medium into four 2L Erlenmeyer flasks. Preheat the flasks with the medium at 37ºC for 30 min.

Step 6.

Inoculate each Erlenmeyer flask with 11mL of the **mini-culture 2.** Let the cultures grow at 37°C with agitation. Initial OD should be around 0.06.

Step 7.

1h 40m later check the OD of the cultures. When the culture reach OD of 0.6, add 350 µL of **IPTG 1M** into the Erlenmeyer flask. Let the cultures grow at 37°C with agitation until an OD of 1.5 - 2.0 is reached. This OD is normally reached after 3h 30m of IPTG addition.

Pelleting the cells

Step 8.

Immediately after growth, transfer all bacterial cultures evenly into 500mL centrifuge bottles and centrifuge at 5000 x g for 12 min at 4 °C to pellet bacterial cells.

Step 9.

While centrifuging, complete S30A buffer preparation by adding 1 ml of 1 M DTT to 1L of previously prepared S30B. Mix and maintain buffer on ice.

Step 10.

When centrifuging is finished, completely remove supernatant by decanting and blotting the centrifuge bottles on a sterile paper towel. Keep pellets and bottles on ice. (Note: Though not recommended, pellet can be stored at 4 ºC overnight to continue with the following steps next day.)

Wash the pellets

Step 11.

Add 50 mL of chilled S30B buffer to each of the four centrifuge bottles, and shake the bottles vigorously until pellet is completely solubilized with no remaining clumps. Centrifuge the four bottles at 5,000 g for 12 min at 4 °C.

Wash the pellets

Step 12.
Completely remove supernatant from previous step by decanting and blotting the centrifuge bottles on a sterile paper towel.

Step 13.

Repeat steps 10 and 11

Step 14.
Add 10 ml S30A buffer at 4 °C to each centrifuge bottle. Transfer each pellet and S30A combination into the chilled previously weighted Falcon tube.

Step 15.

Centrifuge the Falcon tube at 2,000 g for 8 min at 4 °C. Remove supernatant by decanting.

Step 16.

Re-centrifuge the Falcon tube at 2,000 g for 2 min at 4 °C. Completely remove residual supernatant by pipette Keep on ice.

Step 17.

Weigh the four Falcon tubes with pellet and record mass in **Table 1**. Calculate pellet mass, S30A buffer volume needed, and mass of beads needed based on the specific formulas in **Table 1**.

Step 18.
Add the amount of S30A buffer calculated in Table 3 to each Falcon tube, vortex until homogenous, and return to ice.

Step 19.
While keeping the other Falcon tubes on ice, add beads intermittently to a single Falcon tube in three aliquots, each using 1/3 of the total beads. After addition of each aliquot of beads, vortex for 30 sec. Place Falcon tube on ice between vortex steps and after final vortex. After last aliquot is added, ensure beads are uniformly distributed. A thick paste should be formed.

Step 20.

Prepare a 5 ml pipette tip by cutting off the end using a sterile razor blade to create a 3-4 mm opening. Dial pipette to 2 ml.

Step 21.
Place 20 bead-beating tubes on ice.

Step 22.
Verify high viscosity of cell-bead solution using modified pipet. It should be viscous to the point of barely exiting the pipette tip during ejection.

Step 23.

Remove bead-cell solution from Falcon tube using modified pipet, and transfer into a sterile bead-beating tube, filling it three-quarters full with bead-cell solution. Spin extremely briefly (1s) on a counter mini-centrifuge to remove air bubbles without redistributing beads.

Step 24.
Finish adding bead-cell solution to form a concave meniscus.

Step 25.

Add a very small drop of bead-cell solution onto the inside of a bead-beating tube cap, being careful to not impede the outside lip of the cap; otherwise, the bead-beating tube will not close sufficiently.

Give small taps to the cap on a

Step 26.

Cap the bead-beating tube with the bead-beating cap from the previous step. Hand to assistant for bead beating. If done correctly, the cap should be tightly sealed, no air bubbles should be visible, and little (if any) bead-cell solution should overflow. Redo the loading process if air bubbles are visible or the cap does not fully close.

Step 27.

Vortex Falcon tube often while filling beat beatter tubes to ensure even distribution of beads during the whole process.

Step 28.

Conduct steps 23-27 simultaneously. Have assistant take filled bead-beating tubes and place on ice. Once two filled bead-beating tubes have been collected and have been on ice for at least one minute, begin bead beating.

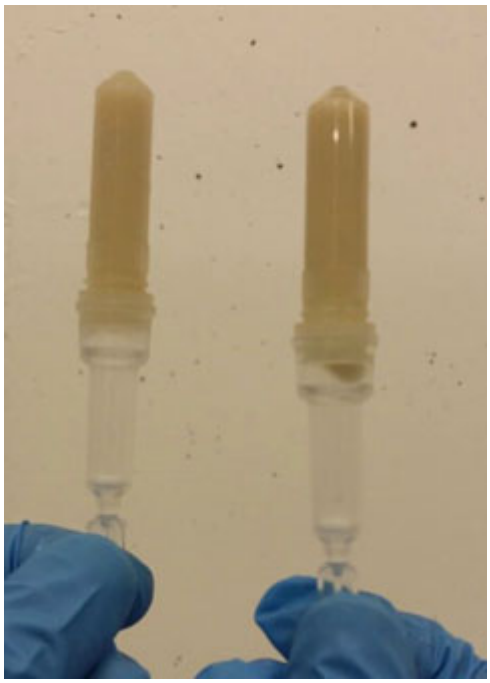
Step 29.
Beat one tube for 30 sec at 46 rpm. Place upside down on ice for 30 sec while beating the other tube.

Step 30.
Repeat previous step such that each filled bead-beating tube has been beat for 1 min total.

Step 31.

Once all the bead-cell solution from the Falcon tube had be passed to the bead-beating tubes and beated already.Then, construct filter apparatus from 15 ml Falcon as follows.

Add a new bead-beating cap, flat-part face up, to the bottom of a 15 ml Falcon. Then, remove cap from processed bead-beating tube and press micro-chromatography column firmly onto end of processed bead-beating tube until completely sealed. Snap off elution end of micro-chromatography column, and place micro-chromatography column, elution end down, into empty bead-beading tube. as in the figure.



Place this complex into 15 ml Falcon. Repeat for all the filled bead-beating tubes; keep on ice when

complete.

Step 32.

Centrifuge the filter apparatuses, Falcon tube uncapped, at 6,000 g for 5 min at 4 °C to separate extract and pellet from beads.

Step 33.
Verify each bead-beating tube has produced viable extract. Properly beat extract will not be turbid, and the pellet will have two distinct layers. Discard all turbid tubes, and transfer the supernatant from non-turbid tubes into individual 1.75 ml micro-centrifuge tubes, taking as little pellet as possible.