TEV-mCherry from C100

Introduction

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Demonstrator: Maike told Jei and Zoe F how to do it, and showed us as well
Paper we based it on is here: http://journals.iucr.org/f/issues/2011/10/00/fw5320/fw5320sup1.pdf
Edit Log:

Noah 12/09 - Created File and added basic protocols based on info from Zoe. F
Noah 13/09 - Added some more based on having done a fair amount of the protocol
Zoe F. 13/09 - Added rest of the protocol
Noah 15/09 - Printed

Materials

Day One

- Plate with your cells on
- Inoculation stuff
- 1L Baffled Plastic flask from Maike, so the cells grow happily O/N, or just a 1L glass flask should be ok, they have plenty of time

Day Two

- 2L 1L Baffled Plastic flask from Maike, so the cells grow happily, otherwise you may wait all day for them to reach the correct OD
- Arabinose Stock Solution
- Booked Avanti Centrifuge for around 5pm

Day Three

- Buffers for purification (see seperate protocol)
- Nickel column (either prepacked or get the beads and column)
- Pump for column
- Booked cell disruptor and asked Maike to help you with it (can't do it alone) for around 10ish
- Booked avanti centrifuge for around 10:30-11

Procedure

Day One

1. Inoculate transformation of C114 in 5ml of LB+CMR

   Incubate at 37°C all day

2. Add this 5ml to 100ml of LB+CMR in a 0.5L (preferably plastic baffled - Ask Maike)

   Incubate at 37°C O/N

Day Two

3. Take OD600 of 100ml culture in flask

    In 1ml cuvette, blank with 0.9ml of LB before adding 0.1ml of cells
    OD will then be this OD x10

4. Dilute down these cells to a level of 1L in a 2L baffled flask to an OD of 0.1

    Plastic ones, ask Maike to use
    Work near a flame, but do not set self on fire!
    Add 1L of LB to the flask, with 1ml of CMR
    Add your cells using a glass pipette
    (For reference, the first one we did we had an OD of 3.21 in the original mixture so we added 31ml of cells to the 1L LB)

5. Incubate cells at 37°C for 2 hours, then check the OD

    If it is not 0.6-0.8, continue to next step, if it is, proceed to 7

6. Continue to incubate, checking OD every half hour until it is between 0.6-0.8

    (The first time we did this the cells took 3 hours to reach the desired concentration

7. Incubate cells at 30°C for 5-10 minutes, then induce with arabinose - we started with 0.01% arabinose but probably should be 0.1%

8. Incubate at 30°C for 3 hours - We started with this but it will vary for different proteins

9. Centrifuge the cells in the big centrifuge in the prep room - Need to prebook this! -

    Make sure to weigh empty centrifuge bucket so that you know the weight of the pellet afterwards
    8000g, 10 minutes, 4°C
    Keep it cold so that the cells aren't able to do any metabolising we don't want them to do e.g. proteolysis

10. Pour away the supernatant from the bucket

    Pellet should be pretty well stuck to the side, so pour carefully but no need to be too careful

11. Freeze the cells at -20°C O/N

Day 3 (The Big Day)

12. Take your cells out of the -20°C and keep on ice at all times

13. Add 5x lysis buffer V/g to the cell pellet and shake it and scrape it etc. until you have a slurry in the bucket

14. Add this to a homogeniser (can borrow from Maike) and make it super-smooth

15. Add DNAase to stop the aggregation of DNA making the lysate viscous

    Just a spatula of Bovine DNAse 1 will do

16. Take this solution and lyse the cells with the cell-disruptor (Maike has to show you this) (see protocol)

17. Centrifuge the lysate for 30 minutes in a JA20, balanced, 15000g.

18. While centrifuge is running, set up your Ni-NTA column. Beads are stored in ethanol so need washing.

19. Wash with a LARGE (at least 50% of the column volume) amount of MilliQ, then equilibrate beads with buffer by adding about half the previous volume of wash buffer (you should have more than half of the wash buffer left!)

    Don't let your beads run dry
    When adding a new solution to the beads, seal both ends of the tube and mix well
    'Wash' means just let the stuff run through the column + collect it in a waste bucket - don't need this wash. DO need wash when you've added protein!

20. Add your supernatant from the centrifuge to the column. Don't get the pellet in (keep the pellet on top and it should be stable)

    Keep 100ul of supernatant aside for SDS analysis

21. Put on the roller in the cold room for two hours. Seal each end with parafilm, and label the column.

22. Set up the column again, but collect the flow-through in a 50ml falcon tube that is on ice. Everything from now on should go onto ice! Buffers should also be on ice.

23. Being careful not the let the column run dry, stop the column (off switch on pump) when there is ~1-2mm of liquid. Add ~20ml of wash buffer, and mix. Then run this on the column.

24. Keep adding wash buffer until the nanodrop (A280) gives a low volume (or you run out of buffer). Nanodrop every so often to see how 'clean' your beads are. Collect the filtrate.

25. Now to elute! Add 2-3ml of elution buffer at a time, mix, then collect in 15ml falcon tubes as small fractions. Again, nanodrop to check concentration.