Protein purification and analysis

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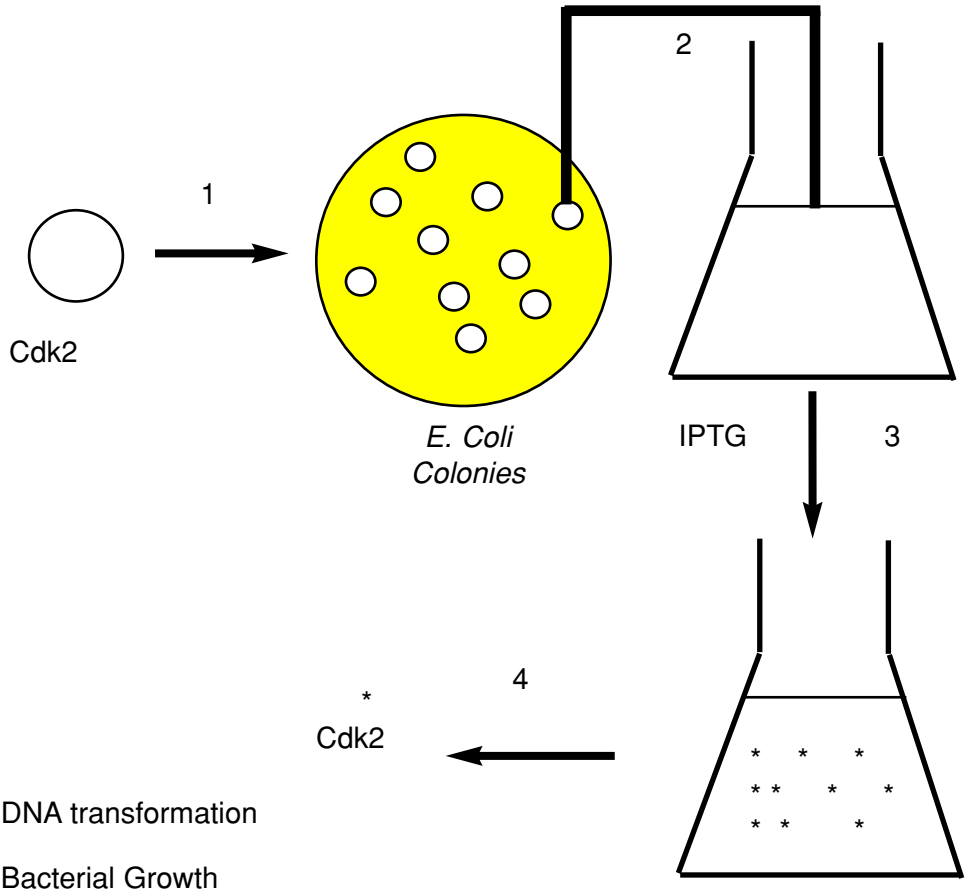
Overview of protein purification



1: DNA transformation

2: Bacterial Growth

3: Protein *induction*

4: Protein *purification*

DNA Transformation

1. Use 1ml DNA (10ng) with 40ml competent cells for each transformation (always include a negative control with no DNA) and 18ml 5mM Tris, 5mM MgCl2 (stock solution kept in the fridge) always add first to the tube.

2. Leave cells, DNA and stock solution on ice for 30 mins. Incubation times of 15-60 mins can be used but 30 mins is the optimum.

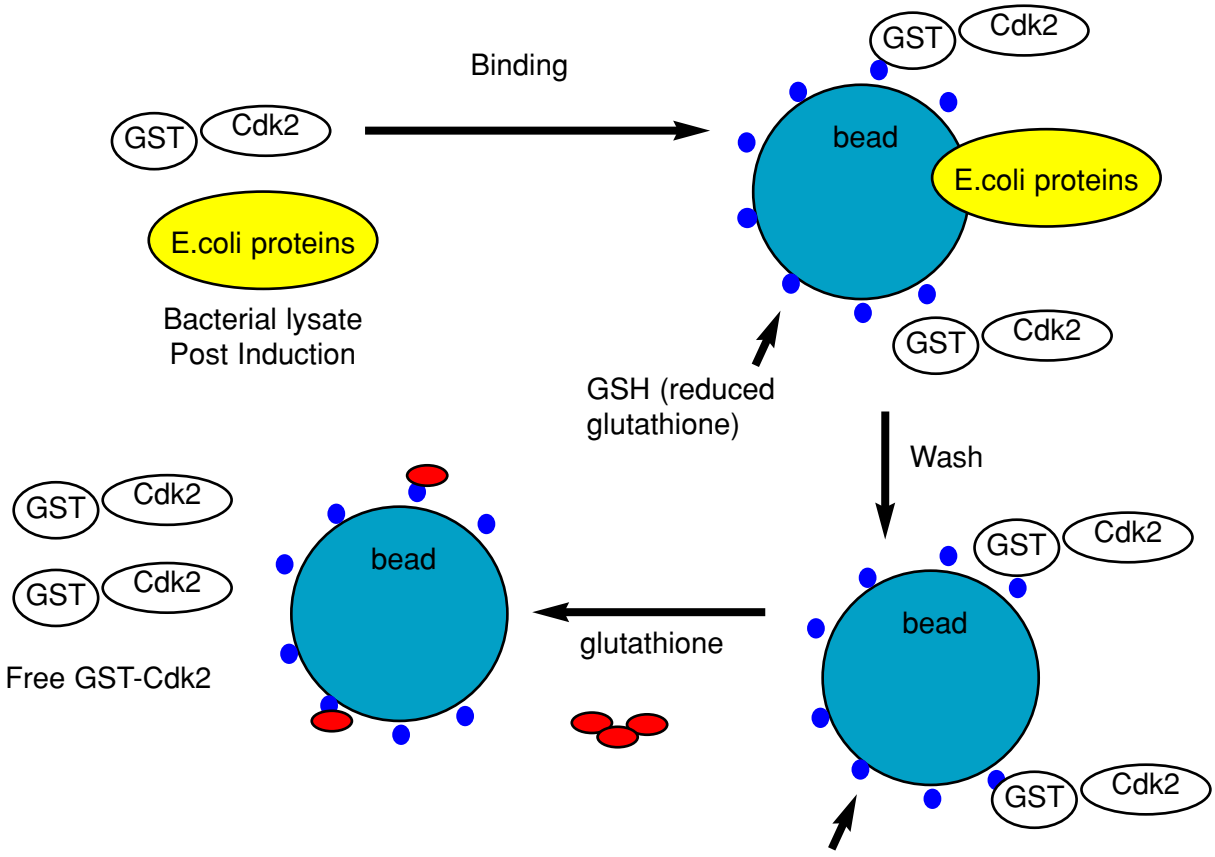
3. Heat shock in the water bath at 42°C for 45 sec.

4. Replace tube on ice and add 160ml LB (no antibiotic) but do not mix.

5. Place on 37°C heating block for 30 mins (also can be left for 30-75 mins).

6. Plate out 100ml and 10ml on LB+Amp plates (previously dried in the oven) and incubate overnight at 37°C

Principle of protein purification



Protein induction

1. Inoculate a 30ml LB+Antibiotic culture with a single colony and leave shaking overnight at 37˚C.

2. Use to inoculate a 500ml culture of LB+Antibiotic and leave to grow shaking at 30˚C until an O.D. of 0.8 is reached.

3. Remove 200microl for the pre-IPTG induction sample, pellet, resuspend in 25microl 2XR, boil 2 minutes/95˚C for run on SDS PAGE.

4. Leave the culture at room temperature for 10 minutes and add IPTG to a concentration of 0.1mM; leave the culture shaking at room temperature for 5 hours (22˚C).

5. Remove 200ml for the post-IPTG induction sample and treat as the pre-IPTG sample for PAGE.

6. Pellet the bacteria 4000rpm, 10 minutes, 4˚C and transfer with cold PBS to a 30ml tube. Pellet the bacteria in the tube and leave at -70˚C until ready to start the purification.

Protein purification

7. Resuspend the pellet fully in 13.5 ml cold PBS containing protease inhibitors plus 1mg/ml lysozyme; place the tube on ice for 10 min and sonicate with the following settings:- using the micro-tip, output control - micro-tip limit, 70% duty cycle, pulsed power, 6 X 10 pulses (with a time interval between each 10pulses to prevent the solution from heating up.)

8. Add 1.5ml cold 10% Triton X-100 to give a final concentration of 1%, mix and centrifuge 10,000rpm/20 minutes/4˚C, remove the supernatant. Keep 1microl = supernatant pre-incubation with the beads for PAGE.

9. Incubate the supernatant with 1ml washed Glutathione Sepharose beads(2 x cold PBS x 10ml) in a 50ml Falcon tube for 1 hour on the wheel in the cold room.

10. Centrifuge 1000 rpm/2 minutes/4°C and transfer the supernatant to a clean tube, remove 1ml = supernatant post-incubation with the beads for PAGE.

11. Wash the beads 3 x 10-15 ml cold PBS, 1 x cold 50 mM Tris pH 8.0.

12. Transfer the beads from the 50 ml Falcon tube to 2 x Eppendorf tubes (0.5 ml beads each tube) using cold 50 mM Tris pH 8.0 and centrifuge 1000rpm 1 minute to remove the residual Tris buffer.

Elution of the protein

13. Add 0.5 ml of 10 mM Glutathione in pbs for 2 - 3 minutes at room temperature and centrifuge 1000 rpm/1 minute/4°C.

14. Centrifuge the tubes containing the purified proteins 14000 rpm/

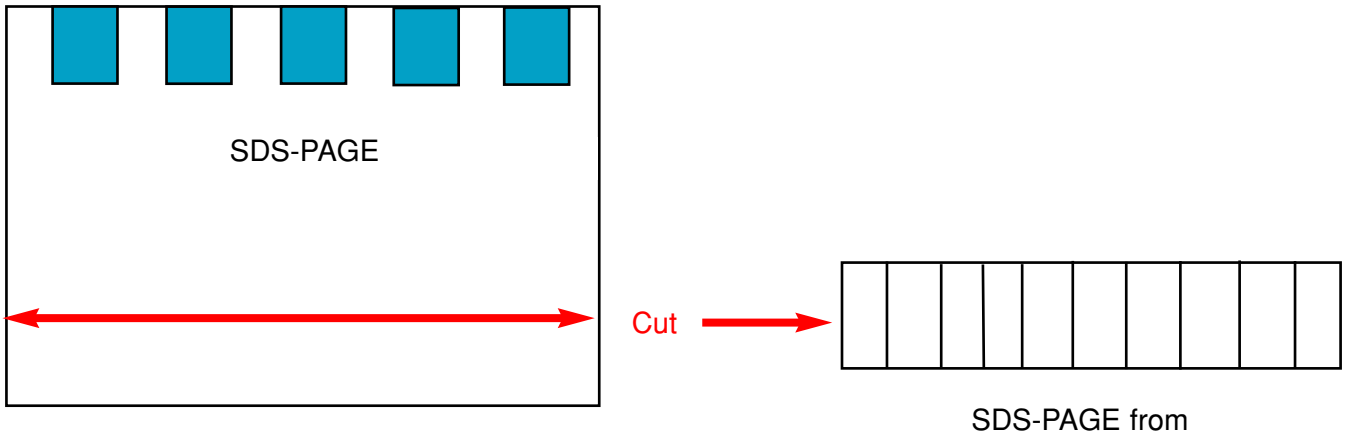
1 minute to remove any trace of Glutathione Sepharose beads and transfer the fractions to clean, pre-chilled tubes.

SDS-PAGE

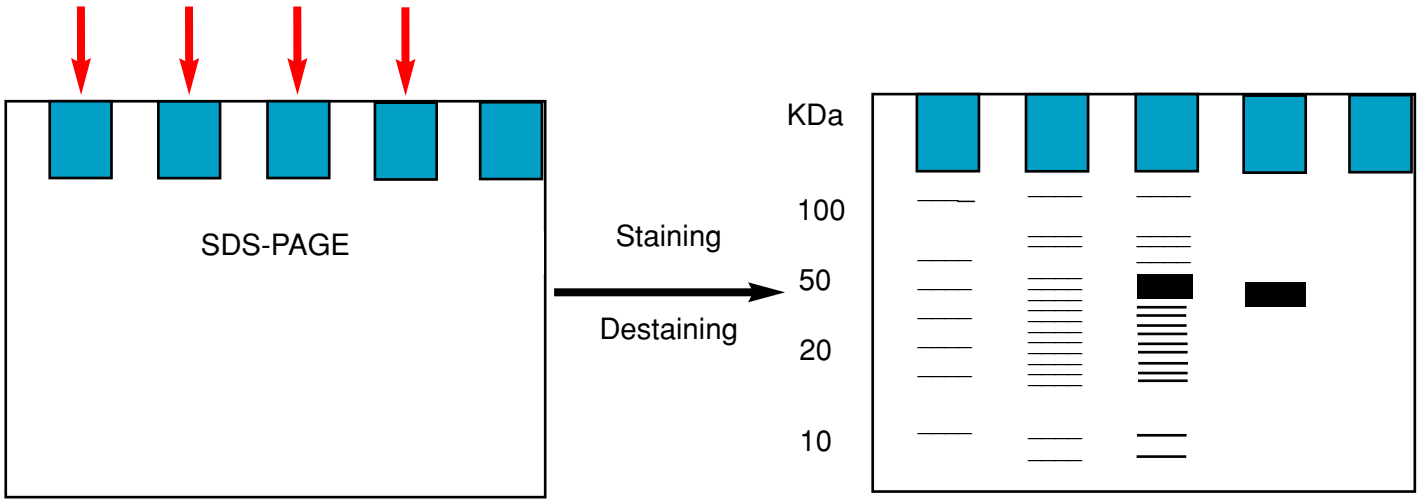
Soduim **D**odecyl **S**ulfate **P**oly**A**crylamide **G**el **E**lectrophoresis

Used to separate proteins according to their size, as a control that the purification was sucessfull. SDS bind the proteins according to their weight, the migration will only depend of the molecular weight of the protein. The lighter the protein is the faster it will migrate. PolyAcrylamide create a network, the size of the latice depend on acylamide concentration.

12% is the usual concentration, it allows a resolution from 10 to 100KDa



Practical example



1. Molecular marker
2. Pre-induction
3. Post-induction
4. Purified

Role of Cdk2