Protocol for plasmid transformation into E. coli and protein expression

Sources: https://www.neb.com/en/protocols/2012/05/21/transformation-protocol

https://www.addgene.org/protocols/bacterial-transformation/

https://www.sigmaaldrich.com/DE/de/technical-documents/technical-article/genomics/cloning-and-expression/competent-cells?srsltid=AfmBOorhs-px3v6sfu4Dn04cQT7N7mu0DgM4LyemL55ENjHY2DBED6hm

Reagents

1. Competent E. coli cells
2. Plasmid DNA with the target protein and the Amp^R marker.
3. LB broth.
4. LB agar with ampicillin (50–100 µg/ml).
5. LB broth with ampicillin (50–100 µg/ml).

Equipment

1. Thermostat 37°C.
2. Water bath 42°C (for heat shock)
3. Laminar flow box.
4. Shaker-incubator 37°C.
5. Pipettes.
6. Loop for inoculating bacteria.
7. Petri dishes + burner

Transformation protocol

1. Preparation

- Thaw competent E. coli cells on ice (15-30 min).
- Dissolve 40 g of prepared LB-agar powder in 1 liter of distilled water.
- Autoclave at 121°C, 15 min to sterilize.
- Cool the solution to 50-55°C (if hotter, the antibiotic will be destroyed).
- Add ampicillin to a final concentration of 50-100 µg/ml (e.g. 1 ml 50 mg/ml per 1 liter).
- Under sterile conditions (preferably in a laminar flow box or near a burner), pour 20-25 ml into each cup, turn the cups upside down.
- Remove the LB-agar cups with ampicillin and bring to room temperature.

2. Heat shock:

- In a sterile tube, add 50 µl of competent cells.
- Add 1-5 µl of plasmid DNA (~50-100 ng).
- Mix gently, incubate on ice for 20-30 min.
- Carry out heat shock: 42°C, 45 sec.
- Transfer immediately to ice for 2-5 min.
- Add 500 µl SOC or LB-bouillon, incubate at 37°C, 45-60 min, shaking (~200 rpm).

3. Seeding and selection of transformants

- Apply 50-200 µl of the mixture to LB-agar with ampicillin, spread evenly.
- Incubate at 37°C, 12-16 hours.

Protein expression in liquid culture

1. Preparation of liquid culture

- Select an individual colony, transfer to 5 ml LB + ampicillin.
- Incubate for 12-16 hours at 37°C, 200 rpm.

2. Main culture and induction

- Dilute overnight culture in 50-500 ml of LB + ampicillin (1:50-1:100).
- Incubate at 37°C until OD_{600} ~0.4-0.6 (2-4 hours).
- Add IPTG (0.1-1 mM), continue incubation:
    - 37°C, 2-6 hours (rapid expression).
    - 16-25°C, 12-24 hours (if soluble protein is needed).

Protocol for the synthesis of round gold nanoparticles (AuNPs) and their functionalization with FMN and tryptophan

Source: https://pubs.acs.org/doi/10.1021/jp061667w

1. Synthesis of spherical gold nanoparticles by Turkevich method

Required reagents

- Gold (III) chloride - $HAuCl_4$-$3H_2O$ (e.g. 1 mM solution).
- Trisodium citrate - $Na_3C_6H_5O_7$ (stabilizer and reducing agent).
- Deionized (Milli-Q) water.
- Thio-β-tryptophan
- 5'-thioflavinmononucleotide (Thiol-FMN)

A note on safety issues: although HAuCl4 is a fairly strong acid, thiol-substituted amino acids are the main problem. They are not biologically very dangerous, but extremely stinky, to work only in a fume hood.

Equipment

- Erlenmeyer flask (100-250 ml).
- Magnetic stirrer with heating.
- Glass pipette or automatic pipette.
- UV-visible spectrophotometer (for particle size control).

Synthesis protocol

1. Prepare 50 ml of a 1 mM solution of $HAuCl_4$ in deionized water.
2. Bring to a boil with constant stirring (~500 rpm).
3. Add 5 mL of 38.8 mM sodium citrate solution by rapid injection.
4. Continue boiling for 10-15 minutes, the solution will turn red (indicator of nanoparticle formation).
5. Cool to room temperature with stirring.
6. Store at 4°C in a dark container.

Quality control:

- Measure absorption spectrum ($\lambda max \approx 520$ nm indicates spherical particles ~15-20 nm).
- If the peak is shifted, vary the concentration of $HAuCl_4$ or citrate.

Hint: In my experience, it also makes sense to check the nanoparticles concentration with DLS method

Functionalization Protocol

Source: https://www.sciencedirect.com/science/article/pii/S0021979723011050

- Separate the colloidal solution into 2 samples.
- Add tFMN and tTrp to the AuNPs colloidal solution (usually 1:10 by molar ratio to gold).
- Incubate at room temperature for 1-2 hours with stirring.
- If necessary, wash the particles by centrifugation (10,000 g, 10 min) and resuspend in buffer.
- Store at 4°C.

Quality control:

- Measure the UV-visible absorption spectrum (a shift in λmax or broadening of the peak will indicate functionalization).
- Conduct a Zeta-potential (check the change in surface charge).