Schedule and Basic techniques for protein expression

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Date and Place

<Core curriculum>

July 9th and 10th, 2007, 13:00-17:00. Building A for Clinical research, 4th floor.

<Advanced Course >

July 9th, 2006, 13:00-18:00. Building A for Clinical research, 4th floor.

Schedule

<Core curriculum>

July 9th, 13:00-13:30: Guidance

Lecture about preparation of plasmid DNA, protein extraction.

14:00-17:00: Practice of mini-prep, Digest DNA, and electropholesis

July 10th, 13:00-13:30: Lecture about protein extraction, SDS-PAGE and cell culture

13:30-16:30: Practice of electropholesis, SDS-PAGE and cell culture

16:30-17:00: Summary

<Advanced Course >

July 13th, 13:00-14:00: Lecture about immunocytochemistry and fluorescent microscopy

14:00-16:30: Practice of immunocytochemistry.

16:30-17:00: Summary

Protocol

<Core curriculum>

A. DNA

Small scale preparation of plasmid DNA (mini-prep by PureLink™)

(Preparing Cell Lysate)

(1. Transfer 1.5 ml of an overnight culture (E.Coli in LB medium) to a microcentrifuge tube.)

(2. Centrifuge for 1 min at 15000 rpm at 4 degree. Decant the supernatant.)

3. Resuspend the pellet with 250 μl of Resuspension Buffer (R3) with RNase A by vortex.

4. Add 250 μl of Lysis buffer (L7) to cells. Mix gently by inverting the capped tube 5 times. **Do not vortex.**

5. Incubate the tube for 5 min at RT. **Do not exceed 5 min.**

6. Add 350 μl of Precipitation Buffer (N4). Mix immediately by gently inverting the capped tube 5 times.

Do not vortex.

7. Centrifuge at 15000 rpm for 10 min to clarify the lysate.

(Binding DNA)

1. Place a Mini column inside a 2-ml Wash Tube.

2. Load the supernatant form above-mentioned step 7 onto the spin column.

3. Centrifuge the column at 15000 rpm for 1 min. Discard the flow-through and place the column back in the tube.

4. Add 500 μl Wash Buffer (W10) with ethanol to the column. Incubate for 1 min at RT.
Centrifuge at 15000 rpm for 1 min. Discard the flow-through and place the column back in the tube.

5. Add 700 μl Wash Buffer (W9) with ethanol to the column.

6. Centrifuge at 15000 rpm for 1 min. Discard the flow-through.

7. Centrifuge at 15000 rpm for 1 min to remove any residual Wash buffer (W9).
Discard Wash tube with the flow-through.

(Eluting DNA)

1. Place the spin column in a clean 1.5-ml Recovery Tube.
Add 75 μl TE to the center of the column.

2. Incubate the column for 1 min at RT.

3. Centrifuge at 15000 rpm for 2 min.

4. The elution tube contains your plasmid DNA. Discard the column.

Digestion of plasmid DNA

Mix reagents described below in a microcentrifuge tube. Incubate it at 37 degree for 60 min.

T7blue-MUC5AC	5 μl
10x buffer (M)	2 μl
SacI	1 μl
SpeI	1 μl
H2O	11 μl
	20 μl

Electrophoresis in an agarose gel

1. Digested DNA 9 μl + 10x loading dye 1 μl
2. Apply the samples to an agarose gel.
3. Run a gel at 100V for 30 min.
4. Visualize the DNA under UV.

B. Protein

Protein extraction and SDS-PAGE

1. Cultured cells are lysed and scraped on ice with 300μl lysis buffer
2. Collect the sample and mix vigorously for 20 sec
3. Centrifuge 15000rpm for 10min
4. Collect supernatant and add 100 μl 4x sample buffer
5. Boil at 100 degree for 2min
6. Apply the 20 μl samples onto an 5-20% acrylamide gel.
7. Run the gel until the dye reaches the bottom.

C. Cell Culture

Reviving frozen cells

1. Place the frozen vial containing NCI-H292 cell at 37C degree waterbath just long enough to thaw contents.
2. Slowly remove the cell suspension and place in 15 ml centrifuge tube that contain 5 ml of pre-warmed RPMI + 10% FBS.
3. Centrifuge for 5 min at 1500 rpm.
4. Aspirate the medium and leave the cell pellet.
5. Gently resuspend the cell pellet with 5 ml of RPMI + 10% FBS.
6. Count the cells in hemocytemeter.
7. Transfer the cells to 60 mm dish. Place the dish in a CO_2 incubator.

Protocol

<Advanced Course >

A. Western blotting

1. Proteins are separated by SDS-PAGE (see Core curriculum B. Protein)
2. After electrophoresis, equilibrate the gels in transfer buffer
3. Transfer proteins electrophoretically to polyvinyliden fluoride (PVDF) membranes
4. Block non-specific binding sites by immersing the membrane in 5% fat-free skim milk in PBS containing 0.005% Tween 20 for 30-60 min at RT
5. Wash with TBS-T 3 times
6. Incubate membranes with appropriately diluted primary antibody* for 40-60min at RT
7. Wash with TBS-T 3 times
8. Incubate membranes with diluted HRP-conjugated secondary antibody** for 40-60 min at RT
9. Wash with TBS-T 3 times and incubate the membrane in the dilution for 30 min at RT on an orbital shaker
10. Drain the excess wash buffer and put the membrane in Ziploc
11. Mix detection solutions A and B in a ratio of 40:1 and pipette it in Ziploc
12. Gently smooth out any air bubbles and place the membrane in chemiluminescence detector.

*We use p44/p42 Map Kinase (Erk1/Erk2) antibody and Pospho-p44/p42 Map Kinase (Thr202/Tyr204) antibody for primary antibody.

**We use HRP-conjugated anti-rabbit IgG whole antibody for secondary antibody.

Pospho-p44/p42 MapK Ab