Schedule of basic technique for gene analysis

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Date
July 3rd, 2007, 13:00 – 19:00
Practical training I (core curriculum)
July 10th, 2007, 13:00 – 19:00
Practical training II (advanced course)

Place: Building A for clinical research, 2nd floor, department of medicine and biological science
Room number MC2-17

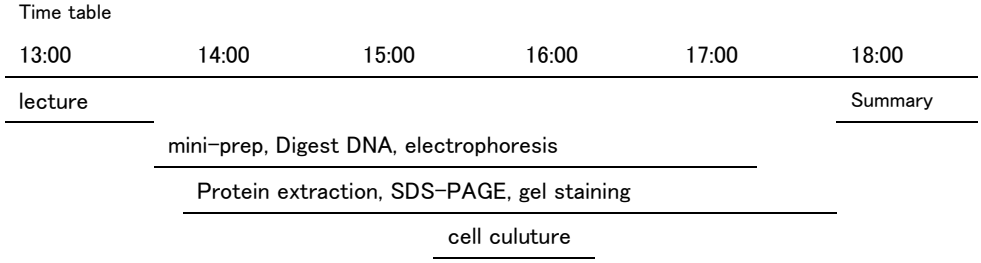
Contents: basic training to handle DNA, RNA and protein

Aim: Protein expression is translated from messenger RNA, which is transcribed from genomic DNA. Profiling of gene and protein expression and functional analysis of the products is basic as well as necessary for biological science. Therefore, we provide basic training program for techniques to handle these materials, or DNA, RNA and protein. The core curriculum includes (A) small scale preparation of plasmid DNA with spin columns (B) crude extraction of protein from cultured cells and SDS-PAGE and (C) basic technique to manipulate cultured cells. The advanced course further includes (D) subcloning and (E) RNA extraction and electrophoresis.

Contents of the core curriculum
A. DNA
(1) Plasmid preparation
Plasmid DNA will be extracted from transformed E. coli with spin columns (Qiagen).
(2) Digestion of plasmid DNA with restriction enzymes
Extracted DNA will be digested with restriction enzymes. Digested DNA will be run in an agarose gel and be visualized under UV.

B. Protein
(1) Crude extraction of protein
Protein will be extracted from cultured cells with lysis buffer.
(2) SDS-PAGE and protein staining
Extracted protein will be size-fractionated in an acrylamide gel and be stained with coomassie brilliant blue.

C. Cell culture
Preparation of cultured cell from frozen stock.
Study basic technique to keep aseptic condition. Thaw frozen stock, count cell number and transfer them to a dish.



Contents of advanced course

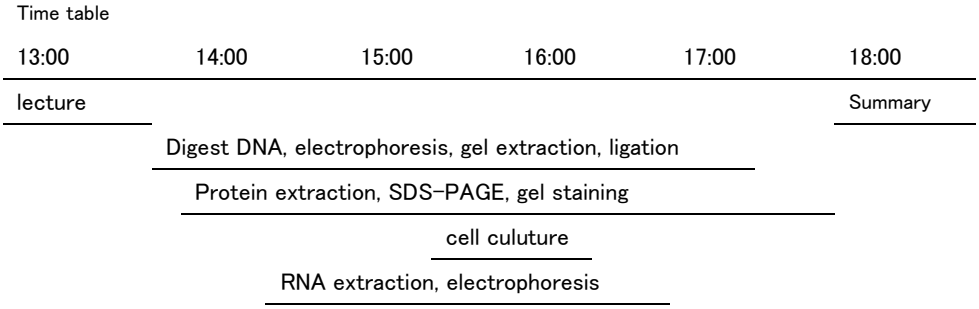
The advanced course includes (A) DNA, (B) protein, and (C) cell culture.
This course further includes (D) and (E).

D. Subcloning

(1) Digest plasmid DNAs with restriction enzymes. The digested plasmid will be size-fractionated in an agarose gel by electrophoresis. The DNA fragments will be extracted from the gel with gel extraction kit (Qiagen).
(2) The extracted DNA (plasmid + insert) will be ligated with T4 ligase. Competent cells (E.coli) will be transformed with the ligated DNA by heat shock method.

E. RNA extraction and electrophoresis

Total RNA will be extracted from cultured cells with Isogen reagenet (Nippon Gene).
The extracted RNA will be size-fractionated in a denaturing agarose gel and be visualized under UV.



Protocol

A. DNA

Small scale preparation of plasmid DNA (mini-prep)

1. Transfer 1 ml of bacterial cells to a microcentrifuge tube.
2. Centrifuge for 2 min at 3000 rpm, Decant the supernatatnt.
3. Resuspend the bacterial pellet with 250 μl of P1 buffer by vortex.
4. Add 250 μl of P2 buffer and gently invert the tube 5 times to mix.
5. Add 350 μl of P3 buffer and invert the tube immediatedly but gently 5 times.
6. Centrifuge for 10 min at 13000 rpm.
7. Apply the supernatant to the QIAprep spin column by pipetting.
8. Centrifuge for 60 sec at 13000 rpm and Discard the flow-through.
9. Wash the column by adding 750 ml of PE buffer.
10. Centrifuge for 60 sec at 13000 rpm and Discard the flow-through.
11. Centrifuge for another 60 sec at 13000 rpm.
12. Place the QIAprep column in a clean microcentrifuge tube.
13. Add 50 ml of H2O and let stand for 60 sec.
14. Centrifuge for 60 sec at 13000 rpm to harvest plasmid DNA.

Digest plasmid DNA

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

TA-vector	10 μl
10x buffer (H)	2 μl
EcoRI	1 μl
H2O	7 μl
	20 μl

Electrophoresis in an agarose gel

1. Digested DNA 20 μl + 6x loading dye 4 μl
   Undigested DNA 5 μl + 6x loading dye 2 μl
2. Apply the samples to an agarose gel.
3. Run a gel at 100V for 20 min.
4. Visualize the DNA under UV.

B. Protein

Protein extraction and SDS-PAGE

1. Aspirate the culture medium. Add 2 ml of PBS and agitate it. Aspirate it again.
2. Add 150 μl of lysis buffer and scrape cells. Transfer the cell lysate to a microcentrifuge tube.
3. Vortex for 30 sec. Centrifuge for 10 min at 13000 rpm at 4℃.
4. Take 5 μl of the supernatant and mix with 5 μl of loading dye.
   Positive control: BSA (1 μg/μl) 5 μl
5. Apply the samples to an acrylamide gel.
6. Run a gel at 150V until the dye reaches the bottom.

Gel staining

1. Remove the gel from the plates and stain it with CBB.
2. De-stain it with de-staining solution until protein bands are visible.

C. Cell culture

1. Thaw a frozen stock tube containing 293T cells at 37℃.
2. Transfer the cells to a microcentrifuge tube containing 5 ml of pre-warmed medium.
3. Centrifuge for 5 min at 1000 rpm.
4. Aspirate the medium and leave the cell pellet.
5. Resuspend the pellet with 5 ml of medium.
6. Count the cell number with hemocytemeter.

7. Transfer the cells to 60 mm dish. Place the dish in an incubater.

D. Subcloning

Digest plasmid DNA and extranct fragmented DNA from an agarose gel

1. Mix reagents described below in a microcentrifuge tube. Incubate it at 37℃ for 60 min..
   Two kinds of plasmids (Vector + insert)

DNA (1.0 μg/μl)	5 μl
10x buffer (H)	2 μl
HindIII	1 μl
XbaI	1 μl
H2O	11 μl
	20 μl

2. Size-fractionate the digested DNA in an agarose gel at 100V. Excise the DNA fragment with a sharp razor.
3. Transfer the agarose block to a microcentrifuge tube. Add 3 volumes of buffer QG and dissolve the agarose at 50℃ for 10 min.
4. Add 1 gel volume of isopropanol to the sample and mix.
5. Place a QIAquick spin column in a collection tube.
6. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.
7. Discard flow-through, place QIAquick column back in the same collection tube and repeat step 6.
8. Discard flow-through. To wash, apply 0.75 ml of buffer PE to QIA quick column and centrifuge for 1 min.
9. Discard flow-through and centrifuge for an additional 1 min.
10. To elute DNA, add 50 ml of H2O to the center of the QIAquick membrane. Let the column stand for 1 min and then centrifuge for 1 min.

Ligation and transformation

1. Mix reagents described below in a microcentrifuge tube. Incubate it at room temperature for 10 min..

Insert DNA	9 μl
Plasmid Vector	1 μl
2x buffer	10 μl
T4 ligase	1 μl
	21 μl

2. Thaw competent cells on ice for 10 min.
3. Mix 60 μl of the competent cells with 6 μl of the ligation mix. Incubate on ice for 20 min.
4. Heat the tube at 42℃ in a water bath for 45 sec, rapidly transfer it to a crashed ice and leave it for 2 min.
5. Add 300 μl of LB medium and incubate for 60 min at 37℃.
6. Transfer the appropriate volume of transformed competent cells onto LB agar gel and incubate at 37℃ over night.

E. RNA extraction and electrophoresis

1. Aspirate medium from cultured cells. Add 500 μl of isogen, scrape cells and transfer it to a microcentrifuge tube.
2. Add 100 μl of chloroform, vortex 10 sec, and centrifuge at 14000 rpm for 10 min at 4℃.
3. Tranfer 200 μl of the supernatant to a microcentrifuge tube and add 200 μl of isopropanol. Vortex briefly.
4. Centrifuge at 14000 rpm for 10 min at 4℃.
5. Decant the supernatant. Add 500 μl of 70% ethanol and centrifuge at 14000 for 3 min at 4℃.
6. Decant the supernatant and dry the pellet briefly.
7. Dissolve the pellet with 30 μl of DEPC treated water.
8. Apply the RNA samples to a denaturing agarose gel. Run the gel at 100V for several hours. Check ribosomal RNA under UV.