Introduction to Common Molecular Biological Techniques Used in Research Laboratories

Dingtong Tan, Qiwen Chen, Winifer Ali, Ruitang Deng
College of Pharmacy, University of Rhode Island

Abstract

The main techniques used in molecular biology are cloning, PCR (Polymerase Chain Reaction), nucleic acid electrophoresis, DNA microarray analysis, in situ hybridization (HIS), sequencing (Sanger or Next Generation Sequencing (NGS)).
Molecular biology techniques are extremely valuable for addressing a wide range of issues impacting the general state of humanity, in addition to investigating fundamental scientific concerns.
Applications for molecular biology techniques include the prevention and treatment of disease, the creation of new protein products, and the modification of plants and animals to produce desired phenotypic traits.

Technique Introduction

Passaging Cells

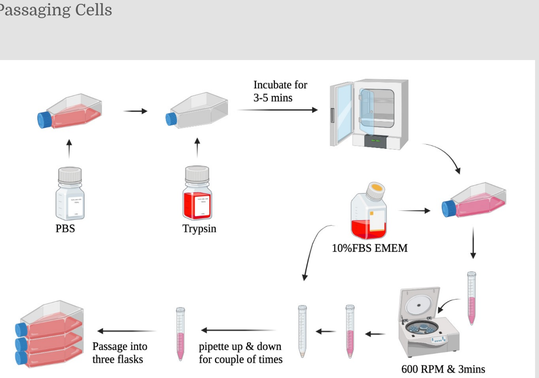


Figure1. Passaging cell workflow
The purpose of passaging cell is to give more space for the cell to grow to avoid confluency. All work must be done in a sterilized hood. All material going into the hood must be sprayed down with 75% ethanol.

Steps to passaging cells
1. Remove old media in the flask.
2. Add 5ml of PBS to wash away old media.
3. Add 3ml of Trypsin to detach the cells from the flask.
4. Incubate the flask for 3-5 mins.
5. Add 3ml of EMEM to stop the Trypsin reaction.
6. Pipette all solution into 15ml tube and centrifuge at 600 RPM for 3 mins.
7. Remove all solution with minimum disturbance to the cell pellet.
8. Add new media to the cell pellet and pipette up and down for couple of times.
9. Add cells into flask, containing fresh media.

Contents:

Technique Introduction

DNA Transfection

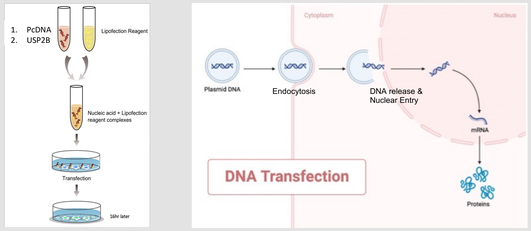


Figure2. DNA transfection workflow
The purpose of DNA transfection is to artificially introduce DNA into cells to enhance or inhibit specific gene expression.

Protein Extraction

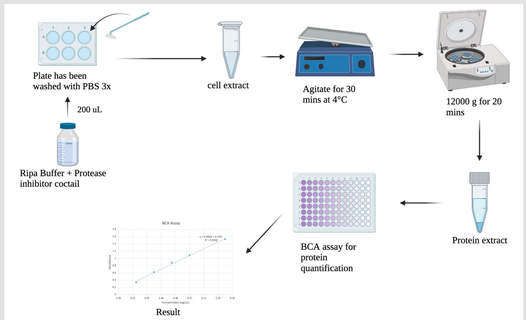


Figure3. Protein extraction workflow

BCA Assay

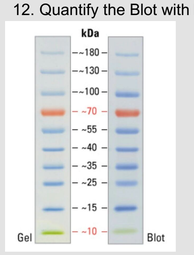
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.8222	1.5323	1.0878	0.8787	0.6109	0.3414	0.211	0.1133	0.0894	0.0469	0.0472	0.0489
B	1.8857	1.5375	1.0751	0.8918	0.6157	0.3335	0.2015	0.1104	0.0872	0.0459	0.0462	0.0467
C	1.9778	1.5209	1.1037	0.8788	0.6294	0.3348	0.2061	0.1123	0.0885	0.0465	0.0471	0.0467
D	0.8204	0.8291	0.8399	0.7949	0.7286	0.8699	0.9546	0.9561	0.9684	0.9015	0.0468	0.047
E	0.8365	0.7724	0.7406	0.7985	0.7315	0.9607	0.9381	0.8891	0.8741	0.8912	0.0472	0.0468
F	0.9011	0.7396	0.7955	0.8115	0.698	0.9871	0.9144	0.8886	0.8898	0.969	0.0474	0.0487
G	0.0469	0.0464	0.046	0.0465	0.0475	0.0473	0.0476	0.0469	0.0483	0.0465	0.0468	0.0652
H	0.0481	0.0473	0.0474	0.0469	0.0475	0.0485	0.0485	0.0479	0.0468	0.0471	0.0475	0.0502

	average	concentration in well	contration in tube	Sample (20ug)	Loading buffer	water
pcDNA 1	0.853	0.076	2.530	47.434	69.000	21.566
pcDNA 2	0.780	0.068	2.275	52.752	69.000	16.248
pcDNA 3	0.792	0.069	2.316	51.817	69.000	17.183
pcDNA 4	0.802	0.070	2.350	51.068	69.000	17.932
pcDNA 5	0.719	0.062	2.060	58.263	69.000	10.737
USP2b 1	0.939	0.085	2.835	42.325	69.000	26.675
USP2b 2	0.936	0.085	2.823	42.512	69.000	26.488
USP2b 3	0.911	0.082	2.737	43.851	69.000	25.149
USP2b 4	0.911	0.082	2.735	43.879	69.000	25.121
USP2b 5	0.921	0.083	2.769	43.331	69.000	25.669

The purpose of BCA Assay is to quantify the protein concentration. The darker purple, the higher concentration. For the BCA Assay curve, the $R^2 = 1$ is the best.

Western Blot

1. Make the acrylamide Gel for Western Blot.
2. Prepare samples for Western Blot based on the calculation.
3. Load 10 uL of protein ladder and 20 uL of samples into the gel.
4. Run gel at 85V for 35 minutes and 110V for 1 hour and 15 minutes for protein separation.
5. Activate the PVDF membrane with methanol for 30 sec.
6. Transfer gel onto PVDF membrane for 2 hours at 0.30 amp.
7. Incubate membrane with 5% of milk for 1 hour.
8. Incubate with primary antibody overnight at 4°C.
9. Incubate membrane with secondary antibody for 1 hour.
10. From step 7 to step 9, wash membrane for 4 time each time with TBST.
11. Detect membrane with the chemiluminescent solution
12. Quantify the Blot with ImageJ.



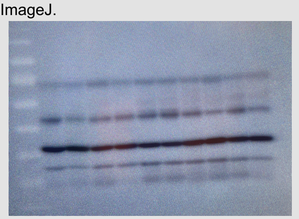


Figure4. Above showed Western Blot image of GAPDH at 35 kda.

DNA Extraction from Mice ear patch and PCR

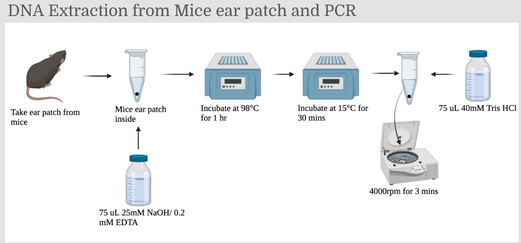
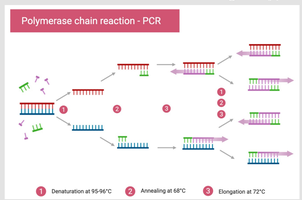
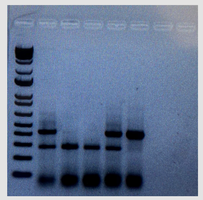


Figure5. DNA Extraction workflow
After centrifuging the tubes, the DNA is in the supernatant. The purpose of DNA Extraction is to obtain the genotypes of the mice used for the test.





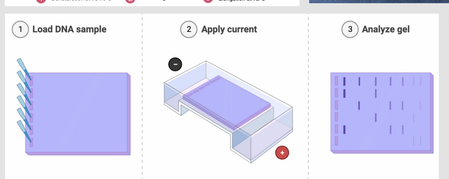
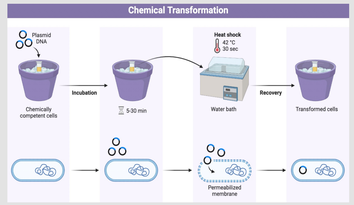


Figure6. Genotyping results

DNA samples are mixed with a master mix containing forward and reverse primer, then the mixture is put into the PCR for denaturation, annealing and elongation. The DNA is loaded onto agarose gel with the DNA ladder, and image with UV light.

Bacteria transformation



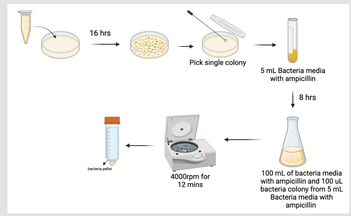


Figure7 and 8. Bacteria transformation workflow

Bacterial transformation is a process of horizontal gene transfer by which some bacteria take up foreign genetic material (naked DNA) from the environment. The purpose of bacteria transformation in this experiment is to express large amounts of proteins and enzymes.

DNA purification from bacteria (midi prep)

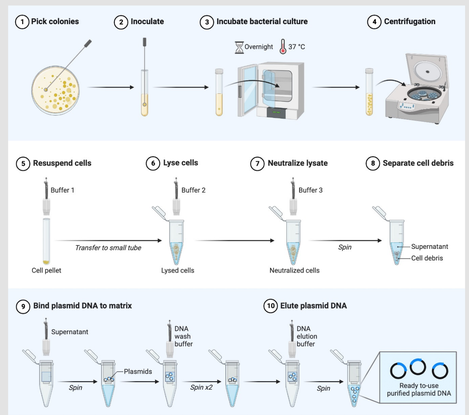


Figure9. DNA purification workflow
The purpose of DNA purification is to help extract genomic and plasmid DNA in the sample quantities that the research requires.

Acknowledgements

Pictures used for illustrating are made on *Biorender* Website.
Abstract information is taken from clinisciences.