

## How to Begin Molecular Research of Metabolic Diseases

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Advancing higher education is an important goal within the scientific and medical communities. The Korean Endocrine Society has worked with medical researchers who hope to conduct molecular research in addition to their clinical education. Based on concepts developed at a 2016 educational workshop, this article summarizes the requirement for a strong foundation in the performance of molecular research. Specifically, recent articles in metabolic research are highlighted to provide examples of commonly used techniques in this field of study.

**Keywords:** Molecular research; Basic experiment; Gene cloning; Metabolic research; Models, theoretical

### INTRODUCTION

Molecular research is an exciting and rewarding component of the medical field, though the decision to pursue this career can be intimidating. Thus, when researchers open molecular biology textbooks, primary emphasis is placed on the central dogma, gene expression, protein folding and modification, and other key concepts. These concepts require careful attention and dedicated study. Unfortunately, it is often difficult to appreciate the link between the textbook concepts and the skills needed for successful basic science research. Skilled laboratory practice is the product of a solid theoretical foundation.

This article focuses on the molecular technologies available for metabolic research, particularly for young scientists who want to gain a greater appreciation for the field of medical science. Of course, researchers may start with making solutions and gels, efficient pipetting technique, and RNA isolation. However, we know that most beginners often wonder what they should study first. There is a seemingly endless supply of books,

articles, and other resources available to scientists, some of which are too broad, while others are too specific. In fact, we do not need to read the *Molecular Cloning* by Sambrook, the classic textbook of molecular work, all the way through. In many cases, it is impossible for us to know everything before we do. Nevertheless, for young scientists interested in molecular research, it is helpful to know how experts developed their educational foundations. This article highlights some of their important advice.

### IMPORTANT CONCEPTS FOR LAB WORK

#### Basic molecular biology

Laboratory work requires understanding of the concepts and mechanisms emphasized in molecular biology. A central concept of molecular biology is the theory of gene expression. This includes gene structure (i.e., promoter, exon, intron), chromosome and epigenetic regulation, transcription, and translation, all of which are related with understanding gene expression. Since

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**Table 1.** Essential and Basic Molecular Concepts

1. Eukaryotic gene structure: promoter, exon, intron, 5'-untranslating region (5'-UTR), 3'-UTR, coding sequence, etc.
2. Gene expression: promoter regulation, codon, transcription factor, transcription, splicing, translation, etc.
3. Protein structure: amino acid sequence, protein domain, protein folding, protein modification (particularly phosphorylation), protein-protein interaction, etc.
4. Gene cloning: recombinant DNA, gene manipulation, vector and plasmid, restriction enzyme, ligation, transformation, competent cells, antibiotic selection, DNA purification, etc.

proteins are often the end product of gene expression, we investigate mutations, genome analysis, and changes in mRNA and protein expression in order to identify relationships between pathologic cellular changes and protein functions. Although gene expression is not always associated with the final physiologic changes, the purpose of molecular work is basically to analyze how cells act in response to various gene expression signals. If you are new to the field of molecular research, important review concepts in molecular biology are summarized in Table 1.

### Basic molecular experiments

Many recent breakthroughs in the field of molecular biology stemmed from the discovery of restriction endonucleases and DNA ligases. These enzymes allowed researchers to manipulate DNA, opening a new era for the life sciences based on molecular biology. Recombinant DNA technology demonstrates a link between theoretical and practical concepts in molecular biology. Using this technique, researchers were able to produce recombinant DNA by the process known as “gene cloning.” As listed in Table 2, basic molecular experiments include handling and manipulating DNA, RNA, protein, cells, and animals. However, many labs are unaware of the importance of gene cloning. Gene cloning is a series of molecular reactions, encompassing DNA cutting or amplification, ligation, DNA preparation, transformation, antibiotic selection, DNA sequencing, and gene expression (Table 1). Gene cloning will teach you how all the molecular work came to be possible, how you can manipulate enzymatic reactions, how you link theory and practice, and that molecular research is fun. Therefore, it is strongly recommended that people who want to emphasize important molecular concepts while learning essential laboratory techniques start with gene cloning. This is also why many laboratory science books discuss the production of recombinant DNA in their first chapter.

There is another key reason for beginners to learn the funda-

**Table 2.** Basic and Advanced Molecular Experiments

1. Molecular cloning
  - Cloning vectors, eukaryotic/prokaryotic expression vectors, plasmid/virus vectors
  - Enzymes (restriction enzyme, ligase, and other useful enzymes)
  - Bacterial transformation, competent cells, antibiotic selection
  - Plasmid DNA purification
  - DNA sequencing
2. Extraction and preparation of DNA, RNA, and proteins
  - Extraction of genomic DNA or plasmid DNA
  - Extraction of total RNA, avoiding degradation
  - Protein preparation, choosing an optimal lysis buffer based on purpose
3. Molecular detection techniques
  - Polymerase chain reaction (PCR) and reverse transcriptase-PCR
  - Real-time PCR
  - (Agarose) gel electrophoresis, (affinity) chromatography, (ultra) centrifugation
  - SDS-PAGE and Western blot analysis
  - Immunoprecipitation
  - ELISA
4. Manipulation of molecules
  - Site-directed mutagenesis
  - siRNA and shRNA technology
5. Introducing molecules
  - Transfection using chemicals, lipofectamine, virus, and electroporation
6. Handling of experimental models
  - Cell culture
  - Animal experiments
  - Genetically modified mouse model (transgenic or knockout)

SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; siRNA, small interfering RNA; shRNA, short hairpin RNA.

mentals of gene cloning technology. The most powerful molecular analysis comes from “gain-of-function” and “loss-of-function” studies in cellular or animal systems. In such studies, researchers observe what happens when a specific protein is absent or present and attempt to identify the gene(s) responsible for a specific disease. A combination of “gain-of-function” and “loss-of-function” tests leads to many of the valuable discoveries in modern life science. Thus, it is important to learn how to use vectors to enhance or repress gene expression.

What skills in molecular techniques are essential? We may have an answer from a hint that many labs look for post-doctorates with a “strong background in molecular biology.” A strong molecular background generally means whether researchers are comfortable performing the following techniques: (1) molecular cloning; (2) extraction and preparation of DNA, RNA, and proteins; (3) molecular detection techniques; (4) manipulation of

**Table 3.** Experimental Model Introduced in 52 Articles Published in the Journal *Cell Metabolism* from January 2016 to July 2016

Model	Frequency, n (%)	Cell lines or animal models
Cell	27 (52)	Primary culture, 3T3-L1, HEK293T, NIH-3T3, hiPSC, hESC, adult mouse ear fibroblast, mouse embryonic fibroblast cell, Huh7, A549, H1975, MIN6, IMR-90, MCH46, HK-2
Animal	47 (90)	Mouse, <i>Drosophila</i> , <i>Caenorhabditis elegans</i> , rat, zebrafish
Human	8 (15)	-

HEK293T, human embryonic kidney cells 293 V40 large T antigen; hiPSC, human induced pluripotent stem cell; hESC, human embryonic stem cell.

molecules; (5) introducing molecules; and (6) handling of experimental models. Table 2 summarizes some of the experiments that should be familiar to a skilled researcher with a strong background in molecular biology.

## SPECIAL EXPERIMENTS IN METABOLIC RESEARCH

Metabolic research incorporates many of the experimental techniques highlighted in the previous section. However, there are certain tools that are specifically important to the field of metabolism. This report discusses recent articles to provide a practical guideline to highlight important techniques for beginners interested in metabolic research.

### Experimental model

We summarized methods and main figures from 52 articles published in *Cell Metabolism*, one of the leading journals in metabolic research, from January 2016 to July 2016 (<http://www.cell.com/cell-metabolism/archive>). Main topics include the following: obesity, insulin secretion/resistance, glucose homeostasis, one-carbon metabolism, circadian rhythm, neuronal response to food or fasting,  $\beta$ -cell, atherosclerosis, appetite, lipolysis, lipid uptake, triglyceride, thermogenesis, browning, beige fat, mitochondrial dysfunction, aging, and lifespan. Regarding the experimental model, 52% of articles studied on cells; in details, primary cell culture was most widely—approximately 40 percents—used. Studies using 3T3-L1 and HEK293T cell lines were the second most common. Animal models were frequently used in these studies; 47 of the 52 articles contained

an animal model, and eight articles involved a human study (Table 3).

### Animal experiments regarding metabolism

As mentioned previously, animal modeling is the most popular experimental design. Researchers should begin by choosing an animal that can successfully model their hypothesis. In our review, mice were used most commonly (42 of 52 articles). *Drosophila* and *Caenorhabditis elegans* were each used in 6% of articles, with rat and zebrafish used in 4% and 2% of articles, respectively. Once an animal model is selected, the details of the experiment should be clarified. For instance, if the study requires a drug injection, determining the age of the animal at the time of injection, injection route, dose, interval of injection, and time of observation need to be decided in advance. When making decisions about drug formulation, it is always important to consider drug solubility.

In metabolism studies, obesity or diabetes mellitus are frequently induced by feeding a high-fat diet (HFD) to C57BL/6 mice (also called B6 mice). Conventionally, development of HFD-induced obesity takes more than 12 weeks of HFD feeding; however, there have been new protocols so that researchers have multiple options. Moreover, knockout mice, transgenic mice, and knock-in mice can be suitable models for metabolic studies.

### Metabolic index and molecular index

Once cells or animals are selected as experimental models, researchers can check many things related to metabolism. For convenience, we define that metabolic index is important parameters for metabolic study. The metabolic index can be categorized into four groups: physiologic index containing whole body morphology and activity, serum index, tissue index, and functional index. Tissue index includes, for instance, morphology or triglyceride content of individual organs such as liver, muscle, pancreas, white adipose tissue, and brown adipose tissue. Metabolic indexes used in the articles in *Cell Metabolism* are summarized in Table 4.

It needs to be considered beforehand if the index should be measured before or after death of the animal. Although it is the best to record each index time to time on live mouse, it has a limit. Luckily, a growing number of imaging technique, which we will introduce in later section, has brought tools to record dynamic change of the animals. Also, there are other advanced experimental techniques such as comprehensive laboratory animal monitoring system (CLAMS) studies or metabolic cage

**Table 4.** Metabolic and Molecular Indexes Shown in *Cell Metabolism* from January 2016 to July 2016

Metabolic index	Molecular index
Physiologic index: Survival, Body weight, Body Mass Composition, Organ weight/ size, Rectal temperature, Food intake/ energy expenditure, Meal size/number, Feces production, Physical activity	Metabolic enzymes or pathway: GK, PFK, PK, PEPCK, G6Pase, FAS, ACC, FABP, FATP, HK1, HK2, PDH, Lipoprotein, Cyto C, Glut1
Serum index: Fasting/fed glucose, TG/cholesterol, Lipoprotein, Insulin, Glucagon, Fatty acid, Amino acids, ALT/AST, Bile acid, Lactate, Citrate, Fumarate, Succinate, Aspartate, Glutamate, Glutamine, Phosphoenolpyruvate	Thermogenic genes: UCP1, Dio2, ELOVL3, Cidea, Cox8b, Prdm16, Ebf2 White fat-selective genes: Tle3, Resistin, Gsta3, Lyz2 Brown/beige fat markers: Cited1, Ear2, Tmem26, Hoxa9, Lhx8, Zic1
Tissue index: Tissue weight, Adipocyte size, Tissue TG content	Transcription pathway: SREBPs, PPARs, PGC, ChREBP, LXR, CREB, 4EBP1
Functional index: Glucose tolerance test (oral/intraperitoneal), Insulin tolerance test, Urinary glucose excretion	Signaling pathway: IR, IRS, PI3K, AKT, ERK, SMAD, AMPK, TSC, mTOR, RAPTOR

GK, glucokinase; PFK, phosphofructokinase; PK, pyruvate kinase; PEPCK, phosphoenol pyruvate carboxykinase; G6Pase, glucose 6-phosphatase; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; FABP, fatty acid binding protein; FATP, fatty acid transport protein; HK1, hexokinase 1; HK2, hexokinase 2; PDH, pyruvate dehydrogenase; Cyto C, cytochrome C; Glut1, glucose transporter 1; UCP1, uncoupling protein 1; Dio2, deiodinase 2; ELOVL3, elongation of very long chain fatty acid-like 3 (fatty acid elongase 3); Cox8b, cytochrome C oxidase 8b; Prdm16, PR domain containing 16; Ebf2, early B-cell factor 2; Tle3, transducin-like enhancer of split 3; Gsta3, glutathione S-transferase alpha 3; Lyz2, lysozyme C-2 precursor; SREBP, sterol regulatory element-binding protein; PPAR, peroxisome proliferator-activated receptor; PGC, PPAR gamma coactivator; ChREBP, carbohydrate response element-binding protein; LXR, liver X receptor; CREB, cAMP response element-binding protein; 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; IR, insulin receptor; IRS, insulin receptor substrate; PI3K, phosphoinositol-4,5-bisphosphate 3-kinase; ERK, extracellular signal-regulated kinase; AMPK, 5' adenosine monophosphate-activated protein kinase; TSC, tuberous sclerosis complex; mTOR, mammalian target of rapamycin; RAPTOR, regulatory-associated protein of mTOR.

studies, which are difficult for individual researchers to set up. Among the 52 summarized articles, CLAMS was used in four studies.

In terms of molecular index, when mice are sacrificed or

**Table 5.** Molecular Research Tools Shown in *Cell Metabolism* from January 2016 to July 2016

Category	Techniques (frequency in the articles, out of 52)
Basic techniques	Western blot analysis (44) RT-PCR (35) siRNA or shRNA (10) ELISA (9) Immunoprecipitation (7) FACS (6) ChIP assay (4)
Screening tools	Metabolomics (9) Proteomics (8) Microarray (7) RNAseq (5) RNA interference screening (2) ChIPseq (1) Phosphoproteomics (1)
Metabolism-related or advanced techniques	ATP assay (4) Patch clamp study (3) Seahorse assay (3) Echo MRI analysis for fat mass (3) CRISPR/Cas9 (2) Thermal imaging (2) cAMP assay (2) Indirect calorimetry (2) Insulin secretion assay (1) Fatty acid oxidation assay (1) PER assay in <i>Drosophila</i> (1) Triglyceride colorimetric assay (1) Enzymatic colorimetric assay (1) Luciferase assay (1) AMPK activity assay (1) 2-Deoxyglucose uptake assay (1) Cycling assay (1)

RT-PCR, reverse transcriptase-polymerase chain reaction; siRNA, small interfering RNA; shRNA, short hairpin RNA; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; ChIP, chromatin immunoprecipitation; RNAseq, RNA sequencing; ChIPseq, ChIP sequencing; ATP, adenosine triphosphate; MRI, magnetic resonance imaging; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; cAMP, cyclic adenosine monophosphate; PER, proboscis extension response; AMPK, 5' adenosine monophosphate-activated protein kinase.

samples are prepared, we can explore various molecular changes related to metabolism. This analysis mostly involves detection of certain mRNA or protein, which can be possibly classified based on function into metabolic, transcription pathway,

and signaling pathway species (Table 4). Metabolic pathways such as glycolysis, gluconeogenesis, lipid synthesis, and lipid oxidation require further attention in studies of metabolism. Representative indexes cited in *Cell Metabolism* are listed in Table 4.

### **Cell experiments in metabolism**

In metabolism research, primary cell cultures are widely used, which include primary hepatocytes, adipose-derived stem cells, and mouse embryonic stem cells (MEF) when analyzing knock-out mice (Table 3). In our review of recent publications, 21 of 52 articles used primary cell cultures. These cultures are relatively difficult to extract, so extra caution and planning are required. In terms of established cell lines, liver studies often use HepG2, Alexander, HII4E, or AML-12 cell lines; but researchers should remind that these cell lines lost some of important characteristics of normal hepatocytes. Muscle studies use C2C12 and L6 cell lines, which require differentiation. Lastly, adipocyte studies largely depend on 3T3-L1 cells, which also require the differentiation of preadipocytes resembling fibroblasts.

Similarly to animal studies, molecular indexes can be measured in cells, or changes in morphology can be observed. Conventional histology, immunohistochemistry, and immunofluorescence staining are all potential methods for such analysis; it is a strength of cell experiments that imaging study with confocal microscope can be performed. Images from confocal microscopy are shown as a main figure in eight of 52 articles in *Cell Metabolism*. Other imaging tools in the articles include Normanski imaging, intravital microscopy, positron emission tomography/computed tomography (PET/CT), microCT, and electron microscopy.

### **Specific experimental methods in metabolic research**

As explained earlier, basic experimental tools include Western blot and RT-PCR. Table 5 summarizes the frequency of these techniques in recent publications. A variety of specialized and advanced imaging, screening, and assay tools are used for anal-

ysis, depending on the specific study aim(s). In terms of imaging, confocal microscopy, often with immunofluorescence staining and electron microscopy, is one of the most popular techniques. MicroCT, PET/CT, and intravital microscopy are also highly effective for use with animal models.

Many papers have identified new genes or proteins using a variety of screening tools. The variety of screening tools (used in 33 of 52 articles) are listed in Table 5. This emphasizes the importance of high-throughput screening analysis for publication in a top-tier journal. In *Cell Metabolism* this year, metabolomics is the most widely used screening tool with PCA scatter plot analysis, gene ontology analysis, tissue specific expression analysis, and evolutionary analysis. Table 5 also summarizes the analysis techniques frequently used in metabolic research.

## **CONCLUSIONS**

In this article, we wanted to provide simple guidelines for a researcher new to the field of molecular research. We know that beginners are always looking for concise guidelines or summaries. While reading this guideline will not make you an expert in the field, time spent in the laboratory can help you to achieve a strong background in molecular biology. Remember, the final aim of molecular work is to explore the unknown. The field focuses on learning and clarifying important concepts. Furthering scientific progress is an inspiring opportunity, so go get started.

## **CONFLICTS OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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