



Ferdowsi University of Mashhad



Institute of Biotechnology

Advanced Training Course in Cell and Molecular Techniques

**Presented by Institute of Biotechnology
Ferdowsi University of Mashhad**

Summer 2018

Stem Cell Derivation & Characterization Workshop
18-23 August 2018

Stem Cell Derivation & Characterization Workshop 18-23 August 2018	
<ul style="list-style-type: none"> • Introduction to biotechnology • Overview of workshops (stem cell derivation and cultivation, induction of stem cell differentiation, stem cell characterization by flow cytometry and immunostaining) • Introduction to stem cells 	Duration
Practice on cell culture principles; cell passage, freeze and defrost	1 day
Practice on stem cell derivation from rat bone marrow	1 day
<ul style="list-style-type: none"> • Introduction to differentiation and characterization of stem cells 	
Inducing differentiation of stem cells toward mesodermal lineages	2 days
Practice on flow cytometry detection of superficial antigens	1 day
<ul style="list-style-type: none"> • Visiting laboratories in Institute of Biotechnology, Faculty of Science, Faculty of Veterinary Medicine, Faculty of Agriculture (Ferdowsi University of Mashhad), and ACECR, Mashhad • Group meeting 	1 day

Gene Expression Analysis Workshop 25-30 August 2018	
<ul style="list-style-type: none"> • Introduction to biotechnology • Overview of workshops (gene expression analysis at mRNA and protein levels) 	Duration
Practice on RNA extraction from human cells	1 day
<ul style="list-style-type: none"> • Principles of quantitative RT-PCR 	
Synthesis of cDNA followed by PCR and electrophoresis	1 day
Real time RT-PCR for quantitative analysis of gene expression	2 days
<ul style="list-style-type: none"> • Introduction to gene expression analysis at protein level 	
Practice on immunohistochemistry	2 days

Expression Analysis of RNA-seq Data Workshop 1-3 September 2018	
<ul style="list-style-type: none"> • Introduction to next generation sequencing (NGS) 	Duration
<ul style="list-style-type: none"> • Data input (raw sequence, gene annotation, reference genome) • Preprocessing (quality control of raw sequence-FastQC, trimming-Trimmomatic, indexing of genome reference-Bowtie2) 	1 day
<ul style="list-style-type: none"> • Alignment (Tophat2, BAM/SAM, IGV, RseQC) • Transcriptome assembly (Cufflinks, Cuffmerge) • Abundance estimation (Htseq, RPKM, FPKM) 	1 day
<ul style="list-style-type: none"> • Expression analysis (alternative and differential expressions) • Visualization and gene ontology analysis • Final reports (quality summery, significant gene, clustering patterns, pathway enrichment reports, statistic summery) 	1 day



Outlines of Stem Cell Workshop

- **Basic stem cell biology**
 1. General introduction to embryonic and adult stem cells
 2. *In vitro* maintenance of embryonic stem cells
 3. Inducing differentiation of embryonic stem cells
 4. Niche and function of adult stem cells
 5. *In vitro* maintenance and differentiation of adult stem cells
 6. Somatic cell reprogramming
- Applications of stem cells
 1. Clinical use of stem cells
 2. Stem cells and cloning
 3. Claims against therapeutic use of stem cells
 4. Stem cell theory of cancer
- Challenges to stem cell research
- Stem cell isolation and cultivation
 1. History of stem cell extraction
 2. Development of embryonic stem cell cultivation
 3. Culture conditions of tissue-specific stem cells
- ***Practice on cell culture principles; cell passage, freeze and defrost***
- ***Practice on mesenchymal stem cell extraction from rat bone marrow***
- ***Inducing differentiation of stem cells toward mesodermal lineages***
- ***Practice on flow cytometry detection of superficial antigens***

Outlines of Molecular Analysis Workshop

- **Introduction to gene expression analysis**

1. Basics of PCR and electrophoresis
2. PCR chemical components, reverse transcription, housekeeping gene
3. Primer design guidelines; primer specificity, length, GC content, 3' end stability, sequence complexity, melting temperature, location in the sequence, amplicon size, cross-exon boundary, primer and template sequence secondary structures
4. RT-PCR vs. blotting and microarray

- **Basics of real time PCR**

1. Practical and theoretical principles underlying real-time PCR
2. Detection of target DNA by binding dye or specific probes; TaqMan, molecular beacon, scorpion
3. Data analysis and reporting
4. Standard curve, amplification curve, melting curve, cycle threshold and base line
5. Relative and absolute expression analysis
6. Normalization standards, dilution method, exponential phase and efficiency calculation
7. Advantages and disadvantages
8. Applications of quantitative PCR

- **Introduction to methods for protein detection**

1. Principles of immunostaining; sample preparation; embedding and fixation
2. Preventing non-specific hydrophobic and ionic interactions
3. Antibody characteristics; selection and optimization
4. Practical aspects of antigen retrieval and blocking
5. Direct and indirect detection of antigen-antibody complex
6. Selection of controls; endogenous tissue, background control, no primary antibody control, isotype control, tissue type control
7. Problems and pitfalls of immunostaining; advantages and applications

- Antigen detection in paraffin-embedded tissue specimens

- ***Practice on RNA extraction from human cells***

- ***Synthesis of cDNA followed by PCR and electrophoresis***

- ***Establishing a real time PCR and followed by data analysis***

- ***Practice on immunohistochemistry***