List of molecular biology skills:

Gene Transcription and Expression

1) Western blot analysis: I have western blotted for a variety of proteins including viral proteins (Coronavirus, HSV, structural proteins, replication proteins), signal transduction proteins (Src family members, UPR proteins, eIF2α, interferon inducible proteins, TRAFs, phosphorylated proteins) and proteins involved in the cytoskeleton (F-BAR, WASp, Src family members).

2) Northern blot analysis: I have performed northern blot analysis for UPR inducible genes, control genes and coronavirus transcripts. Northern blots were performed using radioactive probes I cloned and synthesized.

3) Immunopercipitation and co-Immunopercipitation: I have performed immunopercipitation for radiolabeled coronavirus proteins mutants and proteolytically processed replicase proteins. I have co-immunopercipitated the interacting partners src/WASp/dynamin in different combinations with different mutants

4) real-time PCR: I have performed real-time PCR with the primer probe method and syber green. I have used primer probes for the detection of UPR target genes and coronavirus transcripts. I have used syber green for the detection of HSV transcripts and interferon regulated genes. I have successfully used PCR arrays using syber green to detect changes in NF-κB regulated genes. I have experience developing the primers for real-time assays and validating the use of the primers.

5) Reporter promoter assays (luciferase and β-gal): I have used the luciferase reporter gene to monitor the induction of several promoters including UPR elements, NF-κB and interferon, AP-1 regulated elements. I have also used β-gal expressing HSV viruses to monitor the replication of the virus.

6) Quantitative Flow Cytometry: I have used flow cytometry to measure the absolute number of TNF super family members and cell-adhesion molecules on different cell types. These assays were run using a calibrated standard curve for absolute quantitation.

7) ELISA:ELISAS were used to follow inflammatory genes and quantitate amounts of purified recombinant proteins.

8) Luminex assays: Luminex assays were used to monitor levels of cytokines under different conditions. Luminex assays include single and multi-analyte analysis.

Gene expression and manipulation

1) Generation of siRNA and shRNAs: I have designed shRNA vectors for knockdown of UPR, and F-BAR proteins and cloned the proteins into expression vectors. I have introduced siRNAs into different cell types for knockdown including macrophages, and fibroblasts

2) Knockout cell lines: I have immortalized and grown immortalized knockout cell lines including PERK, F-BAR knockouts, Nectin, and TNF superfamily. These cell lines were used in various functional assays

3) Overexpression/dominant negative expression: I have cloned and over expressed different proteins to either complement knockout cell lines or to examine the effects of a protein on a cell. These cell lines were also selected using eukaryotic antibiotics to create stable cell lines. I have overexpressed a variety of proteins to examine the biochemical interactions of the protein with its partners. I have overexpressed proteins to examine the function of single point mutations or deletions on function of the protein in various assays. I have overexpressed dominant negative version of proteins (lacking transactivator domains) to block transcription of genes from UPR promoters.

4) Cloning: My cloning experience includes cloning of genes into a variety of expression vectors, both eukaryotic and bacterial. I identified the orfs of the protein, designed primers, performed pcr, and inserted the orfs using restriction digestion and ligation. Often, antibody tags were inserted in frame with the orf to allow detection with monoclonal tag antibodies. For soluble recombinant proteins, they were inserted in frame with an affinity tag allowing them to be purified from eukaryotic or bacterial cell lysates.

5) Mutagenesis: PCR mutagenesis was used in a variety of projects to introduce mutations to disrupt protein function or find the minimal unit of function. Point mutations were introduced using site directed mutagenesis PCR and partially overlapping primers. I have used site directed mutagenesis to remove predicted glycosylation sites, catalytic residues in proteases, predicted phosphorylation sites, and areas were ligands interact with receptors. I have also PCR amplification and restriction digestion cloning to produce deletion mutants.

6) Antibody generation/validation: Cloned regions identified to be antigenic into GST expression vectors. These expression vectors were used to induce protein production in bacteria and proteins purified over columns. Recombinant protein were tested for purity and quantity and injected into animals using adjuvents. I then tested the serum from pre-bleeds for reactivity in various assays including western blot, immunofluorescence and immunopercipitation to identify applications for antibody. Proper controls were used to antibody specificity.

7) Recombinant proteins: Recombinant proteins were generated for several purposes including in vitro assays, positive controls, or cytokines. These proteins were produced in both eukaryotic and prokaryotic expression systems using expression tags. The proteins were purified over columns and purity tested by western blot and elisa. Functional assays such as reporter gene induction was used to ensure proper folding and processing of protein.

Microscopy/Fluorescence Microscopy/Flow Cytometry

1) Immunostaining: Immunostaining was used on fixed samples including macrophages, peripheral blood cells, adherent cell lines, primary adherent and differentiate cell types, fixed/section animal tissue and cell lines over expressing proteins of interest. Cells were stained using antibodies, fluorescent markers or fluorescent conjugated phallodin. Immunostaining was used to examine localization of viral proteins (coronavirus, HSV), transcription factors (UPR), cytoskeletal rearrangement (actin, tubulin, actin remodeling proteins), identification of infiltrating cell types, and expression of proteins in different cell types.

2) Geimsa staining: Geimsa staining was used to identify different cell types from peripheral blood.

3) Confocal microscopy: Confocal microscopy was used to provide high-resolution images of immunostained or GFP tagged proteins. Multiple tagged/fluorescent proteins were imaged for co-localization. Confocal microsopy was used to identify cytoskeletal structures including invadapodia in cell types.

4) Flow cytometry: Flow cytometry was used for several applications. I have extensive experience utilizing antibodies to identify immune cell type populations from mice. These markers were used to examine infiltrating cell types at the site of viral infection. These cell types included T cells subtype, B cells, neutrophils, macrophages/monocytes, and NK cells. Markers were also used to determine the purity of cell types extracted from different organs or regions of the mouse. Flow cytometry was also used to identify infected cells utilizing antibodies targeting viral proteins and reporter viruses.

Cell Culture and Differentiation

1) Non-adherent cells: I have cultured a variety non-adherent cell lines including human and mouse. These cell lines were of different lineages including macrophages, NK cells, B cells, and T cells.

2) Adherent cell lines: In my studies, I worked with a variety of immortalized cell lines including but not limited to HeLa, CHO, 293T, HT-29 (gastrointestinal cancer cell line), keratinocyte cell lines, breast cancer cell lines, prostate cancer cell lines, NIH3T3, and immortalized fibroblasts. These cell lines required a variety of growth medias and hormone conditions to allow them to grow efficiently.

3) Cell differentiation: I have grown and differentiated a variety of primary cell lines from humans and mice. I have isolated primary bone marrow cells and differentiated them into macrophages using CSF-1. I differentiated fibroblasts into adipocytes using a variety of hormones including insulin. These cells were shown to have lipid deposits in their cytoplasm. I have grown and differentiated keratinocytes and induced them to form adherence junctions using Mg and Ca.

4) Virus propagation/infection: During the course of my projects, I learned to grow a variety of viruses. Some of these viruses were used for studies on their replication and spread, while other viruses were used to introduce genes into cells. These viruses include vaccinia virus, coronaviruses, Herpes viruses, lentivirus, retrovirus and VSV. These viruses were grown in large batch cultures to provide high titer stocks for experiments. All of these viruses were handled in appropriate biosafety levels and all precautions were taken to avoid exposure. Virus titer was measured by plaque assay on Vero cells and other permissive cell lines. One-step and multi-step growth curves were also done to examine viral kinetics of mutants and viruses grown under anti-viral conditions. Experience pseudo-typing viruses with different receptors and receptor mutants to examine entry of viruses.

Functional Assays

1) Phagocytosis Assays: Macrophages isolated from mice or cell lines were induced or grown under conditions to enhance phagocytosis. I have experience with both opsinized red blood cells and beads. Opsins have included antibodies and zymogens. Readouts include enzymatic colormetric assays or fluorescent flow cytometry counting.

2) Fluid phase endocytosis/Antigen uptake: Several types of assays were used to measure fluid phase endocytosis and antigen uptake in cultured macrophages. Fluorescent assays include Lucifer yellow, fluorescent dextrans, and fluorescent ovalbumin. To enhance uptake, antibody/ovalbumin complexes were also used.

3) Migrations assays: Utilized several different assays to monitor migration of adherent and non-adherent cell types. Assays include transwell assays were chemokines were included in the lower channel and migration assessed as cells which have migrated through. Wound assays were used to measure migration rates of adherent cell lines.