UMMC Department of Pathology

Molecular Pathology Rotation

The molecular pathology rotation is scheduled for one month during the residency period. Molecular diagnostic testing has become standard of care within many areas of pathology; hence, you will find molecular integrated into many other rotations and a strong working knowledge of molecular pathology is essential for all pathologists in training. On completion of the month, the resident should form a broad, foundational understanding of molecular pathology.

General competencies are focused in the following major subject areas (based off recommendations by the Association of Molecular Pathology, J Mol Diagn 2016, 18: 153-162; http://dx.doi.org/10.1016/j.jmoldx.2015.10.006):

1. Basic molecular pathology goals/laboratory management
2. Basic concepts in molecular biology and genetics
3. Technology (shared with Cytogenetics rotation)
4. Inherited disorders (shared with Cytogenetics rotation)
5. Oncology (shared with Surgical Pathology rotation)
6. Infectious disease (shared with Microbiology rotation)
7. Pharmacogenetics
8. Histocompatibility and identity (shared with Immunology rotation)
9. Genomics
10. Information management

Checklist items:

1. Read current procedures of molecular tests performed in-house and observe sample throughput
2. Interpret patient test results
3. Attend genetics clinic and/or genetic counseling session with Dr. Abdulrahman or Dr. Maher and staff
4. Review quality controls and proficiency testing
5. Reading or other assignments as provided by faculty
6. Discuss/read about as many of the required and recommended goals for each of the 10 major subject areas as possible

Basic Molecular Pathology Goals/Laboratory Management Goals

Required:

1. Assist clinicians regarding appropriate test ordering/use (including appropriate sample selection and use of molecular testing at autopsy)
2. Define the test designations RUO, IUO, ASR, IVD, and LDP
3. Explain the regulatory requirements governing molecular diagnostic testing and compare the differences and similarities among the various test types (RUO, IUO, ASR, IVD, and LDP)
4. Describe quality assurance and quality control for molecular testing and discuss any differences among the various test types
5. Explain unidirectional flow and effective strategies for contamination prevention, with respect to the appropriate design and workflow to reduce the risk of carryover contamination
6. Explain the important legal, ethical, and social implications with regards to larger-scale or genomic testing and the ability to blind analytes to prevent unintended data analysis (e.g., next-generation sequencing for cancer discovering an autosomal dominant condition)

Recommended:

1. Be familiar with the CAP checklist related to molecular pathology and explain ways to implement these recommendations in relation to testing practices, quality, safety, and other areas
2. Be familiar with the training requirements of qualified molecular pathology laboratory personnel and how they differ from other clinical laboratory settings

Basic Concepts in Molecular Biology and Genetics Goals

Required:

1. Use the official gene nomenclature for communicating gene symbols and descriptive names via the HUGO Gene Nomenclature Committee (http://www.genenames.org)
2. Use mutation or sequence variant nomenclature based on the Human Genome Variation Society rules (http://www.hgvs.org/mutnomen)
3. Give examples of epigenetic changes and describe their physiologic function, e.g., changes in expression due to methylation and acetylation patterns
4. Describe human genetic variation and define variant polymorphisms, locus, allele, genotype, and phenotype, and copy number variation
5. Describe the major classes of potentially pathogenic variants, e.g. point, insertion, deletion, duplication, translocation, and inversion
6. Define missense, nonsense, synonymous, null, and frameshift in the context of mutation

Recommended:

1. Define other types of advanced –omes, such as epigenome, transcriptome, microbiome, and interactome
2. Explain contiguous gene syndromes
3. Describe the primary types of nucleic acid vectors (e.g., plasmid, cosmid, BAC, YAC, defective virus) and explain their function and potential importance in terms of diagnostics and therapeutics
4. Describe mitochondrial DNA and its inheritance pattern and define the term heteroplasmy

Technology Goals

Required:

Pre-analytical considerations

1. Describe the applications and limitations of specimen types and different methods of receiving specimens, e.g. heparin vs. EDTA vs. other anticoagulants for peripheral blood, as well as collecting samples from bone marrows and buccal swabs
2. List advantages and limitations of cell enrichment methods
3. Describe the minimum percentage of tumor cells needed depends on the method of analysis

Preparation techniques

4. List limitations of DNA, RNA, and miRNA isolated from FFPE, including safety issues for solvents
5. Describe preparative methods for karyotyping and for FISH
6. List common inhibitors of molecular analytical applications, especially PCR
7. Describe the preanalytical variables, such as fixation or overfixation, necrosis, and contamination, and the effects of tissue processing, fixatives, and stains on DNA and RNA integrity
8. Discuss storage conditions and requirements for primary samples and nucleic acids
9. List strengths and weaknesses of several methods for quantifying nucleic acids
10. Describe how to assess nucleic acid quality using spectrophotometry, electrophoresis, and PCR

11. Compare and contrast different methods for different variant types, including analysis and potential pitfalls, e.g. which methods are best suited for detecting a deletion of 2 bp, 100 bp, or 100,000 bp?
12. Explain gel electrophoresis, including capillary electrophoresis, for size analysis and Sanger sequencing
13. Define the activities and application of restriction enzymes, polymerases, and reverse transcriptases
14. Compare the methods and applications of Southern blot and Northern blot analyses

PCR methods

15. Describe basic PCR, including common variations, e.g. RT-PCR, allele-specific PCR, real-time PCR, and quantitative PCR

Real-time PCR

16. Describe and compare at least three real-time detection formats of PCR, i.e. dye binding, hydrolysis probes, and hybridization probes
17. Explain how real-time PCR data can be analyzed quantitatively

Non-PCR methods for nucleic acid analysis

18. Describe the differences between target amplification and signal amplification
19. Distinguish differences among forms of *in situ* hybridization, e.g. fluorescence, chromogenic, dual

Microarrays

20. Explain applications of microarrays, including determining gene copy number, gene expression, and single-nucleotide polymorphism detection

Sanger sequencing

21. Describe how the Sanger sequencing reaction works, including the detection of reaction products
22. Recognize and analyze common chromatogram patterns, such as heterozygous bases and insertion-deletions
23. Recognize common patterns of unacceptable sequence chromatograms
24. Describe the approximate analytic sensitivity of detection for a mutation (varies with type of mutation)
25. List pitfall primarily related to PCR that can lead to a false-negative result

Next-generation sequencing

26. Describe how performance characteristics of next-generation sequencing platforms compare to Sanger sequencing
27. Describe applications of next-generation sequencing to genetics, infectious diseases, prenatal medicine, and oncology, including circulating nucleic acids
28. Describe how the clinical impact of a sequence variant can be determined and classified, according to the scheme that best suits the testing type (i.e. ACMG nomenclature for describing germline variants)

Recommended:

Preparation techniques:

1. Outline preparative methods for DNA/RNA from various sample types and explain rationale of each step
2. Explain how to concentrate and how to dilute DNA/RNA specimens in a manner that does not compromise their use for molecular assays
3. Describe how whole genome amplification works on intact and degraded DNA samples and the associated pitfalls
4. Explain how DNA cloning works

PCR Methods

5. Describe PCR followed by single base pair extension
6. Define digital PCR and provide an example of its application and, in broad terms, the statistical underpinning

Microarrays

7. Describe different microarray formats, e.g. by probe type or one-color vs. two color
8. Describe the advantages and limitations of each microarray format, such as the ability of cytogenetic arrays to detect balanced translocations
9. List the factors that determine sensitivity and specificity, such as probe density, pseudogenes, and alternative splicing
10. Define issues in the analysis of gene expression arrays, including normalizing data, the multiple testing hypothesis problem, the distinction between training and validation data sets, and leave one out analysis

Next-generation sequencing

11. Contrast advantages and disadvantages of whole genome, whole exome, targeted gene panel, and transcriptome sequencing
12. Describe schematically the work flow for at least one next-generation sequencing method, including library preparation
13. Compare and contrast pull-down (or capture) and amplicon technology relative to resequencing assays
14. Broadly outline the steps in data pipeline and distinguish resequencing from *de novo* assembly
15. Explain the significance of coverage requirements and, qualitatively, the levels needed to investigate Mendelian traits, acquired somatic mutations, and hypermutable targets

Inherited Disorders Goals

Required:

1. Explain modes of inheritance for single-gene (Mendelian), mitochondrial, and complex disorders, e.g. autosomal dominant, autosomal recessive, X-linked, mosaicism, imprinting, qualitative vs. quantitative traits, genetic modifiers, and environmental modifiers
2. Describe the nucleic acid alterations that cause heritable disease and the most appropriate methods to detect those alteration, e.g. point mutations, splice site mutations, insertions/deletions, trinucleotide or unstable repeat expansions, copy number variation, methylation/imprinting defects, frequency within populations of different ethnicity, and nomenclature considerations
3. Discuss the impact of sample type for analyzing for the various alterations, e.g. blood serum, cultures cells (fibroblasts, amniocytes, chorionic villus sampling), preimplantation genetic diagnosis, cord blood, maternal cell contamination, patients with bone marrow or organ transplant, and DNA vs. RNA

Recommended:

1. Explain risk analysis, including Baysian analysis and odds ratios
2. Discuss the factors that affect the interpretation of the consequence of mutations in the context of anticipation, imcomplete penetrance, variable expressivity, X-inactivation, new (*de novo*) mutation rate, and consanguinity

Molecular Oncology Goals

Required:

1. Describe the distinction between tumor suppressor genes and oncogenes and how detection of aberrations in these genes is related to tumor biology

2. Discuss the major molecular mechanisms for inherited predispositions to tumors
3. Explain the indications for molecular testing in oncology, including applications in screening, diagnosis, treatment monitoring, prognosis, predict response to therapy, evaluation for underlying inherited conditions
4. Describe the major distinctions among assays used for diagnosis vs disease monitoring for hematologic malignant tumors (e.g. qualitative vs. quantitative testing for BCR-ABL1)
5. Discuss major translocations in leukemias and lymphomas and the appropriate testing methods for detection
6. Explain the difference between testing used for prognostic and predictive value and the clinical trials methods to establish these findings
7. Describe application of methylation analysis in oncology
8. Discuss predictive markers in various tumor types, e.g. lung carcinoma, colorectal carcinoma and melanoma

Recommended:

1. Describe major testing methods for methylation analysis

Infectious Diseases Goals

Required:

1. Explain species and genus-specific sequences
2. Explain the benefits and pitfalls of multiplexed systems for the detection of infectious agents
3. Explain the potential impact of sample matrix on assay performance and result interpretation
4. Explain the concepts involved in suing sequence data for organism identification
5. Explain the concepts involved in using mass spectrometric data for organism identification
6. Interpret the detection of the nucleic acids of infectious agents in clinical samples using both end-point and real-time PCR methods
7. Interpret the meaning of infectious agents detected in clinical samples that may or may not be pathogens
8. Interpret the results of a method designed to quantify infectious disease burden in clinical samples
9. Interpret the results of a molecular assay in which the goal of testing is to classify a microbe based on phylogenetic analysis
10. Interpret the results of *in situ* hybridization in the context of morphological features and clinical data
11. Interpret method validation results for qualitative and quantitative infectious organism testing

Recommended:

1. Explain the genetic basis of pathogenesis and drug resistance
2. Explain the generation of quantitative molecular data using quantitative standards
3. Interpret the results of a molecular assay in which the goal of testing is to determine variants associated with antimicrobial or antiviral resistance

Pharmacogenetics Goals

Required:

1. Define the terms used to classify metabolizer status
2. Discuss how polymorphisms of CYP family genes can alter the clearance of pharmaceuticals
3. Compare polymorphisms related to altered drug clearance with those associated with prodrug activation

4. Discuss polymorphisms that alter pharmacodynamics compared with those associated with changes in pharmacokinetics

Recommended:

1. List examples of drugs with altered clearance due to polymorphisms in the genes for their metabolic pathways (e.g. warfarin, SSRIs, opiates, irinotecan)
2. List examples of drugs with decreased prodrug activation due to genetic polymorphisms (e.g. clopidogrel, tamoxifen)

Histocompatibility and Identity Determination

Required:

1. Explain how genetic variation in the HLA genes is used to asses compatibility of solid organ and bone marrow transplants
2. Define chimerism and its role in hematopoietic cell engraftment analysis
3. Discuss the use of molecular identity testing in specimen source identification

Recommended:

1. Discuss the advantages and limitations of molecular-based HLA typing compared with serologic typing
2. Describe the basic structure of HLA nomenclature (e.g. HLA-A*30:14L, low resolution, high resolution)
3. List the typical molecular methods used for HLA typing (SSOP, SSP, sequencing)
4. Explain how STRs are used for engraftment analysis
5. Describe the use of informative peak intensities for calculating engraftment percentage
6. Describe STR testing in forensic identity and parentage testing

Genomics Goals

Required:

1. Define genomics
2. Understand the application and limitations of genomic analyses in heritable disease, oncology, infectious diseases, and preventive medicine and give examples
3. Explain the rationale for and composition of multidisciplinary teams needed for communication of genomic data to patients
4. Discuss the ethical considerations associated with genomic analysis
5. Define the unique regulatory challenges associated with genomic analyses
6. Use the major, publicly available databases to facilitate analysis of genomic data for both constitutional and tumor-specific indications
7. Compare quality control for genomic tests to quality control for single analyte tests
8. Compare reporting of genomic tests to reporting of single analyte tests

Information Management Goals

Required:

1. Discuss the basic concept of and limitations associated with a reference sequence
2. Understand the publicly available tools to identify potential clinical significance of novel variants in different contexts, e.g. tools to evaluate variants in heritable conditions vs. in somatic testing
3. Discuss the unique challenges associated with data storage and management for –omic analyses
4. Describe the unique regulatory issues related to the large data sets from genomic analyses
5. List several online software tools for molecular analysis and describe their utility, e.g. UCSC genome browser, dbVar

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Reading material and Resources:

1. *Diagnostic Molecular Pathology in Practice: A Case-Based Approach* by Iris Schrijver – you can borrow a hard copy from Dr. Chastain or an electronic copy is located on the R: drive under the Molecular folder
2. *Molecular Pathology in Clinical Practice* by Debra Leonard –Dr. Chastain has a copy of this book
3. *Molecular Genetic Testing in Surgical Pathology* by John Pfeifer
4. *Clinical Genomics* by Shashi Kulkarni and John Pfeifer – Dr. Chastain has a copy of this book
5. *Thompson and Thompson Genetics in Medicine* by Robert Nussbaum – Dr. Chastain has a copy of this book
6. *Gemonic and Personalized Medicine* by Geoffrey Ginsburg and Huntington Willard – electronic copy on R: drive, Molecular folder
7. *Molecular Diagnostics for the Clinical Laboratorian* by William Coleman and Gregory Tsongalis –electronic copy on R: drive, Molecular folder
8. 2013 AMP MGP review course – PDF and audio files in R:/Molecular
9. Association for Molecular Pathology (AMP) website http://amp.org/
10. CAP website with cancer protocols http://www.cap.org
11. Stanford University Genomic Medicine lectures: https://www.youtube.com/playlist?list=PLfTljtR5bxMcTg8hgQp9sA4YQwicpSAQv
12. NCCN guidelines http://www.nccn.org/ Practice guidelines including a ton of information about recommended genetic testing. Free but you have to have a login to access information.
13. ClinVar http://www.ncbi.nlm.nih.gov/clinvar/ Public database for specific clinically important mutations.
14. COSMIC http://cancer.sanger.ac.uk/cosmic Catalogue of Somatic Mutations in Cancer, maintained by Sanger Institute. Contains database of variants that have supposedly been identified as somatic cancer mutations (i.e. not germline); however, several errors in the database. Still contains abundant useful information.
15. dbSNP http://www.ncbi.nlm.nih.gov/SNP/ Database of short genetic variations occurring in normal individuals.
16. Exome Variant Server http://evs.gs.washington.edu/EVS/ Contains some SNPs that are not in dbSNP.
17. IARC TP53 database http://p53.iarc.fr/ Catalog of variants in TP53, including information on which ones have been reported in Li-Fraumeni syndrome
18. My Cancer Genome https://www.mycancergenome.org/ Summaries on specific mutations in specific cancers, maintained by Vanderbilt University.
19. USCS Genome Browser http://genome.ucsc.edu/ Genome viewer that can be used to explore a region of interest for SNPs or other features
20. Online Mendelian Inheritance in Man http://omim.org/
21. Training Residents in Genomics (TRIG) http://www.pathologylearning.org/trig -- also saved on R: drive

Prerequisite knowledge to Molecular Pathology Rotation

Many fundamental concepts of molecular diagnostics are taught during medical school or in undergraduate courses. Understanding of these concepts is essential to building further knowledge. Glance through the following list and quickly review any items that need refreshing.

Basic Molecular Pathology Goals/Laboratory Management:

1. Perform basic mathematical and statistical calculations used in the molecular laboratory, including, but not limited to, molarity, logarithmic and exponential conversions, regression, CIs, sensitivity, specificity, LOD, and linear range, etc
2. Confirm acceptability of new reagent lots

Basic Concepts in Molecular Biology and Genetics:

1. Define genome, exome, metabolome, and proteome

2. Explain the basic organization of the human genome and contrast with bacterial, viral, and other organisms
3. Describe and distinguish the unique properties of nucleic acids
4. Identify purines and pyrimidines
5. Describe Watson-Crick base pairing
6. Describe the structure of chromosomes
7. Define histones, chromatin, and heterochromatin
8. Describe replication of double-stranded DNA
9. Describe normal DNA repair processes
10. Explain basic eukaryotic and prokaryotic gene structure
11. Define transcription and contrast the process between prokaryotes and eukaryotes
12. Describe RNA processing eukaryotes
13. Define and describe the process of translation
14. List several posttranslational modifications
15. Define the various functional types of RNA, e.g. mRNA, tRNA, rRNA, snRNA and miRNA
16. Describe regulation of gene expression including induction, repression, and enhancers
17. Define *cis* and *trans*
18. Define transition and transversion in the context of point mutation
19. Describe how mutations may affect RNA splicing or stability
20. Explain how a mutation may alter transcription
21. Describe the basic pathways and signaling networks

Technology for Molecular Pathology:

1. Describe high content nucleic acid analysis, i.e. next-generation sequencing, addressing benefits and limitations, including multiple gene analysis, detection of mutations in genes unrelated to the disease state, detection of variants of unknown significance in multiple genes, systematic vs. random error of selected assay chemistries, potential biases introduced by the bioinformatics pipeline

Molecular Oncology:

1. Explain microsatellite instability testing and its clinical implications
2. Discuss major translocations in sarcomas and methods for testing

Infectious Disease:

1. Explain the basic organization of the DNA/RNA genomes of infectious agents
2. Explain the primary evolutionary associations among microorganisms

Pharmacogenetics:

1. Describe the clearance of pharmaceutical agents and prodrug activation through metabolic pathways and give some examples of the molecular variants associated with them