World Federation for Hemophilia Genetics Course

Aims;
The aim of the course (generally 4 weeks duration) is to acquaint the WFH follow with genetics of both haemophilia A and B and to deliver lab based experience in mutation detection and its interpretation and reporting for both disorders.

Materials;
Where possible, DNA samples of patients and their family members from the fellows own haemophilia centre are studied. This enables them to return home with some families already analysed.

Tutorial materials;

Lab techniques;
Genetic analysis for mutations, dependant on initial plans for establishment in the home laboratory and samples bought to Sheffield. The Fellow will be guided through this analysis whilst in Sheffield, as appropriate to their requirements.

1. DNA extraction from blood; manual and automated
2. Long PCR for F8 intron 1 and 22 inversions. Inverse PCR.
3. PCR amplification, scanning for mutations (by the centres’ own scanning technique, if used) and DNA sequencing of the coding region and splice junctions of the F8 gene.
4. PCR amplification, scanning for mutations (by the centres’ own scanning technique, if used) and DNA sequencing of the promoter, coding region and splice junctions of the F9 gene. Dependant on samples bought to Sheffield.
5. Dosage analysis: MLPA IF available to use in home lab.

Reading materials;
For reading and discussion whilst in Sheffield.

1. Review articles on haemophilia and its genetic analysis;
   Several chapters in Textbook of Haemophilia, second edition, 2010
   Goodeve A. Molecular genetic testing of haemophilia A, Seminars in Thrombosis and Haemostasis 2008
   Oldenburg et al. Molecular basis of haemophilia A. Haemophilia 2004
2. CSGE mechansim
3. Large F8 and F9 deletions/duplications and their analysis
4. Patients with no mutation
   Graw et al. T&H 2002
5. Mutation spectrum in a whole patient cohort.
6. Mutation and inhibitor relationship

Data interpretation;
1. Interpretation of sequence alterations, reading electrophoretograms, use of Staden for sequence analysis, interpretation of nucleotide sequence, prediction of amino acid sequence. Understanding effect of splicing errors.
2. Use of the F8 & F9 internet mutation databases & HGMD to seek previously reported mutations.
3. How to analyse pathogenicity; cross-species amino acid conservation, splice site prediction etc
4. Unclassified variant documentation
5. Mutations that result in the 1 and 2 stage FVIII:C discrepancy.
6. Example reports sent to referring clinician, important details to include.
7. Importance of prior family history of haemophilia.
8. Reporting of mothers of haemophilia males who are apparently not carriers (possible germline mosaics).
9. Human Genome Organisation (HUGO) gene names
10. Human Genome Variation Society (HGVS) sequence recommendations

Theoretical background;
The following will be discussed with the Fellow:
1. Primer design & SNP
2. Mutation spectrum in haemophilia A, notably F8 inversions
3. Mutation spectrum in haemophilia B, notably promoter mutations and haemophilia B Leiden
4. Association of mutations with disease severity
5. Association of mutation types with inhibitor development, particularly in haemophilia A
6. Inheritance of haemophilia, frequency of new mutations (particularly in severe haemophilia), frequency of founder effect in mild haemophilia
7. 2N VWD masquerading as mild haemophilia A
8. Dependent on plans for analysis in home laboratory; Intragenic polymorphism in the F8 gene- what is available (STR and SNP), ethnic variation, selection of a panel of markers for a particular population.
9. Intragenic polymorphism in the F9 gene- what is available (SNP and ins/del), ethnic variation, selection of a panel of markers for a particular population.
10. Limitations on the use of linkage analysis (lack of prior family history, lack of all family members, lack of informativity of polymorphisms)
11. Prenatal diagnosis, options of chorionic villus sampling and amniocentesis. Testing only males at PND and females later in life.
12. Age of testing for possible female carriers.
Information required;
Equipment and reagent suppliers available to the home lab. In some cases, agents for certain suppliers are not available.

Splicing prediction sites;

https://splice.cmh.edu/
http://www.fruitfly.org/seq_tools/splice.html
http://www.cbs.dtu.dk/services/NetGene2/
http://violin.genet.sickkids.on.ca/~ali/splicesitefinder.html
http://www.umd.be/SSF/
http://www.ebi.ac.uk/asd-srv/wb.cgi

Protein alignment etc;

http://agvgd.iarc.fr/agvgd_input.php
http://genetics.bwh.harvard.edu/pph/
http://www.ebi.ac.uk/Tools/clustalw2/index.html
http://blocks.fhcrc.org/sift/SIFT_aligned_seqs_submit.html
http://msms.usc.edu/msrv/
http://www.russell.embl-heidelberg.de/aas/
http://ngrl.man.ac.uk/snpcheck/index.html
http://www.igs.cnrs-mrs.fr/Tcoffee/tcoffee.cgi/index.cgi