TECHNIQUES PRESENTATIONS: WRITE-UP TEMPLATE

1. Names and contributions of group members:

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2. Technique chosen:

In-Situ Hybridization: cDNA, cRNA and Oligonucleotide Probes

3. What does this technique 'do'?

In situ hybridization traces regional and cellular sites of gene expression within tissues (Gee, C.E. *et al.* 1983)

4. What applications is this technique employed for? (Mitchell)

In situ hybridization (ISH) technologies can be used to probe for the location of DNA or RNA in cell or tissue samples. To do this ISH takes advantage of the hybridization that occurs between two complementary strands of DNA or RNA. The probe strand can be labelled with either radioactive isotopes or fluorochromes to identify the localization of the target strand of DNA or mRNA. Once the probe strand has been success fully annealed to the target strand, a visualization system such as an epifluorescence microscope may be utilized to view the results. Use of a fluorochrome to label the nucleic acid probe is known as fluorescence in situ hybridization (FISH). FISH makes use of NTP's that have been conjugated to biotin or digoxigenin, these tags can then be labelled with fluorescent antibodies (Garimberti and Tosi.). As mentioned above, this family of techniques is primarily used for identifying the location of a nucleic acid sequence of interest. If for example, one was interested in the localization of a specific mRNA during embryonic development, tissues could be fixed and probed using a sequence complementary to your mRNA of interest. Additionally, in more recent RNA studies a "locked nucleic acid" (LNA) probe with a covalent bond connecting the 2' hydroxyl and the 4' carbon has been developed for use in the study of microRNA localization as it provides a more stable bond with the target strand (Urbanek et al.). For DNA related studies, ISH can be used to identify chromosomal copy numbers and the localization of specific genes on a chromosome. (Younis et al.) To this end, a specific type of ISH used to study whole genomes has been developed. Known as genomic in situ hybridization (GISH) this technique uses whole genomic DNA from one organism to label that of another (Younis et al.) This can be used to examine conserved sequences of DNA between parent and offspring or as mentioned above, to study meiotic events as it is capable of examining the entire genome (Younis et al.) In summary, in site hybridization techniques can be used as a method for visualization of RNA and DNA in cells or tissue.

5. What questions relating to gene regulation and/or development can be addressed using this technique? Provide two examples (peer-reviewed papers) that use this technique. (James)

This technique allows us to study gene regulation and development by looking at tissue specific gene expression. By hybridizing probes to different tissue samples, we can observe differential expression of genes in specific tissue types. This also greatly helps in studying development as you can take the same tissue type at different life stages and observe the gene expression temporally.

By using a specific technique of in situ hybridization, namely whole mount in situ hybridization, we can study the spatial and temporal expression of genes of interest in

developing embryos. One study that used this technique revealed the translational control of segmentation gene in Drosophila, hunchback, via analyzing the gene's spatial distribution during different stages of the embryo (Tautz). Another study concerning hematopoietic stem cells used in situ hybridization assays to determine the expression pattern of Thrombopoietin, the main growth factor for hematopoietic stem cells (Sungaran).

6. What critical reagents are required to use this technique? (Garry)

- Wash, detection and block buffer;
- Labeled probe (RNA, DNA or nucleic acid);
- RNase or DNase;
- Pepsin;
- Antibound or detection compound (present in blocking buffer);
- Hybridization mix solution (dependent on probe: biotin nick translation = biotin-16dUTP, DIG nick translation = digoxigenin-11-dUTP, nick translation); and,
- DAPI for FISH.

7. What critical information is required to be able to employ this technique?

Although efficient and insightful, the follow information must be known to employ in situ hybridization:

| Probe Type | Labeling Technique | Applications | Advantages/Disadvantages |
|-----------------|---|---|--|
| cDNA | - Random priming using Klenow DNA polymerase - Nick translation | Viral detection Interphase cytogenetics | Poor penetration of section Large probe sequence High specific label |
| cRNA | - Bacteriophage RNA polymerase | - Detection of mRNA of low copy numbers | High specific label Label hybrids formed |
| Oligonucleotide | - end labeling with terminal transferase T4 polynucleotide kinase | - Allele-specific investigations | Custom synthesis Penetrates tissue sections easily Lower specific label |

a. The type of probe and labeling technique required based on the application

(Woodroofe, M.N. et al. 1994)

- b. the amount and concentration of reagents (i.e. tailing buffer) to use, the length of time the sample should sit in the reagent and temporal conditions based on the sequence of the focal DNA, RNA or protein (Wisden and Morris, 1994). There are a lot of redundancies based on the type of tissue used (Lewis, *et al.*, 1988).
- c. the sequence of the focal DNA, RNA and nucleotides (Sambrook, et al., 1989)

8. References:

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