**Generally asked question for the**

**Molecular Cytogenetics and Epigenetics Shared Core facility**

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| **Question** | **Response** |

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| How to submit cell lines for karyotype, FISH, and spectral karyotype (SKY) | Grow the cells preferably in T-25 flasks in duplicate. Cells should be about 25~40% confluent at the time of submission. Sub-culture the cells 2~5 days before the submission. Provide about 50ml of growth medium for 2 T-25 flasks along with the cell line. |
| What probes to use for FISH | If you already know the target gene and if a commercial probe is available, we may have the probe in our holdings. If a probe is not available with us, we need to find out where and how to get it. If no commercial probe is available, we can discuss the option of making a probe in house, which involves a bit of effort and time. |
| How many colors/probes can be used to do FISH | Two probes are generally used. In some situations a 3-color probe can be designed. |
| How many metaphases will be analyzed by karyotype | A total of 20 metaphases will be analyzed for both Structural rearrangements and copy number changes. |
| How many cells will be analyzed by FISH | Depending on the type of probe and the tissue, up to a total of 200 interphase cells will be analyzed. If required additional cells will be analyzed. |
| What type of tissue is needed for generating a primary tumor cell line | We need fresh tissue (a minimum size of 0.5cm2) be collected in sterile conditions and placed in a sterile 15 ml centrifuge tube with 1~2 ml of RPMI. Transport the specimen to the shared core facility lab as soon as possible before 24 hours. |
| How much DNA is needed to label the probe | We need at least 5ug of DNA at a minimum concentration of 100ng/μl.  |
| What quality of DNA is required for FISH labeling | The DNA should be run on agarose gel, after spectrophotometric estimation, to estimate the concentration and to see the DNA doesn’t contain RNA. If there is lot of RNA, the DNA sample needs to be purified with RNAase treatment |
| How to prepare formalin fixed paraffin-embedded (FFPE) tissue sections | A single paraffin section at 4 micron thickness is mounted on coated (+) slides |
| How to prepare tissue microarray (TMA) | A single array at 4-micron thickness is mounted on a coated (+) slide. TMA should be designed in a way the rows and columns can be easily identifiable on microscope.  |
| What is needed for PAC/BAC clones to grow and prepare DNA | If you already know which clone you want to use for growing, provide us the bacteria containing the PAC/BAC. If you do not know what clones to select, we will be able to help you choose the clones relevant to your target of FISH. |