

EMBO practical course:

Advanced Techniques in Molecular Medicine

June 13-20, 2006, Uppsala University, Sweden

Practical Exercises

- Single nucleotide polymorphism analysis by minisequencing with microarrays;
- analysis of expression array data; single gene analysis with padlock probes in situ,
- ultrasensitive protein detection by proximity ligation.

Lab 1a

In situ genotyping of mitochondrial point mutations using padlock probes

Instructor: Chatarina Larsson

In this lab you will use circularizable oligonucleotide probes, so called padlock probes, for determining the distribution of normal and mutated mitochondrial genomes in the cytoplasm of different cell lines by performing genotyping of a mitochondrial point mutation. Padlock probes are useful for in situ genotyping applications for several reasons. The probes are equipped with target complementary sequences at both ends, and they are designed to hybridize end to end on the target sequence. The variant base in the target sequence is positioned at the 3' end of the probe. If there is a perfect match between the probe and the target sequence, a DNA ligase can join the ends, turning the probe into a circle, while mismatched 3' ends will inhibit ligation. In this manner single-nucleotide substitutions can be discriminated. Two padlock probes, specific for the normal and the mutant sequence at the MELAS locus, respectively, will be hybridized to their targets, then ligated, and finally detected through rolling circle amplification followed by hybridization of fluorescence-labelled oligonucleotides. The distribution of fluorescence in the cells will be recorded using a fluorescence microscope equipped with a digital camera.

Reference:

Larsson, C. et al. In situ genotyping individual DNA molecules by target-primed rolling-circle amplification of padlock probes. *Nature Methods* 1, 227-232 (2003).

Lab 1b

Detection of endogenous protein-protein interactions in situ using proximity ligation

Instructor: Malin Jarvius

This lab is analogous to Lab1a, since this technique also uses the formation of circular DNA as a tool for detection, but here you will study interactions between proteins instead of DNA molecules. The model system is the well-characterized interaction between the proto-oncogene c-Myc and its partner Max, and you will study this in human fibroblast, grown and fixed on glass slides. Proximity probes, consisting of antibodies with attached oligonucleotides, are added to the samples.

With the addition of circularization probes and ligase, the proximity probes template the formation of circles, if c-Myc and Max are in close proximity. DNA circles are then replicated by rolling circle amplification and detected using fluorescence-labeled probes. The products will be visualized using an epi-fluorescence microscope or a confocal microscope.

Lab 2

Single nucleotide polymorphism analysis by minisequencing with microarrays

Instructor: Snaevar Sigurdsson

A promising approach towards high-throughput genotyping of single nucleotide polymorphisms (SNPs) is to use arrays of immobilised oligonucleotides in miniaturised assays. Significant advantages of performing the assays in microarray formats are the reduced costs of genotyping due to the simultaneous analysis of many SNPs in each sample, and the small reaction volumes employed.

In the laboratory exercise the participants will learn to use a tag-array minisequencing system. The minisequencing reaction uses a DNA polymerase to extend detection primers that anneal immediately adjacent to the sites of the SNPs. The primers are extended with fluorescently-labelled nucleotide analogues that are complementary to the nucleotide at the SNP site. The reactions are performed in solution using detection primers with 5'-"tag" sequences. Each SNP has its own specific tag that is complementary to one of the "cTags" that are immobilised to the microarray. When the extended detection primers are applied to the microarray, the tags will hybridise to their corresponding "cTags". From the known locations of the "cTags" on the microarray, the genotypes of the SNPs can be deduced.

The aim of this exercise will be to genotype a panel of genomic SNPs in a number of individual [DNA samples](#) by using the four-colour tag-array minisequencing primer extension method. Some SNPs will be analysed in further detail.

Reference:

Lindroos, K., Sigurdsson, S., Johansson, K., Ronnblom, L. & Syvanen, A.C. Multiplex SNP genotyping in pooled DNA samples by a four-colour microarray system. *Nucleic Acids Res* 30, e70 (2002).

Lab

Proximity ligation for highly sensitive detection of the platelet-derived growth factor protein

3

Instructors: Sigrún Gústafsdóttir, Edith Schallmeiner

This experiment involves a novel strategy for highly sensitive and specific detection of proteins, where the coincident binding of two affinity probes to individual protein molecules results in an amplified detection signal. The assay will be used to detect PDGF-BB in serum and plasma. Participants will incubate samples with pairs of proximity probes. The probes used are aptamers, that is DNA molecules selected through the SELEX procedure for high affinity to the PDGF protein. In a next reaction step oligonucleotide extensions from pairs of aptamer probes that have bound to the same antigen molecules are joined by ligation. This is immediately followed by amplification of the ligation products with real-time detection using the TaqMan assay.

Lab 4

Computer exercise - Analysis of cDNA microarray expression data

Instructor: Hanna Göransson

The laboratory exercise will give an introduction to the analysis of DNA microarray expression data, in particular cDNA microarray data. The course participants will be given hands-on experience in handling the data from microarray experiments. The computer exercise will cover the process of normalization. The purpose of normalization is to identify and remove systematic variation present in microarray data. It is an essential step in the analysis process, to make sure the differences in measured intensities on the microarrays are indeed due to differential expression, i.e. true biological variation.

The analysis will also include statistical tests for identification of genes that are differentially expressed between for example different conditions or tumour classes. The data can also be visualised using clustering or further analysed using multivariate techniques as classification.

The computer exercises will give experiences using BASE, a database environment for secure storing of data from microarray experiments. We will perform analysis using algorithms implemented in the user-friendly interface to the system, the LCB Data Warehouse at the Linnaeus Centre for Bioinformatics.