The hybridization and nucleic acid probes

The nucleic acid hybridization is the process wherein two DNA or RNA single chains from different biological sources, make the double catenary configuration, based on nucleotide complementarity and of contingent sequence homology of the two sources, resulting DNA-DNA, RNA-RNA or DNA-RNA hybrids. In most cases, the purpose of the hybridization techniques is identification or localization of certain nucleic acid sequences in the genome of some species. Two basic notions are used: the target molecule representing the DNA, RNA or protein sequence that should be identified and the probe molecule that identify the target, by hybridization. When hybridization takes place on a solid carrier is named blotting and is divided in 3 categories:

* Southern blotting whereby DNA molecules are identified using DNA or RNA probes;

* Northern blotting whereby RNA molecules are identified using RNA or DNA probes;

* Western blotting whereby proteic sequences are identified using specific antibodies.

The general characteristics of nucleic acid probes:

1- To show a chemical marking to allow viewing the target probe molecular hybrid;

2- To have a dimension of 10-10000 nucleotides. Too short probes hybridize very quickly (some minutes), but they show non-specific hybrids, too and are difficult to trace. Too long probes hybridize very slowly (some hours), but hybrids are more stable and more specific;

3- Not to show intracatenary complementarity areas, not to hybridize with itself or with non-targets but to have high sequence homology specificity with the target molecule.

Hybridization stages, these include;

1. Probe synthesis 2. Probe marking 3. Target DNA processing 4. Target DNA denaturation 5. Target DNA transfer to solid carrier 6. Molecular hybridization

1. Probe synthesis

1.1. Probe synthesis by "nick-translation" Usually starts from a target molecule copy figure 1:

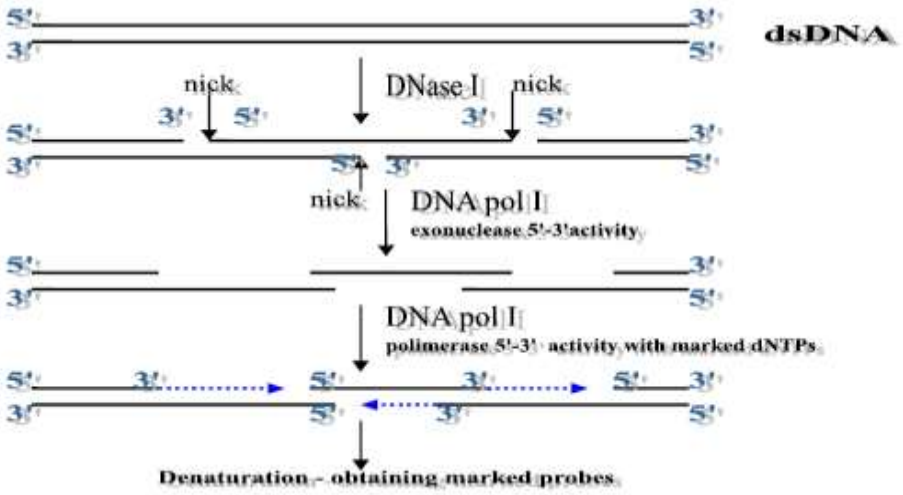

***Figure 1**. Probe synthesis b y "nick-translation"*

1.2. Probe synthesis using randomized primers. Randomized primers are heterogeneous sequence oligonucleotides that can hybridize in many sites of the matrix chain. (Figure 2)

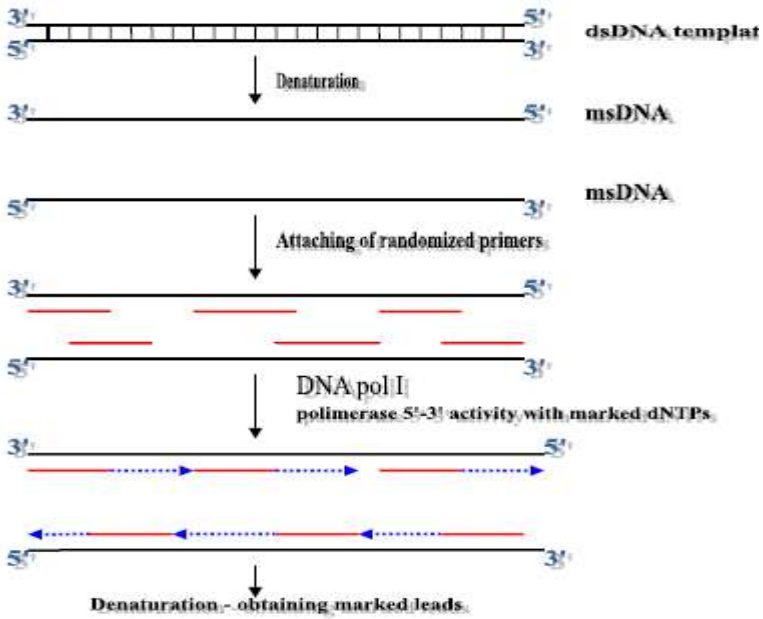

***Figure 2.** Probe synthesis using randomized primers*

1.3. Total DNAc probe synthesis (complementary DNA with an m.s.RNA population) can be done in two versions:

1-Total mRNA is isolated from cells and secondary structures are taken to pieces by heating at 70°C, for 5 min (figure 3).

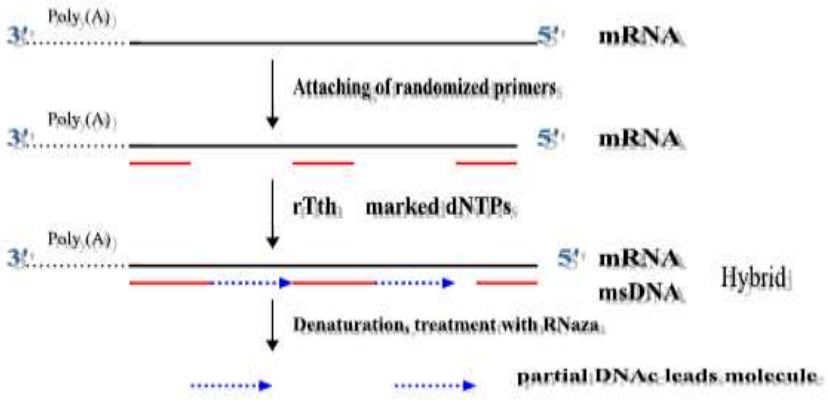

Figure 3. *DNAc probe synthesis with randomized primers*

2- As regards a total mRNA isolated from cells and denaturated, oligo(dT) primers are attached based on complementarity to poly ends (A) from 3'ends of mammalian mRNA molecules (figure 4).

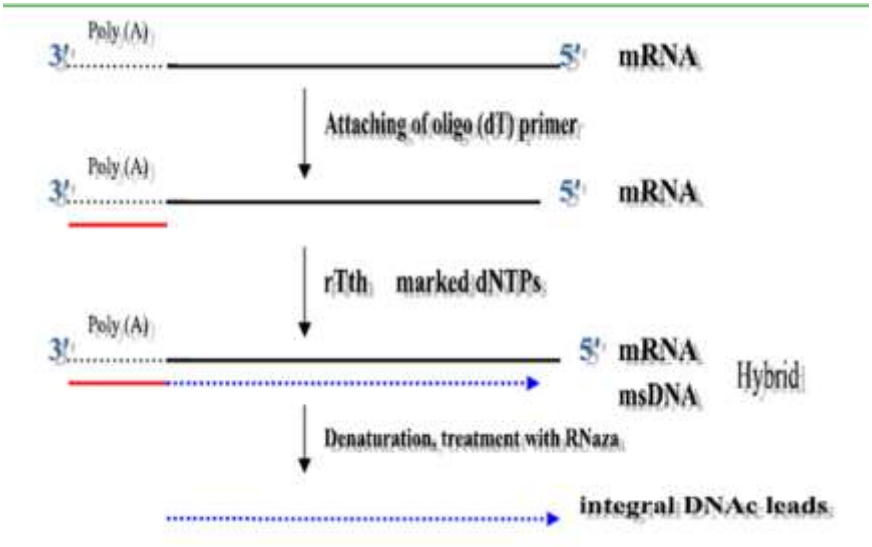

Figure 4. *DNAc probe synthesis with oligo(dT) primers*

1.4. RNA probe synthesis by *in vitro* registration.

It requests exogenous DNA cloning (based on it obtaining of RNA probes is requested) in clonation vectors that contain strong promoters, highly specific such as SP-6, T3 or T7 phage promoters. These promoters are recognised extremely specific by RNA polymerase from phages concerned (and that it does not recognize neither chromosomal bacterial or plasmidial promoters nor eukaryote promoters recognized by other RNA polymerase). Vectors containing these promoters are plasmidial type (pGEM 3/4 or pGEM 3z/4z) or phagemides: pBluescript (figure 5) or vectors derived by the λphage (λZAP)

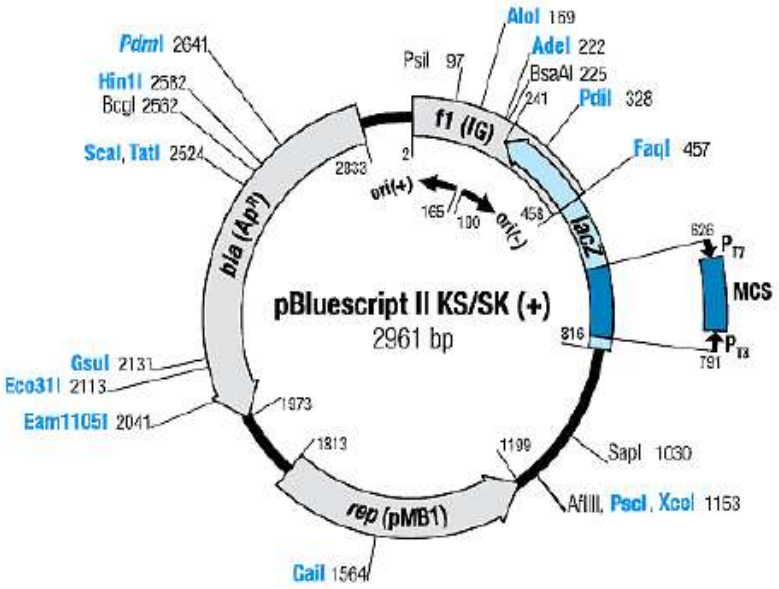

Figure 5. *Genetic map of pBluescript phagemide*

2. Probe marking is carried out several times **radioactively** during the probe synthesis using 32P or 35S. The method has the advantage that very small amounts of nucleic acids can be detected. The following disadvantages are mentioned: risk of handling radioactive materials, imbalance of isotopes and long exposure times. Apart the incorporation of radioactively marked nucleotides in new-synthesized chains, are marked the 3'OH smooth or cohesive ends of d.s.DNA sequences, resulted after restrictases activity.

3. Target DNA processing assumes the source DNA isolation and purification and its digestion with a suitable restrictase so as the target oligonucleotidic sequence is obtained as a linear, double-stranded molecule. Sequences obtained by the restriction reaction are separated by electrophoresis in the agarose gel

4. Target DNA denaturation in order to get single chain (mono-stranded) molecules (by breaking hydrogen bridges between complementary nucleotides) means the introduction of electrophoresis gel in NaOH 0.5N solution. The breaking of phosphodiesteric bundles between complementary nucleotides of the two chains of DNA can be made by HPLC (***H****igh* ***P****erformance* ***L****iquid* ***C****hromatography*), the accurate but laborious and expensive method that can provide errors by mixing the nuclear DNA with the mitochondrial one (in case of eukaryota). Another method is the chromosomal DNA ultracentrifugation in gradient of CsCl when the floating density of the sample is compared with the reference DNA sample and resulted value depends on %G +C : Floating density =1.66 + 0.098 · % mole GC The most common denaturation method is thermal denaturation. Increasing the temperature of the reaction mixture between 20-100°C, absorbance variation at a wave length of 260nm (A260) is checked by spectrophotometry 260nm (A260). During denaturation absorbency can raise by 40% as well. This process is called hyperchromic shift. Tm is the melting temperature where half of DNA is as a single chain type and half is still bicentenary

5. Target DNA transfer to solid carrier (nitrocellulose paper, nylon membrane) and be carried out in three ways

1- Transfer of DNA sequences from the gel, through capillarity by an SSC buffer, in a sandwich system (kitchen paper laid on and under the solid carrier). The transfer rate depends on the size of sequences and agarose concentration.

2- Electrophoretic transfer is carried out only on nylon membranes that are electrically charged and this method is also effective for small DNA molecules up to 60pb.

3- Vacuum transfer is much more effective and faster than the other versions. It assumes the electrophoresis gel is laid directly on the solid carrier, and the entire assembly is laid on a

spongy carrier under which there is a vacuumed chamber. The buffer is poured over the assembly and then it is extracted by vacuum and takes over the nucleic acid from the gel and stores them on the solid carrier. The solid carrier, with nucleic acids stored on it, is exposed to UV radiations, and as a result, are created cross-links between nucleic acids and carrier structure, thus provide the fastening.

6. Molecular hybridization consist in immersion of the solid carrier with the target single chain DNA molecules in 5x SSC buffer containing the marked probe as well and are left in contact 14-16h. After hybridization probe remained non-hybridized is removed by serial washing with an SSC buffer. The process is influenced by 5 factors:

1- Length of target DNA sequences (the bigger the higher renaturation rate is, but a long length drives a higher solution viscosity, and as a result, renaturation is suppressed; a compromise was made.

2- Ionic strength of incubation buffer (Na^+ ions from reaction buffer affects denaturation and renaturation as it influences nuclease activity);

3- DNA preparation purity (RNA, carbohydrates, solution proteins interferes with hybridization);

4- Initial concentration of DNA molecules (C0) and incubation time (t): $C/C0= 1/(1+ k \cdot C0t)$;

5- Optimum incubation temperature of the reaction mixture is 25°C. At lower temperatures nonspecific hybrids are formed, by reassociations between non-homologue sequences, at high temperatures, the reaction rate decreases a lot.