User Manual:

Read all the procedures before using Super Hybridization Buffer for experiment!!

User Manual (for membrane hybridization only):

1. Thaw the Superhyb solution at 60^{o}C (Repeat freezing and thaw is OK)
2. Shake the solution to mix it well and add solution to membrane in the hybridization tube or bag (**For 10cm x10 cm membrane, use 10 ml of SuperHyb solution).**
3. Move hybridization bag or tube so that the solution can cover all surface of the membrane.
4. After prehyb for 30 min at 60^{o}C, add certain amount probes (100ng/ml for non-isotopic probes and 1x10^{5}cpm/ml for isotopic probes) directly to the hybridization tube or bag without changing hybridization solution.
5. Hybridization is performed at 60^{o}C for >50nt RNA probes or at 50-50^{o}C for >50b DNA probes. (2-8h hyb is sufficient for most abundant genes. Overnight hyb is necessary for low abundant genes such as transcription factors, receptor genes, etc.).
6. After hybridization, wash membrane with 1XSSC at hybridization temperature for 2X10 min following by wash in 0.2XSSC for 2X10 min at 5-10^{o}C below the hybridization temperature.
7. Air-dry membrane after washes and detect the hybridization signals by exposure to film or image box (isotopic labeled probes) or by immunocytochemistry (non-isotopic labeled probes).

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Troubleshootings:

1. **Weak hybridization signals**: following reasons may contribute to the weak signals

   - Hybridization temperature is not optimized

     Suggestion: change the hybridization temperature based on the size of the probe. Use 60^{o}C for >100nt probe and 50^{o}C 50-100nt probes.

   - Lower quality of the probes.

     - Probes are hydrolyzed after synthesis or probes
     - The radioactivity of labeled probes is too low, less then 10^{6}cpm/μl.
     - Probes added into the hybridization solution are not enough for maximal hybridization.

     Suggestion: the radioactivity of isotopic probes in the hyb solution should be ~$10^{5\text{-}6}$cpm/ml and for the no isotopic probe, it should be ~100ng/ml.

   - The abundance of target genes are low: the most common reason for weak signals.

     Suggestion: increase the amounts of target genes on hybridization membrane.

2. **High background**

   - Parts of the membrane are dried out during hybridization step or washing step so that the probes adhere to the membrane.
   - Labeled free nucleotides are not completely removed from the probes:

     Suggestion: after generating probes by *in vitro* transcription, use NeuBiogene RNA cleaning-up kit to remove labeled free nucleotides from the probes.

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