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Nucleic Acid Hybridization

Sibylle Herzer and David F. Englert

PLANNING A HYBRIDIZATION EXPERIMENT

Hybridization experiments usually require a considerable investment in time and labor, with several days passing before you obtain results. An analysis of your needs and an appreciation for the nuances of your hybridization event will help you select the most efficient strategies and appropriate controls.

The Importance of Patience

Hybridization data are the culmination of many events, each with several effectors. Modification of any one effector (salt concentration, temperature, probe concentration) usually impacts several others. Because of this complex interplay of cause and effect, consider an approach where every step in a hybridization procedure is an experiment in need of optimization. Manufacturers of hybridization equipment and reagents can often provide strategies to optimize the performance of their products.

What Are Your Most Essential Needs?

Consider your needs before you delve into the many hybridization options. What criteria are most crucial for your research—speed, cost, sensitivity, reproducibility or robustness, and qualitative or quantitative data?

Visualize Your Particular Hybridization Event

Consider the possible structures of your labeled probes and compare them to your target(s). Be prepared to change your labeling and hybridization strategies based on your experiments.

results are unsatisfactory, a point at which it might be too late to determine incorporation efficiency.

Before skipping any control steps, consider the implications. Minimally, measure incorporation efficiency when working with a new technique, a new probe, a new protocol, or a new kit. Radiolabeled probes need to be purified or at least Trichloroacetic acid (TCA) precipitated to determine labeling efficiency, as discussed in Chapter 7, "DNA Purification." Determining the efficiency of nonradioactive labeling reactions can be more time-consuming, often involving dot blots and/or scanning of probe spots. Follow manufacturer recommendations to determine labeling efficiency of nonradioactive probes.

Is It Necessary to Purify Every Probe?

Unincorporated nucleotides, enzyme, crosslinking reagents, buffer components, and the like, may cause high backgrounds or interfere with downstream experiments. Hybridization experiments where the volume of the probe labeling reaction is negligible in comparison to the hybridization buffer volume do not always require probe cleanup. If you prefer to minimize these risks, purify the probe away from the reaction components.

While there are some labeling procedures (i.e., probes generated by random primer labeling with ^{32}P-dCTP), where unpurified probe can produce little or no background (Amersham Pharmacia Biotech, unpublished observations), such ideal results can't be guaranteed for every probe. When background is problematic, researchers have the option to repurify the probe preparation. Admittedly, this approach wouldn't be of much use if the experiment producing the background problem required a five day exposure. (Purification options are discussed in Chapter 7, "DNA Purification.")

HYBRIDIZATION MEMBRANES AND SUPPORTS

What Are the Criteria for Selecting a Support for Your Hybridization Experiment?

Beyond the information listed below and your personal experience, the most reliable approach to determine if a membrane can be used in your application is to ask the manufacturer for application and or quality control data. Whether a new membrane formulation will provide you with superior results is a matter that can usually be decided only at the bench, and the results can vary for different sets of targets, probes, and detection strategies.

Physical Strength

Nitrocellulose remains popular for low to medium sensitivity (i.e., screening libraries) applications and for situations that require minimal handling. The greater mechanical strength of nylon makes it superior for situations that require repeated manipulation of your blot. Nylon filters may be probed 10 times or more (Krueger and Williams, 1995; Li, Parker, and Kowalik, 1987). Even though nitrocellulose may be used more than once, brittleness, loss of noncovalently bound target during stripping, and decreased stability in harsh stripping solutions make nitrocellulose a lesser choice for reusable blots. Glass supports and chips can be stripped, but stripping efficiency and aging of target on these supports may impair reuse of more than two to three cycles of stripping and reprobing. Supported nitrocellulose is sturdier and easier to handle than pure nitrocellulose, but remember that it needs to be used in the proper orientation.

Binding Capacity

Nylon and PVDF (polyvinylidene difluoride) membranes bind significantly more nucleic acid than nitrocelluose; hence they can generate stronger signals after hybridization. Nucleic acids can be covalently attached to nylon but not to nitrocellulose, as discussed below. Positively charged nylon offers the highest binding capacities. As is the case with detection systems of greater sensitivity, the greater binding capacity of positively charged membranes could increase the risk of background signal. However, optimization of hybridization conditions, such as probe concentration and hybridization buffer composition, will usually prevent background problems. If such optimization steps do not prevent background, a switch to another membrane type, such as to a neutral nylon membrane, might be required. If your signal is too low, try a positively charged nylon membrane. Positively charged nylon is often chosen for nonradioactive applications to ensure maximum signal strength. The quantity of positive charges (and potential for background) can vary by 10-fold between manufacturers. The lower binding capacity of nitrocellulose decreases the likelihood of background problems under conditions that generate a detectable signal.

Thickness

Most membranes are approximately 100 to 150 μm thick. Thickness influences the amount of buffer required per square

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