

Analysis of Single Stranded Tail Formation using Slot Blots

DNA samples isolated from *GAL::HO* induction experiments can be analyzed by slot or dot blots to quantitate the amount of single stranded tails that are formed. Three sets of samples are loaded in a typical slot blot experiment described below. When using native samples, only the single stranded tails bind to the membrane. The denatured samples can be used for assessing the degradation of the 5' to 3' strand. Finally various controls can be included to assure accuracy.

1. Native samples. Dilute 1 µg of yeast genomic DNA from the time course samples to 200 µl adjusting the final concentration to 10x SSC. This will be enough for two slot blots, one for each strand-specific probe.
2. Denatured samples. Denature 0.1 µg of genomic DNA by adding NaOH to a final concentration of 0.4 N NaOH. Neutralize with 3 M sodium acetate pH 5.7. The amount needs to be determined empirically for each new solution of sodium acetate (pH indicator strips are useful for this). In our experiments the reaction induced by the HO endonuclease produces only a small amount of ssDNA in the native samples. Therefore we use more native DNA (10x) than denatured DNA in order to obtain comparable signal strengths.
3. Quantitation samples and other controls. Digest a plasmid containing the target sequence with a restriction enzyme and prepare samples ranging in amount from 0.05 to 1.0 pg of hybridizable sequence. Denature the samples with 0.4 N NaOH and then neutralize the samples using sodium acetate as described in the previous step. These samples will be useful in deriving a calibration curve if the signals need to be quantitated using light-based densitometry.

For other controls include a sample with non-denatured plasmid DNA (0.01 ng of hybridizable sequence), samples of non-radioactive RNA transcripts complementary to the RNA probes that will be used (0.001 ng of hybridizable sequence) and if possible, denatured genomic DNA lacking hybridizable sequences as a negative control.

One blot is prepared for each strand-specific probe. A small amount of signal can sometimes be detected using the non-complementary strand-specific probe. For this reason, single-stranded probes are preferred to denatured double-stranded DNA probes. Load the samples into the wells of the slot blot or dot blot apparatus and transfer the DNA to two nylon membranes—one for each strand-specific probe. After the samples have transferred to the membrane, rinse the sides of each well with 500 μ l of 10x SSC. The DNA is then crosslinked to the membrane with UV light and hybridized according to the protocol of Church and Gilbert or other suitable hybridization protocol.

The most recent version of this protocol may be found at the Haber Lab web page:
<http://www.bio.brandeis.edu/haberlab/jehsite/protocol.html>.