

Optimizing Oligo GEM Array Results

Many aspects of microarray performance have already been optimized on the Oligo GEM Array®, such as specificity and hybridization stringency. However as with any biological assay system, the researcher must optimize a few experimental parameters themselves in order to achieve optimum results. These factors, which all depend on one another, include input RNA and labeled cRNA quantity and quality, the amount of cRNA used in hybridization, exposure time, and data normalization. This article discusses each of these factors in turn.

Anticipated Raw Data

The initial result (or the real raw data) from an Oligo GEM Array experiment is a grayscale digital image of the array spots. The relative level of gene expression is directly related to the spot intensity. Dark spots represent genes expressed at a high level; light spots, genes at a lower level; and blank spots, genes at an undetectable level. Figure 1 displays three hypothetical array images: an ideal result and two extreme substandard results. In the ideal image, the four spots of varying intensity span the complete distance between the background and the maximum signal level. All positive spots are significantly darker than the blank spot and significantly lighter than the most intense spot. Thus, the ideal array image utilizes the full dynamic range. The goal of optimizing the microarray experimental parameters is to obtain an image as close to the ideal as possible.

GEM Array Signal Intensity Guidelines

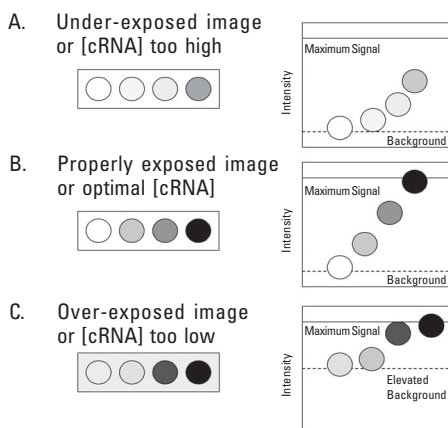


Figure 1: Suggested guidelines for GEM Array signal intensity. Three hypothetical microarray images are shown with spot intensities corresponding to various exposure times or amounts of labeled cRNA target in the hybridization.

Optimizing Total RNA and Labeled cRNA Target Yield

1. Always and only use certified RNase-free reagents and labware. Check pipette tips and tubes to ensure that they are RNase-free.
2. To isolate RNA in sufficient quality and quantity from:
 - Transformed cultured cell lines
Use ArrayGrade™ Total RNA Isolation Kit (GA-013).
 - Mammalian tissues
Use TRIzol® reagent from Invitrogen first followed by a clean-up step with the ArrayGrade™ Total RNA Isolation Kit (GA-013).
3. To insure RNA quality and quantity:
 - Determine yield by A_{260} of a 1:50 dilution in TE pH 8.0.
 - Determine A_{260}/A_{280} and A_{260}/A_{230} ratios.
Ratios greater than 1.8 indicate a lack of protein and salt contaminants, respectively.
 - Characterize RNA by agarose gel electrophoresis or with an Agilent 2100 BioAnalyzer.
Results should yield two sharp 28S and 18S ribosomal RNA (rRNA) bands with roughly a 2:1 intensity ratio.
4. Synthesis and characterization of cRNA:
 - Use 3 μg RNA in TrueLabeling-AMP with 4-h incubation.
 - Determine yield by A_{260} of a 1:50 dilution in TE pH 8.0.
 - Characterize cRNA by agarose gel electrophoresis.
The cRNA should yield a 0.5 to 1.5-kbp smear.
 - Characterize cRNA by dot blot of a serial dilution.
Biotin should be detectable at a 1:1000 dilution.

Optimizing the cRNA Amount Used in Hybridization

- For lower-density, tetra-spotted Oligo GEM Arrays microarrays (Catalog numbers 401-999):
Use more (5-20 μg) cRNA.
- For higher-density, single-spotted Oligo GEM Arrays: (Catalog numbers 000-400):
Use less (2-5 μg) cRNA.

Optimizing the Exposure Time

Generate a series of exposures with varying time to help guarantee that one of the exposures should be optimal.

Properly Normalizing Microarray Results

Option 1: Choose ONE housekeeping gene.

The chosen housekeeping gene MUST:

- a. Maintain constant expression despite the occurrence of other biological changes. The raw data for that gene must not change more than 10 percent (the reproducibility of the Oligo GEM Array®) between each array compared.
- b. Have an expression level that does not lie too close to the either extreme end of the dynamic range. Its expression must not be saturated or be called absent by the data analysis software in order for its response to experimental conditions to be observable.

Option 2: Use the Interquartile.

The Interquartile calculates the median value of the middle 50% of all of the intensity values on the array. It ignores 1/4 of the least intense signals and 1/4 of the most intense signals preventing them from skewing or biasing the relative gene expression profile. The Interquartile has been shown to respond to the systematic variation of the microarray experiment.

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