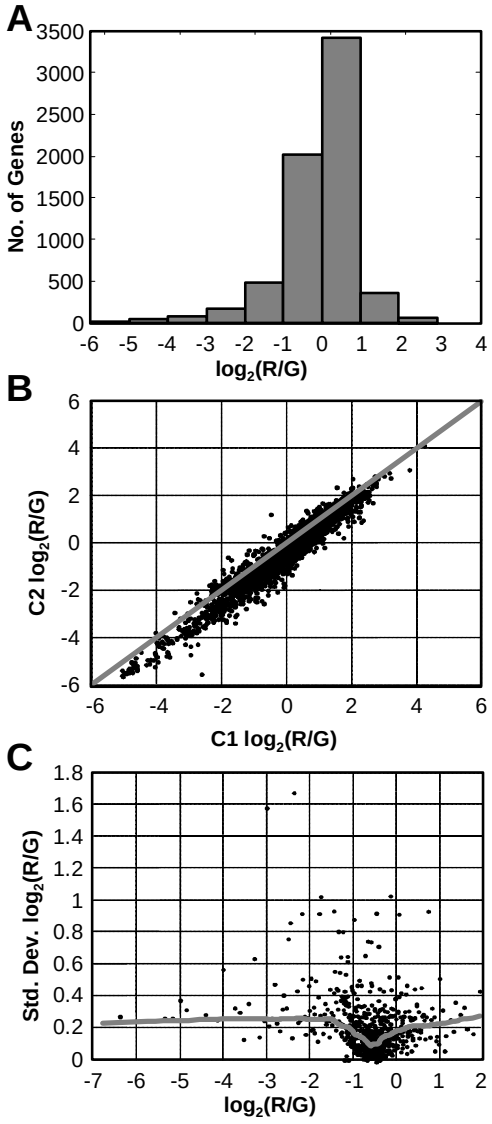
**Correlation Between Biological and Technical Replicates.** To evaluate reproducibility of our experimental technique as well as between $T_0$ samples, a statistical measurement model was generated from microarray data from six independent $T_0$ samples. The measurement model represents the observed $\log_2$ (R/G) values in terms of specific effects due to gene, culture, and replicate. Let $Y_{ijk}$ denote the measured $\log_2$ (R/G) value of the $i^{th}$ gene for the $k^{th}$ replicate slide within the $j^{th}$ culture. The model is given by $Y_{ijk} = \mu_i + \alpha_j + \beta_{k(j)} + \varepsilon_{ijk}$, where $\mu_i$ represents the underlying true/idealized (but unknown) value of the log-ratio for the $i^{th}$ gene, $\alpha_j$ denotes a global effect (across all genes) that is present during the measurement of slides from the $j^{th}$ culture, $\beta_{k(j)}$ denotes an effect on the measurement (across all genes) associated with the $k^{th}$ replicate slide within the $j^{th}$ culture, and $\varepsilon_{ijk}$ represents the specific effect of the $k^{th}$ replicate slide within the $j^{th}$ culture on the $i^{th}$ gene. The $i^{th}$ gene is said to be up-regulated or down-regulated (treatment versus control) if the estimate of $\mu_i$ ($\hat{\mu}_i$) is significantly different than zero. Examination of $\mu_i$ shows data between 1 and -1 $\log_2$ demonstrating no significant differences between the $T_0$ samples (Fig. 1A).

The other effects ($\alpha_j$, $\beta_{k(j)}$, and $\varepsilon_{ijk}$) are regarded as random measurement errors and thus hinder the ability to determine whether a gene is up- or down-regulated. In this case, the $\alpha_j$ effects are relatively large ($\hat{\sigma}_\alpha = 0.57$) and are possibly a consequence of unique PMT settings for each culture. The $\beta_{k(j)}$ effects are smaller ($\hat{\sigma}_\beta = 0.08$). In combination, the $\alpha_j$ and $\beta_{k(j)}$ effects introduce a specific global shift (across all genes) in log-ratios for each slide when comparing $T_0$ measurements averaged from culture two versus averaged from culture one (Fig. 1B). Presumably, these global effects can be effectively removed via

proper normalization. The other measurement error ($\varepsilon_{ijk}$) is gene-specific and much smaller than the $\alpha_j$ and $\beta_{k(j)}$ effects. A LOWESS smooth [54] of the raw standard deviations suggests that $\hat{\sigma}_\varepsilon$ is about 0.30 when $|\hat{\mu}| \geq 1$ (Fig. 1C). Thus, disregarding the $\alpha_j$ and $\beta_{k(j)}$ effects, any change greater than 1.6 fold can be viewed as statistically significant with a Type I error (or false positive rate) of about 0.01.

**Fig 1.** (*A*) Histogram of estimated values of $\mu$ $\log_2$ ratio. (*B*) Mean $\log_2$ ratio of culture 2 (C2) slides versus culture 1 (C1) slides (replicate $T_0$ samples). (*C*) Standard deviation of the $\varepsilon_{ijk}$'s as a function of $\hat{\mu}_i$ across the various genes with LOWESS smooth.




References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