Noise model

Since CV and VMR are the two most commonly used metrics of noise, we used them for our analyses. Figure 4B shows that the strong TATA curve appears slightly offset from the no TATA and weak TATA curves. In a seeming contradiction in Figure 4A it appears that the no TATA curve is slightly offset from the weak and strong TATA curves. On balance it suggests that TATA has no effect on noise.

To support this idea we plotted the log of the mean expression vs. the log of the variance (Figure S1).

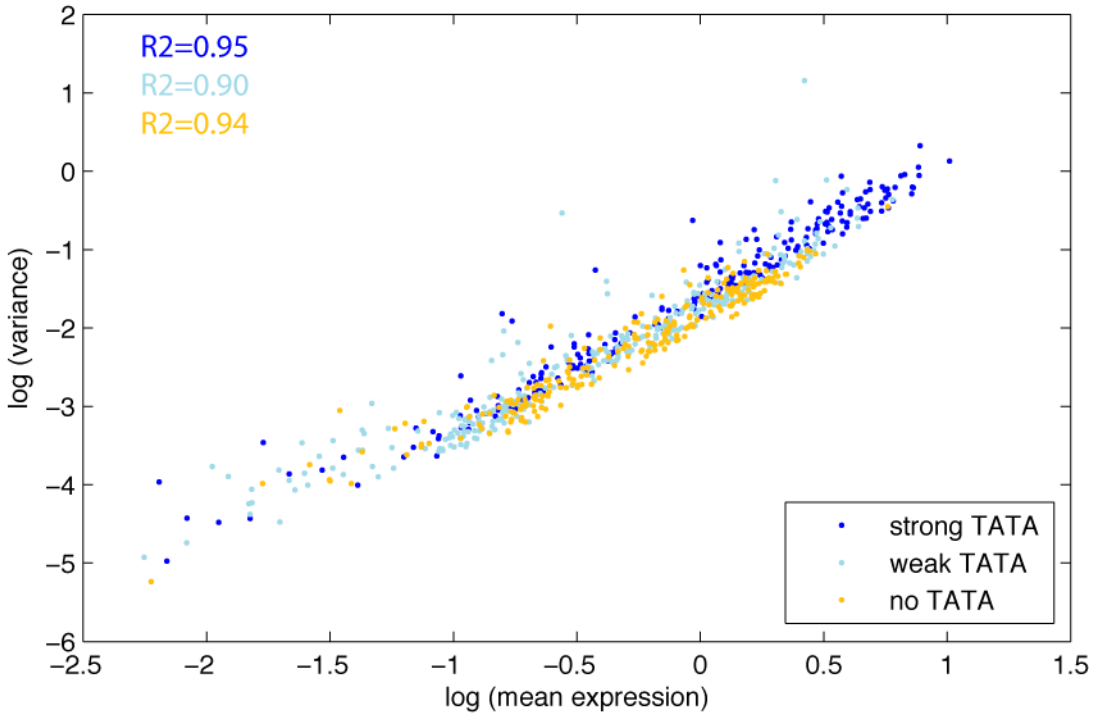

Figure S1: log-log scatter plot of mean vs. variance. The plot revealed that the log of the variance is in a linear relationship with the log of the mean. The three TATA libraries are not different from each other.

The data from all three libraries overlap with no significant differences between the different data sets. All of the data also lies along a straight line in this plot. This justified the use of a new noise model, which assumes that the relationship between mean expression and the variance of expression follows a power law of the form mean=C*variance^k, where C is a constant and k is the degree of the power law (k is also the slope of the line in Figure S1). Using the CV as the metric sets k=0.5, while using the VMR sets k=1. However, if we simply fit our data to a power law the actual value of k is around 0.6 in all three libraries (0.59 for the strong TATA, 0.59 for the weak TATA and 0.62 for the no TATA library), and the value of C is around 1 (0.9 for the strong TATA, 0.9 for the weak TATA and 1.1 for the no TATA library). This explains the apparent contradiction between Figures 4A and 4B. Using the CV (in Figure 4A) skews the no

TATA data (the lowest expressing clones) slightly downward because k is too small. Using the VMR (in Figure 4B) skews the strong TATA data (the highest expressing clones) slightly upwards because k is too large. When we graph the relationship between the mean and variance using the new noise model (Figure S2), all three curves are on top of each other and there are no statistical differences between any of the curves (Student t-test, *P*-value > 0.36).

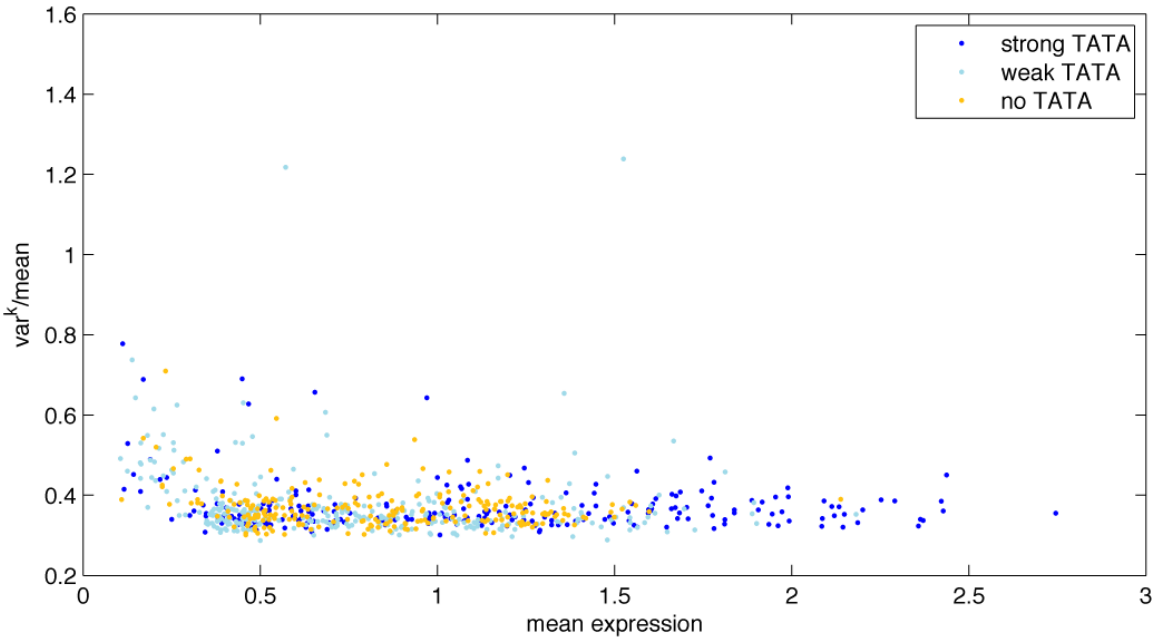

Figure S2: scatter plot of the mean expression vs. the new noise model.

Filtering extrinsic noise

Variation in transcription is usually caused by two sources of noise: intrinsic and extrinsic (Bar-Even et al. 2006; Newman et al. 2006; Paulsson 2004; Raser et al. 2004; Volfson et al. 2006). To better estimate transcriptional variation, extrinsic sources of noise should be filtered out. The major source of extrinsic noise is the cell volume (Di Talia et al. 2007; Nachman et al. 2007; Skotheim et al. 2008), thus dividing the fluorescence measurement by the volume for each cell allowed us to retain mainly intrinsic noise. Previous works have gated the cells selecting only for cells in G1 phase using forward scatter and side scatter (Newman et al. 2006). Figure S3 shows normalized fluorescence (fluorescence divided by volume) vs. CV in a log-log scale for our three TATA libraries. The trend shown in this figure is similar to Figure 2G from (Newman et al. 2006), showing that we efficiently filter out extrinsic sources of noise without the need for gating on G1 cells.

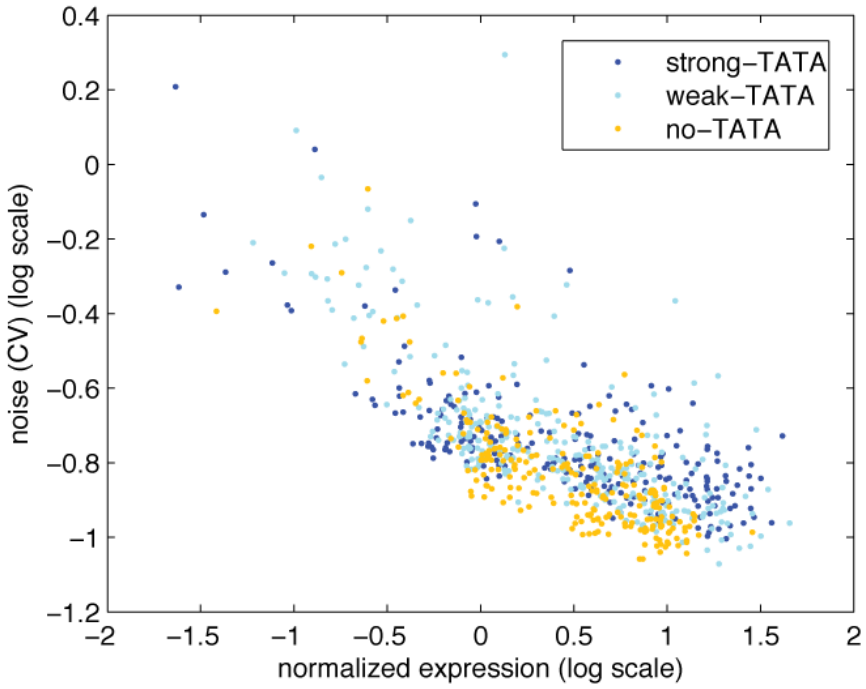

Figure S3: Normalized expression (fluorescence divided by volume) vs. CV in log-log scale for each sample in the three TATA libraries.

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