Additional File 5.

Let the expression of gene i at experiment t, $X_i(t)$, follow a Normal distribution with mean $\lambda(t)\theta_i$ and variance $k\lambda(t)\theta_i$, where k is a constant. We want to show that the $\widehat{\theta}_i$ in (2) is an unbiased estimator of θ_i and $\hat{\lambda}(t)$ in (2) is a consistent estimator of $\lambda(t)$ under this normal model. By (2), $\widehat{\theta}_i$ and $\hat{\lambda}(t)$ can be computed by

$$\widehat{\theta}_i = \sum_{t=1}^{T} X_i(t),\ \hat{\lambda}(t) = \frac{\sum_{i=1}^{n} X_i(t)}{\sum_{i=1}^{n}\sum_{t=1}^{T} X_i(t)}. \tag{S4}$$

Statement 1. $\widehat{\theta}_i$ is unbiased.

Proof: $E\left(\widehat{\theta}_i\right) = \sum_{t=1}^{T} E\left(X_i(t)\right) = \sum_{t=1}^{T}\left(\lambda(t)\theta_i\right) = \theta_i \sum_{t=1}^{T}\lambda(t) = \theta_i$. So $\widehat{\theta}_i$ is an unbiased estimator of θ_i. $\blacksquare$

Statement 2. $\hat{\lambda}(t)$ is a consistent estimator of $\lambda(t)$.

Proof: For $\hat{\lambda}(t)$ to be a consistent estimator of $\lambda(t)$, it is sufficient to show that $\hat{\lambda}(t)-\lambda(t)$ converges to 0 in probability. By (S4), we have

$$\hat{\lambda}(t)-\lambda(t) = \frac{\sum_{i=1}^{n} X_i(t) - \lambda(t)\sum_{i=1}^{n}\sum_{j=1}^{T} X_i(j)}{\sum_{i=1}^{n}\sum_{j=1}^{T} X_i(j)} = \frac{\frac{1}{n}\left[\sum_{i=1}^{n} X_i(t) - \lambda(t)\sum_{i=1}^{n}\sum_{j=1}^{T} X_i(j)\right]}{\frac{1}{n}\sum_{i=1}^{n}\sum_{j=1}^{T} X_i(j)}. \tag{S5}$$

We first consider the numerator (M_n) of (S5).

$$\begin{aligned} E\left(M_n\right) &= \frac{1}{n}\sum_{i=1}^{n}\left[\lambda(t)\theta_i - \lambda(t)\left(\sum_{j=1}^{T}\lambda(j)\theta_i\right)\right] \\ &= \frac{1}{n}\sum_{i=1}^{n}\left[\lambda(t)\theta_i - \lambda(t)\theta_i\right] \\ &= 0 \end{aligned},$$

and

$$
\begin{aligned}
Var(M_n) &= \frac{1}{n^2}\sum_{i=1}^{n}\left[\left(1-\lambda(t)\right)^2 k\lambda(t)\theta_i + \lambda(t)^2\left(\sum_{j\neq t}^{T} k\lambda(j)\theta_i\right)\right] \\
&= \frac{1}{n^2}\sum_{i=1}^{n}\left[\left(1-\lambda(t)\right)^2 k\lambda(t)\theta_i + k\lambda(t)^2\left(1-\lambda(t)\right)\theta_i\right] \\
&= \frac{1}{n^2}\sum_{i=1}^{n}\left[k\left(1-\lambda(t)\right)\lambda(t)\theta_i\right] \\
&= \frac{k\left(1-\lambda(t)\right)\lambda(t)\theta_i}{n}
\end{aligned}.
$$

So the numerator M_n converges to 0 in probability as n goes to infinity. Now we consider the denominator (D_n) in (S5). It is reasonable to assume that θ_i's are uniformly bounded. That is that there exists a positive real value A and B, such that $A \le |\theta_i| \le B$ for any i. Then we have

$$
0 < A \le E(D_n) = \frac{\sum_{i=1}^{n}\theta_i}{n} \le B,\quad Var(D_n) = \frac{\sum_{i=1}^{n}k\theta_i}{n^2} \le \frac{kB}{n} \text{ and } \lim_{n\to\infty} Var(D_n) = 0.
$$

Consequently, for any $\varepsilon > 0$, we have

$$
\begin{aligned}
\lim_{n\to\infty} P\left(\left|\tfrac{M_n}{D_n}\right| > \varepsilon\right) &= \lim_{n\to\infty}\left(P\left(\left|\tfrac{M_n}{D_n}\right| > \varepsilon, |D_n - E(D_n)| > \tfrac{A}{2}\right) + P\left(\left|\tfrac{M_n}{D_n}\right| > \varepsilon, |D_n - E(D_n)| \le \tfrac{A}{2}\right)\right) \\
&\le \lim_{n\to\infty}\left(P\left(|D_n - E(D_n)| > \tfrac{A}{2}\right) + P\left(\left|\tfrac{M_n}{A/2}\right| > \varepsilon, |D_n - E(D_n)| \le \tfrac{A}{2}\right)\right) \\
&\le \lim_{n\to\infty}\left(\frac{Var(D_n)}{(A/2)^2} + P\left(|M_n| > \tfrac{A\varepsilon}{2}\right)\right) \\
&= \lim_{n\to\infty}\frac{Var(D_n)}{(A/2)^2} + \lim_{n\to\infty} P\left(|M_n| > \tfrac{A\varepsilon}{2}\right) \\
&= 0
\end{aligned},
$$

So $\hat{\lambda}(t) - \lambda(t) = \frac{M_n}{D_n}$ converges to 0 in probability as n goes to infinity, and then $\hat{\lambda}(t)$ is a consistent estimator of $\lambda(t)$. ∎