Standardization

A comparison of in vitro transcribed RNA verses synthetic oligo DNA standards
RNA vs DNA Standards

**Human ER\(\alpha\) - sDNA vs sRNA**

\[ y = -3.234x + 38.037 \]

\[ R^2 = 0.9981 \]

**Unknown values:**
- sRNA - \(1.86 \times 10^4\) molecules
- sDNA - \(1.75 \times 10^4\) molecules
RNA vs DNA Standards

Human β-Actin - sDNA vs sRNA

\[ y = -3.4374x + 39.26 \]

\[ R^2 = 0.9995 \]
The term “absolute quantification” is a misnomer - it does not exist with the technology available today.

For “absolute quantification, we will need an independent method to quantify how many molecules in a reference standard will work in real-time qPCR, e.g., mass spectrophotometry.

All quantification is ‘relative’ to the standard being use whether it is a standard curve or a reference sample for a ddCt calculation.