

## **Primer Normalization**

### **1. Make cDNA standards A → E**

2. Select the cycle and fix the annealing temperature.
3. Run Q-PCR with standards and Blank
4. Check the melting curve, whether it has single product or not.
5. Check 3.3 CT value difference between 1:10 dilutions
6. Check whether blanks amplified or not.
7. Check whether it has non-specific amplification.
8. Confirm the single product by running gel.