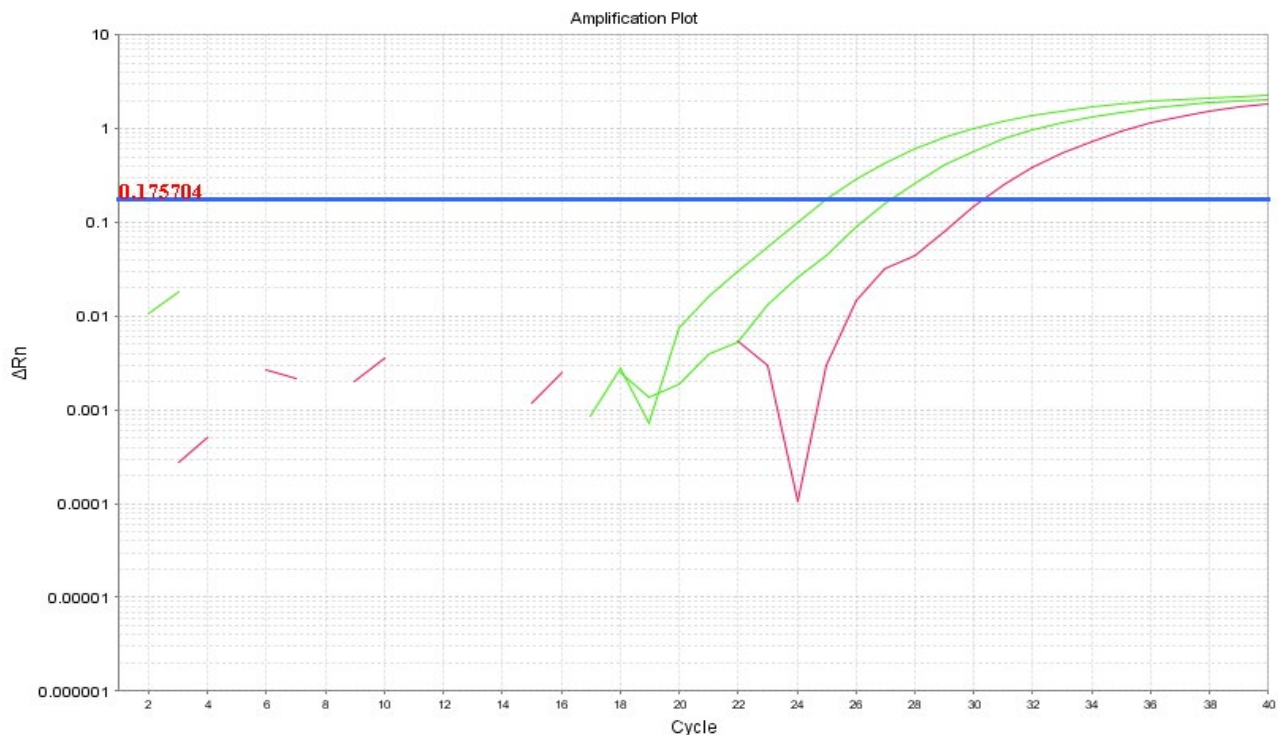


### Understanding PCR Ct values

The PCR Ct (cycle threshold) value refers to the number of cycles needed to replicate enough DNA/RNA to be detected (crosses a threshold line). A Ct value of 20 means it took fewer cycles to produce enough DNA/RNA than a Ct of 30. The lower Ct value means there was more DNA/RNA in the sample to begin with.

**Large amount of DNA/RNA in sample → Fewer cycles needed for detection → Low/Hot Ct**

**Smaller amounts of DNA/RNA in sample → More cycles needed for detection → Higher/Cooler Ct**



Looking at the plot above, the **red line** on the right has a Ct ~30 (crosses the **blue** threshold line), the middle line has a Ct ~27 and the furthest left **green line** has a Ct ~25 according to the figure. (Don't worry, the program does calculate exact Ct's, which we use for reporting.) In this case, the sample associated with the **red line** had the *least* amount of DNA/RNA. Whereas the sample associated with the line furthest to the left, had the most DNA/RNA. When a Ct is recorded as 0, no DNA/RNA of the target was detected.

Johne's example: An animal with a Ct less than or equal to 29 is considered a high Johne's shedder, because fewer cycles were needed to detect the DNA. This correlates with a large number of bacteria. An animal with a Ct of 36-37 took more replication cycles, meaning there is less DNA. This correlates with fewer bacteria in the sample and therefore is considered a low shedder.

Figure and text by B.Stenger, 2022