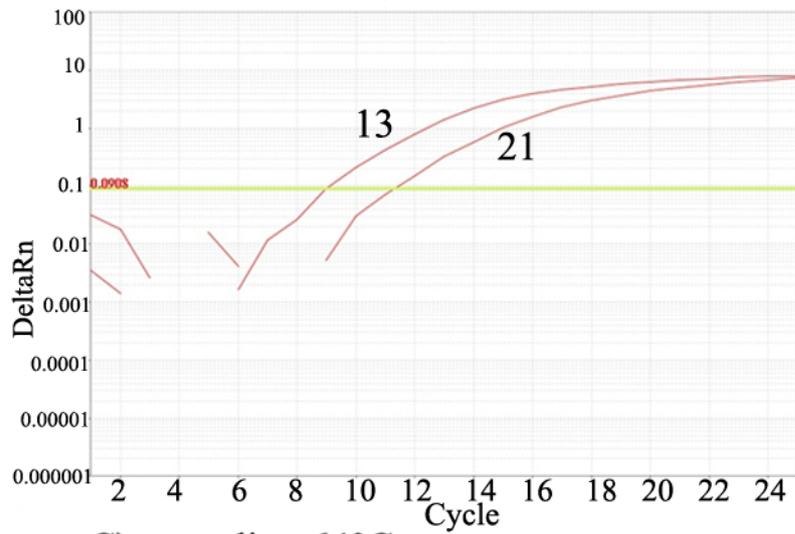
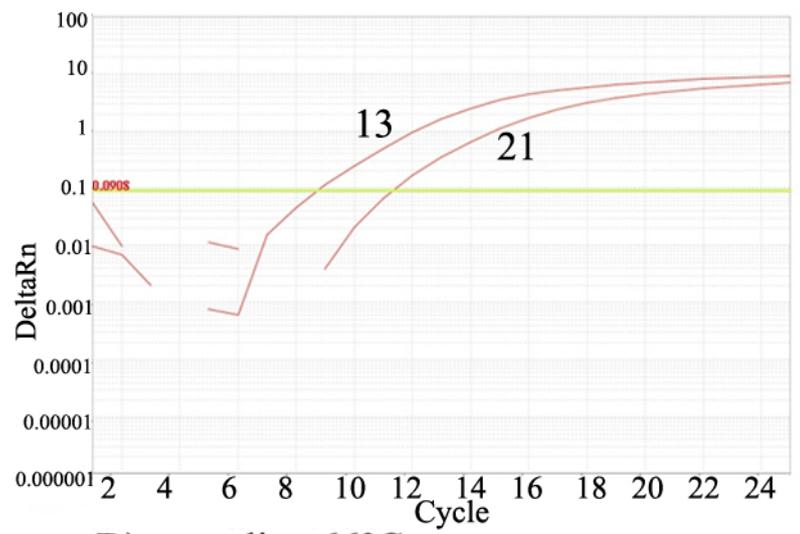


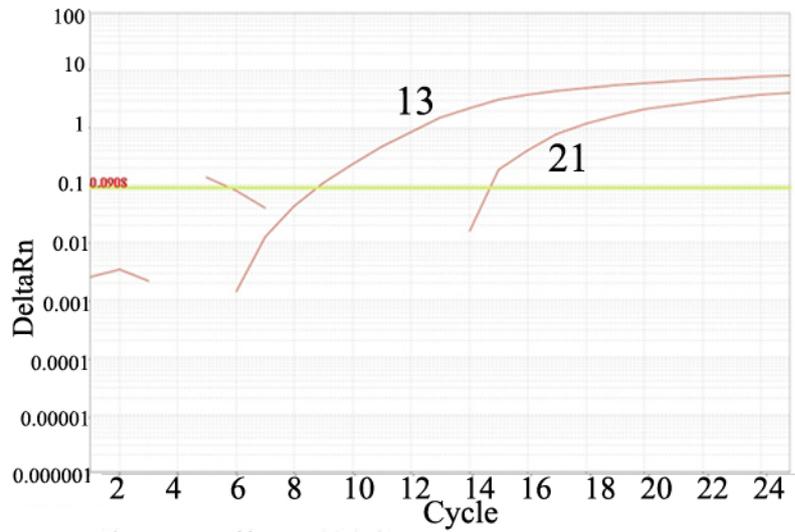
a A) annealing 60°C



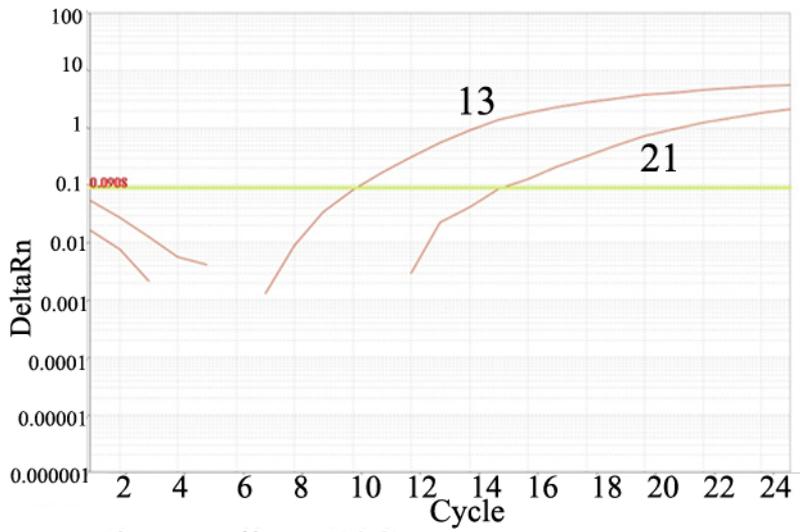
B) annealing 62°C



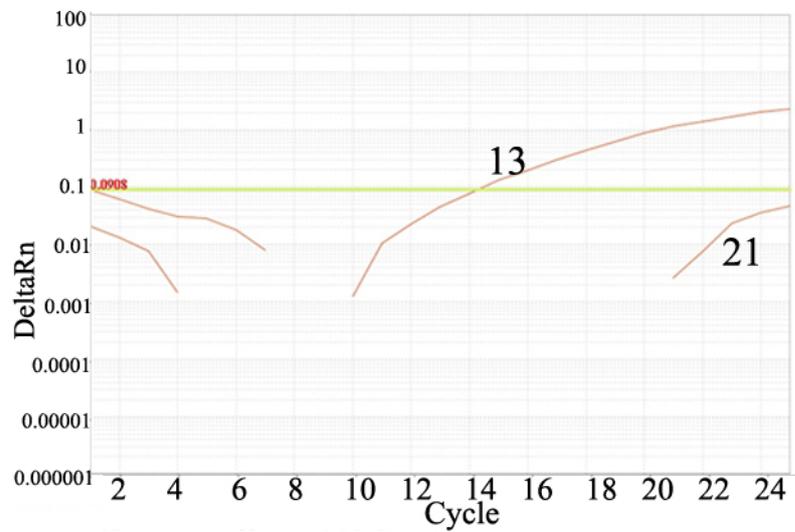
C) annealing 64°C



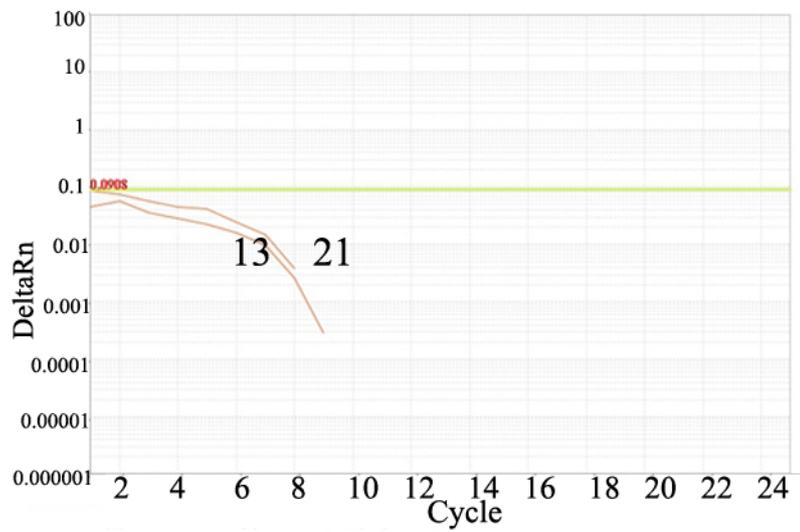
D) annealing 66°C



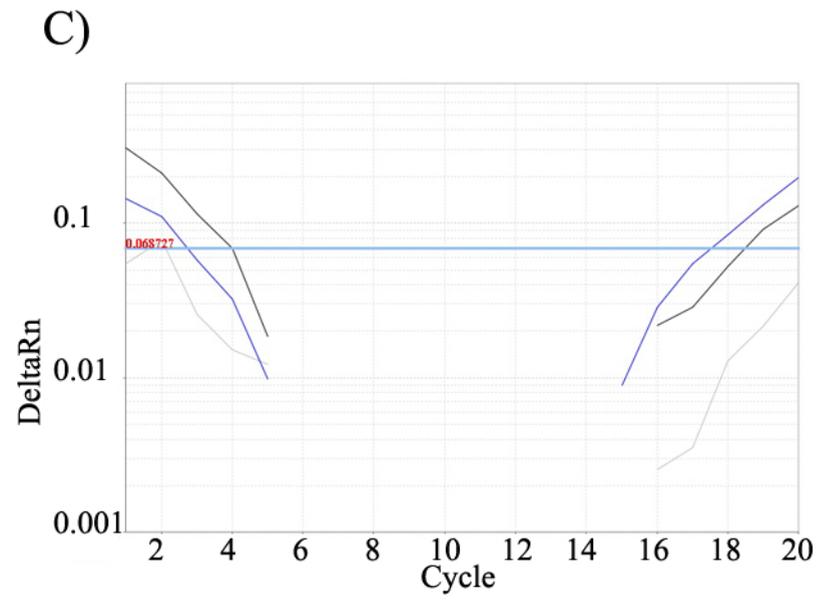
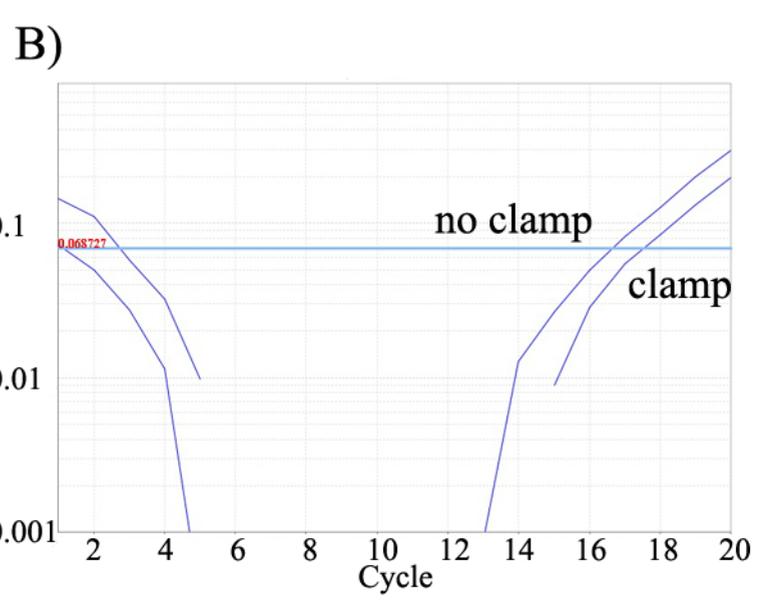
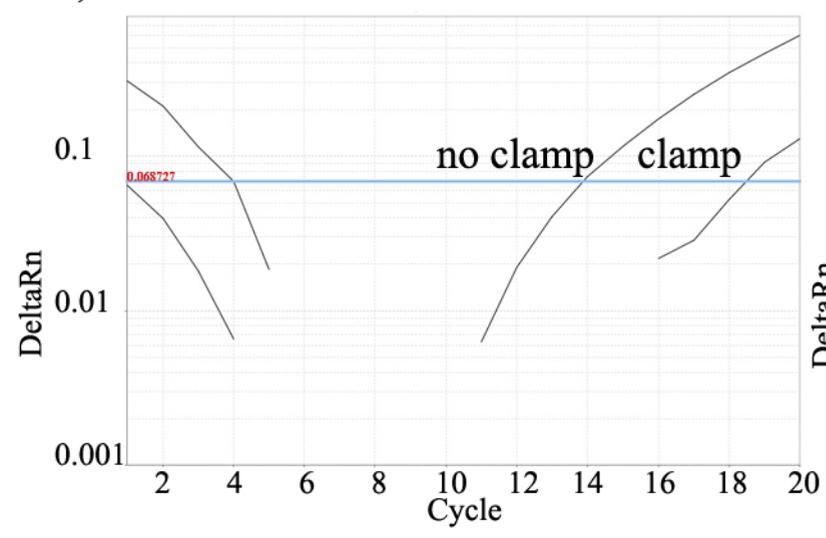
E) annealing 68°C



F) annealing 70°C



b A)  $\blacksquare$  Y  $\blacksquare$  13  $\blacksquare$  21



**Figure S5. PCR optimization to detect a nucleotide substitution in centromeres 13 and 21.**

The homologue arrays D13Z1/D21Z1, present in centromere 13 and 21, are almost 100% identical. An A/T substitution, however, has been shown to exist in centromere 13 in the D13Z1 array (see also Fig. S4). a) A qPCR assay was developed to detect the “T” substitution present in D13Z1 using a primer with locked nucleic acid (LNA) modifications at the nucleotide substitution and one base before and after it. This primer, in combination with a reverse primer that binds to both D13Z1 and D21Z1, was included in a PCR reaction in order to specifically detect the D13Z1 array. This combination of primers was tested in a PCR reaction with DNA isolated from human/rodent hybrid cells that contain human Chromosome 13, or hybrids that contain other chromosomes, using an annealing/extension temperature gradient (60-70 °C). Detection of D13Z1 sequences was confirmed by sequencing. Increasing annealing temperatures in a PCR reaction showed that at 68 °C, the D13Z1 LNA primer exclusively detected D13Z1, while the D21Z1 array is not detected. At 70 °C, the PCR assay detected neither array. b) A qPCR was developed to detect these “C and G” substitutions in D21Z1 using primers with locked nucleic acid (LNA) modifications at the substitutions and one base before and after them. These forward and reverse primers in combination with a D13Z1 primer clamp, which is the same LNA primer used to detect D13Z1 but phosphorylated at the 3' site in order to inhibit the amplification of D13Z1, was included in a PCR reaction in order to specifically detect the D21Z1 array. The combination of primers and clamps was amplified in a temperature gradient reaction (60-70 °C) using DNA isolated from human/rodent cell hybrids that contain Chromosome 21, or hybrids that contain other chromosomes. A) An annealing temperature of 64 °C in a PCR reaction showed that amplification of the centromere 13 array D13Z1 is substantially inhibited with the D13Z1 clamp, and B) the D21Z1 LNA primers preferentially

detect the D21Z1 array. C) Assessing the DNA from human/rodent hybrids shows that the PCR conditions favor the amplification of the centromere of Chromosome 21 rather than that of Chromosomes 13 or Y. By running the assay at 64 °C for 17 cycles and adjusting the fluorescent threshold in the Real-Time analysis, we are able to detect a substantial signal from Chr 21, but not from Chr 13. Water was used as a negative control.