


The use of chimeric DNA/RNA primers in quantitative PCR for microbial detection

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- Genaphora Ltd.
 - Tick born pathogens
 - *Ehrlichia canis*
 - *Babesia canis*
 - Chimeric primers for qPCR





- Genaphora Ltd. is a privately held company founded in February 2008, leading the way to next generation real-time PCR by developing advanced-computational-biology-based **molecular diagnostics assays**.

- Genaphora has developed a proprietary platform technology to provide supersensitive qPCR assays using:
 - Unique DNA/RNA chimeric primers

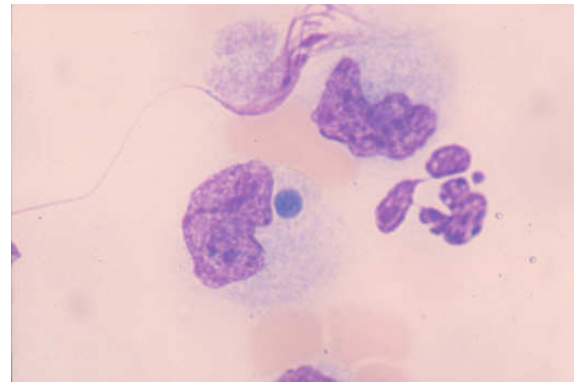
 - The Singleplexer software for searching the most suitable and complex primers & probes for qPCR

 - The Multiplexer software for searching the best primers & probe combinations for multiples qPCR

Ehrlichia canis

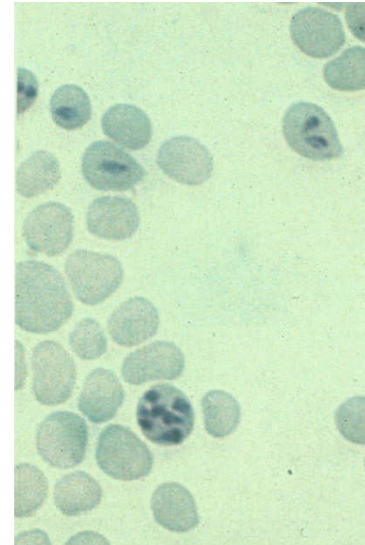


- Small gram-negative bacteria
- Tick-born canine pathogen
- Infecting monocytes
- Clusters called morulae – hard to visualize
- Disease prevalent worldwide
- The bacteria can persist in infected dogs for months & years
- Variable clinical signs
- May be fatal



Babesia canis

- *Babesia* spp. are protozoa infecting erythrocytes, causing hemolysis > resulting in anemia
- *B. canis canis*, *B. canis vogeli* *B. canis rossi* and *B. gibsoni* are commonly known organisms that infect dogs



Non-specific byproducts are inevitable?

- PCR non-specific byproducts seem to be inevitable

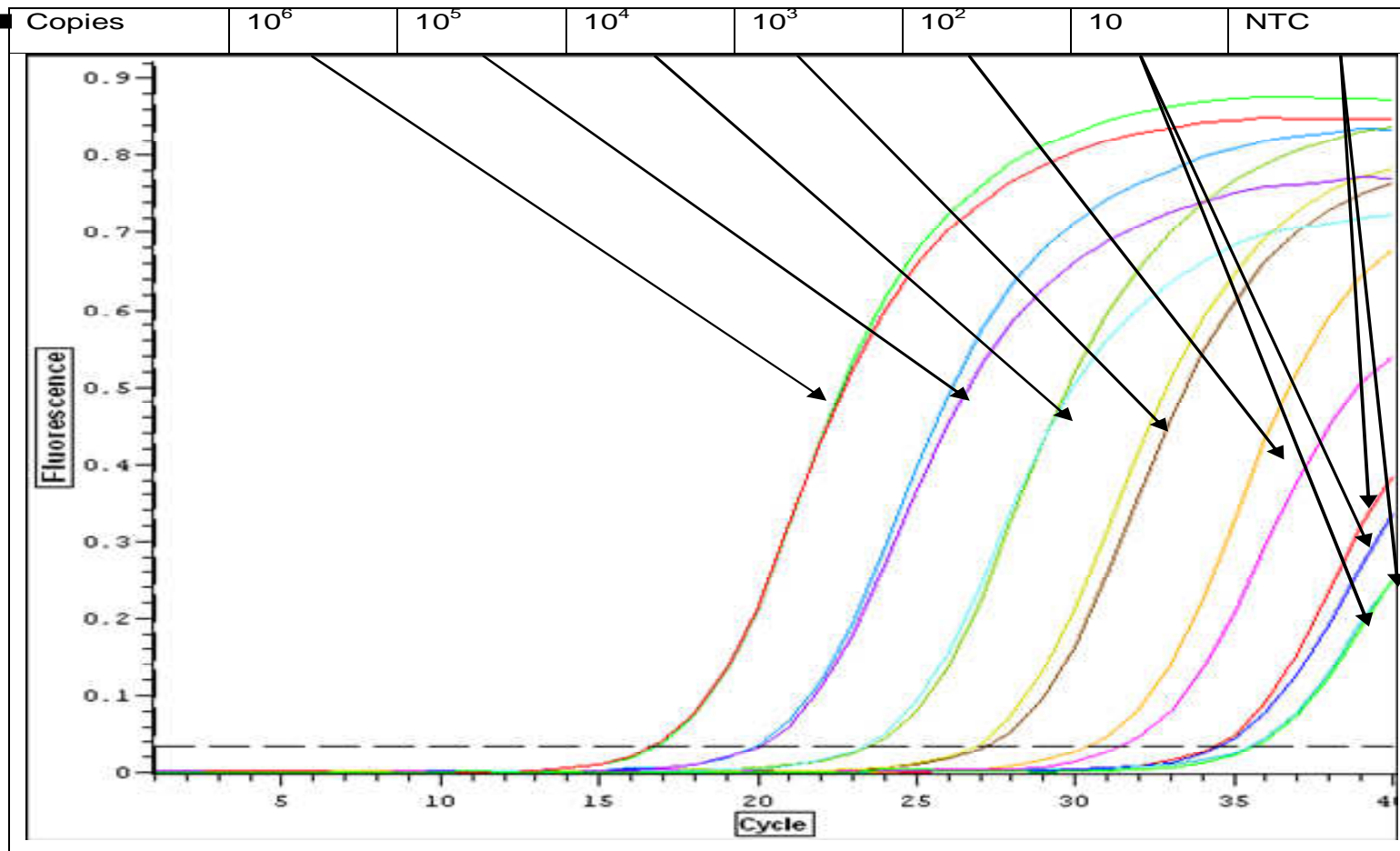
“Non-specific byproducts at low concentrations of target sequence appear

after 30 cycles when complementarity of at least one nucleotide at the 3' end is present, and

after cycle 40 when no 3' end complementarity present” (Brownie et al. 1997)

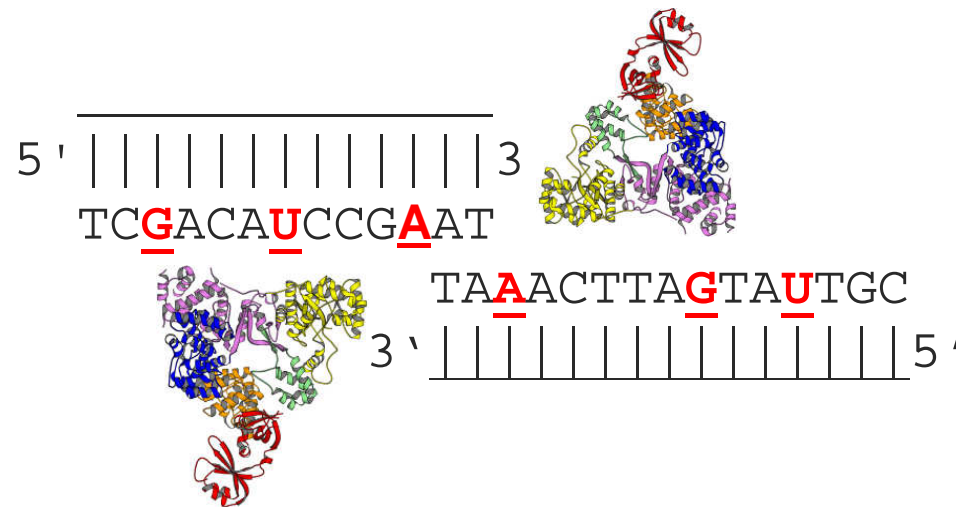


E. Canis 16s rRNA DNA primers



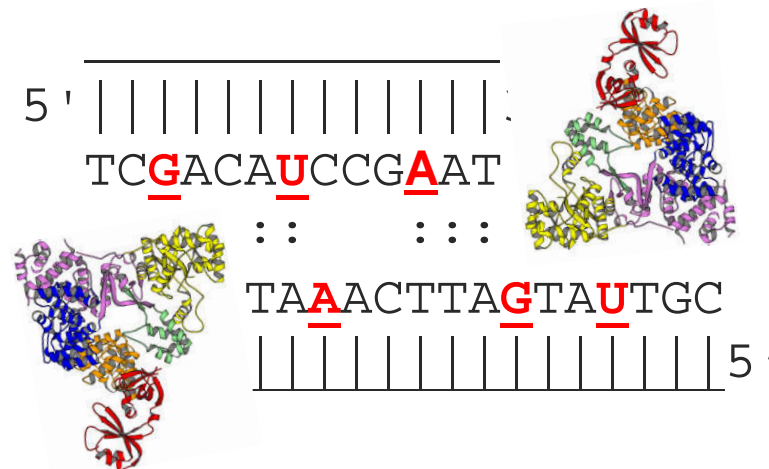
New DNA/RNA chimeric primers concept

“Initiation speed bumps”

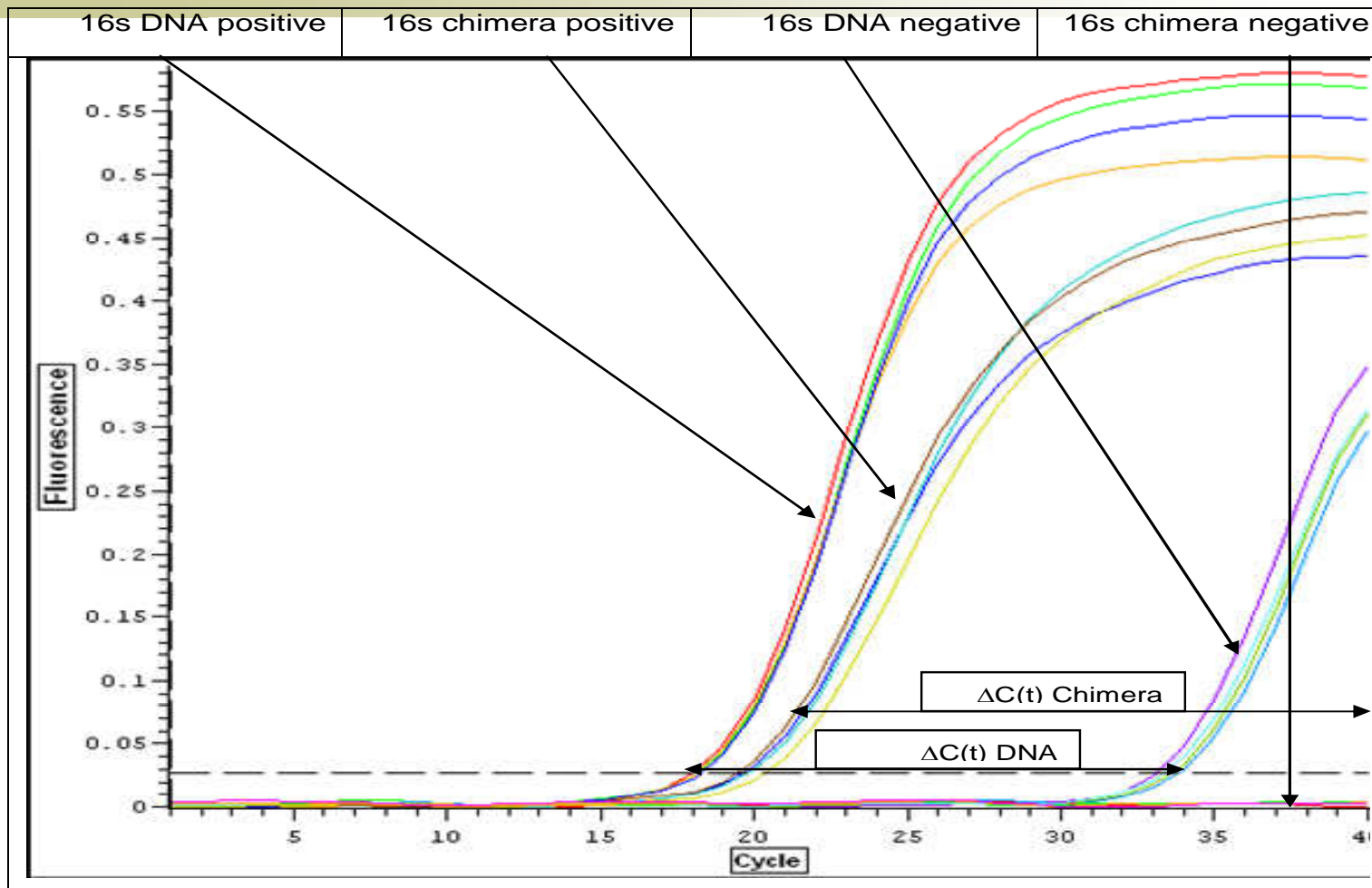


New DNA/RNA chimeric primers concept

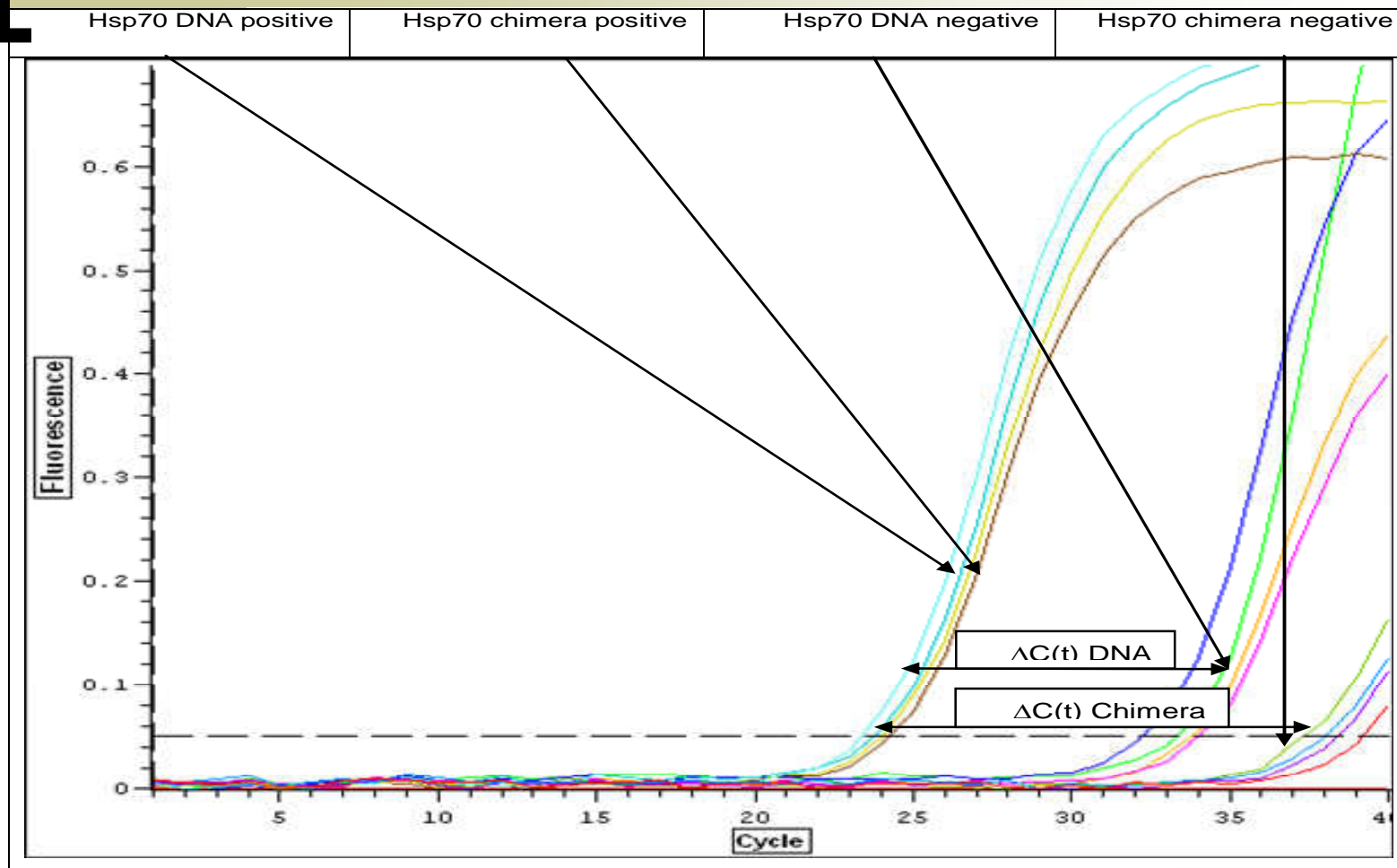
“Chimeric junction thermodynamics”



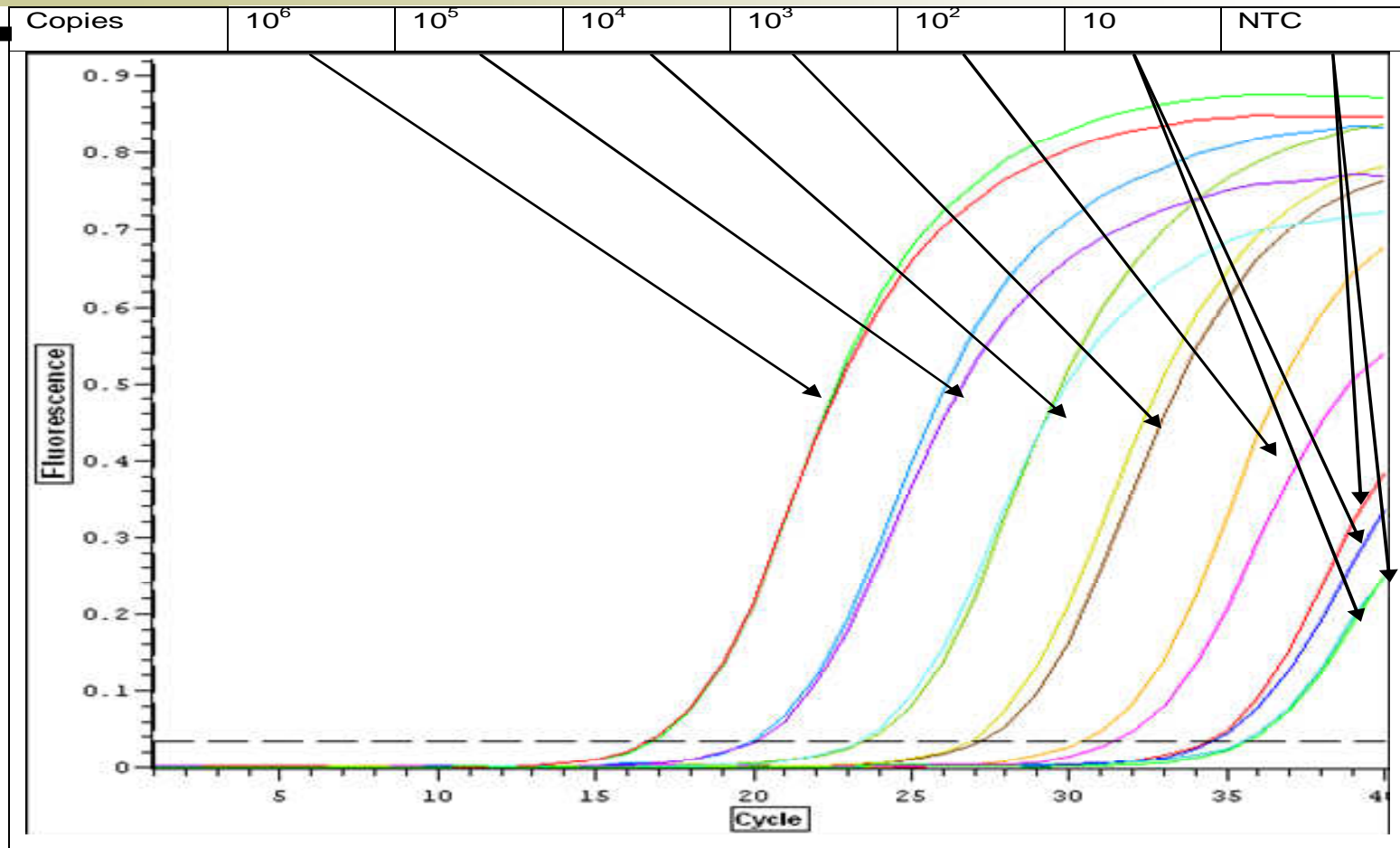
E. canis 16s rRNA gene DNA and chimeric primers



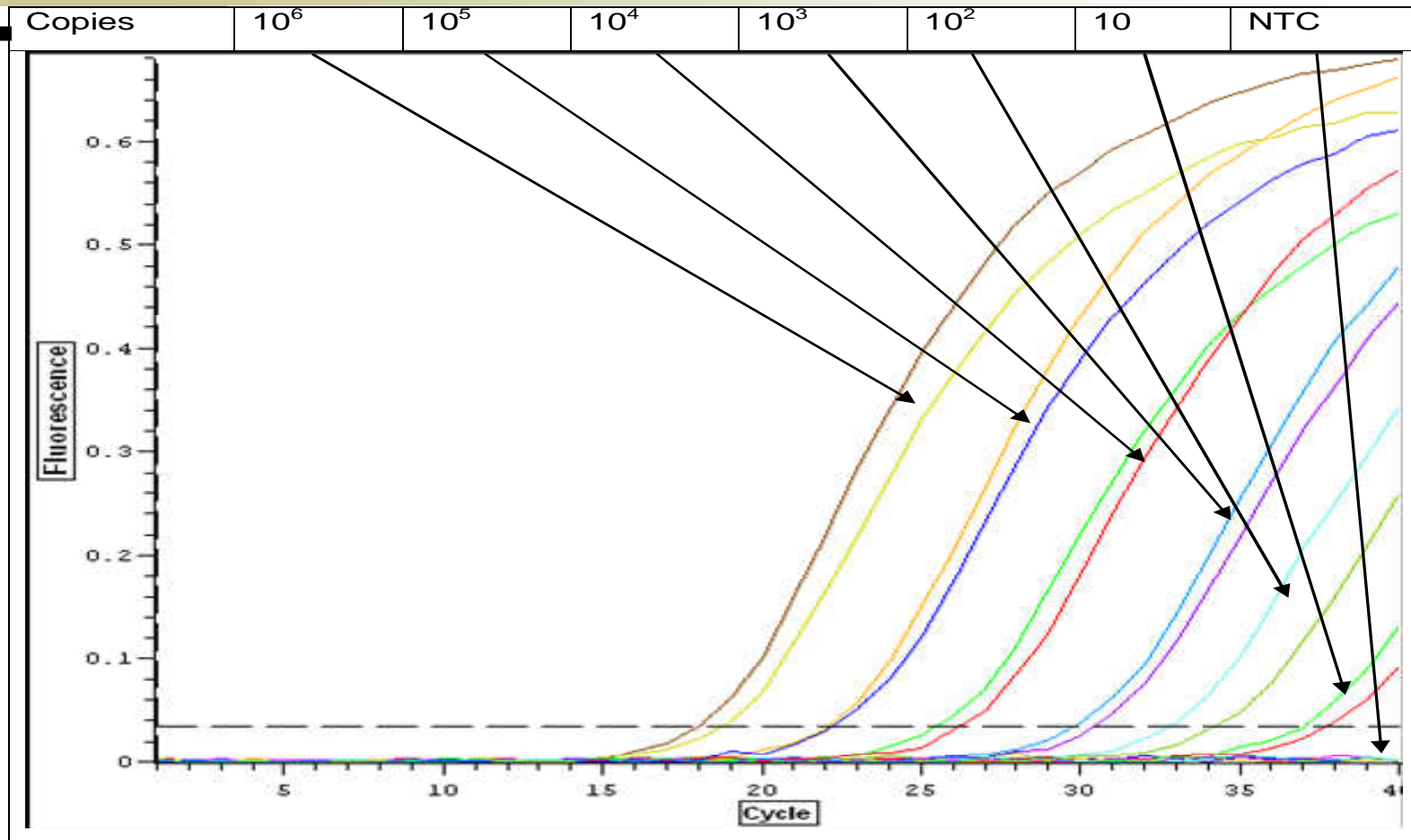
B.canis Hsp 70 DNA and chimeric primers



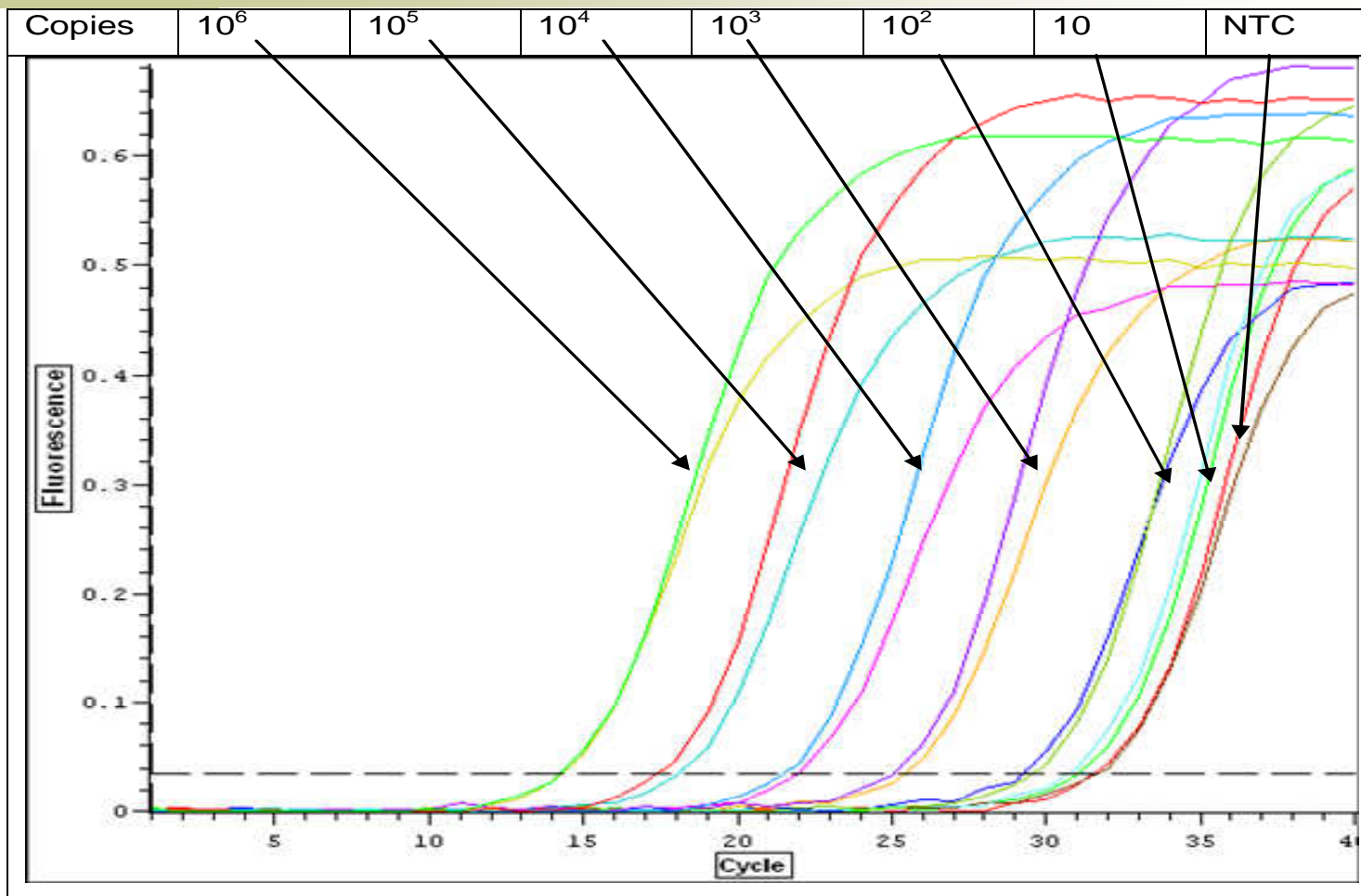
E. Canis 16s rRNA DNA primers



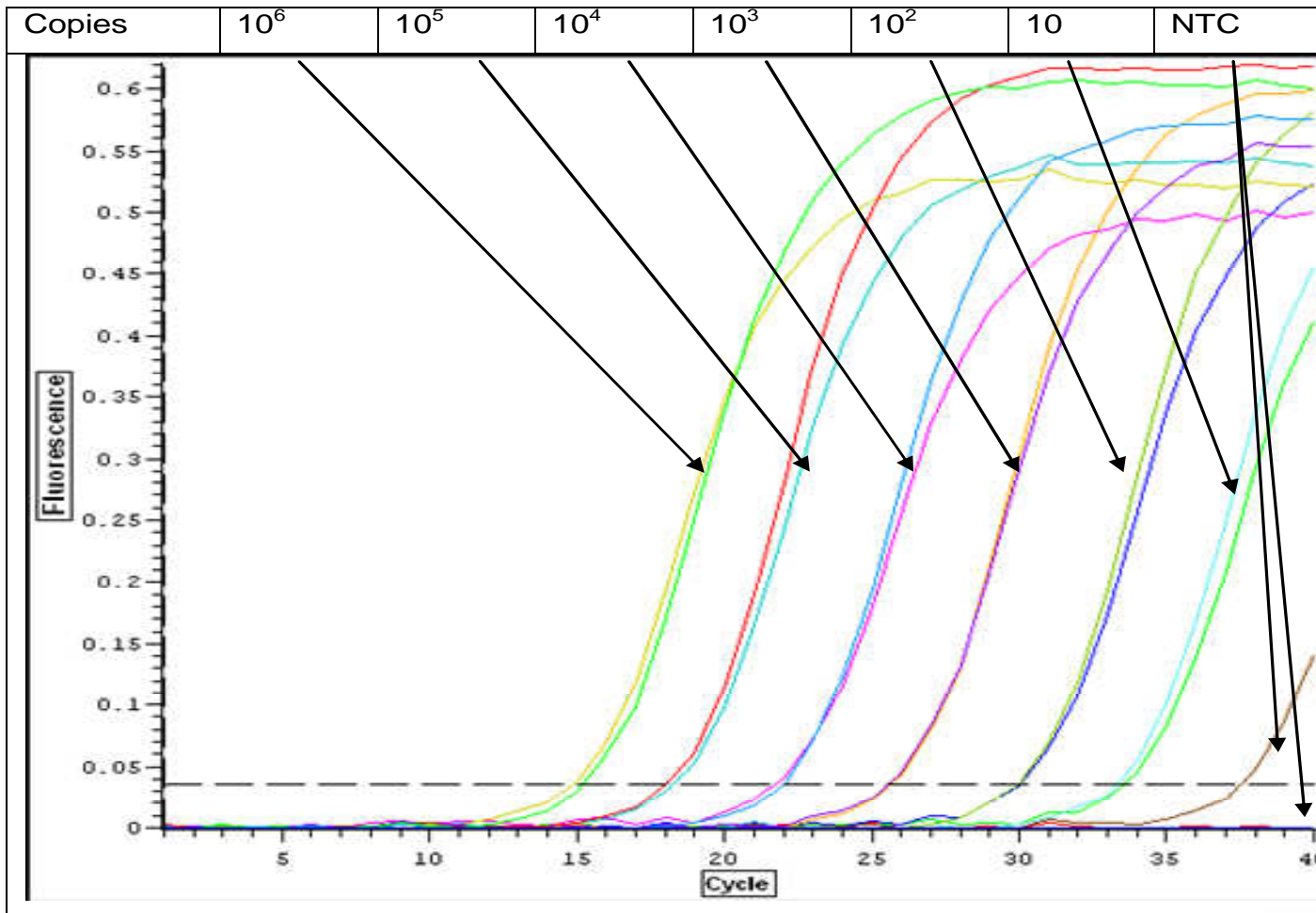
E. canis 16s rRNA chimeric primers



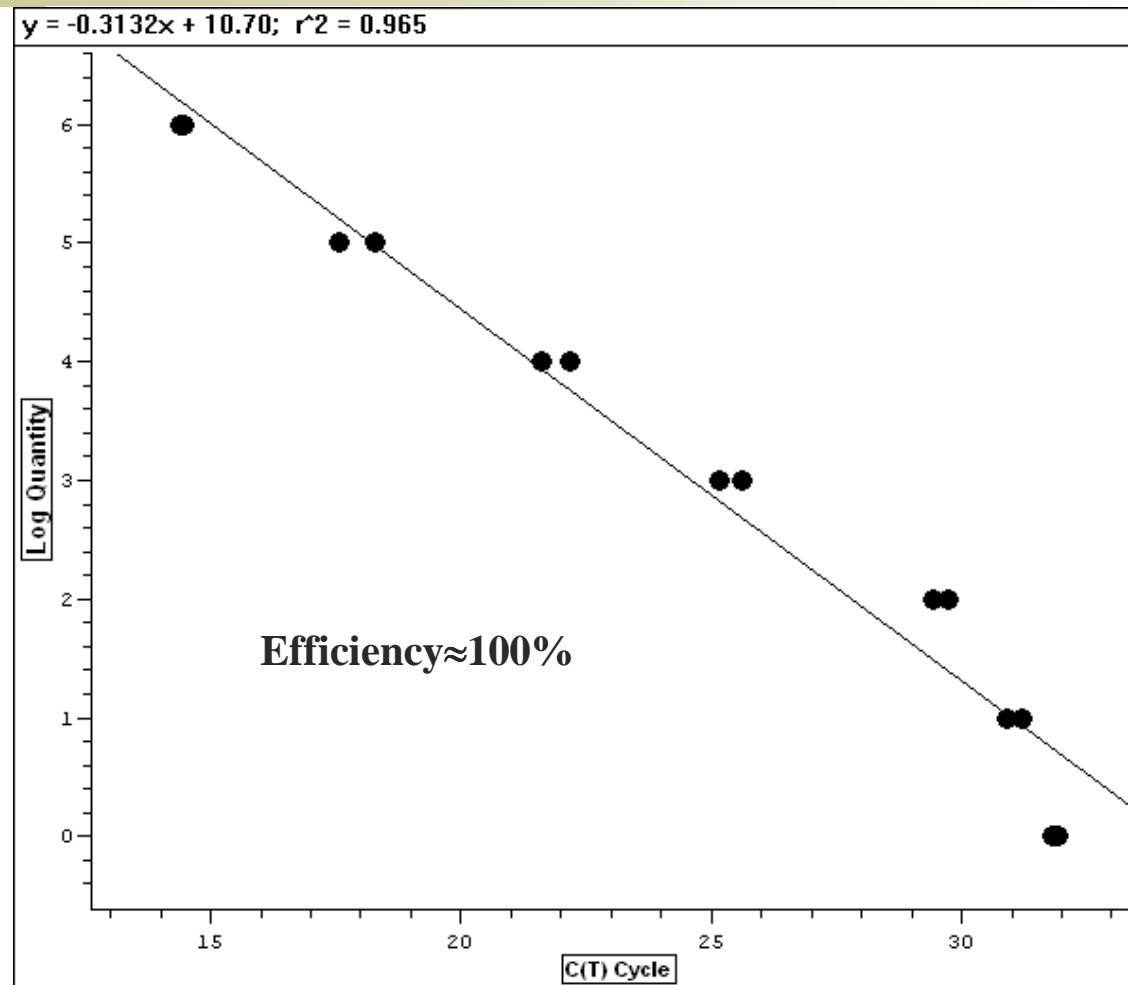
B. canis Hsp70 DNA primers



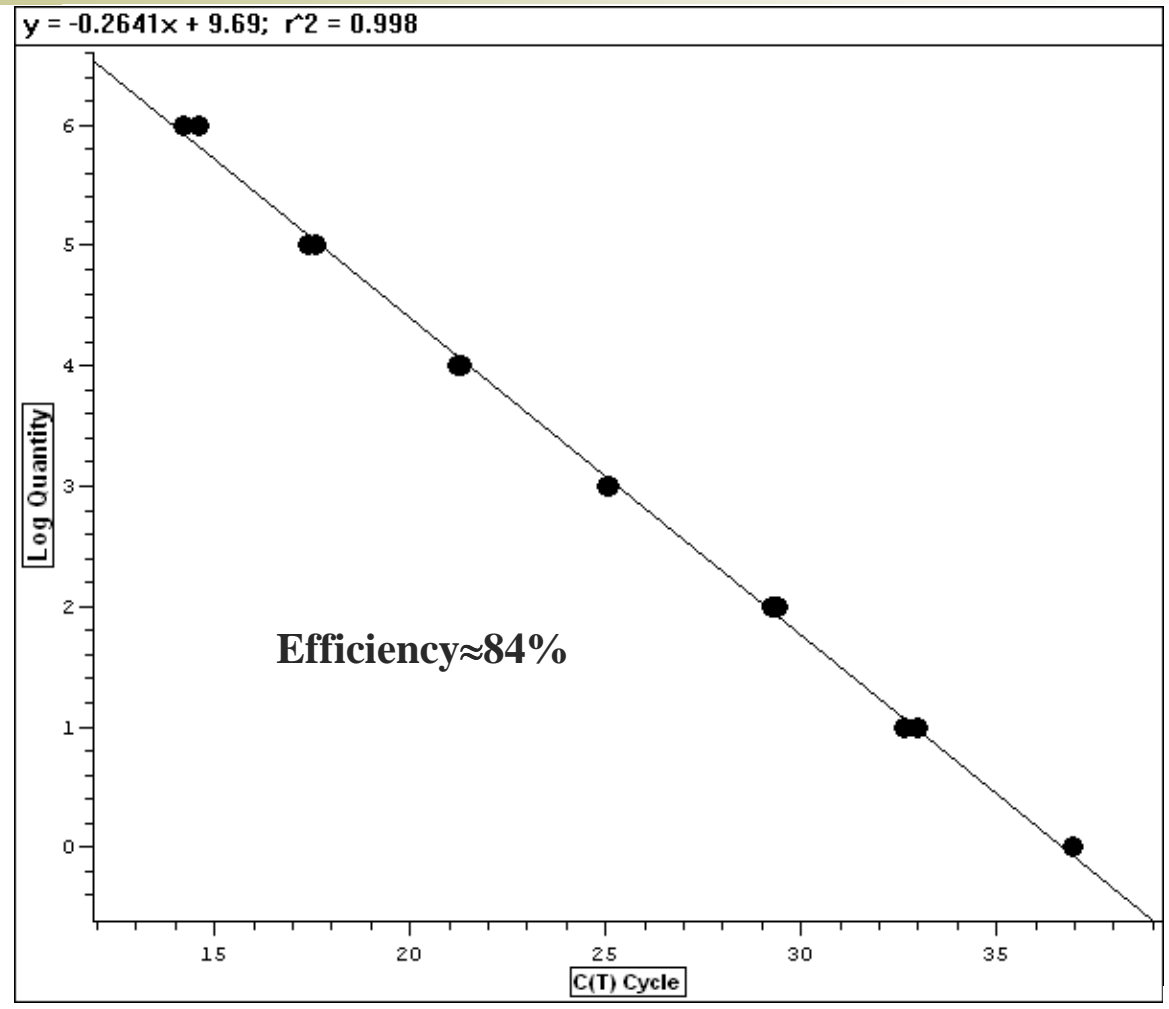
B. canis Hsp 70 Chimeric primers



Standard plot of *B.canis vogeli Hsp70* gene using DNA primers



Standard plot of *B.canis vogeli Hsp70* gene using Chimeric primers

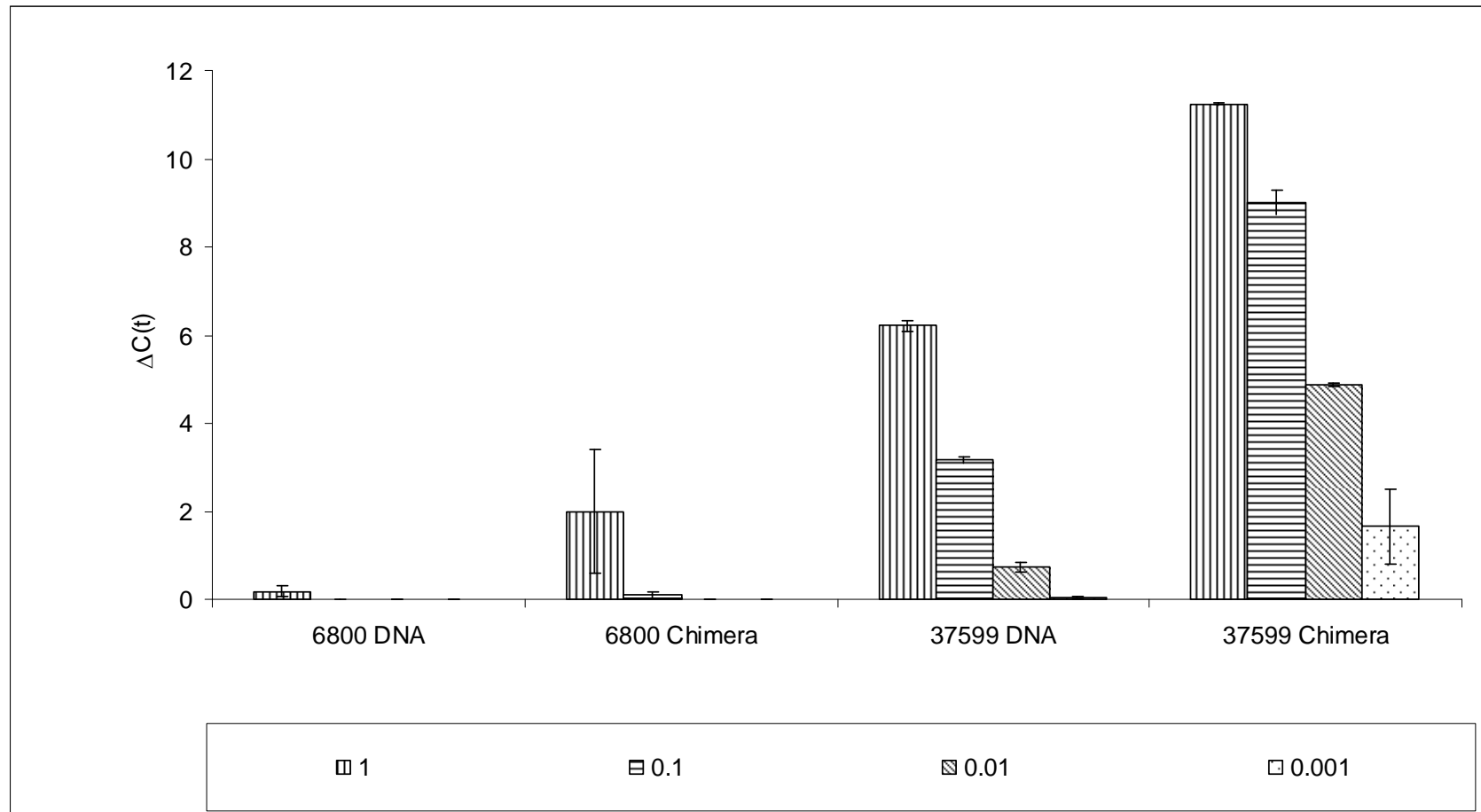


Chimera mechanism

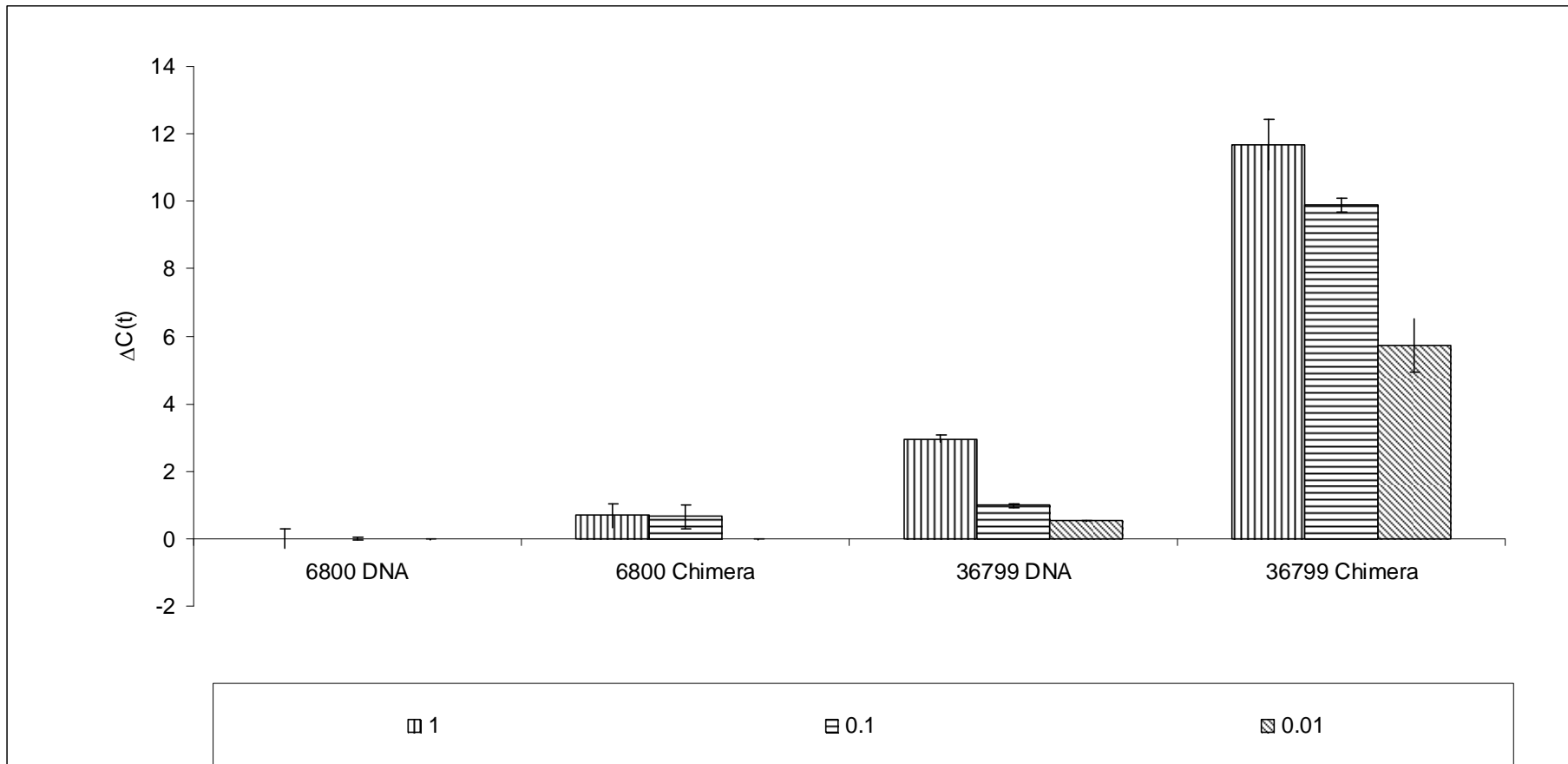
Second cycle



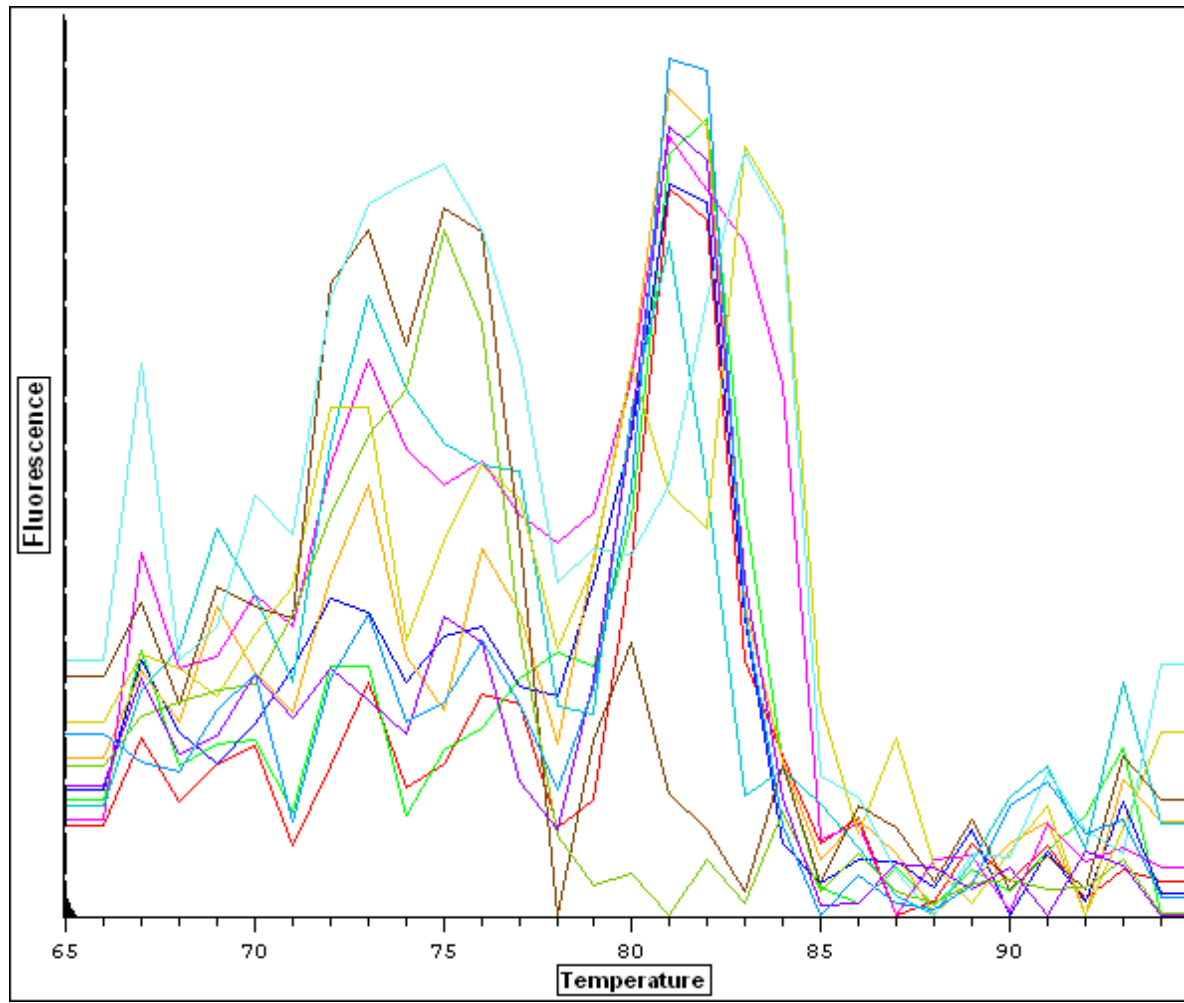
Serial decimal dilutions of DNA samples extracted from blood of naturally infected dogs with *Ehrlichia canis*



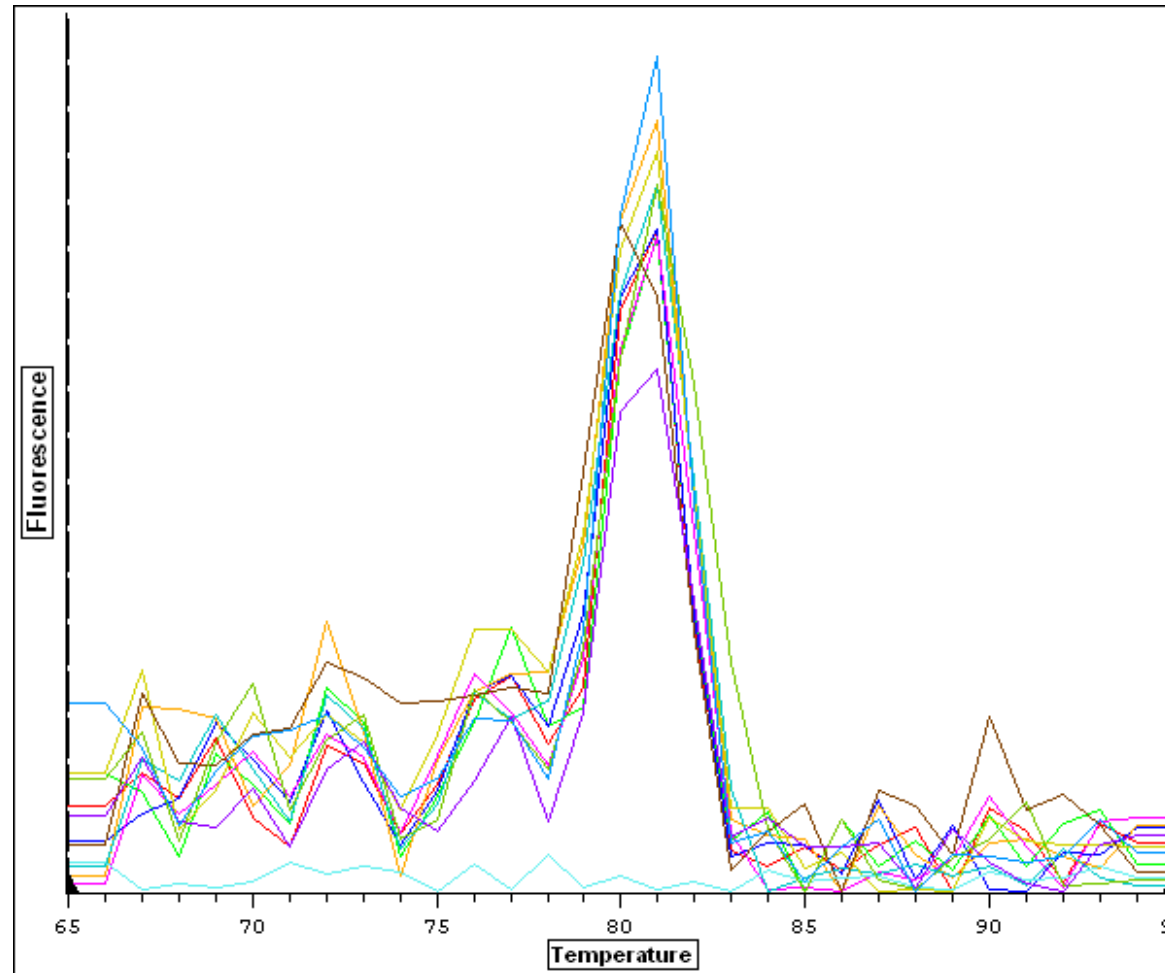
Serial decimal dilutions of DNA samples extracted from blood of naturally infected dogs with *Babesia canis*



T_m analysis of *β actin* from DNA samples extracted from blood of a healthy puppy using DNA primers



T_m analysis of *β actin* from DNA samples extracted from blood of a healthy puppy using chimeric primers

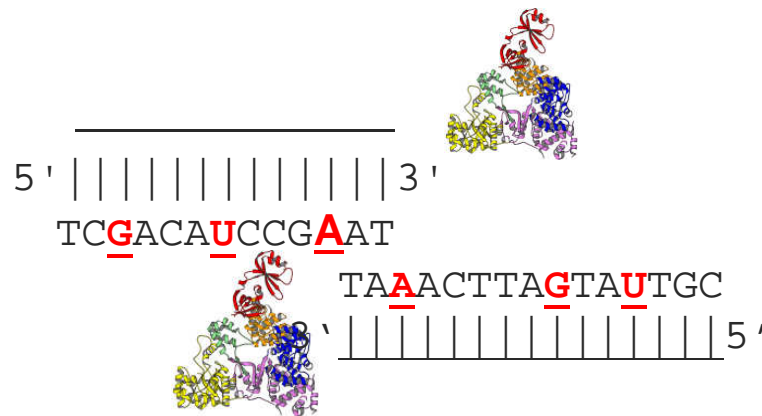


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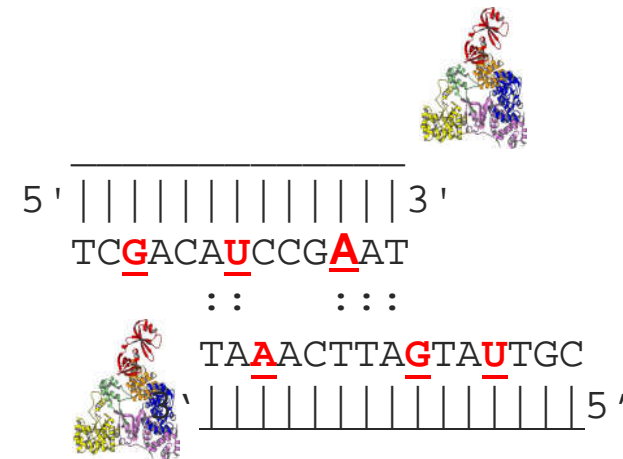


Putative mechanisms

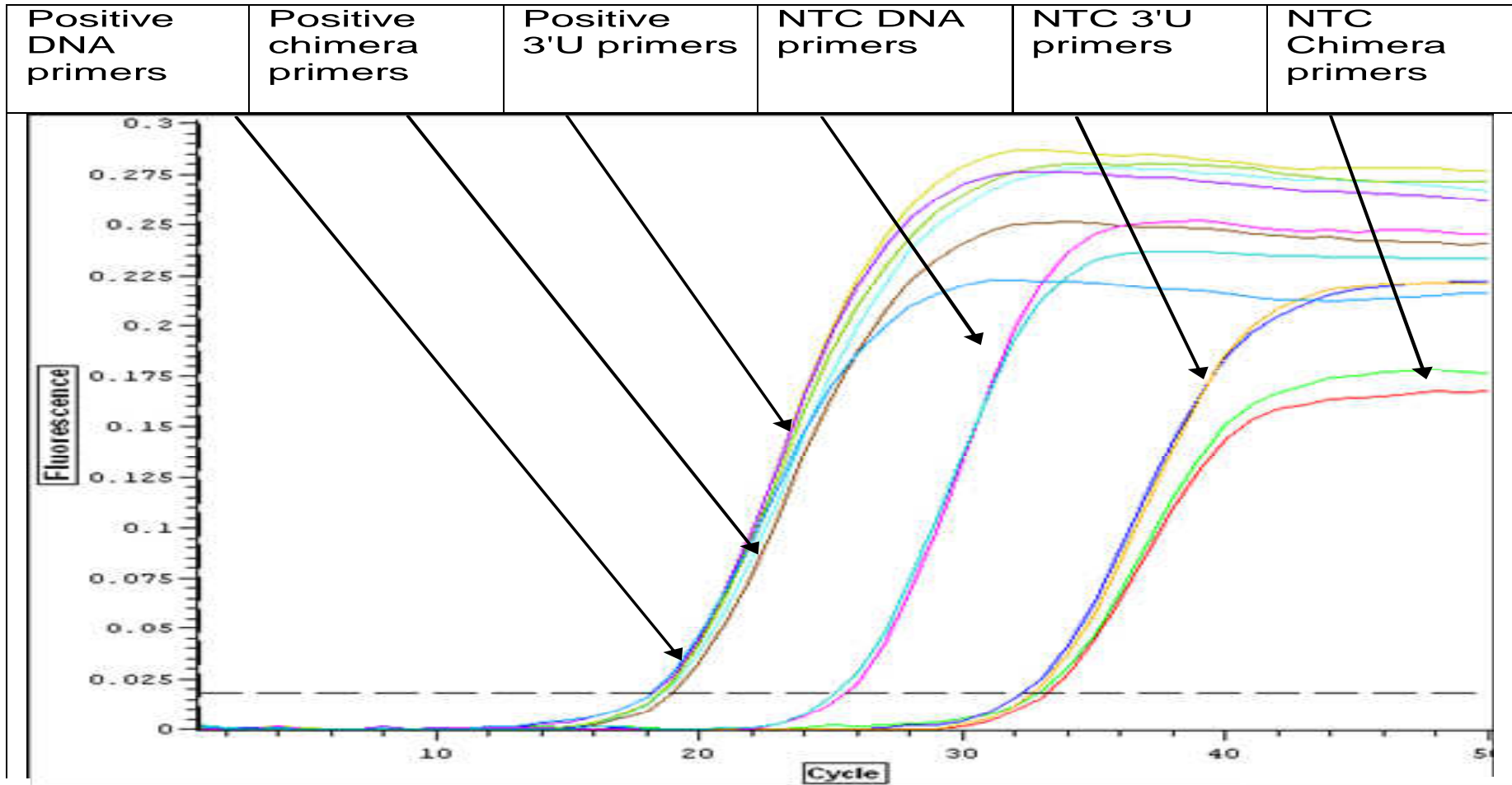
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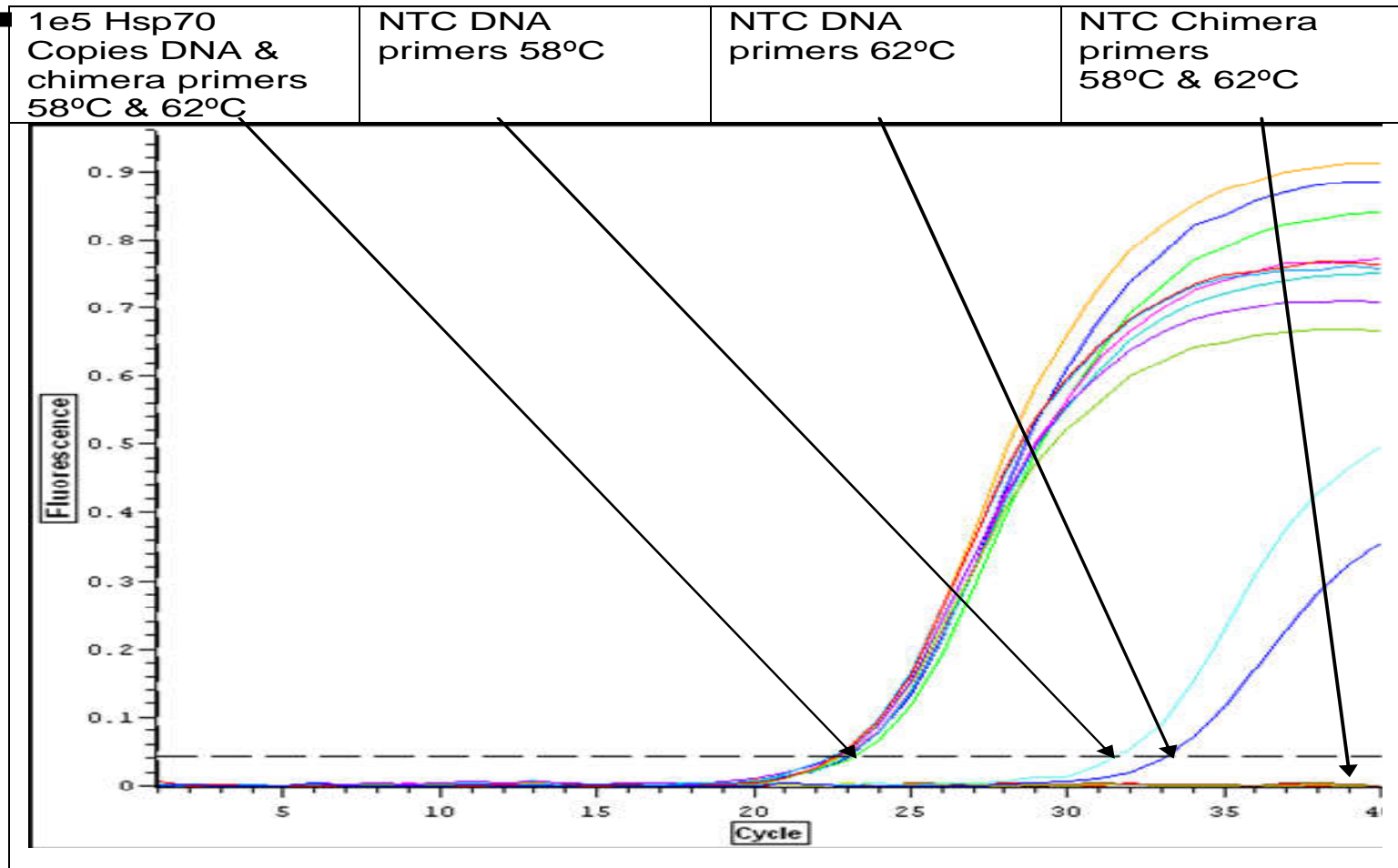
“Chimeric junction thermodynamics”



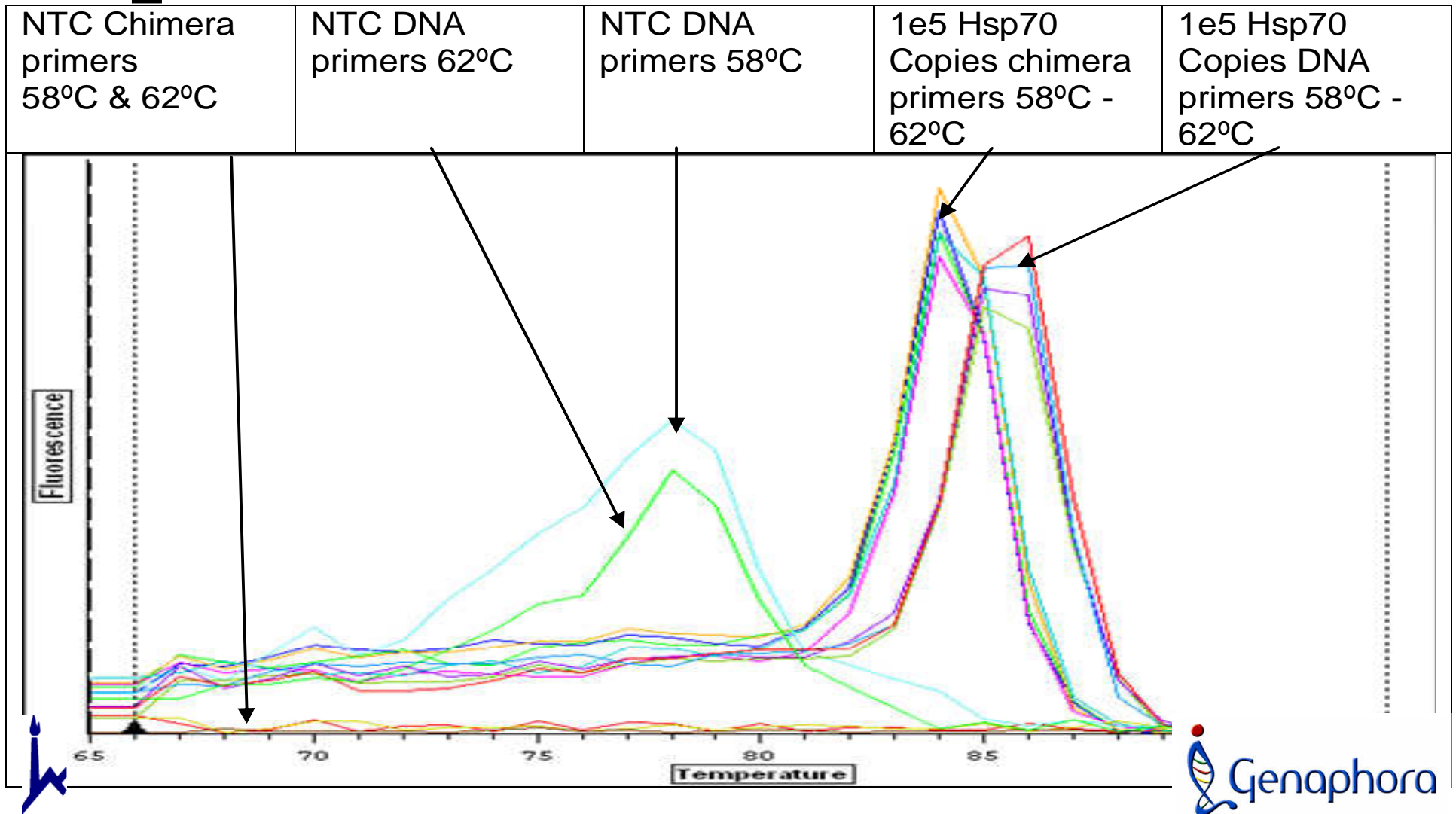
Chimera vs 3'U vs DNA primers



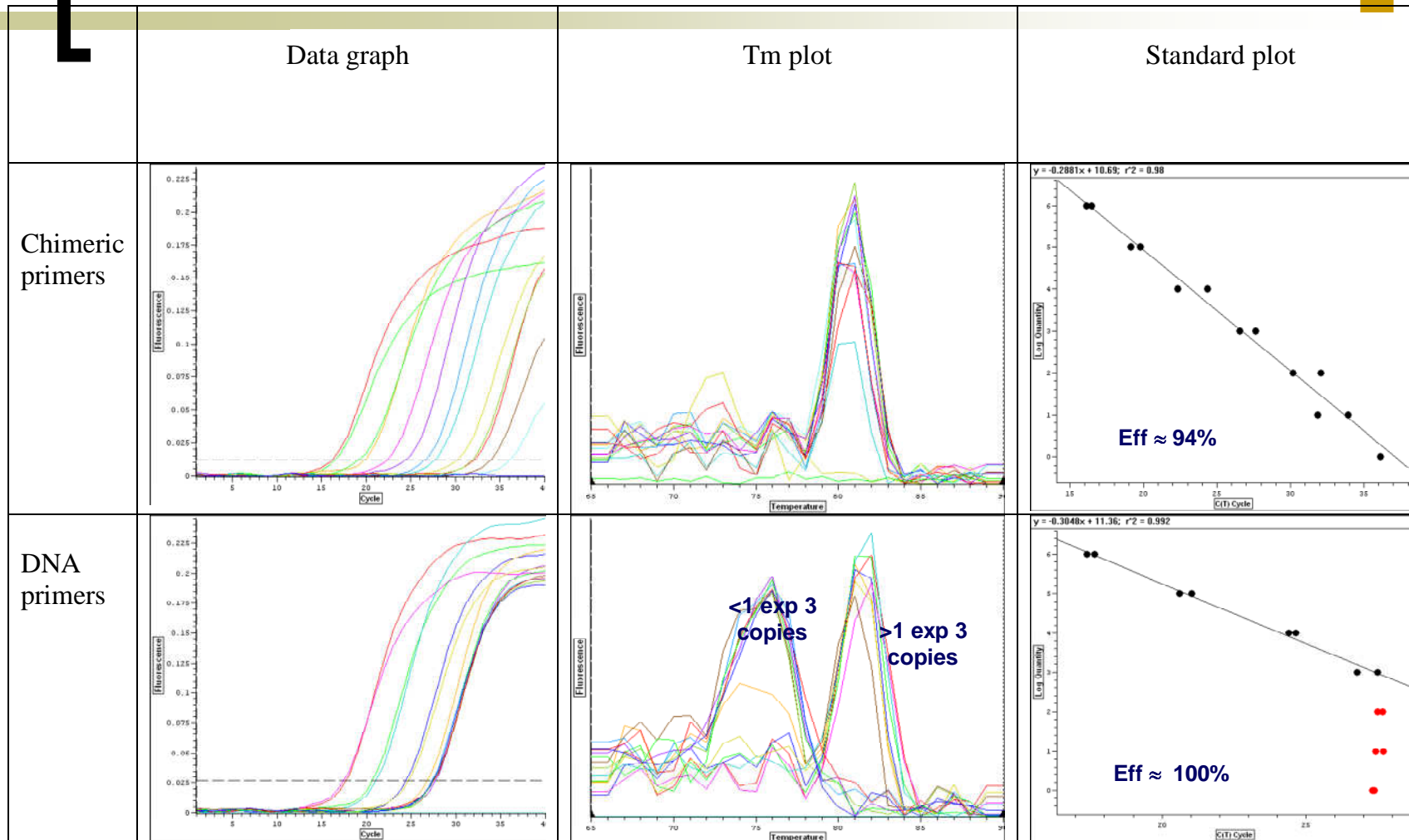
B.canis HSP70 Gradient 58°C-62 °C Eva Green (Jena) DNA vs Chimera



Tm plot Gradient 58°C-62 °C Eva-Green DNA vs Chimera



SyBR green, NTP - dUTP, *Canin ACTB*



Summary

- **Genaphora's unique DNA/RNA chimeric primers**
 - **Minimize the formation of non specific products**
 - **Reduce threshold and elevate assay sensitivity**
 - **Enable low copy number detection using non specific dye**
 - **Enable HRM analysis of low copy number of target**
- **The deduced mechanism is probably thermodynamic**
- **Using chimeric primers for HRM analysis is underway**
- **Using chimeric primers with specific probes is underway**



[Acknowledgements]

- Dr. Osnat Eyal
- Dr. Kobi Inbar
- Prof. Gad Baneth
- Prof. Shimon Harrus



My beloved negative control





Thank you

