Real Time PCR

eppendorf

Improved Real Time PCR Applications using Novel Eppendorf Amplification Technologies

> Andrés Jarrin Application Specialist Eppendorf AG



Specific Challenges of TaqMan like qPCR

Real time qPCR with cleavable signaling probes is a multiplex assay by design, even with a single target

Target Amplification (PCR) Enzymatic Signal Release (Probe Cleavage) Physical Signal Release & Detection (Excitation, Emission, & Fluorescence Detection)

Signal Amplification

All 3 processes run simultaneaously, but have diffrerent optimal reaction conditions.

Challenge: - To find the most efficient compromise.





Specific Challenges of TaqMan like qPCR

Approach to improve qPCR results:

Novel Hot Start Technology

Optimize Buffer Systems









DNA Substrate

≥ **60°C**



Front View



Front View



Competitive Affinity Ligand



- Monovalent Ion Concentration
- Inhibitor Conc.
- Taq DNA Pol. Conc.









Characteristics:

- Competitively Binding Affinity Ligand Mimiking the DNA Substrate of Polymerases
- Inhibitor Binding and Dissociation Are Reversible and Temperature-dependent
- Provides "Hot Start" and "Cold Stop" (Brake): Permanent Control of Primer Extension Specificity throughout PCR cycling
- Mode of Action: Retardation of the DNA Polymerase below Permissive Temperature (60°C) through Reduction of the Extension Rate and Processivity
- No Heat-activation of the Polymerase Necessary
- Universal Inhibitors for Thermophilic DNA Polymerases
- Inert to Intercalating Fluorescent Dyes used for Detection
- No Interaction with Primers and Template DNA





5 Minutes Primer Extension with 2 U Taq DNA Polymerase with & without HotMaster Inhibitor







Hot Start - Cold Stop Feature







Amplification of a 2 kb ß-globin target on human genomic DNA





Amplification of a 131 bp $TNF\alpha$ target on human genomic DNA



eppendorf



Specific Challenges of TaqMan like qPCR

Approach to improve qPCR results:

Novel Hot Start Technology

•Optimize Buffer Systems





Influence of the Buffer on C_t





Influence of the Buffer on Signal and ΔRFU



eppendorf



Primer Dimer Supression by Non-canonical dNTPs

dNTP Analogs (Melt Comparison) 87 86.5 86 [emperature (°C) 85.5 85 84.5 84 83.5 83 82.5 dNIP dUTP dTTP deaza-dGTP dUTP/deaz: dUIP/ dITP dITP/dUTP/deaza

200 150 19/ (n=1) 19/ -50 76 Temperature, Celsius 200 150 / (ILH) / 50 80 86 88 90 92 94 60 62 -64 66 68 70 72 -74 76 78 82 84 Temperature, Celsius





Primer Dimer Supression by Non-canonical dNTPs



NTC w/ RealMaster dNTPs

HotMaster Taq w/ STND dNTPs





Reproducibility Across 96 Wells





Storage Stability at Various Temperatures









Freeze/Thaw Storage Stability



Thanks to:



R&D, Boulder, Colorado: Ryan Westberry Jessica Goodrich Melissa Garner Allan Roberts

Eppendorf AG, Hamburg Andreas Gigler Andrés Jarrin

Product Applications Wilhelm Pluester JaNae Grutt Jennifer Halcome Gerry Huitt

