



# The Effect of Consumables on the Sensitivity and Reproducibility of QPCR

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# Factors Affecting Variance

- Plastic Consumables
  - White, Natural or Frosted
- Sealing Materials
  - Effect of Evaporation
  - Transmission
- QPCR Reagents
  - dTTP vs dUTP
  - Enzyme Activation

# Plastic Consumables

- Comparison of three plate types for QPCR
  - Natural
  - Frosted
  - Opaque white
  - Mirror plate



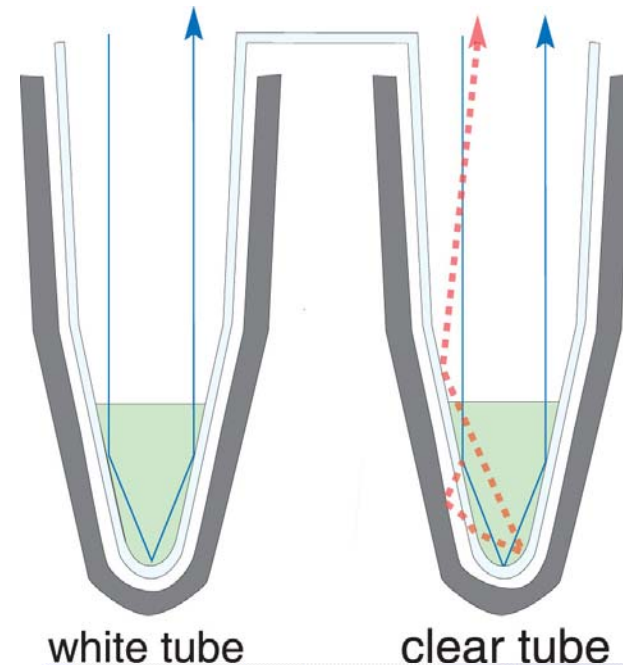
# Natural Plates



Clear material allows light to be transmitted through to the block where it is either absorbed (Black) or reflected (Silver)

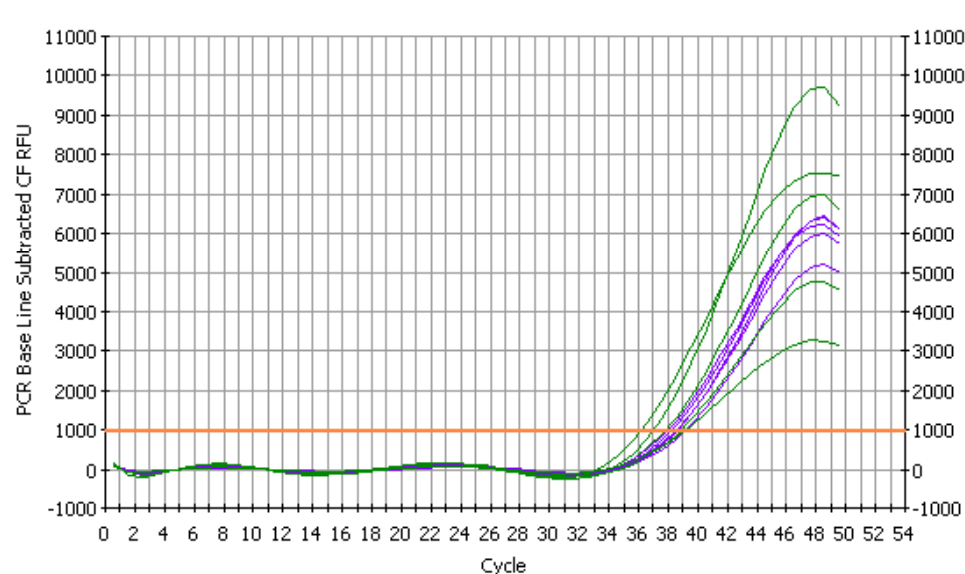
- Well-to-well cross talk can occur
- Light also reflects off outside of the tube

**Results in  
Refraction and High  
Variance**



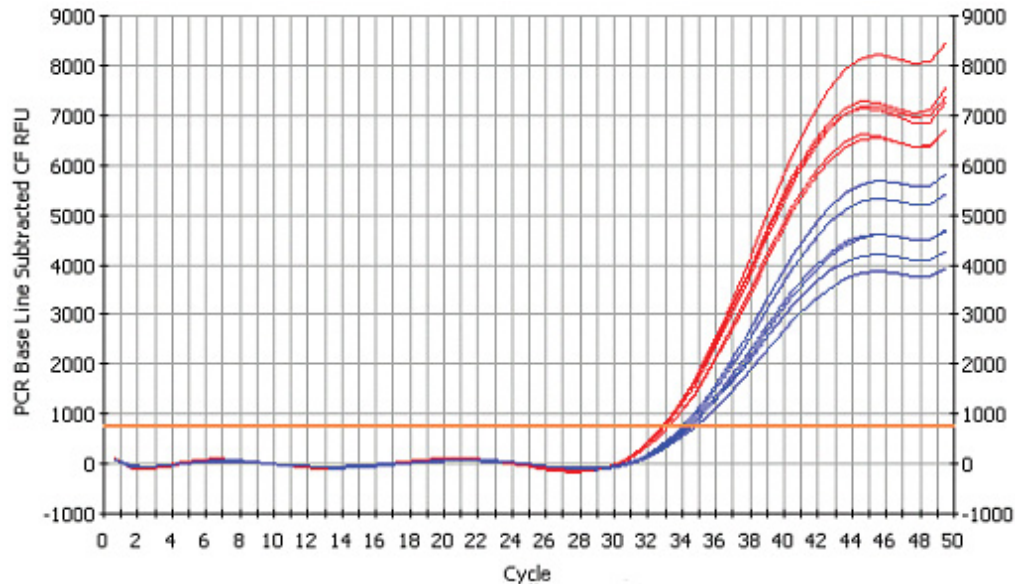
# Frosted Plates

- Frosted plates have an uneven surface on outside causing inconsistent reflection & refraction



GAPDH amplification using 0.2ng Human genomic DNA SYBR® Green Mix in frosted plates (Green) and in white plates (Blue)

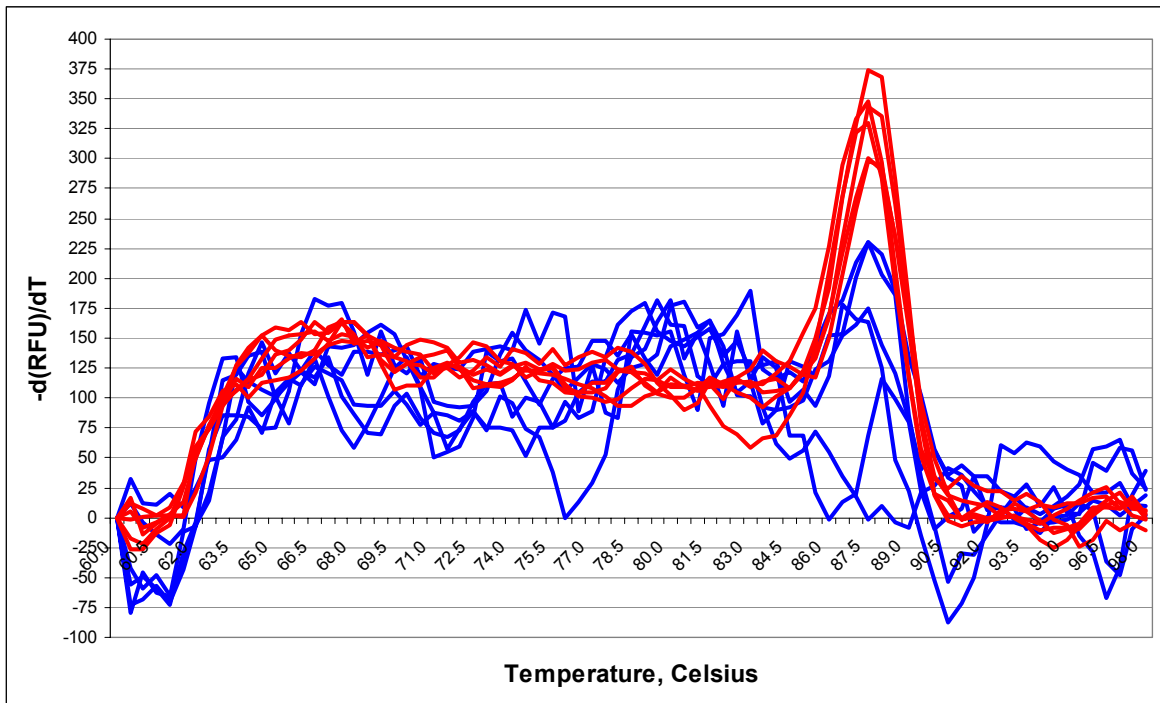
# White Plates



GAPDH amplification using 2ng Human genomic DNA SYBR<sup>®</sup> Green Mix in Natural plates (Blue) and in white plates (Red)

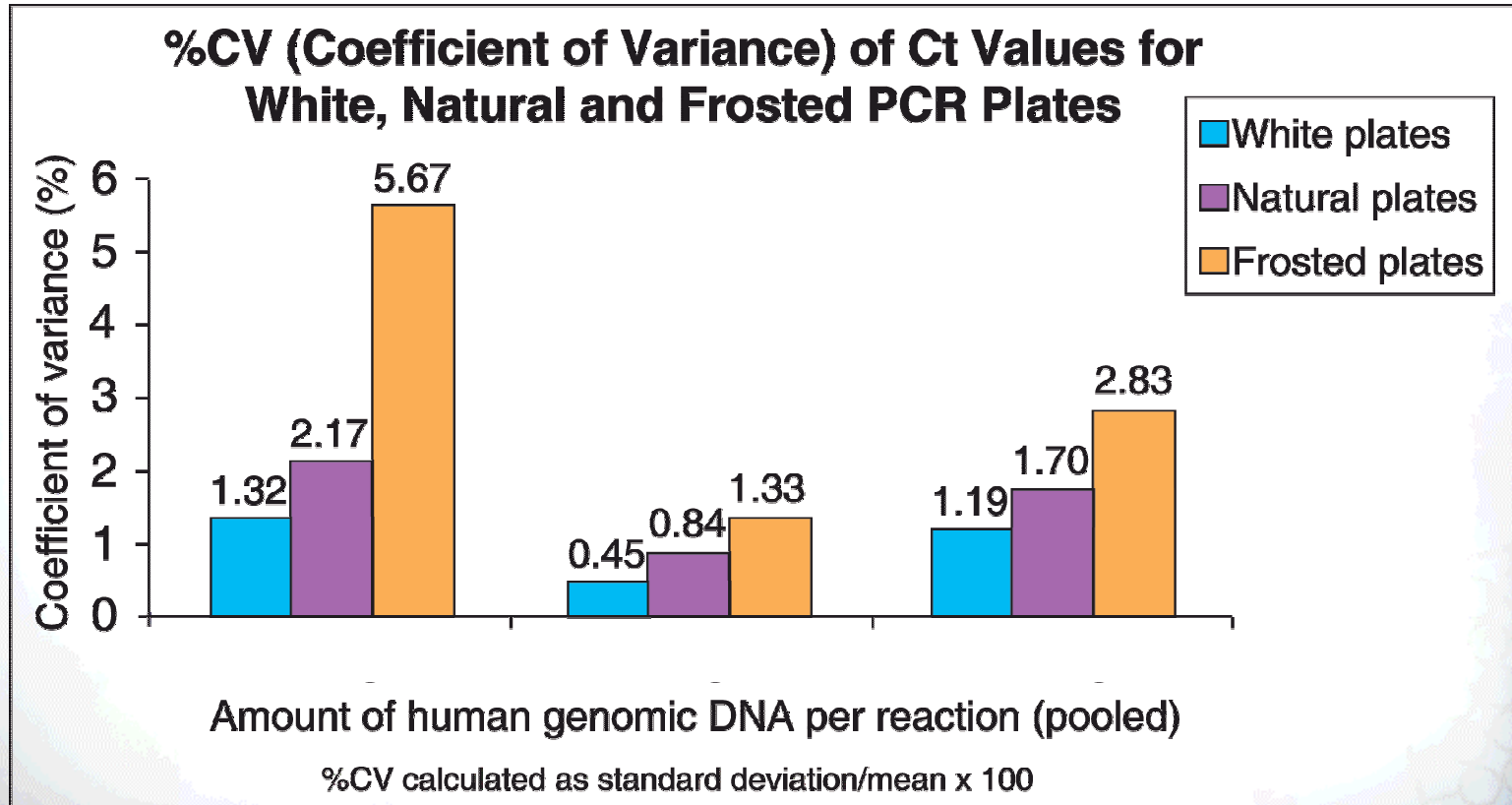
- More consistent and even reflection (no refraction)
- Higher signal to background ratio
- Eliminates possibility of well to well cross talk

# Low Copy Number

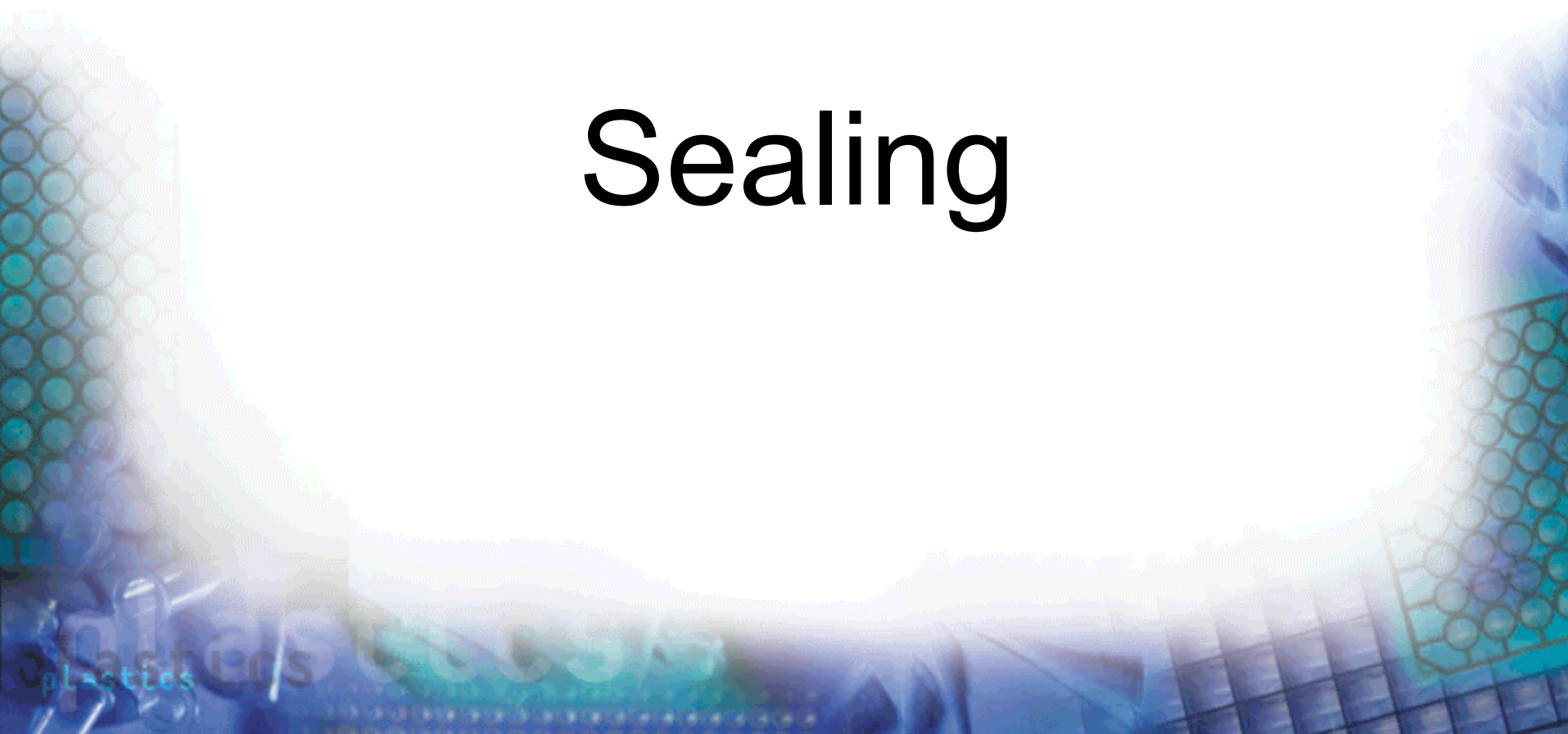


Melt Curve comparing 10 copies of Human Genomic DNA simultaneously amplified using ABSolute™ QPCR SYBR Green Fluorescein mix (AB-1219) in a white plate (Red) and in a natural plate (Blue)

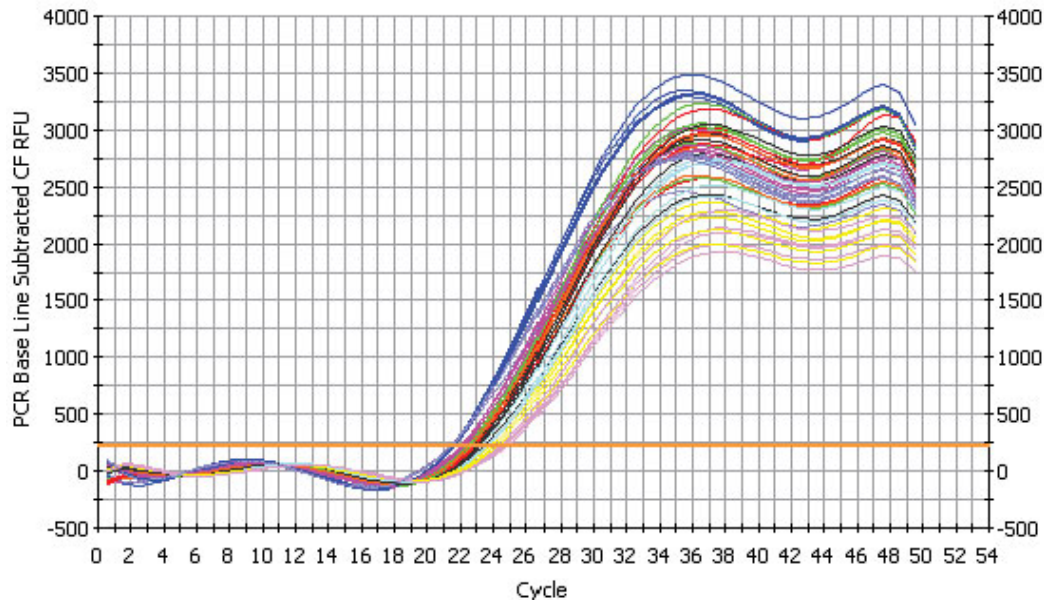
# Plate Choice



# Sealing



# Effect of Evaporation



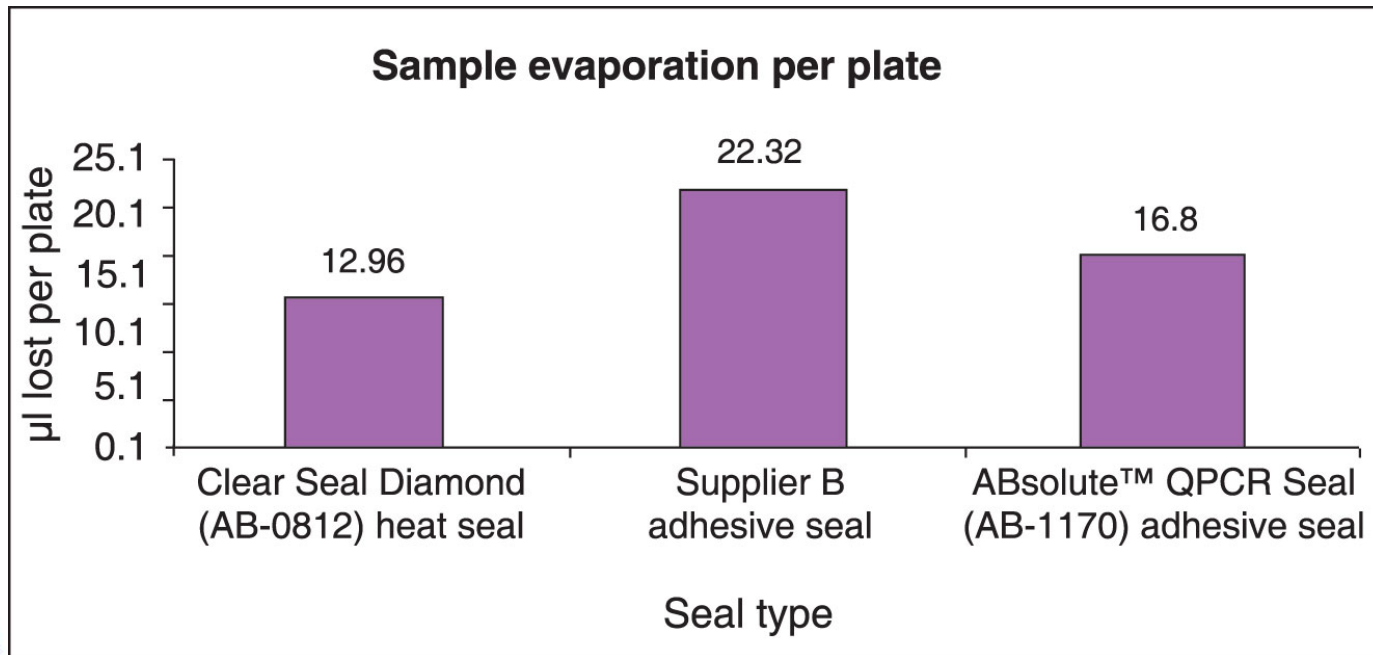
GAPDH amplification using 20ng Human Genomic DNA SYBR® Green Mix. 25µl starting volume with 1-10µl water loss.

- **Sensitivity** reduced by 0.5Ct-values for each µl evaporated
- **Endpoint** values reduced by 200RFU for each µl evaporated

# Evaporation

- Trials performed comparing mass loss after QPCR (due to evaporation)
  - 25 $\mu$ l reactions
  - Plates loaded and weighed before & after assay
- Three seals compared:
  - ABgene<sup>®</sup> Clear Seal heat seal
  - ABgene<sup>®</sup> ABsolute<sup>™</sup> QPCR Seal
  - Supplier B adhesive Seal

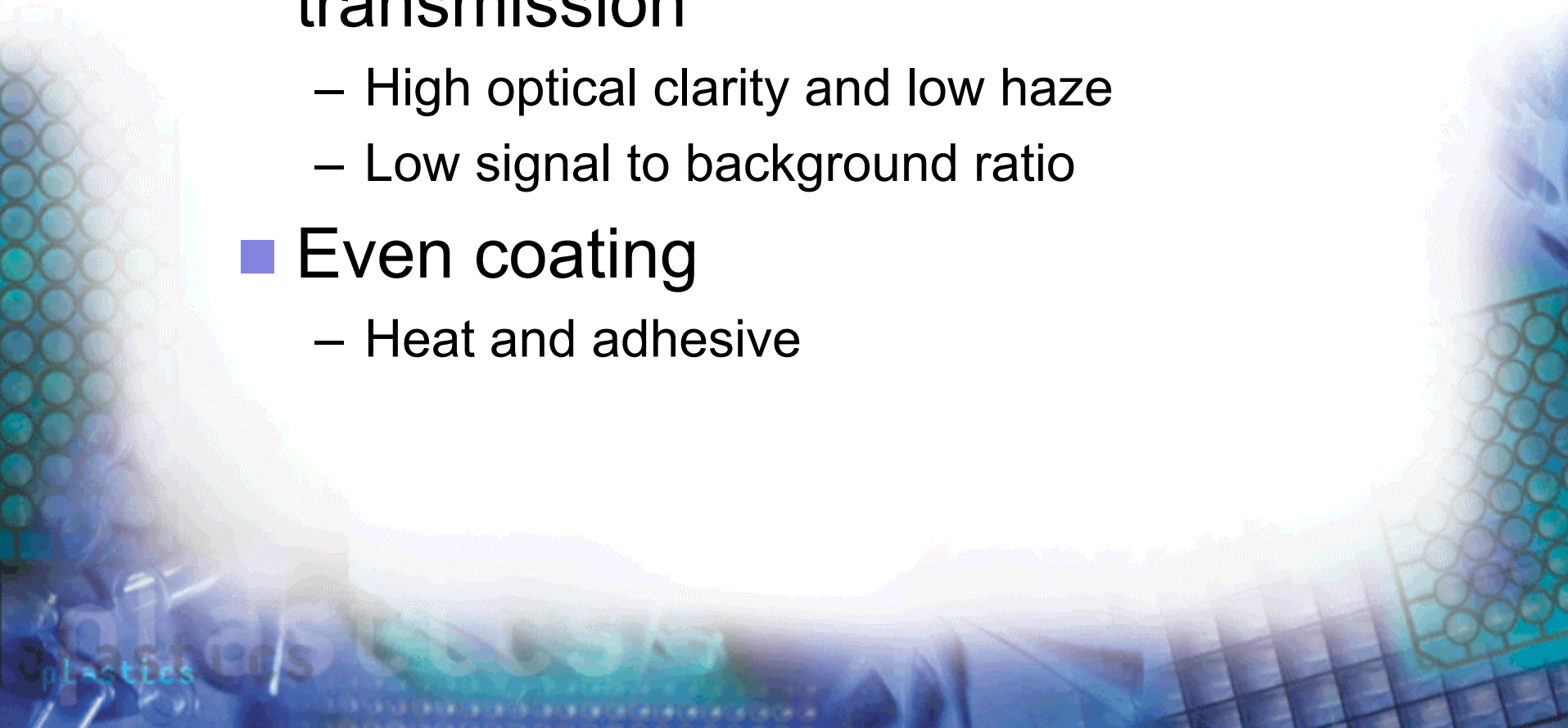
# Evaporation



- Evaporation not uniform across the plate

# Transmission

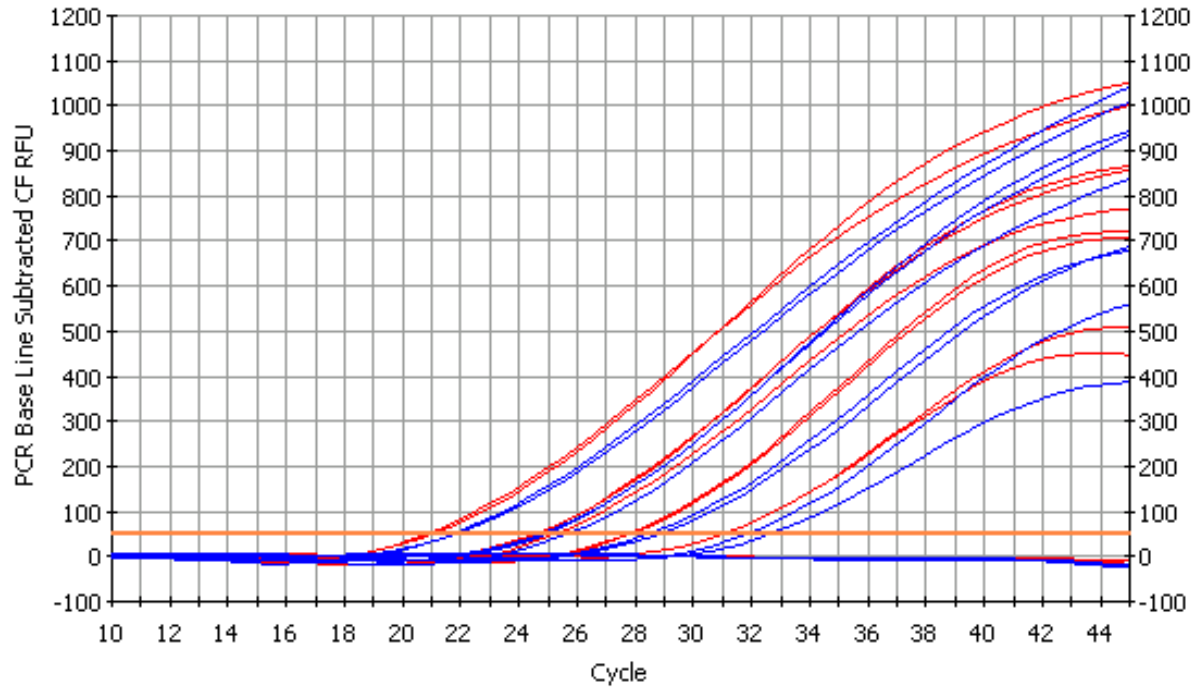
- Low auto fluorescence/ Maximal transmission
  - High optical clarity and low haze
  - Low signal to background ratio
- Even coating
  - Heat and adhesive



# Reagents

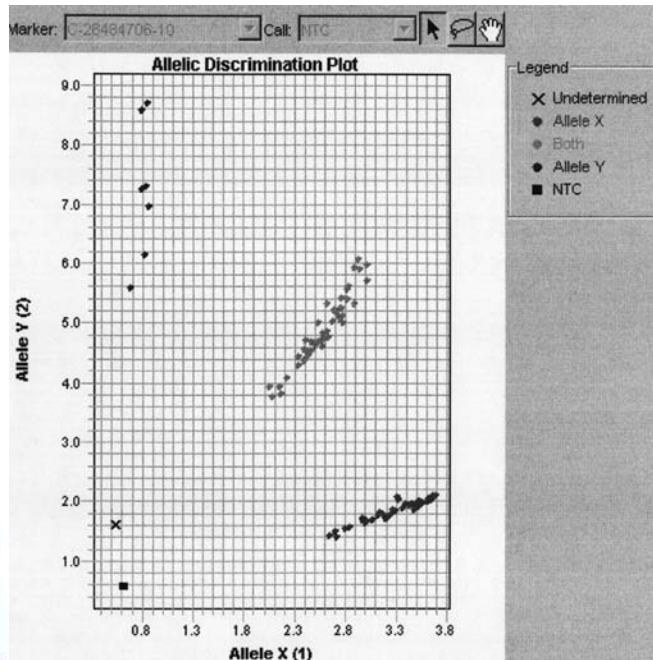
The background of the slide features a laboratory setting. On the left, there is a rack of test tubes with blue caps. In the center, a petri dish is visible, containing a white substance. On the right, another rack of test tubes is partially visible. The overall color scheme is dominated by light blues and whites, with a soft, glowing effect around the central text.

# Reagents

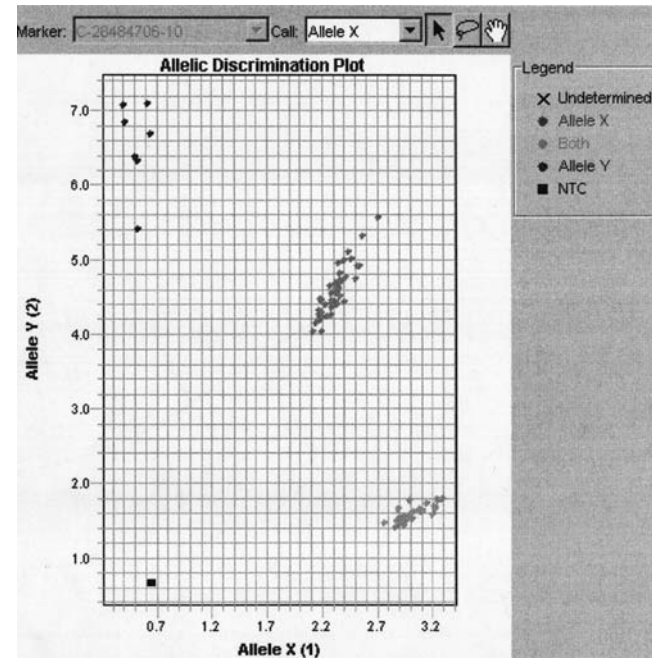


The substitution of dUTP (Blue) with dTTP (Red) resulted in lower Ct values

# SNP Analysis



ABsolute™ QPCR ROX  
dUTP Mix

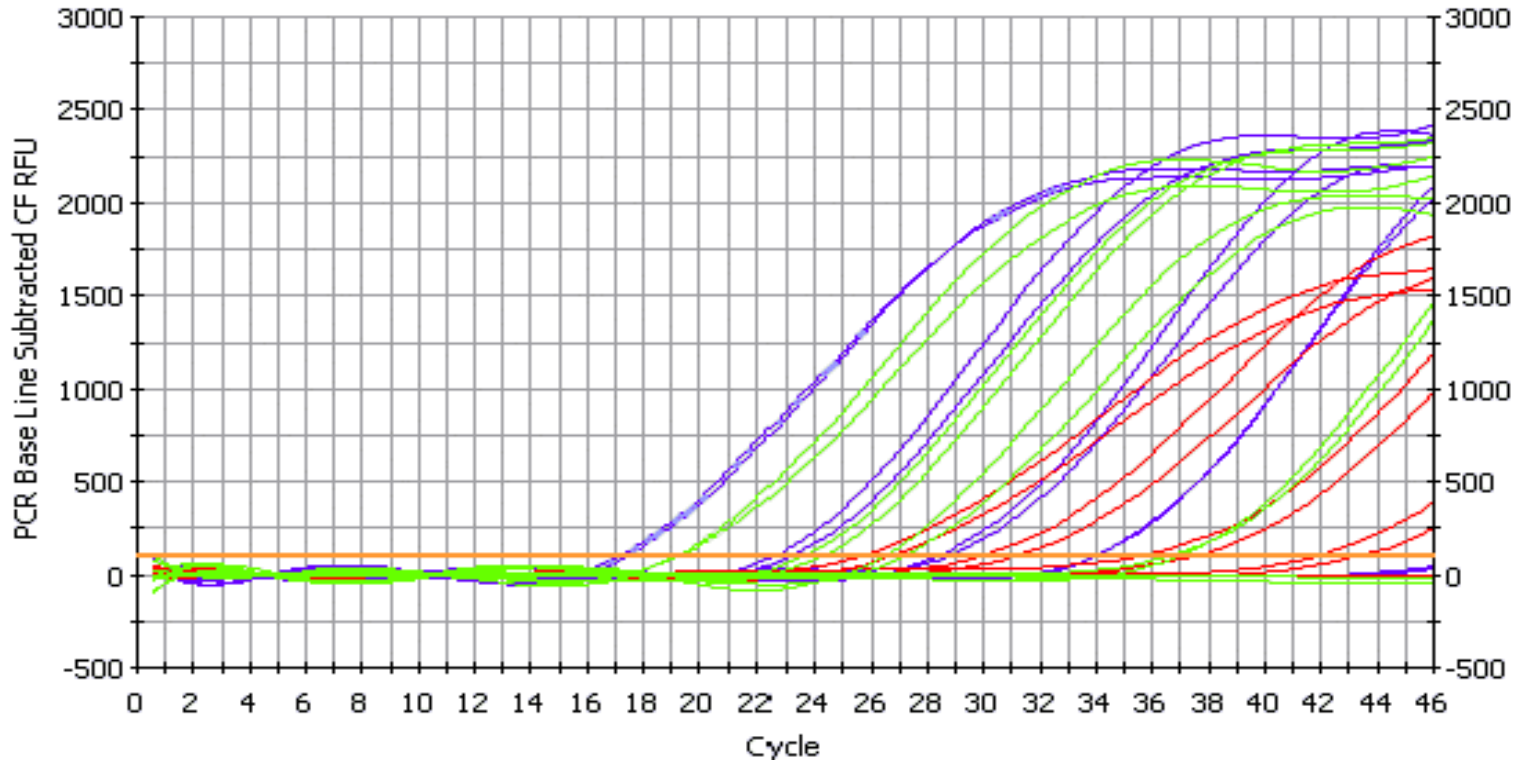


ABsolute™ QPCR ROX  
dTTP Mix

- More consistent end-point readings

External Data - A Belgian University on ABI Prism®  
7900HT

# Enzyme Activation



Amplification of a serial dilution Human Genomic DNA.

Blue: Fully activated Thermo-Start®.

Green: Partial activation (10min)

Red: Supplier A's chemically modified enzyme for comparison

# Summary



- For minimising variation in QPCR applications, ABgene<sup>®</sup> recommend the following:
  - Cleanroom manufactured consumables to minimise risk of contamination
  - White plastic consumables for highest sensitivity and consistency
  - Optically clear heat seals or high-quality adhesive QPCR seals for highest sensitivity and reproducibility
  - ABsolute<sup>™</sup> QPCR Master Mixes containing dTTP.

# Acknowledgements



- Anne van der Valk
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- Simon Ng

