

Session 5

Pete Vallone

Cristián Orrego



Outline

Buel/Nicklas Alu SYBR Green I (nuclear)

ABI Quantifiler (nuclear/IPC)

CAL DOJ (nuclear/mito)

CAL DOJ Triplex Degradation Assay



Buel/Nicklas Alu qPCR Assay

Nicklas J, Buel E. J Forensic Sci 2003; 48:936-944

SYBR Green I based assay

Alu, Ya5 Subfamily

Multi copy locus (~2,500 copies/genome)

124 base pair amplicon

Dynamic range 10 pg to 10 ng

Originally developed on the Rotor-Gene instrument

Buel/Nicklas Alu qPCR Assay

Reduced cost since the assay uses SYBR Green I instead of a TaqMan probe

Increased sensitivity due to the use of a multi copy locus
– One cell will still have ~2,500 copies of the target

Limited dynamic range (on the high end)

Is there any variance between the unknown and a Calibrant in terms of number of Alu copies/cell?

Does not use a specific probe

Buel/Nicklas Alu qPCR Assay

Since this is not a commercial assay you will have to:

- Purchase the PCR primers (hydrate/quant)
- Purchase a SYBR green PCR mastermix kit
 - Various choices

Flexible with multiple options, but optimization and troubleshooting are up to you (follow the paper protocol)

Buel/Nicklas Alu qPCR Assay

SYBR Green PCR Mastermix Kits

Typically contain

- Enzyme
- SYBR Green I
- dNTPs
- ROX (to normalize for non-PCR related fluorescence variation) not required with Rotor-Gene instrumentation

Buel/Nicklas Alu qPCR Assay

Thermal Cycling Conditions

95°C for 2 min

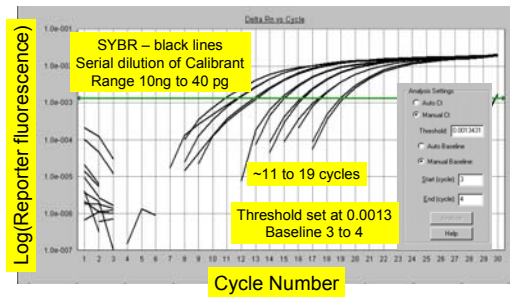
30 cycles of:

- 95°C for 15 sec
- 68°C for 35 sec (read fluorescence)
- 72°C for 30 sec

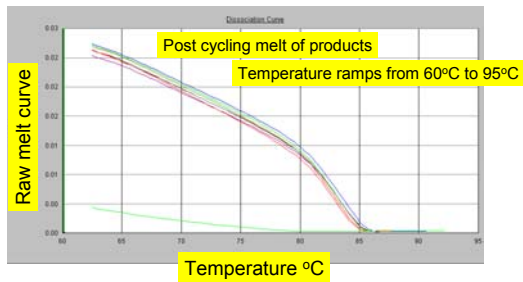
Melt

- 60°C to 95°C

Buel/Nicklas Alu qPCR Assay

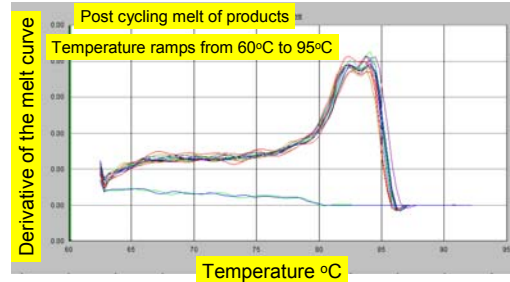


Buel/Nicklas Alu qPCR Assay



The data is better viewed by taking the first derivative of the curves...

Buel/Nicklas Alu qPCR Assay



Melts may give an indication if multiple amplicons were produced

Applied Biosystems Quantifiler

Quantifiler™ Human DNA Quantification Kit

- Commercial kit (reagent QC)
- Contains an Internal PCR Control (IPC) for the detection of PCR inhibitors
- hTERT probe FAM-MGB/NFQ
- IPC probe VIC-MGB/NFQ
 - Duplex assay
- Validation paper published
- Green et al J Forensic Sci 2005; 50: 809-825



Applied Biosystems Quantifiler

Quantifiler™ Human DNA Quantification Kit

- Autosomal specific
- Single copy target
- hTERT gene (human telomerase reverse transcriptase gene)
- Located on chromosome 5 (at 5p15.33)
- 62 base pair amplicon
- Dynamic range 23 pg to 50 ng



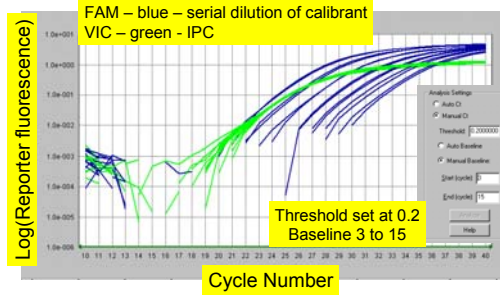
Applied Biosystems Quantifiler

Thermal Cycling Conditions
95°C for 10 min
40 cycles of:
– 95°C for 15 sec
– 60°C for 1 min (read fluorescence)

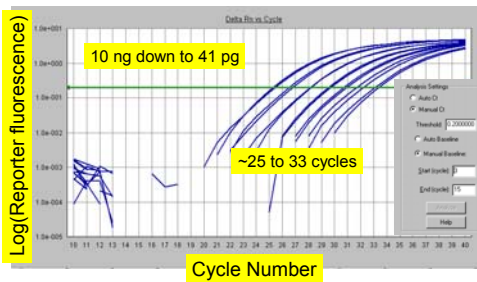
No melt curve



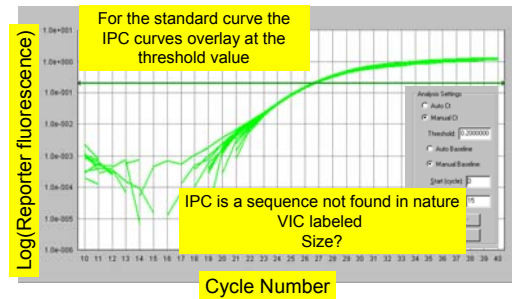
Applied Biosystems Quantifiler



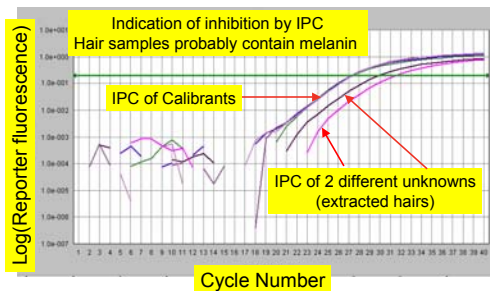
Quantifiler Calibrant Data



Quantifiler IPC



Quantifiler IPC



Real-Time qPCR Efforts

Marie Allen – nuclear and mtDNA assay (BioTechniques 2002, 33: 402-411) Uppsala, Sweden

Centre for Forensic Sciences – nuclear; TH01 flanking region (JFS 2003, 48:1041-1046) Toronto, Canada

Mark Batzer - nuclear, mtDNA and Y (Anal. Biochem 2005, 337:89-97) LSU

Summary

Alu

- SYBR Green I chemistry
- ~\$0.80 per sample
- Good sensitivity due to multi copy locus

Quantifiler

- TaqMan chemistry
- IPC (duplex) inhibition
- Specific probe
- ~\$5 per sample

