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

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

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

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

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

Quantifier™ 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

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
- 95°C for 15 sec
- 60°C for 1 min (read fluorescence)

No melt curve

Applied Biosystems Quantifiler

FAM – blue – serial dilution of calibrant
VIC – green - IPC

Threshold set at 0.2
Baseline 3 to 15

Log(Reporter fluorescence)
Cycle Number

Quantifier Calibrant Data

10 ng down to 41 pg
-25 to 33 cycles

Log Reporter fluorescence
Cycle Number

Quantifier IPC

For the standard curve the IPC curves overlay at the threshold value

IPC is a sequence not found in nature
VIC labeled
Size?

Log Reporter fluorescence
Cycle Number

Real-Time qPCR Efforts


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

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