Session 5D

Quantitative PCR (qPCR) Tools for the DNA Analysis of Challenging Samples: the CAL DOJ Triplex Degradation Assay

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How To *Identify* Challenging Samples and Which Tool(s) to Use?

- experience (analyst, intra-lab, inter-lab, literature)
- unsuccessful analysis using routine methods
  - *i.e.*, partial or null typing results
    - ✓inefficient use of analyst time
    - ✓inefficient use of reagents and kits
    - ✓inefficient use of (possibly limited) DNA extract
    - ✓increased documentation and review

Goal

- to develop real-time qPCR tools to help identify challenging samples so that optimal strategies can be attempted at the outset of analysis
  - measure human DNA ***quantities***
    - total nuclear DNA
    - mitochondrial genome copies
    - total male DNA (Y-chromosome)
  - assess DNA ***quality***
    - degree of degradation
    - presence of PCR inhibitors

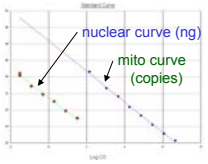
qPCR Assays at CA DOJ

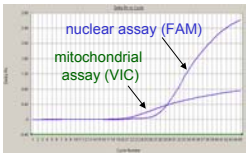
- duplex nuclear-mitochondrial qPCR assay
  - developed for Missing Persons DNA Program (MPDP-Richmond), but also used for non-MPDP cases
  - on-line for ABI 7000; >20 qualified analysts
- triplex qPCR assay to assess nuclear DNA quantity and quality (DNA degradation and presence of PCR inhibitors)
  - developed for general casework (non-mito)
  - DOJ-wide (Richmond DNA lab and DOJ field labs)
  - validated on ABI 7500; analysts in training

nuTH01-mtND1 Duplex qPCR Assay

(development & validation: J.Forensic Sci. (2005) v.50, pp. 1044-60)

- on-line at Richmond DOJ DNA Lab ~1 year (~200 runs)
- custom primers and TaqMan(MGB) probe oligonucleotides to co-amplify and detect nuclear and mitochondrial target sequences
- validated on ABI 7000 SDS qPCR instrument
  - ABI Universal TaqMan Master Mix (+ BSA (0.16 ug/uL))
  - 20 uL PCR with up to 4 uL of sample
  - DNA Standard: Promega Genomic Human DNA (male)

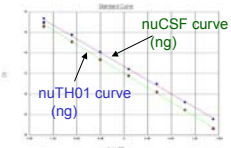


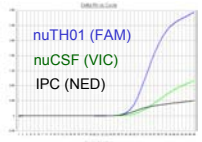


nuTH01-nuCSF-IPC Triplex qPCR Assay

(development: Forensic Sci. International (2006), v. 158, pp. 14-26)

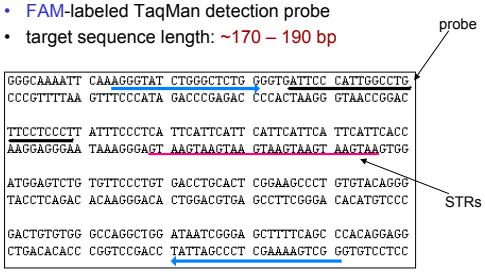
- nuTH01 assay (long nuclear target) **plus**:
  - nuCSF assay (short nuclear target) – for assessing degradation
  - IPC (internal PCR control) – for assessing PCR inhibition
- validated on ABI 7500 qPCR instrument
  - 5-color instrument with improved NED detection and a Cy5 channel
  - - ABI Universal TaqMan Master Mix (+ BSA, 0.16 ug/uL)
  - + 2.5 U AmpliTaq Gold)
  - - DNA Standard: Promega Genomic Human DNA (male)





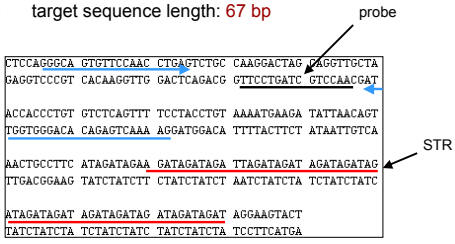
Nuclear nuTH01 qPCR Target

- target sequence spans TH01 CODIS STR locus (2 copies/diploid genome)
- FAM-labeled TaqMan detection probe
- target sequence length: ~170 – 190 bp



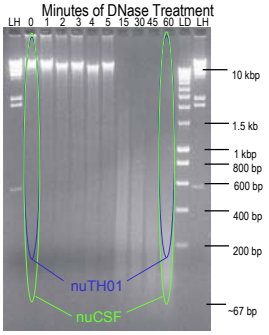
Nuclear nuCSF qPCR Target

- target sequence flanks the CODIS CSF STR region – (2 copies/diploid genome)
- VIC-labeled TaqManMGB detection probe
- target sequence length: 67 bp



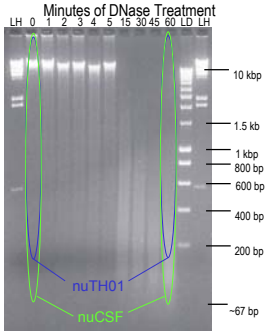
Using Short and Long Nuclear Targets to Assess DNA Fragmentation

- nuCSF assay – detects and quantifies DNA fragments larger than ~67bp
- nuTH01 assay – detects and quantifies DNA fragments larger than ~180bp

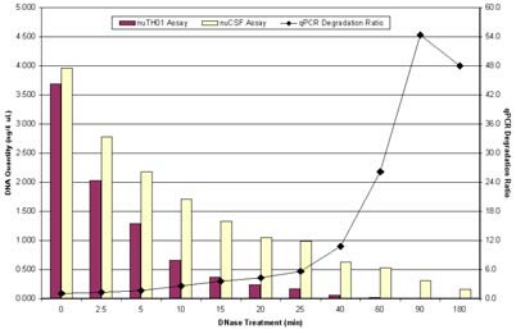


qPCR Degradation Ratio = nuCSF Quantity (ng) / nuTH01 Quantity (ng)

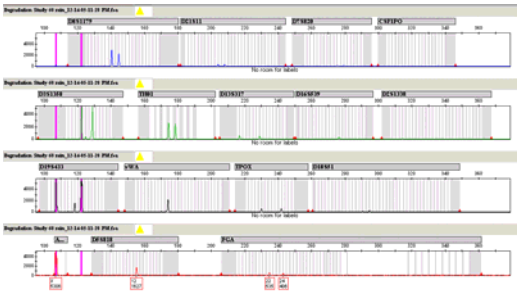
- For high-molecular weight DNA, expect the Degradation Ratio to be ~ 1.
- For highly-degraded DNA, expect the Degradation Ratio to be > 1.
- The bigger the qPCR Degradation Ratio, the more fragmented the DNA.



Triplex Quantifications and Degradation Ratios for DNaseI-Degraded Samples



qPCR Degradation Ratio ~ 25: "1 ng" (nuTH01) Identifiler STR Results

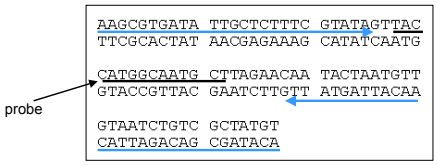


Interpreting the qPCR Degradation Ratio

Degradation Ratio	STR Implications
1 – 3	none
3 – 5	"wedge" effect, possible cross-dye pull-up
>5 (>10 ⇒ artifacts expected to be significant)	increasing "wedge" effect, pull-up, dropped-out alleles at larger loci, off-scale peaks, called stutter peaks, -A shouldering

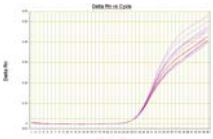
IPC qPCR Target

- template is a 77 nt synthetic oligonucleotide
- 'artificial sequence' - designed to be non-homologous to GenBank sequences
- oligo spiked into primer-probe mix (~90,000 copies/sample)
- NED-labeled TaqManMGB detection probe

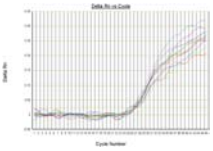


IPC for Non-Inhibited Samples Should Amplify to Give (Nearly) Identical Ct values

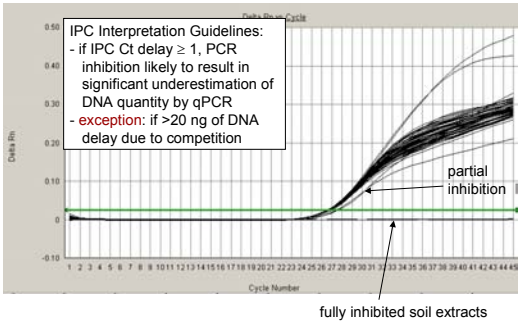
- ABI 7500 instrument
  - (assay **implementation**)
  - clean NED signal
  - identify PCR inhibition by
  - delay in Ct



- ABI 7000 instrument
  - (assay **development**)
  - poor NED signal-to-noise
  - easy to identify fully inhibited samples, hard to assess weak PCR inhibition



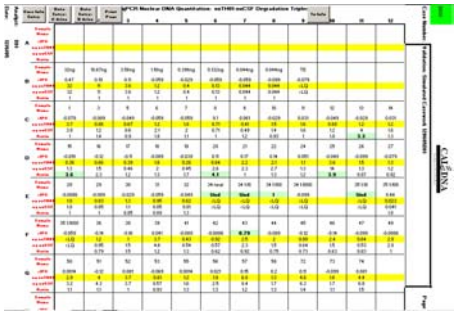
IPC qPCR Validation: Casework Type Samples



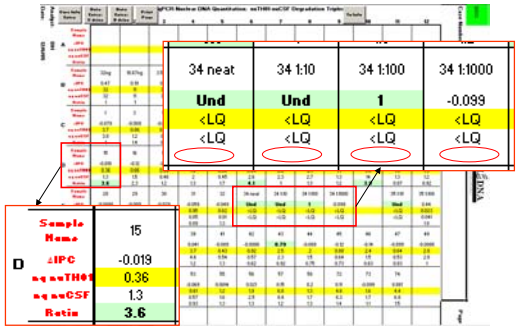
Semi-Automatic Calculation of Degradation Ratio and Delay in IPC Ct

- Excel Worksheet (Steven Myers, CA DOJ DNA Lab)
- for each qPCR run, Results are exported from
  - ABI 7500 analysis software and loaded into worksheet:
    - calculates the Degradation Ratio for each sample
    - "flags" samples with Degradation Ratio > 3 to alert for possibly degraded sample
    - calculates the delay in IPC Ct ("ΔIPC") for each sample (= delay in Ct relative to the average IPC Ct for DNA standards < 32ng)
    - "flags" samples with ΔIPC > 0.75 cycles to alert for possibly inhibited sample
    - generates single-page report (values rounded to two significant figures)

Semi-Automatic Calculation of Degradation Ratio and Delay in IPC Ct



Semi-Automatic Calculation of Degradation Ratio and Delay in IPC Ct



Multiplex qPCR Analysis of Challenging Samples

nuTH01-nuCSF-IPC Triplex Assay

- IPC to detect PCR inhibition
  - re-quant with dilution series ?
  - achieve non-inhibited downstream analysis by:
    - » dilution
    - » augmented STR amp (extra Taq/BSA)
    - » sample clean-up
- Degradation Ratio to detect DNA fragmentation
  - MiniSTRs (use nuCSF quant for input)
  - mitochondrial analysis