Development of a forensic DNA quantification assay using digital PCR

Quentin Gauthier, Kristen Price, Tiffany Layne, Mark F. Kavlick
Research & Support Unit, FBI Laboratory Division

Yotam Blech-Hermoni, John Chuckalovcak
QIAGEN

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Disclosures

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This work is the result of a forthcoming Collaborative Research and Development Agreement (CRADA) between the Federal Bureau of Investigation Federal Laboratory and QIAGEN Sciences Inc.

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Quantification of nuclear and mito DNA

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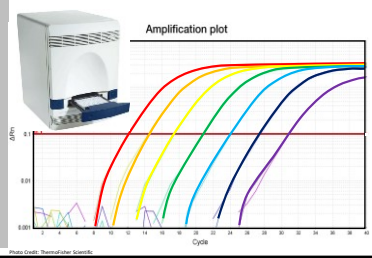
- Currently, two separate protocols in the DNA Casework Unit of the FBI Laboratory



Double

- Standard curves
- Instrument Time
- Reagent Costs
- Quality Control

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dPCR

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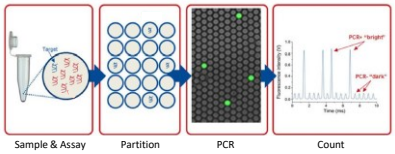
- Fundamentally still PCR
- Thousands of ~0.8nL endpoint reactions



- Signals (+/-) counted and concentration determined by Poisson statistics

Photo Credit: Integrated DNA Technologies

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QIAcuity instrument and plates

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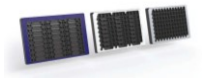
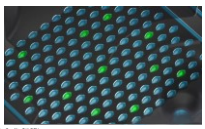
- Nanowell plates
- QIAcuity dPCR instrument





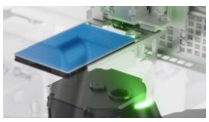


Photo Credit: QIAGEN

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A combined mitochondrial and nuclear assay

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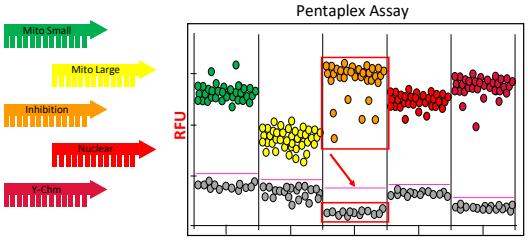

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Initial development

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- Mito Short
  - Mito quantification
  - 105bp
  - FAM
- Mito Long
  - Mito degradation
  - 316bp
  - VIC
- IPC
  - Assay inhibition
  - 65bp
  - NED


Mitochondrion 46 (2019) 41–50

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Development of a triplex mtDNA qPCR assay to assess quantification, degradation, inhibition, and amplification target copy numbers

Mark F. Kavlick*

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Singleplex comparison to qPCR

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	QIAcuity		7500	
	Copy/µL RXN	Ct Value	Copy/µL Sample	Copy/µL RXN
FAM – Mito Small	46.8	27.88	971.5	48.6
VIC – Mito Large	43.5	30.8	~900	~45
NED – IPC	53	27.95	~1000	~50

FAM – Mito Small

- Increase separation
- Change orange fluorophore

NED – IPC

Yellow Channel | Orange Channel

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Optimized mitochondrial triplex on dPCR

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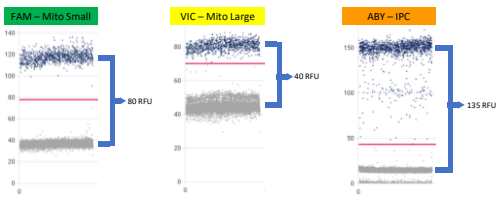
- Forward and reverse primers and probe concentrations were optimized for each target
- Optimized triplex conditions resulted in RFU separations seen below



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Sensitivity Study – Mito Short

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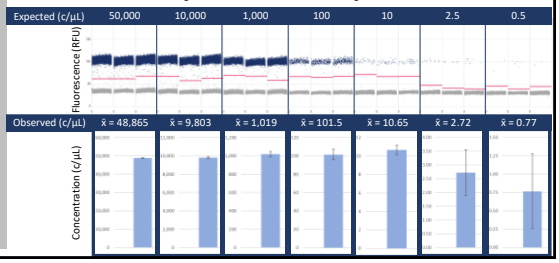
- NIST SRM 2372a Component B measured in triplicate



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Inhibition studies – 3 Targets

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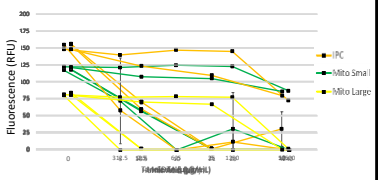
- Inhibitors with dPCR
  - EDTA
  - Melanin
  - Tannic Acid
  - Humic Acid
  - Hematin



- Increasing inhibitor concentration decreases RFU

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Inhibition vs Quantification

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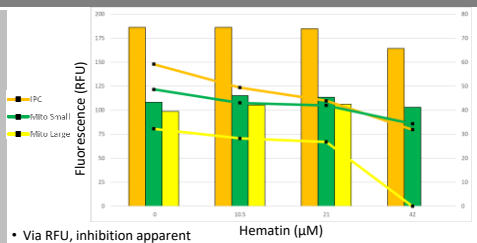

- Via RFU, inhibition apparent
- Concentration and RFU not always concordant
- Careful interpretation required

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Interpretation of Results

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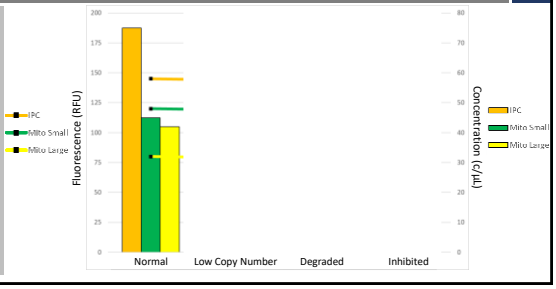

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Conclusions

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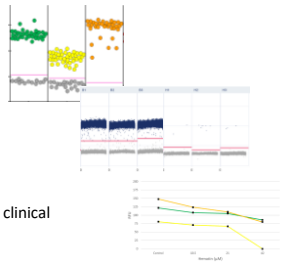
- Mitochondrial triplex on dPCR
- Highly sensitive down to 0.5 c/µL
- Sensitive to 5 of 5 inhibitors tested
- Digital PCR works for forensics and clinical



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Future Directions

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- The ultimate goal is a pentaplex assay
  - Add small Nuclear and Y targets
  - Difference between nuclear and mito copy number
  - Saturation of mito assay on instrument
- Developmental validation
- Test on casework type samples
  - Hair
  - Bone

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Acknowledgements

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Special Thanks to:

Federal Bureau of Investigation
- Tom Callaghan, LD
- Susannah Kehl, LD
- Gene Peters, LD
- James Robertson, LD
- Jonathan Spielman, ORTA
- Jascity Hutchison, ORTA

QIAGEN
- Jeff Durocher
- Mark Guilliano
- Carole Cummings
- Keith Elliott

Oak Ridge Institute for Science and Education
FBI Laboratory Visiting Scientist Program

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Questions

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Quentin Gauthier
QGauthier@FBI.gov

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References

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- QIAGEN, Inc – QIAcuity Product Brochure
- Kavlick MF. Development of a triplex mtDNA qPCR assay to assess quantification, degradation, inhibition, and amplification target copy numbers. Mitochondrion. 2019 May;46:41-50.
- Hudlow WR, Chong MD, Swango KL, Timken MD, Buoncristiani MR. A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: a diagnostic tool for STR typing. Forensic Sci Int Genet. 2008 Mar;2(2):108-25.
- Romsos E, Kline M, Farkas N, Duewer D, Toman B. Certification of Standard Reference Material® 2372a Human DNA Quantitation Standard, Special Publication (NIST SP), National Institute of Standards and Technology, Gaithersburg, MD. 2018. [online].
- Rodrigues de Almeida P, Demoliner M, Antunes Eisen AK, Heldt FH, Hansen AW, Schallenberger K, Fleck JD, Spilki FR. SARS-CoV2 quantification using RT-dPCR: a faster and safer alternative to assist viral genomic copies assessment using RT-qPCR. BioRxiv. 2020 preprint [online].
- Basu AS. Digital Assays Part I: Partitioning Statistics and Digital PCR. SLAS TECHNOLOGY: Translating Life Sciences Innovation. 2017;22(4):369-386.

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