Dried Blood Spot DNA Extraction Guidelines to Ensure Robust Performance in NBS Molecular Assays

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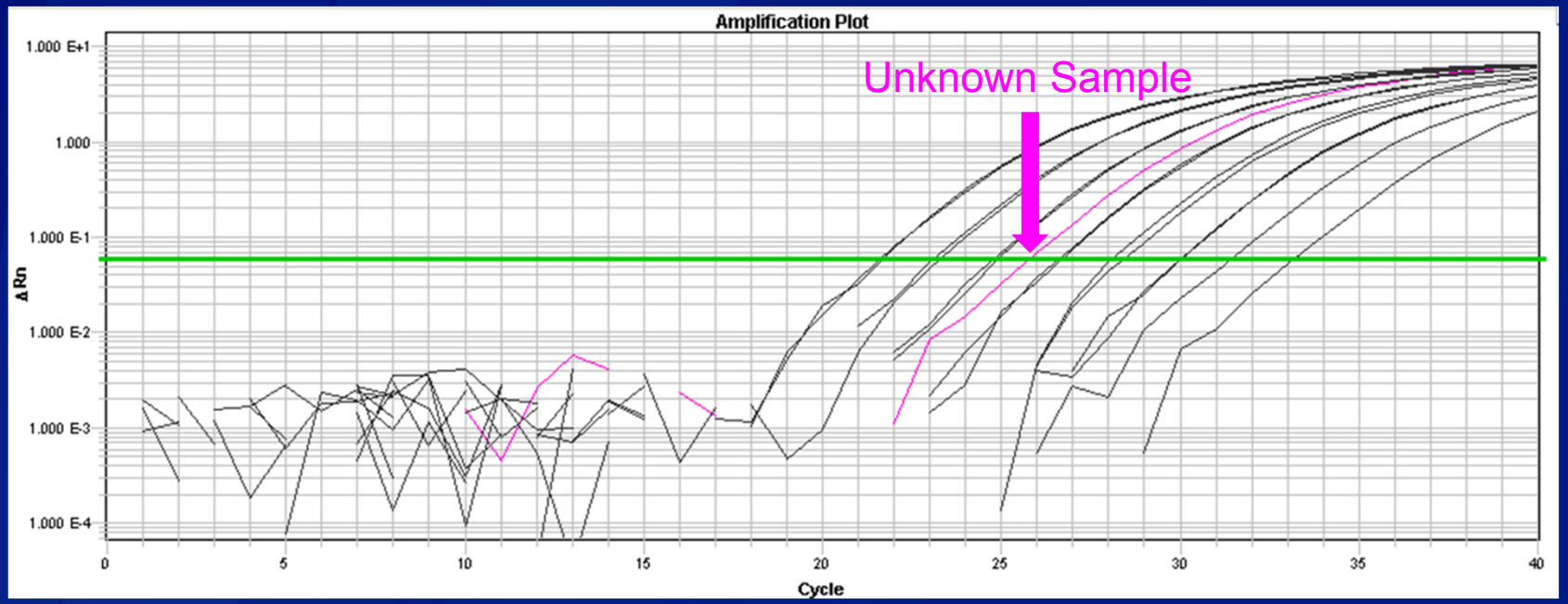
Validating DNA Extracted from DBS

- How much DNA do you need and what extraction is appropriate?
- Are there inhibitors present in your extract?
- Is your DNA fragmented and does it matter?
- Testing DNA for contamination and sample identity
- Next Generation sequencing… Will DBS work?

DBS DNA Extraction Methods Used in Newborn Screening

- **Qiagen Lysis Method - Ex: Solutions 1 & 2**
  - Multiple wash steps, followed by boil
- **Boil Prep Method**
  - No wash, followed by prolonged boil
- **Methanol Boil Prep Method**
  - Fixation of proteins, followed by prolonged boil

Measuring DNA Concentration
quantitative PCR



- Concentration represents amplifiable DNA
- Unknown concentrations are calculated relative to a standard curve

DNA Yield from 3 mm Adult DBS Punch

	Lysis (Qiagen)	Boil	Methanol Boil
Sample*	DNA yield (ng)	DNA yield (ng)	DNA yield (ng)
Adult CF PT (low)	44.50	6.05	4.05
Adult CF PT (med)	122.50	32.51	8.75
Adult CF PT (high)	289.50	54.59	19.60

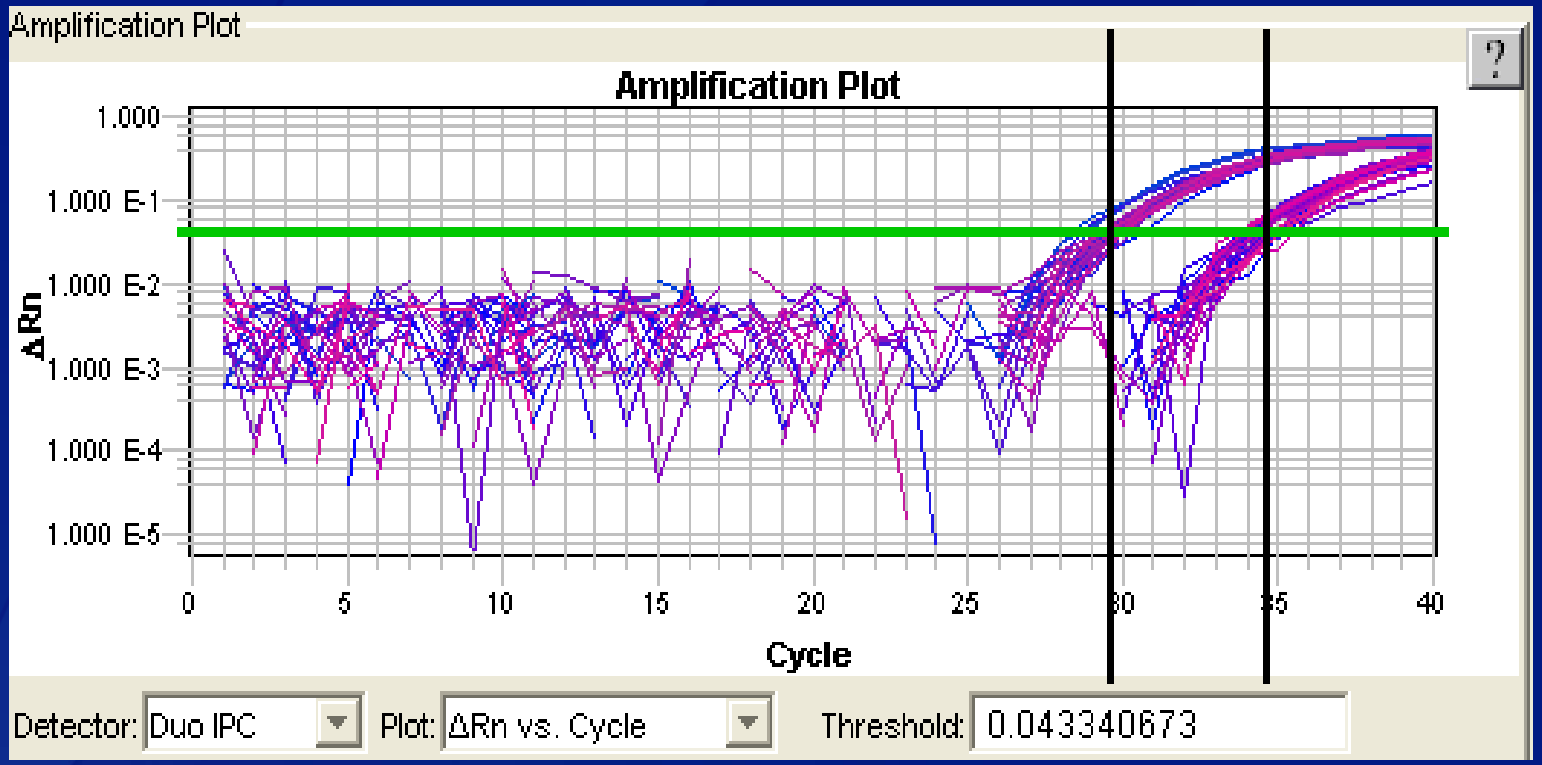
* Extracted from NSQAP's Cystic Fibrosis PT specimens with known high, medium and low concentrations

DNA extracted using the Boil and Methanol Boil Methods is significantly lower than the Qiagen Lysis Method

qPCR to Detect Inhibitors
Quantifiler Duo Assay

- Detect PCR inhibitors using an internal positive control (IPC)
- IPC is an artificial template simultaneously amplified with human DNA
- IPC C^T values ≥ 31 indicate an extract may be inhibited

qPCR to Detect PCR Inhibition
Internal Positive Control (IPC)

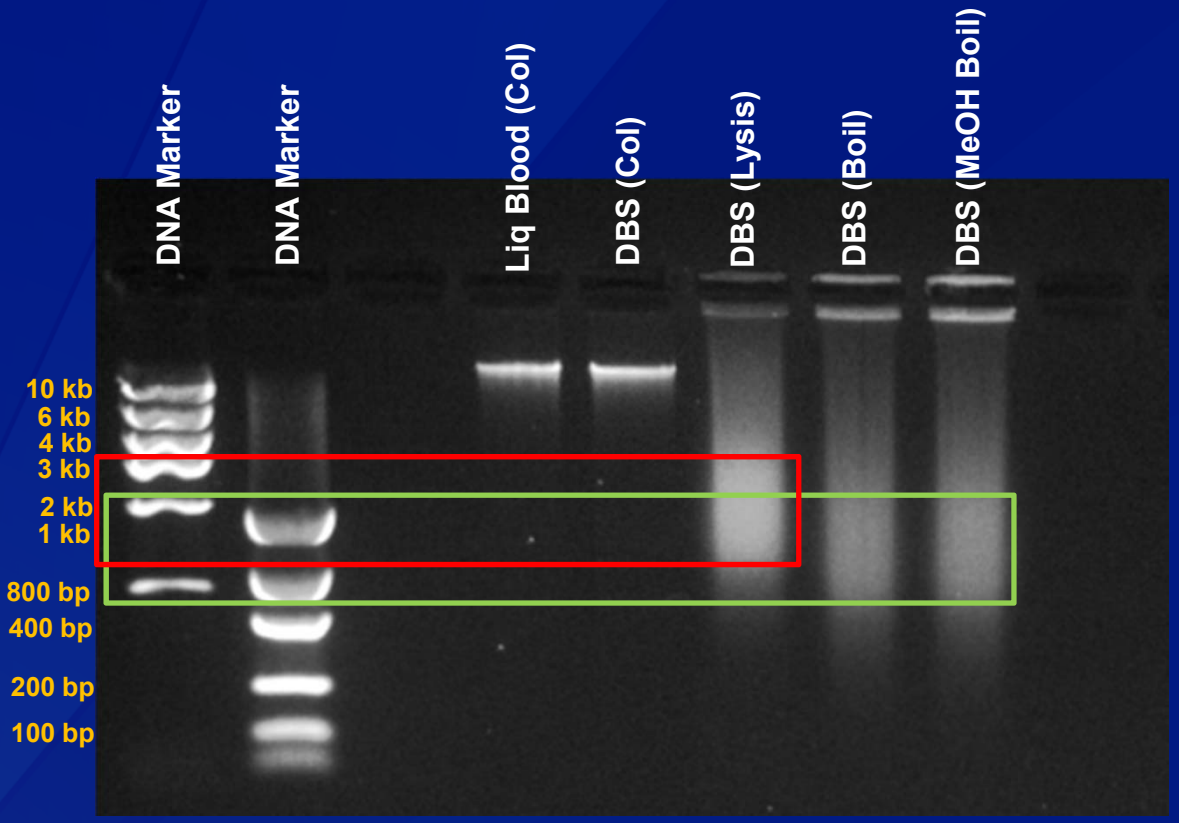


- 1st cluster amplifies as expected (IPC Ct<31)
- 2nd cluster amplifies later indicating inhibition (IPC Ct>31)

How DNA Becomes Fragmented

- Exposure to prolonged high temperatures
- Mechanical shearing – pipetting, mixing, etc.
- DNAse enzyme activity

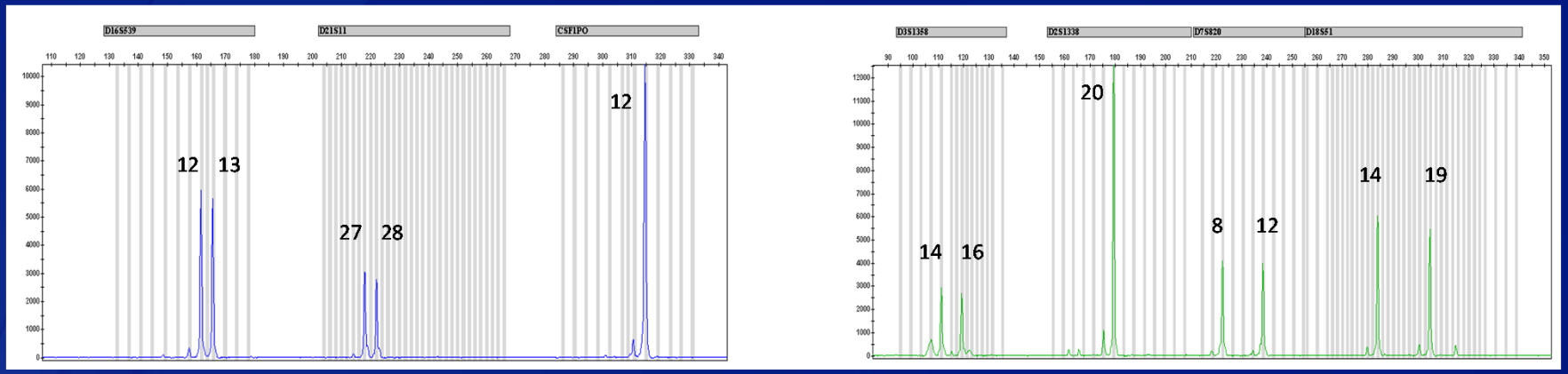
Genomic DNA Fragmentation



- Most NBS assays are small target sizes (< 1kb)
- Fragmented DNA often results in better amplification
- Can amplify 6 kb fragment from Lysis Prep

DNA Contamination and Identity

- "DNA fingerprinting" uses repeats or microsatellites (2 - 6 bp) found in the genome
- Microsatellite length is variable and inherited
- If marker has >2 alleles, may indicate contamination
- Also useful for identity testing between discrepant samples

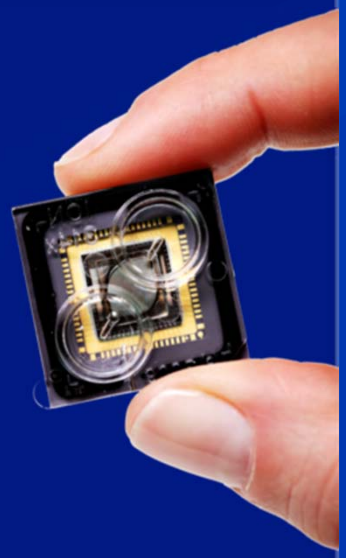


Individual Sample Profile of 7 markers

See Poster P-17 for more information

DBS DNA Work and Next Gen Sequencing
Ion Torrent

- **Uses semi-conductor technology in high-density array**
  - Massive parallel DNA sequencing
- **Target(s) amplified using highly multiplexed primer pool**
  - DNA input recommended - 10 ng per pool
- **Barcoding allows multiple samples per chip**



Ion Torrent Next Generation Sequence Data from DBS

- Goal 1: ≥ 90x coverage for all amplicon targets
- Goal 2: 100% concordance with identified mutations/ variations

		Ion Torrent Next Generation Sequencing Results			
		Target coverage ≥ 90x		% Concordance	
Cystic Fibrosis Sample Mutations	variants*	Liq Blood (Column)	DBS (Qiagen Lysis)	Liq Blood (Column)	DBS (Qiagen Lysis)
3905insT	8	100%	100%	100%#	100%#
E60X / delF508	10	100%	100%	100%	100%
2055del9insA	5	100%	97%	100%	100%
R75X / R74W / D1270N	0	99%	84%ǂ	100%	100%#
R1158X	11	100%	92%	100%	100%
delF311	13	100%	100%	100%#	100%#
G551D / R117H	8	100%	92%	100%	100%
delF508 / delI507	11	98%	100%	100%#	100%#

* Excludes the TG_X/T_Y repeat in Intron 9 which is still in development by Life Technologies

ǂ Library preparation not optimal

Samples for the 3' UTR 8/9T region (c.*133delT) were manually called

DNA extracted from liquid blood (column) and DBS (lysis) performs well

Conclusions

- **Not all DBS DNA extraction methods are created equal**
  - Boil prep and Methanol Boil prep methods give significantly lower yields than the Qiagen Lysis method
- **qPCR can quantify DNA and identify PCR inhibitors**
- **DNA extractions with prolonged heat steps result in fragmented DNA**
- **Short tandem repeats used to identify contamination and resolve discrepant results**
- **DNA from DBS can be used for Next Gen Sequencing Ion Torrent platform (CFTR)**

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