# Capillary Electrophoresis in DNA Analysis

## Real-time qPCR and miniSTRs

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## Outline for Workshop

- Introductions
- STR Analysis
- Introduction to CE and ABI 310
- Validation and Interlaboratory Studies
- Real-time qPCR and miniSTRs
- Stats and Higher Throughput Approaches
- Y-Chromosome Analysis
- Troubleshooting the ABI 310
- Review and Test

## Why is Accurate DNA Quantitation Important in Forensic DNA Testing?

- Optimal signals lead to quality data!
- Concentration range: (usually 0.5-2 ng)
  - Too much DNA leads to off-scale peaks, split peaks (due to noise or +A), stutter effects, enhanced baseline noise and bleed through from other dye colors.
  - Too little DNA leads to allele dropout and other stochastic effects (poor peak balance and drop in )
- Limited amount of DNA available
  - Usually cannot perform multiple tests for quantity
  - Want to preserve DNA for STR testing (tests should be efficient)

## Calculation of the quantity of DNA in a cell

1. **Molecular Weight of a DNA Basepair = 618g/mol**
   A =: 313 g/mol; T: 304 g/mol; A-T base pairs = 617 g/mol
   G = 329 g/mol; C: 289 g/mol; G-C base pairs = 618 g/mol
2. **Molecular weight of DNA = 1.85 x$10^{12}$ g/mol**

There are 3 billion base pairs in a haploid cell ~3 x 109 bp
(~3 x $10^{9}$ bp) x (618 g/mol/bp) = 1.85 x $10^{12}$ g/mol

3. **Quantity of DNA in a haploid cell = 3 picograms**
   1 mole = 6.02 x 1023 molecules
(1.85 x 1012 g/mol) x (1 mole/6.02 x 1023 molecules)
   = 3.08 x $10^{-12}$ g = 3.08 picograms (pg)
A diploid human cell contains ~6 pg genomic DNA

4. ***One ng of DNA contains the DNA from 167 diploid cells***

1 ng genomic DNA (1000 pg)/6pg/cell = ~333 copies of each locus (2 per 167 diploid genomes)

## So What's the bottom line?

- You need to sample sufficient cells to avoid stochastic effects –
  - 167 cells = 1ng total DNA
  - 1pg of DNA is 1/6 of a cell
  - 100 pg is 17 cells
- PCR can amplify fractions of a cell-
  - Just increase the cycle number
  - But would you want to?
- You also cant overload the system-
  - Stutter goes up
  - Noise increases

## Introduction

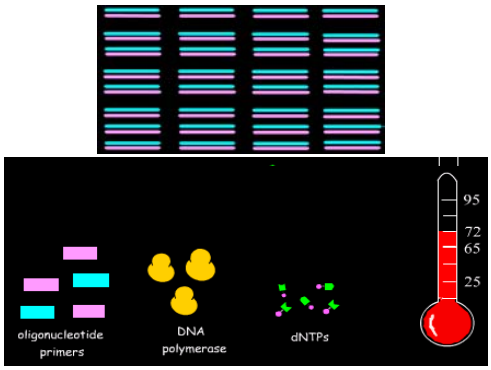
- What is rtPCR or qPCR?
- How does it work?
- How does it compare to traditional methods of Human DNA quantitation?
- What techniques are available?
- What systems are available?

## History

- RtPCR is a very recently developed technique
  - Developed by Higuchi in 1993
  - Used a modified thermal cycler with a UV detector and a CCD camera
  - Ethidium bromide was used as intercalating reporter As [dsDNA] increased fluorescence increased
- Higuchi, R.; Fockler, C.; Dollinger, G.; Watson, R. "Kinetic PCR analysis: real-time monitoring of DNA amplification reactions" Biotechnology (N Y). 1993 Sep;11(9):1026-30

**Figure 3. Mechanism of PCR**

*D. Voet, J. Voet, and C. Pratt, Fundamentals of Biochemistry, 1999*




## PCR amplification

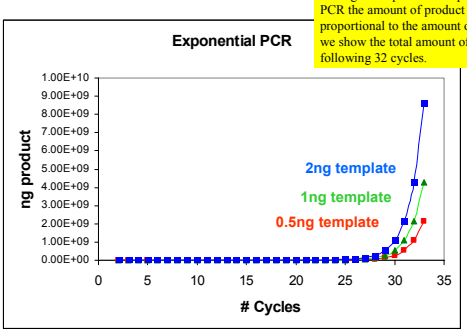
- Theoretically the quantity of PCR template T doubles with each cycle.
- After 2 cycles the quantity of product is 2T
- After N cycles the quantity of product is

$$P = (2)^n T$$

  - Thus there is a exponential relationship between the original quantity of product and the amount of template

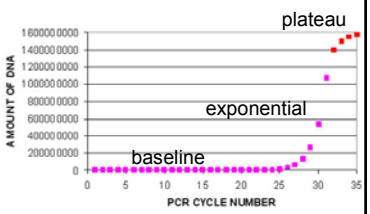
PCR Product Amount is Proportional to the Amount of Input DNA Template

During the exponential expansion of the PCR the amount of product produced is proportional to the amount of template. Here we show the total amount of product following 32 cycles.




## What is qPCR?

- To use PCR as a Quantitative technique, the reaction must be clearly defined
- In fact there are several stages to a PCR reaction
  - Baseline stage
  - Exponential stage
  - Plateau stage

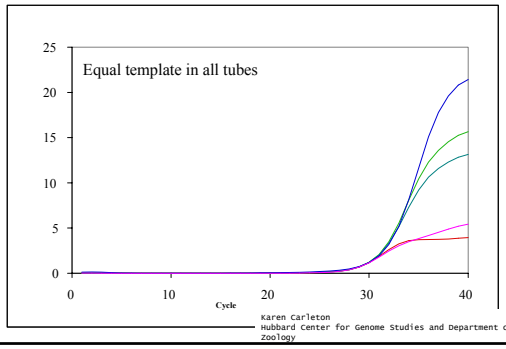



## PCR plateaus

- PCR product can not double forever
  - Limited by
  - Amount of primer
  - Taq polymerase activity
  - Reannealing of product strands
- Reach plateau
  - No more increase in product
- End point detection
  - Run for fixed # cycles and then quantify on agarose gels

## Problem #1: End point plateau does not depend on T

Even if same amount of template, different tubes will reach different PCR plateaus

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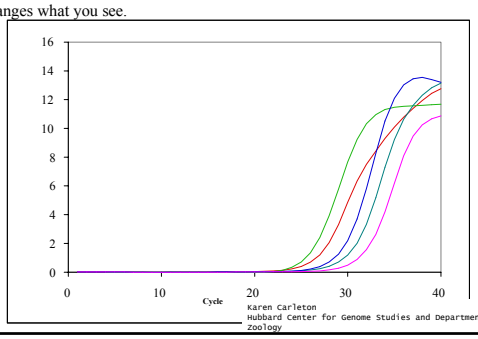
Hubbard Center for Genome Studies and Department of Zoology

## Problem #2: For endpoint detection, how many cycles should you do?

Different wells reach plateau at different cycle numbers. When you look changes what you see.

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## Issues for quantitation by non RT-PCR methods

- In spite of its use in mixture resolution, PCR is not technically a quantitative technique
- The time and rate at which plateau appears varies with temperature, tube position, inhibitors, matrix
- Once plateau appears, increase in product concentration is non linear
- Standards can be added but they must have the same primer binding sites and similar sequence to target


## Solution

- Use data when still in exponential phase
  - PCR product proportional to initial template
- Need to look at PCR product each cycle
  - Use fluorescent detection, where fluorescence is proportional to PCR product
- Use real time PCR machine which records fluorescence for each well at each cycle

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## Quantitation using the PCR Reaction

- PCR proceeds exponentially doubling each cycle:

$$Y_n = Y_{n+1}(1+E_c)$$

Where $E_c$ is the efficiency ($E_c$ = 1 for a perfect amplification) and $Y_n$ is the yield of product for a particular cycle

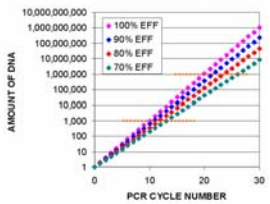
- During the exponential stage of the reaction

$E_c$ is relatively constant and the reaction yield Y is a function of the quantity of input DNA, X

$$Y = X\,(1+E_c)^n$$

## Effect of efficiency on [DNA]

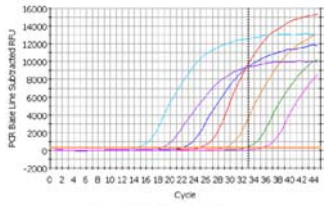
- $E_c$ is a function of:
  - Hybridization efficiency
  - Quantity of reactants/target DNA
  - Temperature




http://www.med.sc.edu:85/pcr/realtime-home.htm

## Real Time PCR

- Quantitation of DNA is a based on the number of cycles required to reach a threshold intensity, $C_t$.
- The greater the amount of starting DNA, the sooner this threshold value is reached.




$C_t$

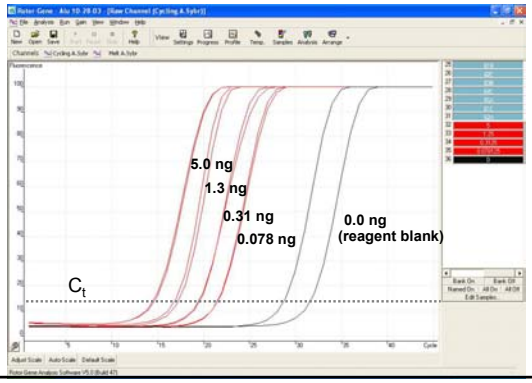
SERIES OF 10-FOLD DILUTIONS

http://www.med.sc.edu:85/pcr/realtime-home.htm

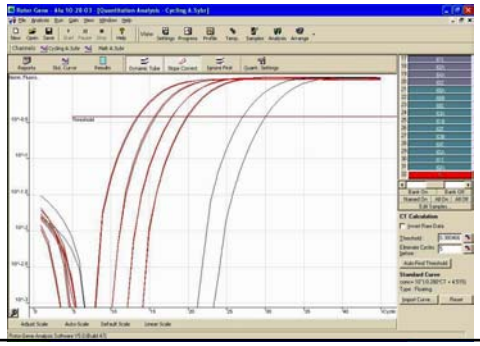
## Quantitation using $C_t$

- The log of DNA template concentration vs $C_t$ is plotted using a series of stds yielding a calibration curve
- The unknown is then run and the number of cycles required to reach threshold, $C_t$ is compared to the calibration curve.

## Development of a standard curve

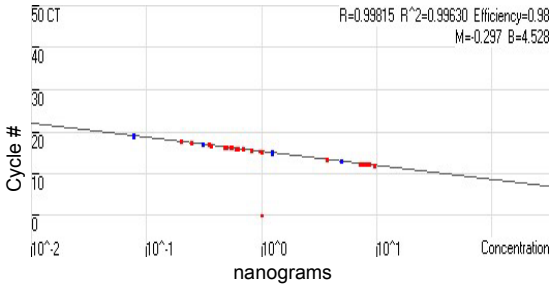



**The output data is plotted on a log scale and the fractional # cycles required to reach $C_t$ is measured**



## Standard curve

**Plot the cycle # at threshold CT vs concentration**



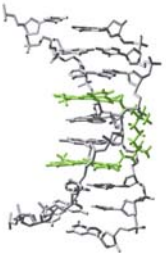

Concentration = 10^(-0.297*CT+ 4.528)

## Detection Methods

- Fluorescent intercalating dye - SYBR Green
  - Fluorescence increases with concentration of dsDNA
- Taqman probes
  - Fluorescence increases as quenched probe is digested
- Molecular beacons
  - Fluorescence increases as quenched probe hybridizes to template

## SYBR green product detection

- Easy
  - Fluorescence only with dsDNA
  - Use with existing PCR primers
- Generic,
  - Detects all double stranded products, including primer dimers
  - However, can be very specific with proper primer design
- Singleplexed
  - Multiple probes cannot be used



dsDNA Intercalation

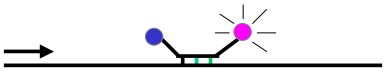
http://www.probes.com/handbook/figures/1557.htm

## Molecular beacons

- Consist of ssDNA with an internal complementary sequence that keeps reporter and quencher dyes close → No fluorescence

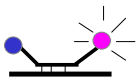
Molecular beacon  Reporter Quencher

- Following denaturation, beacon anneals to template, separating both dyes and yielding fluorescence proportional to PCR product concentration



## Molecular Beacons

- Improved specificity and multiplexing
  - Non-specific amplification will not produce a signal
  - Can multiplex several probes (quantify nuclear, Y, int std.)
- Can be tricky to design
  - Loop portion – binds to DNA template
  - Stem portion – must be complementary to other stem
  - Probe must denature from template below 72° so Taq polymerase does not chew it up during extension step



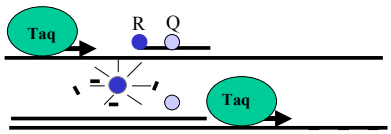
$T_{anneal} < T_m < T_{ext}$

Above $T_m$ loop structure reforms and probe leaves template

## Taqman

Probe also binds to PCR product during extension but is always quenched

- 5'-3' exonuclease activity of Taq polymerase digests probe and frees reporter dye from quencher
- Free dye accumulates with PCR product




## Probes vs SYBR Green

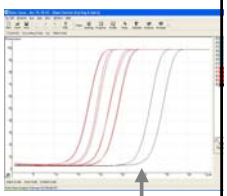
- SYBR Green
  - Singleplex probes (Alu)
  - If no sample, amplification of contaminants occurs at high cycle #
  - If inhibition, no result or poor efficiency curve
- Probes (Taqman, Mol. beacons)
  - Multiplex targeted probes – Quant Y, nuclear DNA, int. std
  - Inhibition and no sample can yield no result (if single locus probe)
  - to check for inhibition, an internal std. is used
- Choice: Simplicity (SYBR green) vs Multiplexing (probes)

## Single vs Multilocus Targets

- SYBR Green – Multilocus Probe
  - Alu inserts occur at multiple locations throughout the genome - sensitive
  - If no sample, amplification of contaminants occurs at high cycle #
  - Syber green requires no special kit –Inexpensive
- Probes (Taqman, Mol. beacons)
  - Single location in genome
  - an internal std. is used to check for amplification and correct for changes in efficiency
  - Lower sensitivity due to noise at low copy number
- Choice: Sensitivity (SYBR green) vs Internal Standard Precision (probes)

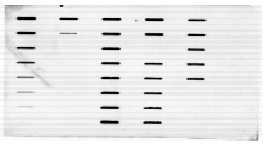
## Effects of Inhibitors on Alu Assay

- Use Alu sequence, present at 1,000's of copies/cell
  - Assay is sensitive to ambient human DNA in air and water
  - Normal Reagent blanks have a Ct at about 27-29 cycles
- If inhibitors are present – no amplification occurs or efficiency is altered
  - Thus low level ambient DNA serves as an internal control for inhibitors
- For non Alu based RtPCR, an internal standard is required to detect inhibition




## Slot blot versus real time PCR






## Slot Blot

- 2 days of rinses, incubations, pipettings, washes, exposures, and developments
- Semiquantitation by manual comparison or through scanner
- Quantity obtained may not reflect final result due to variations in PCR efficiency

- 2 hours setup and run time **RtPCR**
- Automated quantitation
- Quantity obtains reflects amplifiable result

## Big issue – sensitivity and dynamic range

**Quantiblot-ECL 40 pg - 2.0 ng**

**ACES 2.0 40 pg - 4.0 ng**
No longer available
(ACES tended to work better on degraded DNA)

**Real Time PCR 1.0 pg - 16 ng**

**RTPCR has lower detection limit and larger dynamic range**


## Comparison Studies Slot blot vs RtPCR

| | Reference | RTi-PCR | Quantiblot |
|---|---|---|---|
| 1 | 5 | 5.38 | 6.25 |
| 2 | 1.25 | 1.14 | 0.56 |
| 3 | 0.3125 | 0.29 | 0.56 |
| 4 | 0.078125 | 0.08 | 0.12 |
| 5 | 5 | 4.92 | 8.75 |
| 6 | 1.25 | 1.32 | 0.63 |
| 7 | 0.3125 | 0.30 | 0.81 |
| 8 | 0.078125 | 0.09 | 0.23 |

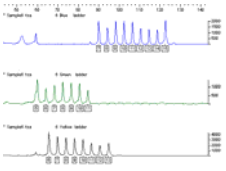
Calibration studies in our lab with experimental primers

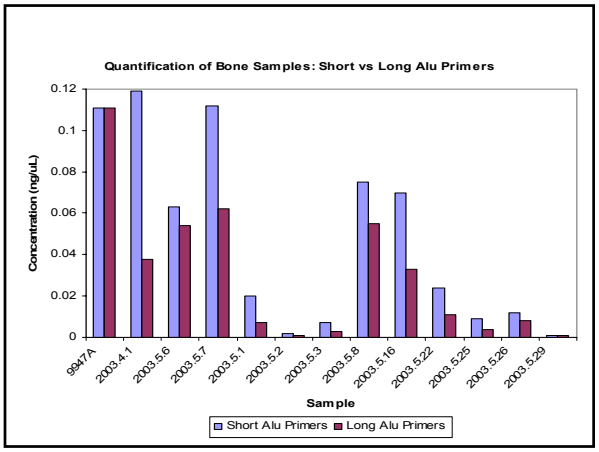
| sample | rtPCR | slot blot | Tho1 Allele |
|---|---|---|---|
| blood on stick | 0.32 | 0.50 | 1880 |
| blood on metal | 0.40 | 0.50 | 1890 |
| blood on concrete | 0.40 | 0.50 | 1860 |
| blood on leaves | 0.08 | 0.20 | 1540 |
| blood on cardboard | 0.27 | 0.24 | 1450 |
| blood on cloth | 0.04 | 0.05 | 577 |
| blood on denim | 0.25 | 1.00 | 1240 |

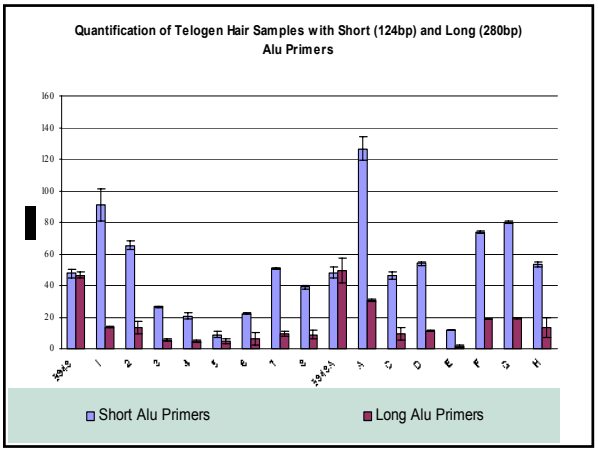
Validation work of Jan Nicklas and Eric Buel
Nicklas, J.; Buel, E., J. Forens. Sci. 2003, 48(5) pp. 936-944

## Work in OhioU/FIU Laboratory

- Development of miniplex STRs for degraded DNA typical sizes 60-120 bp.
- Slot blot works poorly on these samples
- Current assay has 124bp Alu product – reduction to 84bp testing in progress




## Other potential applications of RtPCR

- Rapid sex typing
- Simultaneous Mt, Y, Human DNA
  - If differences in copy# can be resolved
- Determination of degradation by multiplex amplification of different STR probes

## RT-PCR Instruments Cited

- Corbett Research Rotorgene
  - Phenix Research, Hayward, CA
- ABI 7000 Sequence Detection System
- ABI 7700 (discontinued)
- ABI 7900HT Sequence Detection System
  - Applied Biosystems Foster City, CA

## Real-Time PCR Efforts

- Marie Allen – nuclear and mtDNA assay (BioTechniques 2002, 33(2): 402-411)
- Eric Buel – Alu system (JFS 2003, 48(5):936-944)
- Centre for Forensic Sciences – nuclear; TH01 flanking region (JFS 2003, 48(5):1041-1046)
- John Hartmann – Alu system (SWGDAM Jan 2003)
- CA-DOJ – TH01 assay (NIJ DNA Grantees June 2003)

- SYBR Green assay – human-specific with right PCR
- Quantifiler kit (ABI) – separate nuclear and Y assays

## NIST Lessons Learned from Real Time-PCR Assays

We are using ABI 7000 (some work also with Roche LightCycler)

- Results are RELATIVE to standards used
- Single source and mixed source samples with same UV concentrations differ with RT-PCR assays
- Need to keep instrument clean to avoid background fluorescence problems
- Assay reagent costs:
  - Quantifiler: $2.46/sample (only permits 2 µL/sample)
  - SYBR Green: $0.80/sample (up to 10 µL/sample)
  - QuantiBlot: $0.54/sample (5 µL/sample)

## Conclusions


- RTPCR is a homogeneous PCR based method for human specific quantification
  - Is easily automated, provides electronic storage of data
  - SYBR green or targeted probes can be used
- Results give quantity of amplifiable DNA – not necessarily overall quantity
  - Inhibition can be detected
  - Multiplexing can be used
- Big advantages are speed and dynamic range

## Acknowledgements


- Jan Nicklas and Eric Buel - Vermont Crime Laboratory
- Jiri Drabek
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- Nancy Tatarek
- John Butler, Yin Shen

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- The OU Provost's Undergraduate Research Fund
- Ohio University Research Incentive Fund


Chemistry & Biochemistry
OHIO UNIVERSITY

## References


On-line

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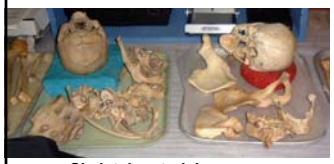
Trends in microbiology 2000, 12, pp. 61-66


# miniSTRs

STR Size Reduction
Through Moving Primer Positions
Closer to the Repeat Region

## Large Multiplex Kits provide Efficient and Rapid Analysis of Convicted Offender Samples

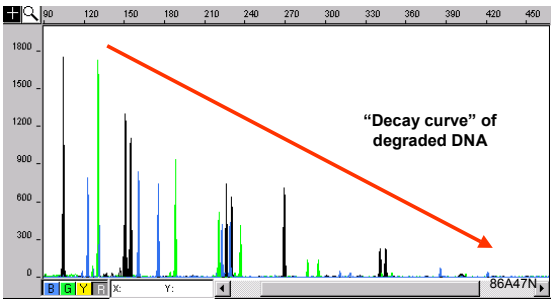

**But what about degraded DNA ?**



**Skeletal material being preped for extraction**

**Such samples present a special challenge**

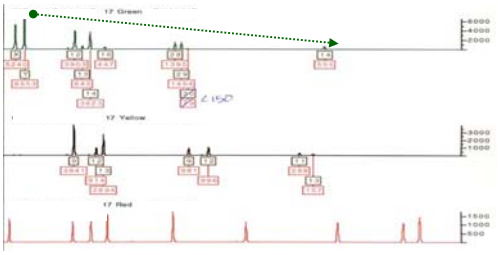
PowerPlex 16 Result on Aged Blood Stain
(15 years at room temperature storage)




NIST

## Case work example:
**Efficiency loss at larger sizes**

**Sample: Fingernail Scraping**

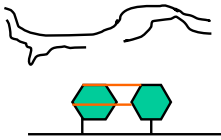


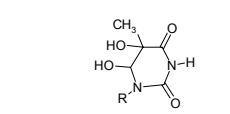
*Results with Profiler+ (ABI)*

*Lynne Helton MSP*

## DNA Degradation

1. **Strand breakage**
2. **Pyrimidine dimers**
3. **Chemical oxidation and hydrolysis**
4. **Bacterial degradation and metal contamination**

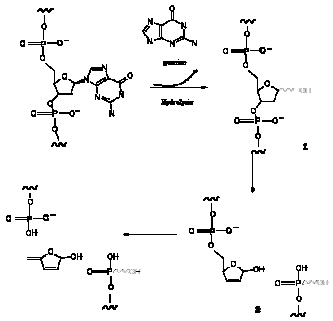






**Thymine glycol**

## Hydrolytic Damage to DNA



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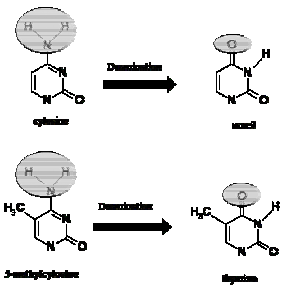
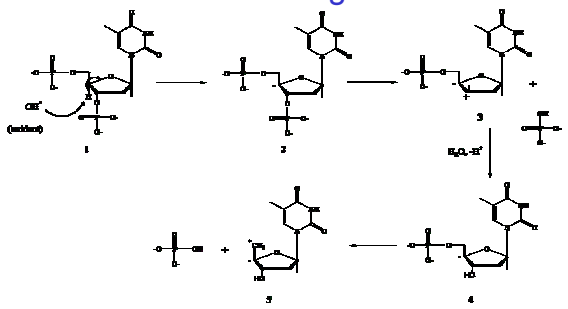
## Deamination



Griffiths, A.J.F.; Gelbart, W.M.; Lewontin, R.C.; Miller, J.H. Gene Mutation: Origins and Repair Processes. *Modern Genetic Analysis*, 2nd Edition; W.H. Freeman: New York, NY 2002; Chapter 10

## Oxidative Damage to DNA



Sonntag, C. V. Polynucleotides and DNA. *The Chemical Basis of Radiation Biology*, Taylor & Francis, Inc. Philadelphia, PA 1987; Chapter 9

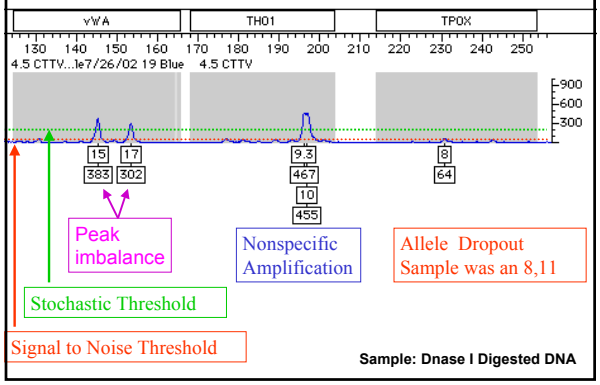
## Degraded DNA

1. **Fragmentation due to the environment**
2. **The presence of PCR inhibitors**

**Result**

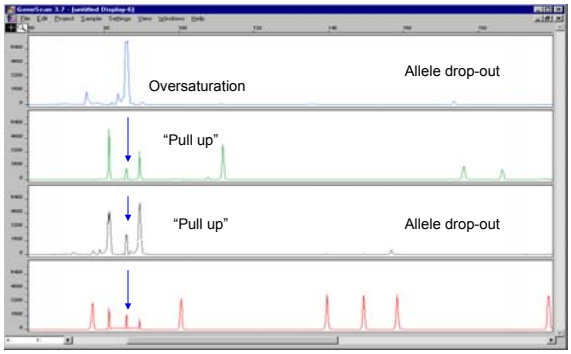
1. **Poor amplification efficiency**
2. **Peak imbalance and allele dropout**

## The Problem with Degraded DNA




Sample: Dnase I Digested DNA

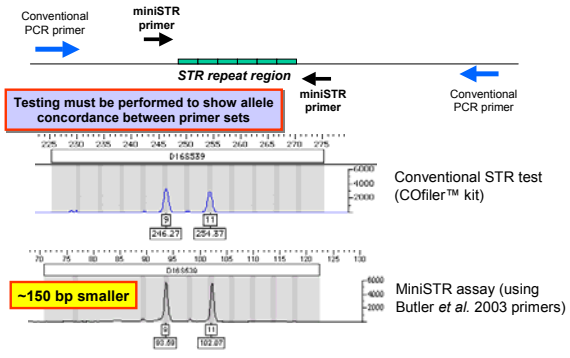
## Loss of Larger Loci Intensity




## The Miniplex Approach to Degraded DNA

1. Redesign primers to make each STR amplicon as short as possible.
2. Avoid overlap by having only 1 STR locus in each dye lane.
3. Provide an alternative to mtDNA for degraded DNA template.
4. Develop of specialized STR systems for degraded DNA.

A miniSTR is a reduced size STR amplicon that enables higher recovery of information from degraded DNA samples




Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 7.2, ©Elsevier Science/Academic Press

## MiniSTR Work

- miniSTRs (*a.k.a. BodePlexes*) are being used successfully in WTC effort
- Collaboration between John Butler and Bruce McCord (NIJ-funded) to further develop reduced size STR amplicons
- Mike Coble (NRC postdoc) at NIST is developing new miniSTR loci that are unlinked to CODIS loci
- Kerry Opel (FIU grad student) is performing validation studies on the new kits

*J. Forensic Sci.* 2003 48(5): 1054-1064

John M. Butler,[1] Ph.D.; Yin Shen,[2,3] Ph.D.; and Bruce R. McCord Ph.D.[2]

The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA*

Describes new primer sequences for all CODIS loci and initial assays developed

## Current Miniplex Loci

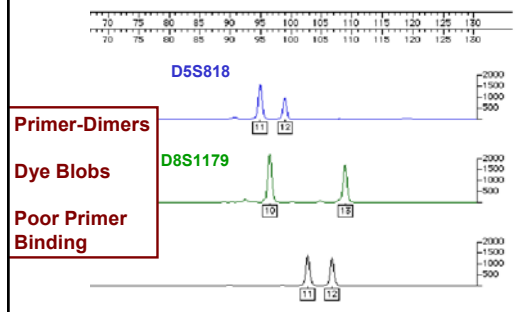
and reduction in size relative to ABI kits

| Dye Label: | Blue | Green | Yellow |
|---|---|---|---|
| Miniplex 1 | TH01 -105 | CSF1P0 -191 | TPOX -148 |
| Miniplex 2 | D5S818 -53 | D8S1179 -37 | D16S539 -152 |
| Miniplex 3 | FGA - 71 | D21S11 -33 | D7S820 -117 |
| Miniplex 4 | vWA -64 | D18S51 -151 | D13S317 -105 |
| Miniplex 5 | Penta D -282 | Penta E -299 | D2S1338 -198 |

## Miniplex Primer Sets

| | | FAM | VIC | NED |
|---|---|---|---|---|
| Big Miniplex | Miniplex 1 | TH01 | CSF1PO | TPOX |
| | Miniplex 3 | FGA | D21S11 | D7S820 |
| Miniplex 2 | | D5S818 | D8S1179 | D16S539 |
| Miniplex 4 | | vWA | D18S51 | D13S317 |

## MINI 2 PROFILE - BLOOD



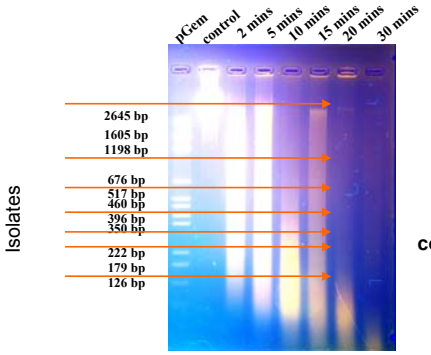

## The Big Question:

How does it work with degraded DNA?

Approach: Examine the effect of template size on DNA amplification

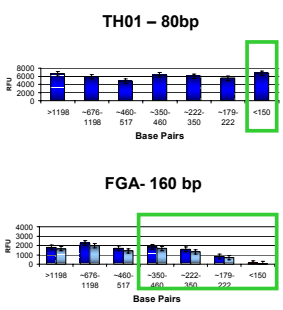
1) Extract large quantities of DNA from liquid blood
2) Digest with DNaseI
3) Cut sections at different size ranges and amplify
4) Compare with a commercial multiplex kit

## DNA Degraded With DNase I

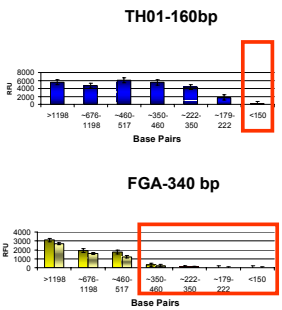



Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

## "Big Mini"

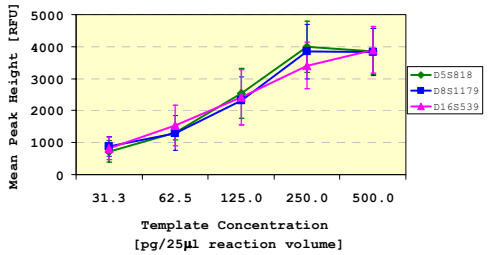



## PowerPlex 16

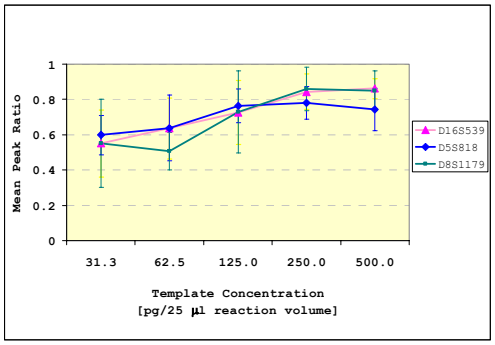



Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.

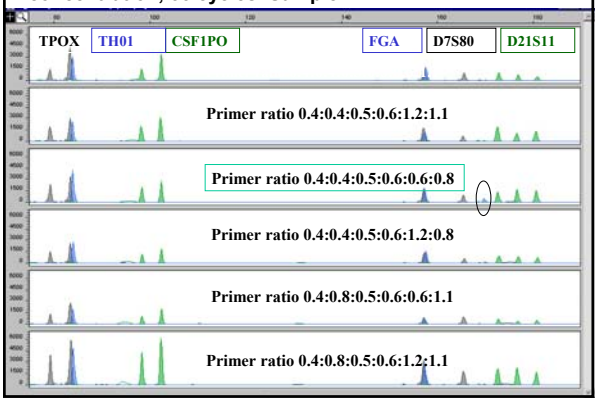
## Mini 2: Sensitivity



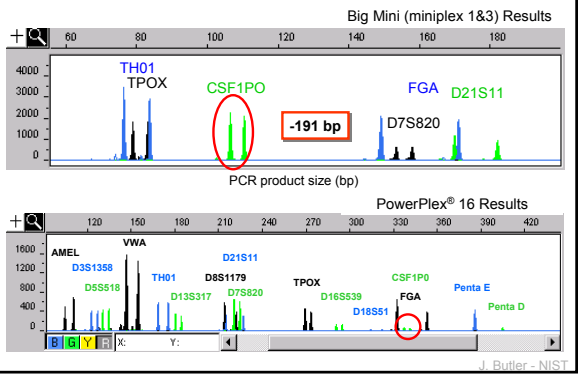
## Mini 2: Peak Balance




## Big Mini Primer Set, Cell line K562, 0.1ng/25 uL concentration, 33 cycles. Sample 1




## Comparison between MiniSTR and Commercial Kit Results Degraded DNA Template - NIST



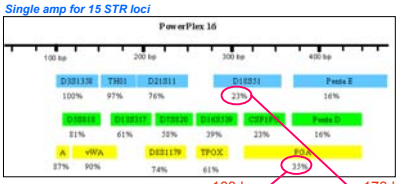

## Experiments with bone samples UT Forensic Anthropological Center

Surface Full Sun

Surface Partial Shade

## Comparison of PCR Amplification Success Rates with Commercial Kit vs. miniSTR Assays

*Single amp for 15 STR loci*

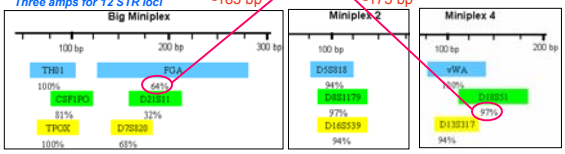



Study with 31 bones from the "Body Farm" (Knoxville, TN) and Franklin County Coroner's Office (OH)

*Three amps for 12 STR loci*

-183 bp

-173 bp




*Opel, et al., The application of miniplex primer sets in the DNA profiling of human skeletal remains, submitted*

## Results

Samples from the Anthropological Research Facility

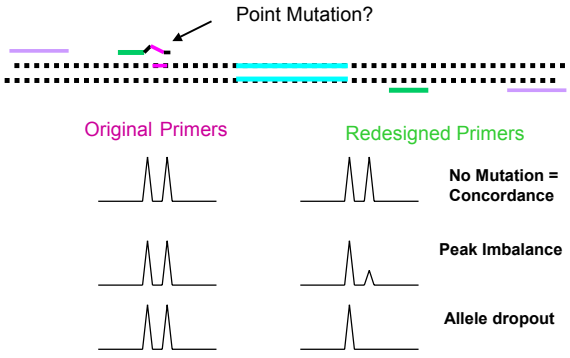
| Primer Set | Samples tested | Full Profile | Partial Profile |
|---|---|---|---|
| Miniplex 2 | 25 | 23 (92%) | 2 (8%) |
| Miniplex 4 | 25 | 22 (88%) | 3 (12%) |
| Big Miniplex | 25 | 6 (24%) | 19 (76%) |
| *Miniplex 1* | | 20 (80%) | 5 (20%) |
| *Miniplex 3* | | 7 (28%) | 18 (72%) |
| PowerPlex 16 | 25 | 3 (12%) | 22 (88%) |

Samples from the Franklin County Coroner's Office

| Primer Set | Samples tested | Full Profile | Partial Profile |
|---|---|---|---|
| Miniplex 2 | 6 | 6 (100%) | 0 |
| Miniplex 4 | 6 | 6 (100%) | 0 |
| Big Miniplex | 6 | 3 50%) | 3 (50%) |
| *Miniplex 1* | | 5 (83%) | 1 (17%) |
| *Miniplex 3* | | 3 (50%) | 3 (50%) |
| PowerPlex 16 | 6 | 2 (33%) | 4 (67%) |

*Chung, et al., The application of miniplex primer sets in the DNA profiling of human skeletal remains, submitted*

## Primer Design- Concordance?




## Results of Allele Concordance between Miniplex Primers and Commercial STR Kit Primers

Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.

- 532 U.S. population samples (Caucasians, African Americans, and Hispanics) examined with four miniplexes (including all CODIS loci except D3S1358)
- Results compared to Identifiler STR kit (6,384 genotypes compared)
- 15 discrepancies (0.23%) – most of which occur in 2 loci (D13S317 and VWA) and involve deletions between the two primer sites
- Representative samples from each discrepant locus have been sequenced to locate the polymorphic nucleotides causing the allele dropout

## Allele Discordance Resulting from Mutations in Miniplex Primer Binding Site

| | Locus | Origin | Miniplex | Identifiler | PP16 | Likely Cause |
|---|---|---|---|---|---|---|
| 1 | D13S317 | AA | **11**,13 | 10,13 | 10,13 | deletion outside of allele 11 |
| 2 | D13S317 | H | **9**,14 | 8,14 | 8,14 | deletion outside of allele 9 |
| 3 | D13S317 | AA | **10**,11 | 9,11 | 9,11 | deletion outside of allele 10 |
| 4 | D13S317 | H | **10**,11 | 9,11 | 9,11 | deletion outside of allele 10 |
| 5 | D13S317 | H | **10**,14 | 9,14 | 9,14 | deletion outside of allele 10 |
| 6 | D5S818 | AA | 11,**11** | 11,12 | 11,12 | primer binding site mutation |
| 7 | vWA | AA | **16**,16 | 12,16 | 12,16 | primer binding site mutation |
| 8 | vWA | AA | **18**,18 | 13,18 | 13,18 | primer binding site mutation |
| 9 | vWA | AA | **15**,15 | 14,15 | 14,15 | primer binding site mutation |
| 10 | vWA | AA | **15**,15 | 14,15 | 14,15 | primer binding site mutation |
| 11 | vWA | AA | **17**,17 | 14,17 | 14,17 | primer binding site mutation |
| 12 | vWA | AA | **17**,17 | 14,17 | 14,17 | primer binding site mutation |
| 13 | vWA | AA | **19**,19 | 14,19 | 14,19 | primer binding site mutation |
| 14 | vWA | AA | **19**,19 | 14,19 | 14,19 | primer binding site mutation |
| 15 | vWA | AA | **19**,19 | 14,19 | 14,19 | primer binding site mutation |

## Proposed mechanism

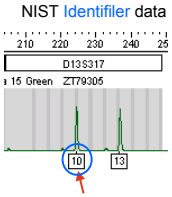
**Deletion outside Miniplex primers-limited region (samples 1-5)**

**Mutation/polymorphism in Miniplex primer binding region (samples 6 - 15)**

## Examination of Concordance:

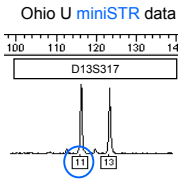
African American sample ZT79305

NIST Identifiler data




Really "11-1" allele

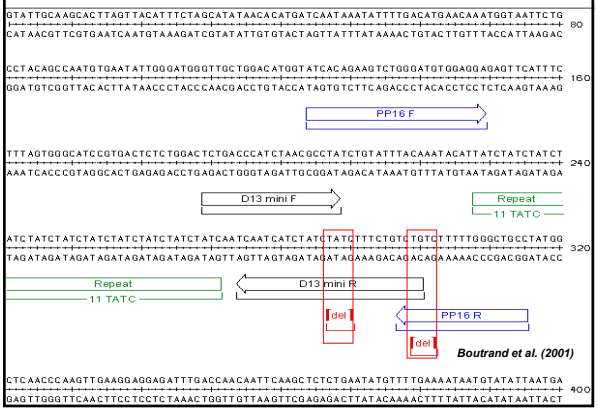
Ohio U miniSTR data



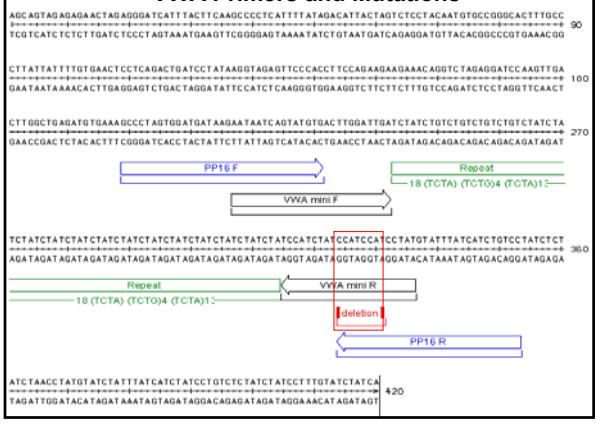

*This problem has been seen multiple times by NYC OCME review of WTC BodePlex data*

A deletion *outside* the miniSTR primers causes the kit produced allele to appear one repeat smaller…

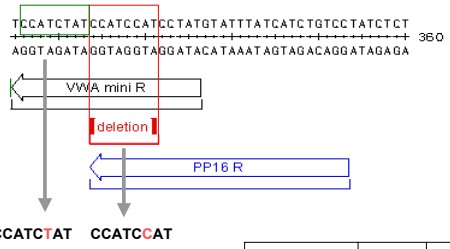
## D13S317 Primers and Mutations



## VWA Primers and Mutations




## VWA Mutation Region




CCATCTAT CCATCCAT

- Allele drop out with Miniplex primer
- PPlex 16 primers still bind because there is only one base pair difference in the adjacent sequence
- ABI reported this as a C-T polymorphism (Lazaruk et al. 2001)
- There is actually 2 repeats (8 bp) more than the actual allele called in commercial kits

| Sample | Miniplex | PP16 |
|---|---|---|
| PT83871 (AA) | **16**,16 | 12,16 |
| PT84216 (AA) | **18**,18 | 13,18 |
| MT95095 (AA) | **19**,19 | 14,19 |

## New miniSTR (non-CODIS) Loci Under Investigation

| STR Locus | Sequence Motif | Allele Range | Size Range (bp) | Observed Heterozygosity |
|---|---|---|---|---|
| **D1S1677** | $(GGAA)_n$ | 9-18 | 81-117 | 0.75 |
| **D2S441** | $(TCTA)_n$ | 9-17 | 78-110 | 0.76 |
| **D4S2364** | $(GAAT)(GGAT)(GAAT)_n$ | 8-12 | 67-83 | 0.53 |
| **D10S1248** | $(GGAA)_n$ | 10-20 | 83-123 | 0.78 |
| **D14S1434** | $(GATA)_n(GACA)_n$ | 13-20 | 70-98 | 0.68 |
| **D22S1045** | $(TAA)_n$ | 5-16 | 76-109 | 0.77 |

Coble, M.D. and Butler, J.M. (Jan 2005) *J. Forensic Sci., in press*

## Conclusions

**1. For degraded or environmentally challenged samples, Mini-STRs can provide a result that is compatible with larger Multiplexes. Sensitivity is enhanced.**

**2. The technique is fully capable of analyzing degraded DNA in both the laboratory and in environmentally challenged samples**

**3. PCR inhibitors are minimally affected by amplicon size. Inhibitors must be removed prior to analysis. Treatment with EDTA and/or BSA greatly improves success**

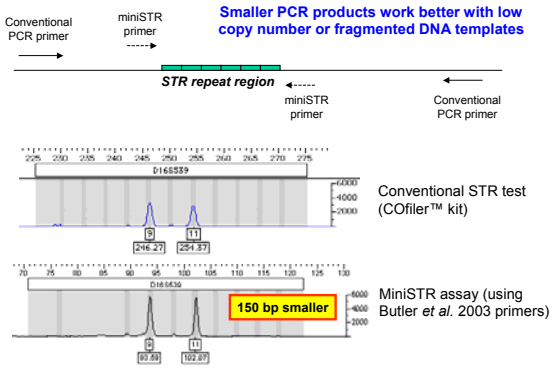

## Acknowledgements

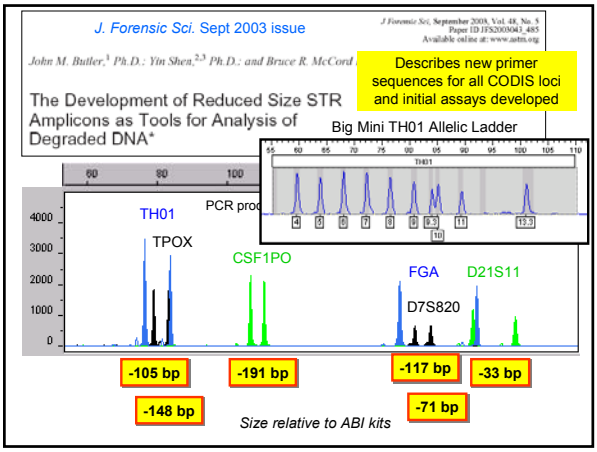
***National Institute of Justice, National Science Foundation***
***Ohio University Undergraduate Research Fund***
***Dr. John Butler, Dr. Nancy Tatarek, Dr. Lee Meadows Jantz, Dr. Janice Nicklas, Dr. Eric Buel, Dr. Mike Coble***


**McCord Research Group**

# miniSTRs

## miniSTRs: new tool for degraded DNA



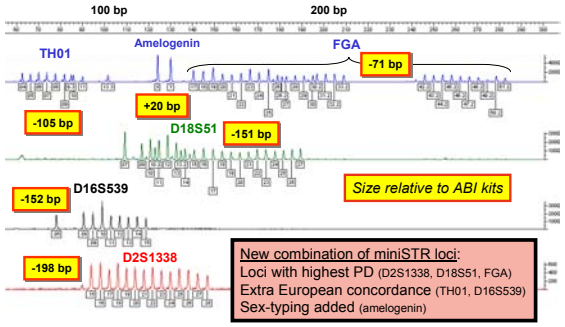

## Recent Publications on miniSTRs

- Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5): 1054-1064.
- Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M., McCord, B.R. (2004) A study on the effects of degradation and template concentration on the efficiency of the STR miniplex primer sets. *J. Forensic Sci.* 49(4): 733-740.
- Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. (2004) Concordance study between miniplex STR assays and a commercial STR typing kit, *J. Forensic Sci.* 49(4): 859-860.
- Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA., *J. Forensic Sci.*, 50: 43-53.

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm

## New miniSGM miniplex assay

Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)




Retains same miniSTR primers from Butler et al. (2003) *J. Forensic Sci* 48(5): 1054-1064

## Many CODIS Loci Make Poor miniSTRs

- Large allele range (e.g., FGA)
- Large alleles (e.g., D21S11 and FGA)
- Poor flanking regions prohibiting reliable primer annealing immediately adjacent to the repeat region (e.g., D7S820)

## Why go beyond CODIS loci

"STRs have proven to be highly successful [for mass disasters] in the past e.g. Waco disaster and various air disasters. However, even if the DNA is high quality there are occasions when there are insufficient family members available to achieve a high level of confidence with an association."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

## Why go beyond CODIS loci

"To achieve this purpose, either **new STRs could be developed**, or alternatively, existing STRs could be supplemented with a SNP panel."

"There are also efforts for modifying existing STR panels by decreasing the size amplicons by designing new primers."

Gill, P., Werrett, D.J., Budowle, B. and Guerrieri, R. (2004) **An assessment of whether SNPs will replace STRs in national DNA databases-Joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDAM).** *Science&Justice*, 44(1): 51-53.

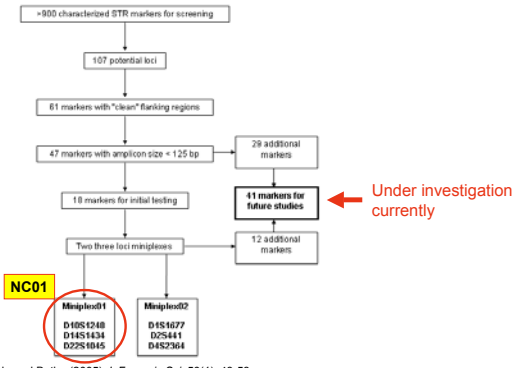
## Why go beyond CODIS loci

- Desirable to have markers unlinked from CODIS loci (different chromosomes) for some applications
- Small size ranges to aid amplification from degraded DNA samples
- **New miniSTR loci will benefit missing persons investigations and paternity testing (and perhaps national databases in the future)**

## Characterization of New miniSTR Loci

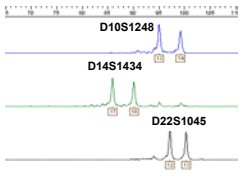
- Candidate STR marker selection
- Chromosomal locations and marker characteristics
- PCR primer design
- Initial testing results
- Population testing
- Allelic ladder construction
- Miniplex assay performance

## Initial Testing Results with Potential miniSTR Loci

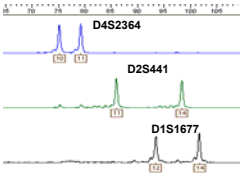



Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

Miniplex NC01




Miniplex NC02



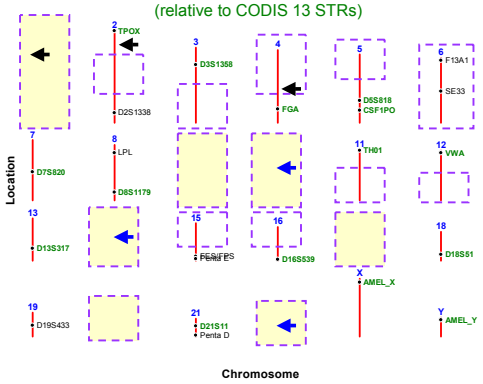

Some Marker Characteristics

| Chr. | Marker Name | (Motif) | Ref. Repeat | Amplicon Size | Primer distance from repeat |
|---|---|---|---|---|---|
| 10 | D10S1248 | TETRA | 13 | 102 | 1 |
| | GGAA23C05N | GGAA | | | 0 |
| 14 | D14S1434 | TETRA | 10 | 88 | 1 |
| | GATA168F06 | GATA | | | 0 |
| 22 | D22S1045 | TRI | 13 | 105 | 3 |
| | ATA37D06 | ATA | | | 6 |
| 1 | D1S1677 | TETRA | 15 | 103 | 0 |
| | GGAA22G10N | GGAA | | | 0 |
| 2 | D2S441 | TETRA | 12 | 92 | 0 |
| | GATA8F03 | GATA | | | 0 |
| 4 | D4S2364 | TETRA | 7 | 78 | 2 |
| | GAAT1F09 | GAAT | | | 1 |

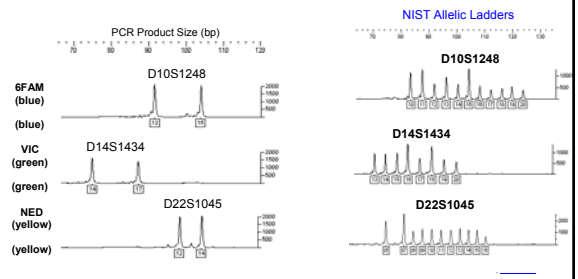
Coble and Butler (2005) *J. Forensic Sci.* 50(1): 43-53

## Locations of Focus for New miniSTR Loci

(relative to CODIS 13 STRs)



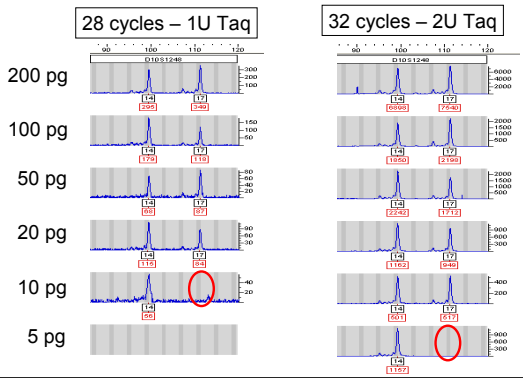
## Miniplex "NC01"



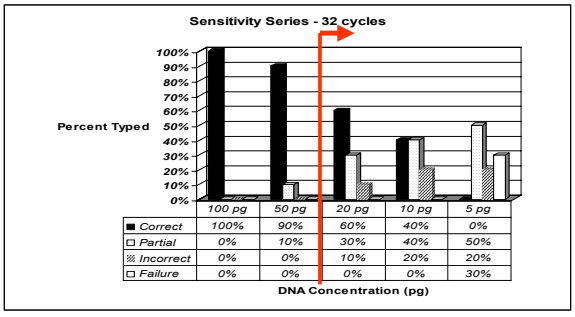

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm

Coble and Butler (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA *J. Forensic Sci.* 50(1): 43-53

## miniSTR Assay Sensitivity (D10S1248)



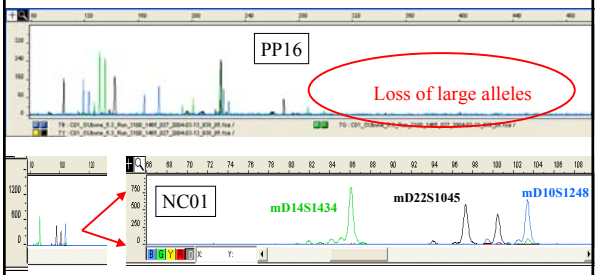

## Problems with Obtaining Correct Allele Calls at Low DNA Levels




| | 100 pg | 50 pg | 20 pg | 10 pg | 5 pg |
|---|---|---|---|---|---|
| Correct | 100% | 90% | 60% | 40% | 0% |
| Partial | 0% | 10% | 30% | 40% | 50% |
| Incorrect | 0% | 0% | 10% | 20% | 20% |
| Failure | 0% | 0% | 0% | 0% | 30% |

Coble, M.D. and Butler, J.M. (2005) *J. Forensic Sci.* 50: 43-53

## Sensitivity - Degraded DNA from an OU Bone Sample



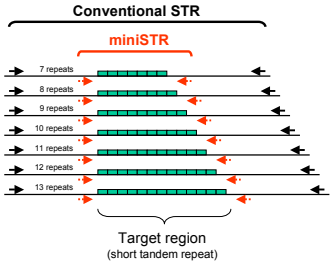

## Peter Gill Recommendations to EDNAP and ENFSI (April 2005, Scotland)

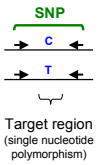
- **"miniSTRs are the best way forward for stain work for the foreseeable future…"**
- miniSTRs and 34 cycle PCR seems to be the best option to maximise sensitivity (note importance of minimising cycle number to avoid stochastic effects).
- Recommended to the ENFSI group that miniSTRs are the best way forward.
- Suggested NIST NC01 loci as additional European markers that are being advocated to manufacturers for future STR kits.

## Status of Additional STR Loci

- **D10S1248, D14S1434, D22S1045** are chromosomally unlinked to all CODIS STR loci
- Full locus characterization, allelic ladders constructed, population studies completed and published (Coble and Butler JFS Jan 2005)
- Demonstrated success in EDNAP degraded DNA interlab study coordinated by Peter Gill
- EDNAP/ENFSI newly recommended loci to commercial manufacturers for future STR kits
- Being adopted in multiple U.S. paternity testing labs (BRT Labs and Orchid Cellmark East Lansing)

## Comparison of STRs and SNPs




**Larger target region** (miniSTR targets same region)
**More possible variants than SNPs**
**Only need a moderate number of STR markers**
**Range of sizes examined** (e.g., 28 bp spread if 4 bp/repeat)

**Smaller target region**
**Fewer possible variants**
**Need more SNP markers**
**Constant size examined**