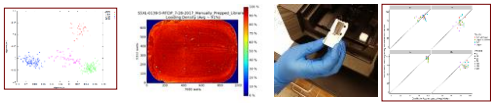
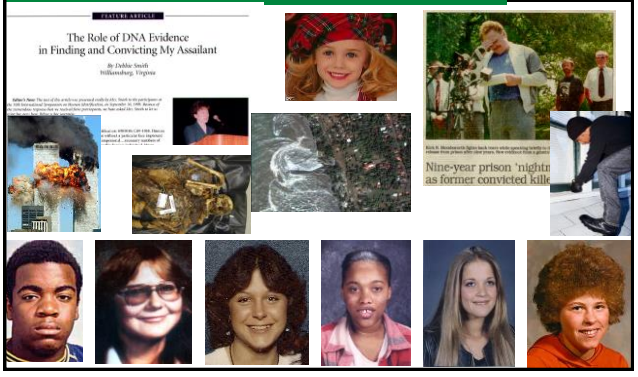
UNT HEALTH SCIENCE CENTER

Massively Parallel Sequencing Enhances Forensic Genomics Applications

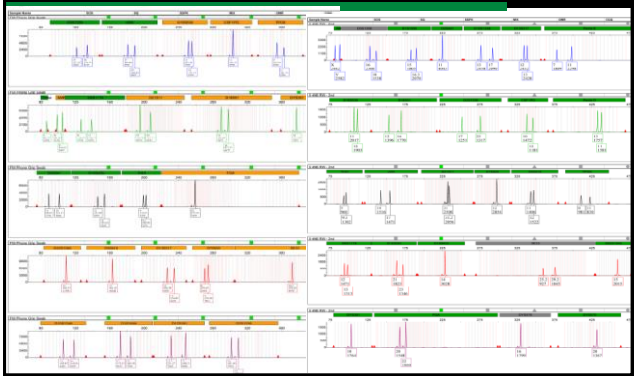


Bruce Budowle
Center for Human Identifications
Department of Microbiology, Immunology, and Genetics,
University of North Texas Health Science Center
Fort Worth, Texas USA

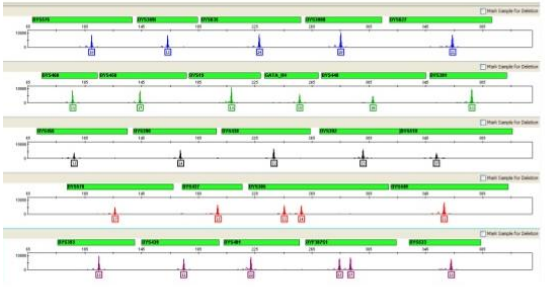
The Motivation



Mega Autosomal STR Kits

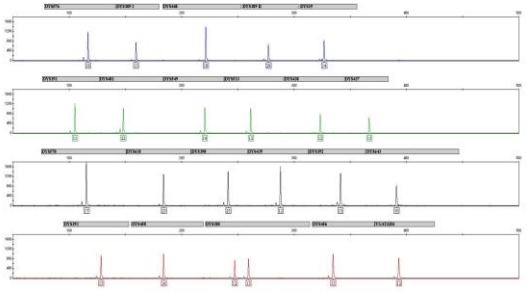


Yfiler Plus Profile

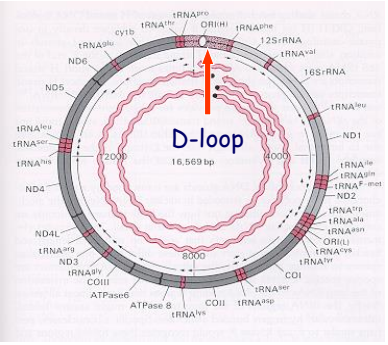


1ng of male 007 control DNA amplified for 30 cycles

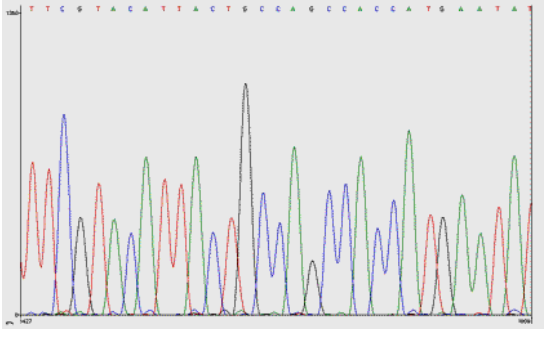
PowerPlex® Y23 System



Mitochondrial DNA



Sanger Sequence Electropherogram



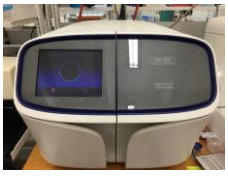
Current Forensic DNA Workflows

- CE-based systems are the mainstay of DNA typing
  - Well validated
    - Well understood
    - Robust
  - Well established

- But are they sufficient for today's needs?

Massively Parallel Sequencing



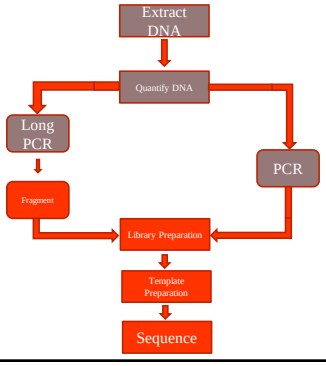



Areas of Interest

- STRs
- Mitochondrial DNA
- HID SNPs
- Ancestry SNPs
- Phenotype SNPs
- Mixtures
- Pharmacogenetics
- Microbial Forensics
- …going to need a bigger boat


Image courtesy: geek.com

Generic MPS Workflow



Library Preparation

- Getting the DNA ready for sequencing

1. Genomic DNA (Nuclear/Mitochondrial)
2. Get the DNA to the right size
3. Add adapters, priming sites, and barcodes
4. Normalize and pool libraries
5. Cloning

Library Preparation

Adapters:

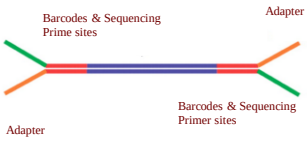
short pieces of DNA for DNA fragment to attach to a solid support for clonal amplification

Barcodes:

short pieces of DNA necessary for identifying multiplexed samples bioinformatically

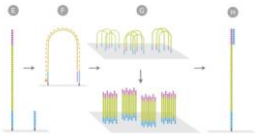
Priming sites:

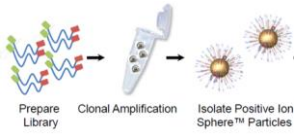
Short sequences for primers to bind to initiate sequencing by synthesis



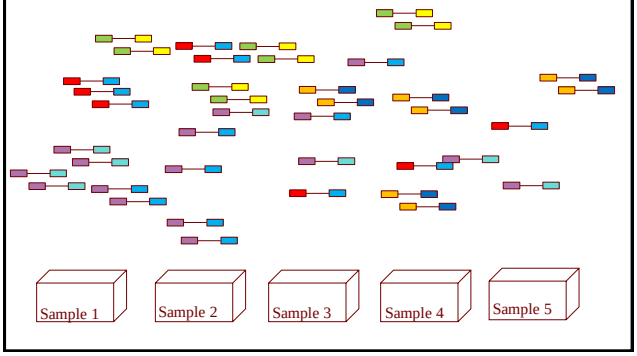
Amplification of Library Fragments

- Bridge Amplification
- Emulsion PCR

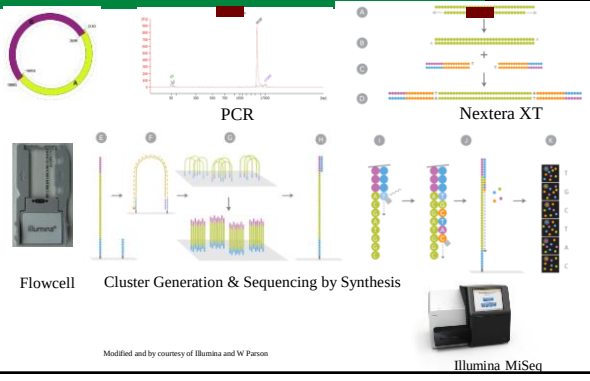




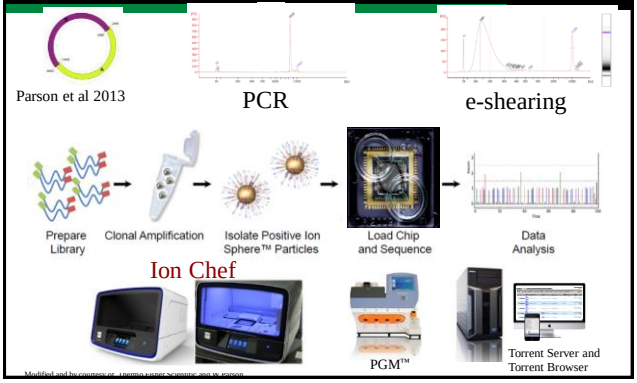
Multiplexing/Demultiplexing Barcoding/Index Sequences



Illumina MiSeq Workflow



PGM/Ion Torrent Workflow

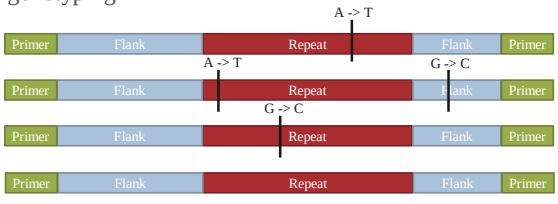


MPS Technologies

- Increasingly user-friendly
- Highly automated workflow
- Compatible with numerous genetic marker types
- Available data analysis options
- Accurate, reliable data

STR Sequence Variation

- MPS provides nucleotide level data for high resolution genotyping



- If ignore the deeper information, can generate size-based allele calling

Types of SNPs

- Individual Identification SNPs:
  - SNPs that collectively give very low probabilities of two individuals having the same multisite genotype; individualization, High heterozygosity, low Fst
- Ancestry Informative SNPs:
  - SNPs that collectively give a high probability of an individual's ancestry being from one part of the world or being derived from two or more areas of the world
- Lineage Informative SNPs:
  - Sets of tightly linked SNPs that function as multiallelic markers that can serve to identify relatives with higher probabilities than simple di-allelic SNPs
- Phenotype Informative SNPs:
  - SNPs that provide high probability that the individual has particular phenotypes, such as a particular skin color, hair color, eye color, etc.
- Pharmacogenetic SNPs – molecular autopsy

Massively Parallel Sequencing

- Generates immense amount of data

Bioinformatics

- Unprecedented access to biological data
  - data acquisition
- Managing biological databanks with numerous contributors and users
  - store, organize, networks
- Extracting useful information from large and dense biological data
  - manipulate, visualize
- Assembling molecular pieces into predictive models of biological systems for *in silico* experiments
  - modeling, inference
  - scientific computing: multiprocessor, faster processors

Objectives

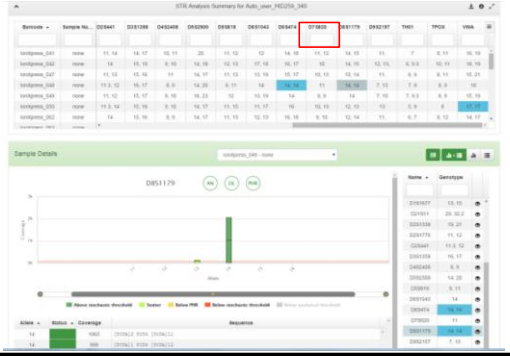
- Sample to answer
  - Simplicity
  - Flexibility
  - Data and information management
- What do the data mean?

STR Calls

- FASTQ/FASTA Files
- Assign reads to loci
  - Loci library (file) with configurations
- Reads grouped based on sequence similarities
  - Quality assessment
- Identify alleles
  - Allele library (file)
- Report Allele candidates plus stutter, etc.
- Evaluation by analyst

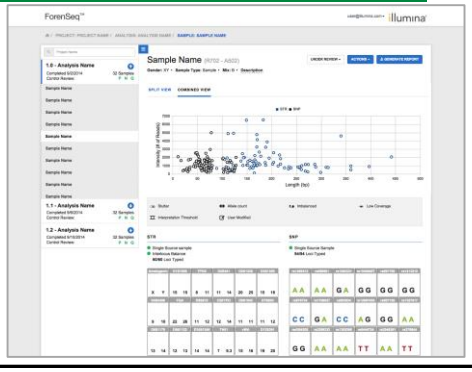
Main Analysis Page of STR Genotyper

(Ion Torrent)

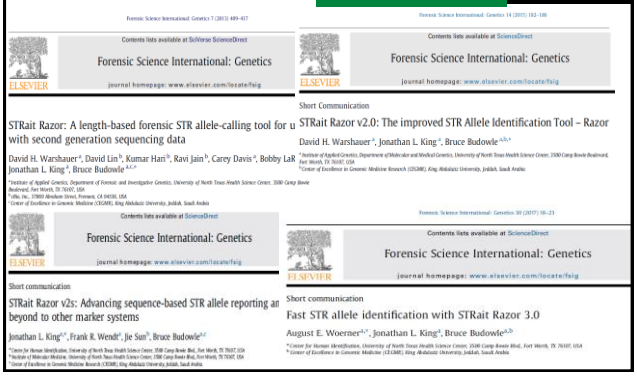


Universal Analysis Software Interface

Sample Results Display: STRs and Identity SNPs



Our Efforts to Address Data Analyses



Forensic Science International: Genetics

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

STRait Razor: A length-based forensic STR allele-calling tool for u with second generation sequencing data

David H. Warshauer[a], David Lin[b], Kumar Hari[b], Ravi Jain[b], Carey Davis[a], Bobby LaR Jonathan L. King[a], Bruce Budowle[a,c,*]

Short Communication

STRait Razor v2.0: The improved STR Allele Identification Tool – Razor

David H. Warshauer[a], Jonathan L. King[a,*], Bruce Budowle[a,b,*]

Short communication

STRait Razor v2s: Advancing sequence-based STR allele reporting an beyond to other marker systems

Jonathan L. King[a,*], Frank R. Wendt[a], Jie Sun[b], Bruce Budowle[a,c]

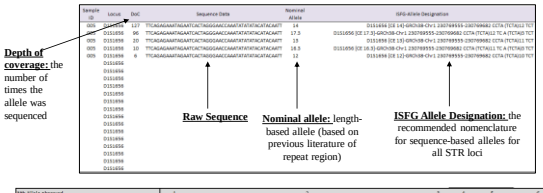
Short communication

Fast STR allele identification with STRait Razor 3.0

August E. Woerner[a,c], Jonathan L. King[a], Bruce Budowle[a,b]

STRait Razor Output

Sample 005, D1S1656 locus

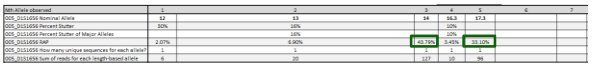


Depth of coverage: the number of times the allele was sequenced

Raw Sequence

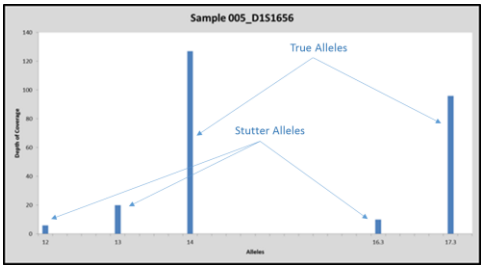
Nominal allele: length-based allele (based on previous literature of repeat region)

ISFG Allele Designation: the recommended nomenclature for sequence-based alleles for all STR loci



Images taken from modified version of STRait Razor 2.0; Warshauer *et al.* (2015)

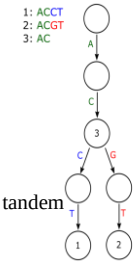
STRait Razor Output



Images taken from modified version of STRait Razor 2.0; Warshauer *et al.* (2015)

STRait Razor 3.0

- ~660x faster allele identification
- Perform "fuzzy" (approximate) string matching of anchor sequences
- Portable compiled language, C++
  - functions on all major operating systems
- Multithreading capabilities
  - exploit multi-cpu computer architectures
- Enhanced descriptions of anchor sequences
  - for proper extraction of loci that are duplicated in tandem



Variation Example at D21S11 Locus

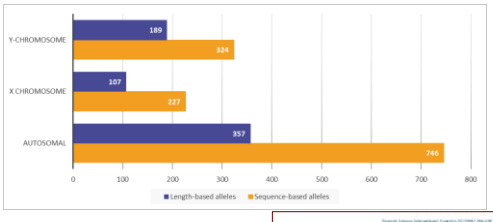
Allele 30
TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATGGATAGATAGATGATA
GATAGATAGATATAGATAGATAGACAGACAGACAGACAGACAGATAGATAGATAGATAGAT
AGATAGA

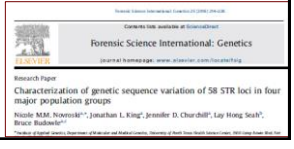
Minus Stutter Allele 31
TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATGGATAGATAGATGATA
GATAGATAGATATAGATAGATAGACAGACAGACAGACAGACAGATAGATAGATAGATAGAT
AGATAGA

Allele 31
TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATGGATAGATAGAT
GATAGATAGATAGATATAGATAGATAGACAGACAGACAGACAGACAGATAGATAGATAGAT
AGATAGATAGA

Plus Stutter Allele 30
TAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATGGATAGATAGAT
GATAGATAGATAGATATAGATAGATAGACAGACAGACAGACAGACAGATAGATAGATAGAT
AGATAGATAGA

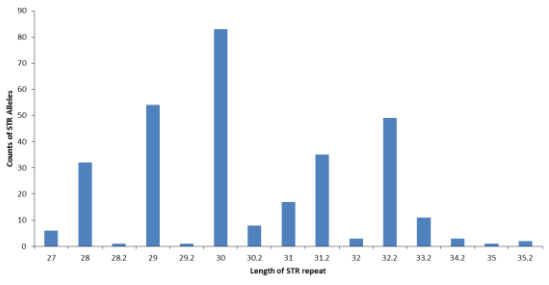
Population Study Results





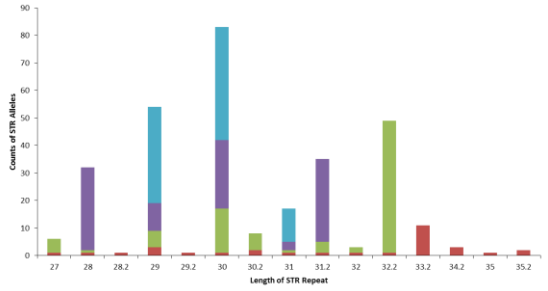
D21S11 Example

[TCTA], [TCTG] Repeat Motif

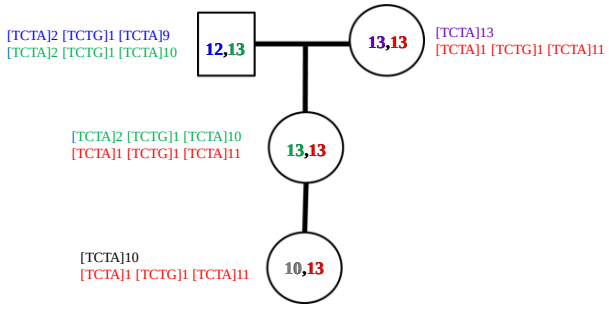


D21S11 Example

[TCTA], [TCTG] Repeat Motif

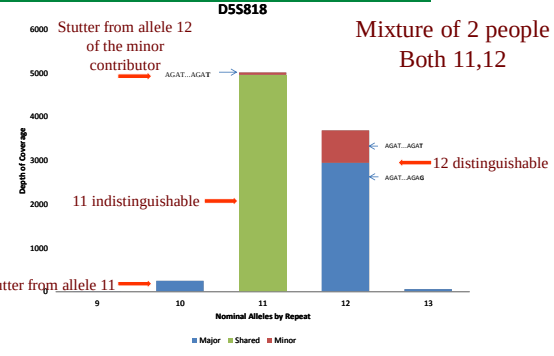


Identifying Relationships

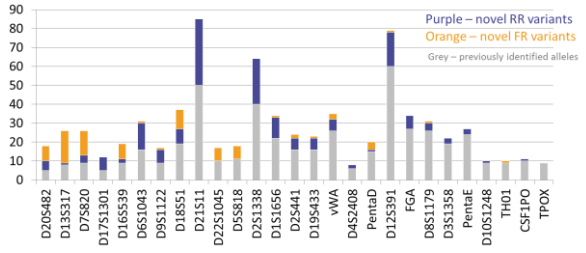


Sequence variants present within the D8S1179 alleles

Minor and Major Contributor Alleles

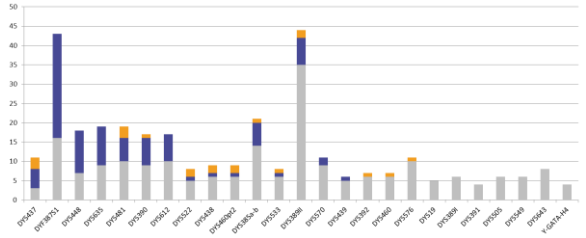


Novel Autosomal STR Variants



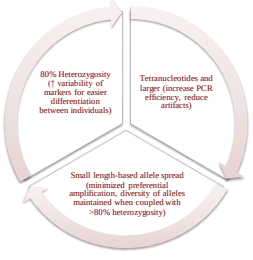
RR – Repeat Region
FR – Flanking Region

Novel Y STR Variants



Search for More Resolving STRs

- 1000 Genomes Project (raw sequences, unsorted)
- STR Catalog Viewer
  - Summary of human STR variation compiled using lobSTR software




Forensic Science International: Genetics
Research paper
Potential highly polymorphic short tandem repeat markers for enhanced forensic identity testing

More Informative STRs Mixtures

Phase 1 with >3000 Candidates → 793 Candidates → Design and Redesign → 248 Candidates

↓

Illumina TruSeq Custom Amplicon Preliminary Panel (248 Candidates)

↓

Sequencing Performance → STR Classified on Read Depth (Depth of Coverage) and Chemistry Compatibility

↓

From the 248 Candidates → 55 Failures; 58 Poor Performers; 72 Fair Performers; 63 Good Performers

↓

Considering (some) Poor, (all) Fair, (all) Good Performing Candidates → Heterozygosity and Allele Spread Assessed and Ranked

↓

53 STR Candidates that met criteria of high heterozygosity (>80%), reduced length-based allele spread (≤10), and a repeat size of four or more nucleotides

↓

Using top ranked candidates for heterozygosity and chemistry compatibility → 73 Candidates Selected for Refined (DECoDE) Panel

↓

Phase 3: DECoDE Panel Assessment and *in silico* mixtures

Exploratory Multiplex

- 73 highly heterozygous loci using MPS chemistry
  - 15 loci previously described by Phillips (2016) and others
- 451 unrelated individuals from three U.S. populations
  - Caucasian (CAU; n=155);
  - Hispanic (HIS; n=148);
  - African American (AFA; n=148)
- Each STR locus was characterized and reviewed manually for diversity using in-house Excel workbooks
  - Alleles characterized by length and sequence
  - Population genetics analyses (heterozygosity; Hardy-Weinberg equilibrium (HWE); linkage disequilibrium (LD); random match probabilities (RMP))

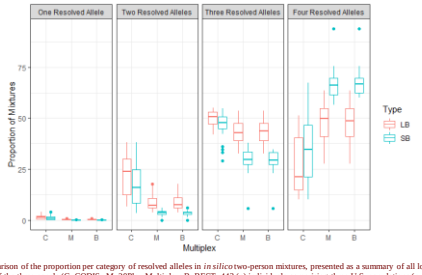
In silico Mixtures

- A **subset of 20 loci** selected for comparison to the CODIS core loci
  - The current requirement is **20 CODIS core loci** for upload into the national DNA database
  - high heterozygosity (>90%)
  - Operationally problematic loci (even if heterozygosity >90%) were excluded
- 443 U.S. population samples
  - African American, (AFA; n=140, 8 incomplete profile samples removed)
  - Caucasian, (CAU; n=155)
  - Hispanic, (HIS; n=148)

CODIS Panel Loci	Exploratory Panel Loci	BEST Panel Loci
D2S1338	D3S2406	D3S2406
D12S391	D2S1360	D2S1360
D1S1656	D7S3048	D7S3048
D21S11	D8S1132	D8S1132
D8S1179	D11S2368	D11S2368
vWA	D15S822	D15S822
D3S1358	D2N2	D2N2
D18S51	D1N10	D1N10
FGA	D12N15	D12N15
D19S433	D1N16	D1N19
D13S317	D1N19	D1N21
D5S818	D1N21	D8N23
D16S539	D8N23	D15N26
D22S1045	D15N26	D14N56
D7S820	D14N56	D3N61
D2S441	D3N61	D12S1338
CSF1PO	D4N70	D4N70
D10S1248	D11N52	D2S1338
TPOX	D17N32	D1S1656
TH01	D2N43	D11N52

Shaded cells reflect the CODIS core loci.

In silico Mixtures Locus Resolution

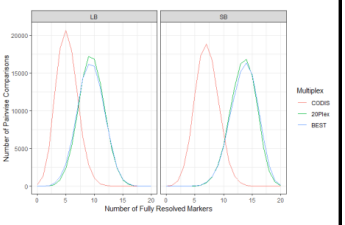


Comparison of the proportion per category of resolved alleles in *in silico* two-person mixtures, presented as a summary of all loci for each of the three panels (C=CODIS; M=20Plex Multiplex; B=BEST; 443 (n) individuals comprising three U.S. populations (n=140 African American; n=155 Caucasian; and n=148 Hispanic); two-person (k) mixtures; 97,903 (s) comparisons per locus (n=20) in each panel; total comparisons (N) = 1,958,060 for each DNA profile type (LB=length-based; and SB=sequenced-based)).

In silico Mixtures
Top 20 Performance

N = 443 individuals representing three U.S. populations (n=140 African American; n=155 Caucasian; and n=148 Hispanic samples)

Full Resolution	CODIS (LB)	Multiplex (LB)	BEST (LB)	CODIS (SB)	Multiplex (SB)	BEST (SB)
0 Loci	155	0	0	3	0	0
1 Locus	1330	6	7	104	0	0
2 Loci	5001	27	76	663	0	0
3 Loci	11715	211	359	2541	0	1
4 Loci	18200	839	1181	6333	8	4
5 Loci	**20574**	2320	2885	12056	14	41
6 Loci	17881	5242	5971	17179	128	139
7 Loci	12141	9592	10134	**18791**	440	496
8 Loci	6514	14203	14249	16929	1204	1275
9 Loci	2889	**17073**	**16079**	11656	2801	2682
10 Loci	1063	16806	15882	6966	5379	5230
11 Loci	317	13764	13121	3169	9287	8794
12 Loci	98	9305	8937	1253	13194	12117
13 Loci	18	5005	5235	431	16127	15129
14 Loci	5	2398	2475	119	**16747**	**16270**
15 Loci	2	823	919	22	14580	14779
16 Loci	0	226	322	6	10125	10755
17 Loci	0	53	62	1	5266	6560
18 Loci	0	10	8	1	2005	2765
19 Loci	0	0	1	0	539	777
20 Loci (Full Profile)	0	0	0	0	49	93
Total	97,903	97,903	97,903	97,903	97,903	97,903

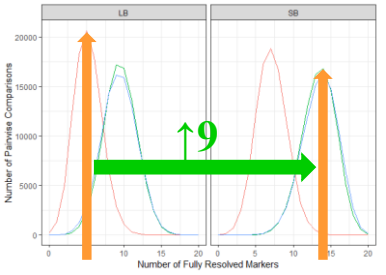


Highlighted in **BOLD** is the mode - the number of loci with the greatest number of counts for which four alleles at each locus were resolved.

LB = length-based; SB = sequence-based

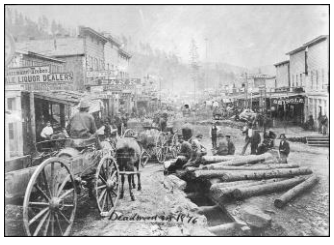
In silico Mixtures
Top 20 Performance

N = 443 individuals representing three U.S. populations (n=140 African American; n=155 Caucasian; and n=148 Hispanic samples)



LB = length-based; SB = sequence-based

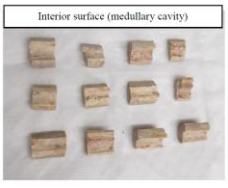
History of Deadwood, SD

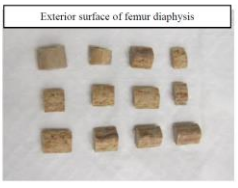


- 1874 → Expedition led by General George Custer discovered gold in the Black Hills of S.D.
- Growth out of "Black Hills Gold Rush"
- 1876 → City of Deadwood established as a primitive gold mining camp
- Wild, lawless frontier town in its early days; transitioned to a prosperous commercial center as a railroad was built to connect it to the outside world

Bone Sections

- Right femur
- Diaphysis surface-sanded w/Dremel® 4000 Rotary Tool and sterile grinding stone
- Sectioned with Stryker® autopsy saw





Phenotype Probabilities

HIrisPlex hair & eye colour prediction tool

	SNP ID	Minor allele		No.
1	N29insA	1	A	0
2	rs11547464	2	A	0
3	rs885479	3	T	0
4	rs1805008	4	T	2
5	rs1805005	5	T	0
6	rs1805006	6	A	0
7	rs1805007	7	T	0
8	rs1805009	8	C	0
9	Y152OCH	9	A	0
10	rs2228479	10	A	0
11	rs1110400	11	C	0
12	rs28777	12	C	0
13	rs16891982	13	C	0
14	rs12821256	14	G	0
15	rs4959270	15	A	1
16	rs12203592	16	T	1
17	rs1042602	17	T	0
18	rs1800407	18	A	0
19	rs2402130	19	G	0
20	rs12913832	20	T	1
21	rs2378249	21	C	1
22	rs12896399	22	G	2
23	rs1393350	23	T	0
24	rs683	24	G	1

HIrisPlex: Walsh et al. (2013)

Hair color	
Brown	0.19
Red	0.69
Black	0.04
Blond	0.09

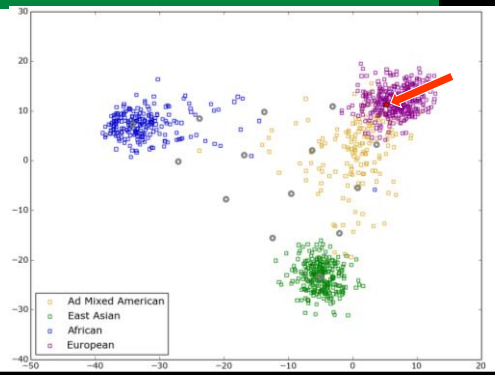
Hair Color Shade	
Light	0.71
Dark	0.29

Eye Color	
Brown	0.51
Intermediate	0.22
Blue	0.27

http://hirisplex.erasmusmc.nl
Accessed on 10-07-2014

European Ancestry

ForenSeq Aims



HID-Ion AmpliSeq™ Ancestry Panel

Sample	Biogeographic Ancestry
1	European
3	European
4	Asian
5	European
6	European
7	Asian
10	European
13	African Americans
14	African admix
15	African admix
16	African
17	European

Mitochondrial DNA

- mtDNA data used to characterize the maternal lineage of the Deadwood skeletal remains
  - Halogroup: H1+16189
    - Most common haplogroup in Western Europe

Haplogroup Predictor

Yfiler Results Table

Haplo-group	Fitness score	Proba-bility (%)
E1a	2	0.0
E1b1a	4	0.0
E1b1b	5	0.0
G1	3	0.0
G2a	3	0.0
G2b	1	0.0
I1	2	0.0
I2a	10	0.0
I2b	2	0.0
J1	3	0.0
J2a	5	0.0
J2b	1	0.0
L	7	0.0
N	7	0.0
O	10	0.0
Q	10	0.0
R1a	11	0.0
R1b	29	100.0
R2	6	0.0
T	5	0.0

ForenSeq Results Table

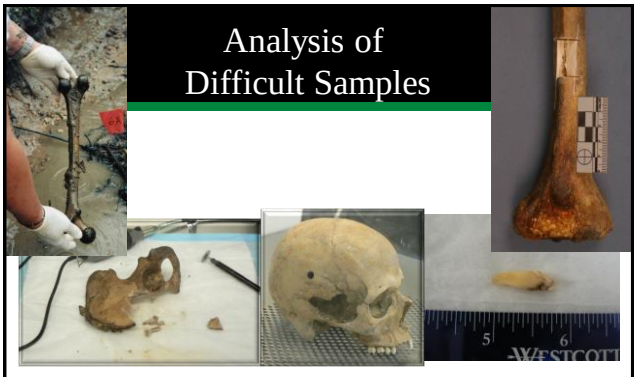
Haplo-group	Fitness score	Proba-bility (%)
E1a	2	0.0
E1b1a	29	0.0
E1b1b	18	0.0
G1	8	0.0
G2a	11	0.0
G2b	0	0.0
I1	6	0.0
I2a	35	0.0
I2b	10	0.0
J1	11	0.0
J2a	27	0.0
J2b	9	0.0
L	21	0.0
N	13	0.0
O	26	0.0
Q	26	0.0
R1a	31	0.0
R1b	79	100.0
R2	9	0.0
T	17	0.0

Combined Results Table

Haplo-group	Fitness score	Proba-bility (%)
E1a	1	0.0
E1b1a	8	0.0
E1b1b	9	0.0
G1	4	0.0
G2a	6	0.0
G2b	0	0.0
I1	3	0.0
I2a	18	0.0
I2b	4	0.0
J1	5	0.0
J2a	10	0.0
J2b	3	0.0
L	9	0.0
N	7	0.0
O	14	0.0
Q	14	0.0
R1a	17	0.0
R1b	40	100.0
R2	5	0.0
T	10	0.0

http://www.hprg.com/hapest5/

Analysis of Difficult Samples

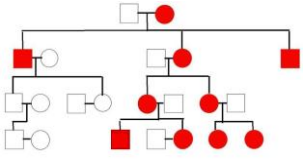


mtDNA is the most successful marker

Mitochondrial DNA

- Higher copy number per cell compared with the nuclear genome

- Maternal inheritance



Sanger Sequencing

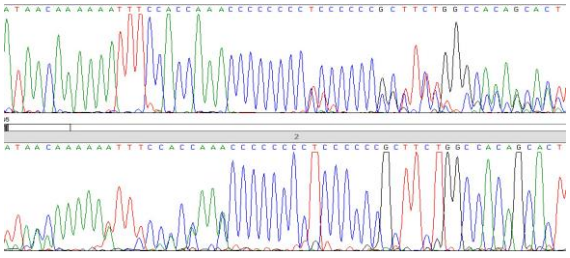
- Current Standard for forensic mtDNA analyses
  - Reliable
  - Casework
    - Bones
    - Teeth
    - Hair
    - Not used for mixtures
      - However exclusions could be made
  - Databases
- CE is current standard for most forensic marker systems

Limitations of Sequencing Technology

- Sequencing is labor intensive
- Analysis of results is time consuming
- Costly (prices range from $1000 to $3000 per mtDNA sample and only HV1 and HV2)
- Variation in intensity of peaks
- Not quantitative– impacts mixture interpretation
- Heteroplasmy difficulties

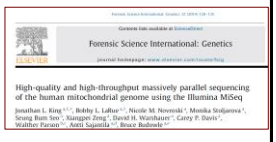
Length Heteroplasmy

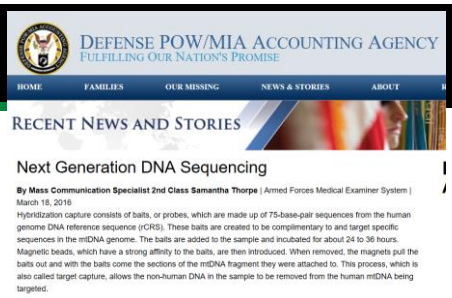
...CCACCAAACCCCCCCCTCCCCCCGCTT… 8 C's
...CCACCAAACCCCCCCCCTCCCCCCGCTT… 9 C's
...CCACCAAACCCCCCCCCCTCCCCCCGCTT… 10 C's



MPS - Population Studies Become Easy

- Sequenced 283 whole genomes on MiSeq following protocol
- Three populations – African American, Caucasian, SW Hispanics
- Multiple software
- mitoSAVE
- Haplogrep
- First upload of MPS mtDNA data to EMPOP





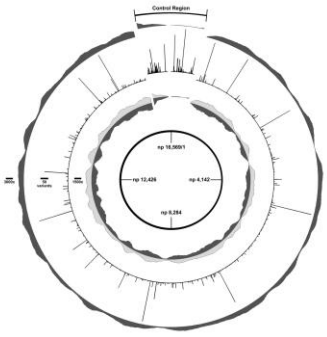
AFDIL is first to use MPS ---

Hybridization capture…allows the non-human DNA in a sample to be removed from the targeted human mtDNA

MPS on the MiSeq

Summary Representation of mtGenome Data

- Mean coverage (outer circle; n=24)
- Variants distribution(middle circle; n=283)
- Mean strand coverage - reverse (dark) and forward (light) strand (inner circle; n=24)
- Disproportionally low coverage observed in HVII is likely an artifact of alignment to a linear reference



Variation Across the mtGenome
(n=283)

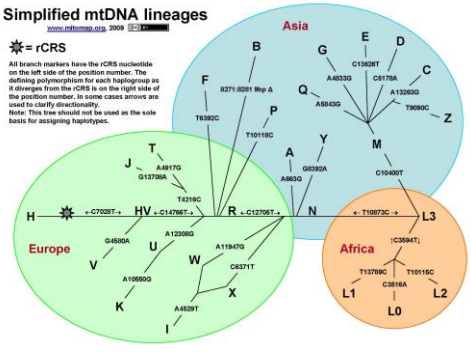
- 11,607 variants
  - defined in relation to the rCRS
- Polymorphism density clustered in HVI/HVII
  - 2,938 of the variants (25.3%)
- ~75% of variation in coding region
- Increase the value of mtDNA

Comparison of Haplogroups and Haplotypes Generated by HVI/HVII and Genome Sequence Data

	HVI/HVII			mtGenome		
	AFA	CAU	HIS	AFA	CAU	HIS
Number of Individuals	87	83	113	87	83	113
Unique Haplogroups	55	70	56	70	79	70
Unique Haplotypes	76	77	96	85	83	111

Assigned by Haplogrep and Phylotree

Phylogenetics

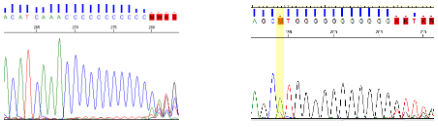


Clade Assignment
mtGenome vs HVI/HVII

Sample	HVI/HVII		mtGenome	
	HaploGroup Assignment	Quality %	HaploGroup Assignment	Quality %
USA_TX_0028	N11a	80.3	L2a1c3	93.1
USA_TX_0052	M73'79	95.1	L3b1a+!16124	95.1
USA_TX_0057	G3	89.3	L3b1a7	97.2
USA_TX_0063	N2	95.2	L3e1f	95.1
USA_TX_0108	HV0	93.9	V2	95.4
USA_TX_0132	R0+16189	87.7	H4a1a1a1a1	97.8
USA_TX_0174	M33c	83.6	A2+64	90.3
USA_TX_0175	D4e1	82.8	A2+64	91.8
USA_TX_0257	P5	95.9	H32	92.5

Assigned by Haplogrep and Phylotree

Length Heteroplasmy
Tissue Sample 400



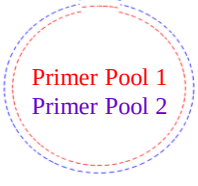
Sanger sequencing of homopolymer C stretch (forward and reverse strands)

rCRS	A	A	A	A	C	C	C	C	C	T	C	C	C	C	
Length type I	A	A	A	C	C	C	C	C	C	C	C	C	C		
Length type II	A	A	A	C	C	C	C	C	C	C	C	C	C	C	
Length type III	A	A	A	A	C	C	C	C	C	C	C	C	C	C	C
Length type IV	A	A	A	C	C	C	C	C	C	C	C	C	C	C	C

Length heteroplasmy sequences detected using MPS

Mitochondrial Genome Panel

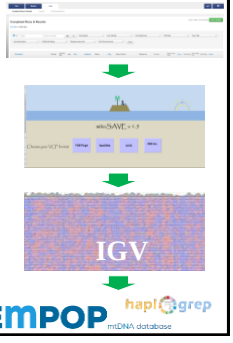
- Multiplex short amplicon system
  - Applied Biosystems Precision ID mtDNA Whole Genome Panel
- Spans entire mitochondrial genome
  - Two multiplex panels
  - Each panel contains 81 primer pairs (plus degenerate primers)
  - Tiled, overlapping pattern
  - Amplicons are ≤ 175 bps in length



Bioinformatic Pipeline

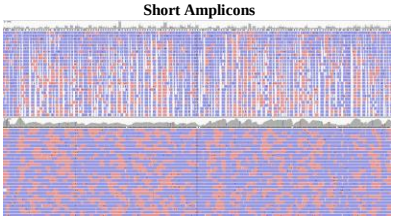
- Raw data analyzed with Ion Torrent Suite Software
  - Downloaded BAM and BAI files
  - Variant calls made with variantCaller plugin; downloaded VCF files
- mitoSAVE
  - Nomenclature
- Manual curation in IGV
  - Phase information
- Verify haplotypes in Haplogrep and Empop
  - Phylogenetic check

Evaluate concordance, read depth, strand balance, and noise



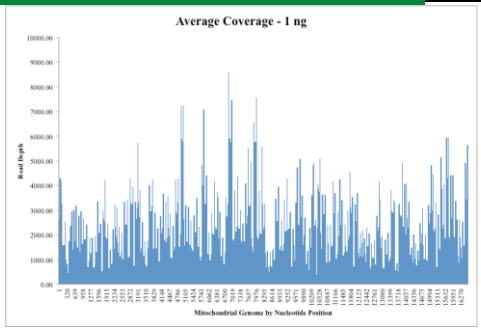
Concordance

- 108 reference samples sequenced
- Haplotype calls concordant with previous data
  - Long PCR on PGM[1] and MiSeq[2]



[1] Churchill et al 2016
[2] King et al. 2014

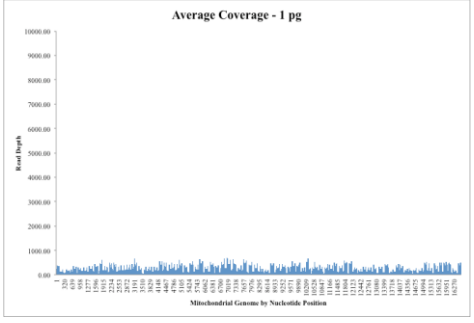
Sensitivity Study



N=3

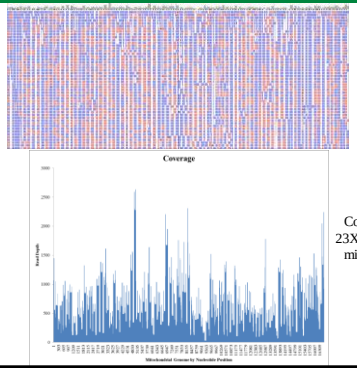
Ranged from 286X to 8,574X

Sensitivity Study



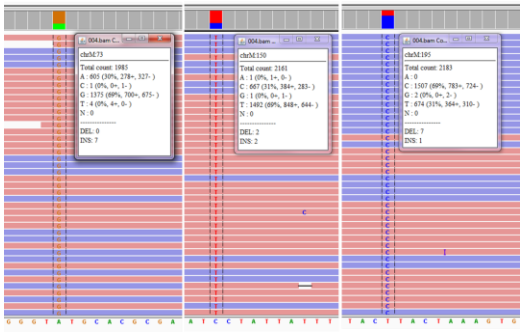
Ranged from 32X to 683X

Hair Shaft

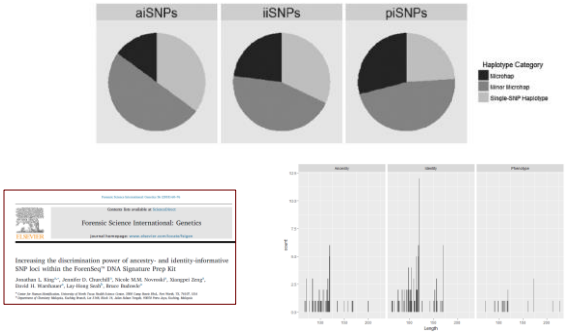


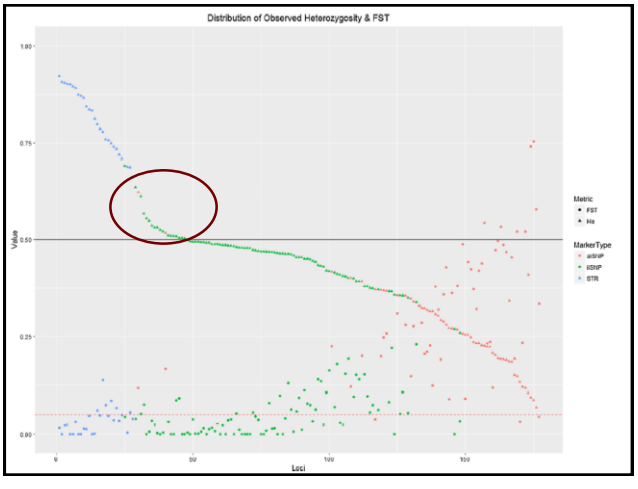
Coverage ranged from 23X to 2,634X across the mitochondrial genome

Mixtures

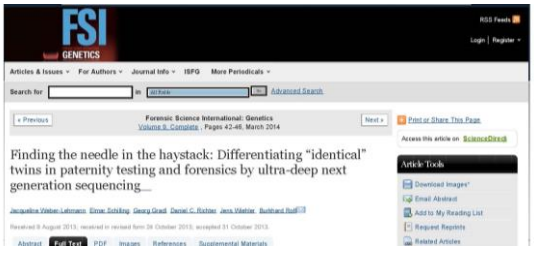


Microhaplotypes

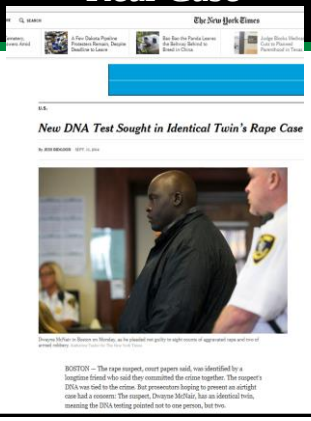




Identical Twins



Real Case



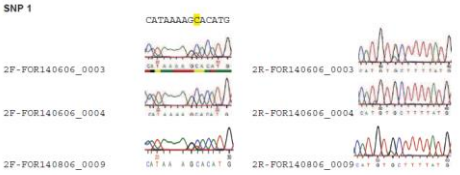
The New York Times

New DNA Test Sought in Identical Twin's Rape Case

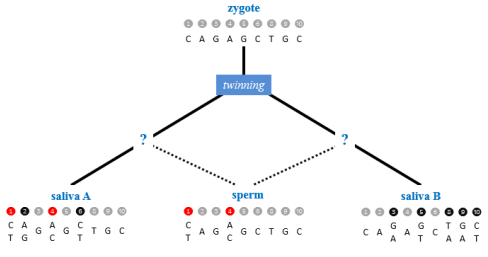
BOSTON -- The rape suspect, court papers said, was identified by a longtime friend who said they committed the crime together. The suspect's DNA was tied to the crime. But prosecutors hoping to present an airtight case had a concern: The suspect, Dwayne McNair, has an identical twin, meaning the DNA testing pointed not to one person, but two.

MPS and Sanger Sequencing

SNP1								
			C		T			
			fwd	rvs	fwd	rvs		
chr2	50,732,108	C/T	36	33	27	20	40.52%	TWIN A
chr2	50,732,108	C	27	50	0	0	0%	TWIN B



no.	zygote	saliva A	sperm	saliva B
1	C	C/T	C/T	C
2	A	A/G	A	A
3	G	G	G	G/A
4	A	A/C	A/C	A
5	G	G	G	G/T
6	C	C/T	C	C
8	T	T	T	T/A
9	G	G	G	G/A
10	C	C	C	C/T



Genetic Genealogy

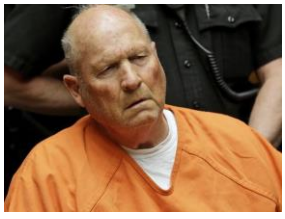
How DNA led to the elusive 'Golden State Killer'

April 27, 2018

https://phys.org/news/2018-04-dna-elusive-golden-state-killer.html

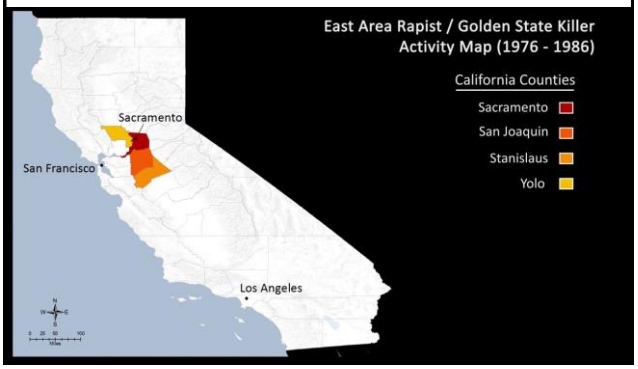
Genetic Genealogy

Joseph James DeAngelo
One of the most notorious serial killers in California



Years active 1974 – 1986

East Area Rapist (N Cal) 1976-79



The Original Night Stalker (S Cal) 1979 - 1986

DNA testing in 2001 connects EAR to ONS "Golden State Killer"

120+ Burglaries
50+ Sexual Assaults
12+ Homicides

NEWS

Former California cop arrested as Golden State Killer

By Natalie O'Neill

April 25, 2018 | 5:20pm | Updated

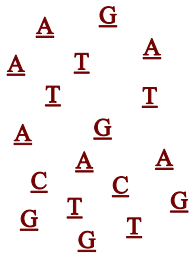
Joseph James DeAngelo

https://nypost.com/2018/06/01/cops-got-dna-of-suspected-golden-state-killer-from-tissue/

SNPs vs. STRs

- SNPs are mostly bi-allelic (e.g., A or T)
  - Require more SNPs for identification
  - ~3 SNPs = 1 STR
- SNPs have very low mutation rates compared to STRs
  - Great for kinship and genealogy
- SNPs have fewer artifacts than STR data
- SNPs can provide ancestry and phenotypic (outward appearance) information
- Technology allows for typing hundreds of thousands of SNPs

SNPs for Identification



ACTTACCGTTCCTGAAGG

ATTTACCGCTCCTGAGGG

ATTTACCGTTCCTGAAGG

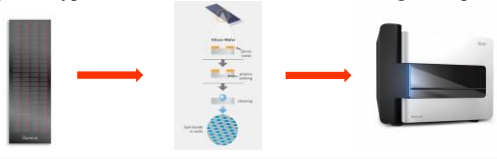
ACTTACCGTTCCTGAGGG

~60 'random' SNPs = Full STR Profile

SNPs in close proximity = Genealogy

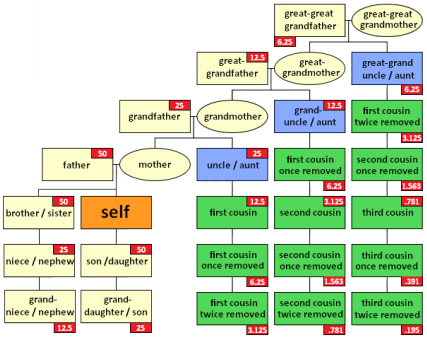
SNP Typing

- MPS can generate hundreds of thousands to millions of SNPs for a single sample
- Arrays can type 600,000 – 1,000,000 SNPs for a single sample

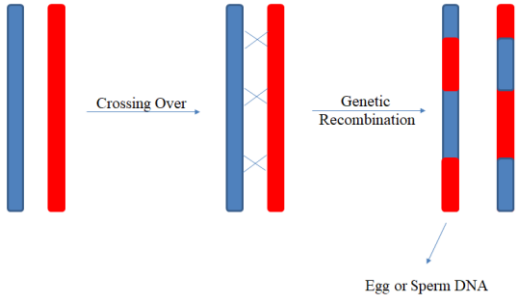


Company	23andMe	Family Tree DNA's Family Finder test	Ancestry.com's AncestryDNA test
Price (as of 11 January 2018)	$199 for the lifetime of the platform in the U.S.	$79 for the lifetime of the platform	$69 in the U.S.
SNP chip used for testing	Customized Illumina GSA chip	Illumina OmniExpress	Customized Illumina chip
Number of autosomal SNPs tested	630,132	about 690,000	637,639
Number of people in the database (as of 5 May 2018)	**5,000,000**	**About 850,000**	**over 9,000,000**

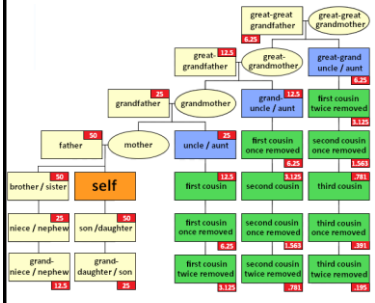
Relationships
(with %DNA Shared)



Genetic Recombination



Genetic Genealogy



ISOGG wiki statistics:

Parent/child: 3539-3748 cMs

1st cousins: 548-1034 cMs

1st cousins 1R: 248-638 cMs

2nd cousins: 101-378 cMs

2nd cousins 2R: 43-191 cMs

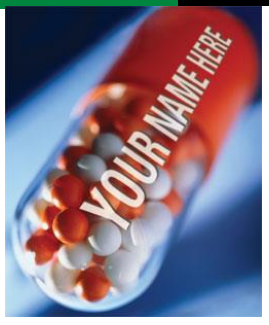
3rd cousins: 43-ca 150 cMs

3rd cousins 1R: 11.5-99 cMs

More distant cousins: 5-ca 50 cMs

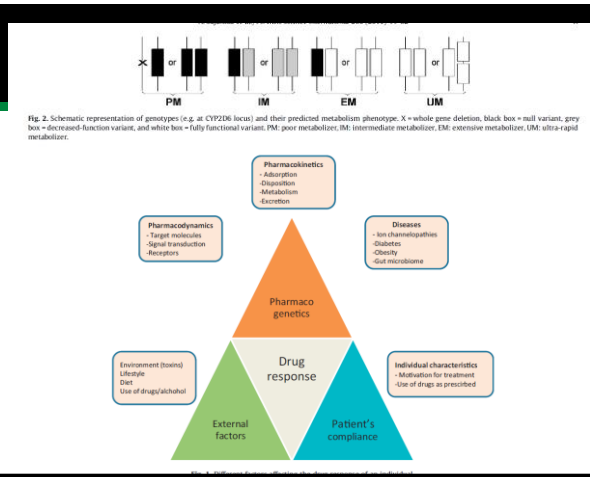
What is Pharmacogenomics?

- Also referred to as "Personalized Medicine"
- Melding of classical pharmacology + human genetics
- Using a patient's genotype to optimize drug therapy and minimizing toxicity



Pharmacogenentics

- Ancient Greece, Egypt, Rome
  - Favism – common to Central Africa and Southwest Asia
- 1930s – Phenylthiocarbamide accident and taste blindness
- 1950s – Technology and assay development
- 1975 – Debrisoquine accident and description of cytochrome p450 mono-oxygenase



Four Different Phenotypes

- Poor metabolizer (PM)
  - No active copies
- Intermediate metabolizer (IM)
  - 1 active copy
- Extensive metabolizer (EM)
  - 2 active copies (normal)
- Ultrarapid metabolizer (UM)
  - More than 2 active copies

Negative Autopsies

- After complete forensic autopsy
  - Cause of death unknown 0.3 - 0.6 % (~1/200)
  - Manner of death unknown 3 - 6 % (~1/20)

Genetics of Death

Predisposition to Sudden Unexpected Death

Genetic variants

Developmental stage
Neonates
Infants
Juveniles
Adults
Elderly

Triggering event
Medication
Physical stress
Mental stress
Climate change
Nutritional change

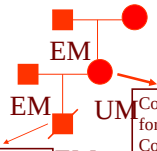
Forensic Example

- Codeine
- Infant died of morphine overdose at 13 days old
  - Mother was prescribed Tylenol #3 (acetaminophen and codeine)
  - Codeine is metabolized into morphine
  - Mother was an ultra rapid metabolizer

http://www.ilike.com/user/codeine0vr

Morphine Poisoning in a Breastfed Neonate

- Genetics
- Mother: CYP2D6*2A / *2x2 (UM)
- Grandfather, father, son: CYP2D6*1/*2 (EM)



Consistent with increased formation of morphine from Codeine and with somnolence and constipation

Clinical picture and laboratory tests are consistant with death due to morphine intoxication

poor (PM), intermediate (IM), extensive (EM), or ultrarapid (UM) metabolizer

Koren et al. The Lancet, 2006

Cytochrome p450

- Phase I metabolism enzymes
- Increase hydrophilicity of drugs and endogenous compounds
- Influence on drug
- Extensively studied
- Metabolizer phenotype

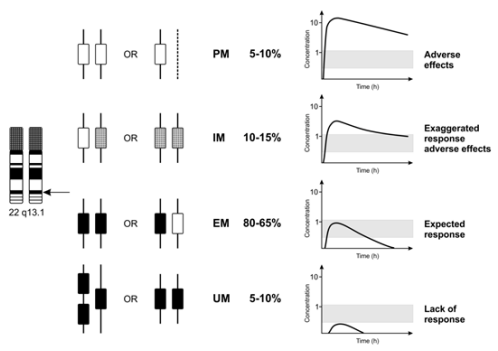
Diversity CYP450 Enzymes

- More than 50 enzymes
- CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 enzymes metabolize 90% of drugs
- Most are expressed in the liver, but can occur in the small intestine, lungs, placenta, and kidneys

CYP2D6

- The only constitutively expressed CYP
- Accounts for ~30% of marketed drug metabolism
- Implicated in accidental overdose and idiosyncratic drug response
  - PM, IM, EM, UM
- 2015:
  - >120 alleles
  - >18 full gene duplications

*CYP2D6** alleles

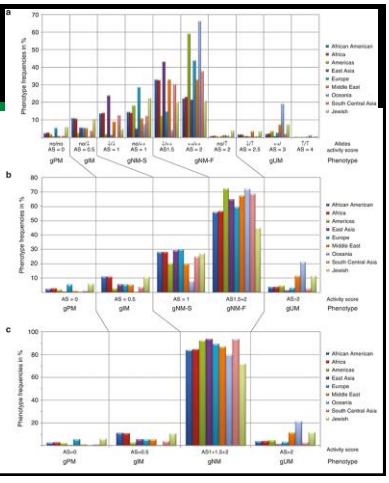


CYP2D6 Genotyping

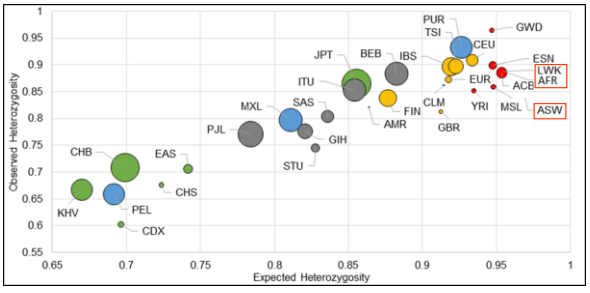
- Drug Reaction
  - Targeted massively parallel sequencing (MPS)
  - Genome wide association studies (GWAS)
  - Single nucleotide polymorphism (SNP) arrays
- No potential for discovery of new/novel variants
- MPS of whole gene provides best prediction

CYP2D6

- Recent studies reveal intra-metabolizer status variability

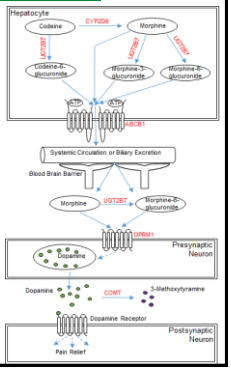


CYP2D6 Genotyping

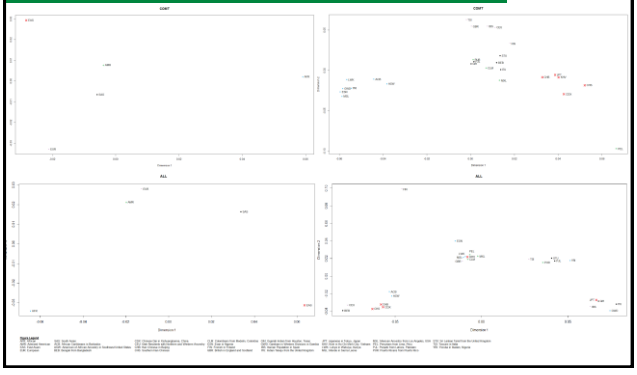


Other Enzymes in Opiate Pathway

- CYP2D6 – ~30% marketed drugs
- UGT2B7 – phase II metabolism
- ABCB1 – ATP dependent transporter p-glycoprotein
- OPRM1 – morphine and M6G receptor
- COMT – enzyme in the synaptic space



UGT2B7, ABCB1, OPRM1, COMT



Multigenic Affects

Gene	SNP	Enzyme Activity
CYP2D6	-	*1A, Wild type, considered fully functional
	rs16947, rs1135840	*2D, Normal function except when duplicated
	rs35742686, rs1135824	*3A, Nonfunctional, frameshift mutation
	rs3892097, rs28371733	*4, Nonfunctional, splicing defect
	-	*5, Nonfunctional, complete gene deletion
	rs5030655	*6, Nonfunctional, frameshift mutation
	rs5030656	*9, Partially functional
	rs1065852	*10, Partially functional
	rs28371706, rs16947	*17, Partially functional
OPRM1	rs1799971	Decreased
UGT2B7	rs7439366	Increased
	rs62298861	Increased
ABCB1	rs2229109	Unsure
	rs1128503	Decreased
	rs2032582	Decreased
	rs1045642	Decreased
COMT	rs4633	Not independently associated with activity
	rs4818	Not independently associated with activity
	rs4680	Decreased
	rs2239393	Not independently associated with activity
	rs165728	Not independently associated with activity
	rs165599	Not independently associated with activity

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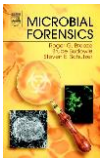
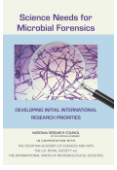
	rs165728	Not independently associated with activity
	rs165599	Not independently associated with activity

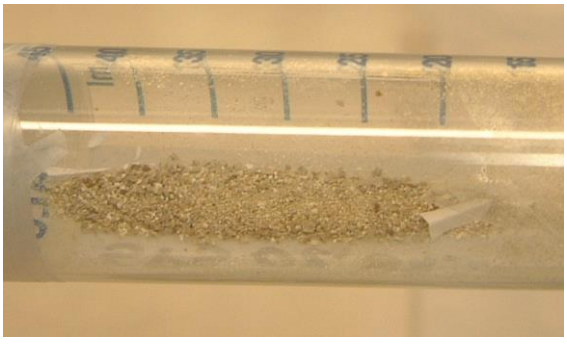
Microbial Forensics

- The use of scientific means to characterize microorganisms and their products for attribution purposes of a biological terrorist attack, biocrime, hoax, or accidental release of a biological agent.
- Now expanded due to advancements in massively parallel sequencing (MPS), metagenomics, and bioinformatics





New York Post Letter Anthrax



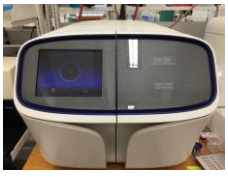
Random Shotgun Sequencing

- ABI Prism 3700 sequencer - 7 run per day 96-well blocks\
- (672 sequences)
- Applied Biosystem PRISM Big Dye™ terminator
- Cycle Sequencing Ready Reaction Kits
- Average trimmed sequence read length: 650 bp

- Applied Biosystem 3730xl sequencer - 96 capillary array - 2 hours run time (1200 sequences/day)
- 50% higher capacity than ABI 3700
- POP-7™ separation matrix
- Sequence reads up to 1,100 bases
- Integrated auto-sampler and plate stacker
- Internal bar code reader
- Automated basecalling
- Reduced reagent & DNA consumption
- Actively temperature controlled oven (18ºC–70ºC)

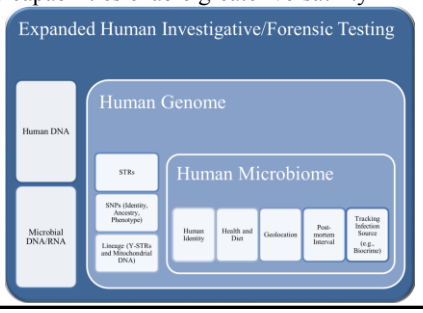
Instruments





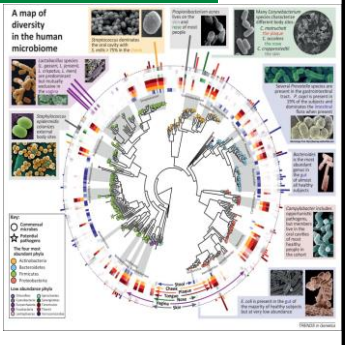

Expansion of Microbial Forensics

- Broader Definition
- Today's capabilities enable greater versatility



More Nonhuman Than Human

- ~10 microbial cells for every human cell
- ~5 million genes
- Single swab
  - ~10,000 bacteria/cm^2
- **The microbiome is a high copy number genetic marker!**



Morgan et al. *TIG*. 2013

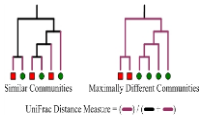
Personal Microbiomes

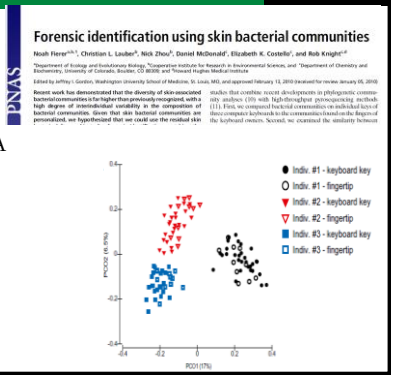
- Evidence of "personal microbiomes" has been demonstrated
- Potential forensic applications
  - 16S rRNA or WGS metagenomic sequencing
    - No species resolution, susceptible to stochastic effects
  - Mainly use of unsupervised methods to demonstrate that skin microbiome signatures from touched items associate with their donors
  - Few studies have utilized supervised methods for the purposes of classification
    - Prediction of individual identification
    - High accuracy (> 96%)
    - One time point
  - A method had yet to be described using supervised learning approaches with strain-level features stable over time intervals

https://www.sciencedaily.com/releases/2010/03/100315161718.htm
(Photo credit: Steve Miller)

Hand Microflora

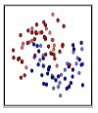
- 3 individuals
  - Pairs of samples
    - finger tips
    - touched keyboard
  - Sequence 16s rRNA



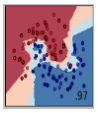


Methods of Analysis

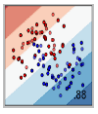
- Many metagenomic studies use *unsupervised* methods
  - These methods know nothing about class labels
    - i.e., whose metagenomic sample is whose
  - e.g., PCA, cluster analysis
- This study uses *supervised* methods.
  - Used for **prediction**
  - Allows models to find salient features that differ between individuals



Original dataset



Nearest neighbor decision boundaries

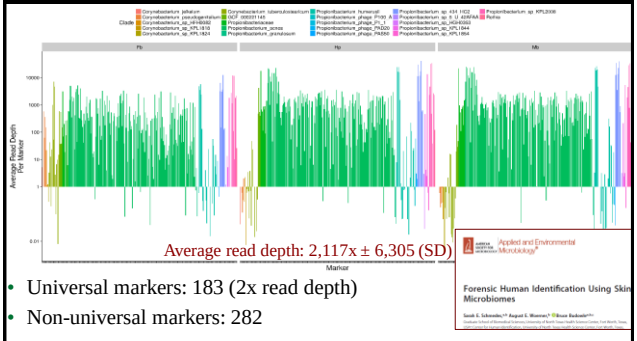


Logistic regression decision probabilities

Collect Skin Samples with 4N6FLOQSwabs® Genetics

Also see Frederike et al: Human-associated microbial populations as evidence in forensic casework. FSI Genetics (in press)

Skin Microbiome Profiling hidSkinPlex

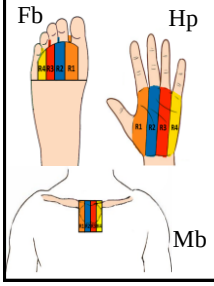


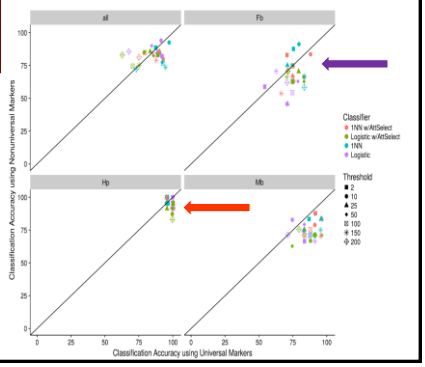
- Universal markers: 183 (2x read depth)
- Non-universal markers: 282

The average read depth at each hidSkinPlex marker present in eight individuals from the toe web/ball of the foot (Fb), palm of the non-dominant hand (Hp) and manubrium (Mb). Markers are ordered by clade then amplicon size on a log scale.

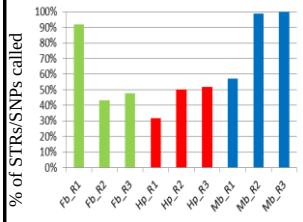
Classification Accuracy

Universal and Nonuniversal Markers



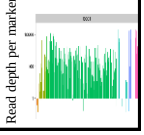


hidSkinPlex Profile and Human-Specific STR and SNP Profile



Percentage of ForenSeq STR/SNP alleles detected from skin swabs collected from subject S001 from the toe web/ball of the foot (Fb = green), palm of the non-dominant hand (Hp = red), and manubrium (Mb = blue).

- 1 skin swab:
  - Low biomass samples
  - < 0.5 pg/µL (Hp, Mb)
  - ~70 pg/µL (Fb)
- Hp
  - 32-52% alleles detected
  - hidSkinPlex classification
    - Up to 100%

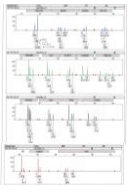


Benefits of STR/SNP/mtDNA Typing by MPS

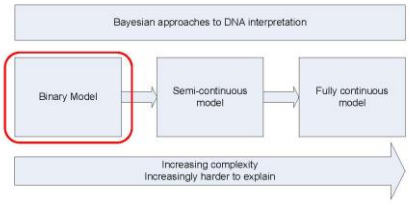
- Many Applications
- Multiplexing
- Whole mtGenome
- Mixtures
  - Better resolution/distinguish allele v stutter
- Can vary thresholds based on noise
- -A not an issue
- STR data are backward compatible
- Efficient Workflow
- Data analysis tools

Hypothesis Driven Methodologies

- Gold standard limitation is most evident in mixture interpretation
- Substantial subjectivity
- But good sign is substantial discussion
  - The real strength of the field
- Present and future issues will be in hypothesis formulation, interpretation of results, documentation and communication
  - Education and training



Models



Allows us do address uncertainty

From J Bright Presentation 2013

Allele Drop-out and Drop-in Rates

- D: the probability of drop-out of one allele of a heterozygote ($\overline{D} = 1 - D$)
  - Depends on locus and DNA quantity; from 0.0 to 0.66 have been reported
  - Can be as high as 100% in a specific case
- D_2: the homozygote drop-out probability
  - $D_2 \approx ½ D^2$;
  - $\overline{D_2} = 1 - D_2$
- C: drop in probability
  - Some include both stutter and contamination together

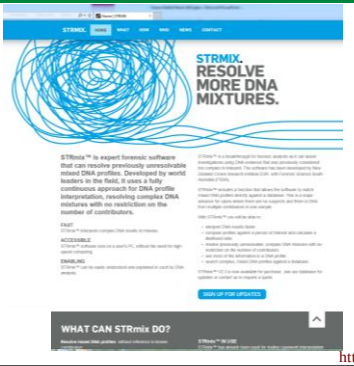
Balding and Buckleton. Interpreting low template DNA profiles. Forensic Sci Int Genet. 2009 Dec;4(1):1-10.

H_1: V + S = E

$$\Pr(ABC \mid AB, CD, H_1) = \overline{D}\,\overline{D}\,\overline{D}\,D\,\overline{C}$$

- A, B, C are not dropped out → $\overline{D}$
- D is dropped out → D
- No drop-in allele → $\overline{C}$

STRMIX



http://strmix.esr.cri.nz/

MCMC – maths

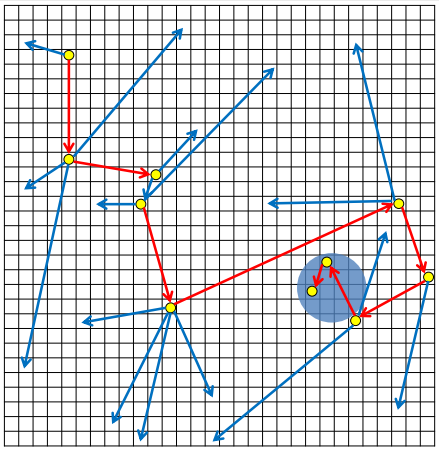
best answer

A possible answer

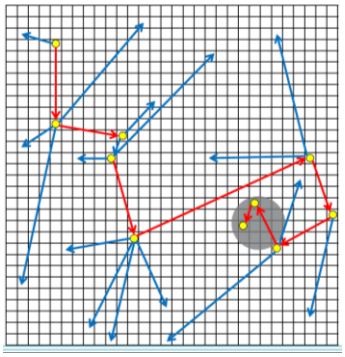
MCMC 'chain'

Hot guess

Cold guess

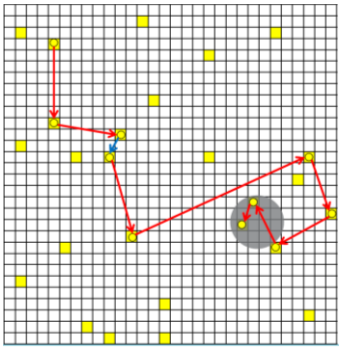


MCMC Process

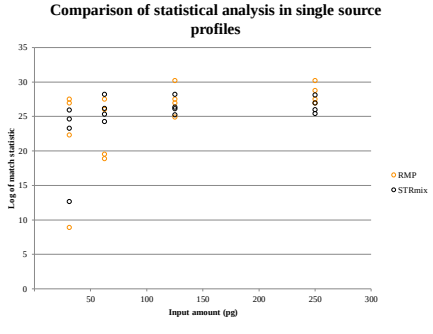


MCMC Process

- 10 MCMC moves
  - Only small fraction of all possible answers need to get to best explanation
- If ran again
  - Probably different starting points
  - Would still reach best explanation
- Can run for greater number of steps



Comparison to RMP



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