The state of Illinois has a grand tradition of corrupt politicians
(Re: Former governor Rod Blagojevich…….)

Ex-Gov. Ryan of Illinois Reports to Prison

By CATRIN EINHORN
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CHICAGO, Nov. 7 — Former Gov. George Ryan, who drew international attention for halting the death penalty in this state, reported to a federal prison in Wisconsin on Wednesday to begin his six-and-a-half-year sentence for racketeering and fraud.

Justice John Paul Stevens of the Supreme Court on Tuesday denied Mr. Ryan’s request to remain free on bail while he continued his appeal.

Mr. Ryan, 73, told reporters that he faced prison with a clear conscience.

“I have said since the beginning of this 10-year ordeal that I am innocent,” he said. “And I intend to prove that.”

Mr. Ryan, who in 40 years in public office became one of the most powerful Republicans in the Midwest, was convicted last year of a long list of corruption charges stemming from his tenure as secretary of state and governor of Illinois, including using public money for campaign work and exchanging state business for money and gifts, among them an island vacation.

Outside Illinois, he was better known for his moratorium on the death penalty and commuting more than 160 death sentences to life in prison just before leaving office after one term, in 2003. To some, Mr. Ryan’s prison term should be cautionary in a state where making deals and giving favors have long been viewed as politics as usual. He is the third former Illinois governor convicted of wrongdoing.
Gov. George Ryan of Illinois, whose state has a bad record of sentencing innocent people to death, declared a moratorium on executions a few years back. Now, in his final months in office, he is considering commuting the sentences of everyone on death row. His willingness to do so may have been tested last month, by televised hearings that underscored the horror of the crimes for which these inmates were sentenced. But despite the bad publicity, Governor Ryan should do the right thing, and commute all the sentences to life in prison.

Illinois has been at the center of the death penalty debate since it was revealed, through DNA evidence, that 13 of the people sent to its death row since capital punishment was restored in 1977 had been wrongly convicted. That's more than the 12 people who were actually executed. The co-chairman of a blue-ribbon commission appointed to study the system noted that it was unlikely that any doctor "could get it wrong over 50 percent of the time and still stay in business." In one case, a convicted murderer who had spent 16 years on death row was exonerated just two days before his scheduled execution.

The investigations into the Illinois exonerations have made it clear how a person who is innocent of a capital crime could nevertheless wind up on death row. Witnesses, from jailhouse snitches to police officers, have testified falsely. Prosecutors, whether out of incompetence or bad motives, have ignored evidence that they were trying the wrong person. Lawyers assigned to represent capital defendants were often not qualified, or failed to conduct investigations that could have cleared their clients.

Governor Ryan, a conservative Republican who voted for the death penalty as a legislator, has said repeatedly that he is considering a blanket commutation, which would reduce death sentences to life in prison. But last month's hearings, which received wide attention across the state, appear to have slowed the momentum. The testimony, much of it from families of murder victims, was often heart-wrenching. But as effective as they were as a reminder of the pain that crime causes, the hearings did not refute the fact that Illinois's use of the death penalty is tragically flawed. Governor Ryan, who has made fairness in administering the death penalty a hallmark of his governorship, will end his tenure on a high note if he takes one last stand for justice and issues a blanket commutation.
What is so compelling about the science underlying DNA fingerprinting that it can be used to overturn a conviction of an individual? Or to unequivocally convict an individual of a serious crime?

Great web sites on DNA forensics

http://www.dnai.org/d/

Innocence Project
http://www.innocenceproject.org/
• After serving 14 years in prison, a DNA fingerprinting test showed that Thomas Webb had been wrongly convicted of rape.
• To date, there have been 245 *post-conviction DNA exonerations* in the United States.
• And in almost 40 percent of the cases profiled on the Innocence Project site (link on previous page), the actual perpetrator has been identified by DNA testing.
What is so compelling about the science underlying DNA fingerprinting that it can be used to overturn a conviction of an individual? Or to unequivocally convict an individual of a serious crime?

To address this question, we need to examine

- the structure of our genome
- the nature of PCR (a molecular methodology used in fingerprinting)
- basic issues in population genetics
What can you tell me about the size and structure of the human genome?
Chromosome 11 “Flyover”
http://www.dnalc.org/ddnalc/resources/chr11.html
The human genome provides a rich source of genetic variability especially in non-coding regions (see also last page of these notes)

**Single nucleotide polymorphisms (SNP’s)**
Frequency in genome: ~1/1250 base pairs
Number per genome: ~ 2-3 million
Mutation rate per site per gamete: $1 \times 10^{-9}$
98% of genetic diversity is in this category: but SNP’s have fewer possible alleles than micro and mini satellites

**Microsatellites:** also called **STR (simple tandem repeats)**
*repeat unit:* typically 2-5 bp
10’s-100’s tandem repeats
Mutation rate per site per gamete: $1 \times 10^{-3}$
Also called: *Simple Sequence Repeats* or *Simple Tandem Repeats*
Nature 409: 888 2/15/01

**Minisatellites**
*repeat unit:* ~15-100 bp in length
10’s-1000’s of tandem repeats
Mutation rate per site per gamete: $10^{-3}$

**VNTR**= variable number of tandem repeats (old name for minisatellites)

Gene mutation rate: $10^{-4} - 10^{-5}$ per gene per gamete
An example of a microsatellite or STR polymorphism

* STRs are short sequences of DNA, normally of length 2-5 base pairs, that are repeated numerous times in a head-tail manner.
* The 16 bp sequence of "gatagatagatagata" would represent 4 head-tail copies of the tetramer "gata".
* Example: D7S280

```
1 aathtttttgta ttttttttag agacgggttt tcaccatgtt ggtcaggtgctg tctatggagtt 
61 tattttttaag gtttaatatata taaaaggtatat gatagaaacac ttgtcatagct ttagaagcga
121 ctaacgatag atagatatag atagatatag atagatatag atagatatag atagatatag atagacagat
181 tgatagtttt tttttatctc actaaatagt ctatagtaaa catttaatta ccaatatttg
241 gtgcaattct cttcaatgagg ataaatgtagg aatcgttata attcttaaga atatatattc
301 cctcttgagtct ttgtatacct cagatatttaa ggcc
```

* The polymorphisms in STRs are due to the different number of copies of the repeat element that can occur in a population of individuals.

- Why are micro and mini satellite regions so mutable?
- What mechanisms of mutation could explain this?
Polymorphisms in mini and microsatellites are used for DNA fingerprinting

- easy to assay using PCR (or restriction enzymes and Southern blots)
- highly polymorphic*
- under no obvious selection pressure -- “anonymous site”
- codominant Mendelian alleles

*Estimated mutation rate at a given mini/microsatellite site is $1 \times 10^{-3}$ /gamete
- This means 1 change in every 1000 gametes
- Results in lots of variation between unrelated individuals in a population
- But mutation rate is low enough that within a family allele changes do not occur readily

The D1S80 repeat unit is 16 base pairs (bp) in length and there are dozens of known alleles ranging from approximately 350 to 1,000 bp.

We want to determine the genotype of an individual for the D1S80 microsatellite site which is located on chromosome 1.

First: We would prepare genomic DNA from an individual

But Then:

- How would we go about determining the repeat length for this particular site?
- How would we “look” at this site without the rest of the genome interfering or obscuring our view?
Our locus of interest is about 350-1000 bp depending on the particular allele(s)

The DNA preparation that you generate from a tissue sample is a complex, heterogeneous mixture of sequences containing the entire human genome

Short sequence embedded in a background of 3 billion basepairs

In a prep of total genomic the D1S80 locus is about 1 part per 10 million!
WANT A TUBE LABELED:  
**PURIFIED DS180 sequences**

How to purify the gene sequences that you want to study?

If you were trying to purify a protein what would you do?
Can take advantage of natural systems to amplify specific DNA sequences

Use recombinant DNA techniques to generate a molecular clone of the DNA: use a cell such as *E. coli* to make lots of copies of your gene -- put it in a DNA molecule that is easy to recover in a pure form from the cell.
Amplify the gene or sequence using PCR -- an in vitro process

PCR = polymerase chain reaction

• Very sophisticated molecular technologies have developed based on our understanding of the enzymology of DNA replication, transcription and translation
• PCR is an in vitro DNA replication technology that has revolutionized basic research in molecular biology and genetics
• PCR involves exponential amplification of a specific gene or region of DNA from a complex mixture of DNA
How do we target amplification to our specific sequences of interest?

How come only the red sequence is amplified from the starting template:
Specificity of amplification is controlled by the primers added to the reaction

WHY?
**PCR animations**

http://www.youtube.com/watch?v=_YgXcJ4n-kQ  
http://www.dnalc.org/ddnalc/resources/animations.html  
http://www.dnai.org/text/mediashowcase/index2.html?id=582  
http://www.maxanim.com/genetics/PCR/PCR.htm

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**Melt** = denature DNA with heat  
**Anneal** = allow primer to hydrogen bond with complementary sequences on the template DNA  
**Replicate** = allow DNA polymerase to extend primer and synthesize complementary copy of template

- **What is temperature scale in °C?**
How to choose primers to amplify the Microsatellite site **D7S280**

**IMPORTANT TO NOTE:**
- A DNA sequence is always given in the 5’ to 3’ direction unless indicated otherwise.
- And, typically the complementary strand is not shown

```
5' 1 aatttttgta ttttttttag agacggttt tcaccatgtt ggctaggctg actatggagt
  61 tatttaaagg ttaatatatata taagggatat gatagaacac ttgtcatagtt ttagacgaa
  121 ctaacgataag atagatagat agataagatag atagatagat agataagatag atagacagat
  181 tgataagtttt tttatatcct actaaatagtt ctatagtaaa catttaatta ccaatatttg
  241 gtgcataatct gtaaatgagg ataatgtgg aatcgatattaa tttttaaga atatatatcc
  301 cctctgagtcttt gttataacct cagatattaaa ggcc 3'
```

Choose left and right primers that match unique (single-copy) sequences that flank the repeat units

**How to choose primers:**

**Choosing primers**
Because DNA polymerase can add subunits (nucleotides) only to the 3’ end of the primer, the primer has to be situated “upstream” — i.e., more 5’ than — the sequence to be copied:

If this segment is to be copied...

...the primers have to be chosen from this sequence...

5’-CTAGATATGAAACCTATATAGCTACGCTGGCCATTCTATGTCGGACACTACCTACAGAA-3'
3’-GATCTTTRATCTTTGATATCCATGCGACCCTGAGATACAGACTGGGCGATGATGAGATGCTT-5'...and this sequence
What do you do with your PCR product once you make it?

How can you determine the genotype of the DS180 locus using your PCR product?
The D1S80 repeat unit is 16 base pairs (bp) in length and there are dozens of known alleles ranging from approximately 350 to 1,000 bp.

Alleles are distinguished by the size of PCR products generated with primers that match unique (single-copy) sequences that flank the repeat units.
Must use more highly resolving gels to legitimize inferences about band sizes

**D1S80 STR site**
PCR based analysis of 6 individuals (C and 1-5)
L = size standards

Which individuals are heterozygous at the D1S80 STR site?

AmpliFLP™ D1S80 PCR Amplification Kit

The D1S80 repeat unit is 16 base pairs (bp) in length and alleles range from approximately 350 to 1,000 bp.
STR loci used for DNA fingerprinting by the FBI

CODIS= Combined DNA Index System

- typically 13 core CODIS loci plus Amelogenin to determine sex of individual
- Amelogenin is found on both the X & the Y chromosomes. But, the Y linked copy results in a PCR product 6 bps longer than the X linked copy

13 CODIS Core STR Loci with Chromosomal Positions
How are the data produced, analysed and displayed in a DNA fingerprinting lab?

Three or four different polymorphisms are labeled with each of four fluorescent dyes.

- **BLUE:** D8S1179, D21S11, D7S820, CSF1PO
- **GREEN:** D31358, TH01, D13S317, D16S539
- **RED:** SEX, D5S818, FGA, D18S51
- **YELLOW:** vWA, TPOX, D18S51

http://www.dnai.org/d/
http://www.dnai.org/d/

*Note: dye color systems will vary from profile to profile –*
- Multiplex STR (simple tandem repeat) profile including X and Y specific products as analyzed by capillary gel electrophoresis
- Multiplex means that a single PCR reaction is performed with more than one set of primers -- 11 primer pairs in this case
- Numbers below STR peaks indicate allele sizes in repeat units.
- The primer pairs are tagged with one of three fluorescent dyes - yellow, blue or green
- The STR profile is displayed in the green, blue and yellow channels of a four-color fluorescent system with the red channel being used for size markers (not shown).
- Standard number of PCR cycles used is 28. 34 cycles are used when little DNA is available: typically <100 pg or <17 diploid genomes!
DNA fingerprint: the multi-locus* pattern produced by the detection of genotype at a group of unlinked, highly polymorphic loci

Comparing two DNA fingerprints to determine if they represent the same person:

**Exclusion:** if the patterns do not match at every micro/minisatellite locus tested, then the DNA must have come from different individuals

**Inclusion:** if the pattern of bands match at every locus, then the DNA may have come from the same source
**Exclusion:** if the patterns do not match at every micro/minisatellite locus tested, then the DNA must have come from different individuals

http://www.dnai.org/d/
click on Innocence project
**Inclusion:** if the pattern of bands match at every locus, then the DNA *may have* come from the same source

Are the data sufficient to conclude identity between the suspect and the forensic sample?

If matches appear in multiple tests*, a statistical conclusion can be reached by calculating the probability of a chance match.

*What sort of information do we need to calculate the probability of such a random match?
A fundamental measure used in population genetics is called *allele frequency*:

Allele frequency:
- the measure of commonness of an allele in a population
- the proportion of all alleles that are of a specific type
How many individuals in a given population carry these specific alleles: in other words, what is the overall frequency of such a genotype?

For loci that are assorting independently, the overall probability of the genotype is the product of the frequency in the population of each individual genotype.

If the DNA fingerprint exhibits a series of rare alleles, a chance match is much less likely than if the alleles are common in the population.
Let's first look at D8S1179. The table below shows some of the allele frequencies for D8S1179 in different populations. The frequencies vary for different groups.

<table>
<thead>
<tr>
<th>D8S1179 (Alleles)</th>
<th>Asians (N = 196)</th>
<th>African American (N = 210)</th>
<th>Caucasians (N = 203)</th>
<th>Hispanic (N = 209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.0024</td>
<td>0.0056</td>
<td>0.0102</td>
<td>0.0025</td>
</tr>
<tr>
<td>10</td>
<td>0.0119</td>
<td>0.0250</td>
<td>0.1020</td>
<td>0.0936</td>
</tr>
<tr>
<td>11</td>
<td>0.1214</td>
<td>0.0361</td>
<td>0.0587</td>
<td>0.0616</td>
</tr>
<tr>
<td>12</td>
<td>0.2905</td>
<td>0.1083</td>
<td>0.1454</td>
<td>0.1207</td>
</tr>
<tr>
<td>13</td>
<td>0.3071</td>
<td>0.2222</td>
<td>0.3393</td>
<td>0.3251</td>
</tr>
<tr>
<td>14</td>
<td>0.2000</td>
<td>0.3333</td>
<td>0.2015</td>
<td>0.2463</td>
</tr>
<tr>
<td>15</td>
<td>0.0548</td>
<td>0.2139</td>
<td>0.1097</td>
<td>0.1158</td>
</tr>
<tr>
<td>16</td>
<td>0.0048</td>
<td>0.0444</td>
<td>0.0128</td>
<td>0.0246</td>
</tr>
<tr>
<td>17</td>
<td>0.0048</td>
<td>0.0083</td>
<td>0.0026</td>
<td>0.0074</td>
</tr>
</tbody>
</table>


**How can we calculate allele frequency from genotype frequency?**
Again, using the Hardy-Weinberg equation, let's calculate the frequency of a Caucasian being homozygous for D7S820 for allele 9.

<table>
<thead>
<tr>
<th>D7S820 (Alleles)</th>
<th>Asians (N = 196)</th>
<th>African American (N = 180)</th>
<th>Caucasians (N = 196)</th>
<th>Hispanic (N = 203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0025</td>
<td>0.0024</td>
</tr>
<tr>
<td>7</td>
<td>0.0102</td>
<td>0.0071</td>
<td>0.0172</td>
<td>0.0215</td>
</tr>
<tr>
<td>8</td>
<td>0.1684</td>
<td>0.1738</td>
<td>0.1626</td>
<td>0.0981</td>
</tr>
<tr>
<td>9</td>
<td>0.0816</td>
<td>0.1571</td>
<td>0.1478</td>
<td>0.0478</td>
</tr>
<tr>
<td>10</td>
<td>0.2168</td>
<td>0.3364</td>
<td>0.2906</td>
<td>0.3062</td>
</tr>
<tr>
<td>11</td>
<td>0.3138</td>
<td>0.2238</td>
<td>0.2020</td>
<td>0.2895</td>
</tr>
<tr>
<td>12</td>
<td>0.1760</td>
<td>0.0905</td>
<td>0.1404</td>
<td>0.1914</td>
</tr>
<tr>
<td>13</td>
<td>0.0255</td>
<td>0.0190</td>
<td>0.0296</td>
<td>0.0383</td>
</tr>
<tr>
<td>14</td>
<td>0.0077</td>
<td>0.0048</td>
<td>0.0074</td>
<td>0.0048</td>
</tr>
</tbody>
</table>
• Hardy Weinberg equation can be used to calculate genotype frequencies from allele frequencies (and vice versa)
• It is valid for genes or loci that have multiple alleles

<table>
<thead>
<tr>
<th>Micro/minisatellite Locus</th>
<th>Genotype ( f_{a_x}/f_{a_y} )*</th>
<th>Frequency of genotype (HW)</th>
<th>Combined frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 such as DS123</td>
<td>het for 2 different alleles: 0.08/0.02</td>
<td></td>
<td>-------</td>
</tr>
<tr>
<td>2 such as DS456</td>
<td>het for 2 different alleles: 0.15/0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 such as DS789</td>
<td>homozygous ( f_a = 0.09 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each locus
\( f_{a_x} = \text{frequency of allele } X \quad f_{a_y} = \text{frequency of allele } Y \)

LOCUS = site on a chromosome
• These calculations appear deceptively trivial, but they are not.
• Initially there was tremendous contention and disagreement among population geneticists about how these calculations should be done
• They are only valid and useful to determine the probability of a chance match if there is reliable information on allele frequency and if the population database used is appropriate

**Consider the case of Josiah Sutton**

In 1999 Josiah Sutton, then 16 years old, was sentenced to 25 years in prison for rape. The evidence seemed airtight: the victim had spotted Sutton walking down a Houston street five days after her attack, and crime lab analysis from Houston's police department showed his DNA was an exact match with semen from the crime.

The DNA evidence was convincing: the analyst testified that the probability of a chance match was 1 in 694,000.

But a later analysis showed that one in 8 black men would have had a similar match.
Validity of conclusions drawn from DNA fingerprinting test depends on
• the number of loci tested
• the number of possible allele variations at each site examined
• the integrity of the population database used to determine allele frequencies -- is it large enough and does it reflect differences in ethnic and racial groups?
• The quality of the laboratory work

DNA evidence must always be considered within the framework of other evidence of many types
Population frequency distribution of alleles at the VNTR locus D14S13 in North American black and white populations. Comparison of the relative frequencies of the 1.4 kb allele illustrates the importance of accounting for inter-population differences: the frequency of that allele is approximately 5 times greater in the white population than in the black population.
Figure 1 | Sources of human genetic variation used in forensic analysis. Further details of the properties of different loci can be found in the text. Heteroplasmy describes the presence of two or more different mitochondrial DNA sequences in the same cell, or individual. FBI CODIS, US Federal Bureau of Investigation Combined DNA Index System; HVS, hypervariable site; Mb, megabase; mtDNA, mitochondrial DNA; SGM, second generation multiplex; STR, short tandem repeat.