DNA analysis and the identification of the human remains at Oaklawn cemetery, a possible strategy.

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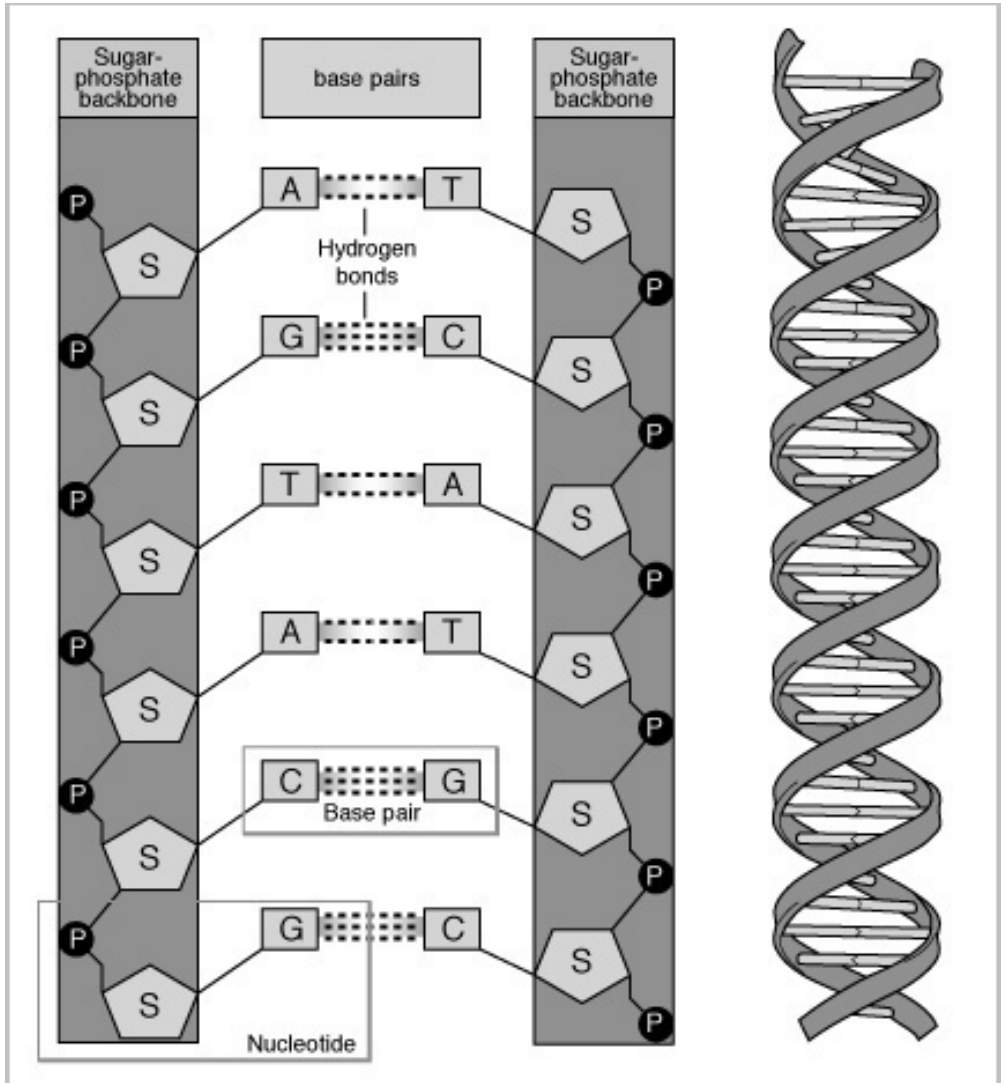
First, a little about me…

- A Tulsa native (except for 5 years spent in Venezuela).
- Educated in Tulsa public schools and University of Tulsa.
- Received my PhD from Purdue University in West Lafayette, IN followed by post graduate training in La Jolla, CA
- Worked for the American Red Cross in St. Louis where I began my career in DNA-based identification (mid-1980s). Did parentage testing and forensic DNA analysis for St. Louis County courts and police department.
- Moved back to Tulsa in 1992 and joined scientific staff at Children's Medical Center (H.A. Chapman Institute of Medical Genetics).
- Performed some early DNA testing for law enforcement in Tulsa.
- Moved to the School of Forensic Sciences at OSU-CHS in 2001 and developed a training program for forensic DNA analysts.
- Have considerable experience in identifying human remains through kinship testing.

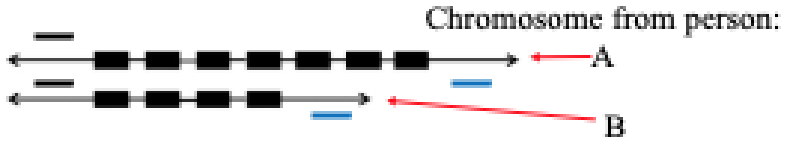
The structure of DNA



DNA exists in the form of a double helical structure composed of simple chemical building blocks called nucleotides (A,C,G, and T) that associate in the middle of the helix to hold it together. The majority of the nucleotides are identical in DNA from two different individuals. It is estimated that only 0.6% of the human genome varies among individuals.

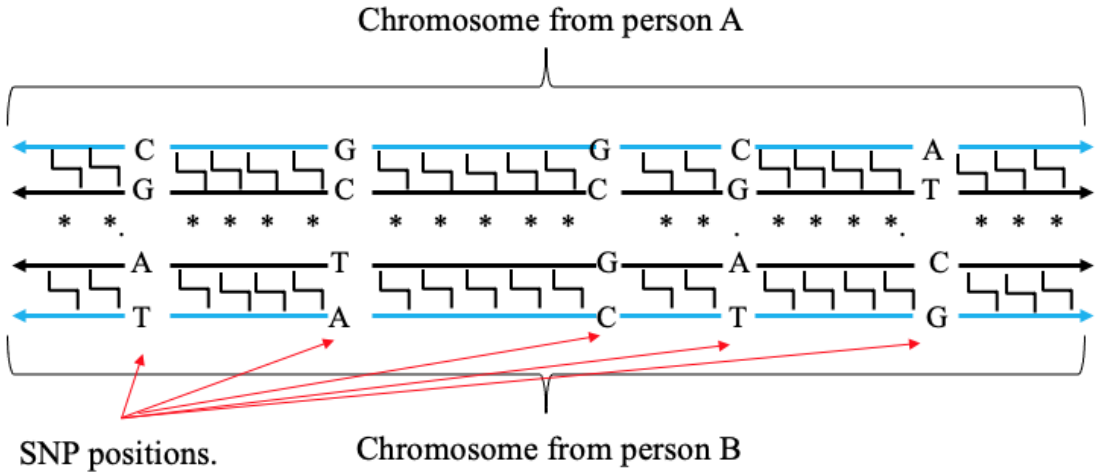
Variability in human genomic DNA can come in two forms relevant to our task.

- Genetic markers consisting of tandem repeats (known as STR markers).



This type of genetic marker is often used for parentage testing or criminal investigations.

- Genetic markers consisting of single nucleotide substitutions (SNPs).



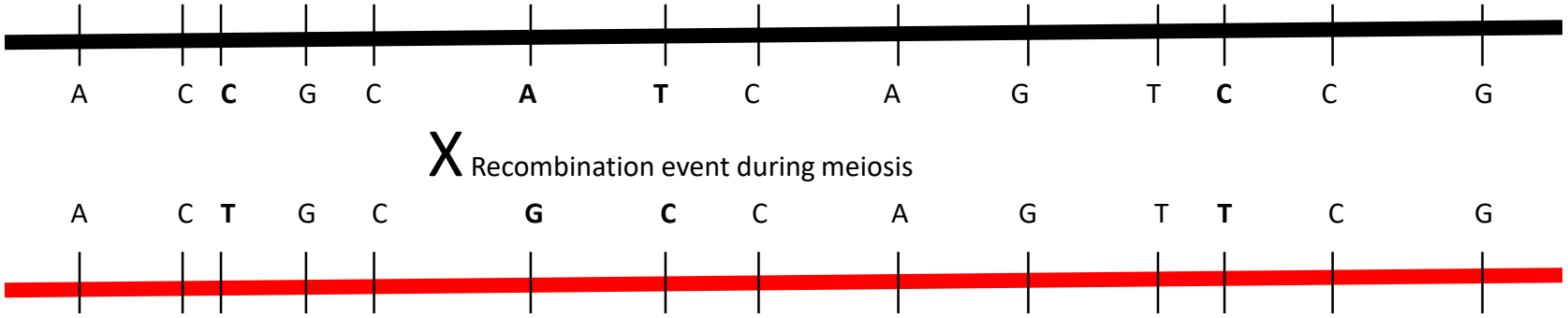
This type of genetic marker is often used to predict ethnic background, physical traits (eye color, hair color, etc.), and distant familial relationships.

Both types of genetic marker exist widely in nature.

Everyone’s DNA is different, but members of the same family will share portions of their DNA (i.e., pieces of their chromosomes) depending upon the relationship.

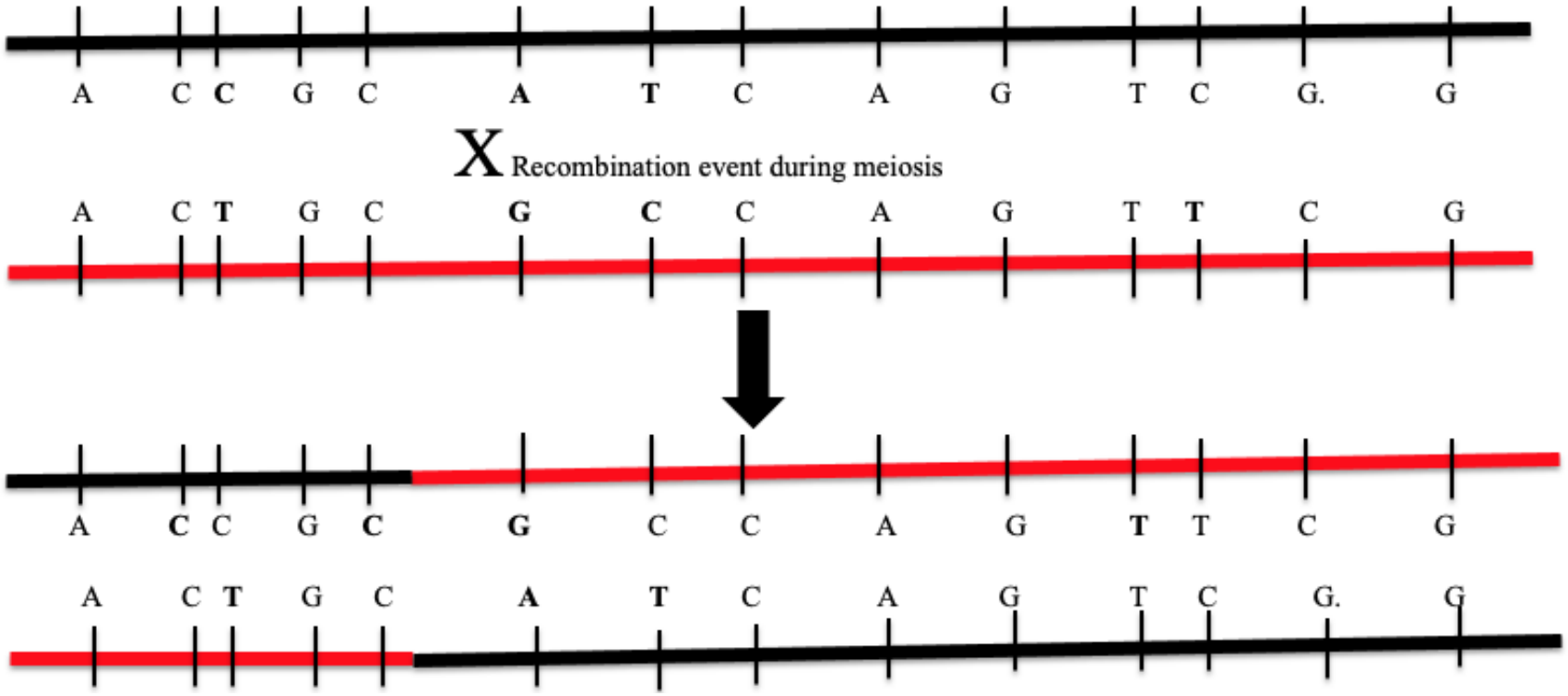
SNPs are abundant in human DNA.

- 4-5 million SNPs scattered over ~3 billion nucleotides of sequence (22 pairs of autosomes, XX/Y, and ~500 mitochondria/cell).
- There is a lot of shuffling of SNPs in the genome during the process of forming reproductive cells.
- Even with the shuffling, the sequence of SNP genotypes on a piece of chromosome (known as a haplotype) can be unchanging within a large family pedigree and therefore can be considered “family DNA”.
- As the family traces back to a common set of parents, the amount of family DNA shared between two individuals within the pedigree will increase.



Consider that this is a diagram of a pair of chromosomes in the nucleus of a cell that will give rise to sperm or egg.

The result of a recombination event is the shuffling of pieces of a chromosome.

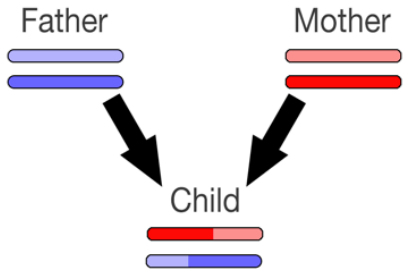


The recombination alters the sequence of SNPs in the chromosome (known as the “SNP haplotype”. Regions of the chromosome that did not recombine retain the SNP haplotype “signature” for the DNA shared within the family.

The shuffling of “family DNA” in developing reproductive cells.

Chromosome Inheritance

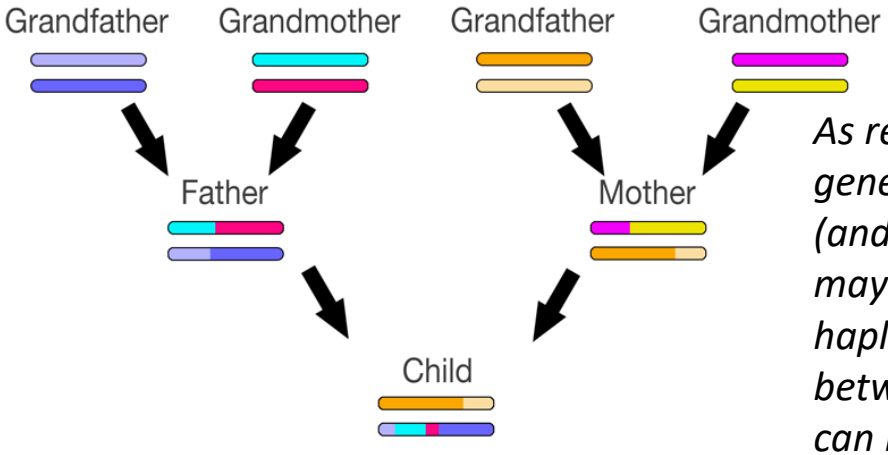
One autosomal chromosome from each pair comes from your mother and the other comes from your father. This means you get half of your DNA from your mother and half from your father. Each chromosome they pass on to you is a combination of their own pair of chromosomes which they got from their parents (your grandparents).



Through recombination, it is possible to transmit chromosomes that differ between parent and child

The image above depicts how one pair of chromosomes may be passed from your parents to you. The colors don't mean anything special - they simply depict the individual chromosomes and chromosome sections.

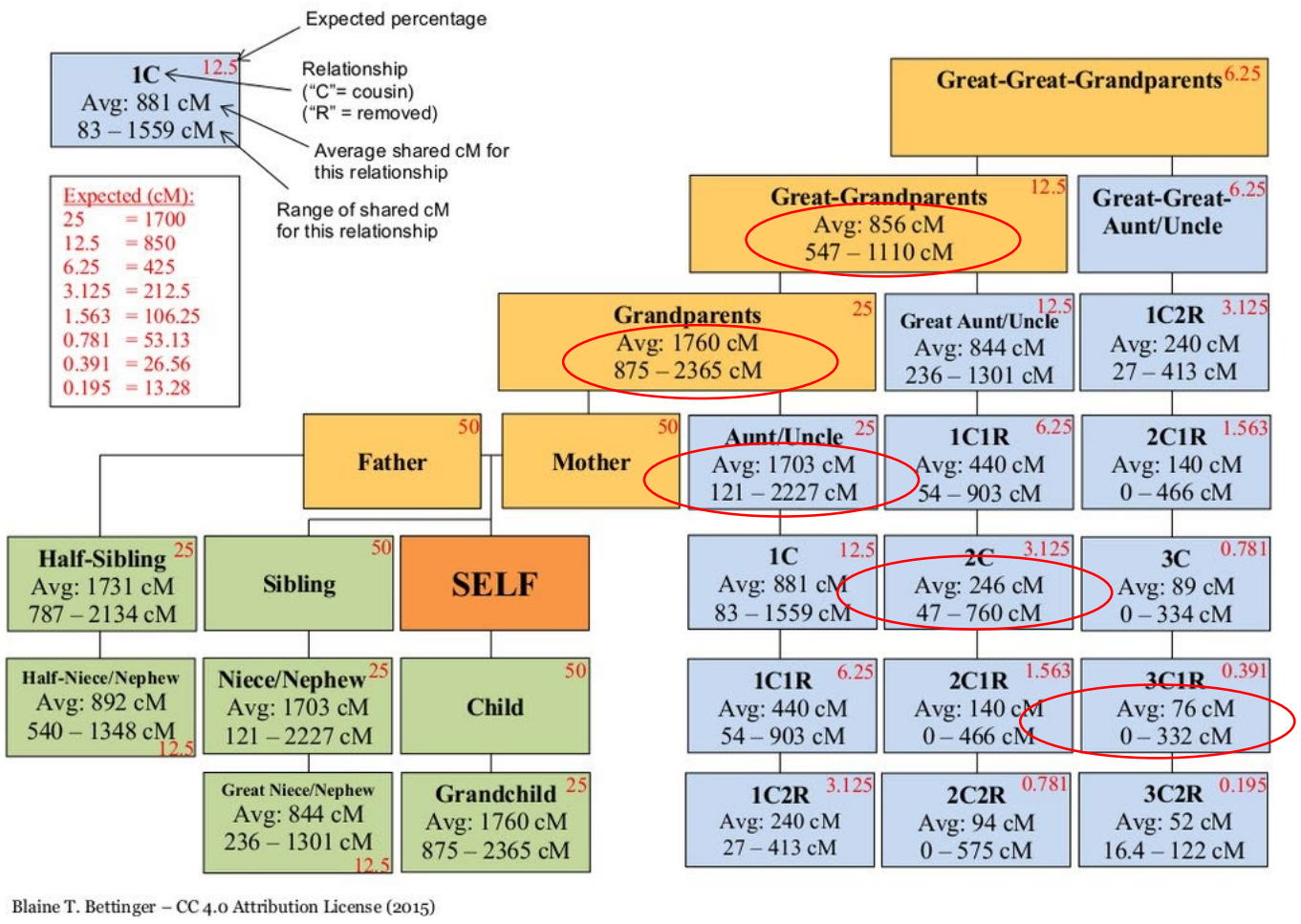
You'll notice that the chromosome passed to you from each parent may not be an exact 50/50 combination of their own chromosomes. This means that you might have a bigger portion of one of their chromosomes than the other - you might be more related to one of your grandparents than another on that chromosome. In fact, you might have an exact copy of one of your parent's chromosomes, and thus you'll get no portion of their other chromosome.



As recombination occurs through the generations, the amount of shared chromosome (and SNP haplotypes) between the generations may decrease continuously. By identifying SNP haplotypes that are shared, relationships between distant parts of an extended pedigree can be suggested.

Figure taken from: https://smithplanet.com/stuff/gedmatch.htm

Expectations for sharing of family DNA within a pedigree.



A centimorgan (cM) is indirectly related to the length of a piece of chromosome, generally considered to be 1,000,000 nucleotides.

Notice how the amount of sharing decreases as relationships become more distant.

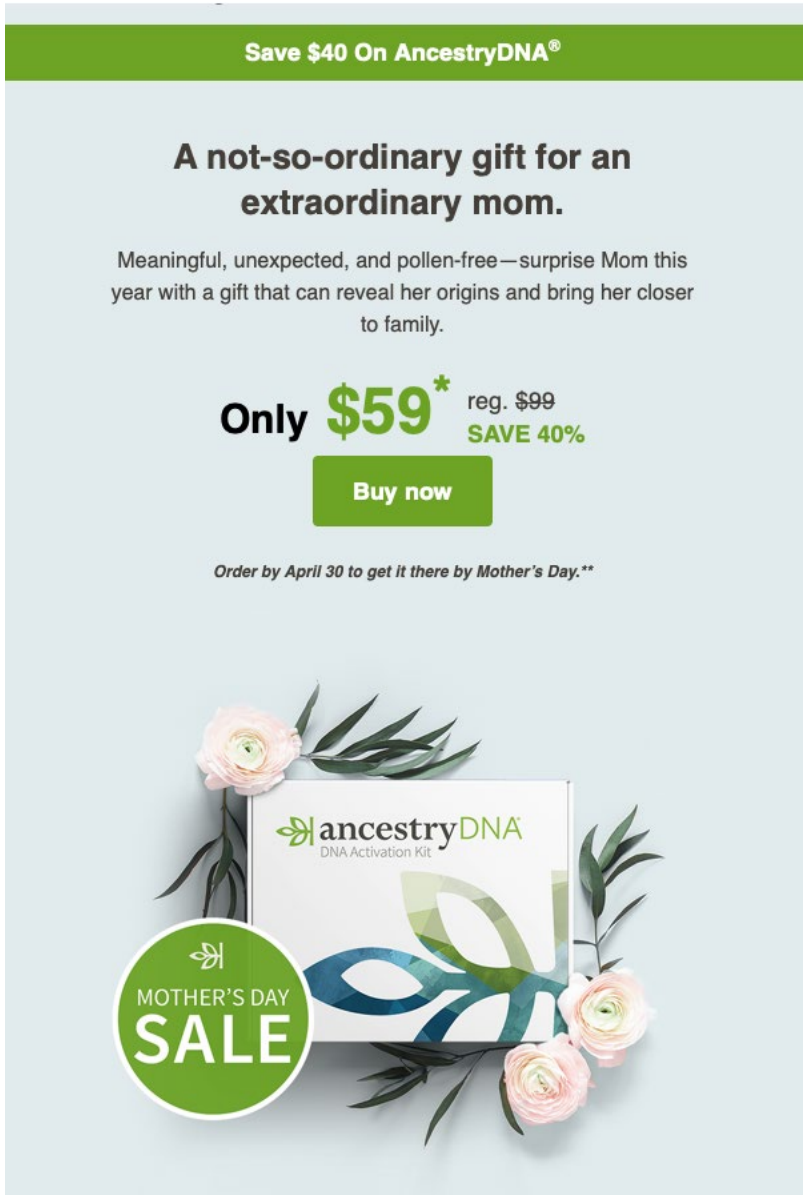
The first mass application of SNP genotyping, searching one's roots.

23andMe

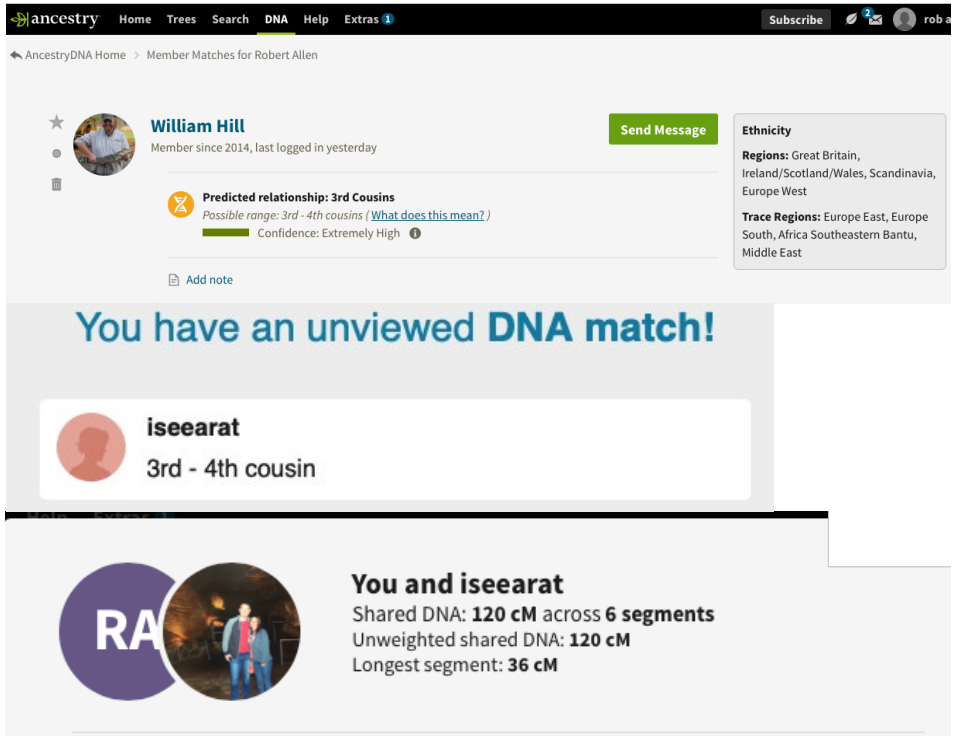
Biotechnology company

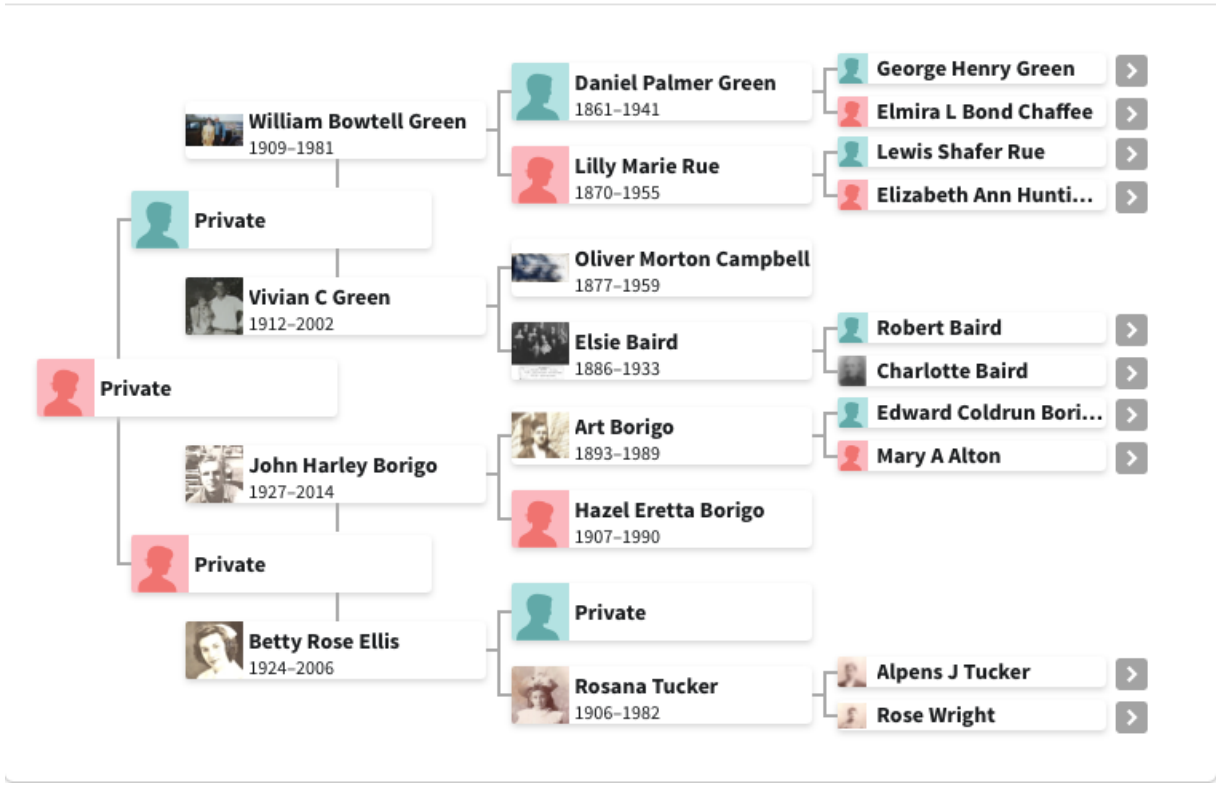
The direct-to-consumer DNA testing industry produces millions of SNP profiles every year. Their service includes searching their database looking for possible kinship relationships among voluntarily submitted samples. If your sample has an above average degree of chromosome sharing with another in their databank, you will be notified and have the option of contacting that person to perhaps extend both family trees.

An ad from Ancestry.com from yesterday..



Some results on me (from Ancestry)





The family tree recorded in Ancestry for Iseearat's family pedigree.

Because this technology is so powerful and has shown success it detecting distant family relationships, I believe our strategy should begin with the “Ancestry.com” process. There are options for contract testing, and I believe some companies might be very interested in this project.

What could test results give us to advance our cause?

1. A prediction of ethnicity.
2. Information about possible family relationships among the buried.
3. Possible familial links to folks in the databases who have submitted samples in the past (or would be willing to submit them in support of this project).

The questions we face:

1. What type of sample will be used for DNA recovery?
    1. Teeth
    2. Bones
2. Can we extract useful DNA from the available sample(s)? Our experience.
    1. Bones (femur, tibia, skull)
    2. Teeth (primarily molars)
    3. In my history, some sources of DNA successfully used for testing were >20 years in the environment before submission for analysis.
3. How will we determine the quantity of DNA recovered?
    1. We have several methods with which to quantify both nuclear and mitochondrial DNA.
    2. The methods available are used in crime laboratories all over the world.
4. Can we assess the integrity of the DNA recovered?
    1. DNA degrades with time, especially in a wet environment, continuously fragmenting into smaller and smaller pieces.
    2. We have methods with which to assess the integrity of any DNA we recover from the remains.
5. Answers to these questions will allow us to make informed decisions on how best to proceed.

The proposed workflow:

1. We work systematically through those interred, extracting DNA from a tooth and from a weight bearing bone to assess the quality/quantity characteristics of DNA recovered from each skeleton. It may be that some individuals will yield DNA in higher amounts or with differing integrity…"one size may not fit all".
2. All DNA samples from remains can be quantified and subjected to integrity analysis in the OSU Human Identity Testing Laboratory.
3. Simultaneously with #1 would be an open call to the community for sample donation, SNP genotypes thus assured of being in the database.
4. DNA quantity/quality data will direct our selection of a genotyping service (like Ancestry.com, 23&Me, or other). Considerations:
    1. Cost
    2. Searching capabilities (i.e., what will be reported back).
    3. Accreditation, other quality assurance practices, and guarantee of data privacy/security.

If potential kinship relationships are discovered…

Additional selective DNA testing could be performed if the DNA recovered is sufficiently intact:

1. STR type markers on the Y-chromosome.
    1. Very useful for identifying family relationships within the male family lineage.
    2. Analysis can be applied to sets of male remains recovered as well as to potential male relatives suggested from the ancestry testing.
2. Mitochondrial DNA analysis.
    1. Very useful for identifying family relationships existing within the female family line.
    2. Analysis can be applied to sets of female remains recovered as well as to potential female relatives suggested from the ancestry testing.
3. My laboratory could perform the Y chromosome analysis, but the mtDNA would have to be contracted.
4. I believe the project will require the services of a genealogist, especially one used to combining the DNA record with that constructed with traditional sources (public records, etc.)

Questions??