# Chimeric Fluidity: A Case Study of a Male Stem Cell/ Bone Marrow Bone Marrow Transplant Patient

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## Introduction:

In 2014 Washoe County employee, Chris Long, was diagnosed with AML/MDS Leukemia. Chris underwent a stem cell/bone marrow transplant and volunteered to participate in a case study led by the Washoe County Sheriff's Office Forensic Science Division. This case study is ongoing and monitors the fluctuation of DNA profiles from both the recipient and donor in various bodily fluids and tissues. Over the last four years Chris exhibited a full uptake of the donor's DNA within his blood and dynamic fluidity of chimerism within other tissues. This study highlights the potential effects of chimeric fluidity in forensic DNA interpretation. Chris's story is one of both medical success and the resilience of the human spirit. Recently celebrating four years cancer free, he strives to inspire others to work hard at enjoying what they have; by taking what life gives you and doing the best with it.

## Methods:

Sample Extraction was completed with the Qiaamp Blood Mini Kit both manually and on the Qiacube. Differentials were unnecessary for this case study. Quantitation was perfomed utilizing Plexor HY on Applied Biosystem 7500s. Amplification was performed utilizing both Plower Plex 16 HS and GlobalFiler. Capillary Electrophoresis was run on Applied Biosystems 3130s. Samples were analyzed with. Genemapper IDX. STRmix was utilized to calculate mixture percentages only and were conditioned on the reference samples. DNA analyst manually performed mixture deconvolution.

## Diagnosis:

In 2014 Chris was training for a ½ marathon. Unexpectedly, his times began to suffer and he found himself struggling to breathe on long runs. He was hospitalized with pneumonia for four days. During his stay his doctors noted that his blood counts were unusually low. Unable to account for this, the doctors continued to do blood tests for eight weeks. With no answers the doctors performed a bone marrow biopsy to "rule out any other issues". On September 3rd, 2014 Chris was diagnosed with Acute Myeloid Leukemia / Myelodysplastic Syndromes (AML/MDS). AML/MDS is the most common leukemia in adults. At this stage approximately 40% of Chris's marrow was affected (which accounted for his unusual blood results as the cancer had yet to spread to his blood stream.)

## Treatment:

Chris underwent six rounds of chemotherapy to destroy the cancerous blood cells. During that time his doctors searched for a bone marrow donor. Based on 10 protein markers, matches are sometimes found within the patient's family. Chris had no eligible donor candidates within his family. His doctors turned to 'Be The Match' Bone Marrow Registry. The largest and most diverse registry, Be The Match, was able to find three perfect matches for Chris. Donor information is not released to recipients until the recipient survives for at least two years post-transplant. This is to help prevent guilt of the donor should the transplant not be a success, and to protect the donor from being contacted in the future if the recipient needs a second transplant. The doctors did inform Chris that of the three donors they chose the donor who had the same blood type as Chris and who was the youngest, and healthiest.

Chris received his transplant on March 27th, 2015 at UC Davis (which he now refers to as his new birth day). Chris holds the record at UC Davis for the quickest release time from the hospital post-transplant: eighteen days. After his procedure Chris moved next door to the hospital in his parent's fifth wheel. For the next thirty days he underwent daily monitoring, this often included blood work and transfusions. Life post-transplant is largely spent in isolation. With a brand new immune system illness must be avoided. Crowds, sick family members, pets, and restaurants cannot be part of the recipient's life for a year following treatment. Chris describes this part of his journey as one of the toughest.

### Aftermath and Case Study:

Prior to his illness, Chris had become friends with the previous Washoe County Crime Lab Director. After learning about his transplant she asked if Chris would be willing to allow the Crime Lab to monitor his DNA profile within the blood. Chris agreed and the study began. Chris collected a small sample of blood onto cotton balls for the first eighteen days post-transplant. He labeled each sample by the day and placed them into Ziploc baggies, and gave to the lab for analysis. Following this initial sampling Chris agreed to also collect left and right cheek swabs, tongue swabs, blood, hairs (chest and head), and semen samples.

### Chimerism:

As it pertains to this study a chimera is an organism in which cell populations house entirely different genomes. This can occur naturally in fraternal twins in utero when one twin absorbs the other. This can also occur naturally with fetal maternal transfusion. Chimerism can be created artificially with blood transfusions and stem cell transplants. Prevalence is increasing because these types of treatments are being used more frequently, not only cancers of the blood and bone but also aplastic anemia, inborn errors of metabolism, auto immune diseases, trauma and iron deficiencies.

### Blood Results:

As expected, Chris's blood DNA profile started to become that of the donor almost immediately. Within four months (and ever since) Chris's blood DNA profile completely became that of the donor. (graph 1)

### Cheek Results:

Previous literature has shown that recipients often have a mixture of both the donor and recipient DNA within buccal swabs. Chris's cheek swabs exhibited the same behavior. It interesting to note that the amount of mixture varied over time  and was not stagnant (graph 2).

### Lips and Tongue Results:

The lips swabs showed no mixtures until 2019. In 2019 the sample indicated a mixture and approximately 34% of the mixture was attributed to the donor. The tongue samples always indicated mixtures with slight fluidity between sampling years. (graph 3).

### Why Mixtures In Mouth Samples?

Initially it was assumed that perhaps mouth lesions and/or cracked lips could have attributed to these results. Perhaps some blood had contained the buccal samples. Upon further investigation literature indicated that white blood cells within salvia are likely the cause of these mixtures.

### Hair results:

From 2015-2018 no mixtures were indicated in any of the hair samples. In 2019 a few stray alleles could be attributed to either stutter or the donor. Literature indicated that aggressive pulling may have caused some blood and or leukocytes to be attached to the follicle therefore resulting in a slight mixture.

### Semen Sample:

Unexpectedly the semen sample from 2015 indicated a mixture with 87% being attributed to the donor. It should be noted that Chris is vasectomized therefore only seminal fluid is present in these samples. In 2016, 64% of the mixture is attributed to the donor. In 2019, 100% of the sample is now the donor profile (graph 4). As white blood cells are present in seminal fluid observing a slight mixture would not be too farfetched. However, high amounts of white blood cells in seminal fluid usually indicate infection and/or sterility issues. Given that Chris's body accepted the graph in 2015, and within four months his blood completely became that of his donor, the 2015 and 2016 samples in which Chris's original DNA profile accounts for large portions of the mixtures were surprising. We currently have no explanation for these findings but are continuing to research them further.

### Forensic Implications:

Obviously, the fact that a recipient's buccal swab would not match their other bodily fluids has a large implication in DNA forensics. A potential suspect may not match the evidence and this could be misleading to a case. The non-obvious is the fact that most of the samples from this study resulted in mixtures. This too can be misleading for a case as authorities may be under the assumption that there are two suspects. It should be noted that, although not ideal, men and women can donate marrow to each other. Which then could cause authorities to believe the suspect is of a different sex.

### Mixture Interpretation:

Mixture interpretation has been the topic of forensic DNA typing for years. Anytime mixtures are involved there is the opportunity for difference of opinion. In this study, mixtures were given to DNA analysts and they were tasked with the deconvolution of the samples. While each analyst's interpretation fell within the laboratory's SOPs, there was variation amongst the interpretations. Some analysts utilized a CPI stat while others determined partial majors and partial minors.

### Implications for CODIS:

CODIS eligibility is also often a topic of discussion. Determining whether or not a sample may be entered into the database depends on whether or not a crime has occurred, if it was developed from a crime scene, and if the profile can be attributed to the perpetrator. Using the 2016 semen sample as an example, one could argue that if only one male suspect is expected, then obtaining a mixture would have alleles not attributed to the suspect. In cases of consuenal intercourse prior to assault this could be handled with an elimination sample for the consensual partner. However, in a case where consensual intercourse prior is not the fact is the sample eligible? Without knowing that the suspect is a chimera it would be assumed that the extra alleles in the mixture are attributed to a non-perpetrator and could make the profile non-eligible for searching purposes.

### Implications For Databasing:

Often times when we see unexpected mixtures we think contamination. In databasing samples should be single source. With a chimera the sample has the potential to be a mixture. At first glance it's reactionary

for analysts to assume that contamination has occurred. When the mixture is reproducible over and over what do you do? More recently the multiallelic offender index was added to CODIS to handle such samples.

### Forensic Conclusions:

If a chimera is assumed in a case, we suggest asking for hair samples as references. Hair samples always remained the recipient's DNA profile throughout the study. We also suggest simply requesting an additional buccal sample, as depending on the time of collection, there may be less of a mixture. We further suggest that as we move to automated mixture deconvolution, analysts remain unbiasedly involved in the process. When results don't fit and defy logic one may want to keep an open mind to an idea that, while it's not something you see daily, a chimera could pop up in your own casework.

### Personal Conclusions:

Chris has a new outlook on life. For Chris, and many others who have come face to face with life threatening illnesses, life is far too short to sweat the small stuff. Being grateful for the time you have and the people and things you have is key. Chris now volunteers with his church, was recently married, and recently got to witness his daughter get married. He mentors current cancer patients and has made lifelong friends with his nurses and doctors. He is grateful to be here today and strives to encourage others to feel the same. He plans to meet his donor next year in Germany with his new wife.


### Special Thanks:

Kerri Heward (Crime Lab Director), Darby Stienmetz, Dr. Lisa Smyth-Roam, Renee Romero and the Washoe County Sheriff's Office


### Dedication:

In appreciation of her work on this study, this presentation is dedicated to the memory of Dr. Brittany Baguley. She knew what it meant to "not sweat the small stuff". Her example of leadership and her love of forensic science lives on in us.

### References:


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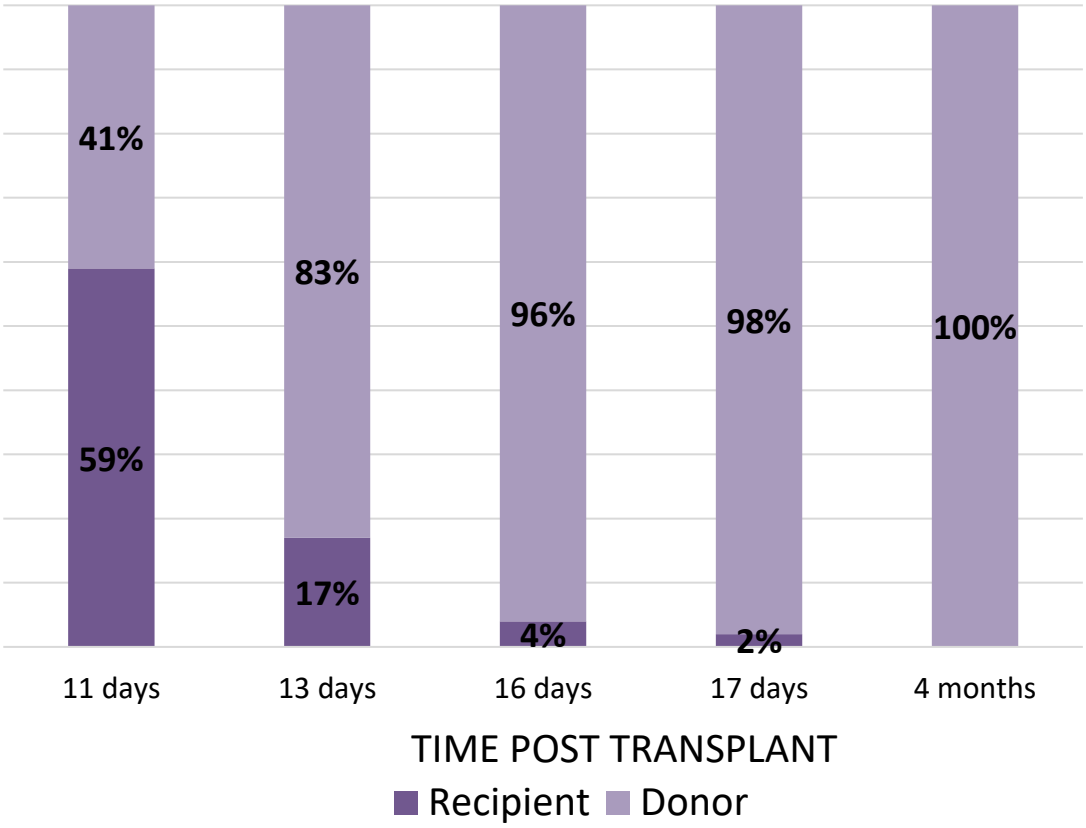
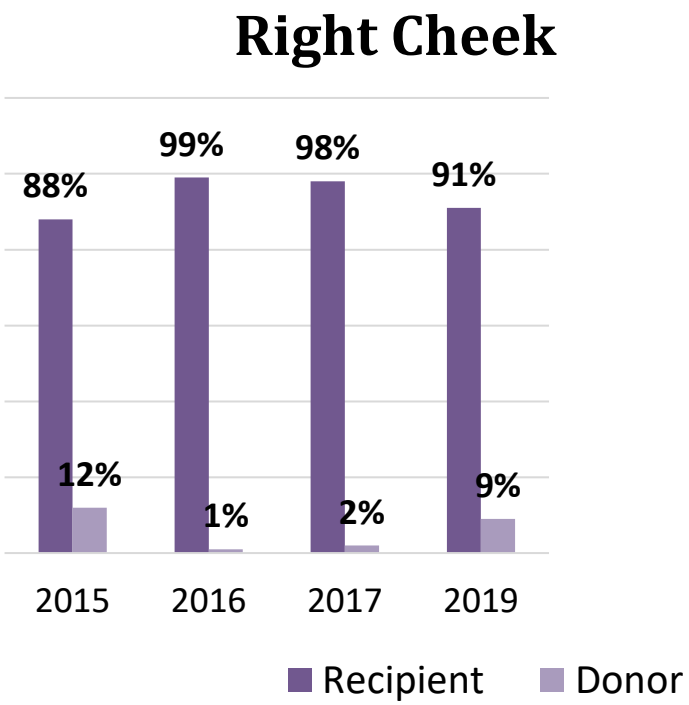
**Graphs:**

**1 Blood**

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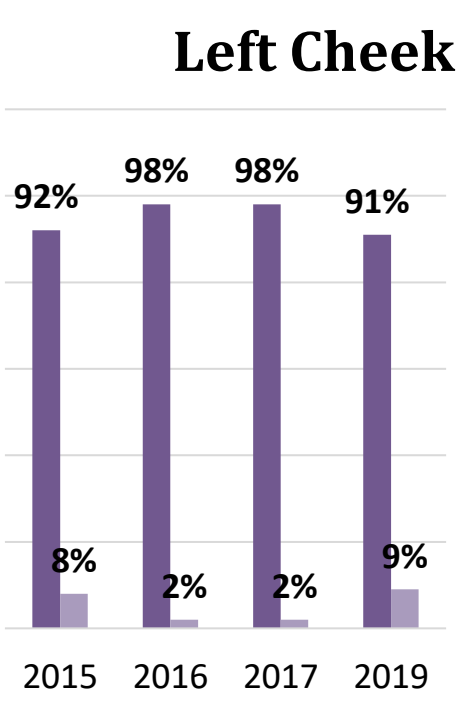
2017
2019
Recipient
Donor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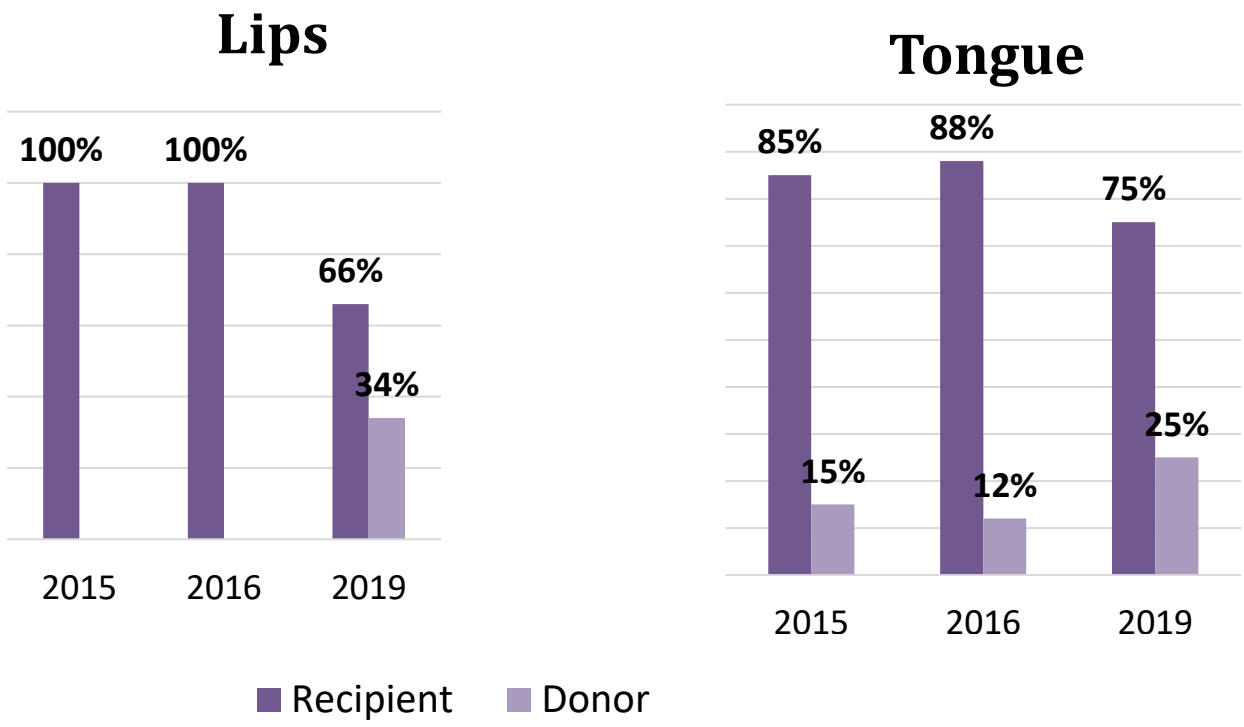
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**4 Semen**