

CHIMERIC FLUIDITY:

A CASE STUDY OF A MALE STEM CELL/BONE MARROW TRANSPLANT PATIENT



Presented by Chris Long and Brittney Chilton
Washoe County Sheriff's Office Forensic Science Division



DIAGNOSIS

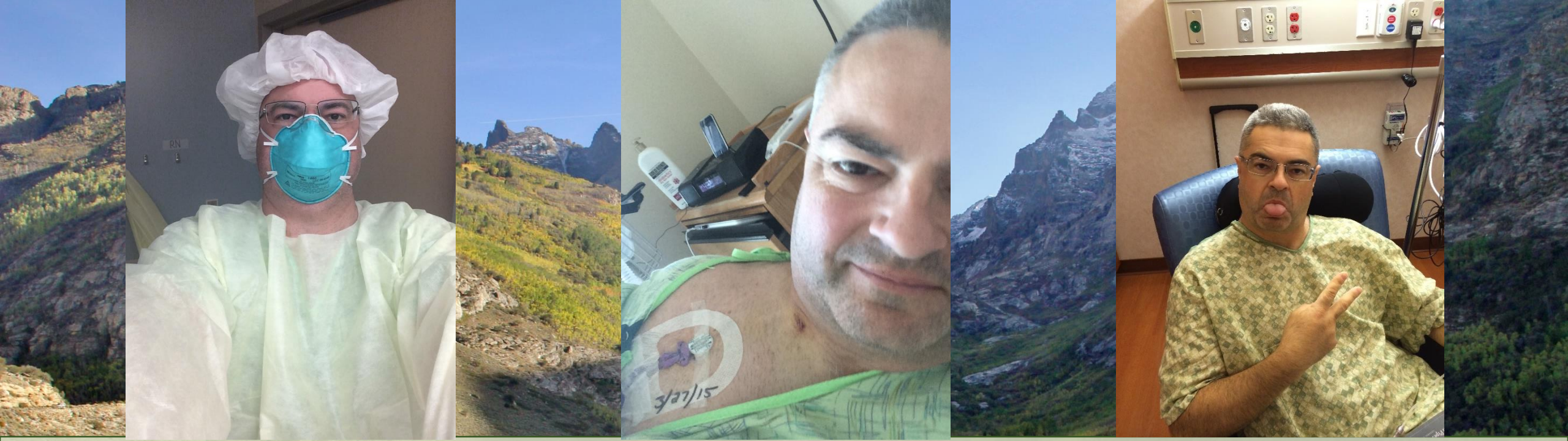
2014 began training for a ½ marathon

- Training was halted with the onset of poor training times and difficulty breathing
 - Became hospitalized with pneumonia
 - At this time red and white blood cell counts were low without a reasonable explanation
 - 8 weeks of blood work

September 3rd diagnosed with Acute Myeloid Leukemia / Myelodysplastic Syndromes (AML/MDS)

AML/MDS

- Characterized by over production of immature white blood cells, which fill up the bone marrow and prevent normal production
- Most common leukemia affecting adults
 - At this stage 40% of marrow was affected



TREATMENT

Six rounds of chemotherapy

- Daily monitoring of immune system and blood

Bone Marrow Transplant

- 10 markers
 - No familial match

Be The Match

- Largest and most diverse marrow registry in the world
- No North American matches
- 3 perfect matches in Europe
 - Youngest, healthiest, same blood type

Transplant

- Blu/Flu Chemo treatment
- Stem cell replacement
 - Donor's white blood cells are introduced directly into blood stream
- Stayed for 18 days in the hospital
 - In isolation
- An additional 30 days next door to the hospital
 - Travel trailer



AFTERMATH AND CASE STUDY

New “lifestyle” immediately after

- Digestive issues/ mouth sores
- Isolating
 - Work from home
 - Dining in on overcooked meals
 - Silver lining – weight loss

Through work met the previous Laboratory Director and became friends/training partners

- She suggested the study after learning about the diagnosis
- Requested buccal swabs prior to the transplant for “recipient reference sample”
- Requested blood samples immediately following the treatment

Sample collection

- Previous TL suggested collection of various tissues/fluids at time intervals
 - Left/right cheek
 - Tongue
 - Lips
 - Hair (chest/head)
 - Blood
 - Semen

CHIMERISM

What is it?

- A Chimera is an organism in which cell populations' house entirely different genomes

What Causes it?

- Can occur naturally
 - Fraternal twins in utero
 - Fetal maternal transfusion
- Can be created artificially
 - Blood transfusion
 - Stem cell/ bone marrow transplant

Prevalence is increasing because these type of treatments are being used more frequently

- Cancers of the blood and bone
- Aplastic anemia
- Inborn errors of metabolism
- Auto immune diseases
- Trauma
- Iron deficiencies



METHODS

Sample Extraction

- Qiaamp Blood Mini Kit
- Manually and Qiacube
- Differentials were unnecessary

Quantitation

- Plexor HY on Applied Biosystem 7500s

Amplification

- Plower Plex 16 HS and GlobalFiler

Capillary Electrophoresis

- Applied Biosystems 3130s

Analysis

- Genemapper IDX
- STRmix (utilized to calculate mixture percentages)
 - Conditioned on the reference samples
- DNA analyst manual mixture deconvolution



EXPECTED RESULTS

Blood

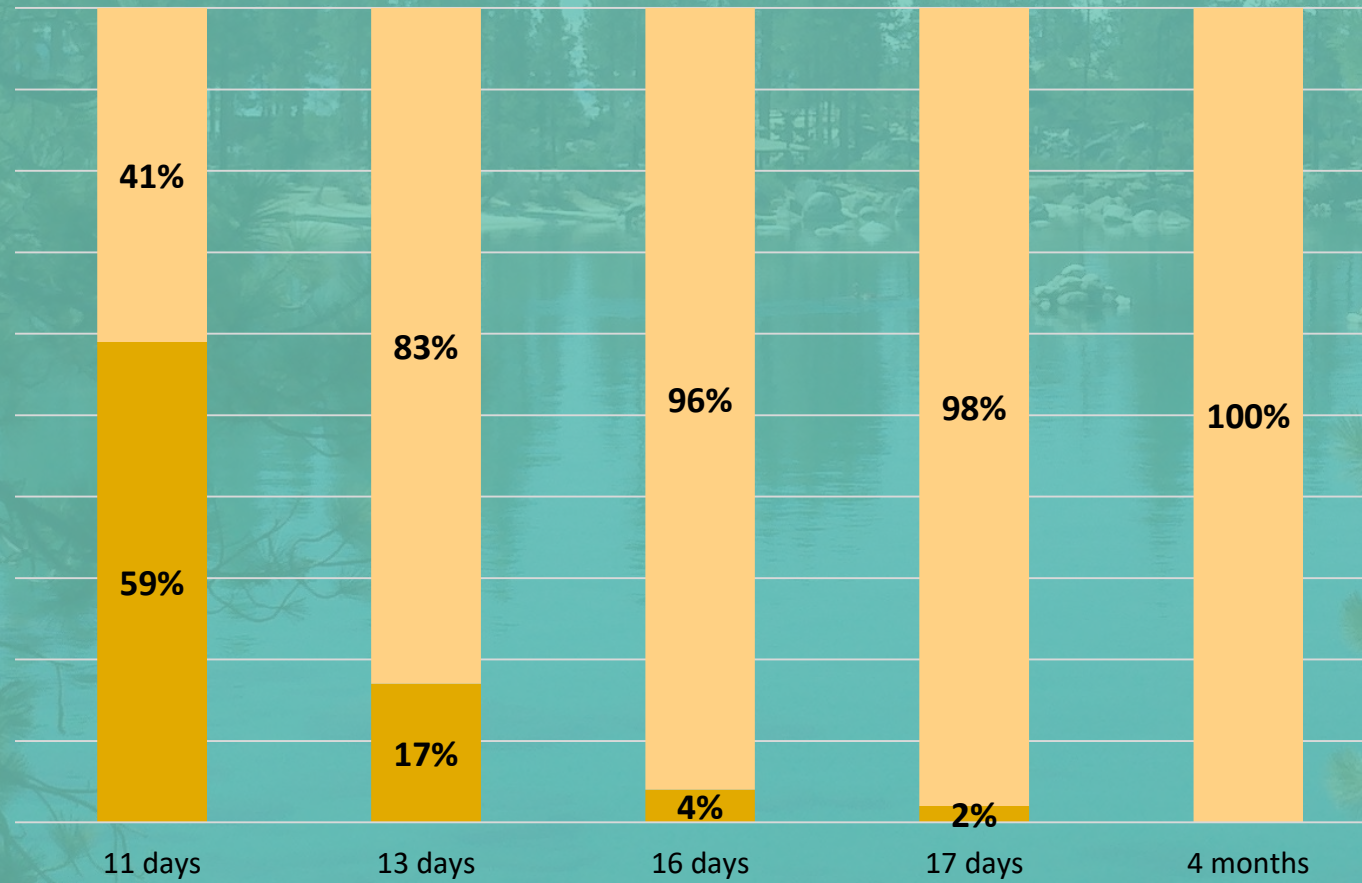
- Expected the recipient's DNA profile to become the donor's profile in 2-6 weeks

Buccal

- From previous literature we expected slight mixtures



BLOOD RESULTS

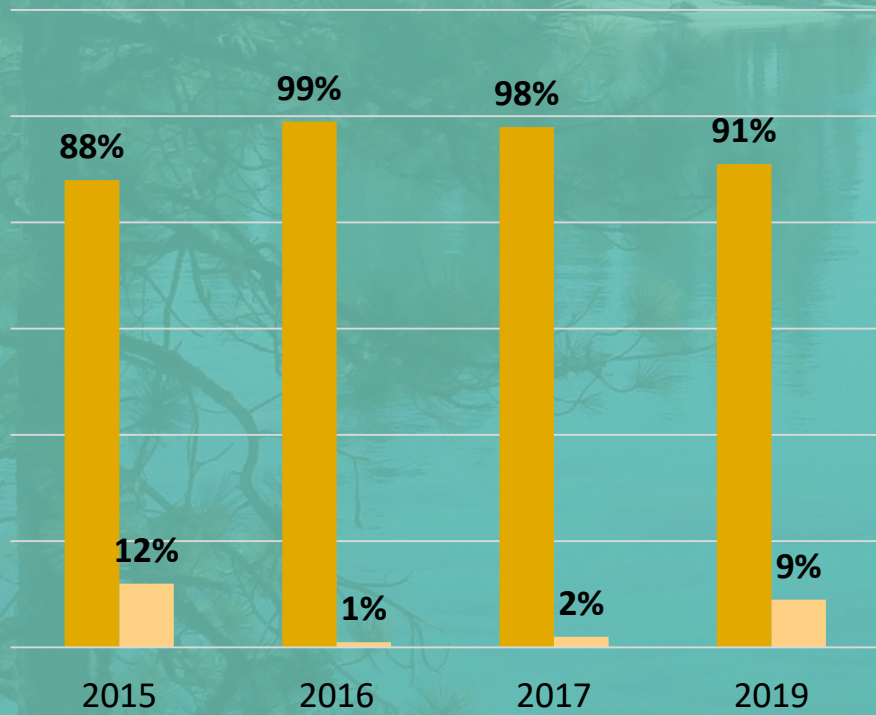


TIME POST TRANSPLANT

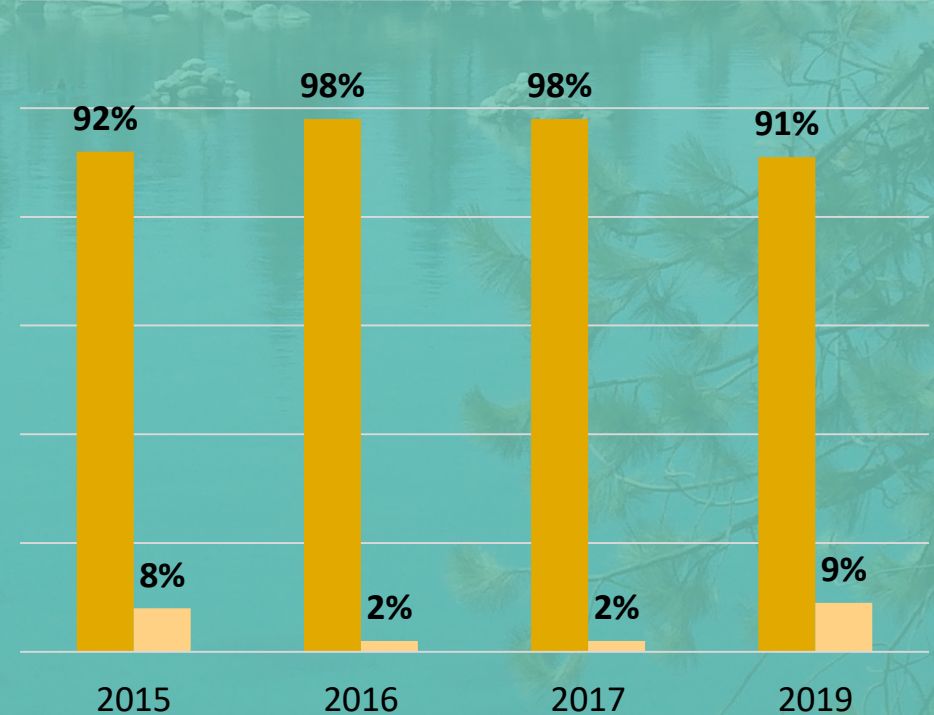
■ Recipient ■ Donor

BUCCAL RESULTS

Right Cheek



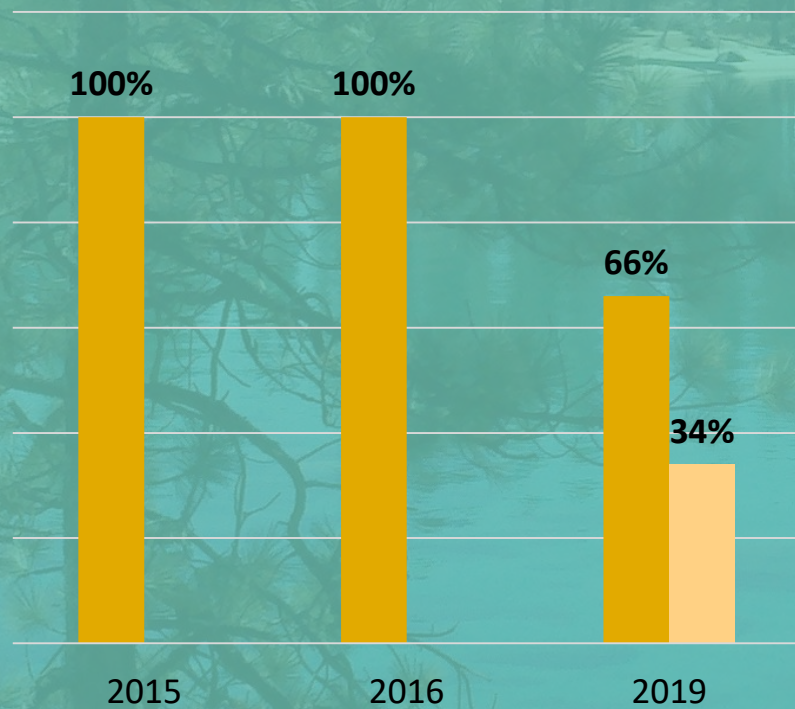
Left Cheek



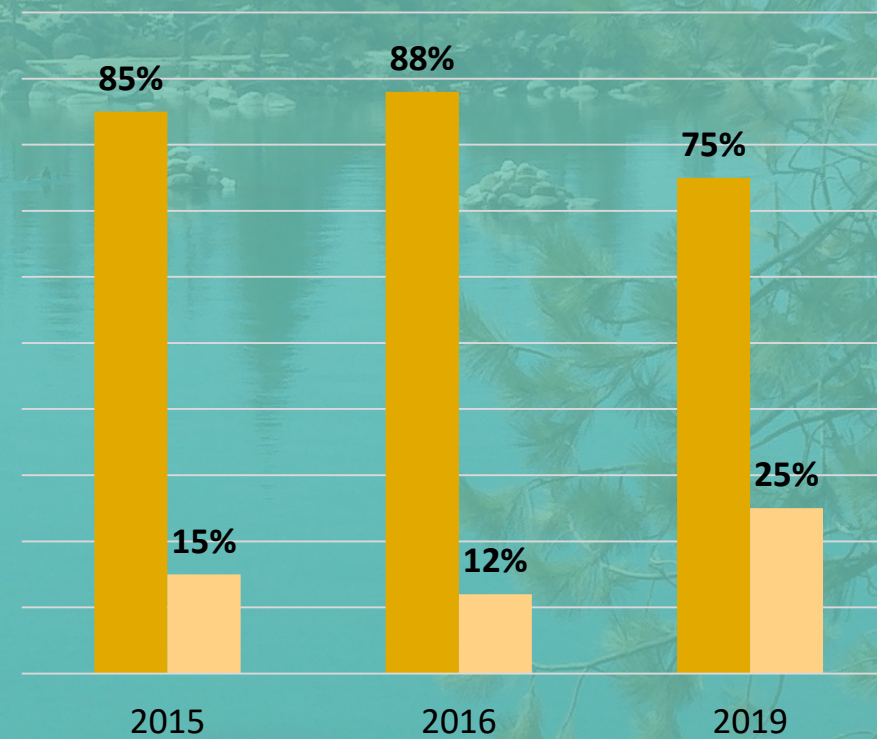
■ Recipient ■ Donor

LIPS AND TONGUE RESULTS

Lips



Tongue



■ Recipient ■ Donor



MOUTH MIXTURES

Hypothesis

Since hematopoietic stem cells should only be able to differentiate into blood cells or components of blood

Our thoughts..

- Injury to the tissues
 - Cracked lips, mouth lesion, etc.

Actual Scientific Findings

- White blood cells present in saliva
 - 2019 lips sample

HAIR RESULTS

2015-2018

- no mixture indicated

2019

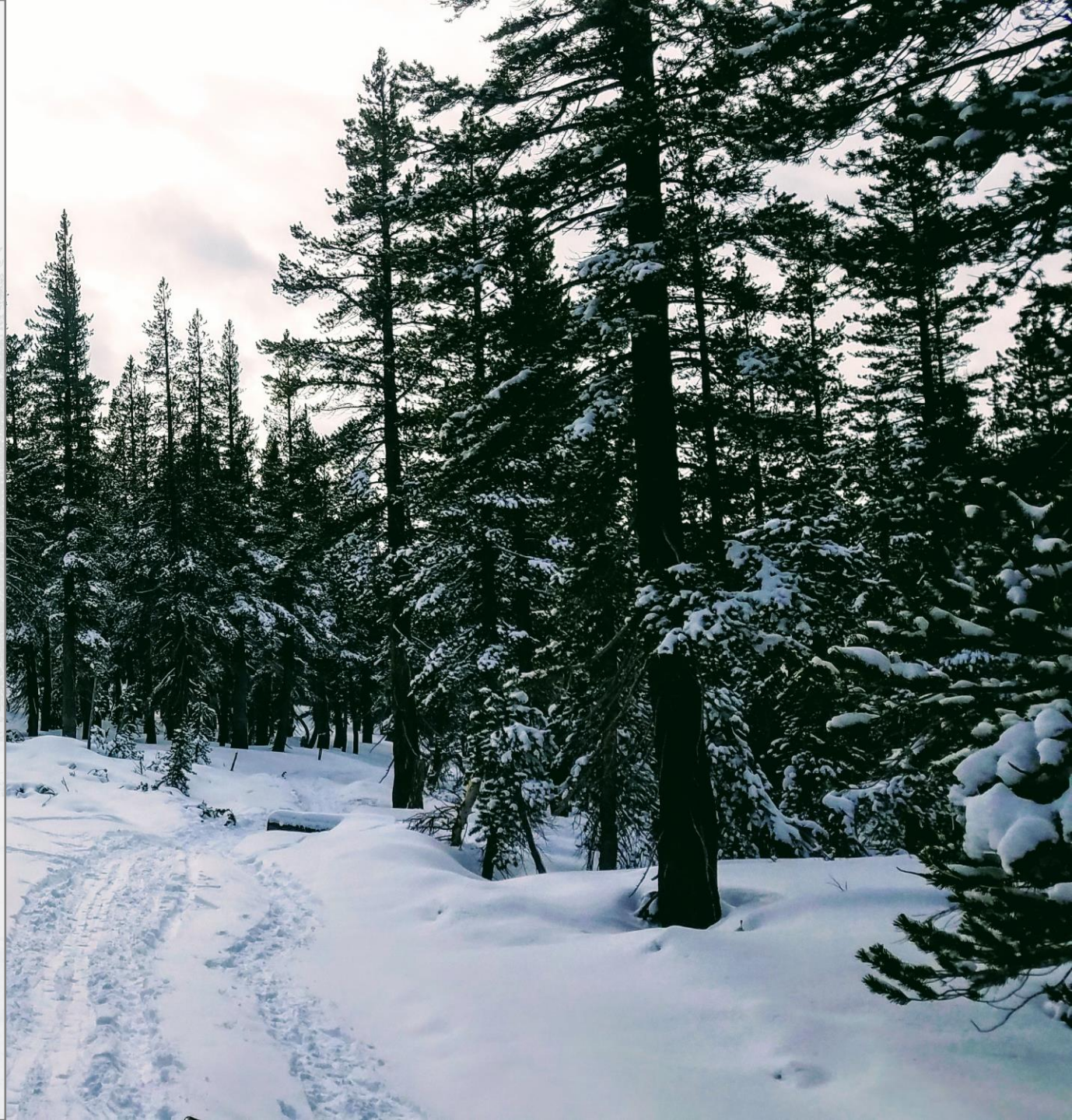
- A few stray alleles were observed that could be attributed to the donor and/or stutter
 - Aggressive plucking



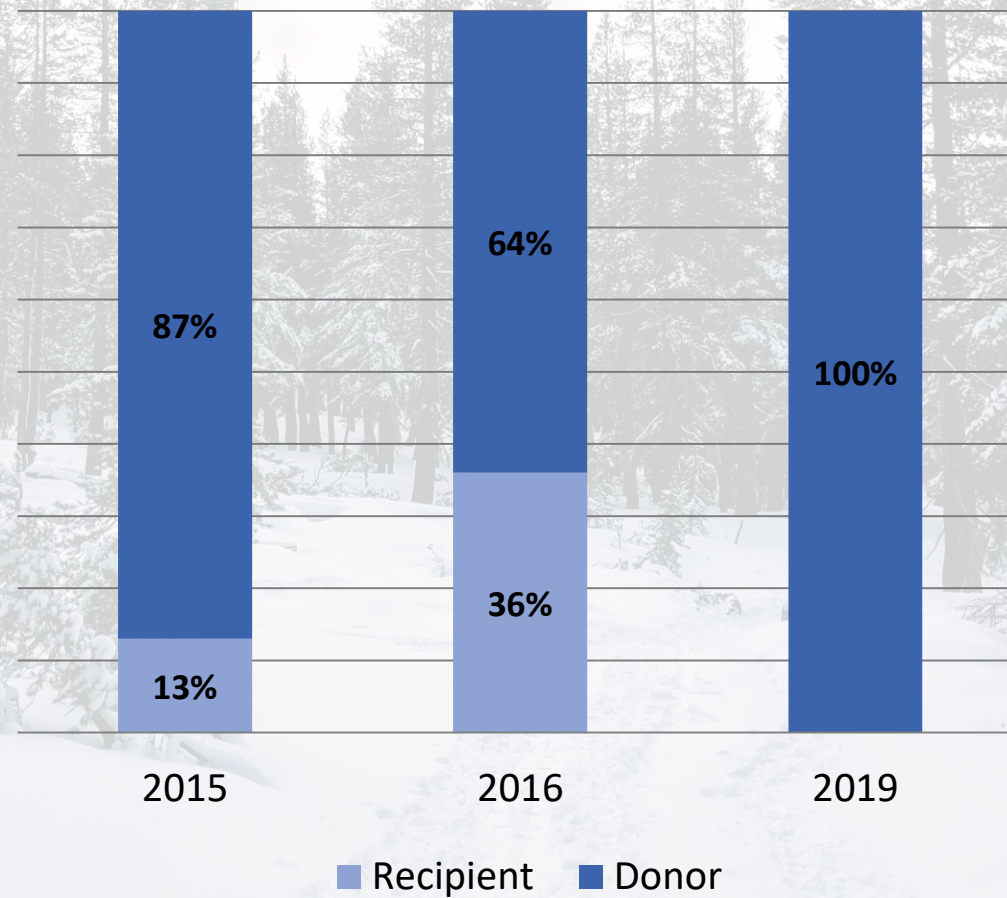
UNEXPECTED RESULTS

Semen

- Due to cancer treatments male patients often become sterile
- Should be noted that the recipient was vasectomized prior to treatment



SEMEN RESULTS



IMPLICATIONS

The most obvious

- DNA profiles from reference samples and evidence samples do not match

The non-obvious

- Mixtures
 - Previously thought of chimeras as an individual with tissues that had distinct genomes. However, many of the results we observed were mixtures. These would have been reported as a mixture of at “least two individuals” which could be misleading for a case*



* Although not preferred, donor and recipients can be different sexes. Which could be greatly misleading for a case



IMPLICATIONS

Effected the interpretation of some samples

- 2019 Tongue: One analyst did a mixed dominant of two sources and would have utilized a CPI statistic. The second analyst deconvoluted a major and minor
- 2016 Semen: Two analysts did a mixed dominant and a third analyst who deconvoluted a partial major and a partial minor



IMPLICATIONS

CODIS

Scenario

- Semen sample
 - Ejaculation occurred, no sperm ID'd, P30 Positive
 - Victim reports single male assailant, she was coherent for the entire assault, has had no consensual partners
 - DNA profile is a two person mixture
 - Contamination?
 - Inquire submitting agency
 - Consult your CODIS tree of knowledge
- Has a crime occurred?
 - Yes
- Is the profile from the crime scene?
 - Yes
- Can the profile be attributed to the perpetrator?
 - **Maybe????**
 - Will the real Doug Hares please stand up? Please stand up? Please Stand up?



IMPLICATIONS

DATABASING

Mixtures when you aren't supposed to get them and you keep getting them

- Contamination during collection or is it really a chimera???

As our databases increase we are likely to see more of this

- Multi allelic offender category





FORENSIC CONCLUSIONS

Ask for a new sample collection

Ask for different sample source

- Like hairs which remained the recipient throughout

As we move to automated mixture deconvolution as an analyst we still have to be unbiasedly aware/ involved in the process

- Looking at the bigger picture/ other info we have been given (if any) when results defy logical explanation
- Having an open mind to situations like this

Yes, this is rare but it can happen

PERSONAL CONCLUSIONS

Life currently

- Got married recently
- Meeting my donor next year
- Periodically still work with the laboratory on this study
- Shares info w/ doctors

Lessons learned

- Mentoring
- New outlook on life
 - Don't sweat the small stuff
 - Be grateful for what you have





Special thanks to:

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

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



QUESTIONS

References

- “Chimerism in DNA of buccal swabs from recipients after allogeneic hematopoietic stem cell transplantations: implications for forensic DNA testing” Berger, B.; Parson, R; Clausen, J.; Berger, C.; Nachbaur, D.; Parson, W. Int J Legal Med (2013) 127: 49-54.
- “Evaluation of blood , buccal swabs, and hair follicles for DNA profiling technique using STR markers” Chaudhary, G.; Dogra, T.; Raina, A. Croat Med J. (2015) 56: 239-245
- “Fetomaternal microchimerism: Some answers and many new questions” Tan,K.; Zeng, X.; Sasajala, P.; Yeo, A.; Udolph, G. Chimerism. (2011) 2(1):16-18
- “Bone Marrow Transplant.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 11 July 2019, <https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/about/pac-20384854>.
- “Transfusion Associated Microchimerism: The Hybrid Within” Evan M.Bloch, Rachael P.Jackman, Tzong-HaeLee, Michael P.Busch. Science Direct (2013) 27(1): 10-20