The Tech Interactive

Biotinkering Programs for Science Centers

Ancient DNA Stories

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
SUPPORTED BY THE NATIONAL INSTITUTES OF HEALTH

Stanford MEDICINE | Department of Genetics

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Ancient DNA Stories Overview

Science Center Experience

Recent technological advances have made it possible to recover intact DNA from individuals who lived thousands of years ago. In this activity, visitors see how it is possible to go from bones, to DNA sequences, to reconstructing the underlying stories. They can explore the lives of people like Ötzi the Iceman, a 5000-year-old mummy found in a glacier; Cheddar Man, a 10,000-year-old skeleton from England; and Arzhan, a 2500-year-old horse from Siberia. Using DNA sequences, visitors decode genetic puzzles to discover what these individuals looked like and may have eaten, diseases they may have had, and more. As visitors piece together the ancient stories, they can share their discoveries with others and even create a drawing.

Subject
Genetics

Ages
8+

Duration
10-45 min

Key Concepts
Traits, genes, evolution, DNA sequences

Activity Goals

- Enable visitors to uncover the stories of people who lived thousands of years ago using authentic DNA sequences.
- Build visitor confidence with problem-solving and computational thinking through narrative-driven genetics puzzles.
- Highlight that biology can meaningfully intersect with other areas and interests, such as history and archaeology.

Operational Considerations

Base Biology	Format Complexity	Lab Requirements	Cycle Length	Cost
DNA Sequences	Low: Paper-based	None	Single session	$$$

Cycle Details

A full *Ancient DNA Stories* cycle can be completed in a single 10-45 minute visitor experience as the activity uses paper-based representations of biology.

Background Information

What is “Ancient DNA”?

In recent years, the field of Ancient DNA has exploded into one of the hottest areas in biology. It is based on the relatively simple premise that DNA can be found in old bones and mummies and used to discover more about these samples.

We can never fully understand the real-life individual behind a sample. However, it is possible to reconstruct many of the details. This requires combining the genetic interpretation with information from other fields, such as archaeology, history, sociology, and paleontology. Robust analyses of ancient samples combine data and expertise from people across many disciplines and specialties.

Ancient DNA studies are revising our understanding of major historical events, such as the origins of agriculture and domestic animals, diseases and immune system resistance, how our species has evolved and spread around the globe, and even why certain species were vulnerable to extinction.

While going from a bone fragment to DNA code is still difficult, recent advances in sequencing technologies have allowed this dream to become a reality. Scientists now routinely sequence the entire genomes of individuals from long ago.

Going From Biological Sample to DNA Code

1. *Samples processed in special laboratories*
   A sample is taken to a lab that is set up specifically to handle ancient DNA. Since ancient DNA is extremely fragile and typically only present in low quantities, all the early steps are done in a clean room. Scientists wear sterile gowns and are careful to limit access to the clean areas, minimizing the risk of contamination from contemporary DNA sources.
2. *DNA extraction*
   Bones or other fragments are cleaned to get rid of any contamination that may be on their surface. Then small samples are taken from inside the bone (to further reduce contamination risk) and ground into a powder. The sample is then dissolved with chemicals that help to isolate short DNA strands. DNA is nearly always sampled from teeth or the petrous bone (a specific part of the skull), as these preserve DNA particularly well.
3. *DNA barcoding*
   Tiny DNA tags (“barcodes”) are added to the sample. These attach to the ends of the DNA and act like tiny handles, allowing researchers to move the DNA around in later steps. Since the researchers also know the exact sequence of the tag they put in, they can double check that no new DNA has contaminated the experiment after this step.

4. *DNA amplification*
   Ancient bones don't have very much DNA, so the minimal existing DNA needs to be amplified into many more copies. This increases the accuracy of the final data: instead of reading each molecule of DNA a single time, it will be read many times.
5. *DNA sequencing*
   The amplified DNA molecules are put into a special machine called a sequencer. It takes in biological samples, analyzes the DNA, and spits out digital text files that a person (or computer) can read. These sequences are all very short, between 30-200 basepairs long.
6. *Alignment*
   Much like a jumbled puzzle, the short DNA pieces need to be arranged into a complete genetic portrait. Usually, the DNA fragments are matched to an existing reference genome. This is like solving a puzzle with detailed guides. A computer can take each short DNA sequence and compare it to the reference to see where it belongs.
7. *Variant calling*
   The aligned sequences are then compared to the reference to see how they are different. There are many ways that DNA can be different between people:
   - Single Nucleotide Polymorphisms (SNPs): This is a type of variant in which a single DNA basepair is different. Most traitcards in this activity show SNPs.
   - Copy Number Variants (CNVs): In this variant, a piece of DNA is duplicated and present in different numbers in different people. The Starch Digestion traitcard is one example.
   - Insertions and Deletions (indels): A piece of DNA is present in some people but absent in others. The difference may be as small as a single basepair or thousands of basepairs in length. Type O blood is an example of a single letter deletion.

Challenges in Ancient Samples

Time: DNA breaks down and degrades over time, making it hard to recover good-quality DNA from old samples. Old samples of DNA are often degraded, with chemical modifications that make it very fragile. The older a sample is, the less good-quality DNA it is likely to have. Currently, scientists estimate the theoretical maximum lifespan of DNA is slightly above 1 million years. After this point, the DNA has degraded so much that it is completely gone.

Temperature: Heat and exposure make DNA break down even more quickly. This is why many high-quality ancient DNA genomes are from cold areas of the world, where it is possible to find DNA that has been frozen intact for millennia.

Contamination: After time and exposure to the elements, ancient samples only have a tiny fraction of their original DNA. They'll also be covered with DNA from microbes in the soil, plants nearby, and even DNA from the people who found the samples. This last bit is particularly difficult — how do you distinguish the real ancient DNA from the DNA of the archeologist who found it? Luckily, some of those same features that make ancient DNA so difficult to work with make it possible to sort the old DNA from the modern: new DNA contamination won't have any signs of degradation and can be sorted out. Careful handling is critical to recovering high-quality data.

Genetic Principles in the Activity

Dominant and Recessive

This is one of the most classic examples of heredity, where a single gene determines a trait. Such a gene may come in two different versions (alleles). A dominant allele only needs to be present in one copy to determine the trait. A recessive allele needs to be present in two copies to determine the trait. An individual with one of each allele will show the dominant phenotype; someone with this genotype is sometimes called a *carrier*, since they can pass the recessive allele on to a child. This inheritance pattern can be modeled and predicted with a Punnett Square.

As this pattern can only be observed where there are two alleles, it can only be examined in traitcards that require finding two DNA matches. The following traits show dominant/recessive characteristics:

- Red pigment is recessive in horses, dogs, and humans.
- Diluted pigment is dominant in both horses and dogs.
- Brown pigment is recessive in dogs.
- Pinto patches are dominant in horses.
- Bladder stone risk is recessive in dogs (Hatch is a carrier).
- The ambling gait is recessive in horses.
- Lactose tolerance is dominant in humans.
- Shovel-shaped teeth are dominant in humans.

Incomplete Dominance

Similar to simple dominant/recessive traits, a trait with an incomplete dominance pattern is determined by a single gene with two alleles. However, in this case an individual with one copy of each will have an intermediate phenotype.

The following traits show this pattern:

- The leopard complex gene in horses shows incomplete dominance, as a heterozygous individual has spots on a white background while a homozygous individual is closer to pure white (Coat Color 4).
- Body size in horses may be described as incomplete dominance, though as there are four known major genes it could be considered a complex trait instead.

Complex Traits

Complex traits are affected by multiple genes or a combination of multiple genes plus the environment. While genetics is often framed in a dominant/recessive way, complex traits are the norm. In this activity, it is difficult to adequately show the breadth and depth of complex traits, but the following are some of the examples that are present:

- Eye color: While the Sunrise Child traitcard shows that dark eyes are generally dominant to light eyes, each version of this traitcard notes that the reality is more complicated. The back of the Cheddar Man and Sunrise Child traitcards show some of the other genes that contribute to this trait.

- Hair color: This trait is influenced by dozens of different genes. The back of the Cheddar Man traitcard shows some of these other genes.
- Skin color: While the Sunrise Child traitcard shows that skin color is partially an example of incomplete dominance, each version of this traitcard notes that the reality is more complicated. The backs of the Cheddar Man and Sunrise Child traitcards show some of the other genes that contribute to this trait.
- Body fat: While the Sunrise Child traitcard shows that body fat is partially an example of incomplete dominance, it also notes that environmental differences like diet and exercise matter as well.
- Coat color: While the activity examines just four genes related to coat color in horses and dogs, the back of each of the “Final Coat Color” traitcards highlights additional relevant genes.

Risk Variants

Risk variants increase the chances of developing a certain condition or disease. For example, diabetes is a complex trait with a known risk variant. Both genetic and environmental differences affect the chances of developing this disease. In the Sunrise Child diabetes traitcard, the “G” allele is the risk allele and measurably increases the risk of developing this condition. In contrast, the “A” version would be described as the protective allele, which decreases the risk of developing this condition.

Variable Expressivity

Variable expressivity suggests that a single genetic combination can have a range of possible presentations. It is related to the concepts of complex traits (the reason *why* a single combination presents in varying ways could be due to other genes or known non-genetic causes). In horses, the leopard complex gene is a good example of this concept, as “Spots” and “Fewspot” may present in a range of ways.

Pleiotropy

Pleiotropy occurs when a single gene influences two distinct traits that may seem otherwise unrelated. The following traits show this pattern:

- In humans, the same gene that determines tooth shape affects hair texture and milk production (see back of Sunrise Child traitcard).
- In horses, the same gene that determines spot patterning affects night blindness (see back of Arzhan Coat Color 4 card).

Homology

Homology is the similarity that exists between two species due to shared ancestry. The following examples of homology can be observed in this activity:

- Y chromosome sequence: Genetic sex in mammals is largely determined by the presence of a Y chromosome. As the Y chromosome was present in our distant mammalian ancestors, horses, dogs, and humans all have very similar Y chromosomes.

- Starch digestion sequence: As humans and dogs both inherited this gene from a shared ancestor, the sequence is very similar between the two species. However, the actual copy number variation is an example of convergent evolution (see below).
- Pigmentation genes: As humans, dogs, and horses all inherited genes from a shared ancestor, many of the same genes show up across the various pigmentation traits. These genes are involved in the same shared pigment production pathway. However, the actual genetic changes and differences within these genes are examples of convergent evolution (see below).

Convergent Evolution

Convergent evolution is when two species independently evolved a similar trait. In contrast to homology, this similarity is not due to shared ancestry. The following examples can be seen in this activity:

- Red pigment: This trait has arisen independently in many different species. In dogs, horses, and humans, it has arisen due to independent mutations within the same gene (MC1R). This gene is both an example of homology (the gene was inherited from a shared ancestor) and convergent evolution (independent mutations in this gene have led to a similar phenotype). As the mutations happened in different parts of this gene, the portions of sequence shown in horse and dog Coat Color 1 are not the same.
- Pigment dilution: This trait arose independently in both horses and dogs from mutations in two different genes.
- Starch digestion: This copy number variant happened independently in both dogs and humans. Dogs and humans both originally inherited a single copy of this gene from a shared ancestor (homology) and independently duplicated it (convergence).

Useful Vocabulary

Term	Definition
Align, Aligning	The process of matching DNA sequences to a reference.
Allele	A version of a gene.
Ancient DNA	A DNA sample that is very old and likely somewhat poor in quality due to age.
Bioinformatics	The field of biology that interprets digital DNA sequences to try to understand what they mean. It is heavily reliant on computers (“dry lab”) and usually does not involve pipetting work in a laboratory (“wet lab”).
Complex Trait	A trait that is affected by multiple genes and/or environmental influences.
Copy number variant (CNV)	A genetic variant in which a specific section of DNA has been duplicated a varying number of times.
Genomics	The study of the entirety of an organism’s DNA, or genome. It is an interdisciplinary field of biology that combines genetics, computer science, bioengineering, and bioinformatics.

Reads (or DNA reads)	Short sequences of DNA that a sequencing machine produces after interpreting a sample.
Reference	A sequence of DNA that is used as a representative of the DNA sequence for that species. It is often used as a basis for comparison so that results are reported as “this person is different from reference at position X.”
Single Nucleotide Polymorphism (SNP)	A genetic variant of one specific DNA letter that occurs at a precise location in the genome.
Trait	A particular characteristic of an organism. It may be determined by genes, environmental factors, or a combination of both.
Variant	A genetic difference.

Visitor Experience

Operational Summary

Context

Ancient DNA Stories uses paper-based representations of real DNA sequences and allows for very varied lengths of engagement, usually from 10-45 minutes, depending on the number of puzzles solved. It does not depend on visitor-produced material supply chains, so can be operated in any space and is easy to pop up at any frequency or cadence.

This activity was created as a self-directed puzzle-solving experience. Visitors are introduced to the activity by a facilitator but then encouraged to work at their own pace for as long as they choose. A facilitator circulates around the room to answer questions, assist where needed, and guide visitors through the different station options. The activity was designed to run on a rolling basis with new visitors allowed in as space permits (e.g., “beginner level” stations available). Some visitors solve only one or two cards, spending only a few minutes in the space. Other visitors may choose to engage more deeply in the puzzles, the drawing, or both and can spend as long as an hour in the activity.

Activity Outline

1. Introduction and Trait Selection
    - Facilitator gives an overview of the activity.
    - Facilitator briefly introduces the ancient individuals, DNA, and puzzle mechanics.
    - Visitors choose which individual and traitcard to work on.
    - Facilitator directs visitors to the correct station to solve the puzzle.
2. DNA Puzzle Solving Stations
    - Visitors use DNA to solve the traitcard puzzle.
    - Visitors share their answers on the magnetic answer boards.
    - Visitors choose a new traitcard and start again.
3. Drawing Station (optional)
    - Visitors synthesize what they have learned to draw an individual’s story.

Introduction and Trait Selection

Overview

Provide a brief overview of the activity to orient visitors to the nature of the experience. The facilitator should introduce visitors to the ancient individuals, DNA, and puzzle mechanics. Provide a central storage location for the traitcards that organizes them by individual to allow for easy visual selection. Visitors start by choosing a card that they are interested in to work on. The facilitator should recommend a “beginner level” individual for all first-time visitors.

Essential Materials	Shared ● Activity traitcards (see Backend Preparations for details) ● File organizers
Example Setup	

Engagement Strategies

Cultivate Confidence and Agency

- Guide visitors to start with less-complex puzzles to help them gain confidence in their problem-solving skills before tackling more complex cases.

DNA Puzzle Solving Stations

Overview

These stations are the core challenge of the activity. Visitors solve genetic puzzles to discover more about each individual. Arrange six separate stations, one for each ancient individual. Young visitors can engage with DNA-as-information in an authentic way, while more advanced visitors may be able to build on prior knowledge of dominant/recessive traits by exploring genetic complexity. Biosketches are also provided for each individual to provide supplemental information about non-genetic details. Once visitors have solved as many puzzles from as many individuals as they would like, they can move to the Drawing Station.

Essential Materials	Shared ● Printed DNA pieces, answer boards, answer magnets, and biosketches (see Backend Preparations for details) ● Storage containers for DNA pieces ● Magnetic boards
Example Setup	
Key Visitor Steps	1. Follow instructions on the traitcard. 2. Use the strips of DNA code to solve the genetic puzzle. 3. Share findings on the magnetic answer board. 4. Choose a new traitcard and repeat.

Engagement Strategies

Facilitate Constructive Problem Solving

- Facilitators should observe and/or check in at several key moments to promote success:
  - As visitors solve their first card, to ensure they understand what to do

    - As visitors finish their first complete set, to highlight options for deeper engagement (e.g., draw what they learned or work on a different set)
    - As visitors encounter potentially difficult puzzles that may require additional help (see "Tricky Traits" below).

Foster Scientific Curiosity

- Facilitators may circulate around the room to answer common questions, help visitors find relevant deeper content (such as on the traitcard backs or in the biosketches), make connections to real-world relevance, and highlight between-case similarities.
- Expert facilitators can help guide in-depth conversations around the content in the cards, introducing visitors to some of the genetic principles that underlie the activity.

Highlight Authentic Science Practices: Collaboration

- While some visitors may prefer to work individually, others will enjoy working in groups. Visitors may work together on a single traitcard, split up the traitcards, or focus on different portions of the activity (e.g., solving traitcard puzzles vs. reading traitcard backs and biosketches).

Support for Tricky Traits

Cheddar Man (beginner)

- Ancestry: This is broken into two traitcards (Ancestry 1 and Ancestry 2), and both are required to find the full answer.

Stuttgart Woman (beginner)

- Ancestry: This is broken into two traitcards (Ancestry 1 and Ancestry 2), and both are required to find the full answer.
- Starch digestion: This trait is a copy number variant, and the card asks visitors to count how many matches are in the DNA basket.

Hatch and Arzhan (intermediate)

- Getting started: Most of these cards require visitors to find two DNA matches. Some visitors may initially not notice this difference and need additional assistance.
- Final coat color: This puzzle is a flowchart that asks visitors to synthesize the answers from Coat Color 1-4.
- Starch digestion (Hatch only): This trait is a copy number variant, and the card asks visitors to count how many matches are in the DNA basket.
- Sex: This card asks visitors whether or not they can find a matching DNA piece.

Sunrise Child (expert)

- Getting started: Most of these cards require finding two DNA matches, and the matching DNA will not align from end to end. Visitors who have become used to the perfect alignments may require assistance noticing this difference and shifting from a strategy of matching the first letters of the sequence to looking for internal patterns.
- Sex: This card asks visitors whether or not they can find a matching DNA piece. Sunrise Child is particularly tricky because this DNA sequence is absent.

Drawing Station

Overview

This optional station should be set up with markers and blank paper so visitors can draw what they think the ancient individuals may have looked like in real life. This allows visitors to synthesize what they have learned, encouraging them to incorporate information from all aspects of the activity. The answer key is kept here in case visitors would like to use it as a reference. Drawing is not necessarily the end point to the activity; visitors may skip it or choose to return to solving more puzzles.

Essential Materials	Shared • Drawing sheets • Markers and pens • Answer key
Example Setup	

Engagement Strategies

Support Creativity

- Open-ended drawing spaces allow visitors to get creative about how to communicate various traits while showcasing their artistic abilities. As a bonus, these may keep younger visitors in family groups occupied while older visitors spend more time engaged in in-depth puzzle solving.
- Consider creating a location where visitors can leave behind their artwork to share with the community and serve as inspiration for future visitors.

Common Visitor Questions

Visitors often ask unpredictable or incredibly specific questions about the content or process of an activity while they are participating in the experience. Every audience will have different interests or prior knowledge that they bring to the experience. Below are examples of the most common questions we hear from visitors and the types of answers we aim to provide.

Question	Information
Is this real?	*Yes! Or at least mostly real.* These are real people and animals who lived thousands of years ago. What you're solving right now with their DNA is real. This is exactly how scientists have used DNA to predict what they were like back then. For some cases we've changed the exact letters of the DNA to make it possible to solve with just one card and some traits have been simplified a bit. But all of the information about which genes are used to solve these cases is true.
Can we get dinosaur DNA?	*Probably not, it's just too old.* Sadly, we probably can't recreate Jurassic Park. Under absolutely perfect permafrost conditions, DNA only lasts a couple million years or so. As of 2022, the oldest DNA ever successfully recovered is about 2 million years old. The last dinosaurs went extinct 65 million years ago, simply too long ago to recover any DNA. But you can actually get modern dinosaur DNA: from birds!
I thought lactose intolerance was a recent mutation?	*No, being able to drink milk (lactose tolerance) is actually the mutation.* All of our distant ancestors were lactose intolerant. People who are lactose intolerant are able to drink milk as babies, but grow out of it as they are weaned. In fact, all other mammals are also lactose intolerant as adults. A kitten can drink milk, but an adult cat will get sick if it drinks too much dairy.
Does diet change your DNA?	*No, you won't have cow DNA mixed in with yours.* But if we looked at the contents of your stomach, we'd find DNA from whatever you ate most recently. This is what was done to figure out Otzi's last meal.
So DNA *does* determine gender?	*No, it's more complicated than we show here.* The sex traitcards look at chromosomes. Most women have two X chromosomes while most men have an X and a Y. But there are many other types of sex chromosome combinations that aren't shown (XO, XXY, XYY, etc). And while chromosomes influence your physical characteristics, they do not define your gender identity or sexual preferences.
How much fat I have is determined by genetics?	*No, it's a combination of genetic and non-genetic things.* There is a genetic component to body fat and metabolism, but diet and exercise matter a lot too. Your genetics affect how likely you are to be overweight, but they do not determine it entirely.

Backend Preparations

Overview of Components

The biological base of *Ancient DNA Stories* is paper-based representations of real DNA sequences, so once the physical materials are produced, minimal ongoing backend support is needed to operate this activity.

We have provided instructions for two levels of material durability. Materials produced using the "Long-Term Durable Materials" method are able to serve thousands of visitors before needing to be replaced, but the process is more involved and equipment-intensive. Materials produced using the "Quick and Easy Materials" method are less durable but require significantly less prep time, which may make this a preferable choice for certain operational contexts or when first testing out the activity.

Backend preparations for this activity include:

- Production Option 1: Long-Term Durable Materials
- Production Option 2: Quick and Easy Materials

Production Option 1: Long-Term Durable Materials

Materials

Reusable Equipment	
Item	**Notes**
Printer	
Laminator	
Cricut Maker 3	Recommended but not required
12x24 Cricut StandardGrip Mat	Recommended if using a Cricut
12x12 Cricut StrongGrip Mat	Recommended for thick magnets if using a Cricut
Cricut Knife Blade	Recommended for thick magnets if using a Cricut
Scissors or knife	To clean up edges
Paper cutter	If not using a Cricut

Consumable Supplies	
Item	**Notes**
Teslin synthetic paper	Recommended over cardstock as it is waterproof and less likely to peel even with cut edges
Laminate pouches	Matte recommended for a more polished look
Adhesive magnet sheets	Thick magnets recommended as thin ones may not adhere through a laminated sheet

Procedures

1. Print **Traitcards_editable.pdf** double-sided on Teslin.
    - Note: This version includes tabs and cutting bleed.
    - Be sure to print at 100% size or scale consistently if you plan to cut with a Cricut.
    - Manual double-sided printing can sometimes yield more consistent results (print all odd pages, then refeed into printer and print all even).
2. Print **AnswerBoards_editable.pdf** and **Displays_editable.pdf** single-sided on Teslin.
3. Laminate all pages.
4. Cut with a Cricut Maker 3 (see Supplemental Resources for details).
5. Recommended: Run cut pieces through the laminator again to ensure edges are sealed.

Time-saving Tip: Cutting Teslin By Hand?

Cut all laminated pages with a strong paper cutter, except for magnet icons. Magnet icons should be adhered to the magnetic sheet prior to cutting so that the cut edges are aligned. A very heavy-duty paper cutter may be sufficient to cut through these.

Production Option 2: Quick and Easy Materials

Materials

Reusable Equipment	
Item	**Notes**
Printer	
Scissors	
Laminator	Recommended
Paper cutter	Recommended

Consumable Supplies	
Item	**Notes**
Cardstock	
Laminate pouches	Recommended
60 mil adhesive magnet sheets	Recommended

Procedures

1. Print **Simplified_doublesided_materials.pdf** double-sided on cardstock.
    - Note: This version does not include tabs or cutting bleed, and DNA strips are single-sided.
2. Print **Simplified_singlesided_materials.pdf** single sided on cardstock.
    - Note: While magnet files are included, these are time-consuming to make and may not be worth using in prototypes. Consider using adhesive velcro strips as a prototype alternative or making paper worksheets for visitors to fill in.
3. Optional: Laminate all pages.

4. Cut out DNA strips.
5. Optional: Run cut pieces through the laminator again to ensure edges are sealed.

Common Backend Questions

Standard operating procedures for this activity will vary based on the unique context of a given institution. Factors such as physical spaces, programming frequency, equipment availability, staffing models, and audience characteristics will introduce constraints and preferences that the general procedures above can be adapted to accommodate. Below are answers to the most common operational questions and insights from our experience running the activity in the Biotinkering Lab.

Question	Information
Can I use pre-cut magnets?	*Sure! You just might need to modify the print sizes.* Magnets are available in many different sizes and shapes. Find something of a decent size and adjust the files to match. This might be helpful if you do not have the tools to easily cut thick magnet sheets.
Why is the laminated sheet cloudy or wrinkled?	*Check your temperature settings.* Cloudiness may be due to insufficient heat to melt the laminate film. Waves and irregularities are often due to excessive heat.
Why are the edges peeling?	*This may be due to insufficiently laminated edges or long-term wear and tear.* Cutting laminated sheets may cause the surrounding area to peel. We recommend running them through the laminator again to rectify this issue. Pages that are beginning to peel due to age may be salvaged the same way.
Why is the Teslin smearing in the printer?	*Check your printer settings.* If you are having issues with smears, try adjusting your printer settings. While “Teslin” is not a typical option, using settings for “transparency” or other smear-prone materials may lead to cleaner results.
Why won’t the double-sided pages line up?	*This may be due to a poorly aligned printer.* Some printers become misaligned over time and tend to print off-center.
Any tips on drawing paper size and format?	*Blank quarter sheets of paper work, but we recommend providing a prompt.* Paper with a printed prompt yields the most on-topic creations. Separate prompts for each individual are the most effective but require additional organization and supply-level monitoring. We print quarter sheets with the generic header “Ancient DNA Stories” and prune off-topic art after each session.

Activity Files

Downloadable activity files for *Ancient DNA Stories* can be found online on The Tech's "Resources for Biotinkering" website (thetech.org/BiotinkeringResources). The files are organized by which production method is being used and can be accessed directly at: thetech.org/BiotinkeringResources/AncientDNA.

Production Option 1: Long-Term Durable Materials

Choose these files if you plan to cut with a Cricut or want to make modifications. These versions are Adobe Illustrator-compatible, with the cutting bleed and tab layers visible.

- **AncientDNA_Editable_DoubleSided.pdf**
  - All items meant to be printed double-sided (Traitcards and DNA sequences)
  - Warning! This is a large file.
- **AncientDNA_Editable_SingleSided.pdf**
  - All items meant to be printed single-sided (Labels, Magnets, Answer boards, Answer Key, Biosketches)
- **CutLines_individual_svg_files.zip**
  - Individual files ready to load into Cricut Design Space
- **CutLines_all.pdf**
- **DrawingSheets.pdf**

Production Option 2: Quick and Easy Materials

These versions are smaller files that are ready-to-print with minimal cutting requirements. DNA strips are single sided, tabs on traitcards are hidden, and all bleed layers are turned off.

- **AncientDNA_MinimalCutting_DoubleSided.pdf**
  - All items meant to be printed double-sided (Traitcards)
- **AncientDNA_MinimalCutting_SingleSided.pdf**
  - All items meant to be printed single-sided (DNA strips, Labels, Magnets, Answer boards, Answer Key, Biosketches, and display cards)
- **DrawingSheets.pdf**

Supplemental Resources

Full Materials List and Recommendations from The Tech

Reusable Equipment		
Item	**Notes**	**Specific Recommendations**
Printer		
Cricut Maker 3	Recommended but not required	We use in combination with a 12 x 24 Cricut StandardGrip Mat, 12 x 12 Cricut StrongGrip Mat, and a Cricut Knife Blade to produce durable activity materials.
Paper cutter	Recommended if not using a Cricut	
Scissors or knife	To clean up edges	We use an X-Acto knife.
Laminator	Recommended	
File organizers	So visitors can easily see all traitcard options	We use two 3-tier file organizers.
Storage baskets	To hold printed strips of DNA	We use six small plastic baskets.
Magnetic boards	For visitors to share puzzle answers on	We use double-sided tabletop whiteboard easels for answer boards and magnets.

Consumable Supplies		
Item	**Notes**	**Specific Recommendations**
Teslin synthetic paper	Recommended over cardstock as it is waterproof and less likely to peel even with cut edges	We use 10 mil 8.5” x 11” sheets from Brainstorm ID.
Cardstock	If not using Teslin	
Laminate pouches	Recommended	We use 5 mil matte Oregon Lamination Pouches.
Adhesive magnet sheets	Recommended	We use 60 mil 8”x10” sheets from Flexible Magnets.
Paper	For visitor drawings	We use quarter sheets with a printed prompt.
Markers		

Cutting with a Cricut Maker 3

Getting started and cutting laminated Teslin

1. Load each cut file individually into Cricut Design Space.
    a. Upload file. Click “add to canvas” and save each file as a new project.
    b. Change the operation of the corner marks from “basic cut” to “pen.”
        i. This lets you use the corner marks as guidelines, without making any extra cuts. No actual pen tool is required, the lines are only used for alignment.
    c. Select all cuts and pen marks and click “attach.”
        i. This groups everything together so that the pen and cut lines are moved as a single object during the align-to-mat step.
2. Click “Make it” to proceed.
    a. Choose “On Mat” with “12 in x 24 in” size.
    b. Align a corner mark to 1x1 intersection (or other recognizable landmark).
3. Click continue and connect the machine.
4. Define a custom material (“laminated Teslin”) with the following specifications:
    a. Fine-Point Blade
    b. 350 cut pressure
    c. 6x cut
5. Carefully align laminated page on the cutting mat to match chosen digital alignment.
6. Carefully feed mat into Cricut.
7. Cut!
8. Remove project from mat.

Making magnets

1. Load the magnet cut file to Design Space as outlined above.
2. Place an adhesive magnet sheet on a heavy-duty mat. Alignment is less finicky, just make sure that the cuts will happen in the boundaries of the blank sheet.
3. Load the knife blade onto the Cricut.
4. The Cricut does not allow custom materials for the knife blade, but the 1/16” Basswood setting worked well. Each sheet will take ~45 min to cut.
5. We recommend wiping down the cut edges with a damp paper towel to remove any residue.
6. Remove adhesive and carefully add the pre-cut laminated image. Press well to seal.

Tips

- While the Cricut is scanning the mat, check if the mat seems reasonably straight. With bleed, it does not need to be perfectly aligned. However, angled mats will lead to less-than-ideal cuts.
- Clean the blade regularly to remove buildup. Poor cuts are often due to debris accumulation.
- As your mat starts to wear out, flip the mat around and/or align to other landmarks.
- An X-Acto knife or sharp scissors may be required to neaten imprecise cuts.

- As tabs are symmetrical, only unique shapes are included.
- As you can rerun cut jobs, it is most efficient to do all pages with a shared shape sequentially.
- Depending on the size of your magnet sheets, you may be able to squeeze in more cuts per sheet. Additional magnet cut lines can easily be added in the Cricut Design Space as needed.

The Stories Behind the DNA

Ötzi the Iceman

In 1991, hikers found a body face down in a glacier in the Alps. Authorities initially thought it might be a victim of a mountain climbing accident, but further investigation revealed that the body was over 5300 years old. Ötzi was extraordinarily well preserved, in large part due to the frigid conditions that effectively mummified his body while leaving much of the DNA intact.

Ötzi has been extensively studied. Examination of his body revealed he was about 45 years old when he died, 1.6 m tall (5'3"), and 50 kg (110 lb). X-rays revealed an arrowhead embedded in his shoulder, most likely the cause of death. He was found with tools, remnants of clothing, and weapons, which helped to reveal much about how he lived. He is also the oldest known case of Lyme disease.

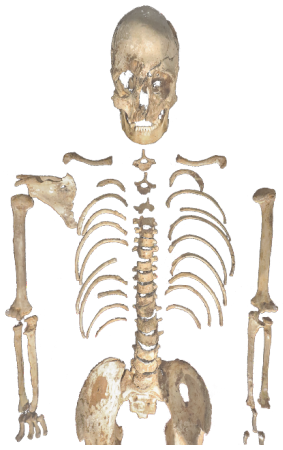
Good-quality DNA was extracted from his left ilium (hip). Additional DNA recovered from his digestive tract helped reveal what he last ate.

Want to learn more?

- Ötzi the Iceman, Museum of Archaeology Bolzano
- Scientists reconstruct Ötzi the Iceman's frantic final climb (with DNA!)

Traitcard	Answer	Actual Genotype	Reference
Eye Color	Brown	OCA2: rs12913832 A/A (ancestral) (but prediction based on many SNPs)	*Keller et al (2012)*
Hair Color	Brown	SLC45a2: rs16891982 C/C (ancestral) (but prediction based on many SNPs)	*Keller et al (2012)*
Skin Color	Light	SLC24a5: rs1426654 A/A (derived) (but prediction based on many SNPs)	*Keller et al (2012)*
Hair Type	Wavy	TCHH: rs17646946 Hair type unreported	N/A
Blood Type	O	ABO: rs8176719 -/-	*Keller et al (2012)*
Lactose Tolerance	Intolerant	LCT: rs4988235 C/C (ancestral)	*Keller et al (2012)*
Disease Bacteria	Borrelia	Based on microbial DNA found (activity does not use real sequence)	*Keller et al (2012)*
Last Meal	Goat	Based on DNA found in stomach (activity does not use real sequence)	*Maixner et al (2018)*

Cheddar Man



In 1903, a skeleton was found in Gough's Cave in Cheddar Gorge, England. At 10,000 years old, Cheddar Man is Britain's oldest complete human skeleton.

Examination of the skeleton revealed he was probably in his early 20s and about 1.66 m (5'5") tall. The cause of the large lesion above the skull's right eye orbit remains in dispute. While this initially led to the hypothesis that Cheddar Man died violently, it may have been caused after the bones were rediscovered.

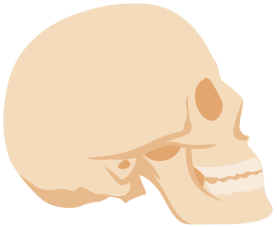
The cool temperature of the cave helped to protect the DNA quality. DNA was extracted from the petrous bone (skull near the ear).

Want to learn more?

- Cheddar Man: DNA shows early Briton had dark skin

Traitcard	Answer	Actual Genotype	Reference
Eye Color	Blue	OCA2: rs12913832 G/G (derived) (but prediction based on many SNPs)	*Brace et al (2019)*
Hair Color	Brown	SLC45a2: rs16891982 C/C (ancestral) (but prediction based on many SNPs)	*Brace et al (2019)*
Skin Color	Dark	SLC24a5: rs1426654 G/G (ancestral) (but prediction based on many SNPs)	*Brace et al (2019)*
Hair Type	Curly	TCHH: rs17646946 G/G	*Brace et al (2019)*
Blood Type	A	ABO: rs8176719 True blood type unreported Hunter-Gatherer allele freq estimated as: 70% O, 30% A	N/A
Lactose Tolerance	Intolerant	LCT rs4988235 C/C (ancestral)	*Brace et al (2019)*
Ancestry	Western HG	Based on many loci (activity does not use real sequence)	*Brace et al (2019)*

LBK380 ("Stuttgart Woman")

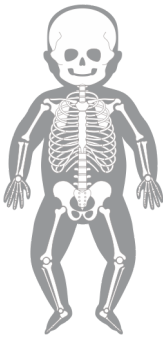


Excavated in 1982 at the Viesenhäuser Hof site near Stuttgart, this ancient individual died around 7,000 years ago. Based on morphology, we know LBK380 was a woman who died between the ages of 20-30.

This site has a large number of well-preserved burials belonging to the Linear Pottery Culture (LBK). She was buried in a specific orientation, aligned East-Northeast to West-Northwest with her skull facing north, a common characteristic for the LBK culture. Detailed pathology studies suggest she may have had primary hyperparathyroidism. Good-quality DNA was extracted from her lower right molar.

Traitcard	Answer	Actual Genotype	Reference
Eye Color	Brown	OCA2: rs12913832 A/A (ancestral) (but prediction based on many SNPs)	Lazaridis et al
Hair Color	Dark	SLC45a2: rs16891982 C/C (ancestral) (but prediction based on many SNPs)	Lazaridis et al
Skin Color	Light	SLC24a5: rs1426654 G/G (ancestral) (but prediction based on many SNPs)	Lazaridis et al
Lactose Tolerance	Intolerant	LCT rs4988235 C/C (ancestral)	Lazaridis et al
Tooth Shape	Not shoveled	EDAR: rs3827760 A/A (ancestral)	Lazaridis et al
Starch digestion	High	AMY1: 16 copies (high)	Lazaridis et al
Ancestry	Eastern Farmers	Based on many loci (activity does not use real sequence)	Lazaridis et al

Xach'itee'aanenh T'eede Gaay ("Sunrise Child")



In 2006, a 6-week-old baby girl who lived 11,500 years ago was found in the Upper Sun River region of Alaska. She was named *Xach'itee'aanenh T'eede Gaay*, which means "Sunrise Girl-Child" in a local Athabascan language. She was found with a second, younger infant whom genetic analysis revealed to be a close relative, such as a cousin.

Her genome is the oldest complete genome of a New World human and revealed a previously unknown population of people who are related to — but older and genetically distinct from — modern Native Americans. This group is now called the "Ancient Beringians."

Traitcard	Answer	Actual Genotype	Reference
Eye Color	Brown	OCA2: rs12913832 A/A (ancestral) Eye color unreported. Prediction based on Native allele frequency.	N/A
Skin Color	Intermediate	SLC24a5: rs1426654 G/G (ancestral) (but prediction based on many SNPs)	Posth et al
Lactose Tolerance	Intolerant	LCT: rs4988235 C/C (ancestral)	Posth et al
Tooth Shape	Shoveled	EDAR: rs3827760 A/G	Posth et al
Body Fat	Intermediate	TBX15: rs2298080 A/G	Racimo et al
Diabetes Risk	Average	SLC16A11: rs13342232 A/A (ancestral)	SIGMA Consortium
Altitude Adaptation	Yes	NOS3: rs1799983 G/G (derived)	Fehren-Schmitz & Georges
Sex	Female	Y chromosome absent	Posth et al

ARZN-1 (“Arzhan”)

The Arzhan sites in Tuva, Siberia, were burial grounds for important Scythians (circa 800 BCE). The Scythians were a nomadic Eurasian group who are best known for their horse-mounted warfare. They were possibly the first people to begin riding horses, and they invented many techniques that made this more feasible. The Scythians believed that their burial goods would follow them into the next life. Because of this, their burial sites are a rich treasure trove of information about their lives and culture. Along with other goods, horses were commonly buried with important people. The more important the person, the more horses.

ARZN-1 was found in the Arzhan-1 location, which contains 29 different archaeological burials and more than 160 horses. All horses buried in this location were stallions older than 12-15 years. DNA was extracted from the metacarpus (lower leg) of ARZN-1.

**This skull image is not actually from ARZN-1.*

Want to learn more?

- Horses: A Scythian’s best friend
- Ancient Horse DNA Shows Scythian Warriors Were Adept Domesticators
- A bit more on horse color genetics

Traitcard	Answer	Actual Genotype	Reference
Coat Color #1	Red pigment	MC1R: e/e	*Ludwig et al*
Coat Color #2	Not diluted	Silver: z/z	*Wutke et al (2016)*
Coat Color #3	No patches	KIT13: KM0/KM0	*Wutke et al (2016)*
Coat Color #4	Spotted	TRPM: LP/lp	*Ludwig et al*
Final coat color	Spotted chestnut		
Sex	Male	Y chromosome present	*Wutke et al (2018)*
Comfortable to ride	No	DMRT: C/C	*Wutke et al (2016)*
Racing Performance	Intermediate	ACTN2: heterozygous (prediction based on multiple SNPs)	*Librado et al*
Body Size	Large	HMGA2: T/T (prediction based on multiple SNPs)	*Librado et al*, *Makvandi-Nejad et al*

Hatch, the Mary Rose Dog

The Tudor warship *Mary Rose* sank off the coast of Britain on July 19, 1545, while fighting a French invasion fleet. The ship was rediscovered in 1971. A small- to medium-sized dog skeleton was found near the ship carpenter's cabin (general dog skull image, not specifically Hatch).

Dogs were often kept onboard ships to catch rats. While Hatch can't be attributed to a specific breed (modern breeds originated after this time period), he was most closely related to the modern Jack Russell terrier. Terrier-type dogs in particular were originally bred to be excellent vermin hunters.

Want to learn more?

- Mary Rose museum website
- DNA testing shows Hatch was male, not female

Traitcard	Answer	Actual Genotype	Reference
Coat Color #1	Has dark pigment	MCR1: E/E	*Zouganelis et al*
Coat Color #2	Brown	TYRP1: b/b	*Zouganelis et al*
Coat Color #3	Diluted	MLPH: D/d Unreported, true genotype unknown	N/A
Coat Color #4	Sable	ASIP: a^y/a^w	*Zouganelis et al*
Final coat color	Light brown sable		
Sex	Male	Y chromosome present	*Zouganelis et al*
Starch Digestion	Medium	AMY2B Unreported for Hatch, but dogs have amylase expansion by this time period	*Ollivier et al*
Breed	Terrier	Prediction based on many loci, though not a perfect correlate to modern breeds	*Zouganelis et al*
Bladder Stones	Low risk	SLC2A9: wt/mutant (carrier)	*Zouganelis et al*

More Details About the DNA Sequences on the Traitcards

Human

1. Eye color: OCA2 variant (rs12913832); estimate of forensic SNPs from HIrisPlex-S
2. Hair color: SLC45a2 variant (rs16891982); estimate of forensic SNPs from HIrisPlex-S
3. Skin color: Two real variants in SLC24a5 shown; rs1426654 is on the right. Left variant is a real SNP, but has <1% incidence of A allele; estimate of forensic SNPs from HIrisPlex-S.
4. Hair type: Two real variants in TCHH shown; rs17646946 is on the right.

5. Blood type: Left SNP (rs8176719) determines Type O. However, the right “T” variant does not exist; Type A and Type B are distinguished by SNPs in other portions of the ABO gene.
6. Lactose: LCT variant (rs4988235)
7. Otzi bacteria: Not real sequence
8. Last meal: Not real sequence
9. Ancestry: Not real sequence
10. Tooth shape: EDAR variant (rs3827760)
11. Starch digestion: AMY1 sequence, but true copy numbers are much higher.
12. Diabetes: SLC16A11 variant (rs13342232)
13. Altitude: NOS3 variant (rs1799983)
14. Body fat: TBX15 variant (rs2298080)
15. Sex: SRY gene. Males typically have one copy, but two are included for easier solving.

Horse

1. Coat color 1: MC1R variant (C901T; equCab2 chr3:36259552; also known as E locus)
2. Coat color 2: KIT13 variant (C786G; equCab2 chr3:77735520)
3. Coat color 3: PMEL17 variant (C1457T; equCab2 chr6:73665304; also known as Silver gene)
4. Coat color 4: TRPM1 variant (rs12913832; equCab2 chr1:108249293; also known as LP gene)
5. Riding: DMRT variant (equCab2 chr23:22999655)
6. Sex: SRY gene, homologous to human sequence. Males typically have one copy, but two are included for easier solving.
7. Body size: HMGA2 variant (equCab2 chr6:81481065)
8. Racing: ACTN2 variant (equCab2 chr1:74842283)

Dog

1. Coat color 1: MC1R variant (C914T; also known as E locus)
2. Coat color 2: TYRP variant (C991T; also known as B locus)
3. Coat color 3: MLPH variant (also known as D locus)
4. Coat color 4: ASIP variants (also known as A locus). Haplogroups for “saddle/sable” (a^y) and “recessive black” (a) are accurate. However, the haplogroups for “agouti” (wolf sable; a^w) and tan points (a^t) are distinguished by variants in other parts of the gene.
5. Sex: SRY gene, homologous to human sequence. Males typically have one copy, but two are included for easier solving.
6. Starch digestion: AMY2B sequence, homologous to human sequence
7. Breed: Not real sequence
8. Bladder stones: SLC2A9 variant