Sequencing a Genome:
Inside the Washington University Genome Sequencing Center

TOUR SCRIPT

Narrator: *The Washington University Sequencing Center is a member of the International Human Genome Consortium and is world renown for its work contributing to the sequence of the Human Genome and the genomic sequences of several other organisms.*

SCENE 1

Exterior/Shuttle Bus: Bryson runs down stairs to catch shuttle bus.

Bryson: *Hold the bus. Hold the bus, thank you.*

CUT TO
Interior/Shuttle Bus: Bryson takes a seat in the middle of the bus. Libby is sitting across the aisle. They nod to each other as they sit.

Bryson: *Thanks. Are you in my genetics class?*

Libby: *Yeah, I thought I recognized you.*

Bryson*:* *I'm sorry; I'm Bryson.*

Libby: *I'm Libby.*

Bryson: *Nice to meet you.*

Libby: So *where are you headed?*

Bryson: I'm on my way to *The Genome Sequencing Center. I'm taking a tour today.*

Libby: Y*ou're kidding, me too.*

Bryson: *Okay, I guess I'm on the right shuttle.*

SCENE 2

Exterior/GSC: Libby, Bryson, and Extras exit shuttle bus on Forest Park Boulevard. Libby and Bryson walk up sidewalk and enter the front door of the GSC building.

Narrator: *Welcome to the Genome Sequencing Center at Washington University. I am Andrea Holmes. On this tour today I'll show you the step-by-step processes involved in sequencing a genome, and I'll also point out the different career opportunities available to someone who's interested in working in a lab facility like the Sequencing Center.*

CUT TO

Interior/GSC: Libby and Bryson meet Andrea in front of the GSC Outreach bulletin board.

Andrea: *Let me give you a little history. We started back in 1990 with a team of 5 people and a budget of about 5 hundred thousand dollars.*

CUT AWAY SHOT: shots of different employees doing different jobs

We currently employ over 150 people and a budget in the millions of dollars. We have jobs ranging from administrative personnel to research technicians, scientists, and engineers. We also employ information technology specialists who are responsible for developing and maintaining all of our computer systems.

Bryson: *That sounds really complicated. How do you keep track of everything?*

Andrea: *The genomes of many organisms can be large*

CUT AWAY ILLUSTRATION: comparison of genome sizes

and keeping track of everything is a huge job. You will notice as we go along that we utilize a bar coding system

CUT AWAY SHOT: close up of technician's hand scanning a barcode on a plate of DNA

to keep track of everything from growth media to the individual DNA samples. We also use powerful servers

CUT AWAY SHOT: GSC Server Room

with large storage capacities for handling terabytes of data.

Libby: *So what other genomes have been sequenced here?*

Andrea: *The Center has sequenced the genomes of a roundworm, C. elegans;*

CUT AWAY PHOTO: photo of *C. elegans*

a plant, Arabidopsis thaliana;

CUT AWAY PHOTO: photo of *Arabidopsis*

and two bacteria, Salmonella and E. coli.

CUT AWAY PHOTO: photo of *Salmonella*

Currently we are sequencing the genomes of Gallus gallus, a wild chicken;

CUT AWAY PHOTO: photo of wild chicken

chimpanzee;

CUT AWAY PHOTO: photo of chimp

and mouse.

CUT AWAY PHOTO: photo of mouse

We also sequenced the genomes of the SARS virus

CUT AWAY PHOTO: photo of SARS newspaper headline

and an E. coli strain responsible for causing the majority of urinary tract infections.

CUT AWAY PHOTO: photo of infectious *E. coli*

Bryson: *So how do you actually sequence a genome?*

CUT AWAY ILLUSTRATION: overview of the sequencing pipeline

Andrea: *Well, the overall system is comparable to an assembly line, but the product is information. There are a number of steps that contribute to the finished product, which is the sequence of DNA base pairs that spell out an organism's genome. As we walk through the various labs, we will follow the process that leads to a sequenced genome. Okay, let's go on to Media Core.*

SCENE 3

Media Core Lab: Andrea and students enter Media Core Lab. Behind and to the left of them, technicians are mixing liquid media on stir plates.

Andrea: *There are two groups that provide support to each of the groups in the Genome sequencing center. Those groups are Media Core and Materials Core.*

CUT AWAY SHOT: footage of technician pouring large agar plates in hood, shots of materials bottles, footage of technician preparing liquid media

In Media Core they prepare all of our liquid growth media as well as pour all of our agar plates. In Materials Core they provide solutions for every single step in the process. In Media Core they prepare over 10,000 plates each week and over 2,000 liters of liquid growth media. They also sterilize all of our glassware.

Libby: *Why is it necessary to sterilize the glassware?*

Andrea: *We have to sterilize the glassware because we reuse it.*

CUT AWAY SHOT: footage of glassware being removed from autoclave

By sterilizing the glassware it ensures that we get rid of all contaminants. If we have contamination it might make us think that DNA from one organism belonged to another organism. And that would be a huge mistake! Okay now we're on our way to Mapping Core.

SCENE 4

Mapping Core Lab: Group enters Mapping Core Lab. Technician is pouring gels in background.

CUT AWAY SHOT: footage of technician pouring gels

Andrea: *As you know, a genome is all of the DNA in an organism. This is way too much to sequence at once – the human genome itself is over 3 billion base pairs long! So, in Mapping Core we receive a genome that has been broken up into pieces that are approximately 2 hundred thousand base pairs long.*

CUT AWAY ILLUSTRATION: genomic DNA being broken up and put into BACs

Each one of those pieces has been inserted into its own cloning vector, creating a huge bacterial artificial chromosome, called a BAC for short.

Bryson: *So you use bacteria to hold DNA from other organisms?*

CUT AWAY ILLUSTRATION: propagation of a bacterial cell containing a BAC

Andrea: *Exactly, and this trick allows us to prepare large amounts of a specific piece of DNA, by starting with a single bacterial cell containing one BAC, and letting that cell grow and divide. When we receive a genome in Mapping Core, we are actually receiving many individual bacterial cell lines, and each cell line carries one of the pieces of the genome in a BAC.*

Bryson: *So if the genome is fragmented how do you know which piece is which?*

Andrea: *Great question! The answer is we don't know. The process that fragments the genome is random. So although a total collection of BACs contains all of the different genomic DNA fragments, we don't know in what order they fit together to make the whole genome.*

CUT AWAY ILLUSTRATION: fingerprinting BACs

Here in Mapping Core, we put the fragments in order. We use restriction enzymes to cut the BACs at specific sequences and then we separate the digested DNA with gel electrophoresis. This gives us "fingerprints," or gel banding patterns of the BAC DNA. Those BACs with similar banding patterns contain overlapping segments of the genome. Knowing where the fragments overlap allows us to construct a map of the fragments in the correct order.

CUT AWAY ILLUSTRATION: mapping BACs

Bryson: *So Mapping Core makes maps of the genomic fragmented DNA.*

Libby: *And now that you know the order, you send all the BACs on to be sequenced.*

Andrea: *No, that would be way too expensive. It's more efficient for the technicians to use a computer program.*

CUT AWAY ILLUSTRATION: clone selection and the Golden Path

This program compares the overlaps and chooses the smallest set of BAC clones possible to cover the whole genome, kind of like stepping-stones. We call this set the "Golden Path." And it is this set of BACs that are sent through our Production Pipeline.

Libby: *I'm guessing this is where the computer bar coding system comes in handy?*

Andrea: *Exactly, it's very important to keep track of which BACs are sent on and how the DNA sequences they carry fit together.*

CUT AWAY ILLUSTRATION: the Golden Path

The first stop in the Production Pipeline is Library Core; let's go there now.

SCENE 5

Library Core: Group enters Library Core Lab and stands in front of incubators.

Andrea: *So now we're in Library Core.*

Libby: *How does the BAC DNA get from Mapping Core to Library Core?*

CUT AWAY SHOT: footage of technician streaking a plate and placing it into incubator

Andrea: *Well, a technician from Mapping Core streaks the bacteria containing the selected BACs onto small agar plates. These plates are placed in a 37˚C incubator and grown overnight.*

CUT AWAY SHOT: footage of different technician removing plate from incubator and inoculating liquid culture.

The next morning, a technician from Library Core removes the plates and picks single colonies to inoculate 250-milliliter liquid cultures.

Libby: *Why do you need such large liquid cultures?*

CUT AWAY SHOT: shot of clear growth media and overnight culture displaying growth

Andrea: *In order to have enough DNA to sequence, we must grow up millions of copies of the BACs.*

Libby: *Oh. So since each bacterial cell contains one copy of the BAC, millions of copies of the bacteria will contain millions of copies of the BAC.*

CUT AWAY ILLUSTRATION: growth of bacterial cells in liquid growth media

Andrea: *You got it. Now the DNA must be extracted and purified from the bacterial culture. This is where the cells are lysed, proteins removed, and clean DNA is precipitated. To be sure that this is the correct BAC, a little of the clean DNA is cut with restriction enzymes and run on a gel.*

CUT AWAY SHOT: footage of technician doing midi prep to isolate DNA

Bryson: *Just like in Mapping?*

Andrea: *Yes, the same procedure. This is actually a checkpoint for the DNA.*

CUT AWAY ILLUSTRATION: test purified BAC DNA with restriction enzymes

We want to make sure the DNA grown up in the culture is the same as the original BAC DNA we chose as part of the "Golden Path" in Mapping Core, so we

compare the banding patterns of this gel with the earlier one from Mapping. Next, the rest of the clean DNA is sonicated.

Libby: *Sonicated?*

Andrea: *Sonicated.*

CUT AWAY ILLUSTRATION: action of sonicator

Sonication is the process of breaking up of DNA into smaller fragments by exposure to intense sound waves. These smaller fragments are run on a gel with sizing markers.

CUT AWAY SHOT: footage of gel rigs and technician cutting bands from gel

Specific size fragments are cut out from the gel and the DNA isolated from the gel matrix. The DNA fragments are then treated with a specialized enzyme called a nuclease that makes them blunt-ended.

Bryson: *What do you mean by blunt-ended?*

Andrea: *Remember, DNA is double-stranded and sonication may cause the DNA to break so that one of the strands is a little longer than the other.*

CUT AWAY ILLUSTRATION: DNA overhang and action of nuclease to make blunt ends

We call this an overhang. We need to fix any overhangs and a nuclease will do this.

Libby: *So now that you have the genome in pieces, what do you do with those pieces?*

CUT AWAY ILLUSTRATION: BAC being broken down into smaller fragments

Andrea: *As you may have guessed by now, we can't take an intact genome and stick it into a sequencing machine. An intact genome is just too big, and a BAC is still too large for a sequencing machine to handle. So each BAC must be broken down again into smaller pieces of about 2 thousand base pairs.*

Bryson: *And that's what you use the sonicator for.*

Andrea: *Correct. And now that the DNA is in smaller pieces, we need to make many copies of these fragments.*

CUT AWAY ILLUSTRATION: ligation

To do this, the fragments are ligated, or attached, to a vector and then put into a competent cell using electroporation. The cells are then spread onto these large agar plates and are incubated at 37 degrees overnight.

Libby: *Can you explain electroporation?*

Andrea: *Well, you know what bacterial transformation is, right?*

Libby: *That's just inserting foreign DNA into a bacterial cell.*

Andrea: *Exactly, electroporation is one technique for doing bacterial transformation.*

CUT AWAY SHOT: footage of technician doing electroporation

CUT AWAY ILLUSTRATION: electroporation

We shock the bacterial cells at high voltage and the electric current creates holes in the cell membranes. These holes are large enough for foreign DNA to pass through and we end up with bacterial cells carrying the DNA that we want to sequence.

Andrea: *Okay, we're off to Picking Core now. I think you will really like it there.*

SCENE 6

Picking Core Lab: Andrea and students enter Picking Core Lab. Technician is at Q-Pix machine in background.

Andrea: *The Picking Core technician retrieves large agar plates from the incubator and then she places them on the Q-Pix machine.*

CUT AWAY SHOT: footage of Q-Pix machine in action

The machine will then scan each of the plates and looks for nice, uniform, isolated colonies. The 96 needle robotic arm will then pick these colonies, place them into glycerol and growth media that are contained in the 384 well plates.

CUT AWAY ILLUSTRATION: procedure for picking clones

The computer software is so sensitive that it will not allow colonies that are too close together to be picked. So each clone that's picked represents a single transformation event.

Libby: *This is a lot better than picking colonies by hand with toothpicks!*

Bryson*:* *So this robot is inoculating mini-liquid cultures?*

Andrea*:* *Exactly, after incubation overnight the 384 well plates are brought to a spectrophotometer, which is a machine that sends a light through the bottom of the culture and checks for cell growth.*

CUT AWAY SHOT: footage of technician at spectrophotometer taking OD readings

Libby: *What happens if it doesn't grow?*

Andrea: *We don't worry if a few wells per plate don't grow. If a significant number did not grow, we discard that plate and then we'll go back to the agar plate and pick new colonies. This is an important quality control and cost saving step.*

CUT AWAY SHOT: footage of technician at Multi-Mek carousel machine in action

In Inoculation Core, they are responsible for taking a small aliquot of these cultures and transferring them into a larger culture, which is a 96 well plate.

Bryson: *So if you are only going to use an aliquot, what happens to the rest of the old culture?*

Andrea: *The plates are archived in freezers.*

CUT AWAY SHOT: footage of technician placing plates in freezer

We always want to be able to go back in case there is an error further along in the Production Pipeline. This way we do not have to start the entire process all over again. Okay, let's go to Prepping.

SCENE 7

Prepping Core Lab: Andrea and students enter Prepping Core Lab and stand behind the DNA Plate Trak machine.

Andrea: *Now, back to those 96-well plates. The new liquid cultures, here in Prepping Core, are picked up by the technician and incubated overnight. The next morning the technicians will remove the plates from the incubator, and they will spin the cells down and pellet them in a centrifuge, and they'll just discard the supernatant.*

CUT AWAY ILLUSTRATION: harvest bacteria

So can you tell me the first step in isolating DNA from cells?

Libby: *First you have to lyse the cells to break open their membranes.*

Andrea: *Exactly. The technicians here in Prepping Core will add a resuspension buffer to those cells.*

CUT AWAY SHOT: footage of technician adding resuspension buffer

The pellets are then resuspended and vortexed and then the plates are placed on the Plate Trak.

CUT AWAY ILLUSTRATION: resuspended bacteria and addition of lysis solution and magnetic beads

CUT AWAY SHOT: footage of DNA Plate Trak machine in action

The robot begins by adding a magnetic beads solution that also contains a lysis solution. Each plate goes through a series of steps. Although the Genome Sequencing Center uses a robot for this process, in many research labs they simply do it by hand. So do you know what comes next?

Libby: *So now you have to remove all the non-DNA stuff, right?*

Andrea: *Exactly. All the proteins and cellular debris must be removed, and this is done by the magnetic beads. They are positively charged and they are porous.*

Bryson: *Since DNA is negatively charged, it binds to the beads.*

CUT AWAY ILLUSTRATION: magnetic bead prep

Andrea: *Exactly. In fact, the plate is gently vortexed to ensure that as much DNA as possible binds to the beads. Next the plate is moved over a powerful magnet, pulling the DNA-bound beads into the bottom of the plate, and then the supernatant is removed by a vacuum. After several washes to remove residual*

proteins, the DNA is clean and ready to use in sequencing reactions. So, now we're ready to go to Sequencing Core.

SCENE 8

Hallway: Students and Andrea walk toward Sequencing Core and meet Dr. Wilson in hall.

Dr. Wilson: *Hey, Andrea. Another tour?*

Andrea: *Yes, this is Libby and Bryson they are two Washington University students and they are interested in our work here at the Genome Center.* (Gestures toward Dr. Wilson) *This is Dr. Rick Wilson; he's our Center's director.*

Dr. Wilson: *Nice to meet you! What brings you guys here today?*

Libby: *I'm researching pathogenic bacteria in one of my classes and so I though I would come see how sequencing works.*

Bryson: *And I'm researching career options for after graduation.*

Dr. Wilson: *Terrific, well I think you guys have come to the right place. As I'm sure Andrea has told you, since we've finished sequencing the human genome, we've moved on to the genomes of other organisms including pathogenic bacteria so we can better understand interactions between hosts and pathogens.*

Bryson: *So will there still be jobs even though the human genome is done?*

Dr. Wilson: *Absolutely. We've gone on not only to sequence the genomes of pathogens, but other animals so we can better understand the human sequence, and we've also started sequencing individual genes from patient DNA, so we can better understand the sequence changes in the genome that cause disease.*

Bryson: *That's good to know!*

Libby: *Thanks for stopping to talk with us.*

Dr. Wilson: *My pleasure. You guys have a good tour. Take care.*

Bryson: *Thank you.*

Libby: *Thank you.*

Andrea: *On to Sequencing.*

SCENE 9

Sequencing Core Lab: Andrea and students enter Sequencing Core Lab.

Andrea*:* *Okay, back to the Production Pipeline.*

CUT AWAY SHOT: shot of DNA-coated magnetic beads in the bottom of a 96-well plate

CUT AWAY ILLUSTRATION: eluting the DNA from the beads with water

The sequencing process begins by eluting the DNA from the beads with water. Once the DNA is in solution, magnetic beads and pulled down to the bottom, because they are placed on another magnet. Now the DNA is clean and ready to be sequenced and this process begins by placing them on the Biomek machines.

Libby: *Another robot?*

CUT AWAY SHOT: footage of the Biomek robot in action

Andrea: *Yes, this robot transfers the clean DNA from the wells into a 384-well plate containing a PCR reaction mix. Including a small amount of each DNA base with different fluorescent dyes attached.*

Bryson: *So, what's the purpose of the PCR reaction?*

CUT AWAY SHOT: shot of PCR machines and technician loading machines

Andrea: *The PCR reaction is where the DNA is amplified and fluorescently labeled bases are added to the ends of each fragment.*

CUT AWAY ILLUSTRATION: (animation with narration) dye terminator reaction

Each reaction requires multiple copies of a double-stranded DNA template, which is a fragment from the genome being sequenced; many copies of a specific single-stranded oligonucleotide primer, which acts as a starting point for DNA synthesis; Taq DNA polymerase, an enzyme that can add nucleotides to a DNA strand during DNA synthesis; free floating single nucleotides; and some fluorescently labeled terminator nucleotides that are designed to stop DNA synthesis.

Changes in temperature are the key to making the dye terminator reaction work. First, the reaction mixture is heated up to 96 degrees Celsius so that the double-stranded DNA template denatures and becomes single-stranded. Second, the reaction mixture is cooled down to 50 degrees Celsius so that the oligonucleotide primer can base pair with, or anneal to, the DNA template. Third, the reaction is warmed up to 60 degrees Celsius, so Taq DNA polymerase can perform DNA synthesis. Taq is able to put free-floating nucleotides into the correct places along the DNA template so that a new complementary strand of DNA is extended

from the primer. However, when a terminator nucleotide is put in place, Taq is no longer able to add more nucleotides and DNA synthesis is stopped.

Multiple rounds of temperature cycling are necessary for the reaction mixture to generate an array of DNA fragments of differing lengths. The PCR machine is able to make rapid transitions between the different temperatures. Each time the reaction mixture is heated for denaturing, cooled for annealing, and warmed for extension, more DNA fragments are created.

The fluorescently labeled terminator nucleotides each have their own specific color. A is green, T is red, G is yellow, and C is blue. After the multiple rounds of temperature cycling, any DNA fragments that can fluoresce green must have an A as the terminating nucleotide. Those that can fluoresce red must have a T at the end. Those that can fluoresce yellow must have a G at the end and those that can fluoresce blue must have a C as the terminating nucleotide. The random assortment of free-floating nucleotides and fluorescently labeled terminator nucleotides ensures that the newly synthesized DNA fragments are each terminated at a different place along the DNA template sequence. The goal is for the reaction to result in an array of fluorescent DNA fragments that have only a single nucleotide difference in length. The sequencing reaction is considered finished when multiple copies of every possible DNA fragment have been generated.

All of the fluorescently labeled DNA fragments created in the dye terminator reaction now need to be separated by size. This is possible with gel electrophoresis. When the fragments are put in order from smallest to largest, and the terminating fluorescent base color is analyzed, then the corresponding terminating nucleotide can be identified.

Bryson: *So the fluorescently labeled bases are the key to reading the sequence?*

Andrea: *Yes, they are critical.*

Libby: *So now we can just read the sequence, right?*

Andrea: *Not just yet. They must be loaded onto sequencing machines.*

CUT AWAY SHOT: shot of the inside of the ABI 3730 sequencer – capillary tubes

This machine has a tiny capillary tube filled with a polymer that allows the DNA fragments to separate by size when an electric current is run through the polymer.

Libby: *So that's just like regular gel electrophoresis!*

Andrea: *Yes, but unlike conventional gel electrophoresis, this machine takes it step further. It not only runs out the DNA fragments - it reads and records the sequence.*

Bryson: *How is that possible?*

CUT AWAY ILLUSTRATION: (animation) sequencing gel process

Andrea: *As a DNA fragment migrates through the polymer, it is hit with a laser beam that excites the fluorescent dye attached to the terminal base of the fragment. A camera captures an image of the fluorescence and a computer converts it to a readable form that we call an electropherogram.*

CUT AWAY ILLUSTRATION: image of good quality electropherogram

The electropherograms, or sequence trace files, are stored on servers and downloaded by employees called finishers.

Bryson: *What do finishers do?*

CUT AWAY SHOT: footage of finishers in front of computers

Andrea: *In Finishing Core they assemble many fragments of BAC sequence into one contiguous, high-quality sequence of DNA. First, the sequences are run through a set of computer programs called Phred and Phrap to assess sequence quality and line up similar sequences. The finisher then uses a program called Consed to look at the assembled data and identify problems. She examines each sequence region and can request that additional data be generated where needed.*

Bryson: *So DNA can be sequenced more than once?*

Andrea: *Yes, we refer to this as "coverage."*

CUT AWAY ILLUSTRATION: multiple reads and consensus sequence

For example, if we sequence a genome four times, we say that our data for that region has 4X coverage. The higher the coverage, the more accurate the final sequence data. As you both know, DNA is a complex molecule. This complexity can result in problems for the finishers.

CUT AWAY ILLUSTRATION: image of poor quality electropherogram

CUT AWAY ILLUSTRATION: conflicting data

Once a finisher conquers all of these problems, the end product is a contiguous sequence of DNA of high quality that can be passed on to public databases.

CUT AWAY SHOT: shot of the National Library of Medicine

We upload all of our DNA sequence data from the databases every day so that the information is freely available to researchers around the world.

Bryson: *So if finishers put the sequence fragments together to form a BAC, who puts all of the BACs together?*

Andrea: *Members of Analysis Core. They are responsible for confirming the placement of the individual BACs in the original map.*

CUT AWAY ILLUSTRATION: assemble finished sequences and annotation

They also annotate the finished sequence, or label specific regions of the sequence with appropriate names or other identifying information.

Libby: *When is the sequencing of a genome considered finished?*

CUT AWAY SHOT: photos of various organisms

Andrea: *Well, depending on the organism being sequenced, there are specific criteria for how the data is presented to other researchers.*

CUT AWAY SHOT: list of the International Human Genome Consortium members

The International Human Genome Consortium ensures that data from the Human Genome Project meets consistent standards.

CUT AWAY ILLUSTRATION: sequence finishing standards

We made sure our data for that project had 10X coverage and an error rate of less than 0.01 percent. That's less than one error per 10 thousand bases!

Bryson: *Interesting.*

CUT AWAY SHOT: shots of WormBase and FlyBase websites

Andrea: *There are consortiums formed for each of the other organisms being worked on and they determine their own criteria for coverage. Okay let's head on to Technology and Development.*

SCENE 10

Technology and Development Lab: Andrea and students enter Tech D Lab and walk toward Dr. Mardis, who is looking over shoulder of technician working at bench.

Andrea: *We are now in the Technology and Development Lab headed by Dr. Elaine Mardis.*

Dr. Mardis: *Hi, nice to meet you.*

Andrea: *Tech D is the group that tests out all our new automation and protocols before they are placed into the Production Pipeline. They are also responsible for keeping costs low.*

Dr. Mardis: *That's right, Andrea. Here in Technology Development what we strive to do is to drive the cost of sequencing ever lower, and we do that by a variety of means including the use of automation to eliminate a lot of manual steps and errors that can creep in. Over the years we've been very successful at what we do to the point where people really talk now about the possibility of having your own personal genome sequence done.*

Libby: *Wow! I didn't realize that was so close!*

Bryson: *Thank you for your time.*

Dr. Mardis: *Sure, it was really nice to meet you both.*

SCENE 11

Hallway: Students and Andrea exit Tech D and stop in front of GSC Outreach bulletin board.

Andrea: *Now that we are at the end of our tour, do you guys have any other questions?*

Libby: *Well, this is a lot of information. Can we check our notes and run it by you and make sure we've got it right?*

Andrea: *Sure!*

CUT AWAY ILLUSTRATION: overview of Preparation

Libby: *Okay, first you decide what you're going to sequence in Mapping Core.*

Bryson: *Using fingerprinting gels.*

CUT AWAY ILLUSTRATION: overview of Production

Libby: *Yeah, then you send the DNA on to Library Core where they generate a collection of specific fragments and ligate them to vectors, and then they send them on to Plating Core to be transformed with bacteria.*

Bryson: *Then we went to Picking Core, where bacterial colonies, each containing a single fragment, were harvested by the Q-Pix machine and then used to inoculate liquid cultures in 384-well plates.*

Libby: *And then they took those 384-well plates, in Inoculation Core, and transferred some to a 96-well plate for Prepping Core, and put the rest in the freezer for an archive.*

Bryson: *Purified DNA samples are sent to Sequencing Core where fragments are copied, and the terminating bases are labeled with fluorescent dyes. Then they are taken to Loading Core where the samples are put into sequencing machines and used to collect data.*

CUT AWAY ILLUSTRATION: overview of DNA Sequence Management

Libby: *And then it goes on to Finishing Core, where they assemble the data and Analysis Core, where they annotate it and send in on to the public databases.*

Andrea: *You guys are good! And remember, genome sequence data has many applications.*

CUT AWAY SHOT: shot of "Genomics and Beyond" poster

Data from the Human Genome Project is already being used by medical practitioners and researchers to improve the diagnosis and treatment of certain diseases.

Bryson: *From what you and Dr. Wilson have said, this seems like important work. Thanks for your time.*

Libby: *Yes, thanks for showing us around. I really learned a lot.*

Andrea: You're welcome, and if you have any questions just give me a call.