Infinium Chemistry Course Narration Transcript

Section	Narration
Welcome	Welcome to the Infinium Chemistry course. This course provides an introduction to the general workflow and chemistry of the Infinium assay. Click Next to continue.
Course Objectives	By the end of this course, you will be able to: • List the steps of the Infinium assay, • Describe the biochemical mechanism of each step, and • Explain how different genotypes produce different fluorescence signals
Assay Introduction	The Infinium assay is a research tool used in genome-wide association studies and analysis of chromosomal aberrations. This powerful assay provides unlimited multiplexing and unparalleled data quality.
General Steps of the Assay	The first steps of the Infinium assay are performed in the wells of a microplate. The sample is transferred from the microplate to an array, which is then imaged on an Illumina scanner. The Infinium assay consists of the following steps: Amplification, Fragmentation, Precipitation, Resuspension, Hybridization, XStain, and Imaging. Let's take a closer look at each step in the Infinium assay.
Amplification	The first step in the Infinium assay is whole-genome amplification of a purified genomic DNA sample. The DNA is denatured then amplified in an isothermal reaction. Using whole-genome amplification eliminates the GC bias of PCR. This step of the Infinium assay uniformly amplifies DNA by up to one thousand-fold.

Section	Narration
Fragmentation	The next Infinium assay step fragments the DNA sample. The purpose of this step is to cleave DNA into fragments of the optimum length for hybridization to an Illumina array. This controlled enzymatic process cleaves DNA into segments of 300 to 600 base pairs. The process uses endpoint fragmentation to avoid overfragmenting the DNA sample. This method is not as time sensitive as other fragmentation methods.
Precipitation and Resuspension	Before hybridizing the DNA sample to an array, the sample is purified by isopropanol precipitation and buffer resuspension. PM1, a blue colored reagent, is added to each sample to visualize the DNA pellet after precipitation. DNA is precipitated by centrifugation, and the supernatant is removed. After the pellet has been allowed to dry, it is resuspended in Hybridization Buffer This buffer provides ideal conditions for the next step: hybridization to the array.
Hybridization	In the hybridization step, the sample is removed from the microplate and applied to an array. An Illumina array contains millions of tiny spherical beads embedded in the array surface. Each bead is coated with hundreds of thousands of copies of a DNA oligonucleotide probe specific to a **locus of interest**. Here is a cut-away view of 3 beads on an Illumina array. Each of these beads has different probe sequences on their surface and perform a different assay. For simplicity, only 2 probe molecules are shown on each bead. The specificity of this hybridization is ensured by the length of the probes, the high stringency buffer conditions, and elevated temperatures.
XStain: Single Base Extension	The next step in the Infinium assay is XStain, which is a conjugation of extension and staining. The extension reaction adds a single labeled base to each probe. The purpose of this step is to discriminate between genotypes. The single base extension reaction uses chain-terminating dideoxynucleotides. The A and T nucleotides are labeled with dinitrophenyl (abbreviated as DNP) and the G and C nucleotides are labeled with biotin. The probes are enzymatically extended by a single base, and the label identifies the genotype. A homozygote will have identical labels bound to the bead and a heterozygote will have a mixture of biotin and dinitrophenyl labels bound to the bead. After the labels have been bound to the probes, the DNA template is washed off the array leaving only the labeled probes on the beads.
XStain: Stain	The second component of the XStain process is staining. The purpose of this step is to apply a specific fluorescent signal to each labeled probe. The first round of staining utilizes green fluorescent streptavidin and red fluorescent anti-DNP antibody. These fluorescent molecules bind specifically to the labeled probes. Next, biotin- and DNP-labeled antibodies are applied to the array. By applying successive rounds of fluorescent molecules and labeled antibodies, the signal is amplified.

Section	Narration
Imaging	After the staining process, the array is ready to be imaged on an Illumina scanner. The purpose of this step is to generate fluorescence intensity data that can be analyzed to make genotype calls for each **loci**. The Illumina scanner uses red and green lasers to excite the fluorophores and measure the fluorescence intensity signal for each bead on the array. In the example on the right, the homozygote TT produces primarily red signal while the homozygote CC produces primarily green signal. The AG heterozygote produces approximately equal red and green signals. Heterozygote bead types appear yellow in intensity images due to the emission of both red and green fluorescence at the same bead location. The Illumina scanner produces fluorescence intensity data files that are used for genetic analysis.
Review	The Infinium assay uses a single bead type and a dual-color channel approach to interrogate an infinite number of loci per DNA sample. The Infinium assay steps that take place on the microplate include: • Whole-genome amplification of a DNA sample to increase the quantity of DNA, • Endpoint fragmentation of the sample to enable access to most of the genome, and • Precipitation and resuspension of the fragmented DNA to purify the sample for hybridization, The next page will cover the Infinium assay steps that take place on an array and on an Illumina scanner.
Review Continued	The Infinium assay steps that take place on the array and on the Illumina scanner include: Hybridization of the DNA sample onto an array to interrogate **up to millions of** loci. Enzymatic single base extension of the probes to confer allele specificity using the sample DNA as a template, Staining the labeled probes on the beads with fluorescent dyes to discriminate between the different alleles for each bead type, The array is aligned in the scanner before it is imaged. Imaging the array on an Illumina scanner measures red and green fluorescence intensities. The intensities are written to intensity data files which Beeline or GenomeStudio convert into genotype calls.
Summary	You should now understand the general workflow and chemistry of the Infinium assay. Take a moment to review the key points from this course.
Quiz	No narration