ExAmp Cluster Amplification Workflow Course Narration Transcript

Section	Narration
Welcome	Welcome to the ExAmp Cluster Amplification Workflow course. Click Next to continue.
Course Navigation	Before we begin, take a moment to review tips for navigating through this training. To view these directions from any page of the course, click Help, in the top right corner of the page.
Course Objectives	By the end of this course, you will be able to: Describe the purpose of ExAmp cluster amplification List the steps in the ExAmp cluster amplification workflow and Identify best practices for preparing the ExAmp reaction.
Patterned Flow Cells	The flow cell used on the HiSeq 3000, HiSeq 4000, and HiSeq X is a patterned flow cell with billions of ordered nanowells. The structured organization of the flow cell allows for even cluster spacing, generation of uniformly sized clusters, and faster imaging due to the known position of each cluster. Using patterned flow cells increases the number of output reads possible from the run and increases the amount of sequencing data generated. Cluster generation on the patterned flow cell is performed using a method called exclusion amplification or “ExAmp.” This course focuses on the steps to prepare your HiSeq patterned flow cell for ExAmp clustering on the cBot.
ExAmp Cluster Amplification	The goal of the ExAmp clustering method is to ensure only a single DNA template binds and forms a cluster within a single well. Amplification efficiency greatly outweighs hybridization efficiency. The almost instantaneous amplification excludes other DNA fragments from hybridizing within a well because there are no longer unoccupied oligos on the flow cell surface for other libraries to bind. The ExAmp clustering method provides maximum data output for patterned flow cells by discouraging poly clonal clusters.
Workflow Overview	The ExAmp clustering workflow consists of five steps. First, you prepare the cBot reagent plate Then, dilute your library and denature it. Next, prepare the ExAmp reaction. Finally, you load onto the cBot reagent plate onto the cBot and start the cBot run. We’ll cover each of the steps in greater detail in the next few pages.

Section	Narration
Dilute Library	The library loading concentration depends on the libraries to be sequenced. Make sure that you dilute to a concentration that is appropriate for the library type. Resuspension Buffer, or RSB, is provided in the kit. You can choose to spike in non-denatured PhiX, if desired, during this step.
Denature Library	Next, denature the library. The library loading concentration depends on the libraries to be sequenced. Make sure that you dilute to a concentration appropriate for the library type.
Prepare cBot Reagent Plate	To prepare the cBot reagent plate, invert the plate several times to mix the thawed reagents. Vortex the plate for approximately 10 seconds to dislodge trapped air bubbles. Tap the reagent plate on a hard surface 5–10 times to collect reagent droplets at the bottom of the tubes. Alternatively, pulse centrifuge the reagent plate. Set the reagent plate aside on ice until you are ready to load it onto the cBot.
Prepare ExAmp Reaction Video	Prepare the ExAmp reaction master mix immediately before use. This video shows you the steps. First, invert each tube of EPX1 and EPX2 and EPX3 several times each to make sure that the reagents are thoroughly mixed. Briefly centrifuge EPX1, EPX2, and EPX3. Do not vortex! Add 210 ul EPX1 to a 1.5 ml tube. Then, add 30 ul EPX2. Using a P1000 pipette set to 200 ul, slowly pipette up and down 10 times to mix the reaction, Do not vortex! The ExAmp reagents, especially EPX2 and EPX3 are very viscous. Make sure to aspirate and dispense reagents slowly to ensure accurate pipetting and to avoid creating bubbles. Add 110 µl EPX3 to the tube. Using a P1000 pipette set to 200 µl, slowly pipette up and down 10 times to mix the reaction. Briefly centrifuge the reaction. Do not vortex. Make sure there are no air bubbles at the bottom of the tube. If the mixture separates into a cloudy portion and transparent portion, repeat the pipette mixing step to make sure that the solution is uniform. Add 35 ul of the master mix into the bottom of each well of the 8-tube strip containing denatured and neutralized libraries. Change tips between samples. Using a multichannel P100 or P200 pipette set to 40 µl, slowly pipette up and down 10 times to mix the reaction. Do not vortex! Make sure that the tubes are capped, and then briefly centrifuge the 8-tube strip. Prior to placing the samples on the cBot, the 8 strip tube must be free of bubbles. Gently flicking the strip tube while holding it vertically followed by quick spins can help remove these bubbles. Set aside the 8-tube strip containing the ExAmp master mix and library solution on ice until you are ready to load it onto the cBot.

Section	Narration
Technique Tips	Technique is very important to the success of the ExAmp reaction. Click the arrows to learn more about the technique tips. Do not vortex the ExAmp clustering reagents. The ExAmp clustering enzymes may be affected. Quick spins are ok. The ExAmp reagents, especially EPX2 and EPX3, are very viscous. Make sure to aspirate and dispense reagents very slowly to ensure accurate pipetting. Aspirate and dispense below the liquid surface to avoid bubbles. Aspirate at 90 degrees and dispense at 45 degrees against the surface of the tube to avoid bubbles. Pause after dispensing to ensure full reagent delivery. Reagents must be thoroughly mixed before proceeding to add the master mix to the denatured DNA. Make sure the solution is uniform. Prior to placing the samples on the cBot, the 8 strip tube must be free of bubbles. Gently flicking the strip tube while holding it vertically followed by quick spins can help remove these bubbles.
Load onto the cBot	When the reaction is prepared, load the reaction onto the cBot. Clustering takes approximately 3 hours. After the flow cell is clustered, load it onto the instrument and set up the run. If necessary, you can store the clustered flow cell in the flow cell storage buffer for up to 48 hours at 2°C to 8°C.
For More Information	For more information on ExAmp cluster amplification, consult the resources listed here.
Summary	You should now understand the ExAmp cluster amplification workflow. Take a moment to review the key points from this course.
Conclusion	Congratulations! You have completed this course.