**DNA Quality Assessment by DNA Ladder PCR**

1. **PRINCIPLE:**
	1. DNA quality is a critical component of quality control in the molecular laboratory. DNA quality may be assessed by amplifying a control DNA sequence which is the same size or larger than the amplicon produced by a positive test result. The quality of a DNA sample can be determined by amplifying an endogenous control gene in the case of some PCR/NGS assays or determined before the sample is tested using the methodology described here.
	2. A polymerase chain reaction (PCR) assay is performed using the Specimen Control Size Ladder by InvivoScribe. This assay uses multiple genes to amplify human DNA of different size products (84, 96, 200, 300, 400, and 600 bp in length). The product sizes produced by a sample in this PCR assay are used to help determine the quality of the DNA before use in other PCR reactions.
	3. This assay will help determine the size of DNA fragments that are present in the sample. Due to the variety of concentrations of DNA used in the assays performed in our lab, different amounts of DNA input are used based upon the final assay to be performed. DNA is tested for DNA quality at the concentration which will be used in the test requested.
2. **SAMPLE:**
	1. Purified DNA isolated from blood, bone marrow aspirate, FFPE tissue block, alcohol-fixed (e.g., CytoLyt) tissue, fresh frozen tissue, CSF, or body fluids.
3. **REAGENTS:**
	1. AmpliTaq Gold DNA polymerase, store at -20°C
	2. Specimen Control Size Ladder master mix, store at -80°C
4. **CONTROLS:**
	1. Positive Manufactured Control: IVS0004 Clonal Control DNA, store at -80°C
	2. Positive Human DNA FFPE Control: Tonsil DNA, store at -20°C or -80°C
5. **PROCEDURE:**
	1. **Create your worksheet: QC Ladder PCR – Test Worksheet Builder**
		1. Log into Soft Molecular.
		2. Open QC Ladder PCR – Test Worksheet Builder using the tile on the dashboard.
		3. Select **Find**.
		4. If applicable, select **New** in the Pending Worksheets window.
		5. Highlight the barcode scanning field. Scan the product label of the patient sample to be added to the worksheet. Select **Enter** on the keyboard. Repeat this step for all pending samples.
		6. Verify the control lot numbers by clicking on the **Sample ID** field.
			1. If the control needs to be changed, click on the dropdown arrow and select the correct control in the window that appears.
		7. Verify the reagent lot numbers by clicking on the vertical **Settings** tab on the left side of the screen.
			1. If the reagent lot needs to be changed, click on the dropdown arrow in the **Stock#** column and select the correct lot in the window that appears.
		8. All possible Blanks will be prepopulated onto the worksheet.
			1. Delete a Blank:
				1. Highlight the appropriate blank.
				2. Select the **Delete** button.
				3. Repeat i-ii for all applicable blanks.
			2. If a Blank was deleted:
				1. Select **Plate View** on the bottom left of the screen.
				2. Adjust for the empty space by multi-selecting the samples/controls following the empty well and dragging them to the correct well.
		9. Mark the **Completed** checkbox and select **Save**.
			1. The worksheet Print Preview screen will automatically generate.
			2. Q Numbers will automatically generate upon saving.
		10. Select the **printer** icon, verify the correct printer is highlighted and click **Print** button.
		11. Close the Print Preview window.
		12. Click on the **Settings** button to open the Select Printer window. Verify the correct printer is selected in the dropdown field. Select **Print**.
			1. **Note**: Select **View** in the Select Printer window to preview the Section Settings.
		13. Select **Back** in the QC Ladder PCR – Test Worksheet Builder.
		14. Exit Soft Molecular application.
	2. Turn on the DNA thermal cycler and hoods.
	3. Using the same diluent that was used in extraction, prepare an appropriate dilution of DNA for the test to be performed. Refer to Quantification of DNA and RNA by Optical Density Appendix A: Selective Testing.
	4. Completely thaw all necessary reagents except AmpliTaq® DNA polymerase.
	5. Briefly and gently vortex reagents except Taq enzyme and flash spin.
	6. Prepare master mix for DNA ladder.
	7. Assemble PCR reactions in appropriate PCR hood using clean gloves, lab coat, and barrier pipette tips, as appropriate.
	8. Label the PCR tubes with the individual reaction # and test.
	9. Mix by carefully pipetting up and down using a barrier tip.
	10. Aliquot 22.5ul of the Master Mix for DNA ladder to the specified tubes, reusing a 30 µl barrier pipette tip.
	11. For samples on which the Clonality, IGH::BCL2, assays were ordered, add 2.5 ul of sample DNA to each reaction tube.
	12. Spin tubes for 30 sec. in PCR tube centrifuge.
	13. On a thermal cycler, initiate File DNA Ladder.
		1. 95°C for 7 min
		2. 35 amplification cycles, as follows:
			1. 95°C for 45 sec. (denaturation)
			2. 60°C for 45 sec. (annealing)
			3. 72°C for 90 sec. (extension)
		3. Final extension reaction for 10 min. at 72°C to complete all products.
		4. Heteroduplexing is not necessary for this assay.
	14. **Process your worksheet:**
		1. Log into Soft Molecular.
		2. Open QC Ladder PCR - Test Worksheet Processing by using the tile on the dashboard.
		3. Scan the QC Ladder PCR worksheet into Worksheet# field and select **Find**.
		4. Use the dropdown under **Thermocycler:** to select the correct instrument.
		5. Complete the QC Ladder PCR activity by marking the **Completed** checkbox and selecting **Save**.
		6. Click **Build Next Worksheet** button.
		7. Verify that the Select all tests, Transfer Controls and QCLADGEL To build checkboxes are marked.
		8. Click on **OK** button.
		9. The system will ask the user if they want to open the new worksheet. Click **No**.
		10. Select **Back** in the QC Ladder PCR – Test Worksheet Processing window.
	15. **Gel Worksheet Builder:**
		1. Open QC Ladder GEL - Test Worksheet Builder by using the tile on the dashboard.
		2. Click **Find**.
		3. If applicable, select the correct worksheet in the Pending Worksheet tab.
		4. Select the **Plate View** tab and adjust the patient samples and controls to reflect how the gel should be loaded.
		5. **Add 50\_BP\_Marker where appropriate.**
			1. Highlight the correct well and select 50\_BP\_Marker from the dropdown next to **Add Control** button.
			2. Click **Add Control** button.
		6. Select the **Worksheet** tab.
			1. Verify 50\_BP\_Marker has a lot number selected. If a lot number is not selected, use the dropdown menu in the Sample ID field to select the correct control lot number.
		7. If you need to make a comment on the worksheet (for example, to add a comment about a blank control), select the comment box in the middle of the screen with an “a” in it.
			1. Enter the lane the blank is being run in.
			2. For Qiagen Blanks, specify if it is QB or QB-2 (first or second extraction run of the day).
			3. Enter the date the blank was made.
		8. Click **Print Plate View** button.
		9. Select **OK** to save before printing.
			1. Q numbers will generate for the 50\_BP\_marker upon saving.
		10. Click on **Print** icon, verify the correct printer is highlighted, and click **Print**.
		11. Click on the **Print Label** button.
		12. Verify the correct printer is selected. Click **Print**.
		13. Select **Back** in the QC Ladder Gel – Test Worksheet Builder.
		14. Exit Soft Molecular application.
		15. Place Gel label and Worksheet next to Thermal Cycler.
		16. Add 4ul of loading dye to each tube and mix well.
		17. Load 20ul into gel.
		18. Analyze by electrophoresis using 6% acrylamide gel at 20 Watts per gel.
	16. **Gel Worksheet Processing**:
		1. Once you have loaded your gel, log into Soft Molecular.
		2. Open QC Ladder Gel – Test Worksheet Processing using the tile on the dashboard.
		3. Scan the QC Ladder Gel Plate View into the Worksheet# field and select **Find**.
		4. Complete the QC Ladder Gel activity by marking the **Completed** checkbox and selecting **Save**.
		5. Select **Back** in the QC Ladder Gel – Test Worksheet Processing window.
		6. Exit Soft Molecular application.
	17. **Refer to Bio-Rad Gel Doc XR Instrument Procedure to capture and edit gel image.**
	18. **QC Ladder Image Capture:**
		1. After you have taken a photo of your gel, log into Soft Molecular.
		2. Open QC Ladder GEL - Test Worksheet Processing by using the tile on the dashboard.
		3. Scan the QC Ladder Gel Plate View into the Worksheet# field and select **Find**.
		4. Verify Worksheet is selected in the Image Type dropdown.
		5. Select **Images** button. On the window that opens, select the **Add File** tab then select the **add file (folder)** icon.
		6. Find and select the file to be added from the Windows Explorer window. Select **Open**.
		7. Choose **Gel Image** from Template dropdown.
		8. Select the **green check** icon to add file(s). Close the window.
		9. Complete the QC Ladder Image Capture activity by marking the **Completed** checkbox and selecting **Save**.
		10. Select **Back** in QC Ladder Gel – Test Worksheet Processing window.
		11. Exit Soft Molecular application.
		12. Deliver worksheets to the Director/Pathologist assigned to QC Ladder Interpretation.
6. **INTERPRETATION:**
	1. A false positive result could be produced by contamination of the PCR reaction with exogenous DNA. The absence of a band in the master mix largely excludes this possibility.
	2. A false negative result could be obtained if the DNA sample is too degraded or is otherwise unable to serve as an adequate PCR template. The presence ofcharacteristic bands in the positive control reaction generated from the clonal control DNA and tonsil DNA is evidence against a false negative result.
	3. If the quality control PCR reaction for a sample does not produce bands greater than a specific size, then the results for certain tests may not be interpretable. For example, if a particular assay produces amplicons of 200 bp, the QC assay should produce bands of at least 200 bp to interpret that assay. See individual PCR procedures to determine the size of the product generated.
	4. If an interfering substance is suspected, refer to: DNA Quality Assessment by DNA Ladder PCR Appendix A: Interfering Substances.

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| Master mix | Target | Control DNA | Product size |
| DNA ladder | Multiple genes | Any DNA | 84, 96, 200, 300, 400, 600 |
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| --- | --- |
| **Result Classification** | **PCR Results** |
| Excellent quality | Strong amplification, largest band = 400 bp or greater |
| Good quality | Strong amplification, largest band = 300 bp  |
| Adequate quality | Moderate amplification of 300 bp band |
| Poor/Suboptimal quality | No amplification of bands > 200 bp |

1. **RESULT REVIEW**
	1. Open QC Ladder GEL - Test Worksheet Processing by using the tile on the dashboard.
	2. Scan the QC Ladder Gel Plate View into the Worksheet# field and select **Find**.
	3. Select the **Images** button on the upper right corner of the screen. This will open the Gel image window. The window can be floated.
		1. **Note:** Leave this window open for input of the QC results as well as the patient results.
	4. Enter results in the QC Ladder Value column (using dropdown) for all patient samples and controls on the run.
		1. **Note:** The 50-base pair marker should be resulted as “50 bp” from the dropdown menu in the QC Ladder Value column.
	5. Complete the Result Review activity by marking the **Completed** checkbox and selecting **Save**.
	6. Select **Back** in the QC Ladder Gel – Test Worksheet Processing window.
	7. Exit Soft Molecular application.
2. **REPEAT TESTING**
	1. During the testing process, testing for some samples must be repeated for a variety of technical or analytical reasons. The specimen can be sent back to one of multiple prior steps in the workflow.
	2. In Soft Molecular, repeating a sample to a prior action can be accomplished from a variety of steps in the test workflow and places in the system. Please see the Soft Molecular Rerun Procedure for the specific steps to perform when requesting rerun testing.
3. **REFERENCES**:
	1. InVivoScribe Technologies IgH Gene Clonality Assay package insert
4. **REVISIONS:**
	1. 3/6/2018: Updated to remove tests that no longer require a ladder to be performed and formatting.
	2. 1/6/2020: Updated with steps for Soft Molecular and updated footer with new lab name.
	3. 10/5/2020: Instructions were added on how to place a comment on the worksheet for blank controls.
	4. 1/16/2024: Clarified controls.