Technical Bulletin

Technical Information for Lyra Parainfluenza Assay

Quidel Corporation has verified the performance of the Lyra Parainfluenza Assay on the Cepheid® SmartCycler® II, software version 3.0b. Internal studies performed by Quidel have demonstrated performance on this instrument is substantially equivalent to analytical and clinical performance data found in the package insert.

Due to differences in instrument platforms new programming procedures for the SmartCycler Dx 3.0b are provided. These procedures are detailed on pages 2-4.

Please contact Quidel Technical Support at 800.874.1517 (in the U.S.), 858.552.1100 (outside the U.S.) or technicalsupport@quidel.com if you have any questions regarding any Quidel product. Our hours of operation are Monday through Friday, 8:00 a.m. to 5:00 p.m. Eastern Time.

You may also visit our website at **quidel.com** for information on Quidel's line of Molecular Diagnostics, Rapid Diagnostics, Cell Culture and Specialty Products (Bone Health and Autoimmune & Complement). Other product information available on our website includes: CPT codes, CLSI procedure guides, MSDS, and Package Inserts.

Lyra Parainfluenza Assay

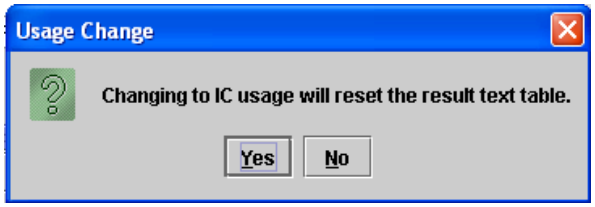
Supplemental Instructions: Creating a SmartCycler Dx Protocol Template

Purpose: The following supplemental instructions will aid in programming a template for the SmartCycler II from Cepheid to run the Lyra Parainfluenza assay kit. Seek specific training or guidance if you are unfamiliar with the use of this platform. For assistance with this protocol, please contact Quidel Technical Support directly.

Limitations: The following protocol was developed for use with Lyra Parainfluenza kit specifically. Its suitability for other assays is unknown. Check with Cepheid to ensure software compatibility.

Programming Instructions:

1. Launch the SmartCycler Dx 3.0b software package.
2. Create the Lyra Parainfluenza assay.
    a. Select the **Define Assays** button from the top of the screen.
    b. Name the assay.
        i. Select the **New** button at the bottom left corner of the screen.
        ii. Type in 'Lyra Parainfluenza' and select **OK.**
        iii. 'Lyra Parainfluenza' will be added to the top of the **Assay Name** list located on the upper left-hand of the screen.
    c. Set the analysis values: Under the **Assay Type: Research** section, select the **Analysis Settings** tab, and make sure the following specifications are made:
        i. Select **FATA25** from the **Dye Set** drop-down menu.
        ii. The **Analysis Type** drop-down menu should be set to **Qualitative** (Default setting).
        iii. In the Channel Name column, enter 'PIV-1' for FAM, 'PIV-2' for Alx532, 'PIV-3' for TxR, and 'PRC' for Alx647.
        iv. In the **Usage** column, select **Target** from the drop-down menus for PIV-1, PIV-2, PIV-3 and select **Internal Control** for PRC. When selecting the **Internal Control**, a window below will pop up. Select the **Yes** button.



        v. In the Curve Analysis column, enter **Primary Curve** for each channel (PIV-1, PIV-2, PIV-3, PRC) (Default setting).
        vi. In the **Thresh Setting** column, enter **Manual Threshold** for each channel (PIV-1, PIV-2, PIV-3, PRC) (Default setting).
        vii. In the **Manual Thresh Flour Units** column, enter the following thresholds:
            a. **PIV-1**: 10.0
            b. **PIV-2**: 20.0
            c. **PIV-3**: 20.0
            d. **PRC**: 20.0

viii. In the **Valid Min Cycle** column (scroll to the right if not immediately visible), enter **10** for each channel (PIV-1, PIV-2, PIV-3, PRC).

ix. In the **Valid Max Cycle** column (scroll to the right if not immediately visible), enter **45** for each channel (PIV-1, PIV-2, PIV-3, PRC).

x. In the **Bkgnd Sub** column, use "ON" for each channel (PIV-1, PIV-2, PIV-3, PRC) (Default setting).

xi. In the **Bkgnd Min Cycle** column, enter **5** for each channel (PIV-1, PIV-2, PIV-3, PRC).

xii. In the **Bkgnd Max Cycle** column, enter **45** for for each channel (PIV-1, PIV-2, PIV-3, PRC).

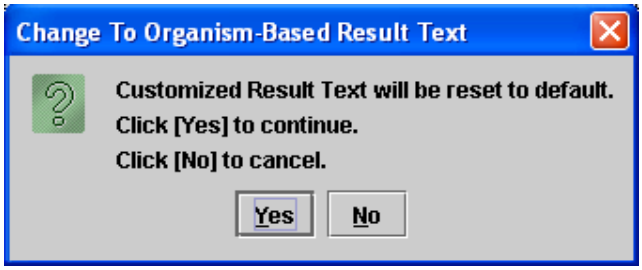
xiii. In the **Boxcar Avg Cycles** column, keep **0** for each channel (PIV-1, PIV-2, PIV-3, PRC) (Default setting).

xiv. In the **End Pt Threshold** column, enter **10** for the PIV-1 channel and **20** for all other channels (PIV-2, PIV-3, PRC).

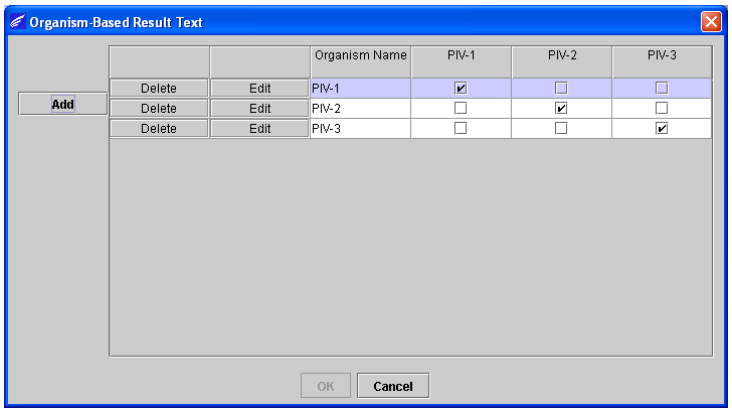
xv. In the **NC IC%** column, keep **"NA"** for channel (PRC) (Default setting).

xvi. In the **IC Delta** column, keep **"NA"** for each channel (PIV-1, PIV-2, PIV-3, PRC) (Default setting).

xvii. In the **Customize Result Text** section (below the table), select **Organism Based Result Text** from the drop-down menu. The warning window below will pop up. Select **Yes**.



xviii. Select the **Customize** button to open the **Organism-Based Result Text** dialog window. Select the **Add** button, enter 'PIV-1' in the **Organism Name** column and check the **PIV-1** box. Select the **Add** button again, enter 'PIV-2' in the **Organism Name** column and check the **PIV-2** box. Select the **Add** button again, enter 'PIV-3' in the **Organism Name** column and check the **PIV-3** box.



Click **OK** at the bottom of the pop up window.

d. Set the RT-PCR cycling times and temperatures at the bottom of the screen as follows:

i. Stage 1

1. Hold
2. Temp: 55.0
3. Secs: 300
4. Optics: OFF

ii. Stage 2

1. Hold
2. Temp: 60.0
3. Secs: 300
4. Optics: OFF

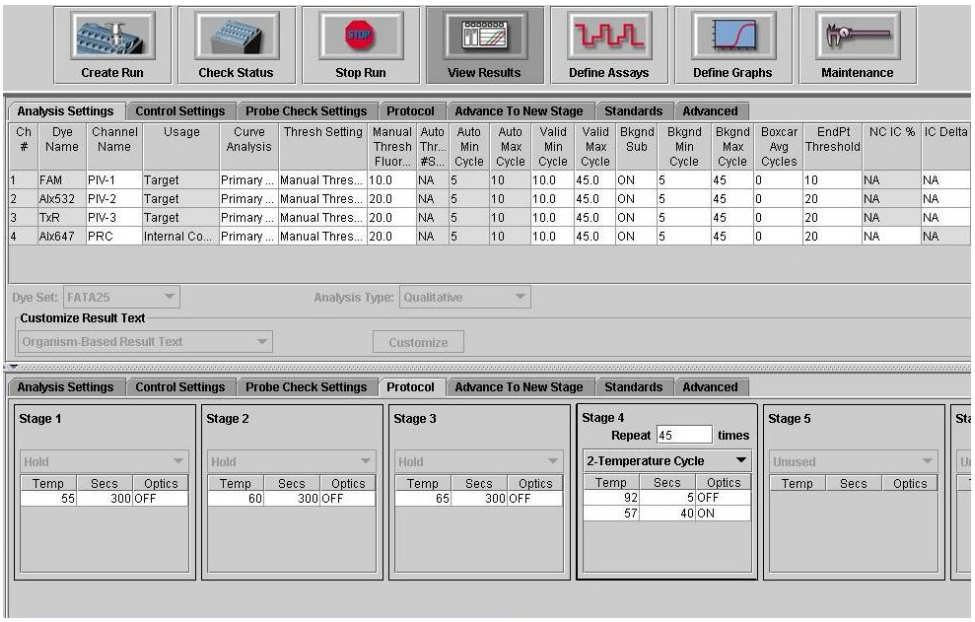
iii. Stage 3

1. Hold
2. Temp: 65.0
3. Secs: 300
4. Optics: OFF

iv. Stage 4

1. 2-Temperature Cycle
2. Times to Repeat: 45
3. First Temperature Row
a. Temp: 92.0
b. Secs: 5
c. Optics: OFF
4. Second Temperature Row
a. Temp: 57.0
b. Secs: 40
c. Optics: ON

3. Save the protocol by selecting the **Save** button at the bottom of the screen.
   Figure of the completed Lyra Parainfluenza Protocol



4. Interpretation of results:

Interpretation of the Lyra Parainfluenza Assay Results using the SmartCycler II				
Detector: PIV-1	**Detector: PIV-2**	**Detector: PIV-3**	**Detector: Process Control**	**Interpretation of Results**
Negative	Negative	Negative	Pass	No Parainfluenza RNA detected
Positive	Negative	Negative	NA*	Parainfluenza 1 RNA detected
Negative	Positive	Negative	NA*	Parainfluenza 2 RNA detected
Negative	Negative	Positive	NA*	Parainfluenza 3 RNA detected
Positive	Positive	Negative	NA*	Parainfluenza 1 and 2 RNA detected
Positive	Negative	Positive	NA*	Parainfluenza 1 and 3 RNA detected
Negative	Positive	Positive	NA*	Parainfluenza 2 and 3 RNA detected
Negative	Negative	Negative	Fail	No Parainfluenza RNA and No PRC detected; invalid test, Retest the same purified sample. If the test is also invalid, re-test another aliquot of the same sample or obtain a new sample and re-test.

*No Ct value is required for the Process Control to make a positive call.

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