



Lyra™ Direct

Technical Bulletin

Technical Information for Lyra Direct Strep Assay

Quidel Corporation has verified the performance of the Lyra Direct Strep Assay on Cepheid's 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 the instrument platform, new programming procedures for the SmartCycler Dx 3.0b are provided. These procedures are detailed on pages 2-5.

The end user will also be required to order a Process Buffer that is specific to running the Lyra Direct Strep Assay with the Smart Cycler II, custom part #M5182.

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, SDS, and Package Inserts.

Lyra™ Direct

Direct Strep Assay

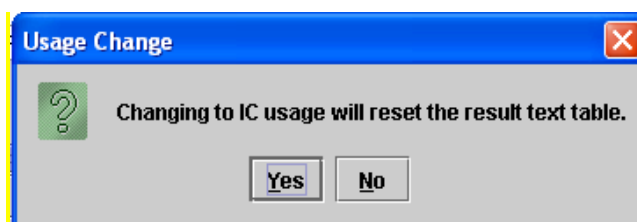
Supplemental Instructions: Creating a SmartCycler Dx Protocol Template

Purpose: The following supplemental instructions will aid in programming a template for the SmartCycler II from Cepheid to run the Lyra Direct Strep 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 the Lyra Direct Strep kit specifically. Its suitability for other assays is unknown. Check with Cepheid to ensure software compatibility.

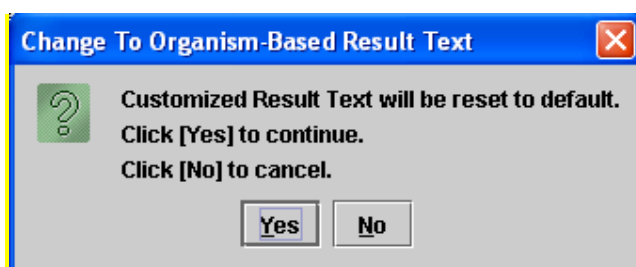
Programming Instructions:

1. Launch the SmartCycler Dx 3.0b software package.
2. Create the Lyra Direct Strep 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 Direct Strep' and select **OK**.
 - iii. 'Lyra Direct Strep' 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 **FCTC25** 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 'GAS' for FAM, 'Strep CG' for TxR, and 'PRC' for Cy5.
 - iv. In the **Usage** column, select **Target** from the drop-down menus for GAS and Strep CG and select **Internal Control** for PRC. When selecting the **Internal Control**, a window below will pop up. Select the **Yes** button.

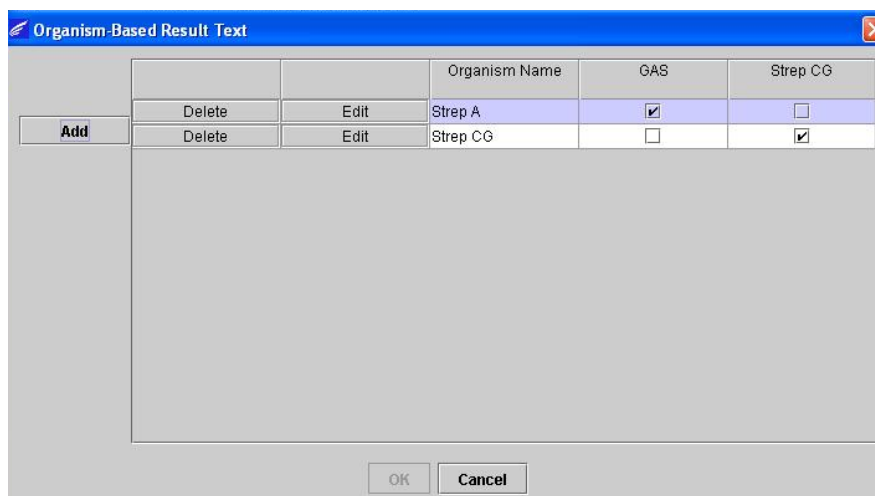


- v. In the Curve Analysis column, enter **Primary Curve** for each channel (GAS, Strep CG, PRC) (Default setting).
- vi. In the **Thresh Setting** column, enter **Manual Threshold** for each channel (GAS, Strep CG, PRC) (Default setting).
- vii. In the **Manual Thresh Flour Units** column, enter the following thresholds:
 - a. **GAS:** 10.0
 - b. **Strep CG:** 10.0
 - c. **PRC:** 10.0
- viii. In the **Valid Min Cycle** column (scroll to the right if not immediately visible), enter **5** for each channel (GAS, Strep CG, PRC).

- ix. In the **Valid Max Cycle** column (scroll to the right if not immediately visible), enter **45** for each channel (GAS, Strep CG, PRC).
- x. In the **Bkgnd Sub** column, use "ON" for each channel (GAS, Strep CG, PRC) (Default setting).
- xi. In the **Bkgnd Min Cycle** column, enter **5** for each channel (GAS, Strep CG, PRC).
- xii. In the **Bkgnd Max Cycle** column, enter **40** for each channel (GAS, Strep CG, PRC).
- xiii. In the **Boxcar Avg Cycles** column, keep **0** for each channel (GAS, Strep CG, PRC) (Default setting).
- xiv. In the **End Pt Threshold** column, enter **10** for each channel (GAS, Strep CG, 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 (GAS, Strep CG, 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 'Strep A' in the **Organism Name** column and check the **GAS** box. Select the **Add** button again, enter 'Strep CG' in the **Organism Name** column and check the **Strep CG** box.



- xix. 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: 92.0
 - 3. Secs: 120
 - 4. Optics: OFF

- ii. Stage 2
 - 1. 3-Temperature Cycle
 - 2. Times to Repeat: 5
 - 3. First Temperature Row
 - a. Temp: 92.0
 - b. Secs: 5
 - c. Optics: OFF
 - 4. Second Temperature Row
 - a. Temp: 57.0
 - b. Secs: 9
 - c. Optics: Off
 - 5. Third Temperature Row
 - a. Temp: 63.0
 - b. Secs: 35
 - c. Optics: Off

- iii. Stage 3
 - 1. 3-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: 9
 - c. Optics: Off
 - 5. Third Temperature Row
 - a. Temp: 63.0
 - b. Secs: 35
 - c. Optics: ON

3. Save the protocol by selecting the **Save** button at the bottom of the screen.

Figure of the completed Lyra Direct Strep Protocol

The screenshot displays the software interface for the Lyra Direct Strep Protocol. At the top, there are several icons: Create Run, Check Status, Stop Run, View Results, Define Assays, Define Graphs, and Maintenance. Below these is a tabbed menu with 'Analysis Settings' selected. The main area contains a table with columns for Ch #, Dye Name, Channel Name, Usage, Curve Analysis, Thresh Setting, Manual Thresh Fluor..., Auto Thr... #S..., Auto Min Cycle, Auto Max Cycle, Valid Min Cycle, Valid Max Cycle, Bkgrnd Sub, Bkgrnd Min Cycle, Bkgrnd Max Cycle, Boxcar Avg Cycles, EndPt Threshold, NC IC %, and IC Delta. The table lists four channels: 1 (FAM, GAS, Target), 2 (Cy3, Unused), 3 (TxR, Strep CG, Target), and 4 (Cy5, PRC, Internal Con...). Below the table are dropdown menus for 'Dye Set' (FCTC25) and 'Analysis Type' (Qualitative), along with a 'Customize Result Text' section. At the bottom, there are five stage configuration panels (Stage 1 to Stage 5). Stage 1 is 'Hold'. Stage 2 is '3-Temperature Cycle' with 'Repeat' set to 5 times. Stage 3 is '3-Temperature Cycle' with 'Repeat' set to 45 times. Stage 4 is 'Unused'. Stage 5 is 'Unused'. Each stage panel contains a table for temperature, seconds, and optics settings.

Ch #	Dye Name	Channel Name	Usage	Curve Analysis	Thresh Setting	Manual Thresh Fluor...	Auto Thr... #S...	Auto Min Cycle	Auto Max Cycle	Valid Min Cycle	Valid Max Cycle	Bkgrnd Sub	Bkgrnd Min Cycle	Bkgrnd Max Cycle	Boxcar Avg Cycles	EndPt Threshold	NC IC %	IC Delta
1	FAM	GAS	Target	Primary...	Manual Thr...	10.0	NA	5	10	5.0	45.0	ON	5	40	0	10	NA	NA
2	Cy3		Unused	Primary...	Manual Thr...	30.0	NA	5	10	13.0	45.0	ON	5	40	0	10	NA	NA
3	TxR	Strep CG	Target	Primary...	Manual Thr...	10.0	NA	5	10	5.0	45.0	ON	5	40	0	10	NA	NA
4	Cy5	PRC	Internal Con...	Primary...	Manual Thr...	10.0	NA	5	10	5.0	45.0	ON	5	40	0	10	NA	NA

4. Interpretation of results:

Interpretation of the Lyra Direct Strep Assay Results on the SmartCycler II Thermocycler				
Assay Result	Detector: GAS	Detector: Strep CG	Detector: Process Control	Interpretation of Results
Negative	Negative	Negative	Pass	No group A Strep and no pyogenic group C/G DNA detected; PRC Detected
Strep A Positive	Positive	Negative	NA*	Group A Strep DNA detected
Strep CG Positive	Negative	Positive	NA*	Pyogenic group C/G DNA detected
Strep A and Pyogenic C/G Positive	Positive	Positive	NA*	Group A Strep and pyogenic group C/G DNA detected
Invalid	Negative	Negative	Fail	No group A Strep or pyogenic group C/G DNA, and no PRC detected. Retest the same processed sample. If the test is also invalid, 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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