



**3' mRNA-Seq Library Prep Kit
on the Hamilton Microlab STAR Workstations**

Installation Guide

Corresponds to Application Release: 2015-12-14

Revision Date: December 14, 2015

1. Introduction

This Installation Guide briefly describes the technical details necessary to install or update the autoQuantSeq STAR protocol on the Hamilton MicroLab STAR Series liquid handler.

Any further support information can be obtained from Lexogen, <mailto:info@lexogen.com>.

2. Installation Prerequisites

The autoQuantSeq STAR can be installed on a Hamilton MicroLab STAR or Hamilton MicroLab STARlet systems (only Hamilton STAR has been tested).

2.1. Deck Layout

The system has to be preconfigured for running the NGS Library prep protocol using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (E7370), implemented by Hamilton Robotics GmbH (referred to as 'NEBNext protocol' in the following). The typical deck layout is shown in Figure 1.



Figure 1: Deck layout for the NEBNext Ultra protocol. The identical deck layout is used for autoQuantSeq.

2.2. Software Setup

The NEBNext protocol has to be installed in the control PC. The autoQuantSeq STAR protocol makes use of some software components (libraries, liquid class definitions, labware definitions, configuration tools) of the NEBNext protocol.

The typical file structure of the HAMILTON folder in the Program Files directory is shown in Figure 2.

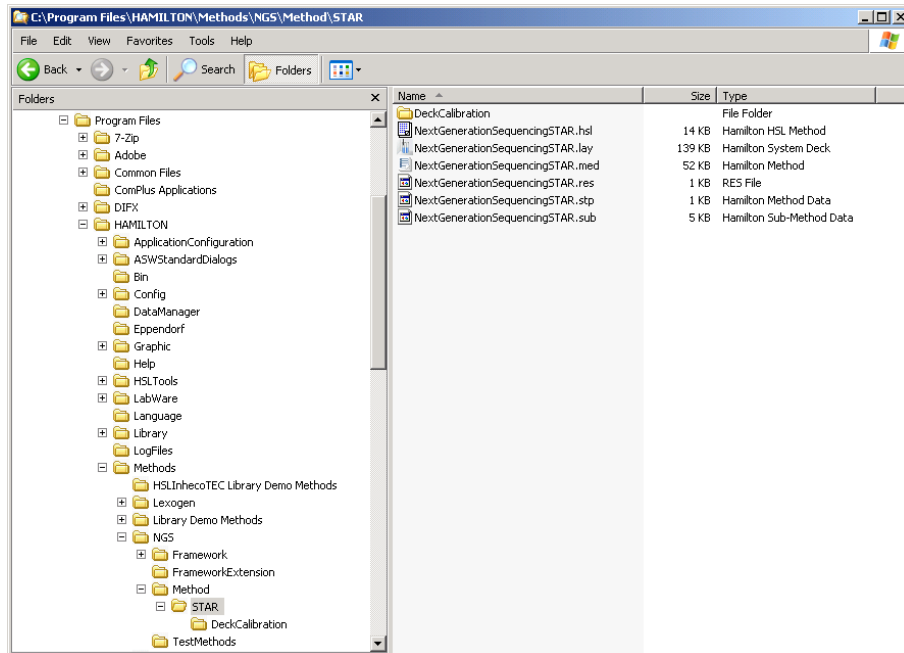


Figure 2: Typical directory structure after installation of the NEBNext protocol.

3. Installation / Update

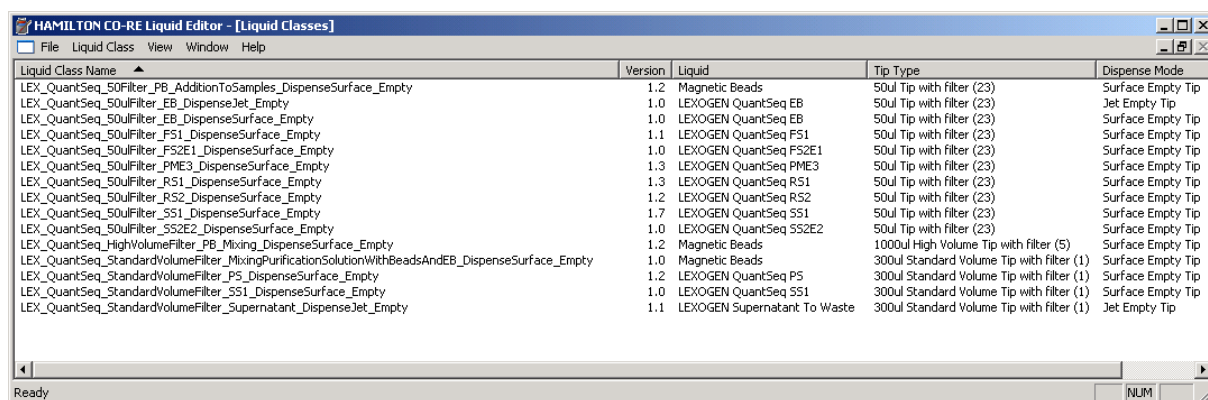
3.1. Installation Package

The software component of the protocol comes in a form of a ZIP archive file (typically named *LEX_autoQuantSeq_YYMMDDn.zip*).

Note:

The standard Hamilton method package solution (.pkg file) is not used since it typically contains all dependent files which would in this situation also include the files of the NEBNext protocol. When installing or updating, this would potentially lead to overwriting of the existing NEXNext files on the target control PC, and thus modifying the HAMILTON protocol.

The folder *Methods_Lexogen_autoQuantSeq* also contains the liquid class definition file *LEX_Liquids.mdb* (see Figure 3).



| Liquid Class Name | Version | Liquid | Tip Type | Dispense Mode |
|--|---------|------------------------------|---|-------------------|
| LEX_QuantSeq_50Filter_PB_AdditionToSamples_DispenSeSurface_Empty | 1.2 | Magnetic Beads | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_EB_DispenSeJet_Empty | 1.0 | LEXOGEN QuantSeq EB | 50ul Tip with filter (23) | Jet Empty Tip |
| LEX_QuantSeq_50ulFilter_EB_DispenSeSurface_Empty | 1.0 | LEXOGEN QuantSeq EB | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_FS1_DispenSeSurface_Empty | 1.1 | LEXOGEN QuantSeq FS1 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_FS2E1_DispenSeSurface_Empty | 1.0 | LEXOGEN QuantSeq FS2E1 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_PME3_DispenSeSurface_Empty | 1.3 | LEXOGEN QuantSeq PME3 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_RS1_DispenSeSurface_Empty | 1.3 | LEXOGEN QuantSeq RS1 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_RS2_DispenSeSurface_Empty | 1.2 | LEXOGEN QuantSeq RS2 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_SS1_DispenSeSurface_Empty | 1.7 | LEXOGEN QuantSeq SS1 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_50ulFilter_SS2E2_DispenSeSurface_Empty | 1.0 | LEXOGEN QuantSeq SS2E2 | 50ul Tip with filter (23) | Surface Empty Tip |
| LEX_QuantSeq_HighVolumeFilter_PB_Mixing_DispenSeSurface_Empty | 1.2 | Magnetic Beads | 1000ul High Volume Tip with filter (5) | Surface Empty Tip |
| LEX_QuantSeq_StandardVolumeFilter_MixingPurificationSolutionWithBeadsAndEB_DispenSeSurface_Empty | 1.0 | Magnetic Beads | 300ul Standard Volume Tip with filter (1) | Surface Empty Tip |
| LEX_QuantSeq_StandardVolumeFilter_PS_DispenSeSurface_Empty | 1.2 | LEXOGEN QuantSeq PS | 300ul Standard Volume Tip with filter (1) | Surface Empty Tip |
| LEX_QuantSeq_StandardVolumeFilter_SS1_DispenSeSurface_Empty | 1.0 | LEXOGEN QuantSeq SS1 | 300ul Standard Volume Tip with filter (1) | Surface Empty Tip |
| LEX_QuantSeq_StandardVolumeFilter_Supernatant_DispenSeJet_Empty | 1.1 | LEXOGEN Supernatant To Waste | 300ul Standard Volume Tip with filter (1) | Jet Empty Tip |

Figure 3: Typical data entries in the liquid class definition files *LEX_Liquids.mdb*.

3.2. Installation Procedure

To install:

1. Unzip the LEX_autoQuantSeq_YYMMDDn.zip package in a temporary folder.
2. Copy the contents of the Methods_Lexogen_autoQuantSeq to (typically)
C:\Program Files\HAMILTON\Methods\Lexogen\autoQuantSeq
 (If the folder already exists, delete its contents completely before copying).
3. Copy the contents of the Library_Lexogen_autoQuantSeq to (typically)
C:\Program Files\HAMILTON\Library\Lexogen\autoQuantSeq
 (If the folder already exists, delete its contents completely before copying).
4. Update the Liquid Class Definition Database by using the Hamilton CO-RE Liquid Editor and importing all liquid classes listed in the *LEX_Liquids.mdb* (if entries with same names already exist in the database, overwrite them).
5. Install desktop shortcut: Copy the file *RUN_QuantSeq.lnk* to your desktop.

3.3. Typical File Structure after Installation

The typical file structure after installation is shown in Figure 4 and Figure 5:

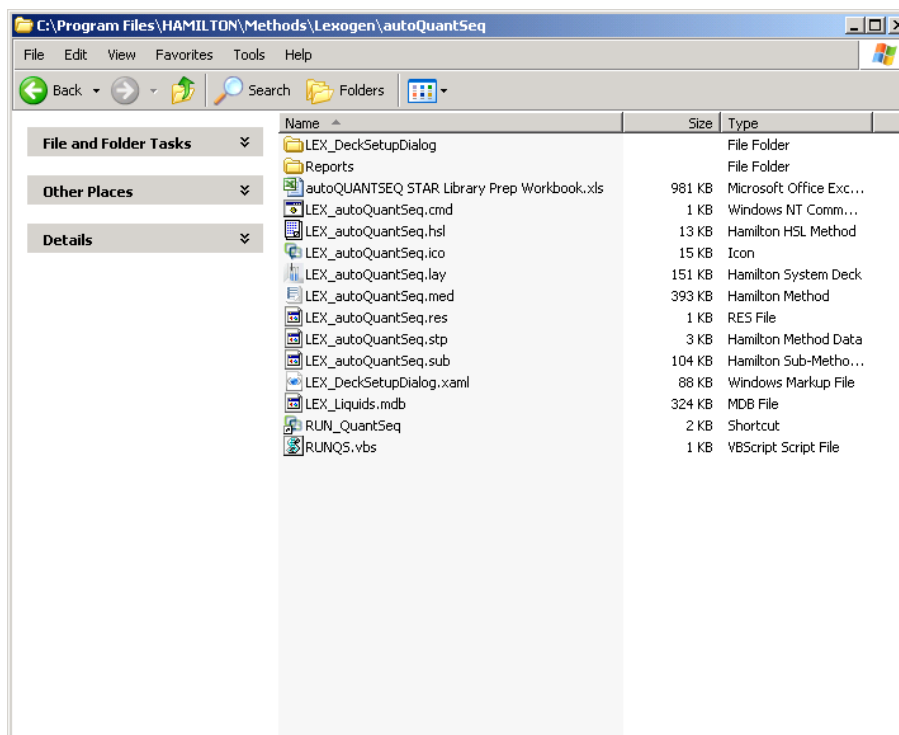


Figure 4: Typical structure of the Methods directory for autoQuantSeq STAR.

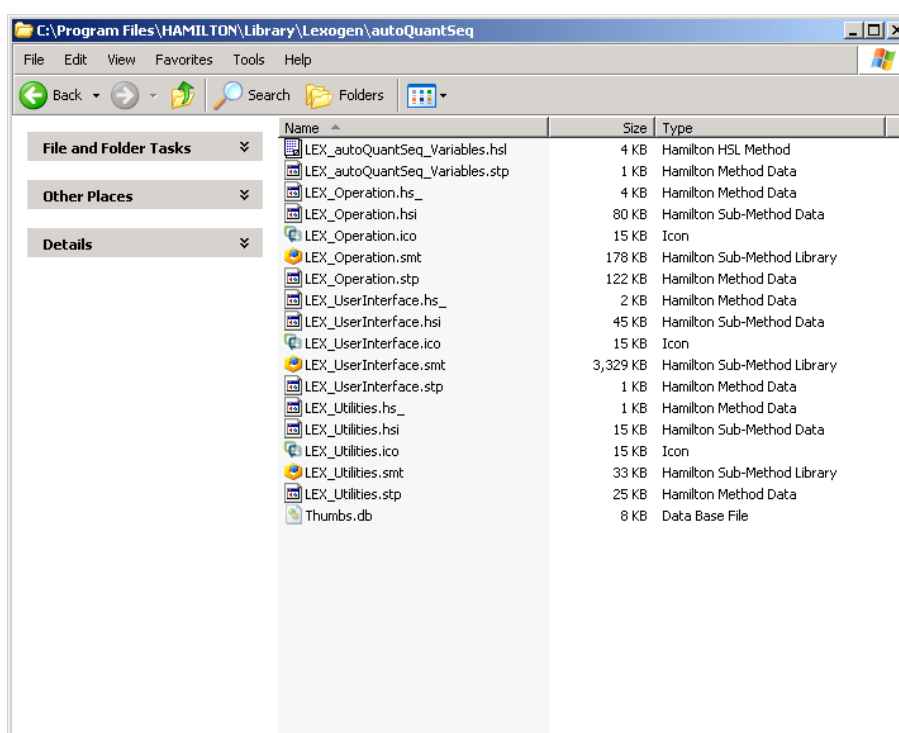


Figure 5: Typical structure of the Library directory for autoQuantSeq STAR.

3.4. Deck Layout Model

The deck layout model (contained in file *LEX_autoQuantSeq.lay*) is shown in Figure 6.

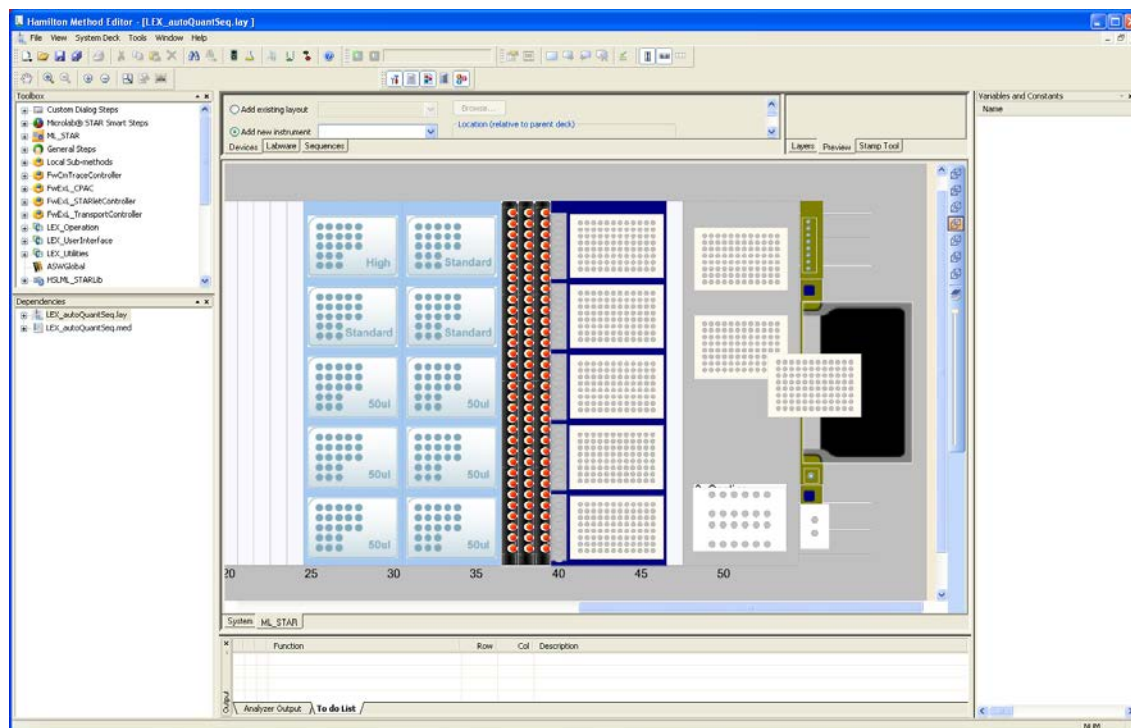


Figure 6: *LEX_autoQuantSeq.lay* layout in the Methods Editor.

4. Testing the Installation

4.1. Software modifications for testing

To facilitate testing and debugging, special features can be enabled. This can be done in the file *LEX_autoQuantSeq_Variables.hsl*. This file has to be opened in the HSL Method editor and the code can be modified.

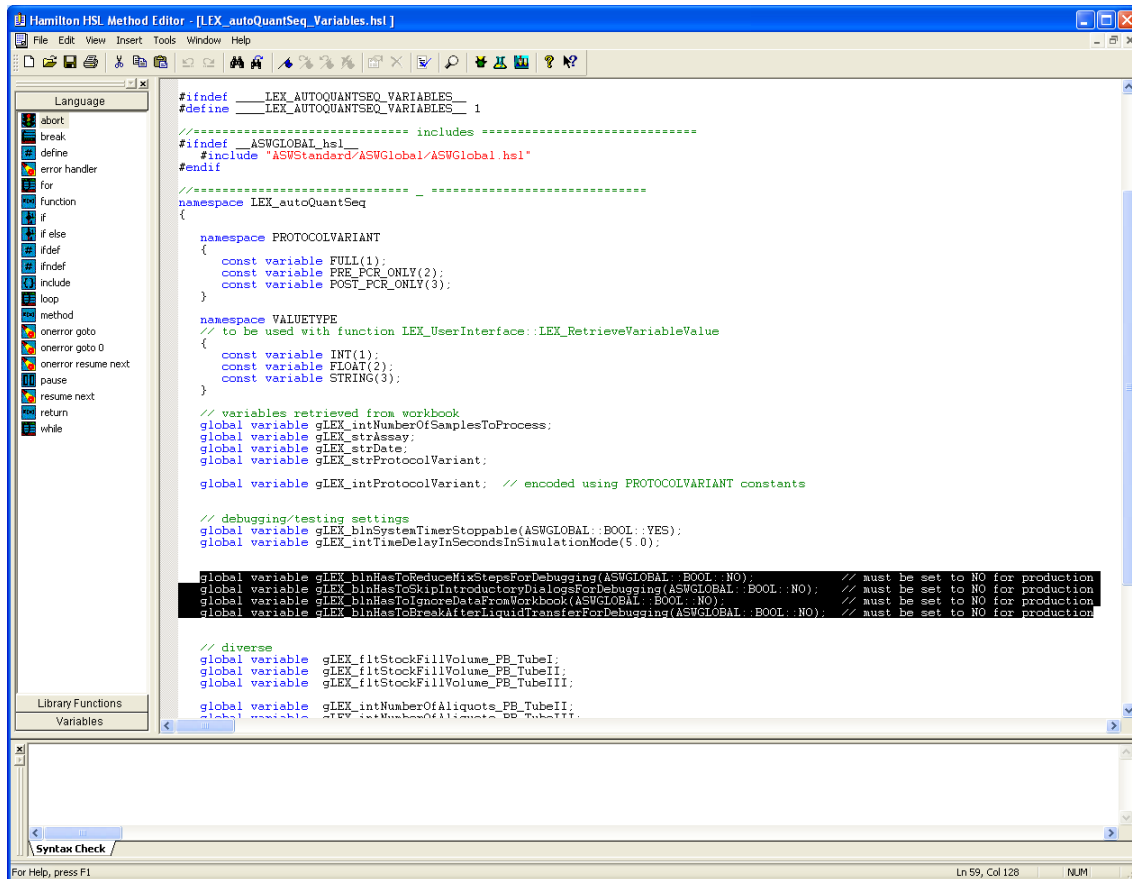


Figure 7: LEX_autoQuantSeq_Initialization submethod. For debugging special setting (overruling the workbook settings) can be edited here.

Following variables can be modified:

global variable **gLEX_blnHasToReduceMixStepsForDebugging**

- If set to `ASWGLOBAL::BOOL::YES`, the number of mixing steps is reduced to speed up testing.
- Set to `ASWGLOBAL::BOOL::NO` for production.
- Note: not actively used in the recent releases.

global variable **gLEX_blnHasToSkipIntroductoryDialogsForDebugging**

- If set to `ASWGLOBAL::BOOL::YES`, some of the standard dialogs at the beginning of the protocol are skipped over, so starting of the protocol is faster. This can be used for debugging.
- Set to `ASWGLOBAL::BOOL::NO` for production.

global variable **gLEX_blnHasToIgnoreDataFromWorkbook**

- If set to ASWGLOBAL::BOOL::YES , the data read from the workbook are overruled by the data assignments made directly in the ode (in the *LEX_autoQuantSeq_Initialization* method). This can be used for debugging.
- Set to ASWGLOBAL::BOOL::NO for production.

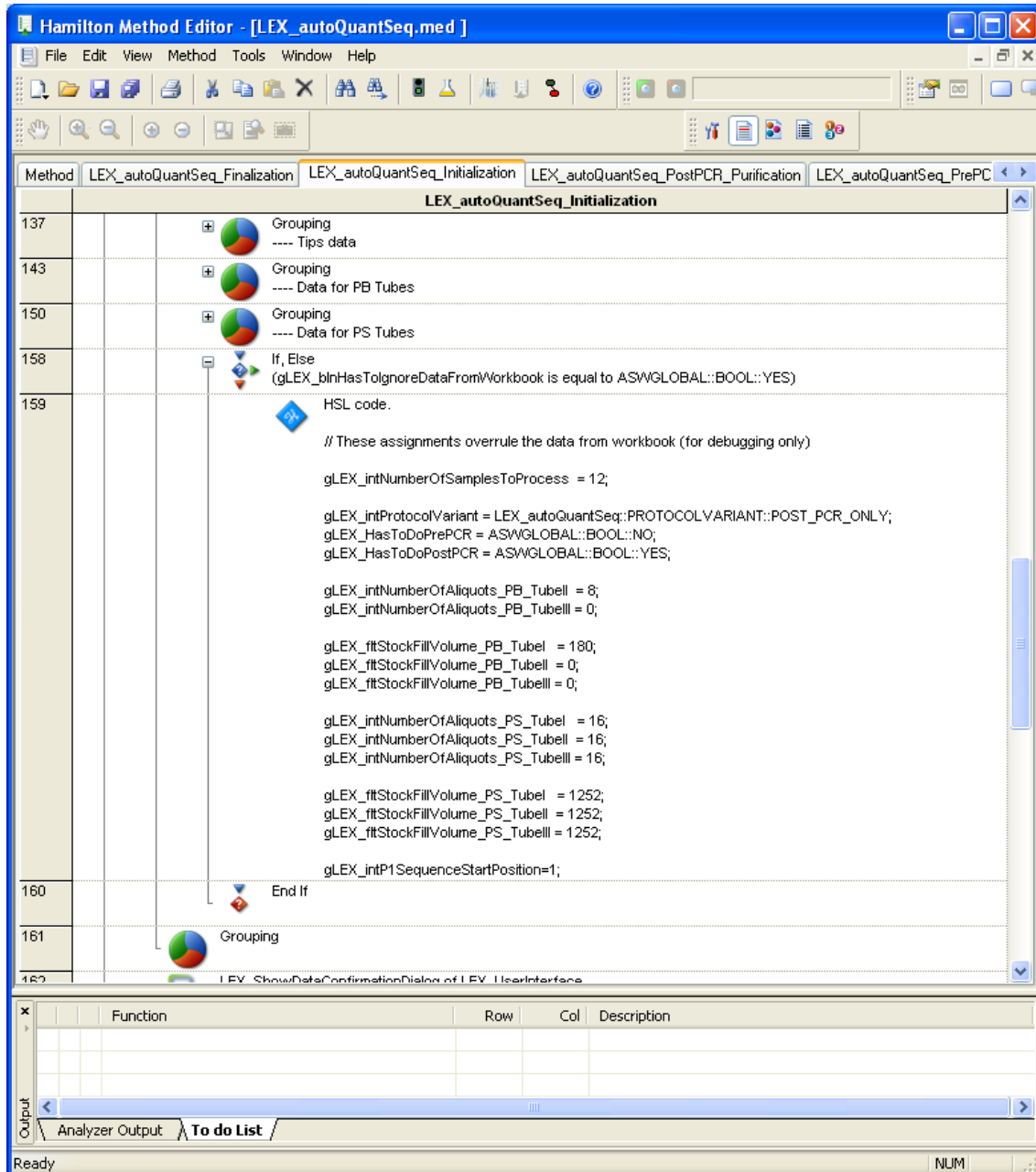


Figure 8: *LEX_autoQuantSeq_Initialization* submethod. For debugging special setting (overruling the workbook settings) can be edited here.

global variable **gLEX_blnHasToBreakAfterLiquidTransferForDebugging**

- If set to ASWGLOBAL::BOOL::YES , the protocol stops after each single liquid transfer command and a dialog is shown on screen. The tester can check the quality of the transfer and resume the run by clicking the button in the dialog. This can be used for debugging.
- Set to ASWGLOBAL::BOOL::NO for production.

4.2. Testing with dummy liquids

Ask Lexogen for an autoQuantSeq Dummy Reagent Kit (Cat.Nr. 019.24). This Kit contains liquids which are chemically inactive but very similar in fluidic properties to the real reagents. They are suitable for wet test of the protocol.

5. Diverse Tips & Tricks

- **To quickly skip over deck layout screen** without having to check all check boxes, press 'TAB' , then 'ENTER' immediately after the dialog appears.