AUTO™ SENSE V2

SENSE mRNA-Seq Library Prep Kit V2
on the PerkinElmer Sciclone/Zephyr NGS Workstations

Installation Guide

Catalog Numbers:

001.24 (SENSE mRNA-Seq Library Prep Kit V2 for Illumina, including Barcode Set 1–3, 24 preps)

001.96 (SENSE mRNA-Seq Library Prep Kit V2 for Illumina, including Barcode Set 1–12, 96 preps)

024.96 (Automation Module for SENSE mRNA-Seq V2, 96 preps)

Corresponds to Application release: 2015-04-06

Revision Date: Apr 6th, 2015

1. PHASE 1–PrePCR on Sciclone

1.1. Installation Prerequisites

1.1.1. Maestro Version

- Application works with Maestro 6.0. with patch level 44.

1.2. Code Details

1.2.1. Global Variables

Name	Type	Typical Value	Comment
g_ApplicationAborted	B	False	Dynamic – GUI stops is this is set to True
g_ApplicationName	S	SENSE_Library_Prep	Constant – DO NOT CHANGE
g_DisposeOfConsumableLocation	S	C1	Constant – DO NOT CHANGE
g_DisposeOfTipsLocation	S	D5	Constant – DO NOT CHANGE
g_ExcelWorkBook	S	C:\ProgramData\CaliperLS\Maestro\LEXOGEN\Workbooks\autoSENSEV2 Library Prep Workbook.xls	Constant – DO NOT CHANGE
g_ExcelWorkSheet	S	Reagent Plates PrePCR(Sciclone)	Constant DO NOT CHANGE
g_IncubationTimer	T	?	Dynamic – used for timer management
g_IsInitializeNumTipBoxPerDeck	B	True	Used by tip management
g_IsSunkenDeckNGS	B	False	Constant – ONLY CHANGE FOR INSTALLATION on NGSx
G_LEX_HasToShowDialog	B	False	Used for debugging – FALSE for real runs
g_LEX_height	N	?	Output from LEX_SUB_VolumeToHeight method
g_LEX_IsGUIActive	B	True	Constant – FALSE inhibits GUI display
g_LEX_IsShortenedTimerDelay	B	False	Used for debugging – FALSE for real runs
g_LEX_IsSimulatedRunWithoutHardware	B	False	Used for debugging – FALSE for real runs
g_LEX_IsWaitingForThermoblock	B	True	Used for debugging – TRUE for real runs
g_MainLayout	S	LEX_SENSE_MainLayout	Constant DO NOT CHANGE
g_NumberofColumnsToProcess	N	?	Constant - Used by tip management
g_NumberOfReservedTipBox	N	?	Constant - Used by tip management
g_NumberOfRowsToProcess	N	?	Constant - Used by tip management
g_NumberOfTipBoxPerDeck	N	?	Constant - Used by tip management
g_NumberOfTipsInRow		?	
g_Offset_A2	N	14.20	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_A3	N	5.45	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_A4	N	13.75	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_actual	N	?	Dynamic
g_Offset_B2	N	14.00	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B2_stack2	N	12.80	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B4	N	14.10	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B4_stack2	N	13.30	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B5	N	38.60	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B5_HJ	N	41.60	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_C4	N	14.60	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_C4_single	N	15.20	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D2	N	13.80	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D2_single	N	14.40	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D3	N	38.75	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D3_HJ	N	41.90	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D4	N	14.00	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_D4_single	N	15.00	TEACHING: see UTIL_SetGlobalOffsets
g_ReservedTipBoxLocation	N	1	Used by tip management

g_ReservedTipBoxYOffset	N	0	Used by tip management
g_ReservedTipBoxZOffset	N	-10	Used by tip management
g_RobotSpeed	N	100	Constant – 100 for full speed
g_ScreenName	S	Phase1	Constant DO NOT CHANGE
g_SENSE_AirGap_L	N	5	Constant – DO NOT CHANGE
g_SENSE_AirGap_M	N	2	Constant – DO NOT CHANGE
g_SENSE_AirGap_S	N	1	Constant – DO NOT CHANGE
g_SENSE_AirGap_XL	N	10	Constant – DO NOT CHANGE
g_SENSE_AirGap_XXL	N	30	Constant – DO NOT CHANGE
g_SENSE_AreBeadsWashedString	S	NO	Constant – from workbook
g_SENSE_AssayID	S	?	Constant – from workbook
g_SENSE_AvailableVolume_Bar_1	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_Bar_2	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_BC	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_BW	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_DSPRI	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_E1T	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_E2	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_EtOH	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_HYB	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_MB	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_PE	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_RTL_1	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_RTL_2	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_SBUF	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_SE_1	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_SE_2	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_ST	N	?	Dynamic – at start from workbook
g_SENSE_AvailableVolume_TRIS	N	?	Dynamic – at start from workbook
g_SENSE_EmptyTipBoxLocationID	N	C2	Constant – DO NOT CHANGE
g_SENSE_GUI_text1	S	?	Dynamic
g_SENSE_IsRNAInputBelow50ng	B	False	Constant – from workbook
g_SENSE_IsRNAInputBelow50ngString	S	NO	Constant – from workbook
g_SENSE_LibrarySizeOptionString	S	A	Constant – from workbook
g_SENSE_MagnetSeparationTime_Standard	T	0:02:00	Constant — DO NOT CHANGE
g_SENSE_NumberOfRowsToProcess	N	8	Constant – from workbook – DO NOT CHANGE
g_SENSE_NumberOfSampleColumns	N	12	Constant – from workbook
g_SENSE_NumberOfSamples	N	96	Constant – from workbook
g_SENSE_StartConfirmed	B	?	Dynamic
g_SENSE_UseOilForSealing	B	True	Constant – from workbook
g_SENSE_UseOilForSealingString	S	YES	Constant – from workbook
g_SENSE_WashBeads	B	True	Constant – from workbook
g_SpareTipBoxIndexHolderLocation	S	A1	Constant DO NOT CHANGE
g_SpareTipsLocationID	S	B3	Constant DO NOT CHANGE
g_ThisApplicationScreensPath	S	C:\ProgramData\CaliperLS\Maestro\LEXOGEN\autoSENSE_Library_Prep\	Constant DO NOT CHANGE
g_TimerWait	B	?	Dynamic - Used by timer management
g_TipsCountMulti	N	?	Dynamic - Used by tip management
g_TipsCountSingle	N	?	Dynamic - Used by tip management
g_WorkingTipBoxLocationID	S	C3	Constant DO NOT CHANGE
g_WorkingTipBoxRemainingCols	N	?	Dynamic - Used by tip management

1.2.2. Set Leg Light Command

If you want to activate the leg lights during the prePCR run on the Sciclone, please add the **Set Leg Light Intensity** commands (recommended settings: 90% intensity for ON; 0% intensity for OFF) to the following points in the code:

UTIL_HardwareInitialization method: after the **Sciclone.Set Speed(…)** command:

Set Leg Light Intensity 90%

LEX_SENSE_07_Finalize method: before the **ThermoLocator3:Temperature On/Off (…)** command:

Set Leg Light Intensity 0%

1.2.3. Diverse

- Always update the text of the **LEX_SENSE_Notes_and_Revisions** methods with most current comments of any changes you make. In particular, this text should contain all specific changes and adaptation for the local installation.
- Always update the 'Last revision' entry in the header of any method you modify.

1.3. Teaching

After teaching, insert the values into the assignments in **UTIL_SetGlobalOffsets**.

Note that it is possible and desirable to attempt to estimate this value to within ±0.05 mm.

Variable Name	Typical Value	Teaching Condition				Comment
		Location	Consumable	No of Tips Loaded	Recommended initial teaching value	
g_Offset_A2	14.20	A2	single HSP-96 plate	96	13.50	
g_Offset_A3	5.45	A3	Corning 384 plate on **INHECO 384**	96	4.50	Taught in the A1 quadrant
g_Offset_A4	13.75	A4	HSP-96 plate on **INHECO 96**	96	13.00	
g_Offset_B2	14.00	B2	single HSP-96 plate	96	13.50	
g_Offset_B2_stack2	12.80	B2	stack of two HSP-96 plates	96	12.00	
g_Offset_B4	13.30	B4	single HSP-96 plate	96	12.50	
g_Offset_B4_stack2	12.80	B4	stack of two HSP-96 plates	96	12.00	
g_Offset_B5	38.60	B5	2mL Deepwell plate	96	38.00	
g_Offset_B5_HJ	41.60	B5	2mL Deepwell plate (Ritter riplate SW)	96	41.00	This is a non-standard consumable used as an optional alternative to Seahorse product

g_Offset_C4	14.60	C4	single HSP-96 plate on **magnet with spacer**	96	14.00	Tips should touch bottom but not push down on magnet.
g_Offset_C4_single	15.20	C4	single HSP-96 plate on **magnet with spacer, 8 tips in a single column 1**	8	14.00	Tips should touch bottom but not push down on magnet.
g_Offset_C5	14.05	C5	single HSP-96 plate	96	13.50	
g_Offset_C5_stack2	13.15	C5	stack of two HSP-96 plates	96	12.50	
g_Offset_D2	13.80	D2	HSP-96 plate on **INHECO 96**	96	13.00	
g_Offset_D2_single	14.40	D2	HSP-96 plate on **INHECO 96, 8 tips in a single column 1 only**	8	13.00	
g_Offset_D3	38.75	D3	2mL Deepwell plate	96	38.00	
g_Offset_D3_HJ	41.90	D3	2mL Deepwell plate (Ritter riplate SW)	96	41.00	This is a non-standard consumable used as an optional alternative to Seahorse product
g_Offset_D4	14.00	D4	HSP-96 plate on **INHECO shaker**	96	13.50	Close to , but not completely at the bottom of the well.
g_Offset_D4_single	15.00	D4	HSP-96 plate on **INHECO shaker, 8 tips in a single column 1 only**	8	14.50	Close to , but not completely at the bottom of the well.

Variable Name	Typical Value	Teaching Condition
l_correction_1_column	0.84	
l_correction_2_columns	0.69	
l_correction_3_columns	0.54	
l_correction_4_columns	0.42	
l_correction_5_columns	0.37	
l_correction_6_columns	0.27	
l_correction_7_columns	0.27	
l_correction_8_columns	0.17	
l_correction_9_columns	0.17	
l_correction_10_columns	0.14	
l_correction_11_columns	0.07	

1.4. Testing at Site

1.4.1. Dry Test

For a dry test, the following variable setting is recommended:

- Set the global variable **g_LEX_IsShortenedTimerDelay** to **TRUE**. This will automatically shorten all longer waiting times to 2 seconds, thus shortening the test run.
- Set the global variable **g_LEX_IsWaitingForThermoblock** to **FALSE**. This will skip all waitings for preset temperature, thus shortening the test run.

Apply these settings directly in the **LEX_SENSE_01_Initialize** method. After debugging, do not forget to reset the values to standard for real runs.

1.4.2. Wet Test

Shortening of the wet test can be achieved by similar variable settings as described above.

Dummy liquids for wet test

Ask Lexogen for a protocol-specific kit with dummy reagents for wet testing.

2. PHASE 1–PostPCR on Zephyr

2.1. Installation Prerequisites

2.1.1. Maestro Version

- Application works with Maestro 6.0 with patch level 44.

2.2. Code Details

2.2.1. Global Variables

Name	Type	Typical Value	Comment
g_ApplicationAborted	B	False	Dynamic – GUI stops is this is set to True
g_ApplicationName	S	SPRI Cleanup	Constant – DO NOT CHANGE
g_BeadVolume	N	22	from input dialog
g_CurrentTipBoxNumber	N	1	Used by tip management
g_ElutionVolume	N	10	From input dialog
g_IncubationTimer	T	?	Dynamic – used for timer management
g_LoopNumber	N	3	Dynamic – used for loop counting
g_NextAvailableTipBox	N	A1	Used by tip management
g_NumberOfColumnsToProcess	N	12	from input dialog
g_Offset_A3_PCR_plate	N	15.2	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B2_PCR_plate	N	14.4	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_B3_PCR_plate	N	14.8	TEACHING: see UTIL_SetGlobalOffsets
g_Offset_C2_PCR_plate	N	14.4	TEACHING: see UTIL_SetGlobalOffsets
g_SampleVolume	N	28	from input dialog
g_ScreenName	S	Setup	Constant DO NOT CHANGE
g_ThisApplicationScreensPath	S	C:\ProgramData\CaliperLS\Maestro\Screens\SPRI\	Constant DO NOT CHANGE
g_TimerWait	B	True	Dynamic – used for timer management
g_TipBoxColumnsAvailable	N	12	Used by tip management
g_TipLoadColumn	N		Dynamic – used for timer management
g_TipsLoaded?	S	No	Dynamic – used for loop counting
g_TotalBindingVolume	N	50	Sum of Bead volume and Sample Volume

2.3. Teaching

After teaching, insert the values into the assignments in **UTIL_SetGlobalOffsets**.

Note that it is possible and desirable to attempt to estimate this value to within ±0.05 mm.

Variable Name	Typical Value	Teaching Condition				Comment
		Location	Consumable	No of Tips Loaded	Recommended initial teaching value	
g_Offset_A3_PCR_plate	14.40	A3	single HSP-96 plate **on magnet (no spacer)**	96	13.50	

g_Offset_B2_PCR_plate	14.40	B2	single HSP-96 plate	96	13.50	
g_Offset_B3_PCR_plate	14.40	B3	single HSP-96 plate **INHECO shaker**	96	13.50	
g_Offset_C2_PCR_plate	14.00	C2	single HSP-96 plate on **INHECO 96**	96	13.50	

Variable Name	**Typical Value**	**Teaching Condition**
l_correction_1_column	0.40	
l_correction_2_columns	0.38	
l_correction_3_columns	0.29	
l_correction_4_columns	0.23	
l_correction_5_columns	0.23	
l_correction_6_columns	0.16	
l_correction_7_columns	0.13	
l_correction_8_columns	0.04	
l_correction_9_columns	0.04	
l_correction_10_columns	0.04	
l_correction_11_columns	0.04	

APPENDIX A: The PE Gold Standard Installation Guide (May 2013)

Installation Guide for Sciclone NGS(x) Workflows that Conform to the Gold Standard Development and Distribution Framework

Background:

SicloneNGS Workflows consist of one or more individual Maestro Applications and supporting files (Excel workbook(s), and text/image files to guide deck setup). Installing a workflow involves copying all support files to the correct directories, importing individual applications into Maestro software, and adjusting specific settings in each application to fine-tune pipetting for a specific SicloneNGS instrument.

All SicloneNGS workflow applications, files, and supporting documents are maintained in a cloud-based repository system known as Assembla. The latest versions of this guide (and all SicloneNGS application files and supporting documents) can be readily obtained by setting up and maintaining a current Assembla repository on your own PC, or with the support of someone who maintains that access themselves (contact us if you need further support).

The following guide is adapted from one created to support installation of the Gold Standard (GS) Nimblegen Workflow. It is intended to provide sufficient guidance to enable the installation of this and any other SicloneNGS workflows on a standard, unmodified SicloneNGS or SicloneNGSx system.

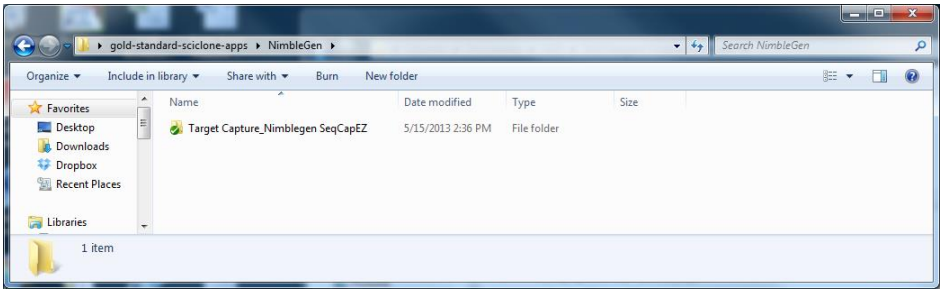
The Nimblegen SeqCapEZ Workflow includes 6 individual applications:

Application Suite (Workflow)	Application
Target Capture_Nimblegen SeqCapEZ	
	1 Nimblegen TruSeq Library Prep
	2 Nimblegen Library Prep Post PCR SPRI
	3 Nimblegen Pre-Capture Normalization
	4 Nimblegen Hyb Setup
	5 Nimblegen Target Capture
	6 Nimblegen Post-Capture Final SPRI

Procedures:

1) Download the Workflow folder from Assembla

For the Nimblegen SeqCap EZ workflow, the folder is in the path: "...gold-standard-sciclone-apps\NimbleGen".
Note that in the future, additional paths and different access controls may be incorporated into Assembla. These will also be updated at your local machine during the normal process of keeping it up to date, or you will be invited to participate by the system administrator.



2) Ensure the Maestro software has patch level >= 37

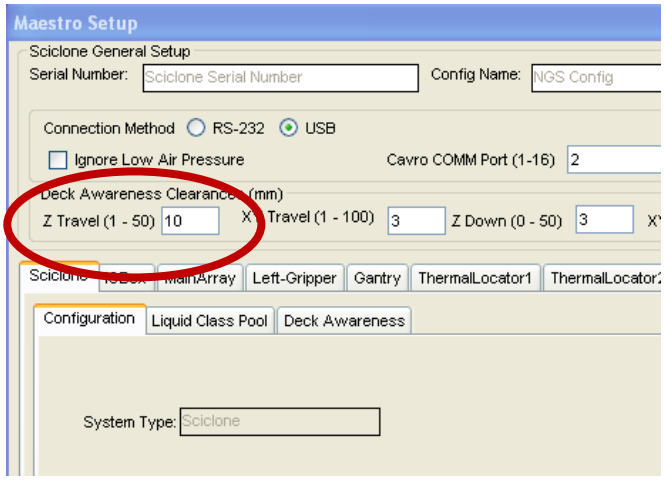
ScicloneNGS applications will not install properly on PC's with Maestro versions prior to 6.0 patch 37. Open Maestro software and navigate to Help/About to see the current software version. Note that if you have a version of Maestro ≥ V6.3 you will not need a patch. It is good practice to always apply the latest patch level when performing a fresh installation, or if there are features of interest (found in the release notes).
To obtain the latest patch version, go to the PerkinElmer website:
http://www.perkinelmer.com/resources/software-downloads.xhtml

Download the folder for Maestro v6.0 – PatchXX. Run the .exe file to install the patch.

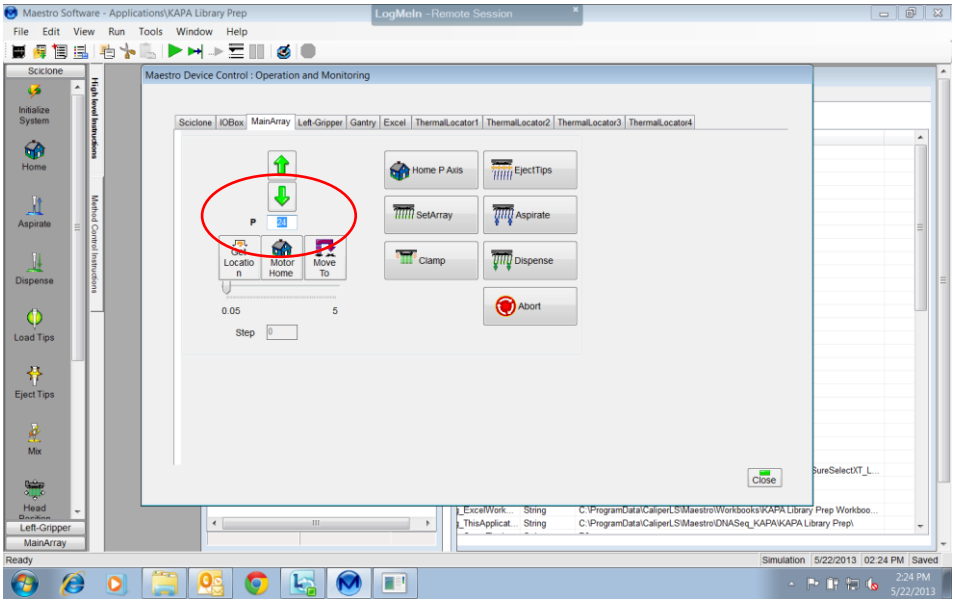
3) Check the configuration file for Maestro

In Maestro, go to **Tools/Setup** to open the configuration window. The configuration file contains settings for liquid handling and consumable handling that apply across all applications.

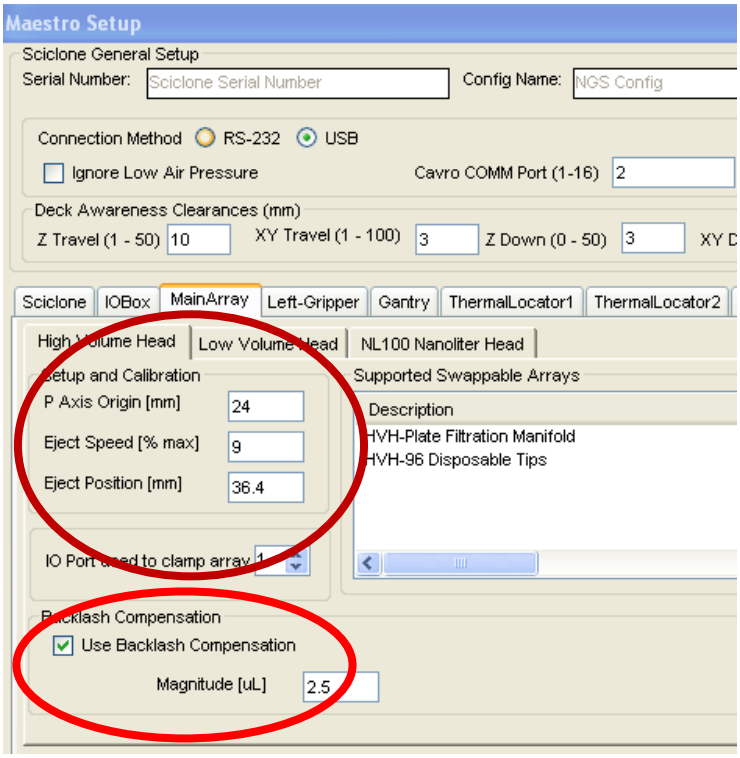
a) Check that the Z Travel for Deck Awareness Clearance is set to 10 mm.



b) Go to the tab for “Main Array”. Check that the P Axis Origin is set to 24 mm, the Eject speed is set to 9%. Eject position should be between 36 and 37 mm in order to ensure the most robust tip separation from mandrels during ejection in order to avoid collisions involving unreleased tips. If the Eject position is found to be less than 36, then change it to 36 mm. Alternatively, the maximum deflection of P can be measured directly using the device control for the P axis (see below), by jogging to the limit and then back my a small amount ~.4mm, and then testing the eject command directly a few times to see that it completes successfully.



c) Check that “Use Backlash Compensation” is Activated.



Note: Backlash compensation is a quality of each instrument that can affect liquid handling accuracy. It is the amount of apparent P-Motor movement that occurs without actual pump movement that occurs when a pipette changes direction from aspirate to dispense or vice-versa. It is measured in the factory and set appropriately for each SciCloneNGS system. It should not be necessary to change this, but it should be checked. The record of the experiment performed in the factory to determine this value is provided in "C:\Caliper Service Folder"

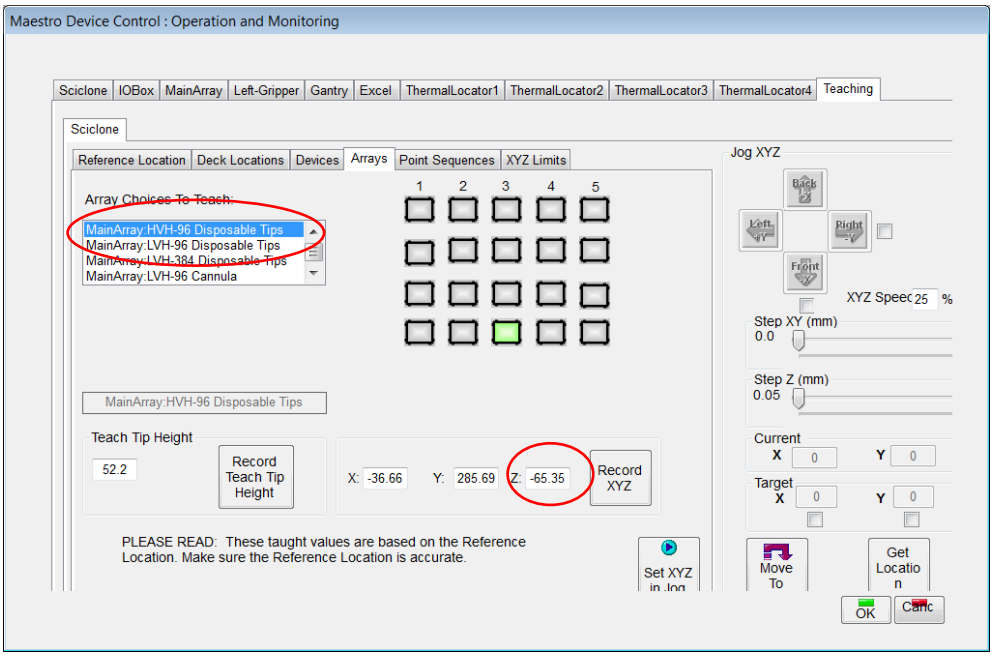
4) Check the teaching of the HVH-96 Disposable Tip array

To achieve robust performance with SciCloneNGS applications, it is necessary to correct for differences in tip height observed when loading variable numbers of tips onto the main array. Proper function of the SciCloneNGS applications requires a specific way of teaching the pipetting array which goes beyond the standard, general purpose Sciclone procedure. This is has been done in the factory in advance for all instruments manufactured after April 2011. Check these values to ensure they are correct after shipping and also to remain familiar with the whole process of ensuring ~.1mm precision in pipetting positioning.

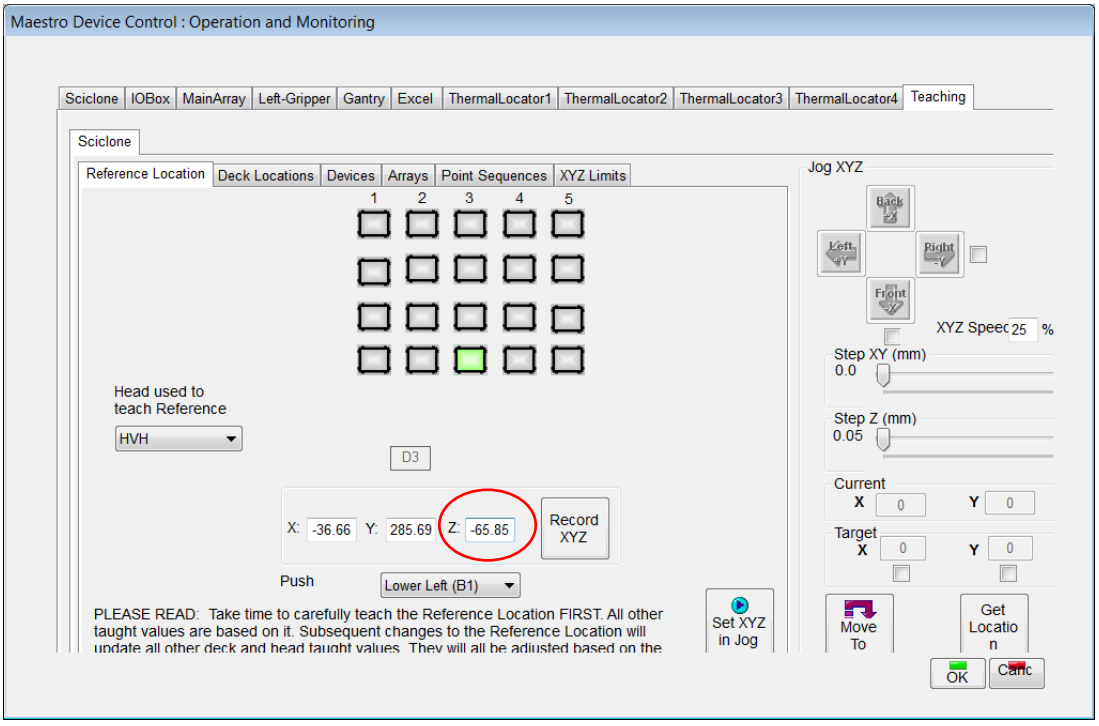
The standard teaching process (four tips in the corners of the array, fully seated against the shuck plate and positioned in the crosshairs at the surface of the metal teach tool) should be used to check/adjust the "reference position". All x,y,z positioning of deck locations, devices and consumable coordinates are calculated with respect to the reference position. It is convenient to teach the Z position by finding the height at which the tips create friction on a piece of paper laid on top of the metal teach plate. The tips should touch the paper, but not pin it down to the plate.

After checking/adjusting the reference position, it is necessary to also teach the Z position for the "Array" with the selection "MainArray:HVH-96 Disposable Tips". This position should be taught with 96 fresh tips which are loaded onto the array from the main tip box location used in most

SicloneNGS applications (C3) immediately after lubricating the mandrel O-rings. Again, find the height at which the tips create friction against a sheet of paper laid on top of the metal teach plate.



When teaching the array, it can be noticed that the Z position is ~0.5 to 0.6 mm higher than when four tips are fully seated in the corners.



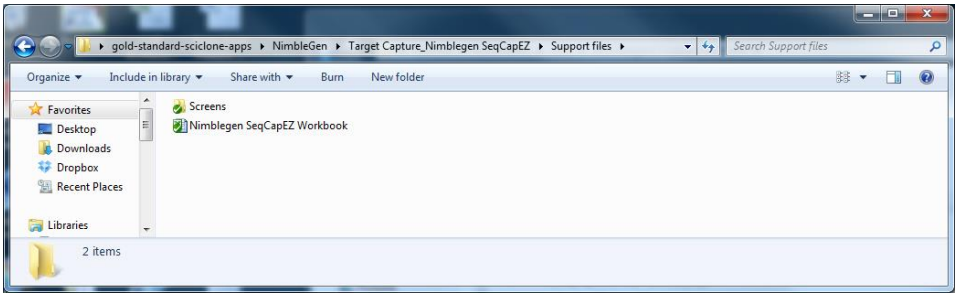
5) Check the teaching of the Gripper in the "Down" position:

The Gripper should line up so that the bottom of the gripper fingers just overlaps the horizontal line on the side of the teach plate. Take care to check the alignment of the gripper in the X direction. Look for equal spacing between the gripper pads and the teach plate. Once aligned by eye, check that the gripper is truly centered over the plate by executing a "grip" command to ensure that the plate does not move when gripped.

6) Install the Workflow files

a) Copy the Excel workbook associated with the workflow to the location:

C:\ProgramData\CaliperLS\Maestro\Workbooks
Do not change the name of the workbook.



Note: C:\ProgramData may be a hidden folder. If it does not appear when exploring, go to My Computer\Tools\Folder Options and select the "View" tab. Under "Hidden files and folders" select the radio button for "show hidden files and folders". (The process of selecting this property may vary in different versions of Microsoft Windows.)

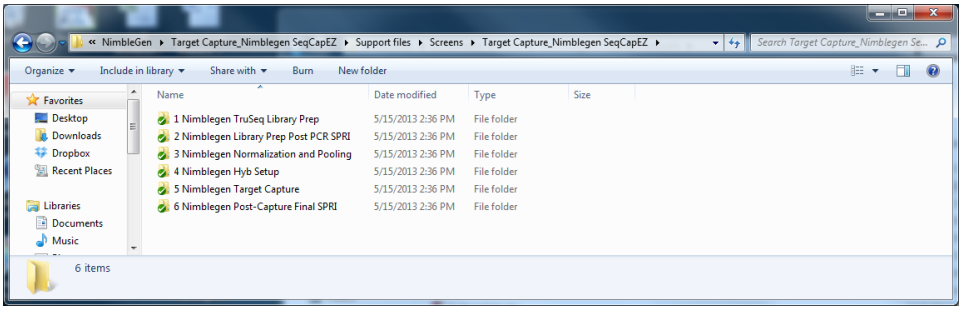
b) Copy the "screens" folders into the correct location:

C:\ProgramData\CaliperLS\Maestro\<Application Suite (Workflow)>\<Application>\

Each application comes with a collection of images and texts that guide the user in setting up the applications. They must be present in the appropriate folders on the target instrument for the startup interface to function properly. For the Nimblegen workflow, there are 6 different screens folders for the 6 different applications. Copy the folders shown above and place them in the correct path.

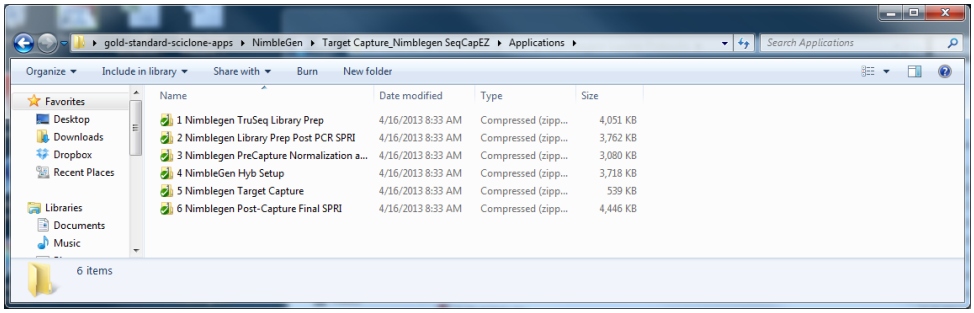
For the Nimblegen SeqCapEZ example, the Application Suite (Workflow) folder is named "Target Capture_Nimblegen SeqCapEZ", and the correct paths are:

C:\ProgramData\CaliperLS\Maestro\Target Capture_Nimblegen SeqCapEZ\Hyb Setup\
C:\ProgramData\CaliperLS\Maestro\Target Capture_Nimblegen SeqCapEZ\LibraryPrep Post-PCR SPRI\
….etc.

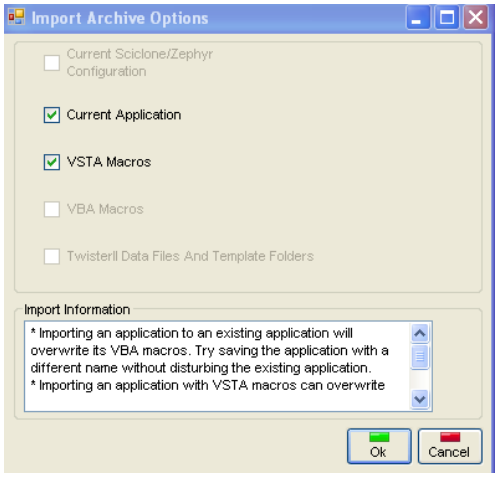


c) Import and save each of the application archive files (6 in this case).

Within Maestro, select File/Import/Import Archive. Browse for the application file and select to import.

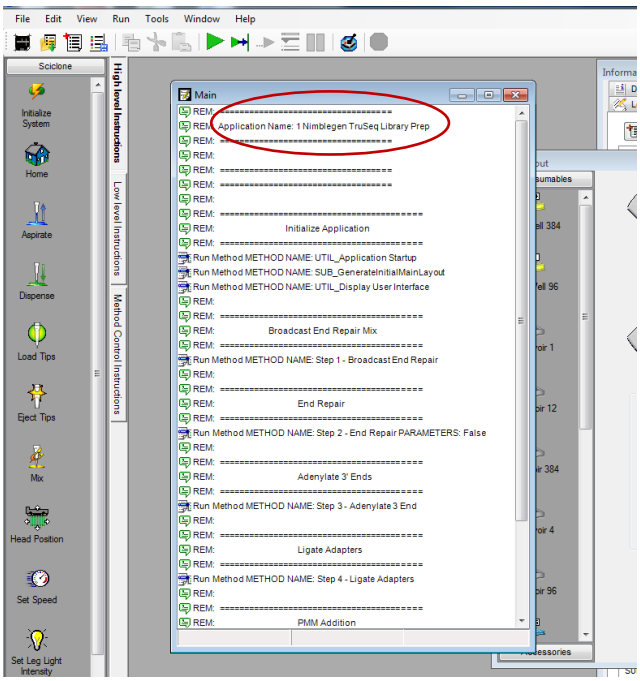


Import the Current Application and the VSTA Macros (Select OK)

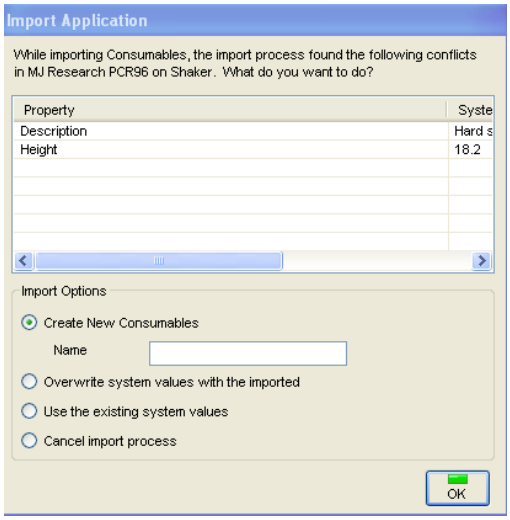


Once the application has imported, you will be prompted to save the application with a new name.

When importing the first application, create a folder with the Workflow name (e.g. Target Capture_Nimblegen SeqCapEZ). Save all the applications together under this folder, using the name displayed at the top of the Main method window. Numbering the applications will allow them to appear in the order in which they are used within the workflow.



*Note: During the application import process, you **may** see a message describing a "conflict" for consumables definitions. This means that there is a difference in the way a consumable is defined in the imported application vs. the way it is defined in the consumables database on the instrument. In the rare instance this occurs, you will have to choose how to proceed to resolve the conflict. This message may appear multiple times for different consumables. You must choose how to proceed each time.*



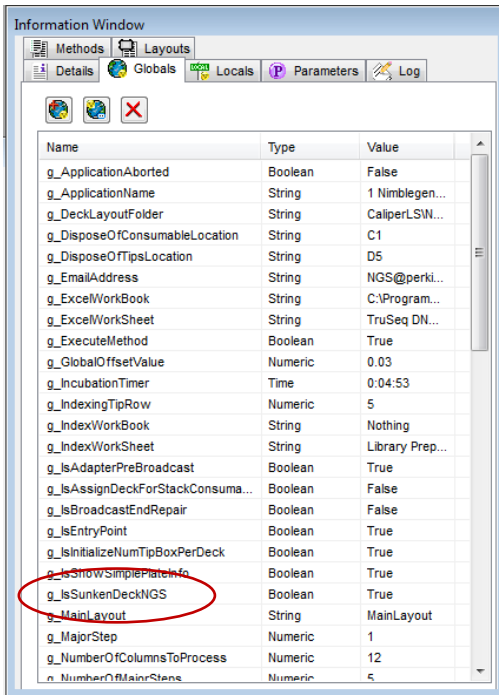
If the description of the conflict indicates a consumable from the Gold Standard Consumable List (e.g. Bio-Rad HSP-96 PCR96), it is recommended to select "Overwrite system values with the imported". The correct consumables definitions for these plates are critical for the Gold Standard applications. If the description of the conflict is for other consumables (e.g. MJ Research PCR96), select "Use the existing system values". This will leave the consumable definition for your instrument unchanged. See the Appendix for the Gold Standard Consumable List.

Warning: Care must be taken to understand the effects of changing consumable definitions on an instrument currently running non-conforming applications. The best path is probably to replace the older applications

present on the system with conforming versions and repeat sample validation studies. This is the only clear way to secure uncomplicated access to all future GS applications for the system.

d) Check the setting for the global variable g_IsSunkenDeckNGS

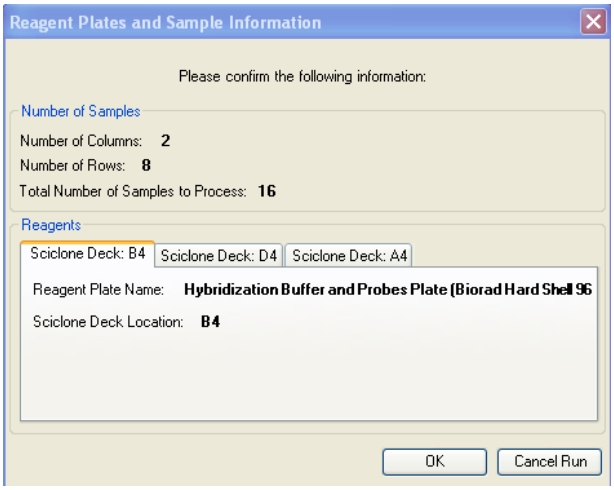
If running a SciCloneNGS instrument (standard deck) this variable must be set to "False". If running a SciCloneNGSx instrument (sunken deck tip storage in columns 0 and 1) this variable must be set to "True". **Failure to match this variable setting to the instrument type will cause crashes in most applications.**



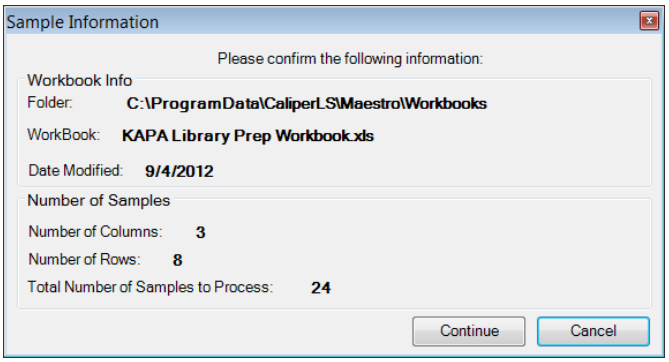
e) Confirm successful import for each application file

After you have saved the application, run the application from the main method. The application should start by initializing the hardware, setting up the deck layout, setting temperatures, etc. This indicates that the VSTA code is working and the support files have been saved in the correct locations.

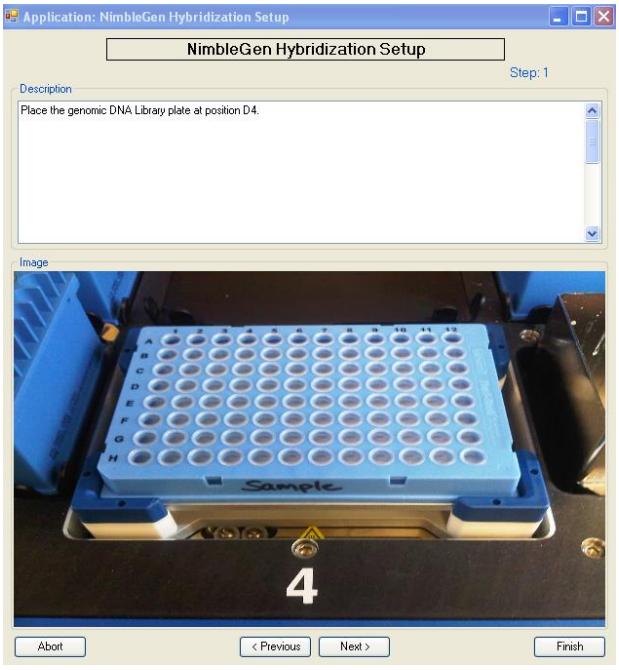
You should see a screen pop up indicating that the Excel worksheet was read correctly. In some cases the only identifiable information will be the number of columns selected and the date (see second example), so it is a good idea to open the spreadsheet, enter a specific value there and save it before starting, to ensure that Maestro is in fact reading from the right copy of the sheet.



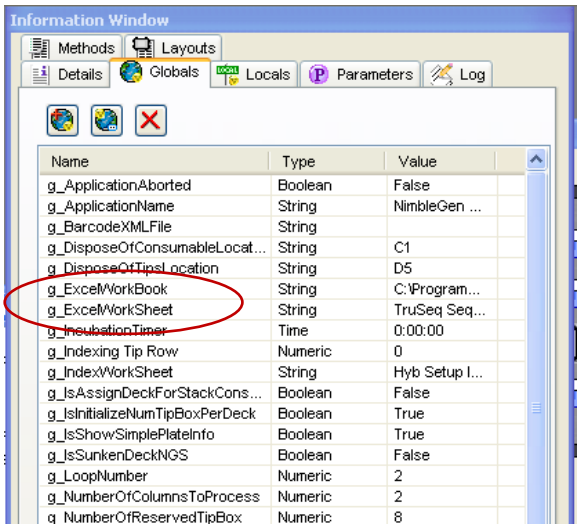
OR



After clicking OK, the setup screens should appear, indicating that the text and picture files have been copied to the correct folder.



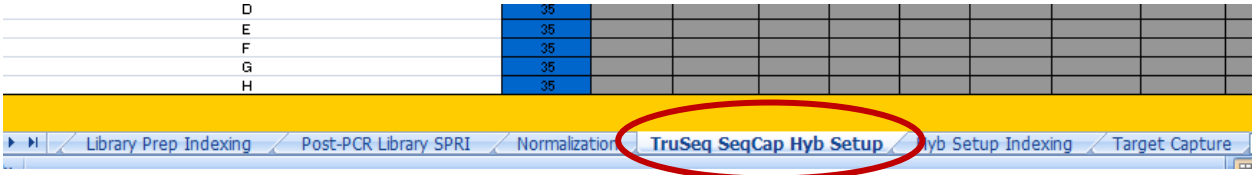
If there is any problem with reading the workbook or displaying the screens, it means that the file path described in the application and the actual file path for the files do not match. Check the following variables in the Maestro Information window:



g_ExcelWorkBook – This is the name and path of the application spreadsheet. It should match the path described in step 4 above.

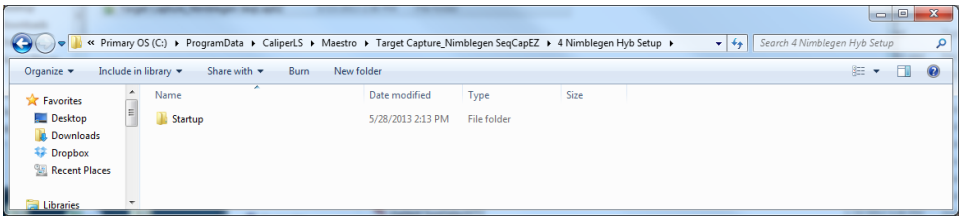
C:\ProgramData\CaliperLS\Maestro\Workbooks\<Workbook Name>.xls

g_ExcelWorkSheet – This is the worksheet within the Excel workbook where we read the data from. The variable should match the name of the worksheet (within the workbook) specific for the application.



g_ThisApplicationScreensPath – This is the path to the files containing the deck setup screens. **Do not include the final folder name** (e.g. "Startup" or "Setup"). There must be a \ at the end of the file path in the string variable: C:\ProgramData\CaliperLS\Maestro\<Application Suite (Workflow)>\<Application>\

g_ScreenName – This is the name of the folder containing the actual text and picture files used for the setup screens. For most applications, g_ScreenName is set to "Startup".

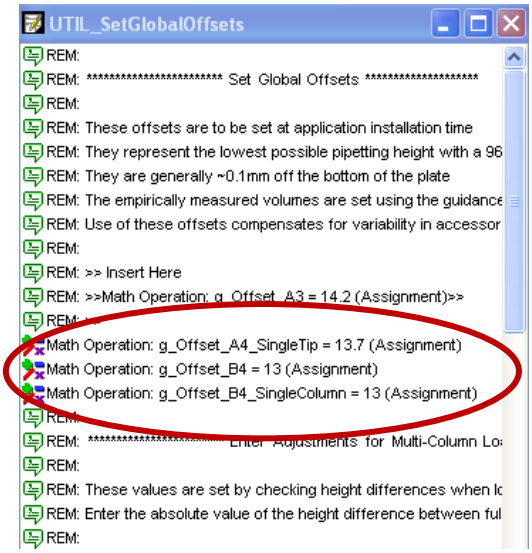


7) Fine-Tune each Application using the method "UTIL_SetGlobalOffsets"

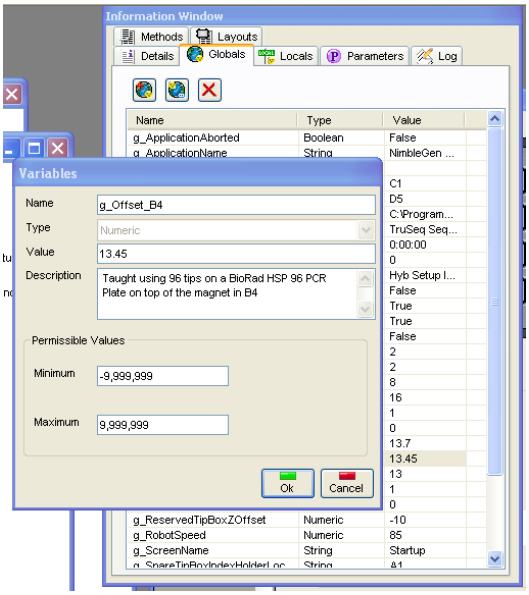
Background: When an application has been developed to conform to the GS framework and practices, completing the tasks within the single method " UTIL_SetGlobalOffsets" should be all that is required to ensure repeatable behavior of the application from one instrument to the next. The application has all critical pipetting heights identified and appropriate variables created to control those pipetting heights. This eliminates the need to identify and optimize critical liquid handling steps for each new installation, and simplifies and accelerates the process.

a) Teach the g_Offset values listed at the top of the UTIL_SetGlobalOffsets method.

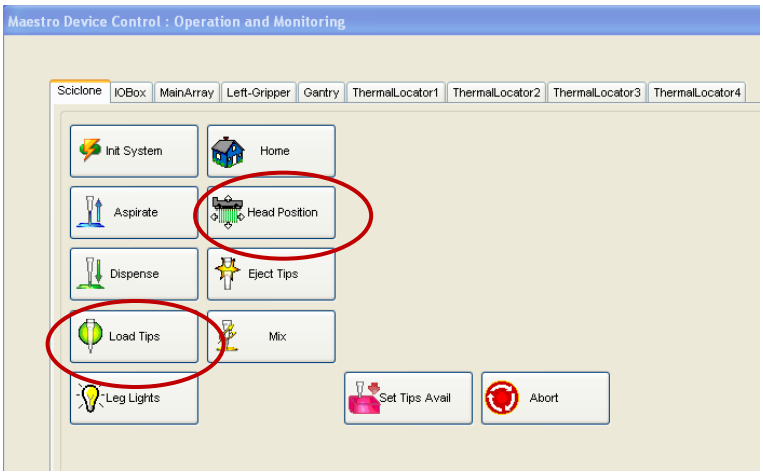
Open the UTIL_SetGlobalOffsets method and find the list of Math Operations defining the g_Offset variables. The number of variables and the names of variables in this list will be different for each application. These variables are used to position tips precisely at the bottom of consumable wells.



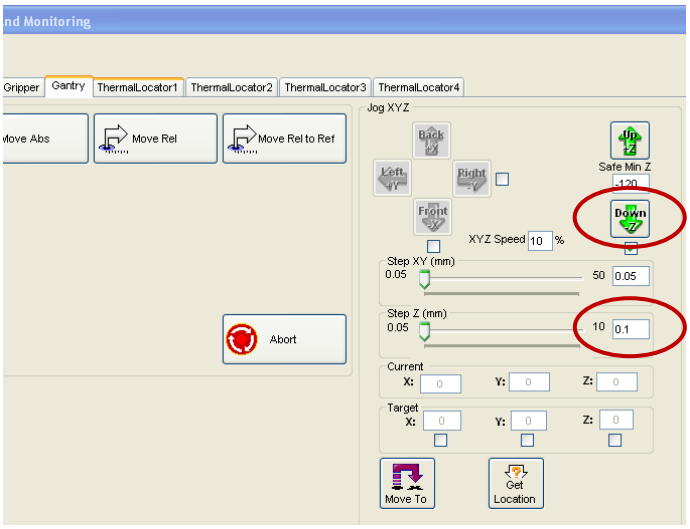
For each g_Offset variable listed, open the variable definition from the information screen. This will give notes on how to find the correct value for the variable.



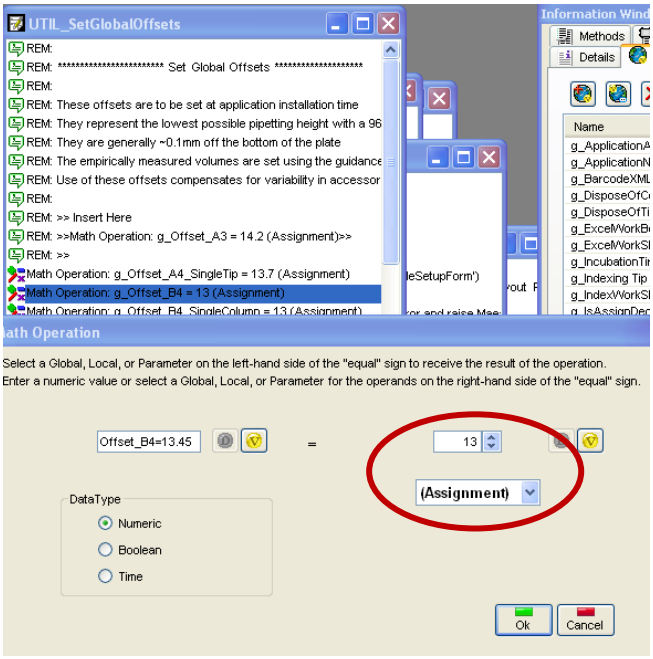
Using Device Control, load the appropriate number of tips, do a “head position” over the appropriate deck location and consumable.



Then, use the gantry control to drive the head down until the tips are at the bottom of the wells, but not pressing the plate down. Count how many mm the head must move down to reach the correct Z position (lift the plate with your hands to judge whether the tips are pressing down against the bottom of the wells), and record the distance. Note that it is possible and desirable to attempt to estimate this value to within +/- 0.05 mm.



Use the **absolute value** for the distance traveled to reach the correct height as the assignment value for the global variable in the UTIL_SetGlobalOffsets method.



Repeat this process for each of the g_Offset variables listed in the UTIL_SetGlobalOffsets Method.

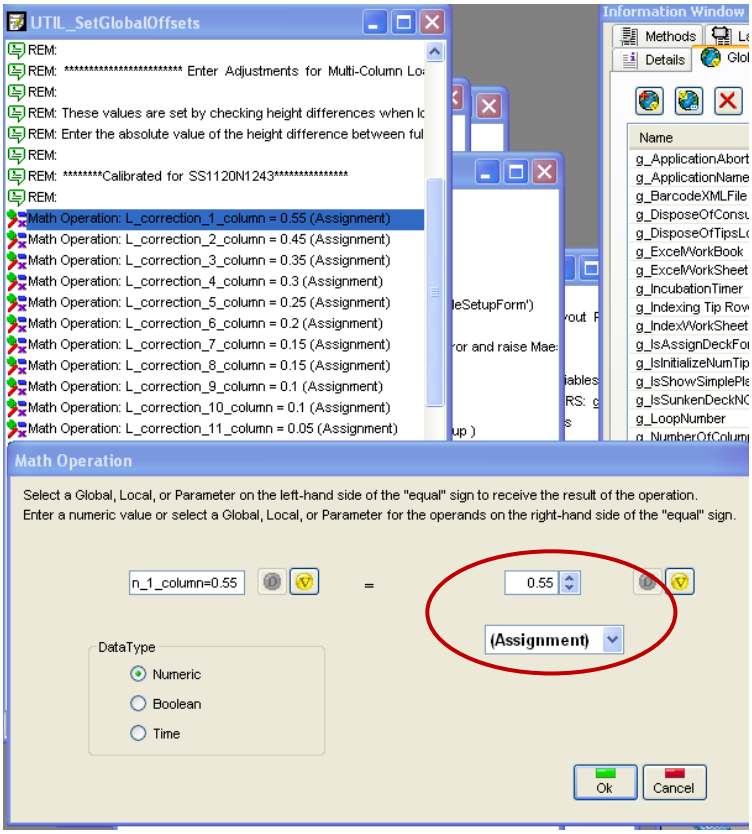
Note: If any problems are seen with X or Y positioning over the consumables, especially those on Inheco locators, see Appendix 4 for instructions on adjusting the positioning.

b) Enter the appropriate L_correction_X_column values for the instrument

Background: There is a small variation in the apparent length of tips depending on the number of columns of tips loaded. We write the Gold Standard applications to allow variable numbers of samples to be run (and hence various numbers of tips to be loaded). The L_correction_X_column variables are used to adjust the appropriate g_Offset values at application startup for the specific number of sample columns to be run that day. This ensures robust performance for steps in which positioning tips exactly at the bottom of the wells is critical.

The L_correction_X_column values are specific for an instrument, but should be the same across all applications run on the instrument. If any Gold Standard SicloneNGS workflows are currently run on the instrument, the values should be copied from these validated applications. Otherwise, the correct values may be determined by following the procedure in Appendix 3.

In the UTIL_SetGlobalOffsets method, scroll down to the section listing the Math Operations defining the L_correction_X_column variables. For each variable, update the value with the value correct for the instrument.



8) Run dry test of applications

Set up the deck with some fresh tip boxes and used plates. Use a number of columns that will require a tip loading offset correction and visit any multicolumn reagent logic (for example 7) so that you can physically ensure that the corrected positioning of tips is similar to those observed when estimating the global offsets earlier. This will ensure that the corrections and the offsets are working together correctly to give repeatable performance across different batch sizes. Select a pattern of indexing adapters from the spreadsheet that produces a pattern that is easily verified by watching the run. Run the applications from **Main** method. Cancel all incubations.

This test will ensure that gripper transports of plates, tips and lids are robust, and that there are no collisions with accessories and consumables. It will also test all VSTA macros that were not run at startup, such as those used to process the indexing sheet of the setup workbook.

9) Run wet test of applications

Use the Excel workbook as a guide for setting up plates with the correct volumes of liquids. Use 10% glycerol or 10% PEG in place of enzyme solutions. Use water in place of wash solutions. If they are available, it is useful to use some Ampure beads (expired is OK) and some 80% ethanol to see that the handling of beads is clean and that beads are not lost during SPRI cleanups (water does not aggregate beads in the same way as alcohol and so does not provide a fully valid test case). If beads

are limited, they can be arrayed in the front row(s) –H and/or G- that are easily observed, and water can be used elsewhere.

Appendix 1 - **Applications included in Gold Standard Workflows**

Application Suite (Workflow)	**Application**
DNASeq_TruSeqDNA	
	1 TruSeqDNA Library Prep
	2 TruSeq Post-PCR SPRI
RNASeq_TruSeqRNA	
	1 TruSeqRNA
	2 TruSeqDNA Library Prep
	3 TruSeq Post-PCR SPRI
Target Capture_SureSelectXT	
	1 SureSelectXT Initial SPRI Cleanup
	2 SureSelectXT Library Prep
	3 SureSelectXT PreCapture Normalization
	4 SureSelectXT Hyb Setup
	5 SureSelectXT Target Selection
Target Capture_Nimblegen SeqCapEZ	
	1 Nimblegen TruSeq Library Prep
	2 Nimblegen Library Prep Post PCR SPRI
	3 Nimblegen Pre Capture Normalization and Pooling
	4 Nimblegen Hyb Setup
	5 Nimblegen Target Capture
	6 Nimblegen Post Capture Final SPRI
RNA Seq_TruSeq Stranded RNA	
	1 TruSeq Stranded RNA cDNA Prep
	2 TruSeq Stranded RNA Library Prep
	3 TruSeq Stranded RNA Post-PCR SPRI
DNASeq_KAPA	

	1 KAPA Library Prep
	2 KAPA Post-PCR SPRI

Appendix 2 - Gold Standard Consumables for Sciclone/Zephyr NGS

For new instruments built after April 2012, these consumables will be present in the consumables database upon shipment. They can be identified as factory installed consumable definitions by the "read only" property. All applications coming out of Hopkinton will be built with these consumables. Pipetting will be optimized based on standard instrument teaching practices used by manufacturing. Please retain these consumables in applications wherever possible. If additional consumables are required please contact the Hopkinton team to add them to the factory installed list.

Bio-Rad HSP-96 PCR96
Bio-Rad HSP-96 PCR96 on CPAC
Bio-Rad HSP-96 PCR96 on Magnet No Spacer
Bio-Rad HSP-96 PCR96 on Magnet With Spacer
Bio-Rad HSP-96 PCR96 on Shaker
Bio-Rad HSP-96 PCR96 Stack 2
Bio-Rad HSP-96 PCR96 Stack 3
Bio-Rad HSP-96 PCR96 Stack 4

Seahorse 2 mL Deepwell 201379
Seahorse 2 mL Deepwell 201379 on CPAC
Seahorse Deepwell Reservoir 201244

Corning 384 on CPAC

Costar-1ml, Polypropylene
Costar-1ml, Polypropylene On CPAC
Costar-1ml, Polypropylene On Magnet
Costar-1ml, Polypropylene On Shaker

Costar-2ml, Polypropylene
Costar-2ml, Polypropylene on CPAC
Costar-2ml, Polypropylene on Magnet

PerkinElmer V-Bottom StorPlate
PerkinElmer V-Bottom StorPlate on Magnet No Spacer
PerkinElmer V-Bottom StorPlate on Shaker
PerkinElmer V-Bottom StorPlate on CPAC

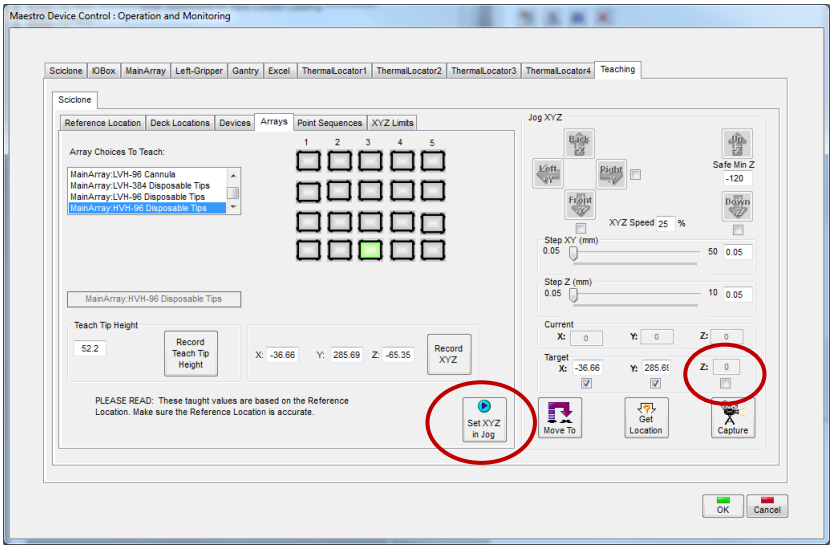
Appendix 3 – Determining values for L_correction_X_column variables

To correct for height differences in tips depending on number of columns loaded, the UTIL_SetGlobalOffsets method in each application applies a correction variable.

Tip: When the offset for the HVH-96 Disposable Tip Array was previously taught (see above), it would have been with 96 FRESH tips loaded immediately after lubricating the mandrel array. This process should be performed immediately after that one -if possible- or, at a minimum make sure that the mandrel array is still recently lubricated and that fresh tips are used.

To determine these correction variables:

a) Place the teach plate in position D3.
b) Place a sheet of paper on top of the teach plate.
c) Load 1 column of tips on the left side of the array.
d) Open the teach window and select the HVH-96 Disposable Tips array from the Arrays tab.



e) Select “Set XYZ in Jog”, then uncheck the Z position box. Select the “Move To” button to position the head correctly with XY coordinates.
f) Repeat the “Set XYZ in Jog”, this time leaving the Z position active. Select “Move To” to bring the tips down over the paper/teach plate.

g) Use the Gantry to drive the head down in final increments as fine as 0.05mm until the tips are touching but not holding the piece of paper against the teach plate and a further 0.05mm increment would capture the paper. (Take care not to crush the tips as they will become shortened as a result and would need to be replaced before proceeding.)
h) Make note of the Z position value where the single column of tips touch the paper.
i) Eject the tips back into the tip box.
j) Load 2 columns of tips and repeat the process. Make note of the Z position value where the two columns of tips touch the paper.
k) Continue for 4-12 columns of tips, recording the Z position at which the tips touch the paper but do not hold it against the teach block.

When values have been found for 1 – 12 columns of tips, calculate the difference between the 12 columns Z height and each of the other column Z heights.

The absolute value of the difference is used as the assignment value for the variables:

L_correction_1_column = 0.65

L_correction_2_column = 0.55

Etc.

<u>Appendix 4</u> – Adjusting XY positioning over Inheco locators

When executing a "Head Position" command over a specific consumable, tips should be centered over the wells of the consumable. Incorrect teaching in the Z direction will cause the tips to go too high or too low, but will be corrected with the g_Offset values. Incorrect teaching in the XY plane, however, will result in poor alignment that should be fixed by re-teaching.

Note: re-teaching involves risk to any currently validated applications. The UTIL_SetGlobalOffsets method must be visited in all validated applications to check that the g_Offset values remain valid after re-teaching.

In the case of most deck positions, the teach plate is placed at the location of interest, and the position is taught by standard procedures (using 4 tips, fully seated on each corner of the array). The Inheco thermoblocks and shaker positions are taught in a specific manner.

For Inheco thermoblocks (positions A3, A4, D2):

a) Remove the CPAC adaptor from the thermoblock.

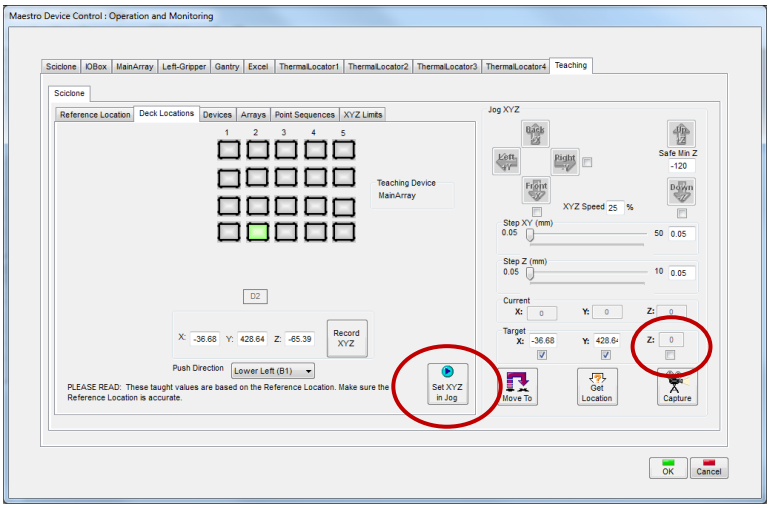
b) Attach the blue Inheco teach plate adapter with the two corner screws (this accessory is supplied with the SciCloneNGS startup kit), then place the teach plate on the adapter.

c) Navigate to Tools/Teaching in the Maestro software.

d) Select the “Deck Locations” window.

e) Select the position to be taught on the deck layout image.

f) Select “Set XYZ in Jog”, then de-select the Z checkbox.



g) Select “Move To” to position the head over the position.

h) Use the Gantry to drive the head down in final increments as fine as 0.05mm until the tips are touching but not holding a piece of paper against the teach plate and a further 0.05mm increment would capture the paper.

i) Select “Capture” then “Record XYZ” to save the position with the correct Z value. Close the teach window to save the new values.

j) Home the Z axis.

k) Remove the blue teach plate locator from the thermoblock, and replace it with a CPAC adapter. You may use a 96-well adapter or a 384-well adapter, depending on which is used in the application(s) in which the XY alignment problem was observed.

l) Execute a “Head Position” command over the position. Be sure that the active layout indicates the same consumable actually placed on the Inheco position.

m) Observe the positioning of tips over the wells.

n) In the teach window, use the gantry to adjust the positioning in the X and/or Y direction, making note of the exact adjustment values (e.g. -2.2mm Y and 1.7mm X)

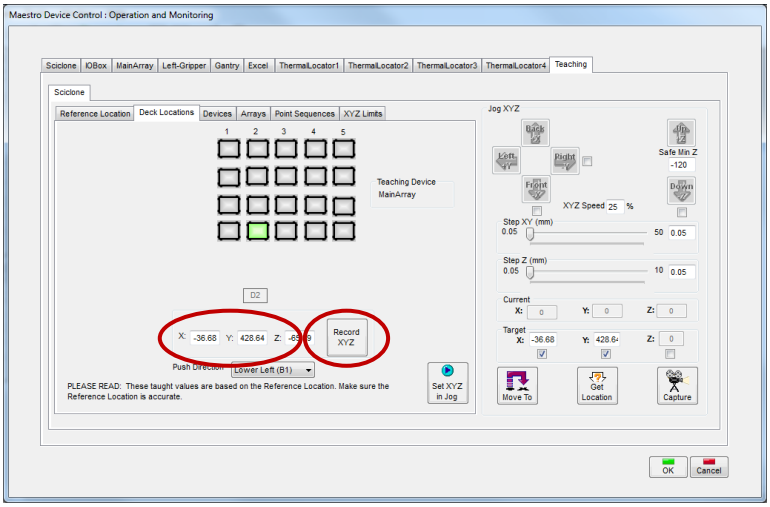
o) In the “Deck Locations” window, select the position to be adjusted by clicking on the deck layout image.

p) Add the exact adjustment values to the X and Y values associated with the position, taking care to mind the sign (positive or negative) of the adjustment direction.

q) Once the corrected values have been entered, select “Record XYZ” to save.

r) Close the teach window to save the new teach values.

s) Execute a “Head Position” command to verify that the tips are now centered over the wells of the consumable.



Note: The coordinates shown to the left of the “Record XYZ” button are the teach values associated with a specific position. They are different from the actual coordinates to which the head will travel when executing a “head position” command. When the “head position” is executed, the consumable definition will be used to determine the correct positioning of tips. This is most obvious when using a 384-well plate—the head position command will send the tips to a specific quadrant of the plate rather than centering the head over the position.

The shaker position should be taught in a similar manner, except the blue teach plate adapter is not used. Instead, the 96-well adapter should be unscrewed from the shaker and the teach plate should be placed on the shaker where the adapter normally sits.