

NimbleScan v2.3 User's Guide Addendum

Sample Tracking Analysis

This addendum guides you with installing and using the Sample Tracking analysis tool within NimbleScan™ v2.3 software. This tool enables you to generate the Sample Tracking report and confirm the sample hybridized to each array in 4-plex arrays.

Analysis	
Auto Align	Ctrl+A
Manually Align	Ctrl+M
Histogram/Profile	Ctrl+B
Reports	▶
CGH	▶
ChIP	▶
Expression RMA...	Ctrl+Alt+E
Methylation	▶
Sample Tracking...	
Sequencing PBC...	Ctrl+Alt+P

Installing the Sample Tracking Analysis Tool

If you do not see a **Sample Tracking** selection within NimbleScan **Analysis** menu, install the Sample Tracking analysis tool:

1. Go to www.nimblegen.com/downloads/software/nimblescan/stc_plugin/install.html
2. Follow the on-screen instructions to download and install the Sample Tracking analysis tool.

Creating and Reviewing the Sample Tracking Report

1. Open NimbleScan. If the images have been gridded, skip to step 7. If the images have not been gridded, proceed to step 2.
2. Burst the scanned image. (See Chapter 2 in the *NimbleScan v2.3 User's Guide*.)
3. Open your microarray image file (.tif), design file (.ndf), and optionally the gene description file (.ngd). (See Chapter 2 in the *NimbleScan v2.3 User's Guide*.)
4. Adjust the image's appearance to better view features. (See Chapter 2 in the *NimbleScan v2.3 User's Guide*.)
5. Align the grid to the array image. (See Chapter 3 in the *NimbleScan v2.3 User's Guide*.)
6. Repeat steps 3 - 5 for all the images to analyze.
7. Generate the Sample Tracking Report by selecting **Analysis -> Sample Tracking**.
8. Click **Add files**.
9. Select all gridded images for the experiment (for example for 4-plex arrays, select four images).
10. Click **Add to List**.
11. Click **Browse** to select destination of output file. To view the output file in Microsoft Excel, save as an .xls file.
12. Open the Sample Tracking report in spreadsheet software, such as Microsoft Excel.
 - a. Confirm that the Image ID, Design ID, and Design Name are accurate.
 - b. Enter the Sample Name and Input STC (Sample Tracking Control) in the appropriate spreadsheet cells. The Input STC is the unique STC number added to each sample before hybridization.

- c. For each array, review the signal intensity and the Absent or Present call for each of the up to 20 STCs that NimbleGen may provide. The STC identified as “Present” should be the same as the Input STC you entered for the sample.
- d. Ensure that a Present call is reported for only the unique STC added to the sample. If more than one Present call is reported, the integrity of your data could be compromised due to cross-contamination that occurred during sample preparation, loading, or hybridization. The extent of cross-contamination that can be tolerated depends on your samples, experimental setup, and experimental goals. We recommend repeating experiments that show cross-contamination.

13. Select **File** -> **Save** to save the changes to the file.

Image ID	109037A01_510_532	109037A02_510_532	109037A03_510_532	109037A04_510_532
Design ID	5010	5010	5010	5010
Design Name	HG18_60mer_expr	HG18_60mer_expr	HG18_60mer_expr	HG18_60mer_expr
Sample Name				
Array Number	A01	A02	A03	A04
Input STC				
STC 1 Intensity	39528	210	210	218
STC 2 Intensity	215	228	253	207
STC 3 Intensity	182	48920	232	206
STC 4 Intensity	191	214	221	250
STC 5 Intensity	195	248	40937	244
STC 6 Intensity	183	242	193	216
STC 7 Intensity	200	252	212	36140
STC 8 Intensity	202	198	212	187
STC 9 Intensity	186	213	208	199
STC 10 Intensity	223	240	203	214
STC 11 Intensity	226	215	216	237
STC 12 Intensity	171	240	185	207
STC 13 Intensity	199	225	206	209
STC 14 Intensity	176	217	264	223
STC 15 Intensity	207	240	233	207
STC 16 Intensity	168	242	206	195
STC 17 Intensity	239	252	268	258
STC 18 Intensity	225	227	212	207
STC 19 Intensity	211	226	219	221
STC 20 Intensity	259	239	228	221
STC 1 Call	Present	Absent	Absent	Absent
STC 2 Call	Absent	Absent	Absent	Absent
STC 3 Call	Absent	Present	Absent	Absent
STC 4 Call	Absent	Absent	Absent	Absent
STC 5 Call	Absent	Absent	Present	Absent
STC 6 Call	Absent	Absent	Absent	Absent
STC 7 Call	Absent	Absent	Absent	Present
STC 8 Call	Absent	Absent	Absent	Absent
STC 9 Call	Absent	Absent	Absent	Absent
STC 10 Call	Absent	Absent	Absent	Absent
STC 11 Call	Absent	Absent	Absent	Absent
STC 12 Call	Absent	Absent	Absent	Absent
STC 13 Call	Absent	Absent	Absent	Absent
STC 14 Call	Absent	Absent	Absent	Absent
STC 15 Call	Absent	Absent	Absent	Absent
STC 16 Call	Absent	Absent	Absent	Absent
STC 17 Call	Absent	Absent	Absent	Absent
STC 18 Call	Absent	Absent	Absent	Absent
STC 19 Call	Absent	Absent	Absent	Absent
STC 20 Call	Absent	Absent	Absent	Absent

Example of a Sample Tracking Report