

LeukoStrat[®] Assays

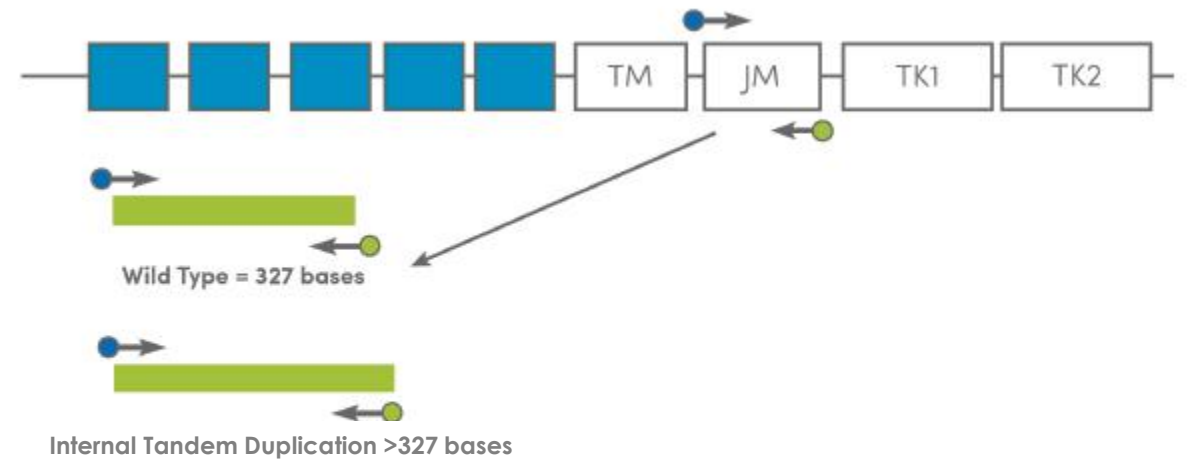
LeukoStrat[®] CDx *FLT3* Mutation Assay
Technical Training

FLT3 Internal Tandem Duplications

- Duplication/insertion in JM region
- Vary in location and length

LeukoStrat® CDx *FLT3* Mutation Assay uses primers in/around the JM region

- Wildtype alleles = 327 ± 1 bp
- Mutated alleles $> 327 \pm 1$ bp



FLT3 Tyrosine Kinase Domain Mutations

- Nucleic acid substitutions/deletions
- Disrupts a wild type EcoRV restriction site

Assay primers target the activating loop of the kinase domain.
Amplified alleles are digested.

- Wildtype alleles = ~79 bp
- Mutated alleles = ~124, ~127 bp
- Undigested amplicon = ~145, ~147 bp



EcoRV digest

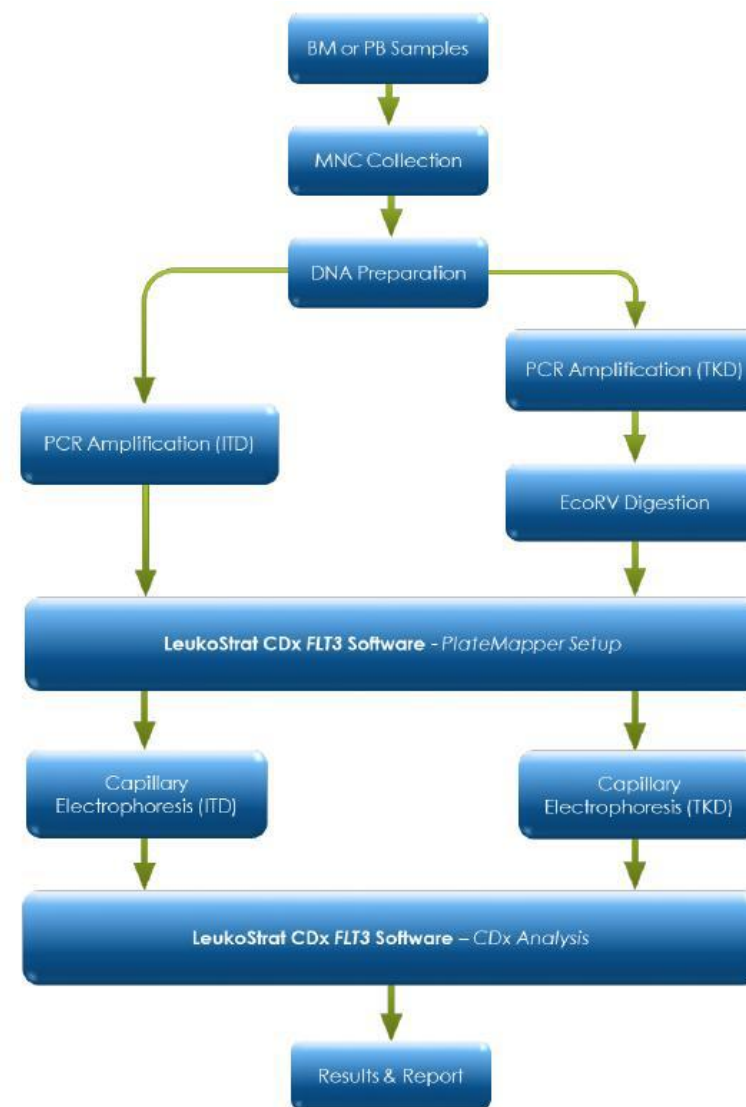
Undigested = 145 bases or 147 bases
Wild Type digested = 79 bases
Mutant digested, point mutation = 127 bases
Mutant digested, deletion = 124 bases

Summary of the Test

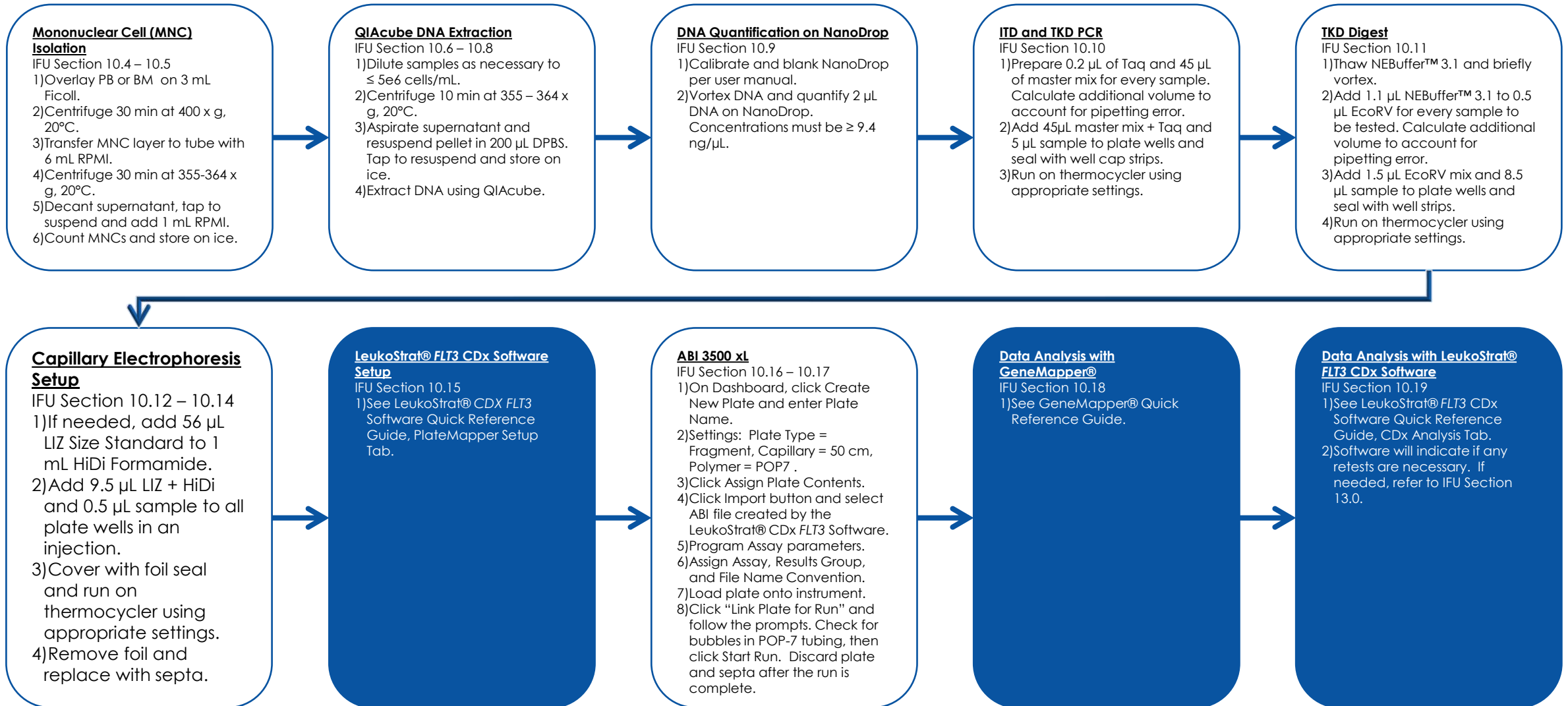
Assay procedures include:

- Isolation of mononuclear cells
- DNA extraction
- PCR amplification (ITD, TKD)
- Enzymatic digestion (TKD)
- Capillary electrophoresis

Included software provides simplified plate setup, transfer of data, and interpretation of results



Detailed Reference Guide



Detailed Reference Guide

LeukoStrat® CDx FLT3 Software Quick Reference Guide

Plate Mapper Setup Tab

General Plate Setup

IFU Section 10.15.2 – 10.15.4

- 1) Open LeukoStrat® CDx FLT3 Software.
- 2) In the PlateMapper Setup tab, enter Plate Name, Results Group, and File Name Convention. Select Assay Type (ITD or TKD) for each injection.

Well Setup

IFU Section 10.15.5.1 – 10.15.5.10

- 1) Enter all controls first (PC, EC and NTC).
- 2) For all wells that will have a sample, click the corresponding well and enter Sample Name, Sample Type, Run Number, and link to EC then click Save Well.
- 3) When all wells have been entered, click Save Plate and choose location to save the ABI and LIVS files generated by the software. Do not modify these files.
- 4) Proceed to **ABI 3500 xL Dx Setup**. See LeukoStrat® CDx FLT3 Mutation Assay Quick Reference Guide.

CDX Analysis Tab

Data Analysis

IFU Section 10.19.1 – 10.19.4

- 1) Select PlateMap File (*.livs) created during PlateMapper Setup.
- 2) Select Results Data File (*.csv) exported from GeneMapper®.
- 3) Select Report Output Directory.
- 4) Click Analyze.
- 5) Run Summary and Sample Summary Reports are located in the directory specified in step 3.

GeneMapper Quick Reference Guide

Data Analysis Setup

IFU Section 10.18.1 – 10.18.3

- 1) Open the GeneMapper® software.
- 2) Under File, Choose New Project and select Microsatellite. Click Ok.
- 3) Under File, click Add Samples to Project and select data files to analyze.
- 4) Click Add or Add & Analyze.

Data Analysis

IFU Section 10.18.4 – 10.18.6

- 1) Ensure Analysis Method = Microsatellite and Size Standard = GS600LIZ+Normalization.
- 2) Set Analysis Method to:
B, G = 100
Y, R, P, O = 50
Polynomial ITD = 3
Polynomial TKD = 5
- 3) Press the green play button to save the file and start analysis.

Data Analysis

IFU Section 10.19.1 – 10.19.4

- 1) Select PlateMap File (*.livs) created during PlateMapper Setup.
- 2) Select Results Data File (*.csv) exported from GeneMapper®.
- 3) Select Report Output Directory.
- 4) Click Analyze.
- 5) Run Summary and Sample Summary Reports are located in the directory specified in step 3.

Software Plate Setup

End User License Agreement (EULA)

User must agree to the EULA to continue with use of the software

End User License Agreement

THIS END USER LICENSE AGREEMENT MUST BE ACCEPTED BY AN AUTHORIZED REPRESENTATIVE OF THE END USER OF THIS PRODUCT PRIOR TO USING THE LEUKOSTRAT® CDX FLT3 SOFTWARE (the "Software").

LEUKOSTRAT® CDX FLT3 SOFTWARE v1.1.1 is Labeled for In Vitro Diagnostic Use.

BY USING THE LEUKOSTRAT® CDX FLT3 SOFTWARE, YOU ASSERT THAT YOU ARE AN AUTHORIZED REPRESENTATIVE OF THE END USER WITH AUTHORITY TO ENTER INTO THIS AGREEMENT. PLEASE READ THIS AGREEMENT CAREFULLY. YOU ARE AGREEING TO BE BOUND BY THE TERMS OF THIS AGREEMENT. IF YOU DO NOT AGREE TO THE TERMS OF THIS AGREEMENT, PLEASE DISCONTINUE USAGE. IF YOU DO AGREE TO THE TERMS OF THIS AGREEMENT ON BEHALF OF YOURSELF AND/OR THE ENTITY YOU REPRESENT YOU MAY CONTINUE USAGE.

This End User License Agreement ("EULA") is made and entered into by and between INVIVOSCRIBE TECHNOLOGIES, INC., a California corporation ("Licensor") and you as the user of the Software (either you as an individual or a legal entity) ("Licensee") for the licensing and usage of the Licensor's Software. Licensee acknowledges and agrees that Licensee's right to use the Software in any manner shall be controlled by this EULA and that such use shall be strictly in accordance with the terms and conditions of this EULA.

Do you agree to these terms of service?

LeukoStrat® CDx FLT3 Software Version v1.1.1

Accept Decline

Home Screen

Enter required fields such as:

- Plate Name
- Results Group
- File Name Convention

These will be used for importing into the ABI Software

Select which injections contain which assays

LeukoStrat® CDx FLT3 Software

Help

PlateMapper Setup | CDx Analysis

LeukoStrat® CDx PlateMapper

Import ABI | Import LIVS

Plate Name* | Results Group* | File Name Convention* | Plate Barcode

2020-01-01-FLT3CDx | LeukoStrat_FLT3 | LS_FLT3_CDX

	1	2	3	4	5	6	7	8	9	10	11	12
A	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12
B	B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
C	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
D	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
E	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
F	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
G	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
H	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12

Assay 1 | Assay 2 | Assay 3 | Assay 4

ITD | TKD

Clear Plate | Save Plate

* Indicates required field

Enter Controls

Select plate wells that contain each control and enter the following information:

- Sample Name
- Sample Type
- Run (Press + to add a run)
- Sample Notes (optional)

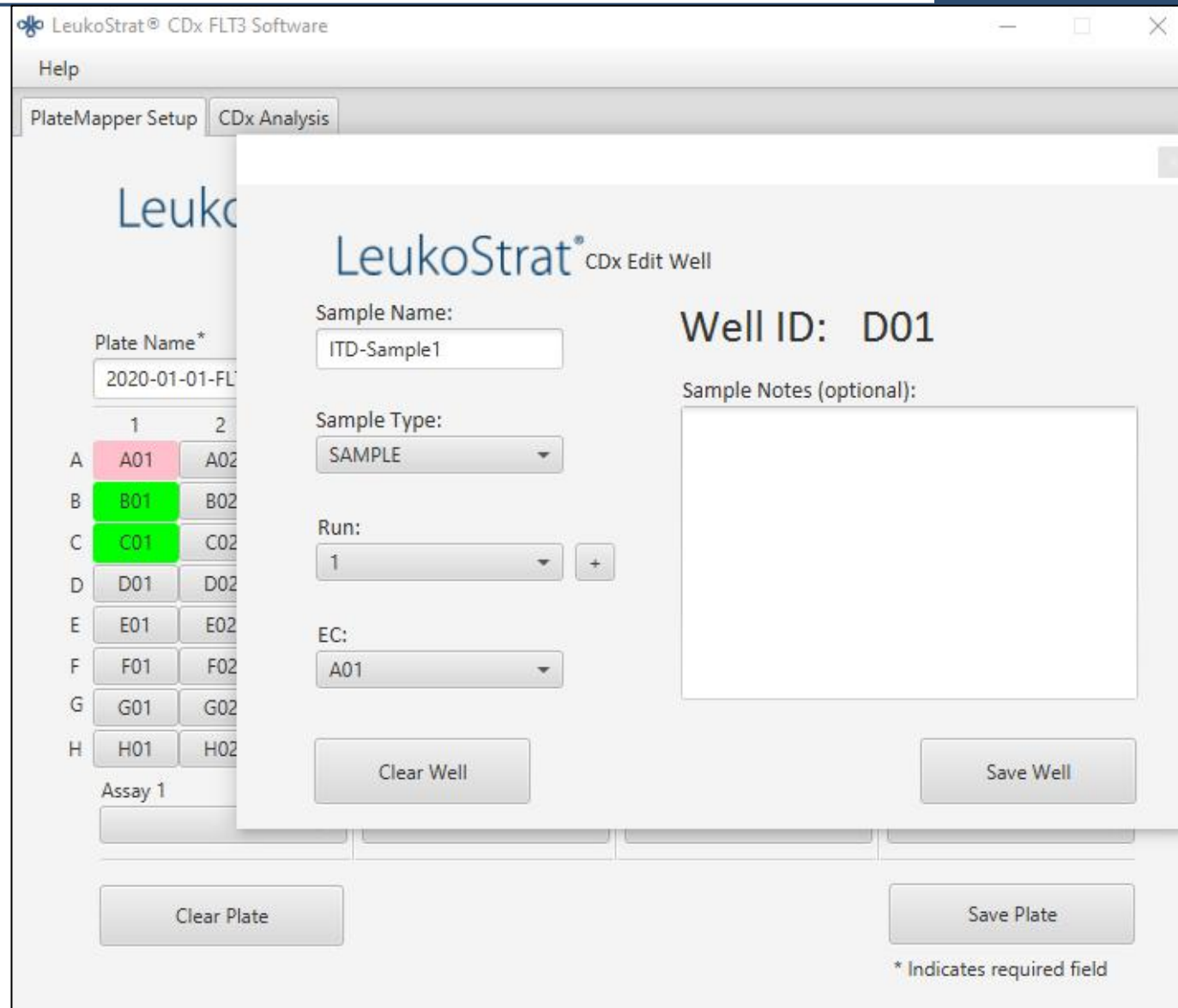
Note that controls do not have an associated extraction control, leave this area blank

The screenshot displays the LeukoStrat CDx FLT3 Software interface. The main window is titled "LeukoStrat® CDx FLT3 Software" and has a "Help" menu. Below the menu are two tabs: "PlateMapper Setup" and "CDx Analysis". The "PlateMapper Setup" tab is active, showing a grid of plate wells labeled A01 through H02. The well A01 is highlighted with a red box. A dialog box titled "LeukoStrat® CDx Edit Well" is open over the grid. The dialog box contains the following fields: "Sample Name:" with the value "ITD-ExtractionControl1"; "Well ID:" with the value "A01"; "Sample Type:" with a dropdown menu set to "EC"; "Run:" with a dropdown menu set to "1" and a "+" button; and "EC:" with a dropdown menu. There is also a "Sample Notes (optional):" text area. At the bottom of the dialog box are "Clear Well" and "Save Well" buttons. Below the grid, there are "Assay 1" dropdown menus and "Clear Plate" and "Save Plate" buttons. A footer note states: "* Indicates required field".

Enter Samples

Select plate wells that contain each sample and enter the following information:

- Sample Name
- Sample Type
- Run (Press + to add a run)
- Sample Notes (optional)
- Associated Extraction Control
- **Each sample must have an EC**
- **Each EC must have one sample**

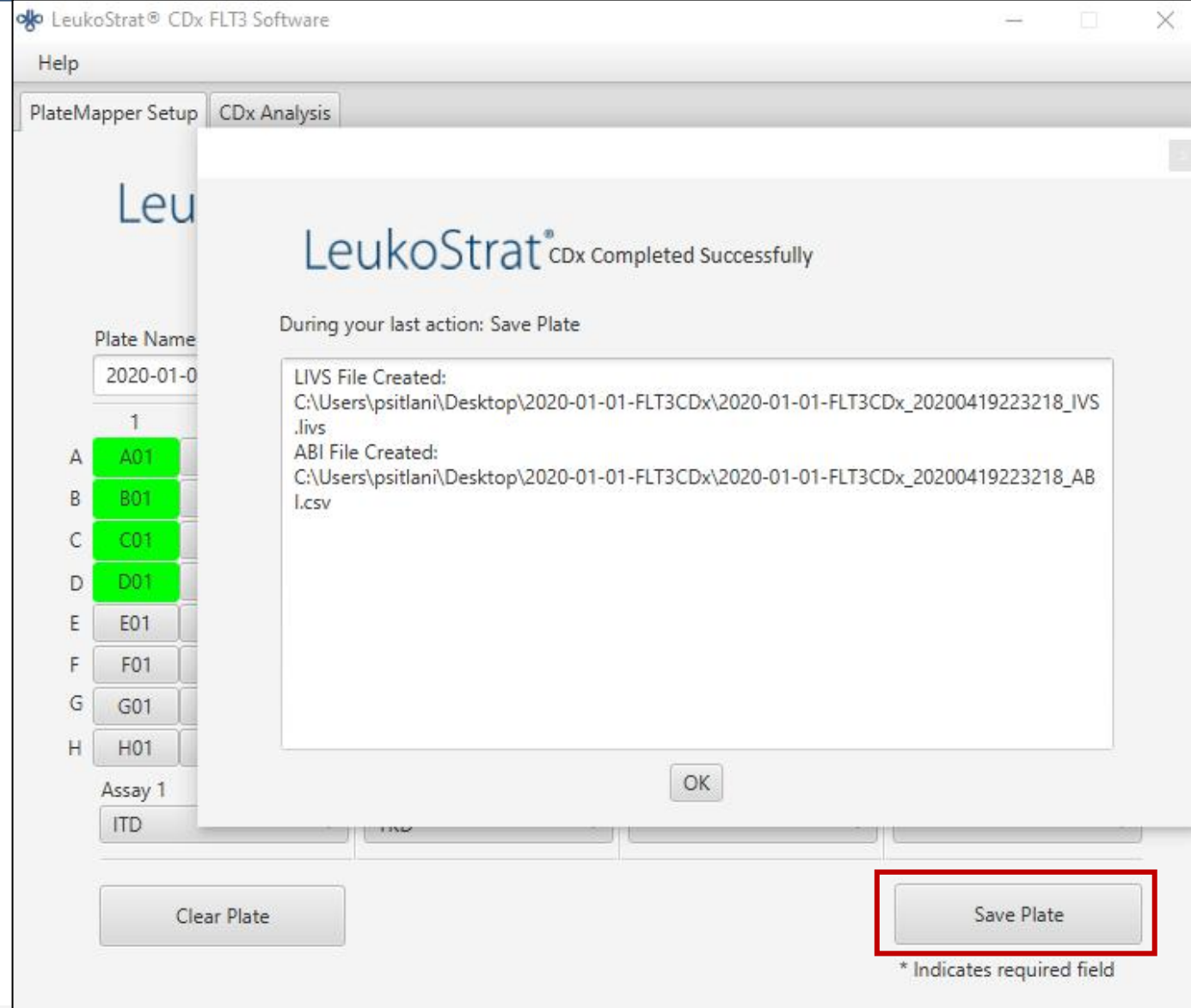


Save Plate

Select Save Plate to generate two files:

- *.livs file is used when analyzing final data output
- *.csv file is uploaded into ABI3500 software run setup

***.csv file MUST be uploaded prior to ABI run, otherwise the data output will have incorrect sample names**



ABI 3500 xL

This is the instrument dashboard. Before beginning a run, verify that all calibrations are up to date and consumables are still valid

Common Operations

- Quick Start Run
- Create New Plate
- Create Plate from Template
- Edit Existing Plate
- View Run Results
- Maintain Instrument

Quick View

Gauges

- POP7 Polymer**: 237 Samples Remaining (8 Injections Remaining)
- ABC - (Anode)**: 11 Days Remaining (38 Injections Remaining)
- CBC - (Cathode)**: 11 Days Remaining (38 Injections Remaining)
- 50cm - 24 cap Array**: 237 Injections Performed

Instrument Status

- Instrument: 3500 Instrument
- Laser: Off
- EP: Off
- State: Idle
- Oven: Off
- Oven Door: Close
- Instrument Door: Close
- Oven Temperature (°C): 28.3
- Detection Cell Temperature (°C): 23.0
- Pre-Heat the Oven: Set Temperature to: 60 (°C) [Start Pre-Heat]

Consumables Information

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	237 Samples Remaining	3	24-Mar-2020 05...	1907186	4393708
Anode Buffer	ABC	11 Days Remaining	3	01-May-2020 0...	1907548	4393927
Cathode Buffer	CBC	11 Days Remaining	3	18-Apr-2020 05...	1907603	4408256
Capillary Array	50cm - 24 cap	0 Injections Remaining	107	28-Jan-2020 04...	25	4404689 - Serial # M519A2515

Maintenance Notifications

Name	Priority	Notification Date	Description	Action

Creating a New Assay

Select 'Library' to access saved 'Plates', 'Assays', and other settings

3500 Data Collection Software

Library Maintenance Tools Manage Preferences Help Log Out

Common Operations

Quick Start Run Create New Plate Create Plate from Template Edit Existing Plate View Run Results Maintain Instrument

Quick View

Gauges

POP7 Polymer: 237 Samples Remaining (8 Injections Remaining)

ABC - (Anode): 11 Days Remaining (38 Injections Remaining)

CBC - (Cathode): 11 Days Remaining (38 Injections Remaining)

50cm - 24 cap Array: 237 Injections Performed

Instrument: 3500 Instrument
 Laser: Off
 EP: Off

State: Idle
 Oven: Off
 Oven Door: Close
 Instrument Door: Close

View Instrument Sensor Details
 Oven Temperature (°C): 28.3
 Detection Cell Temperature (°C): 23.0

Pre-Heat the Oven
 Set Temperature to: 60 (°C) Start Pre-Heat

Refresh

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	237 Samples Remaining	3	24-Mar-2020 05...	1907186	4393708
Anode Buffer	ABC	11 Days Remaining	3	01-May-2020 0...	1907548	4393927
Cathode Buffer	CBC	11 Days Remaining	3	18-Apr-2020 05...	1907603	4408256
Capillary Array	50cm - 24 cap	0 Injections Remaining	107	28-Jan-2020 04...	25	4404689 - Serial # MS19A2515

Maintenance Notifications

Name	Priority	Notification Date	Description	Action

Under Assays, select Create to add a new assay to the library

The screenshot displays the '3500 Data Collection Software' interface. On the left, the 'Library Resources' sidebar is visible, with the 'Assays' section highlighted. The top toolbar contains a 'Create' button, which is indicated by a red arrow. The main window shows a table of assays with columns for Assay Name, Type, Instrument Protocol, Primary Analysis Protocol, Secondary Analysis Protocol, Color, Date Modified, and Is Signed. The table lists 39 assays, including MicroSEQ ID POP7, YF_POP4, CDx FLT3 ITD, and others.

Assay Name	Type	Instrument Protocol	Primary Analysis Protocol	Secondary Analysis Protocol	Color	Date Modified	Is Signed
1 MicroSEQ ID POP7 ...	Sequencing	MicroSEQ ID 50_POP7	MicroSEQ ID PA Protocol			19-May-2009 04:32:31 PM	No
2 YF_POP4_xl	HID	HID36_POP4d_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
3 CDx FLT3 ITD	Fragment	CDx FLT3 ITD	Fragment_Analysis_PA_P...			05-May-2015 09:06:53 AM	No
4 SEF+_Norm_POP4_xl	HID	HID36_POP4d_G5	G5_LS(80-400)+Normaliz...			19-May-2009 04:32:31 PM	No
5 Short_Read_Seq_Ass...	Sequencing	ShortReadSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
6 BDx_Short_Read_Se...	Sequencing	BDxShortReadSeq50_PO...	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
7 Rapid_Seq_Assay_xl...	Sequencing	RapidSeq50_POP7d_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
8 MF+_Norm_POP4	HID	HID36_POP4_G5	G5_3rd(80-400)+Normali...			19-May-2009 04:32:31 PM	No
9 MicroSEQ ID POP6 ...	Sequencing	MicroSEQ ID 50_POP6	MicroSEQ ID PA Protocol			19-May-2009 04:32:31 PM	No
10 YF_POP4	HID	HID36_POP4_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
11 MF+_Norm_POP4_xl	HID	HID36_POP4d_G5	G5_3rd(80-400)+Normali...			19-May-2009 04:32:31 PM	No
12 CO_POP4_xl	HID	HID36_POP4d_F	F_LS(75-400)			19-May-2009 04:32:31 PM	No
13 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			19-May-2009 04:32:31 PM	No
14 PowerPlex16	Fragment	PowerPlex16	Fragment_Analysis_Powe...			03-Jul-2013 02:52:30 PM	No
15 PP_POP4_xl	HID	HID36_POP4d_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
16 YF+_Norm_POP4_xl	HID	HID36_POP4d_G5	G5_LS(80-400)+Normaliz...			19-May-2009 04:32:31 PM	No
17 SGM+_POP4_xl	HID	HID36_POP4d_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
18 FLT3 Signal Ratio Ass	Fragment	FLT3 Signal Ratio Assay	Fragment_Analysis_PA_P...			29-Aug-2014 03:20:24 PM	No
19 Fast_Seq_Assay_xl-...	Sequencing	FastSeq50_POP7d_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
20 MF_POP4	HID	HID36_POP4_G5	G5_3rd(80-400)			19-May-2009 04:32:31 PM	No
21 SEF+_POP4_xl	HID	HID36_POP4d_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
22 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			19-May-2009 04:32:31 PM	No
23 MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID 50_POP6d	MicroSEQ ID PA Protocol			19-May-2009 04:32:31 PM	No
24 BDx_Fast_Seq_Assay...	Sequencing	BDxFastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
25 CO_POP4	HID	HID36_POP4_F	F_LS(75-400)			19-May-2009 04:32:31 PM	No
26 IF_POP4	HID	HID36_POP4_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
27 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			19-May-2009 04:32:31 PM	No
28 IF_POP7	HID	HID36_POP7_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
29 Qualitative-NPM1-FLT3-...	Fragment	Qualitative-NPM1-FLT3-...	Qualitative-NPM1-FLT3-...			01-Jul-2014 02:12:25 PM	No
30 BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP6_1	BDTv1.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
31 SNaPshot Assay	Fragment	SNaPshot50_POP7_1	SNaPshot_PA_Protocol			19-May-2009 04:32:31 PM	No
32 BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
33 PP_POP4	HID	HID36_POP4_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
34 ITD CDx Assay	Fragment	ITD CDx Assay	Fragment_Analysis_PA_P...			09-Sep-2013 12:21:45 PM	No
35 Fast_Seq_Assay-POP7	Sequencing	FastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
36 SGM+_POP4	HID	HID36_POP4_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
37 Biomed	Fragment	Biomed	Fragment_Analysis_PA_P...			19-Aug-2014 02:15:18 PM	No
38 IF+_Norm_POP7_xl	HID	HID36_POP7d_G5	G5_LS(80-400)+Normaliz...			19-May-2009 04:32:31 PM	No
39 NPM1	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			13-Nov-2014 10:19:39 AM	No

Add an Assay Name and change Application Type to 'Fragment'. For Instrument Protocol, select 'Create New'

The screenshot shows the '3500 Data Collection Software' interface. A 'Create New Assay' dialog box is open, titled 'Setup an Assay'. The dialog contains the following fields and options:

- Assay Name:** New Assay (with a 'Locked' checkbox and a 'Color' dropdown set to 'Black').
- Application Type:** Fragment (dropdown menu).
- Protocols:** A section with the question 'Do you wish to assign multiple instrument protocols to this assay?' and radio buttons for 'No' (selected) and 'Yes'.
- Instrument Protocol:** A dropdown menu with 'Edit' and 'Create New' buttons.
- Sizecalling Protocol:** A dropdown menu with 'Edit' and 'Create New' buttons.
- GeneMapper Protocol:** A dropdown menu with 'Edit' and 'Create New' buttons.
- Buttons:** 'Close' and 'Save' buttons at the bottom.

The background table lists various assays with columns: Assay Name, Type, Instrument Protocol, Primary Analysis Protocol, Secondary Analysis Protocol, Color, Date Modified, and Is Signed. A red arrow points to the 'Application Type' dropdown in the dialog, and another red arrow points to the 'Create New' button for the 'Instrument Protocol' field.

Enter the instrument protocol settings as described in the specific IFU provided with the LeukoStrat® CDx FLT3 Mutation Assay. Settings not listed should be left at their default values

Setup an Instrument Protocol

Application Type: **Fragment** → Capillary Length: **50** cm → Polymer: **POP7**

Dye Set: **G5**

Instrument Protocol Properties

- * Run Module: **FragmentAnalysis50_POP7xl**
- * Protocol Name: **NewProtocol** Locked
- Description:

Oven Temperature (°C): **60** Run Voltage (kVolts): **19.5** PreRun Voltage (kVolts): **15** Injection Voltage (kVolts): **1.6**

Run Time (sec.): **1330** PreRun Time (sec.): **180** Injection Time (sec.): **15** Data Delay (sec.): **1**

Advanced Options

Buttons: **Close** **Apply to Assay** **Save to Library**

Assay Name	Type	Instrument	Run Date	Status
10 YF_POP4	HID	HID36		
11 MF+Norm_POP4_xl	HID	HID36		
12 CO_POP4_xl	HID	HID36		
13 Fragment_Analysis_...	Fragment	Fragment		
14 PowerPlex16	Fragment	Fragment		
15 PP_POP4_xl	HID	HID36		
16 YF+Norm_POP4_xl	HID	HID36		
17 SGM+_POP4_xl	HID	HID36		
18 FLT3 Signal Ratio Ass	Fragment	Fragment		
19 Fast_Seq_Assay_xl-...	Sequencing	Sequencing		
20 MF_POP4	HID	HID36		
21 SEF+_POP4_xl	HID	HID36		
22 Fragment_Analysis_...	Fragment	Fragment		
23 MicroSEQ ID xl POP...	Sequencing	Sequencing		
24 BDx_Fast_Seq_Assay...	Sequencing	Sequencing		
25 CO_POP4	HID	HID36		
26 IF_POP4	HID	HID36		
27 Fragment_Analysis_...	Fragment	Fragment		
28 IF_POP7	HID	HID36		
29 Qualitative-NPM1-FLT3...	Fragment	Qualita		
30 BDx_Std_Seq_Assay-...	Sequencing	BDxStd		
31 SNaPshot_Assay	Fragment	SNaPshot50_POP7_1		
32 BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...	19-May-2009 04:32:31 PM No
33 PP_POP4	HID	HID36_POP4_F	F_LS(75-450)	19-May-2009 04:32:31 PM No
34 ITD CDx Assay	Fragment	ITD CDx Assay	Fragment_Analysis_PA_P...	09-Sep-2013 12:21:45 PM No
35 Fast_Seq_Assay-POP7	Sequencing	FastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...	19-May-2009 04:32:31 PM No
36 SGM+_POP4	HID	HID36_POP4_F	F_LS(75-450)	19-May-2009 04:32:31 PM No
37 Biomed	Fragment	Biomed	Fragment_Analysis_PA_P...	19-Aug-2014 02:15:18 PM No
38 IF+Norm_POP7_xl	HID	HID36_POP7xl_G5	G5_LS(80-400)+Normaliz...	19-May-2009 04:32:31 PM No
39 NPM1	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...	13-Nov-2014 10:19:39 AM No
40 IF_POP7_xl	HID	HID36_POP7xl_G5	G5_LS(80-400)	19-May-2009 04:32:31 PM No
41 BDx_Std_Seq_Assay...	Sequencing	BDxStdSeq50_POP6xl_1	BDTv1.1_PA_Protocol-PO...	19-May-2009 04:32:31 PM No
42 CDx FLT3 TKD	Fragment	CDx FLT3 TKD	Fragment_Analysis_PA_P...	05-May-2015 09:03:58 AM No
43 SEF+_POP4	HID	HID36_POP4_G5	G5_LS(80-400)	19-May-2009 04:32:31 PM No
44 BDx_Std_Seq_Assay...	Sequencing	BDxStdSeq50_POP7xl_1	BDTv3.1_PA_Protocol-PO...	19-May-2009 04:32:31 PM No
45 TKD CDx Assay	Fragment	TKD CDx Assay	Fragment_Analysis_PA_P...	13-Nov-2014 10:21:02 AM No
46 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...	19-May-2009 04:32:31 PM No
47 Std_Seq_Assay-POP6	Sequencing	StdSeq50_POP6_1	BDTv1.1_PA_Protocol-PO...	19-May-2009 04:32:31 PM No
48 MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID 50_POP7xl	MicroSEQ ID PA Protocol	19-May-2009 04:32:31 PM No

For Sizecalling Protocol, select 'Create New'

The screenshot shows the '3500 Data Collection Software' interface. On the left is a sidebar with 'Library Resources' and 'Main Workflow'. The main area displays a table of assays. A 'Create New Assay' dialog box is open, showing the 'Setup an Assay' configuration. The 'Assay Name' is 'New Assay' and 'Application Type' is 'Fragment'. The 'Protocols' section has a radio button for 'No' selected. The 'Sizecalling Protocol' field is empty, and a red error message states 'Sizecalling Protocol cannot be empty.' A red arrow points to the 'Create New' button next to this field.

Assay Name	Type	Instrument Protocol	Primary Analysis Protocol	Secondary Analysis Protocol	Color	Date Modified	Is Signed
1	MicroSEQ ID POP7 ...	Sequencing	MicroSEQ ID 50_POP7	MicroSEQ ID PA Protocol		19-May-2009 04:32:31 PM	No
2	YF_POP4_xl	HID	HID36_POP4d_G5				
3	CDx FLT3 ITD	Fragment	CDx FLT3 ITD				
4	SEF+ +Norm_POP4_xl	HID	HID36_POP4d_G5				
5	Short_Read_Seq_Ass...	Sequencing	ShortReadSeq50_POP7_1				
6	BDx_Short_Read_Se...	Sequencing	BDxShortReadSeq50_PO...				
7	Rapid_Seq_Assay_xl...	Sequencing	RapidSeq50_POP7d_1				
8	MF +Norm_POP4	HID	HID36_POP4_G5				
9	MicroSEQ ID POP6 ...	Sequencing	MicroSEQ ID 50_POP6				
10	YF_POP4	HID	HID36_POP4_G5				
11	MF +Norm_POP4_xl	HID	HID36_POP4d_G5				
12	CO_POP4_xl	HID	HID36_POP4d_F				
13	Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...				
14	PowerPlex16	Fragment	PowerPlex16				
15	PP_POP4_xl	HID	HID36_POP4d_F				
16	YF +Norm_POP4_xl	HID	HID36_POP4d_G5				
17	SGM+ _POP4_xl	HID	HID36_POP4d_F				
18	FLT3 Signal Ratio Ass	Fragment	FLT3 Signal Ratio Assay				
19	Fast_Seq_Assay_xl-...	Sequencing	FastSeq50_POP7d_1				
20	MF_POP4	HID	HID36_POP4_G5				
21	SEF+ _POP4_xl	HID	HID36_POP4d_G5				
22	Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...				
23	MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID 50_POP6d	MicroSEQ ID PA Protocol		19-May-2009 04:32:31 PM	No
24	BDx_Fast_Seq_Assay...	Sequencing	BDxFastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...		19-May-2009 04:32:31 PM	No
25	CO_POP4	HID	HID36_POP4_F	F_LS(75-400)		19-May-2009 04:32:31 PM	No
26	IF_POP4	HID	HID36_POP4_G5	G5_LS(80-400)		19-May-2009 04:32:31 PM	No
27	Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...		19-May-2009 04:32:31 PM	No
28	IF_POP7	HID	HID36_POP7_G5	G5_LS(80-400)		19-May-2009 04:32:31 PM	No
29	Qualitative-NPM1-FLT3-...	Fragment	Qualitative-NPM1-FLT3-...	Qualitative-NPM1-FLT3-...		01-Jul-2014 02:12:25 PM	No
30	BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP6_1	BDTv1.1_PA_Protocol-PO...		19-May-2009 04:32:31 PM	No
31	SNaPshot_Assay	Fragment	SNaPshot50_POP7_1	SNaPshot_PA_Protocol		19-May-2009 04:32:31 PM	No
32	BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...		19-May-2009 04:32:31 PM	No
33	PP_POP4	HID	HID36_POP4_F	F_LS(75-450)		19-May-2009 04:32:31 PM	No
34	ITD CDx Assay	Fragment	ITD CDx Assay	Fragment_Analysis_PA_P...		09-Sep-2013 12:21:45 PM	No
35	Fast_Seq_Assay-POP7	Sequencing	FastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...		19-May-2009 04:32:31 PM	No
36	SGM+ _POP4	HID	HID36_POP4_F	F_LS(75-450)		19-May-2009 04:32:31 PM	No
37	Biomed	Fragment	Biomed	Fragment_Analysis_PA_P...		19-Aug-2014 02:15:18 PM	No
38	IF +Norm_POP7_xl	HID	HID36_POP7d_G5	G5_LS(80-400)+Normaliz...		19-May-2009 04:32:31 PM	No
39	NPM1	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...		13-Nov-2014 10:19:39 AM	No

Enter the size calling settings as described in the specific IFU provided with the LeukoStrat® CDx FLT3 Mutation Assay. Settings not listed should be left at their default values

The screenshot displays the '3500 Data Collection Software' interface. A dialog box titled 'New Assay - Create New Primary Analysis Protocol' is open, showing the 'Setup a Sizecalling Protocol' configuration. A red arrow points from the 'Assay Name' column of the background table to the 'NewProtocol' text field in the dialog.

Background Table:

Assay Name	Type	Instrument
10 AB YF_POP4	HID	
11 AB MF+Norm_POP4_xl	HID	
12 AB CO_POP4_xl	HID	
13 AB Fragment_Analysis_...	Fragment	Fragment
14 PowerPlex16	Fragment	PowerPlex16
15 AB PP_POP4_xl	HID	HID36_PC
16 AB YF+Norm_POP4_xl	HID	HID36_PC
17 AB SGM+_POP4_xl	HID	HID36_PC
18 AB FLT3 Signal Ratio Ass	Fragment	FLT3 Sign
19 AB Fast_Seq_Assay_xl-...	Sequencing	FastSeq50
20 AB MF_POP4	HID	HID36_PC
21 AB SEF+_POP4_xl	HID	HID36_PC
22 AB Fragment_Analysis_...	Fragment	Fragment
23 AB MicroSEQ ID xl POP...	Sequencing	MicroSEQ
24 AB BDx_Std_Seq_Assay...	Sequencing	BDxStdSe
25 AB CO_POP4	HID	HID36_PC
26 AB IF_POP4	HID	HID36_PC
27 AB Fragment_Analysis_...	Fragment	Fragment
28 AB IF_POP7	HID	HID36_PC
29 Qualitative-NPM1-FLT3-...	Fragment	Qualitati
30 AB BDx_Std_Seq_Assay-...	Sequencing	BDxStdSe
31 AB SNaPshot_Assay	Fragment	SNaPshot
32 AB BDx_Std_Seq_Assay-...	Sequencing	BDxStdSe
33 AB PP_POP4	HID	HID36_PC
34 ITD CDx Assay	Fragment	ITD CDx A
35 AB Fast_Seq_Assay-POP7	Sequencing	FastSeq50
36 AB SGM+_POP4	HID	HID36_PC
37 Biomed	Fragment	Biomed
38 AB IF+Norm_POP7_xl	HID	HID36_PC
39 AB NPM1	Fragment	Fragment
40 AB IF_POP7_xl	HID	HID36_PC
41 AB BDx_Std_Seq_Assay...	Sequencing	BDxStdSe
42 CDx FLT3 TKD	Fragment	CDx FLT3 TKD
43 AB SEF+_POP4	HID	HID36_PC
44 AB BDx_Std_Seq_Assay...	Sequencing	BDxStdSe
45 TKD CDx Assay	Fragment	TKD CDx Assay
46 AB Fragment_Analysis_...	Fragment	Fragment
47 AB Std_Seq_Assay-POP6	Sequencing	StdSeq50_POP6_1
48 AB MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID PA Protocol

Dialog Box Settings:

- Protocol Name: NewProtocol
- Description: (empty)
- Size Standard: GS600LIZ
- Sizecaller: SizeCaller v1.1.0
- Analysis Settings: QC Settings
- Analysis Range: Full
- Sizing Range: Full
- Size Calling Method: Local Southern
- Primer Peak: Present
- Analysis Start Point: 0
- Sizing Start Size: 0
- Analysis Stop Point: 1000000
- Sizing Stop Size: 100000
- Minimum Peak Height: Blue (175), Green (175), Yellow (175), Red (175), Purple (175), Orange (175)
- Common Settings:
 - Use Smoothing: None
 - Use Baseline (Baseline Window (Pts)): 51
 - Minimum Peak Half Width: 2
 - Peak Window Size: 15
 - Polynomial Degree: 3
 - Slope Threshold Peak Start: 0.0
 - Slope Threshold Peak End: 0.0

Select Save and return to the Dashboard

The screenshot displays the '3500 Data Collection Software' interface. On the left, a sidebar contains navigation options like 'Library Resources', 'Plates', 'File Name Conventions', 'Results Group', 'Analyze', and 'Main Workflow'. The main area shows a table of assays with columns for Assay Name, Type, Instrument Protocol, Primary Analysis Protocol, Secondary Analysis Protocol, Color, Date Modified, and Is Signed. A 'Create New Assay' dialog box is open, titled 'Setup an Assay'. It includes fields for 'Assay Name' (set to 'New Assay'), 'Application Type' (set to 'Fragment'), and 'Color' (set to 'Black'). There are also sections for 'Protocols' with dropdown menus for 'Instrument Protocol', 'Sizing Protocol', and 'GeneMapper Protocol', each with 'Edit' and 'Create New' buttons. A 'Do you wish to assign multiple instrument protocols to this assay?' question is present with 'No' selected. The 'Save' button at the bottom right of the dialog is highlighted with a red arrow.

Assay Name	Type	Instrument Protocol	Primary Analysis Protocol	Secondary Analysis Protocol	Color	Date Modified	Is Signed
10 YF_POP4	HID	HID36_POP4_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
11 MF+Norm_POP4_xl	HID	HID36_POP4x_G5					
12 CO_POP4_xl	HID	HID36_POP4x_F					
13 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...					
14 PowerPlex16	Fragment	PowerPlex16					
15 PP_POP4_xl	HID	HID36_POP4x_F					
16 YF+Norm_POP4_xl	HID	HID36_POP4x_G5					
17 SGM+_POP4_xl	HID	HID36_POP4x_F					
18 FLT3 Signal Ratio Ass	Fragment	FLT3 Signal Ratio Assay					
19 Fast_Seq_Assay_xl-...	Sequencing	FastSeq50_POP7x_I					
20 MF_POP4	HID	HID36_POP4_G5					
21 SEF+_POP4_xl	HID	HID36_POP4x_G5					
22 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...					
23 MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID 50_POP6x					
24 BDx_Fast_Seq_Assay...	Sequencing	BDxFastSeq50_POP7_1					
25 CO_POP4	HID	HID36_POP4_F					
26 IF_POP4	HID	HID36_POP4_G5					
27 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...					
28 IF_POP7	HID	HID36_POP7_G5					
29 Qualitative-NPM1-FLT3...	Fragment	Qualitative-NPM1-FLT3...					
30 BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP6_1					
31 SNaPshot_Assay	Fragment	SNaPshot50_POP7_1					
32 BDx_Std_Seq_Assay-...	Sequencing	BDxStdSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
33 PP_POP4	HID	HID36_POP4_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
34 ITD CDx Assay	Fragment	ITD CDx Assay	Fragment_Analysis_PA_P...			09-Sep-2013 12:21:45 PM	No
35 Fast_Seq_Assay-POP7	Sequencing	FastSeq50_POP7_1	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
36 SGM+_POP4	HID	HID36_POP4_F	F_LS(75-450)			19-May-2009 04:32:31 PM	No
37 Biomed	Fragment	Biomed	Fragment_Analysis_PA_P...			19-Aug-2014 02:15:18 PM	No
38 IF+Norm_POP7_xl	HID	HID36_POP7x_G5	G5_LS(80-400)+Normaliz...			19-May-2009 04:32:31 PM	No
39 NPM1	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			13-Nov-2014 10:19:39 AM	No
40 IF_POP7_xl	HID	HID36_POP7x_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
41 BDx_Std_Seq_Assay...	Sequencing	BDxStdSeq50_POP6x_I	BDTv1.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
42 CDx FLT3 TKD	Fragment	CDx FLT3 TKD	Fragment_Analysis_PA_P...			05-May-2015 09:03:58 AM	No
43 SEF+_POP4	HID	HID36_POP4_G5	G5_LS(80-400)			19-May-2009 04:32:31 PM	No
44 BDx_Std_Seq_Assay...	Sequencing	BDxStdSeq50_POP7x_I	BDTv3.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
45 TKD CDx Assay	Fragment	TKD CDx Assay	Fragment_Analysis_PA_P...			13-Nov-2014 10:21:02 AM	No
46 Fragment_Analysis_...	Fragment	FragmentAnalysis50_POP...	Fragment_Analysis_PA_P...			19-May-2009 04:32:31 PM	No
47 Std_Seq_Assay-POP6	Sequencing	StdSeq50_POP6_1	BDTv1.1_PA_Protocol-PO...			19-May-2009 04:32:31 PM	No
48 MicroSEQ ID xl POP...	Sequencing	MicroSEQ ID 50_POP7x	MicroSEQ ID PA Protocol			19-May-2009 04:32:31 PM	No

Starting a Run

From the instrument dashboard, select 'Create New Plate'

Common Operations

Quick Start Run | **Create New Plate** | Create Plate from Template | Edit Existing Plate | View Run Results | Maintain Instrument

Quick View

Gauges

- POP7 Polymer**: 237 Samples Remaining (8 Injections Remaining)
- ABC - (Anode)**: 11 Days Remaining (38 Injections Remaining)
- CBC - (Cathode)**: 11 Days Remaining (38 Injections Remaining)
- 50cm - 24 cap Array**: 237 Injections Performed

Instrument: 3500 Instrument

Laser: Off
EP: Off

State: Idle
Oven: Off
Oven Door: Close
Instrument Door: Close

View Instrument Sensor Details
Oven Temperature (°C): 28.3
Detection Cell Temperature (°C): 23.0

Pre-Heat the Oven
Set Temperature to: 60 (°C) [Start Pre-Heat]

Consumables Information

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	237 Samples Remaining	3	24-Mar-2020 05...	1907186	4393708
Anode Buffer	ABC	11 Days Remaining	3	01-May-2020 0...	1907548	4393927
Cathode Buffer	CBC	11 Days Remaining	3	18-Apr-2020 05...	1907603	4408256
Capillary Array	50cm - 24 cap	0 Injections Remaining	107	28-Jan-2020 04...	25	4404689 - Serial # MS19A2515

Maintenance Notifications

Name	Priority	Notification Date	Description	Action

Enter plate name, select 96 for number of wells, set plate type to Fragment, Capillary Length to 50cm, and Polymer to POP7. Enter optional settings (Owner/Barcode/Description) if desired.

The screenshot displays the '3500 Data Collection Software' interface. The 'Plate Details' section is active, showing the following configuration:

- Name: TestPlate
- Number of Wells: 96 (selected), 96-FastTube, 384
- Plate Type: Fragment
- Capillary Length: 50 cm
- Polymer: POP7
- Owner: G.Khitrov
- Barcode: (empty)
- Description: (empty)

At the bottom of the window, there is a yellow bar with an 'Assign Plate Contents' button. The Windows taskbar at the bottom shows the time as 9:11 AM.

Use the import feature to import the .csv file generated from the Plate Map setup of the LeukoStrat® CDx FLT3 Software. Verify that the correct Assay is selected and colored for each sample. File Name Conventions and Results Groups should match the values entered in the LeukoStrat® software.

The screenshot displays the 3500 Data Collection Software interface. At the top, the title bar reads "3500 Data Collection Software" and includes a menu bar with "Library", "Maintenance", "Tools", "Manage", "Preferences", "Help", and "Log Out". Below the title bar is a "Plate Name:" field and a toolbar with buttons for "New Plate", "Open Plate", "Save Plate", "Close Plate", "Import", "Export", "Find/Replace", "View Plate Grid Report", and "Print".

The main area shows a plate map with columns 1-12 and rows A-H. The plate map contains the following data:

	1	2	3	4	5	6	7	8	9	10	11	12
A	F	ExtractionControl1_ITD		F	ExtractionControl1_TKD							
B	F	PositiveControl_ITD_PC_I		F	PositiveControl_TKD_PC_I							
C	F	NTC_ITD_NTC_C01_RID0		F	NTC_TKD_NTC_C04_RID0							
D	F	Sample1_ITD_SAMPLE_D		F	Sample1_TKD_SAMPLE_D							
E												
F												
G												
H												

An "Import Plate Record" dialog box is open in the center, displaying the message: "File <Z:\ABI1\Test_20200709\2020-07-09_20200709134419_ABI.csv> has been successfully imported." with an "OK" button.

Below the plate map is a "Fragment" section with "Name: 2020-07-09" and "Barcode:". At the bottom, there are three configuration panels: "Assays", "File Name Conventions", and "Results Groups".

- Assays:** Shows two options: "TKD CDx Assay" (unchecked) and "ITD CDx Assay" (checked). A red arrow points to the "ITD CDx Assay" selection.
- File Name Conventions:** Shows one option: "CDx ABI Sample Name" (checked). A red arrow points to the "CDx ABI Sample Name" selection.
- Results Groups:** Shows one option: "CDx FLT3 Results Group" (checked). A red arrow points to the "CDx FLT3 Results Group" selection.

At the bottom of the interface is a "Link Plate for Run" button. The Windows taskbar at the bottom shows the system tray with the time "11:31 AM".

If Assay, File Name Conventions, and Results Groups created on the ABI instrument do not match the values entered in the LeukoStrat® software, they will need to be 'Added from Library' and then assigned correctly to each sample.

The screenshot displays the '3500 Data Collection Software' interface. The top menu bar includes 'Dashboard', 'Edit', 'Library', 'Maintenance', 'Tools', 'Manage', 'Preferences', 'Help', and 'Log Out'. The main workspace is divided into a 'Plate View' and a 'Table View'. The 'Plate View' shows a 96-well plate grid with the following contents:

	1	2	3	4	5	6	7	8	9	10	11	12
A	ExtractionControl1_ITD_E			ExtractionControl1_TKD_								
B	PositiveControl_ITD_PC_I			PositiveControl_TKD_PC_								
C	NTC_ITD_NTC_C01_RID9			NTC_TKD_NTC_C04_RID;								
D	Sample1_ITD_SAMPLE_D			Sample1_TKD_SAMPLE_I								
E												
F												
G												
H												

Below the plate grid, there is a 'Fragment' section with 'Name: 2020-07-09-2' and 'Barcode:'. At the bottom, three panels are visible: 'Assays', 'File Name Conventions', and 'Results Groups'. Each panel has a red arrow pointing to the 'Add from Library' option. The 'Assays' panel also shows 'Create New Assay'. The 'File Name Conventions' panel shows 'Create New File Name Convention'. The 'Results Groups' panel shows 'Create New Results Group'. A 'Link Plate for Run' button is located at the bottom center.

Link the plate to Position A or Position B, review all settings and consumables, and select Start Run

The screenshot displays the '3500 Data Collection Software' interface. The top navigation bar includes 'Dashboard', 'Edit', 'Library', 'Maintenance', 'Tools', 'Manage', 'Preferences', 'Help', and 'Log Out'. The left sidebar contains sections for 'Plate Name', 'Setup', 'Run Instrument', and 'Review Results'. The main content area is divided into several sections:

- Run Information:** Shows 'Run Name: Run 2020-01-10-09-07-34-290', 'Connection Status: Connected', 'User Name: RD RD', and 'Last Login Time: 10-Jan-2020 08:58:54 AM'.
- Plates on Instrument:** Features two panels, 'Plate A (96 wells)' and 'Plate B'. 'Plate A' has fields for Name (TestPlate), Type (Fragment), and Barcode. A red arrow points to the 'Link Plate' button. 'Plate B' also has 'Link Plate' and 'Unlink' buttons.
- Consumables Information:** A table with columns: Consumable, Name, Status, Days on Instrument, Expiration Date, Lot Number, and Part Number.

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	237 Samples Remaining	3	24-Mar-2020 05...	1907186	4393708
Anode Buffer	ABC	11 Days Remaining	3	01-May-2020 0...	1907548	4393927
Cathode Buffer	CBC	11 Days Remaining	3	18-Apr-2020 05...	1907603	4408256
Capillary Array	50cm - 24 cap	0 Injections Remaining	107	28-Jan-2020 04...	25	4404689 - Serial # M519A2515
- Calibration Information - Capillary Array: M519A2515:**
 - Spatial:** ID: Spatial_Run 2019-09-25-09-01-22 Calibration Date: 25-Sep-2019 09:07:48 AM
 - Spectral:** A table with columns: Dye Set, Chemistry Standard, Calibration Date, and Run ID.

Dye Set	Chemistry Standard	Calibration Date	Run ID
F	Matrix Standard	09-Oct-2019 08:10:14 AM	Run 2019-10-09-07-24-17-303
G5	Matrix Standard	25-Sep-2019 10:28:15 AM	Run 2019-09-25-09-42-22-755

At the bottom of the interface, a yellow bar contains 'Create Injection List' and 'Start Run' buttons. A red arrow points to the 'Start Run' button.

Create GeneMapper* Settings

Within the GeneMapper* application, select 'Tools', then 'GeneMapper Manager'. In the 'Analysis Methods' tab, create one analysis method for FLT3 ITD and one analysis method for FLT3 TKD following the settings provided in the IFU. Note the difference between the Polynomial Degree.

Analysis Method Editor - Microsatellite

General | Allele | **Peak Detector** | Peak Quality | Quality Flags

Peak Detection Algorithm: Advanced

ITD

Ranges

Analysis: Full Range
Sizing: All Sizes
Start Pt: 0
Stop Pt: 10000
Start Size: 0
Stop Size: 000

Smoothing and Baseline

Smoothing: None
 Light
 Heavy
Baseline Window: 51 pts

Size Calling Method

2nd Order Least Squares
 3rd Order Least Squares
 Cubic Spline Interpolation
 Local Southern Method
 Global Southern Method

Peak Detection

Peak Amplitude Thresholds:
B: 100 R: 50
G: 100 P: 50
Y: 50 O: 50

Min. Peak Half Width: 2 pts
Polynomial Degree: 3
Peak Window Size: 15 pts

Slope Threshold

Peak Start: 0.0
Peak End: 0.0

Size Standard Normalization

Enable Normalization
Note: For 35XX series data collection normalization only.

Factory Defaults

OK Cancel

Analysis Method Editor - Microsatellite

General | Allele | **Peak Detector** | Peak Quality | Quality Flags

Peak Detection Algorithm: Advanced

TKD

Ranges

Analysis: Full Range
Sizing: All Sizes
Start Pt: 0
Stop Pt: 10000
Start Size: 0
Stop Size: 000

Smoothing and Baseline

Smoothing: None
 Light
 Heavy
Baseline Window: 51 pts

Size Calling Method

2nd Order Least Squares
 3rd Order Least Squares
 Cubic Spline Interpolation
 Local Southern Method
 Global Southern Method

Peak Detection

Peak Amplitude Thresholds:
B: 100 R: 50
G: 100 P: 50
Y: 50 O: 50

Min. Peak Half Width: 2 pts
Polynomial Degree: 5
Peak Window Size: 15 pts

Slope Threshold

Peak Start: 0.0
Peak End: 0.0

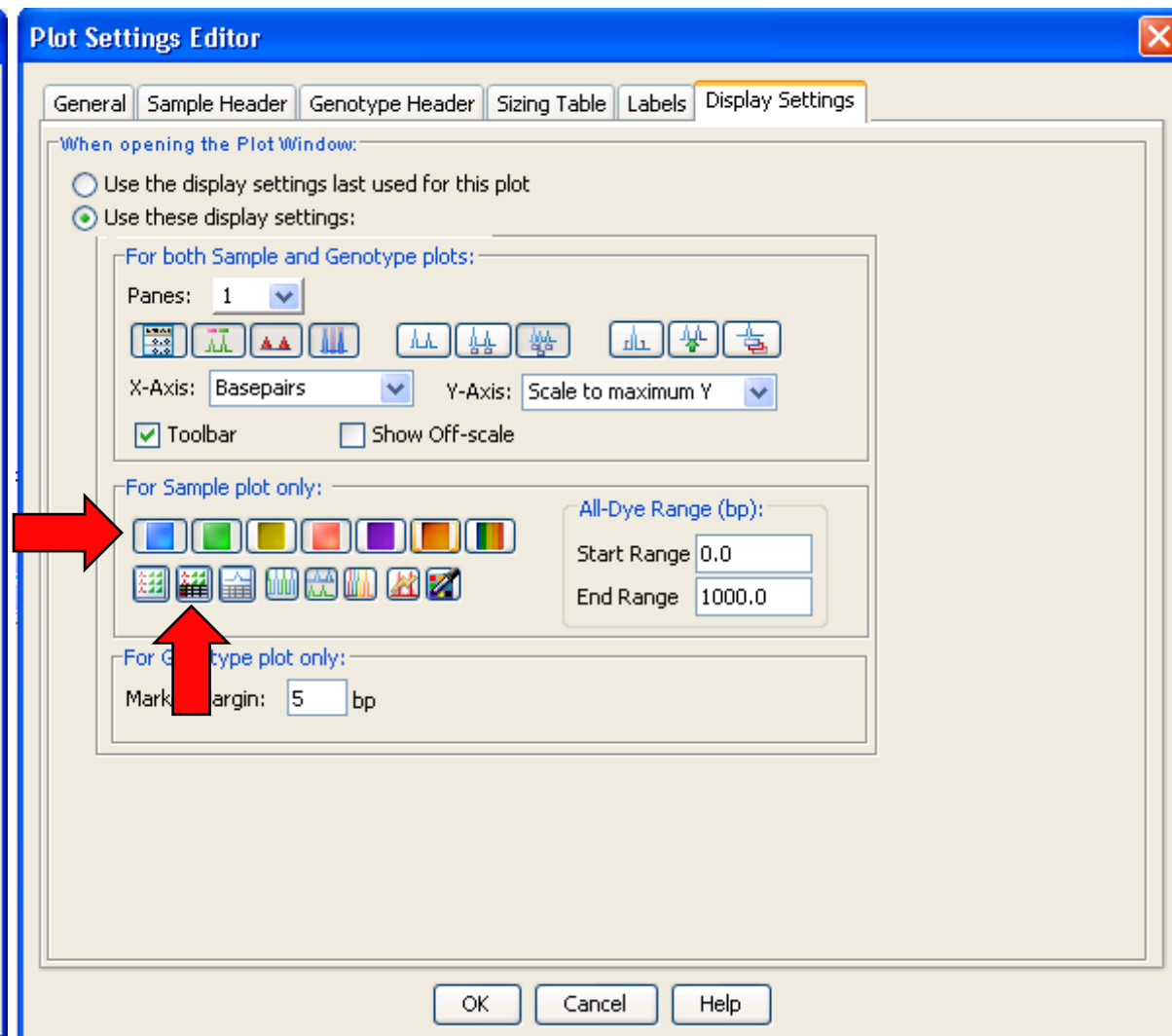
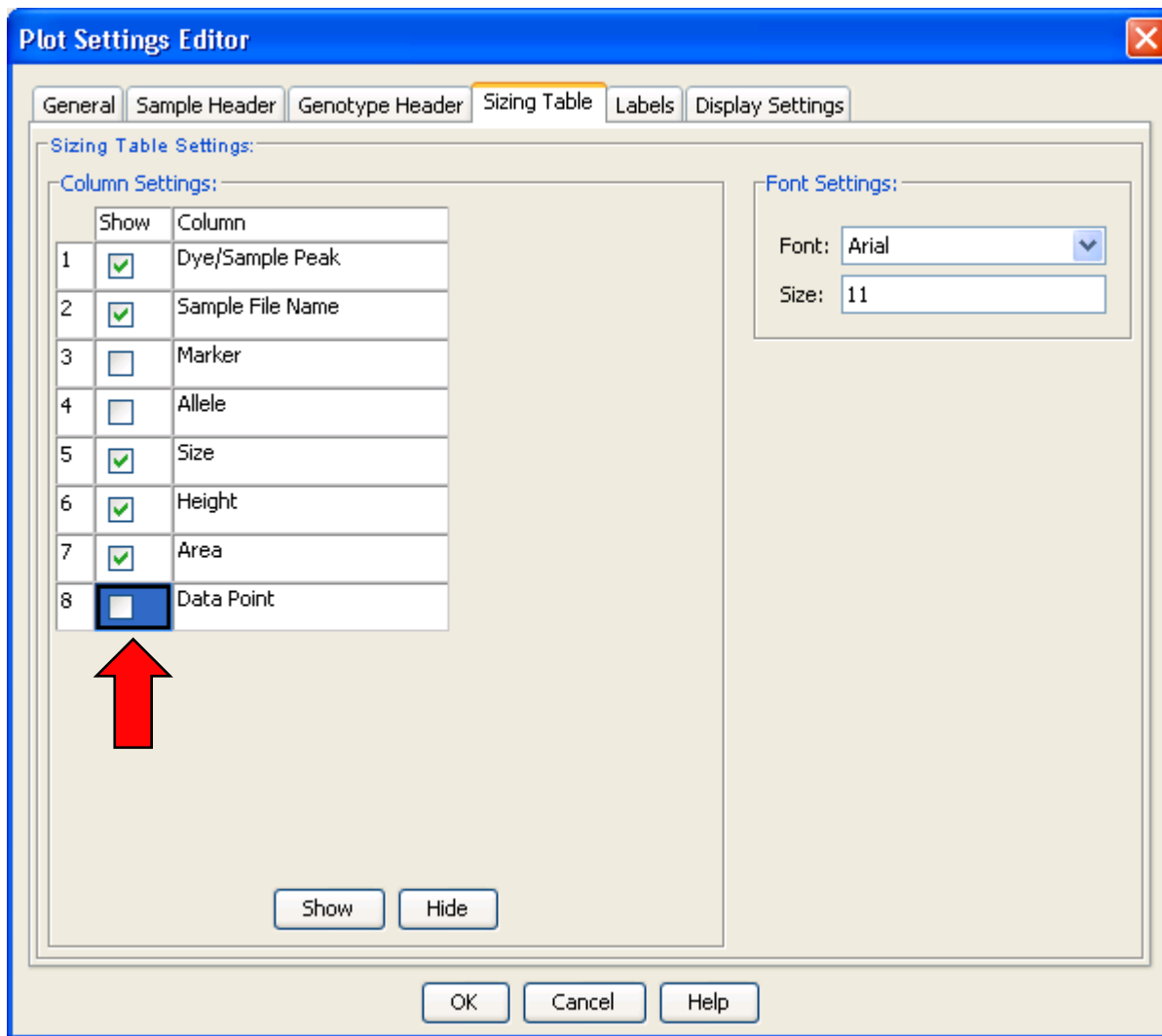
Size Standard Normalization

Enable Normalization
Note: For 35XX series data collection normalization only.

Factory Defaults

OK Cancel

Within the GeneMapper* application, select 'Tools', then 'GeneMapper Manager'. In the 'Plot Settings' tab, create a Plot Setting for *FLT3* assays. In the 'Sizing Table' tab, only checkmark 'Dye/Sample Peak', 'Sample File Name', 'Size', 'Height', 'Area'. In the 'Display Settings' tab, select 'Sizing Table' and only the Blue, Green, and Red dyes.



GeneMapper Data Export

Within the GeneMapper* application, select 'File', then 'New Project', and in the pop-up menu, select 'Microsatellite'. Select 'OK'.

The screenshot displays the GeneMapper software interface. A red arrow points to the 'File' menu in the top-left corner. A 'New Project' dialog box is open in the center, with 'Microsatellite' selected under the 'Project Type' section. Another red arrow points to the 'OK' button in the dialog box. The background shows a table with columns for 'Status', 'Sample File', 'Sample Name', 'Sample ID', 'Comments', 'Sample Type', 'SFN', 'Analysis Method', 'Panel', 'Size Standard', 'Matrix', 'SNP Set', 'Run Name', 'Instrument Type', 'Instrument ID', 'Run Date & Time', 'REF', 'SQI', 'SFNF', 'MNF', 'SNF', 'OS', 'SQ', and 'WELLQ'. The table contains 10 rows, with the first row having a small input field in the 'Status' column.

Select 'File' and 'Add Samples to Project'. Navigate to the ABI injection folders, highlight the *.fsa files and select 'Add to List>>' and then select 'Add'.

The screenshot shows the GeneMapper software interface. The main window displays a table with columns for sample information. An 'Add Samples to Project' dialog box is open, showing a file explorer view of the 'GM Database' folder. The dialog box has a 'Samples To Add:' list on the right and buttons for 'Add To List >>', 'Options...', 'Clear', 'Add', 'Add & Analyze', and 'Cancel' at the bottom. Red arrows indicate the sequence of actions: clicking 'File' in the main menu, navigating to the 'Add to List >>' button, and finally clicking the 'Add' button.

Status	Sample File	Sample Name	Sample ID	Comments	Sample Type	SFN	Analysis Method	Panel	Size Standard	Matrix	Run Name	Instrument Type	Instrument ID	Run Date & Time	REF	SQI	SFNF	MNF	SNF	O5	SQ	UD1	UD2	UD3	Plate	La
1																										
2																										
3																										
4																										
5																										
6																										
7																										
8																										
9																										
10																										

Assign the correct 'Analysis Method' and 'Size Standard' (GS600LIZ+Normalization) for each sample. To simplify the selection of "Analysis Method", select one injection at a time in the left menu to identify all samples that belong to a single assay. Select 'Analyze' and save the project. Once analysis is complete, select all of the samples and select 'Display Plots'.

The screenshot shows the GeneMapper software interface with a table of samples. The table has the following columns: Sample Name, Analysis Method, Panel, Size Standard, Run Name, Instrument ID, Run Date & Time, SGI, SNF, OS, SQ, Lane, and Well. The data rows are as follows:

Sample Name	Analysis Method	Panel	Size Standard	Run Name	Instrument ID	Run Date & Time	SGI	SNF	OS	SQ	Lane	Well
B4120021_E0000026_261452_E01_01_01_ITD CDx Assay.fsa	FLT3 ITD Analysis Meth	None	GS600LIZ+Normalization	Inj1 2014-11-11	3500 Instrumen	2014-11-21 15:00:05		■	■	■	1	A01
B4120021_E0000026_261452_E01_01_04_ITD CDx Assay.fsa	FLT3 ITD Analysis Meth	None	GS600LIZ+Normalization	Inj1 2014-11-11	3500 Instrumen	2014-11-21 15:00:05		■	■	■	4	B01
B4120021_E0000026_R0880050_E01_04_C01_07_ITD CDx Assay.fsa	FLT3 ITD Analysis Meth	None	GS600LIZ+Normalization	Inj1 2014-11-11	3500 Instrumen	2014-11-21 15:00:05		■	▲	■	7	C01
B4120021_E0000026_R0930010_E0000137_D01_10_ITD CDx Assay.fsa	FLT3 ITD Analysis Meth	None	GS600LIZ+Normalization	Inj1 2014-11-11	3500 Instrumen	2014-11-21 15:00:05		■	■	■	10	D01
B4120041_E0000023_261453_E0151_1_A04_01_TKD CDx Assay.fsa	FLT3 TKD Analysis Meth	None	GS600LIZ+Normalization	Inj2 2014-11-11	3500 Instrumen	2014-11-21 15:45:52		■	■	■	1	A04
B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa	FLT3 TKD Analysis Meth	None	GS600LIZ+Normalization	Inj2 2014-11-11	3500 Instrumen	2014-11-21 15:45:52		■	■	■	4	B04
B4120041_E0000023_R0880050_E0000124_C04_07_TKD CDx Assay.fsa	FLT3 TKD Analysis Meth	None	GS600LIZ+Normalization	Inj2 2014-11-11	3500 Instrumen	2014-11-21 15:45:52		■	■	■	7	C04
B4120041_E0000023_R0930010_E0000137_D04_10_TKD CDx Assay.fsa	FLT3 TKD Analysis Meth	None	GS600LIZ+Normalization	Inj2 2014-11-11	3500 Instrumen	2014-11-21 15:45:52		■	■	■	10	D04

Red arrows in the image point to the 'Analysis Method' and 'Size Standard' columns in the table, and to the 'Inj1 2014-11' and 'Inj2 2014-11' folders in the left menu.

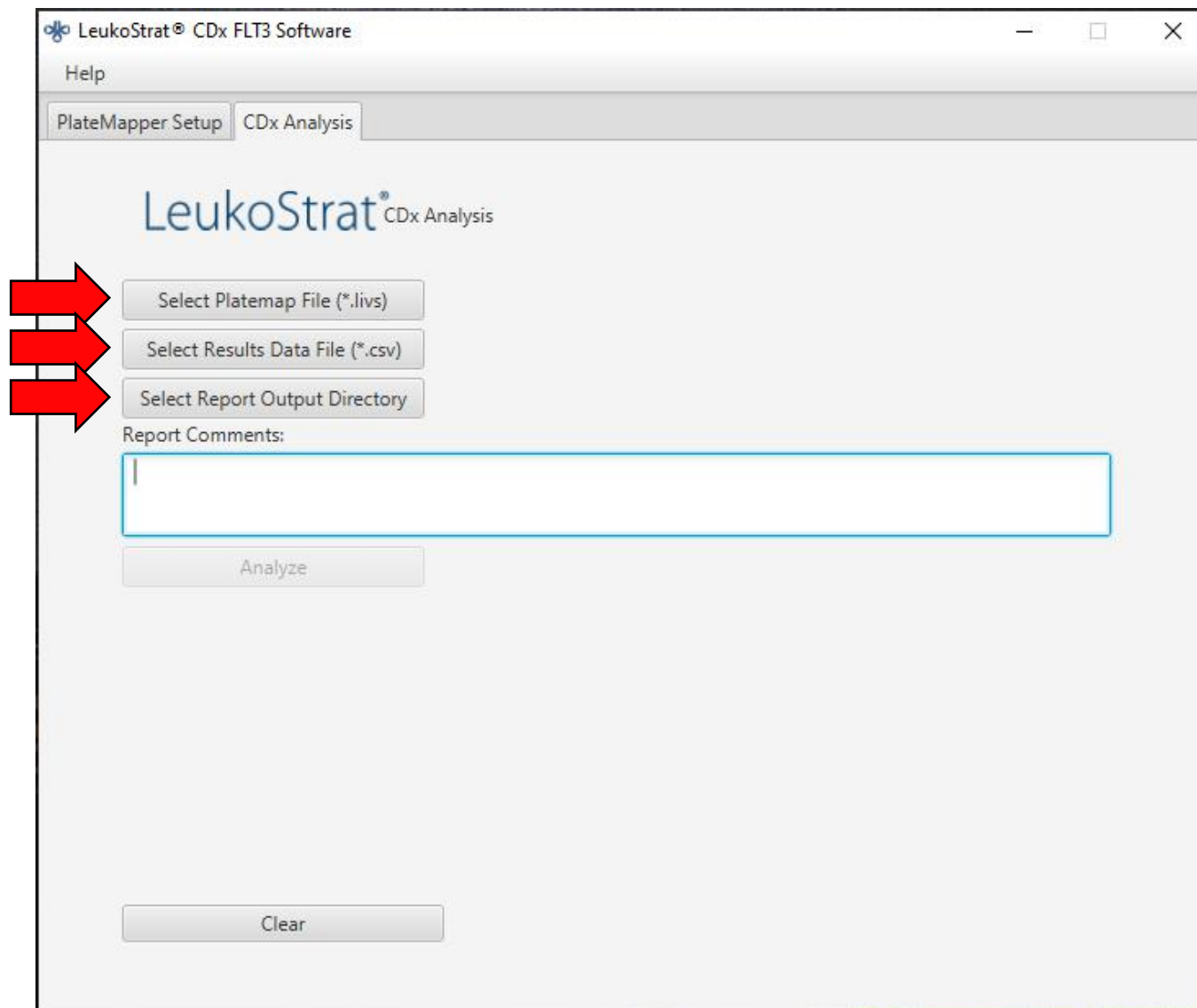
Verify the correct Plot Setting is selected, including the Sizing Table setting and only the Blue, Green, and Red dyes. Select 'File' and then 'Export Table'. Change 'Export File As' to 'Comma-separated values (.csv)'. Export the file and transition to the LeukoStrat CDx FLT3 Software.

The screenshot displays the GeneMapper v4.1.x interface. The main window shows a chromatogram plot with a y-axis ranging from 0 to 32000 and an x-axis from 0 to 900. Below the plot is a table of peak data. An 'Export Table' dialog box is open, showing the 'Export File As' dropdown menu set to 'Comma-separated values (.csv)'. Red arrows highlight the 'Plot Settings' dropdown, the 'Export Table' button, and the 'Export File As' dropdown menu.

Dye/Sample Peak	Sample File Name	Size	Height
B,1	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		129
B,2	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		328
B,3	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		111
B,4	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		446
B,5	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		294
B,6	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		347
B,7	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		211
B,8	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		222
B,9	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		179
B,10	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		282
B,11	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		171
B,12	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		242
B,13	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		193
B,14	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		189
B,15	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		100
B,16	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa		207
B,17	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa	0.71	109
B,18	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa	5.84	338
B,19	B4120041_E0000023_261453_E0151_2_B04_04_TKD CDx Assay.fsa	13.1	960

Final Analysis with LeukoStrat® CDx FLT3 Software

In the 'CDx Analysis' tab, select 'Select Platemap File (*.livs)' and navigate to the .livs file generated during setup. Select 'Select Results Data File (*.csv)' and navigate to the .csv output exported from GeneMapper. Select 'Select Report Output Directory' to select a location to save results and then click 'Analyze'.



The LeukoStrat CDx *FLT3* Software produces PDF reports to simplify the final interpretation. Separate folders are created for both ITD and TKD results. Each folder contains a Run Summary Report for that assay and a sample report for each sample within that assay. Signal Ratio results are reported along with a final call of Positive, Negative, or Fail.

LeukoStrat® CDx *FLT3* Software

Run Report

Run Information			
Run ID	fb170062-996c-4859-90c7-000000000001		
Plate ID	9dd67e4f-d8d0-4016-b72c-f7179eaae829	Assay	ITD
Plate Barcode	01234	Analysis Date	2017-07-24 2:39:56 PM
Plate Name	UnitTestPlate	Run Pass/Fail	Pass

Controls				
Type	Name	ID	Pass/Fail	Fail Detail
PC	PControl1 ITD PC H01	08277bd1d8e5	Pass	
NTC	NTCCControl1 ITD NTC F01	4a6bf004cd22	Pass	
EC	ExtractionControl1 ITD EC E01	4e614e4d9b70	Pass	

Samples				
Sample Name	EC ID	Pos/Neg/Fail	Signal Ratio	Fail Detail
SampleA01 ITD_SAMPLE_A01	4e614e4d9b70	Positive	0.06	

LeukoStrat® CDx *FLT3* Software

Sample Report

Sample and Run Information			
Sample Name	SampleA01 ITD_SAMPLE_A01		
Sample ID	21c1a415-6fad-4f69-af8e-535ad212c275		
Plate ID	9dd67e4f-d8d0-4016-b72c-f7179eaae829	Assay	ITD
Plate Barcode	01234	Analysis Date	2017-07-24 2:39:56 PM
Plate Name	UnitTestPlate		
Run ID	fb170062-996c-4859-90c7-000000000001	Sample Pos/Neg/Fail	Positive

Controls				
Type	Name	ID	Pass/Fail	Fail Detail
PC	PControl1 ITD_PC_H01	08277bd1d8e5	Pass	
NTC	NTCCControl1 ITD_NTC_F01	4a6bf004cd22	Pass	
EC	ExtractionControl1 ITD_EC_E01	4e614e4d9b70	Pass	

Sample				
Sample Name	EC ID	Pos/Neg/Fail	Signal Ratio	Fail Detail
SampleA01 ITD_SAMPLE_A01	4e614e4d9b70	Positive	0.06	

For further information refer to the most current version of the IFU

Ensure that the current IFU is followed every time the assay is run.

