



# **AmplideX<sup>®</sup> PCR/CE *FMR1***

## **Reporter Software**

### *User Guide*

*For Research Use Only.  
Not for use in diagnostic procedures.*

PC-0261



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## Related Products

- P/N: 49402, AmpliEx® *FMR1* PCR kit
- P/N: 49576, AmpliEx® PCR/CE *FMR1* Reporter
- P/N: 49513, AmpliEx® *FMR1* PCR Process Control
- P/N: 49514, AmpliEx® *FMR1* PCR Sensitivity Control

## Related Protocols

- PC-0170v1, AmpliDeX® *FMR1* PCR protocol, available by contacting technical support at support@asuragen.com

Use these protocols in conjunction with the following user guide to successfully execute AmpliDeX® PCR/CE *FMR1* data analysis.

Contact Asuragen Technical Support if any problems are encountered when using this product.

Asuragen Technical Support  
support@asuragen.com  
+1.877.777.1874

## About the AmplideX® PCR/CE *FMR1* Reporter Software

AmplideX® PCR/CE *FMR1* Reporter (P/N: 49576) is a unique data analysis and visualization tool designed to support the rapid analysis of capillary electrophoresis (CE) trace files (.fsa) of the CGG repeat region in the *FMR1* gene, which are produced using the AmplideX® *FMR1* PCR assay.

### Supported AmplideX® PCR/CE *FMR1* Workflows:

The software is currently compatible with the following common CE configurations:

Instrument	Capillary Length (cm)	Run Voltage (kV)
3130	36	15
3500(xL)	50	19.5
3730	36	15
3730	50	15


 **Note:** All application inputs from supported platforms must be configured for English (en-US).

Additional configurations will be supported with software updates.

## Downloading the Software

### LOCATING AND DOWNLOADING THE SOFTWARE:

- Upon software purchase, a software request should be submitted to: <http://software.asuragen.com>. The request should include the following fields:
  - AmplideX® PCR/CE *FMR1* Reporter software (Selection)
  - Company Name
  - Customer Name
  - Account Number
  - Valid email address
- An email will be sent to the email address entered with a link to download the AmplideX® PCR/CE *FMR1* Reporter software package.
- Click the link and then click "Download" to initiate download of the full zip file containing the AmplideX® PCR/CE *FMR1* Reporter software package. Downloading the installer may take several minutes depending on your specific bandwidth, download speed, and other network and computer related factors.

 **Note:** Each software download request will allow a limited number of downloads and will expire after a period of time. To request additional downloads please submit a software download request at: <http://software.asuragen.com>



Welcome to Asuragen software download site.

Please complete the following form and submit the request to receive the requested software by email.

Select a Software

Select a Software

Company Name

Customer Name

Account Number

Email Address

Contact Information

T: 1.877.777.1874

[support@asuragen.com](mailto:support@asuragen.com)

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## Software Installation

The AmplideX® PCR/CE *FMR1* Reporter can be installed on a consumer-grade Windows PC and does not require specialized computing hardware. We recommend the following minimum hardware and software requirements:

### Recommended:

- 4 core Intel i5 processor (or equivalent)
- 100 GB hard drive
- 4 GB RAM

### Required:

- 64-bit Windows 7 SP1 or Windows 8.1 (optionally with KB2919355)
- Port 9002 for localhost must be available
- Hardware assisted visualization is enabled in BIOS



**Note:** Software project creation is not compatible with non-us characters. Please verify OS, keyboard, and browser localization settings are set in US English (en-us).

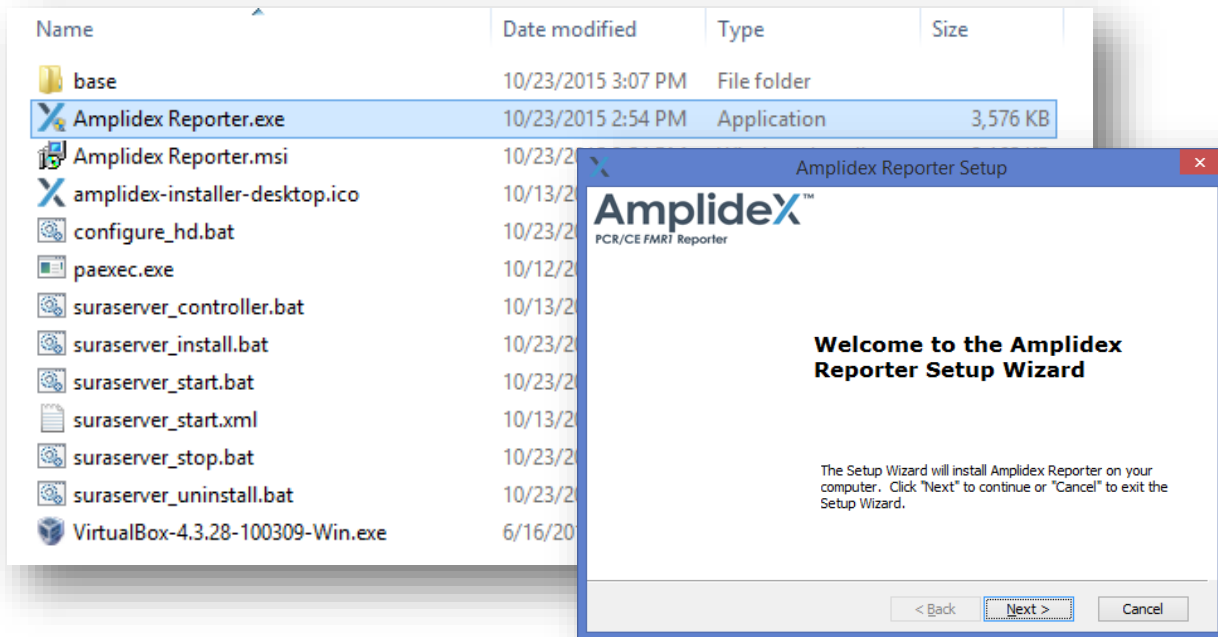
An internet connection is not required to perform an analysis with the AmplideX® PCR/CE *FMR1* Reporter. However, for certain functionality to work (such as error report submission, access to user guide, and software updates) internet access will be required from the machine where the software is installed. Additionally, users installing the software must have Administrator rights on the machine that the software will be installed. Please see the below section titled: ADMINISTRATION & USER MANAGEMENT, for a description of the different user types.

**Browser Support:** Please note the AmplideX® PCR/CE *FMR1* Reporter is only supported on Firefox (v20+) and Chrome (v20+) Browsers, and is NOT supported on Internet Explorer.

**Initiating Software Installation:**

- Following download of the compressed installation package, place zip folder into a unique folder.
- Right click on the zip folder and click “Extract Here”. Following extraction a set of installation files will populate the folder.
- Double click on the file named “AmplideX Reporter.exe”. You will see an installation wizard pop up. Follow the installer dialogs to completion.

**⚠ Note: Disconnecting or switching network connection status may result in a momentary inability to connect to the AmplideX® Reporter.**

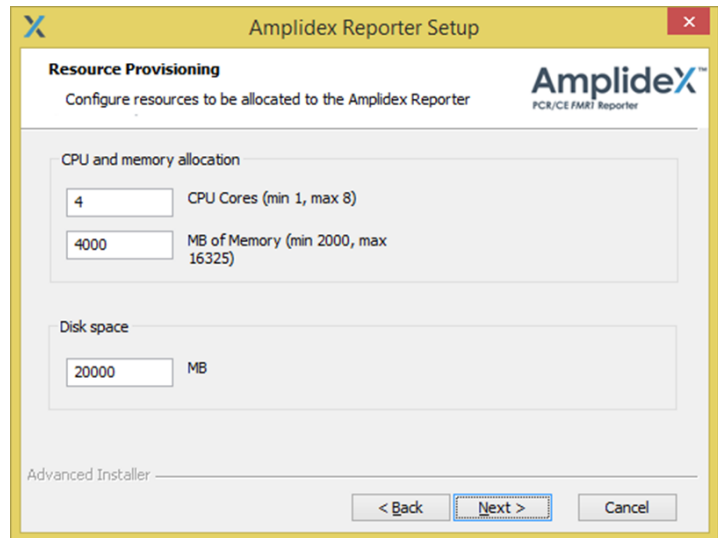


- Following installation a Oracle VM VirtualBox Manager window may open. Close this box.
- Proceed with the AmplideX® PCR/CE FMR1 Reporter Setup Wizard by clicking “Next” and accepting the terms of the License Agreement.

**Provisioning Machine Resources:**

- The amount of host system resources that will be reserved for the AmplideX® PCR/CE FMR1 Reporter is determined during the installation process on the following dialog screen:

- The default values are the minimum recommended allocations to provide the AmplideX® PCR/CE FMR1 Reporter. The CPU and memory allocations should scale together (ie as more CPU cores are reserved, additional memory should be provisioned). Assigning more CPU and memory resources will reduce the total time required to analyze multiple samples or projects. The disk space allocation dictates how many analysis projects can be retained by AmplideX® PCR/CE FMR1 Reporter before needing to export and delete an analysis project. For a full set of 96 samples the software will require approximately 70MB of storage space.



**Note:** The values entered during installation cannot be changed after installation. We recommend careful planning at the time of installation.

#### Completing the Installation Process:

- Following through the installation prompts including provisioning resources, by clicking Next >. Installation may take several minutes. The more disk space that is allocated to the system, the longer the installation process will take. When complete, click “Finish” when prompted.

#### ACCESSING THE AMPLIDEX® PCR/CE FMR1 REPORTER FOLLOWING INSTALLATION

After the installation completes, an option will be presented to launch the AmplideX® PCR/CE FMR1 Reporter. The system can be accessed from the AmplideX® PCR/CE FMR1 Reporter icon on the desktop or by directing a web browser to: localhost:9002 (where 9002 is the port which was assigned to the AmplideX® PCR/CE FMR1 Reporter during installation). The AmplideX® PCR/CE FMR1 Reporter can also be accessed from other machines on the local network by navigating to hostname:9002 (where hostname should be replaced with the hostname of the machine that the AmplideX® PCR/CE FMR1 Reporter was installed to).

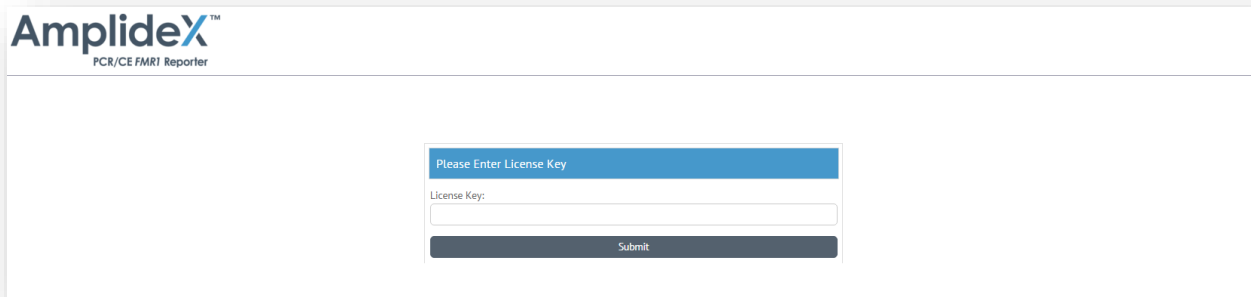
## License Key Input

### License Key:

When launching the software for the first time a product license key screen will appear within your web browser. Please enter the license key provided by Asuragen and click “Submit”. License keys will expire 7 days after the issue date (the date the license key was sent by Asuragen), and will last for 1 year after the date the license is imported into the system. For license key troubleshooting, please contact Asuragen Technical Support.

### License Key Renewal:

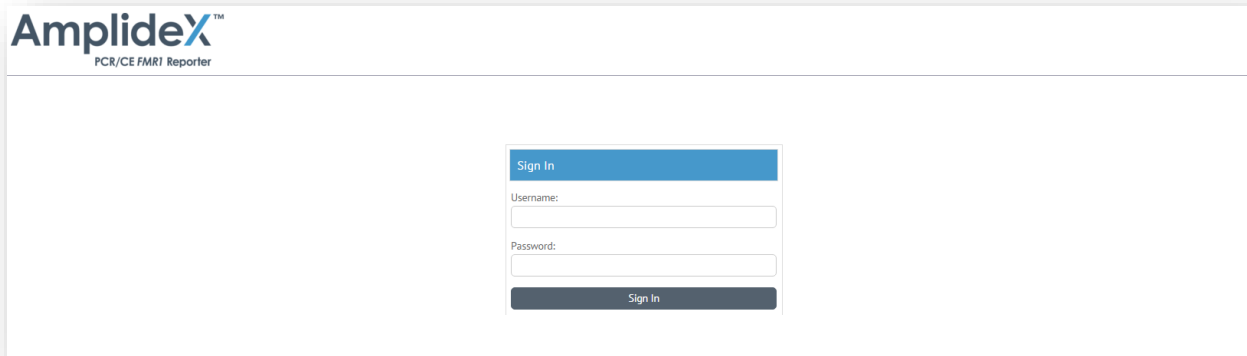
License key should be renewed at the end of usage term in order to allow continual software functionality (See End User License Agreement located in the “About AmplideX® PCR/CE *FMR1* Reporter”) using software license key page. To receive a new license key, please contact Asuragen sales representatives. To input new license key direct the host web browser to: localhost:9002/license or alternatively from other machines use: hostname:9002/license (where hostname should be replaced with the hostname of the machine that the AmplideX® PCR/CE *FMR1* Reporter was installed to).



The screenshot shows the web interface for the AmplideX PCR/CE *FMR1* Reporter. At the top left is the logo "AmplideX™ PCR/CE *FMR1* Reporter". In the center of the page is a form titled "Please Enter License Key" in a blue header. Below the header is a text input field labeled "License Key:". At the bottom of the form is a dark gray button labeled "Submit".

**Login Page:**

A login screen will be presented when navigating to the AmplideX® PCR/CE *FMR1* Reporter in the web browser as shown below:



For first time login, use the following credentials:

**Username: admin**

**Password: admin**

The admin password should be changed on the admin page after the initial login and additional users can be created.

To modify the administrative password:

- Login as administrator using the above credentials
- Click Admin button
- Select admin user from user list
- Click 'Edit User'
- Enter new password
- Confirm password
- Click Save

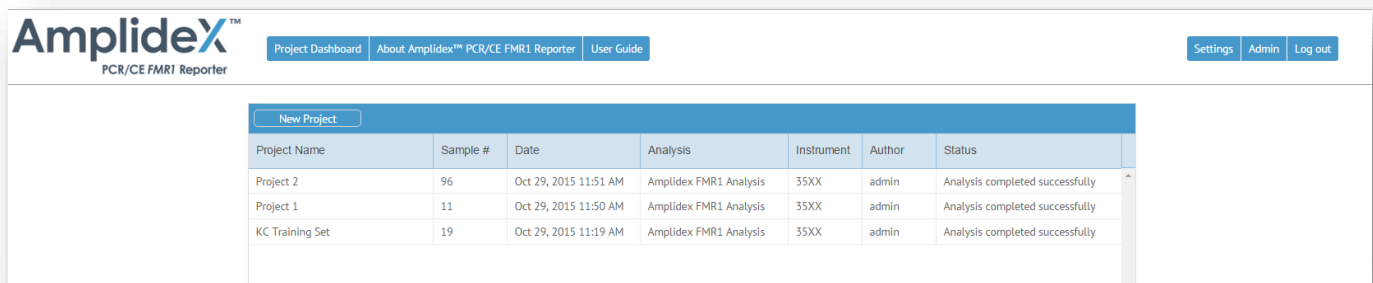


## SIGNING INTO THE AMPLIDEX® PCR/CE FMR1 REPORTER PROJECT DASHBOARD

- Type in user name and password, then click “Sign In”. If no user name has been established (immediately following installation), please see the above section for login credentials.
- After login you will be presented with the Project Dashboard with each table row representing an individual project. Each project represents a container for a specific analysis of a chosen sample set.

### Project Dashboard Features:

Within this view the user can view a listing of previously run projects and projects in the pre-analysis stage. Several features included in this view are as follows:



AmplideX™ PCR/CE FMR1 Reporter						
Project Dashboard   About AmplideX™ PCR/CE FMR1 Reporter   User Guide						
Settings   Admin   Log out						
New Project						
Project Name	Sample #	Date	Analysis	Instrument	Author	Status
Project 2	96	Oct 29, 2015 11:51 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully
Project 1	11	Oct 29, 2015 11:50 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully
KC Training Set	19	Oct 29, 2015 11:19 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully

**Top Dashboard with Project Functions:** Listing of various analysis functions (see below for details on functionality of these features).

**Link to the About the AmplideX® PCR/CE FMR1 Reporter Page:** Provides details on the software including licensure information on 3<sup>rd</sup> party software utilized by the reporter.

**Link to the AmplideX® PCR/CE FMR1 Reporter User Guide:** Opens a .PDF version of the software User Guide (PC-0261).

 **Note:** An Internet connection is required to access the software user guide.

**Settings Page:** System administrators will have the option to configure peak calling threshold and sub-threshold peak reporting (see Configuring Lab Specific Parameters below).

**Admin Page:** For managing users and access to the analysis as well as user names and passwords.

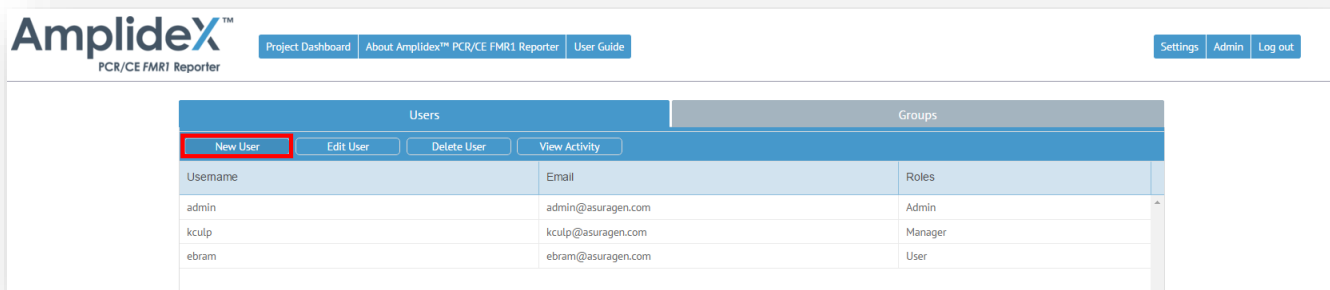
## Administration & User Management

Users with administrator roles will have a link to the admin page available on their project dashboard. From the admin page, an administrator can:

 **Note: These Features Only Available to Users with Admin Roles**

- Create/Delete other administrators, managers, users and groups
- Modify the role of a given user
- Modify a user's password
- View an audit log detailing all user action within the software.

### CREATING A NEW USER



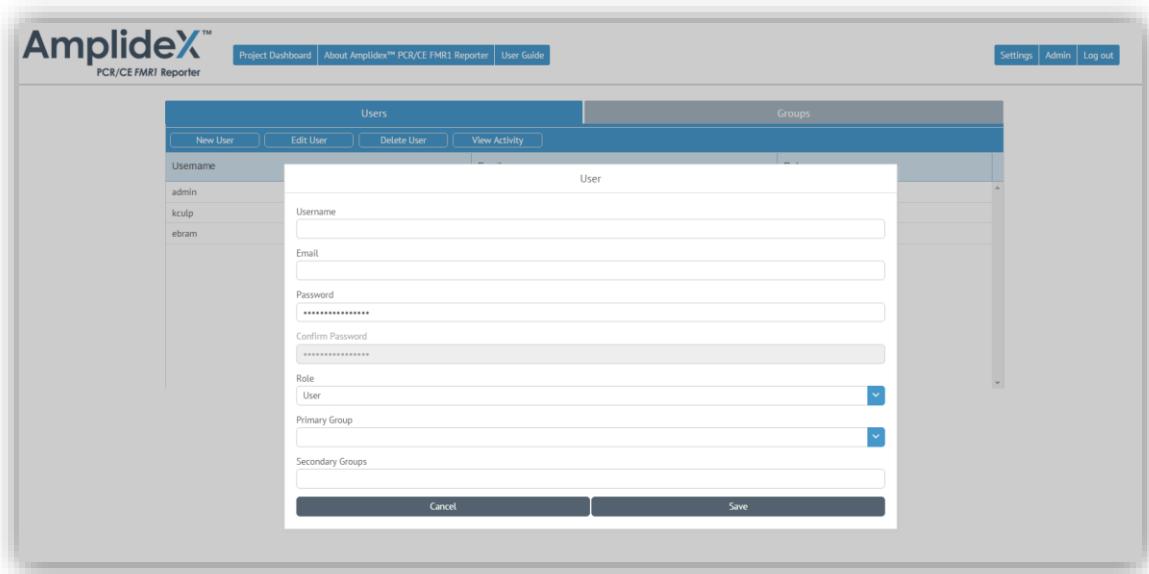
- To create a new user click “New User” from the admin page.
- Enter the username, email address and password and select the user's role (admin, manager, or user). Click “Save” to create the new user.

#### For user management, the following roles and permissions are provided:

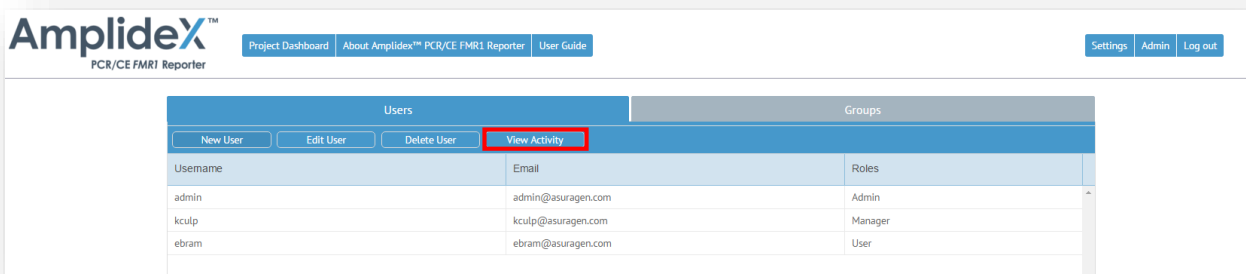
**Admin** – Administrators can view, edit, and delete all users in the system. Administrators can view the system's audit log. Administrators can view, edit, and delete all projects.

**Manager** – Managers can view, edit, and delete any project from groups of which they are a member.

**User** – Users can view, edit, and delete projects they own. Users can also read unowned projects belonging to groups of which they are a member.



- To modify an existing user select the user's row in the table and click "Edit User".
- A dialog box similar to the create user dialog box will appear with current user properties which can be edited. After editing, click "Save".
- Similarly, to remove a user from the system select the user from the table and click "Delete User".
- To view the audit logs of the system click "View Activity".

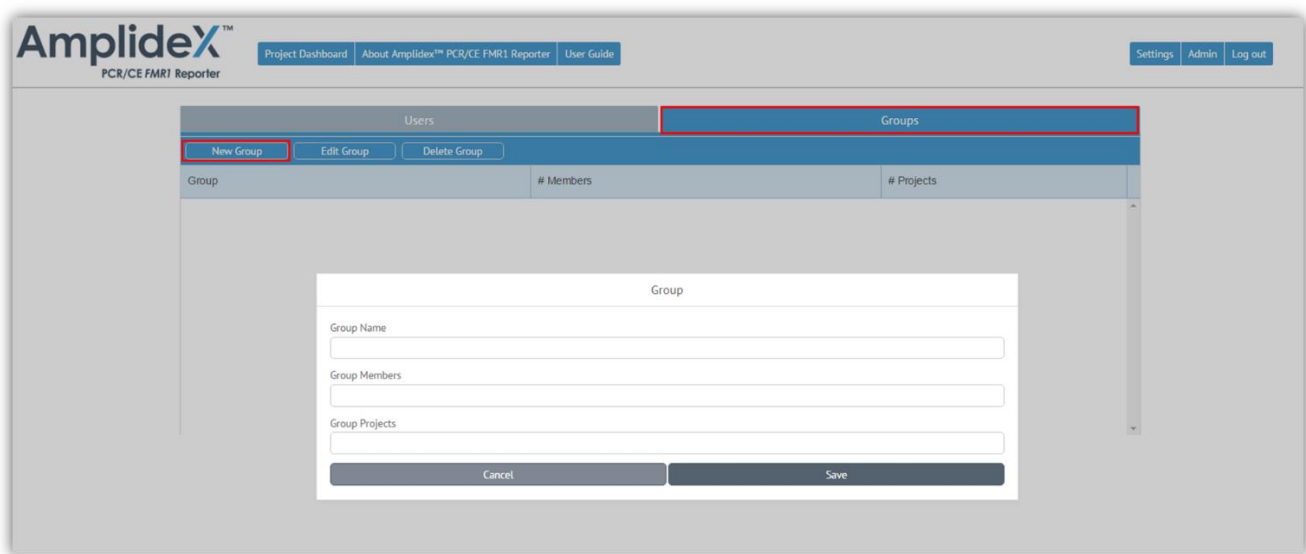


An Excel readable file will be downloaded that contains a list of access events. The file contains 4 columns with the following information: 1) user 2) event 3) event details 4) date and time of event. This information can be used to monitor user activity.

<i>admin</i>	<i>login</i>	<i>NA</i>	<i>6/21/2000 19:00</i>
<i>admin</i>	<i>Created</i>	<i>&lt;User 'testuser'&gt;</i>	<i>6/21/2000 19:00</i>

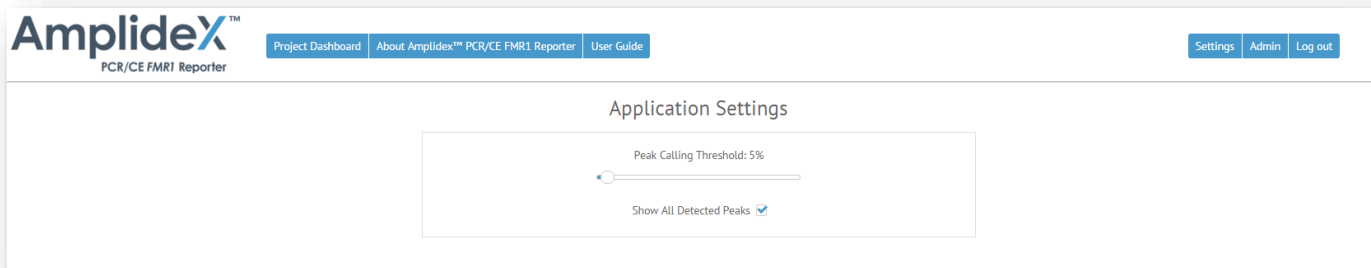
## CREATING A NEW GROUP

To create or modify a group click on the “Groups” tab within the Admin page. Next proceed to enter group name, select from the dropdown menus members and relevant projects to share. Save and exit when done.



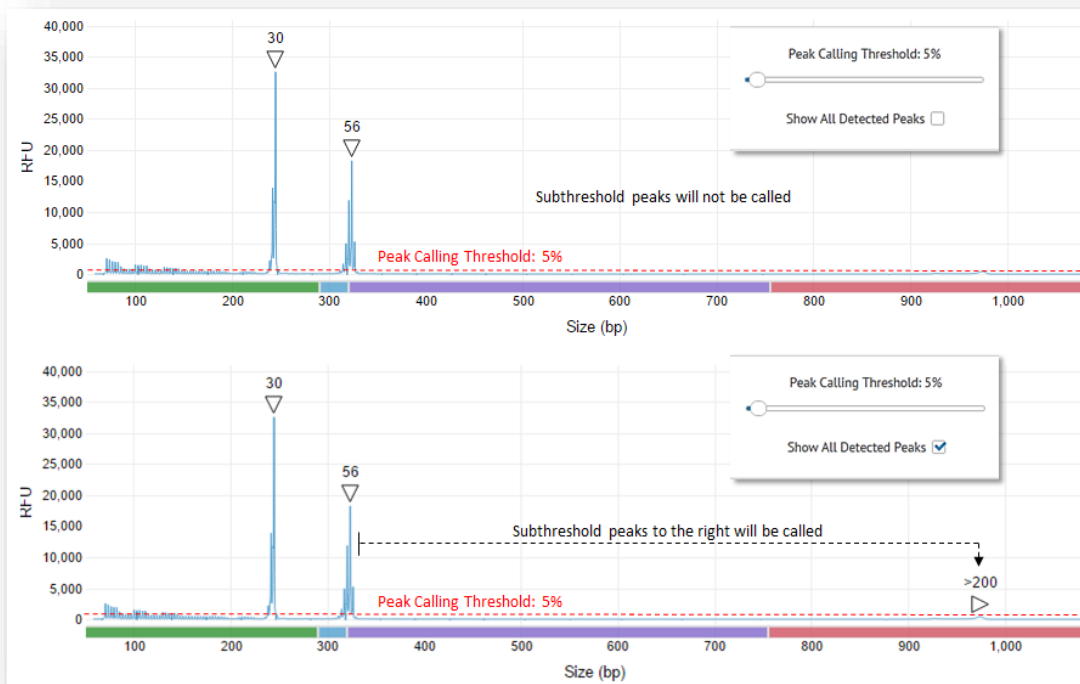
## CONFIGURING LAB SPECIFIC PARAMETERS

System administrators will have the option to configure two analysis and report settings from within the Setting page. To enter the Settings page click on “Settings” located at the top right corner of the page. The following settings can be changed.

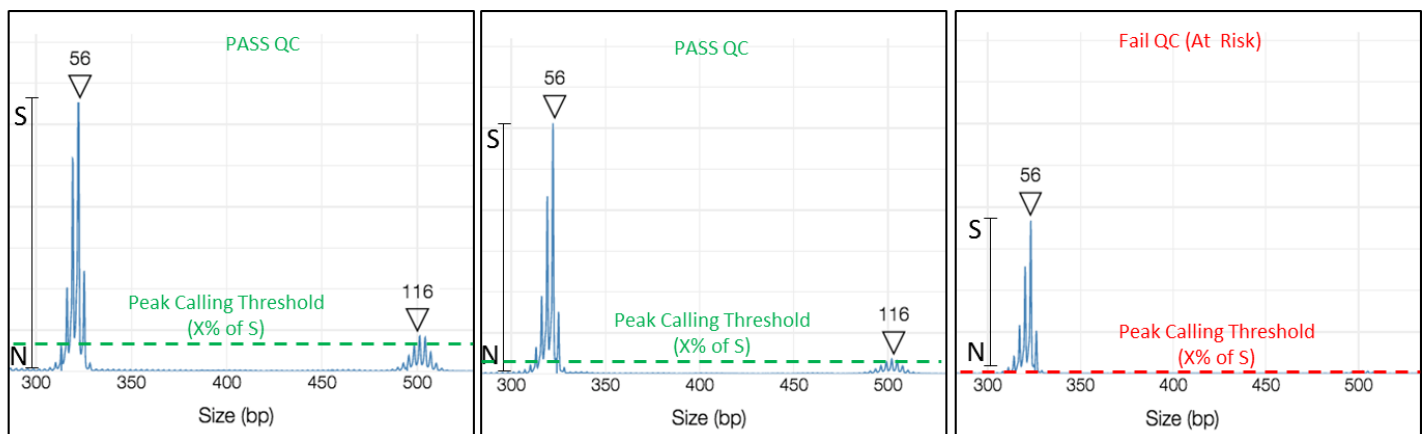


**Peak calling threshold:** This setting allows the laboratory administrator to set a lower bound threshold for reporting of peaks within all lab projects and to provide an adjustable QC metric for insufficient sensitivity (IS). The threshold reflects the minimal signal level above which an identified peak will be annotated in the results table. Accordingly, the threshold also dictates the necessary limit of detection (LOD) to support the corresponding detection of all peaks at or above the defined threshold level. Use the slide bar to change the threshold from its default setting (5%).

**Sub-threshold peak calling checkbox:** When checked, this checkbox allows reporting of low signal peaks detected below the defined threshold that are larger than (in CGG repeat length) the largest gene specific peak.



- Sample specific LOD:** For a given sample, LOD is a function of its signal (S) and noise (N) components and can be reflected as a proportion of the two. The software is designed to support detection of minimal signal ( $S_{min}$ ) down to 2X over the median background noise ( $N_{med}$ ), making  $S_{min} = 2N_{med}$ . The LOD can therefore be represented in relative units as a fraction of the sample's gene-specific highest peak ( $S_{max}$ ), as either:  $[S_{min}/S_{max}]$  or  $[2N_{med}/S_{max}]$ .



### Graphic representation of Peak Calling Threshold functionality:

Administrator-defined Peak Calling Threshold (dotted line) is set at X% of the sample's highest peaks signal. If threshold value (X%) is within the sample's signal – noise range (threshold value (X%) should be higher than the sample specific LOD), the sample will pass QC and all alleles above the threshold will be reported out (left and middle pane). Samples with overall low signal quality inherently present reduced LOD. In a case were a sample's LOD is above the threshold value, samples will be flagged as "at risk" of missing low-level alleles (right pane).

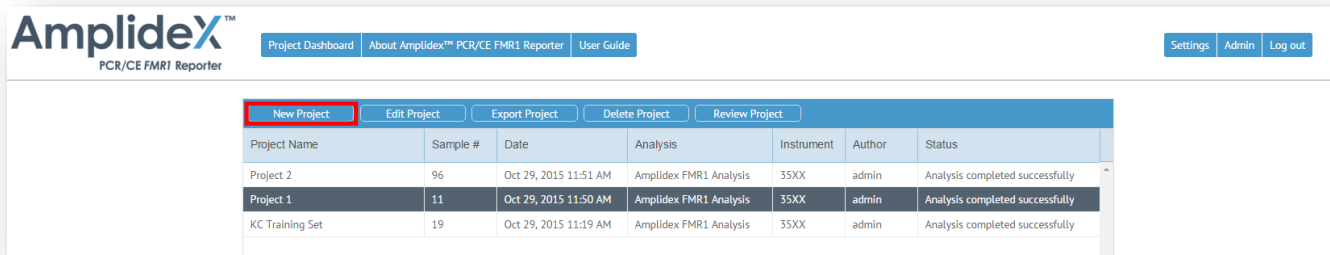
**Note:** Samples with reduced signal levels that do not meet required LOD levels will be flagged as failing the Insufficient Sensitivity (IS) – QC. However, detected peaks will still be reported in the results.

## Creating a New Project & Importing Data

Following an AmplideX® FMR1 PCR/CE batch run, the user will be provided with a set of .fsa files from the CE instrument. These files should then be imported into the reporter for analysis.

- Open the project dashboard in the AmplideX® PCR/CE FMR1 Reporter (see Signing into the AmplideX® PCR/CE FMR1 Reporter Project Dashboard above).
- Click on the “New Project” button at the top left side of the dashboard.

**Note:** .fsa files that are damaged or incorrectly imported cannot be deleted once imported. To resolve this issue, it is recommended that the user deletes the project and creates a new one.



- You will be presented with the Project Editor view. Insert project name, project description, project group (if applicable) and assay type (currently only supporting the AmplideX® FMR1 PCR/CE assay).
- Click “Save and Continue”.

Project Name:

Project description:

Project Group:

Analysis type:

Peak Calling Threshold: 5%

Show All Detected Peaks: ☒

In the following panel titled “Import FSA” the user will select the appropriate .fsa files for the project. Select “Import FSA Files”, click “Add FSA Files”, navigate to the relevant .fsa files and select. Click “Import”. If your CE instrument allows custom filename configuration, ensure that sample filenames begin with a unique identifier. The software will automatically infer a naming convention for the samples based on the sample names provided to the CE within the CE run setup files. Special

**Note:** The system requires that the filenames of the .fsa files are configured such that a unique sample name comes first. (e.g. SampleName-XXXX-XXXX-XXX).

The screenshot shows the 'Import FSA' tab selected in the software interface. The top navigation bar includes 'Project Dashboard', 'About AmplideX™ PCR/CE FMR1 Reporter', and 'User Guide'. On the right, there are links for 'Settings', 'Admin', and 'Log out'. The main content area has four tabs: 'Overview', 'Import FSA' (active), 'Annotate Samples', and 'Review'. Below the 'Import FSA' tab, there is a button labeled 'Import FSA Files'. At the bottom of the panel, there are two buttons: 'Back' and 'Save and continue'.

characters and whitespace will be automatically removed from samples names and hence should be avoided.

### Annotate Samples Tab:

Well information and Instrument type are imported directly from the .fsa file metadata. Gender input data can be entered manually for each sample by double clicking on the cell in the table, entering a value (M/F) and pressing enter. Alternatively, the data can be copy-pasted as two data columns from Excel (sample name and associated gender) into one of the Sample Name cells as long as the sample names are matching what is shown in this table. There is no need to reorder or sort the Excel data to match the ordering in this table. The sample names will be used to match the gender value across the two tables. After entering this information, click “Submit”.

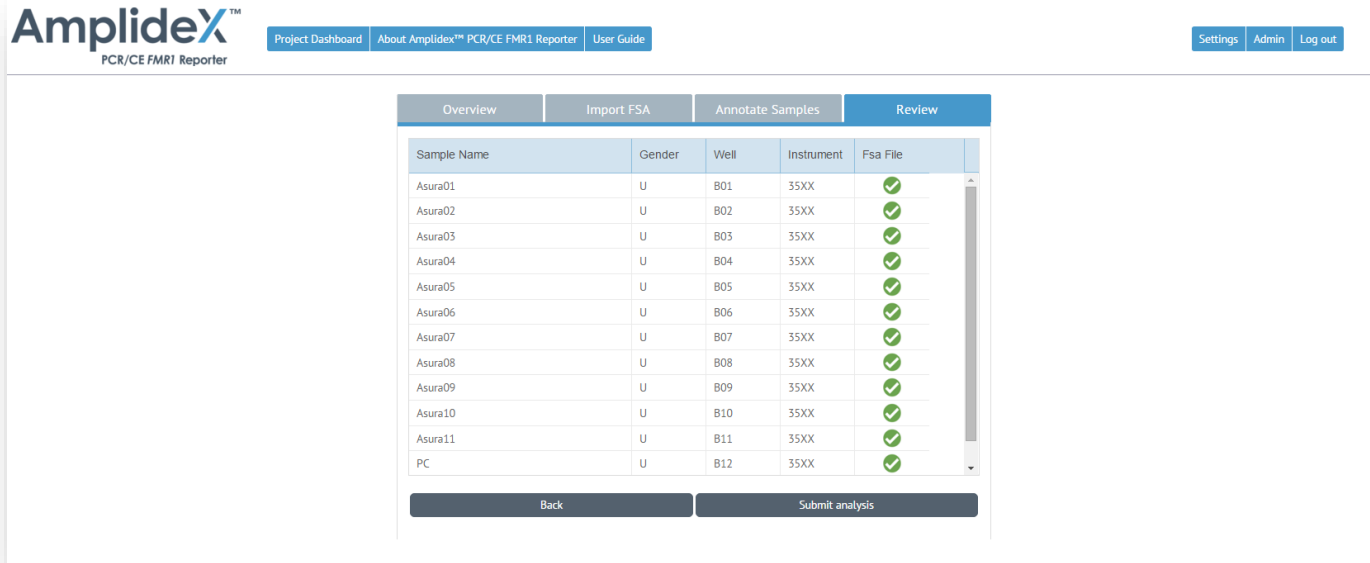
**Note:** Gender input provided as ancillary information will allow accurate zygosity annotation for analyzed samples.

The screenshot shows the 'Annotate Samples' tab selected in the software interface. The top navigation bar is the same as the previous screenshot. The main content area has four tabs: 'Overview', 'Import FSA', 'Annotate Samples' (active), and 'Review'. Below the 'Annotate Samples' tab, there is a table with the following data:

Sample	Gender	Well	Instrument
Asura01	U	B01	35XX
Asura02	U	B02	35XX
Asura03	U	B03	35XX
Asura04	U	B04	35XX
Asura05	U	B05	35XX
Asura06	U	B06	35XX
Asura07	U	B07	35XX
Asura08	U	B08	35XX
Asura09	U	B09	35XX
Asura10	U	B10	35XX
Asura11	U	B11	35XX
PC	U	B12	35XX

At the bottom of the panel, there are two buttons: 'Back' and 'Save and continue'.

- **Review Tab:** After review of the samples and associated information click “Submit analysis”.



## Data Analysis

The software uses a proprietary algorithm to analyze CE trace files of AmplideX® *FMR1* PCR fragments, generating the following sample-specific analytical information:

- Auto scaling and tuning of raw CE data (background process)
- Automated evaluation of signal quality and signal irregularities in both sample and calibration ladder (QC).
- Automated detection of significant *FMR1* alleles
- Automated sizing and categorical classification of all detected *FMR1* alleles

These features were rigorously tested using over 1000 clinical specimens and were found to be fully compliant with ACMG recommended accuracy levels for *FMR1* testing (For more information on *FMR1* size categories please refer to ACMG, Standards and Guidelines for Clinical Genetics Laboratories).

After analysis submission the project dashboard view will be presented. The progress of the newly submitted analysis can be tracked in the “Status” column within the project dashboard page. Once analysis is done status will say: “Analysis Completed Successfully”. Analyzed projects are saved within the system and can be reviewed at any time.



## VIEWING RESULTS WITHIN THE REPORTER SUITE

The user can view the analysis results directly within the reporting suite using the Project Summary Dashboard and Results Table view.

To view specific projects within the Project Summary Dashboard view:

- Select the project of interest in the main project dashboard by clicking on the row containing the relevant project.
- Click the “Review Project” button at the top of the user dashboard. The user will be presented with a project summary view for that project.

AmplideX<sup>TM</sup> PCR/CE *FMR1* Reporter

Project Dashboard | About AmplideX<sup>TM</sup> PCR/CE *FMR1* Reporter | User Guide

Settings | Admin | Log out

New Project | Edit Project | Export Project | Delete Project | **Review Project**

Project Name	Sample #	Date	Analysis	Instrument	Author	Status
Project 2	96	Oct 29, 2015 11:51 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully
Project 1	11	Oct 29, 2015 11:50 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully
KC Training Set	19	Oct 29, 2015 11:19 AM	AmplideX FMR1 Analysis	35XX	admin	Analysis completed successfully

### Project Summary Dashboard Features:

The top dashboard provides a project overview that presents a pie chart distribution of project samples across the following key parameters: Quality Control, Categorical *FMR1* genotype and Gender. The dashboard may be easily concealed/revealed using the arrow button on the right side of the dashboard.

AmplideX<sup>TM</sup> PCR/CE *FMR1* Reporter

Project Dashboard | About AmplideX<sup>TM</sup> PCR/CE *FMR1* Reporter | User Guide

Settings | Admin | Log out

Project Project 1 Summary

Project QC

Categorical Distribution

Gender Distribution

Export Excel | Expand All Rows | Compress All Rows | Reset Genotypes | Edit Project

Sample	Gender	Well	Genotype	RFU	Category	QC	Edited	Rerun
Asura01	U	B01	20	32780	NOR	PASS		
Asura02	U	B02	30	32712	NOR	PASS		
Asura03	U	B03	>200	1236	FM	PASS		

### Project QC:

- **Pass** - Indicates all samples where the analysis detected all alleles down to the designated peak calling threshold (see Configuring Lab Specific Parameters)

- **At Risk**- Indicates all samples where the analysis detected some of the alleles, but due to low sample signal some low-level alleles may be missed.
- **Fail**- Samples in which the overall signal and/or the sizing ladder are insufficient or anomalous.

### Project Positive Control Indicators:

AmplideX® PCR/CE *FMR1* Reporter supports the integration of Asuragen's commercially available set of AmplideX® *FMR1* PCR Controls - The AmplideX® *FMR1* Controls are proprietary mixtures of genomic DNA containing multiple alleles. These well characterized controls can be used as process or batch controls covering multiple size ranges in the *FMR1* gene.

- **PC:** AmplideX® *FMR1* PCR Process Control (P/N: 49513) – A positive control with multiple size peaks spanning the full range of *FMR1* genotypes.
- **SC:** AmplideX® *FMR1* Sensitivity Control (P/N: 49514) - A positive control that includes a low level full mutation allele for analytical sensitivity assessment.

The software will automatically detect the positive control samples based on sample name, using their abbreviated naming convention: "PC" and "SC". The software will evaluate peak size and integrity. The outcome of PC/SC analysis will be reflected within the Project Summary Dashboard PC and SC Indicators:

- Pass – Green
- Fail – Red
- Missing sample– Blank

Positive control samples will also be displayed within project results table. For more information on Asuragen's AmplideX® *FMR1* PCR Controls please visit (<http://asuragen.com/products-and-services/products/amplidex/amplidex-controls/>).

### Categorical Distribution:

Displays the distribution of project samples across the canonical *FMR1* size categories:

- Normal (NOR; 5-44 CGG repeats),
- Intermediate (INT; 45-54 CGG repeats),
- Premutation (PM; 55-200 CGG repeats)
- Full Mutation (FM; >200 CGG repeats).
- NA (Samples that failed QC)

(For more information on *FMR1* size categories please refer to ACMG, Standards and Guidelines for Clinical Genetics Laboratories).

### Gender Distribution:

Represents the distribution of gender within the project based on ancillary information provided by the user during the sample annotation stage.

### Project Results Table

Analysis information for each sample within a project is provided in the Project Results Table. Each line represents a single sample and includes:

- **Sample Name**- Listing of all samples analyzed within the specific project.
- **Gender** – Listing of sample gender, as provided in the sample annotation stage
- **Well** – Sample location in CE plate layout
- **Genotype** – The size (in CGG repeat number) of selected peaks, (For multiple peaks - ordered by size)

- **RFU** – The signal magnitude at the selected peak apex (For multiple peaks - order corresponding to genotype column)
- **Category** – Sample size categories (NOR, INT, PM and FM)
- **QC** – Presented as “Pass” for samples passing QC, or denoting the type of QC failure (as depicted in the table below).
- **Edited** – Indicator denotes that a sample annotation has been manually edited.
- **Rerun** – Samples failing hard QC and/or user defined samples can be selected for rerun.

Export Excel   Expand All Rows   Compress All Rows   Reset Genotypes   ▼									
Sample	Gender	Well	Genotype	RFU		Category	QC	Edited	Rerun
Asura01	M	B01	20	32780		NOR	● PASS	<input type="radio"/>	<input type="radio"/>
Asura02	F	B02	30	32712		NOR	● PASS	<input type="radio"/>	<input type="radio"/>
Asura03	M	B03	200	1236		FM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura04	M	B04	84	31917		PM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura05	F	B05	22, 110, 116	32617, 10589, 2254		PM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura06	M	B06	41, 45, 51, 64, 70	8362, 2026, 2400, 32340, 179		PM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura07	F	B07	17, 112	31922, 4905		PM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura08	M	B08	200	2523		FM	● PASS	<input type="radio"/>	<input type="radio"/>
Asura09	F	B09	30, 32	32617, 32617		NOR	● PASS	<input type="radio"/>	<input type="radio"/>
Asura10	F	B10	30, 200	32617, 4755		FM	● PASS	<input type="radio"/>	<input type="radio"/>

### A summary of software's Quality Control Tests:

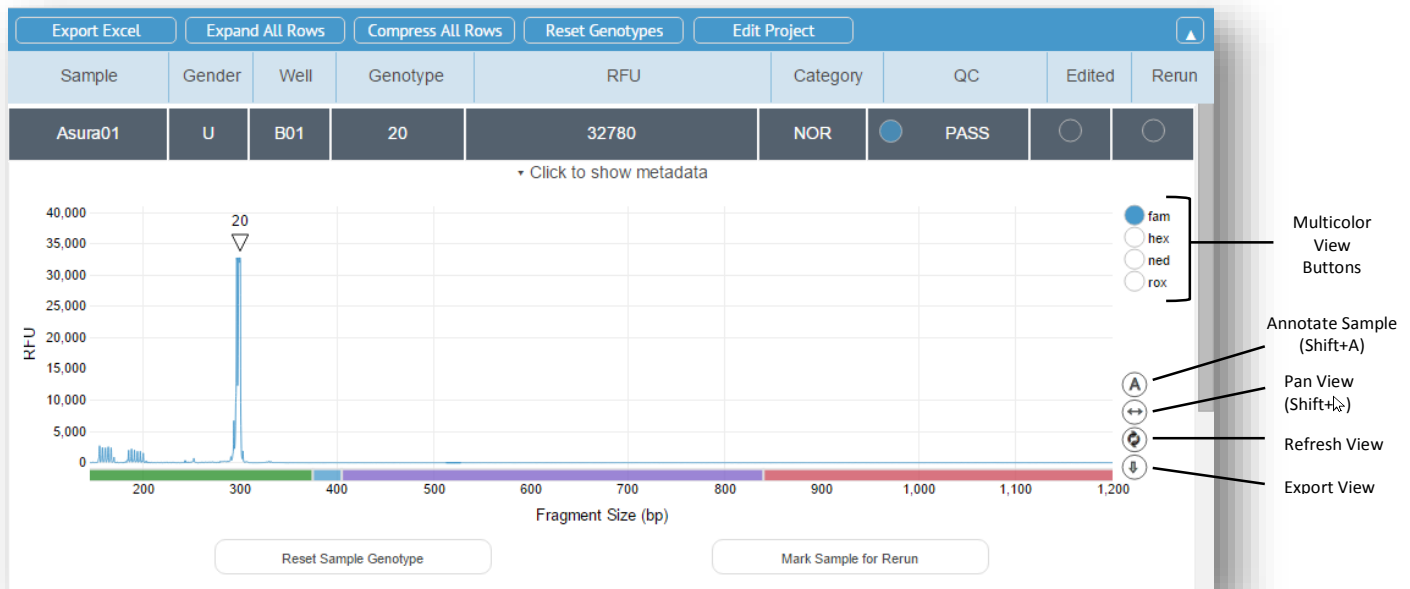
QC Name	QC Abbreviation	QC Type	Cause	Report	Manual Annotation	Resolution
Low Signal	LS	Hard QC (Fail sample)	Extremely low or undetected signal	No genotype annotation	Possible *sizing may be offset	Rerun sample
Ladder Error	LD	Hard QC (Fail sample)	Ladder is abnormal or missing	No genotype annotation	Possible	Rerun sample
Contamination	CT	Hard QC (Fail sample)	One or more contaminating peaks were detected	No genotype annotation	Possible	Rerun sample
Insufficient Sensitivity	IS	Soft QC (At Risk sample)	User defined Peak Calling Threshold is lower than sample LOD	Full genotype annotation	Possible	Proceed with caution – low level peaks may be missing

**Automated Sample Sorting:** The user can sort various rows (Ascending/Descending) by simply clicking each relevant column header. Samples and their respective CE traces will be viewed in the order they appear.

**Edit Project Button:** The user can edit the project by using the edit project button within the Project Results Table.

## VISUALIZATION OF CE TRACES USING THE EXPANDED SAMPLE VIEWING PANE

AmplideX® PCR/CE *FMR1* Reporter provides comprehensive CE trace viewing functionality. For visualization of all CE traces within the project click “Expand All Rows” in the top table bar. Accordingly, click “Compress All Rows” to convert back to the minimized table view.



From within the Project Results Table, click within the sample line to view the corresponding CE trace within an expanded viewing pane. To exit the expanded viewing pane and revert to the results table click again on sample line.

The user may use the following methods to allow panning and zooming within a CE traces:

	Mouse	Hot Key	Optional On-screen buttons
<b>Pan</b>	Click and Drag	+ Shift	“Pan” (Side Arrows)
<b>Boxed Zoom</b>	Click and Drag	+	-
<b>Axis Zoom</b>	Click and Drag on Axis	+	-
<b>Snap back to full view</b>	-	-	“Refresh”
<b>Incremental zoom-out</b>	Double click	-	-

**On-Screen Buttons:** Each CE trace view pane includes a set of glyph buttons to allow multiple pointing device functionalities. These buttons can be toggled on/off and have a green indicator when active.

**Sample Metadata:** All sample metadata is retained and can be viewed within the expanded view pane by expanding the “Click to show metadata” arrow; a second click will collapse the metadata view.

**Multicolor View:** AmplideX® PCR/CE *FMR1* reporter supports viewing of CE traces in either one or all combinations of the four-color dye set (FAM, HEX, NED, ROX). To add or remove color channels from the viewable trace use the color buttons located on the right side of the profile.

**Export Single Trace:** User may export single trace files as .PNG images using the “Export” button (downward arrow).

**Reset Sample Genotype:** Use this button to revert manual sample annotation back to original automated annotation.

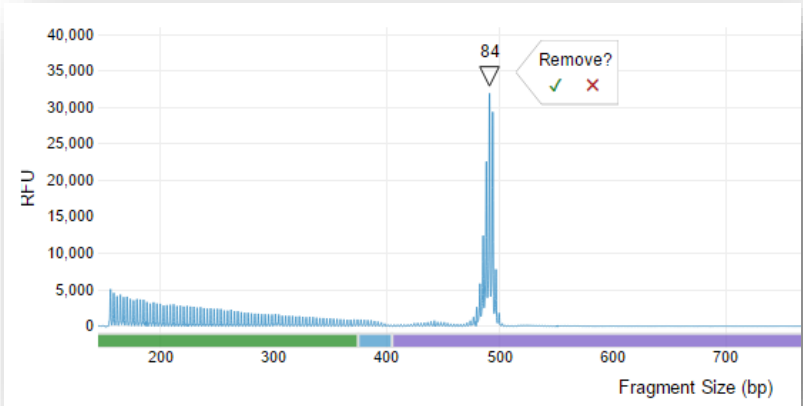
**Mark for Rerun:** User may elect to flag additional samples based on manual interpretation by clicking on the “Rerun” button for a given sample. Rerun flags will automatically transfer into table and exported report.

**MANUAL ANNOTATION OF CE TRACES**

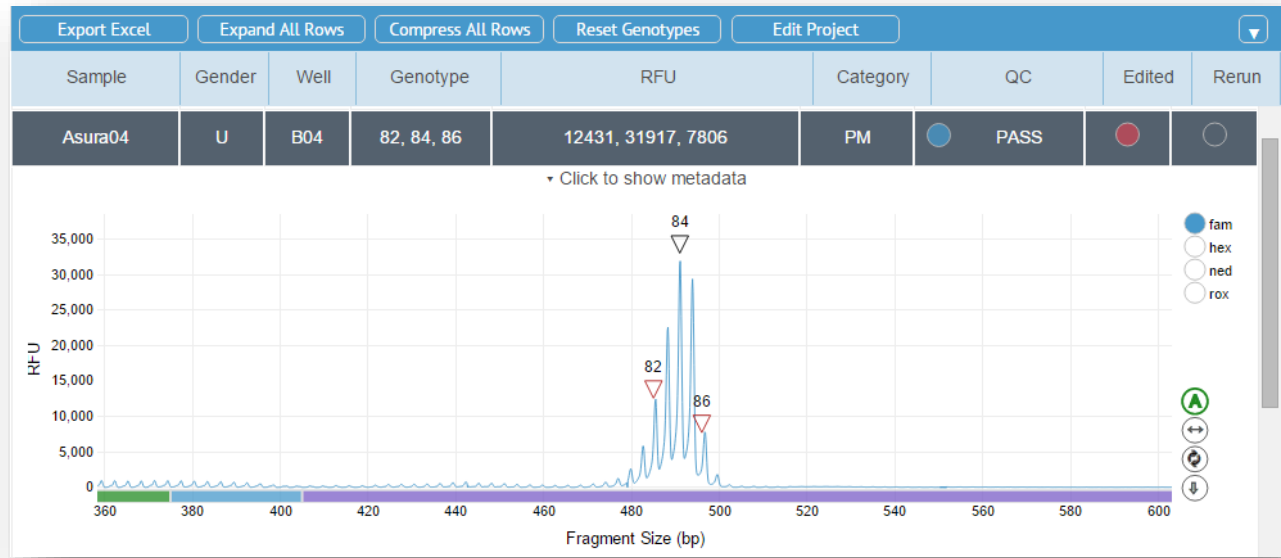
AmplideX® PCR/CE *FMR1* Reporter is designed for automatic trace analysis and annotation, however manual trace annotations are enabled as well.

**Remove Peak Annotation:** Click on peak to open the “Remove” dialog box and then click the checkmark.

**Note:** Any change to the automated analysis of a sample will be recorded in the “Edited” column (red indicator)



**Add Peak Annotation:** To activate the “Add peak” functionality for selected pointing device (e.g. mouse or trackpad), either click the “Annotate” button on the side of the pane, or hold down the [Shift + A] keys together with peak selection clicks. The software will highlight user Annoteselected peaks with a red arrow and a CGG repeat



number will be automatically assigned in both the trace image and the results table.

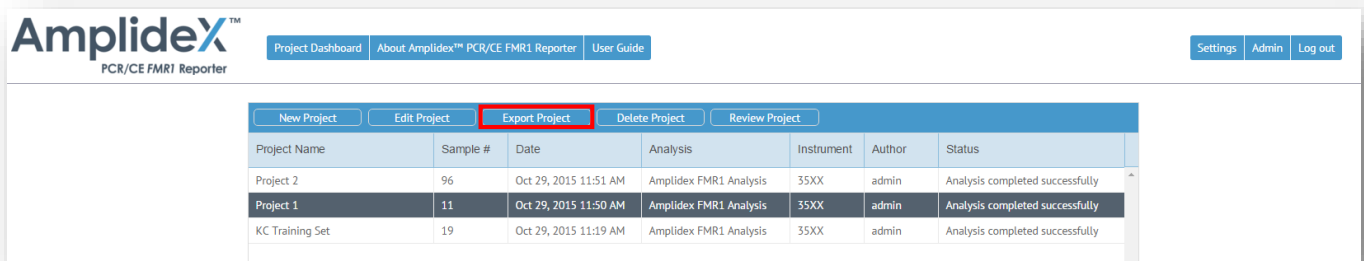
**Reset Genotypes:** Use the “Reset Genotypes” button on the top bar to revert all project annotation back to original annotation.

**Rerun Indicator:** Samples that failed QC will be flagged automatically for rerun using the red “Rerun” indicator. User may elect to flag additional samples based on manual interpretation by clicking on the “Rerun” indicator for a given sample. Rerun flags will automatically transfer into exported report.

## Project Export

It is recommended that after analysis projects are exported, archived and removed from the project dashboard on a regular basis for preserving data in long term storage.


- In the project dashboard, click to select an analyzed project of interest. When selected, click the “Export Project” button at the top of the user dashboard.



- An Excel file containing all analysis information will be exported to the browser default folder. This file can be saved or used for integration into laboratory information management systems (LIMS)

	A	B	C	D	E	F	G	H	I	J	K
1	# Peak Calling Threshold: 0.05										
2	# Showing All Peaks: YES										
3	# Sensitivity Control: MISSING										
4	# Processed Control: MISSING										
5	Sample	Gender	Well	Genotype	RFU	ROXQC	Contamin	SignalMag	Sensitivity	Edited	Rerun
6	Asura01	U	B01	20	32780	PASS	PASS	PASS	PASS		
7	Asura02	U	B02	30	32712	PASS	PASS	PASS	PASS		
8	Asura03	U	B03	200	1236	PASS	PASS	PASS	PASS		RERUN
9	Asura04	U	B04	67 84	764 31917	PASS	PASS	PASS	PASS	EDITED	
10	Asura05	U	B05	22 110 11	32617 105	PASS	PASS	PASS	PASS		
11	Asura06	U	B06	41 45 51	8362 2026	PASS	PASS	PASS	PASS	EDITED	RERUN
12	Asura07	U	B07	17 112	31922 490	PASS	PASS	PASS	PASS		RERUN
13	Asura08	U	B08	200	2523	PASS	PASS	PASS	PASS		
14	Asura09	U	B09	30 32	32617 326	PASS	PASS	PASS	PASS		
15	Asura10	U	B10	30 200	32617 475	PASS	PASS	PASS	PASS		
16	Asura11	U	B11	30 56	32624 326	PASS	PASS	PASS	PASS		

## Uninstalling AmplideX® PCR/CE *FMR1* Reporter

 **Warning:** all user data including analysis projects and AmplideX® PCR/CE *FMR1* Reporter users, passwords and credentials will be deleted during uninstallation. Export any analysis projects prior to uninstalling the software.

### Windows 7 Instructions

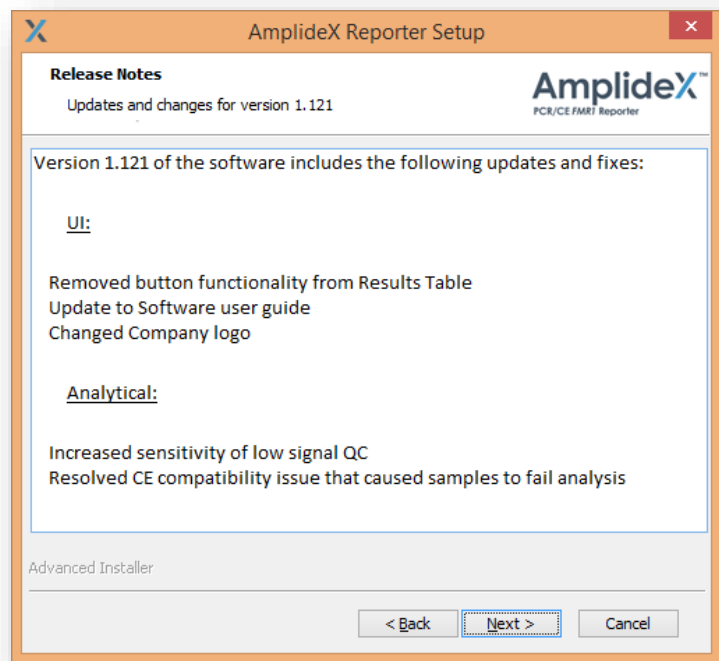
- Within your desktop control panel, navigate start – Control Panel – Programs & Features. Select “AmplideX® PCR/CE *FMR1* Reporter” from the program list and double click.
- A pop up window will ask if you are sure you want to uninstall. Click “Yes”.

### Windows 8.1 Instructions

- Open the control panel and select “Uninstall a Program” from the “Programs” group.
- A list of installed programs will be presented. Select “AmplideX® PCR/CE *FMR1* Reporter” from the list and click “Uninstall”.
- A pop up window will ask if you are sure you want to uninstall. Click “Yes”.

## UPDATING THE SOFTWARE

Software updates will be deployed as necessary and can be installed as described above. Take note of the Release Notes dialogue box presented after the welcome screen during a given software update installation. Release notes will provide details about the nature of updates to the software and will be parsed among the following categories: User Interface (UI), Analytical, Export, and Other.





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**AmplideX® PCR/CE *FMR1* Reporter**

**PC-0261**

**Effective Date: 2015-12-16**

