FastFinder Analysis PCR setup

How to create an Analysis PCR setup file, version 2.1

Supported from FastFinder Analysis 4.6

Purpose of this document

This document describes the format of the PCR setup file that can be provided next to the PCR experiment file to add additional metadata (e.g. sample and target names) and to automate the plate setup, as well as how it can be used in FastFinder. Additionally, some examples are provided.

PCR setup file format

The PCR setup file is a .CSV file that is renamed to .pcrsetup with a header, an empty row, and setup information that can be configured either per target (Option 1) or per well (Option 2).

Header

Line	Description
FastFinder PCR setup*	Fixed file type identifier
Version,2.1*	Version of the PCR setup file, i.e. 2.1
Date,YYYYMMDDhhmmss*	Experiment date, in the format YYYYMMDDhhmmss: - YYYYMMDD: year, month in 2 digits, day in 2 digitis, e.g. 20220308 - hhmmss: hour, minutes, seconds, e.g. 110256 Resulting in 20220308110256
OutputPlateBarcode,Barcode*	PCR plate barcode
InstrumentIdentifier,Identifier*	Instrument ID or serial number

* required fields

Option 1: setup per target

Per well, per target, the following information should be available:

Header	Description
RowIndex*	0-based row index of the well position, e.g. 2 for well C2.
ColumnIndex*	0-based column index of the well position, e.g. 1 for well C2.
TargetExternalReference	Must have a value for all rows having Reporter as DyeTask. This should correspond to the assay's target instrument reference (see Assays > PCR) to facilitate automated plate setup.
SampleName*	Sample name; must have the same value within the same well. In case the sample name is __EMPTY_WELL__, the well cannot be assigned.
SampleType*	PositiveControl, NegativeControl, Regular, QuantificationStandard; must have the same value within the same well.
SampleTypeName*	Name of the sample type, e.g. NTC; must have the same value within the same well. This should correspond to the assay's sample type name (see Assays > General) to facilitate automated plate setup.
ChannelExternalReference*	Must have a value for all rows. This should correspond to the assay's Reporter, Quencher or Channel (for passive reference dyes) instrument reference (see Assays > PCR) to facilitate automated plate setup.
DyeTask*	Reporter, Quencher or PassiveReferenceDye
Barcode*	PCR plate barcode (same one as in the header)
ReplicatesGroup*	Numeric value indicating which wells should be considered as replicates from each other; must have the same value within the same well.
MixExternalReference	Can be empty; must have the same value within the same well. This should correspond to the assay's Mix instrument reference (see Assays > PCR) to facilitate automated plate setup.

* required fields

Option 2: setup per well

Per well per dye, the following information should be available:

Header	Description
RowIndex*	0-based row index of the well position, e.g. 2 for well C2
ColumnIndex*	0-based column index of the well position, e.g. 1 for well C2
TargetExternalReference	Can be empty if MixExternalReference is not empty.
SampleName*	Sample name. In case the sample name is __EMPTY_WELL__, the well cannot be assigned.
SampleType*	PositiveControl, NegativeControl, Regular, QuantificationStandard
SampleTypeName*	Name of the sample type, e.g. NTC. This should correspond to the assay's sample type name (see Assays > General) to facilitate automated plate setup.
ChannelExternalReference*	Must have a value for all rows. This should correspond to the assay's Reporter, Quencher or Channel (for passive reference dyes) instrument reference (see Assays > PCR) to facilitate automated plate setup.
DyeTask*	Reporter, Quencher or PassiveReferenceDye
Barcode*	PCR plate barcode (same one as in the header)
ReplicatesGroup*	Numeric value indicating which wells should be considered as replicates from each other.
MixExternalReference*	This should correspond to the assay's Mix instrument reference (see Assays > PCR) to facilitate automated plate setup.

* required fields

Using PCR setup files in FastFinder

In order to be linked to each other, the setup file should have the same name as the PCR experiment file, e.g. *Filename*.pcrsetup for *Filename.eds*. Both files or multiple PCR experiment files and their corresponding PCR setup files need to be compressed into a .zip package that is renamed to a .pcrexperiment file. It is possible to upload .pcrexperiment files via scripts to import/analysis or upload it directly in FastFinder Analysis.

Examples

Setup per target

An assay with one mix `M1`, with 2 targets (`Target1` in `FAM` and `Target2` in `Cy5`) and `ROX` as passive reference dye.

```
FastFinder PCR setup
Version,2.1
Date,20220228111005
OutputPlateBarcode,PCRPlate01
InstrumentIdentifier,12456

RowIndex,ColumnIndex,TargetExternalReference,SampleName,SampleType,SampleTypeName,ChannelExternalReference,DyeTask,Barcode,ReplicatesGroup,MixExternalReference
0,0,Target1,Sample01,Regular,Regular,FAM,Reporter,PCRPlate01,1,M1
0,0,,Sample01,Regular,Regular,ROX,PassiveReferenceDye,PCRPlate01,1,M1
0,0,Target2,Sample01,Regular,Regular,Cy5,Reporter,PCRPlate01,1,M1
0,1,Target1,PC,PositiveControl,PC,FAM,Reporter,PCRPlate01,2,M1
0,1,,PC,PositiveControl,PC,ROX,PassiveReferenceDye,PCRPlate01,2,M1
0,1,Target2,PC,PositiveControl,PC,Cy5,Reporter,PCRPlate01,2,M1
0,2,,__EMPTY_WELL__,,,,,,,
```

The plate setup describes a regular sample `Sample01` on A1, a positive control `PC` on A2 and a well A3 that cannot be assigned.

Setup per mix

A quantitative assay with one mix `MixReference`, with 3 dyes with the following `ChannelExternalReferences`: `465-510`, `533-580` and `618-660`. The assay also allows 3 replicates per `QuantificationStandard` (`QS1`, `QS2` and `QS3`).

```
FastFinder PCR setup
Version,2.1
Date,20220228111005
OutputPlateBarcode,ABC123
InstrumentIdentifier,12456

RowIndex,ColumnIndex,TargetExternalReference,SampleName,SampleType,SampleTypeName,ChannelExternalReference,DyeTask,Barcode,ReplicatesGroup,MixExternalReference
0,0,,QS1,QuantificationStandard,QS1,465-510,Reporter,ABC123,1,MixReference
0,0,,QS1,QuantificationStandard,QS1,533-580,Reporter,ABC123,1,MixReference
0,0,,QS1,QuantificationStandard,QS1,618-660,Reporter,ABC123,1,MixReference
0,1,,QS1,QuantificationStandard,QS1,465-510,Reporter,ABC123,1,MixReference
0,1,,QS1,QuantificationStandard,QS1,533-580,Reporter,ABC123,1,MixReference
0,1,,QS1,QuantificationStandard,QS1,618-660,Reporter,ABC123,1,MixReference
0,2,,QS1,QuantificationStandard,QS1,465-510,Reporter,ABC123,1,MixReference
0,2,,QS1,QuantificationStandard,QS1,533-580,Reporter,ABC123,1,MixReference
0,2,,QS1,QuantificationStandard,QS1,618-660,Reporter,ABC123,1,MixReference
0,3,,QS2,QuantificationStandard,QS2,465-510,Reporter,ABC123,2,MixReference
0,3,,QS2,QuantificationStandard,QS2,533-580,Reporter,ABC123,2,MixReference
0,3,,QS2,QuantificationStandard,QS2,618-660,Reporter,ABC123,2,MixReference
0,4,,QS2,QuantificationStandard,QS2,465-510,Reporter,ABC123,2,MixReference
```

```
0,4,,QS2,QuantificationStandard,QS2,533-580,Reporter,ABC123,2,MixReference
0,4,,QS2,QuantificationStandard,QS2,618-660,Reporter,ABC123,2,MixReference
0,5,,QS2,QuantificationStandard,QS2,465-510,Reporter,ABC123,2,MixReference
0,5,,QS2,QuantificationStandard,QS2,533-580,Reporter,ABC123,2,MixReference
0,5,,QS2,QuantificationStandard,QS2,618-660,Reporter,ABC123,2,MixReference
0,6,,QS3,QuantificationStandard,QS3,465-510,Reporter,ABC123,3,MixReference
0,6,,QS3,QuantificationStandard,QS3,533-580,Reporter,ABC123,3,MixReference
0,6,,QS3,QuantificationStandard,QS3,618-660,Reporter,ABC123,3,MixReference
0,7,,QS3,QuantificationStandard,QS3,465-510,Reporter,ABC123,4,MixReference
0,7,,QS3,QuantificationStandard,QS3,533-580,Reporter,ABC123,4,MixReference
0,7,,QS3,QuantificationStandard,QS3,618-660,Reporter,ABC123,4,MixReference
0,8,,QS3,QuantificationStandard,QS3,465-510,Reporter,ABC123,5,MixReference
0,8,,QS3,QuantificationStandard,QS3,533-580,Reporter,ABC123,5,MixReference
0,8,,QS3,QuantificationStandard,QS3,618-660,Reporter,ABC123,5,MixReference
```

The plate setup describes 3 quantification standards `QS1` on A1, A2 and A3 that are considered as replicates, 3 quantification standards `QS2` on A4, A5 and A6 that are also considered as replicates, and 3 quantification standards `QS3` on A7, A8 and A9 that are considered as 3 separate quantification standards for `QS3` (see different `ReplicatesGroup` enumeration).