QuantStudio™ 3D Digital PCR System
Data Analysis – AnalysisSuite™ software
Objectives

• Set up analysis studies
• Interpret the results from a digital PCR experiment
QuantStudio™ 3D and AnalysisSuite™ Software

- Image Analysis Software
- Project-based analysis: Combine chips into a project
  - Ability to load up to 100 chips in a project
  - Relative & Absolute Quantification Modules

- Login to Lifetech.com to access AnalysisSuite™ software from the QuantStudio™ 3D system webpage
  - No need to store huge data files on your desktop
  - Hosted on secured Amazon® Server through Lifetech.com
  - Optimized for Chrome™ browser on Mac® OS or PC
How do I access AnalysisSuite™ software

• Final URL: https://dhap.apps.invitrogen.com/quantstudio3d
• All users **must create a new login**, or use pre-existing (validated) Lifetech.com account username and password
• Case sensitive
• Make sure to use **Google® Chrome™ browser**

• Software Downloads Page on Lifetech.com:
  • Firstly login into the webpage
How to Login into AnalysisSuite™ software

• Link will be available on the AnalysisSuite™ Webpage or on Software Downloads in Technical Resources Area:

Use existing Lifetech.com ID

New Users Register Here
Relative Quantification (RQ) Module

• Options for Copy Number Variation and Ratio Analysis:
  • CNV: Enables copy number variation and dual reporter analysis with use of a reference gene (VIC® dye).
  • Taget/total ratio analysis
  • Low fold detection: Gene Expression
Absolute Quantification (AQ) Module

• Ideal for:
  • Viral Load Detection,
  • NGS Library Quantification
  • Generation of Standards and Controls
Software Interface

QuantStudio™ 3D AnalysisSuite™

Current version: 1.0.1-01B11

Relative Quantification
This application allows you to estimate the quantity of a genetic target relative to a reference using digital PCR. Applications include copy number variation and detection of small fold change differences.

Absolute Quantification
Digital PCR-based quantification without the need for standard curves. Applications include viral load determination, quantification of next-generation sequencing libraries, and generation of standards and controls.

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Your profile

- For changing Password and Security Questions
Create a project

- After selecting a type of Quantification (RQ or AQ)...
- You can Create, Export, Import and Delete Projects
Create a Project

- Software will launch a pop-up window in which you should introduce a Project Name
Import Data

- **Import from local source** if the .eds files are accessible from the same computer

- If you have Chip files saved to your cloud account they will appear in the **Available to import from cloud account** option

![Import Data Interface](image-url)
Importing Chips

- Up to 100 chips can be imported
- Name list will appear in the bottom right hand corner
- You can delete chips from here
Define Chips for RQ Study

- Sample Name, FAM™ Assay name and dilution are included here in order to compile the analysis
- Just click in the field and type
Define Chips for RQ Study

- Can assign settings to multiple chips
Define Chips for RQ Study

• RQ always **assumes** two dyes

• BOTH dyes are always used. Setting the target dye only specifies which one is in the numerator of the target/total value.

• You can also include Assay name in the Assay Paragraph
Loading Quality - Using the Chip View: Color by Quality

**Loading Movement**

- **Chip view**
  - **Histogram view**
  - **FAM**
    - Count: 12,989
    - Fluorescence: -2876.74 to 2500.00
    - Adjusted threshold: 4802.03
  - **VIC**
    - Count: 9742
    - Fluorescence: -5911.11 to 25658.42
    - Adjusted threshold: 3337.22

**Adjustment of QV to filter data points to be included in calculation**

- **Quality threshold**
  - Data points above threshold: 0.5 → 17,456 of 17,682

**Number of wells included in calculation**

**Total Number of filled wells**
Review Quality in RQ by Quality

- Option to inspect each chip and determine which data points to include in the analysis
- Chip View Thumbnails available to see all Chips at the same time
Review Quality in RQ by Calls

- Using “color by Call” view to look for uniformity across chip
- Chip View Thumbnails available to see all Chips at the same time
Review Calls for RQ

- Viewing calls in 2D Space for Dual Reporter
- Assign positive or negative calls on a selected plate for each detection channel
- Simple way to view different genotypes
See Results in RQ Study

• In **Show Settings**, Confidence Level and Desired Precision can be established in order to analyse the data.

• **Plot Color** could be set up as Sample, Assay or User Defined.

• Data can be displayed in either Results Plot or Replicate Table View.

• **Target/total** column relates ratio between the target dye versus VIC® dye.
Define Chips for AQ Study

- Sample Name, FAM™ and VIC® Assay names and dilution are included here in order to complete the analysis.
- Just click in the field and type.
- If one sample name is introduced for several chips with same assay names, those chips are considered as replicates.
- Assign Settings to multiple chips is also available as in RQ projects.
Review Quality in AQ by Quality

- **Histogram view** is making an End Point Read Digital Output in order to determine positive and negative reactions.
- Bimodal distribution is expected.
- X-axis denotes florescence intensity.
- Black line denotes the starting point (0 value).
- Red line denotes the median of the two populations.
Review Quality in AQ by Calls

- Using “color by Call” view to look for uniformity across chip
- Select a chip to view and adjust the quality threshold until you have an acceptable balance of data quantity and quality.
- Adjust the fluorescence threshold to maximize the call accuracy.
In **Show Settings**, Confidence Level and Desired Precision can be established in order to analyse the data.

**Plot Color** could be set up as Sample, Assay or User Defined.

Data can be displayed in either Results Plot or Replicate Table View.

Target/total column relates ratio between the target dye versus VIC® dye.

Results shown as **Copies/μl** in the below table.
AnalysisSuite™ Software Help

- Provides guidance on PCR Mix Dilution for follow up experiments if desired target precision is not met.
• Software demo
Export

- Export creates a .csv file.
- All data are exported in a fixed order and format regardless of the sorts and column organization in the tables shown here.
Precision Definition

• Is defined to capture distinguish-ability between two concentrations with a certain confidence

• A precision of X % means you can distinguish between A and B copies/μl as long as: B/A > 1 + X/100, at 95% Confidence

\[ \text{Precision} = \frac{\max(|\lambda_{\text{upper bound}} - \lambda|, |\lambda_{\text{lower bound}} - \lambda|)}{\lambda} \]
Flags in AnalysisSuite™ Software: For Chip Inspection

- AnalysisSuite™ software only displays **Red** and **Yellow** Flags from touchscreen
- By default, FAM™ dye is populated in AQ, hence Flag is for FAM™ (if VIC® Dye was run, then need to change define chip settings)
- If **dual reporter**, then **WORST** flag is shown of two dyes, user should inspect chip
- “**Broken Flag**” indicates that automated analysis has been manually over ridden
Flag Definitions in TouchScreen

- TouchScreen displays **Red**, **Yellow**, **Green** Flags for each Dye.

  - Re-scan or repeat the experiment, visually inspect chip, **not worth bringing in AnalysisSuite™ software**
  - Inspect chip **in AnalysisSuite™ software**
  - Chip passed all screening criteria

![TouchScreen Display](image_url)
Questions
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