Full automated qPCR for small molecule screening of RNA targets

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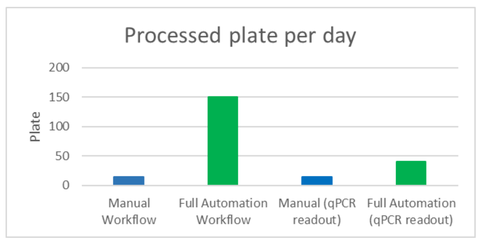
1-Introduction

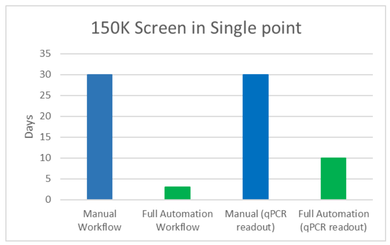
qPCR is a quantitative and reliable method to determine endogenous gene expression and alternative splicing levels. To investigate signalling pathways inducing gene expression or splice modifiers we tend to use indirect assays (i.e. reporter assays) for high throughput screening since they are easier to automate and miniaturize. However, many false positive hits are inherent features of these types of screens.

In order to be able to screen on the actual endogenous RNA target on a large number of compounds (>50K) we have optimized and automated a qPCR screening workflow and data analysis.

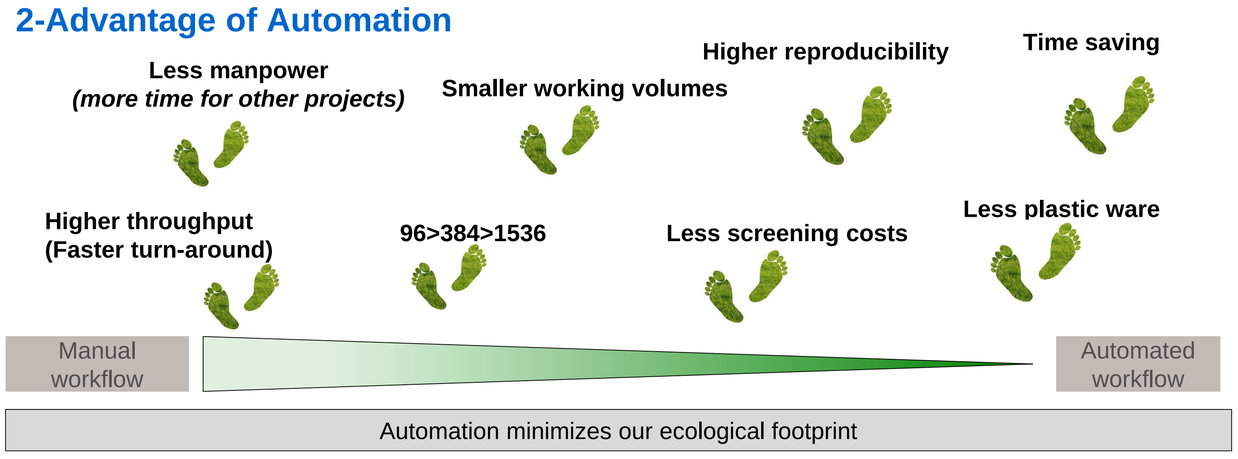
We have established two different workflow for the pre-qPCR steps, one in a automated manner for regular profiling and small screens for up to 15K compounds/day in single point, and one using a fully automated HTS system for up to 50K compounds/day in single point (150 plates/day). For the qPCR step, the capacity is limited by the number of readers available on the system, whereby up to 20 plates can be processed per machine per day (7K compounds/day). In our current workflow we included enough readers redundancy to process up to 40 plates/day. Throughput format from manual is only 14 plates/day.

We are currently expanding integrating the qPCR machines fully into the robotic system to reduce hands-on time. With this we plan to expand into new target spaces and use cases after having conducted these successful screens.





2-Advantage of Automation



Automation minimizes our ecological footprint

3-Automation/Equipment

Fully automated cell seeding,_treatment and_lysis

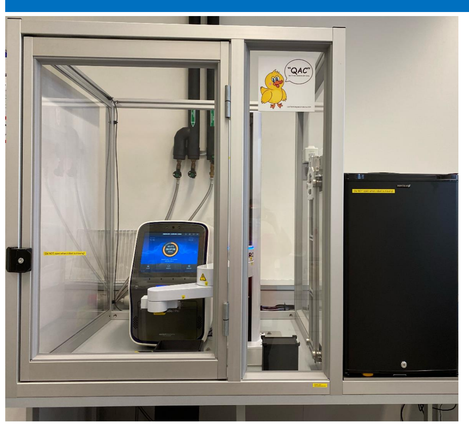
Automated System

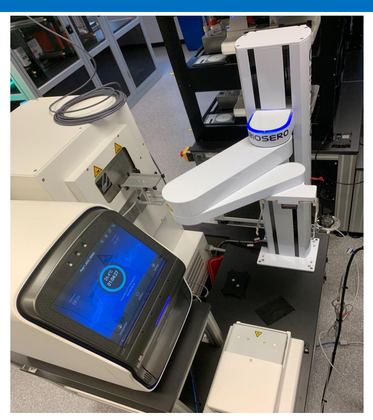
Automated Pipettor

Cell seeding	Treatment	Lysis	Transfer
			
	2 x Echo	1 x Blue-washer	3 x Certus

2x Pipettor 384 +1536, Shaker and Prime

Fully automated qPCR (QAC1 and QAC2)





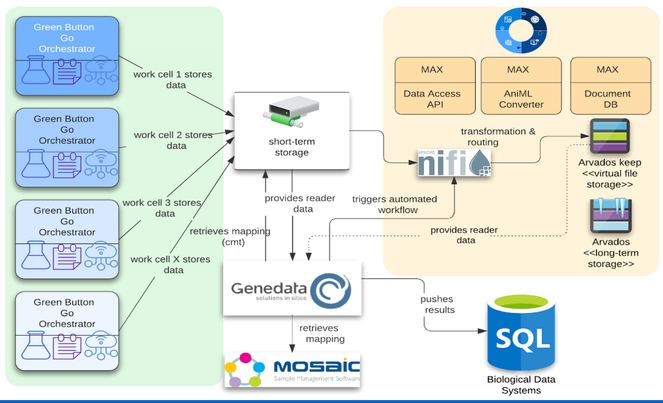
QuantStudio 7 Pro Transfer unit Cooling unit

Automation with different robotic systems to increase the throughput (nearly all steps are now fully automated)

Capacity HiRES system (semi automated or automated seeding) up to 150 plates per day

Capacity QAC 1 and 2 system: up to 40 plates per day

4-Automated raw data transfer



Fully automated capturing of raw data and QC ensures FAIR data and reduces errors in data transfers including automated archiving.

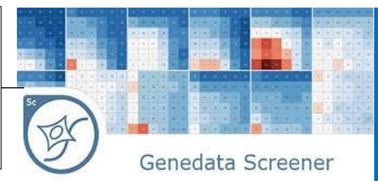
Direct and quick data transfer, no user intervention

5-Optimized Data Analysis

Compound ordering (384/1536 well plates)

Compound mapping

Input data from qPCR machine

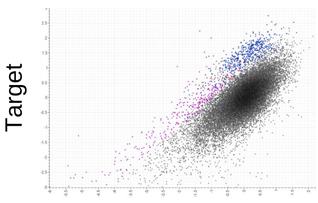


SAR (Structure–activity relationship) Analysis

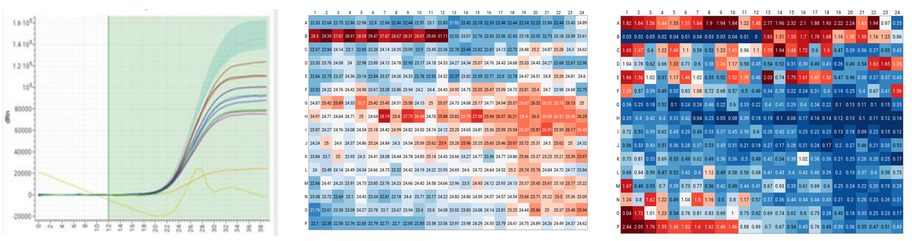
D360

Quality control of each plate
Overview of raw data values, plate patterns
Calculations (CT, ddCT, fold change)
Mark or mask toxic compounds (HKG)
Upload to Roche proprietary data warehouse or export of the final dataset

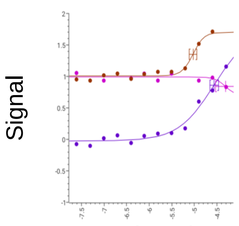
SP hit selection



Raw dRN values → Raw CT values → Fold change calculated



DR fitting /EC50 calculation



Raw dRN values are imported into screener to determine the CTs over all plates with the same threshold per target and to check outliers.

Complete analysis package for screening and profiling in one software

6-Assay Setup

Assay setup checklist

- [x] Good knowledge of the gene of interest?
- [x] Good cell line which highly expresses the GOI? —If possible frozen cellbank---
- [x] Designed a couple of primers and probes to select finally the best combination?
- [x] Good housekeeper selected?
- [x] GOI controls (plasmid, gene block) ?
- [x] Right kit selected?
- [x] Decision about 1 step or 2 step protocol made?
- [x] Amount of cells per well and template input volumes selected for 1 step (lysate) or 2 step (lysate/cDNA)?
- [x] Right fluorochromes selected if heading towards a multiplex assay?
- [x] Primer probe ratios tested? (1:1, 2:1 or 3:1?)
- [x] Titrations on target source (lysate or cDNA) done?
- [x] Efficiency calculation made in singlex and/or multiplex?
- [x] gDNA contamination check done (gDNA primer sets) ?

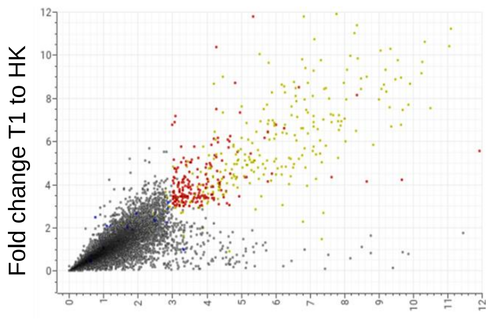
7-Screening Examples

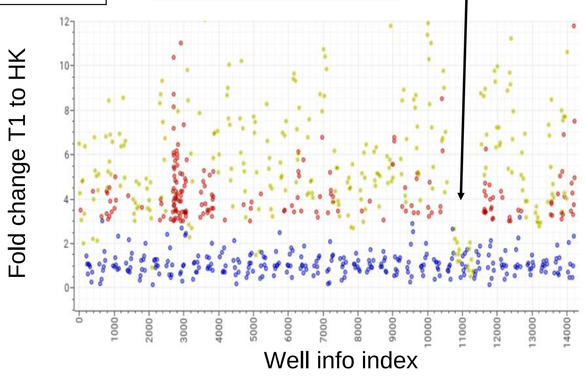
Cmpd Library	Cmpds	Single Point / Dose Response
Screen 1	11k	SP
Screen 1	300	DR in triplicates
Screen 2	33.000	SP
Screen 2	1400	SP in triplicates
Screen 2	28	DR in triplicates
Screen 3	14.000	SP
Screen 3	1100	SP in triplicates
Screen 3	250	DR in triplicates

ddCT		Screen 1				Screen 2				Screen 3			
		T1 to HK		T1 to T2		T1 to HK		T1 to T2		T1 to HK		T1 to T2	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
■	NT	0	0.7	0	0.7	0	0.7	0	0.7	0	0.5	0	0.4
■	ST	2.5	0.9	2.6	0.7	1.9	0.5	1.9	0.5	1.6	0.6	1.6	0.6

■	Stimulator control (ST)
■	Hits
■	Neutral control (NT)
■	Compound

T1=	Target 1
T2=	Target 2
HK=	Housekeeper
M=	Median





Hit rate for the screens: <1%, confirmation rate was here 50%