High-throughput RT-qPCR for small molecule screening assays in advanced cellular models

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Introduction: emergence of advanced models for drug screening

Pharmaceutical development is risky

- $1 billion and 12.5 years to launch a technically successful drug
- 5% average success from first toxicity dose to market approval

To increase effectiveness of drug discovery, new disease-relevant cellular models have been developed

- Patient-derived models: Differentiated iPSCs, primary fibroblast, …
- 3D models: spheroids, organoids, tumoroids, …
- Other complex culture systems: co-culture and organotypic systems

Quantitative reverse transcription PCR (RT-qPCR) represents a method of choice to perform gene expression assays in the most disease-relevant cellular models

- No cell engineering needed / looking at endogenous targets
- Highly sensitive technique / mRNAs or other transcripts (miRNAs, snRNAs, …)
- Suitable for both screening and profiling

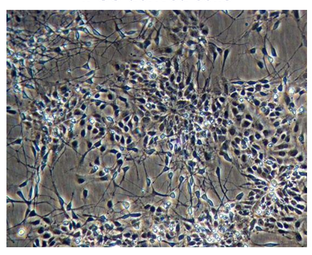
Evotec HTS RT-qPCR plaform has been designed to perform screening campaigns in the most advanced cellular models

- State-of-the-art cell generation and culture platform
- Cost effective approach thanks to an accelerated and optimized PCR protocol
- High-throughput: 384w and 1536w plate formats / up to 400k compound screening

RT-qPCR-based screening in advanced cellular models

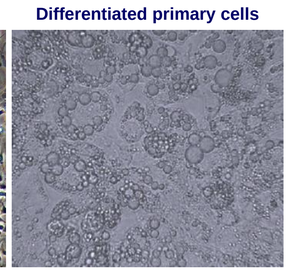
Advanced 2D models

iPSC-derived cells



iPSC-derived neurons

Differentiated primary cells



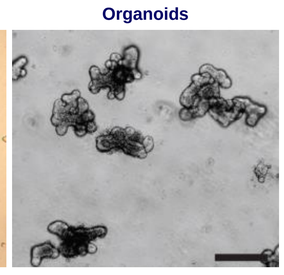
Adipocyte culture

3D models

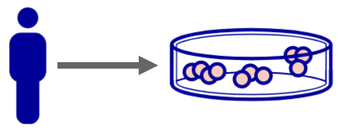
Spheroids

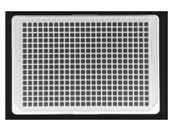
HepG2 sheroids

Organoids

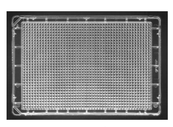


Colon organoids





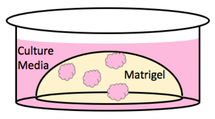
384w plates



1536w plates

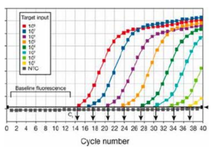
Cell line generation platforms

Scale up and Miniaturization



Culture optimization
Matrigel scaffold

Low attachment Spheroid microplates (Corning)



No cell engineering needed to to perform RT-qPCR assay

Screening can be performed directly in the most relevant possible cellular context (endogenous target)

Track records and recent developments

Track records: HTS campaigns performed at Evotec Toulouse on RT-qPCR platform

- Multiple screens (up 400k compounds) in conventional cellular models
  - 2 targets or 1 target + 1 reference gene
- Multiplex screen: 50k / 4 target genes + 2 reference genes
- Screening in advanced 2D models (primary cultures, differentiated cells)
- 200k compound screen targeting alternatively spliced variants

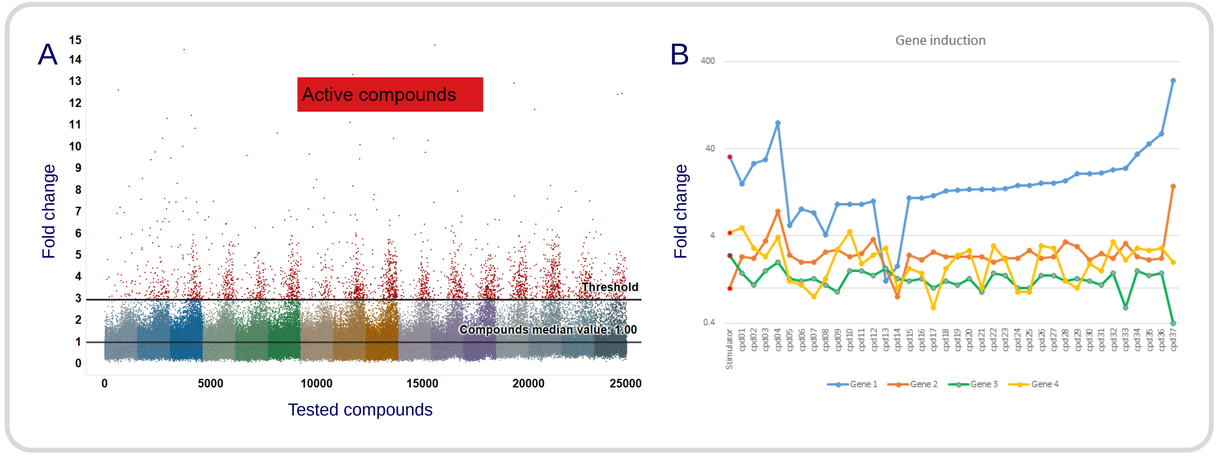


Figure 3: Representative hitID results obtained a Evotec Toulouse using RT-qPCR platform. (A) Gene-induction screen data (fold change vs control) for 25,000 cpds out of a 400,000 cpd screen. Cpds with Fold change above 3 were selected as hits. (B). Representative data (fold change vs control) from a 50k screen, looking at 4 target genes (+ reference genes).

Recent developments of our cell culture and RT-qPCR platforms

- Miniaturization and automation of 3D culture models (spheroids and organoids) down to 384w format: growth in spheroid microplates or matrigel-based systems
- Optimization of cell micro-environment, including coating, scaffold and growth factors
- Profiling of tumor and stem cell markers in patient-derived organoids using RT-qPCR
- RT-qPCR pilot screens have been performed on 3D models

Fully automated RT-qPCR platform at Evotec Toulouse

Regular workflow: Two-step RT-qPCR (RT and qPCR in two separate reactions)

Cell lysis → **Column-based RNA isolation** → **cDNA production (RT step)** → **qPCR amplification**

Accelerated One-step RT-qPCR

Cell lysis → **cDNA production + qPCR amplification**

- Same sensitivity and specificity
- Expeditive process / less error prone
- Low volumes (2uL) / Lower cost per sample
- Highly compatible with HTS automation

Figure 1: We have optimized our RT-qPCR HTS protocol to provide maximal performance and throughput while reducing assay volumes, costs and risk of errors. RT-qPCR is performed directly from cell lysates as a one-step reaction.

ET3 platform (Evotec, Toulouse) – Fully automated plate management system

Throughput of up 20 plates/day in 384-well plate format and up to 20 plates/day in 1536-well plate format

Figure 2: Established screening processes for 384- and 1536-well plate assays. Automated screening system consists of incubators, washer/dispensers, Echo550 acoustic liquid handler, real time thermocyclers 384w and 1536w.

RT-qPCR screening workflow

Cell line generation platform
patient-derived models
3D models

RT-qPCR assay development
Differentiation and SC markers
Target gene(s) for screening

Optimization scale up

Model characterization
PCR reaction validation

Assay miniaturization and robotic adaptation
384w or 1536w format

RT-qPCR primary screening
up to 400k compounds

Up to 80k compounds per week in 1536w

duplex or triplex reactions

n=1

Hit confirmation

n=3

Dose-response evaluation

QC

MedChem review

Small molecule screening libraries
- Evotec collection (450k compounds)
  - 20k fragments
- Aptuit collection (350k compounds)
- Sanofi collection (750k compounds)

Molecular profiling
more genes, TLDA …

Counter-screen

Secondary assays
protein level, HTRF

Integration into Evotec drug discovery platform
Hit to Lead – Lead optimization – INDiGO

Conclusion and Future challenges in drug screening

Upcoming challenges in cell model generation for HTS

- Reproducibility, especially with 3D models (difficult to obtain uniform organoids)
- Miniaturization and optimization of culture for long periods in small volumes (evaporation, media changes, compound addition, …)

Upcoming challenges in gene expression screening

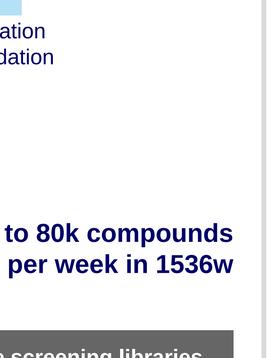
- More targets: triplex and more (384w format)
- Bundling with transcriptomics (full NGS, targeted-RNAseq, …)

RNAs are becoming attractive small molecule targets

- Non-coding RNAs, especially in oncology: miRNAs, snRNAs, …
- Pre-mRNA splicing of disease-causing genes
- RT-qPCR is highly suitable to quantify all kinds of transcripts

One of the main challenge for the next decade will be to find the right balance between model complexity, overall screening throughput and cost per compound

- Demand for very large small molecule screens has been steadily increasing
- In the same time, advanced cellular models require an extensive work prior to reaching the automated platform and miniaturization to 1536w format can be difficult



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