Transferring Evotec's Screening Library to AcoustiX Tubes: State-of-the-Art Technology at the Service of Increased Efficiency and Reduced Compound Consumption

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Overview

Evotec libraries transfer into AcoustiX tubes ®

Evotec is investing in the strategy, to transfer progressively Evotec's collection compounds into acoustic tubes (AT) to support our partner's screening needs.

Compounds will still be distributed from Master stock plates for Assay Validation (AV) and Primary Screen (PS) steps. However, for the Hit Confirmation (HC) and Hit Profiling (HP) phases, compounds will be prepared directly from AT.

This process will allow more flexibility in cherry-picking tubes for high-throughput screening (HTS) with preparation of Assay Ready Plates (ARP) directly from stock solutions. This streamlines the selection process and enhances the efficiency of drug discovery workflows.

Here we describe the rationale behind this strategic investment, highlighting the benefits in terms of cost, flexibility and productivity. Additionally, we will describe the different equipment and adjustments required to support this new process.

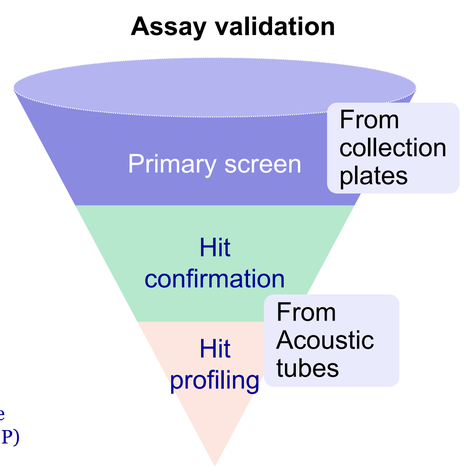

Figure 1. Evotec collection compounds distribution workflow. For Assay Validation (AV) and Primary screen (PS) collection compounds will be distributed from plates. Whereas for Hit Confirmation and Profiling (HC/ HP) compounds will be distributed from AcoustiX tubes ®.

Evotec Collection switching to AcoustiX tubes® : Rational & Gains

Rational

- ✓ **State of the art technology** adopted by pharmaceutical industry
- ✓ **Dedicated facilities** in Evotec Toulouse
- ✓ **Expertise in Evotec**
- → collections of several partners stored and distributed from AT

Gains

1. **Compound consumption** decrease
2. **Turnaround time** decrease
3. **Consumable usage** decrease
4. **Productivity** increase

Demonstrated capacity

- ✓ **Compound consumption** decrease → **Storage time** increase

Poster *(Cuadrado et al.*)* showing that no deterioration of compounds integrity or hydration when at -20 ºC, regardless of the number of free-thaw cycles and the repetitive use of Echo.

Figure 2. Capacity for AT process. Extension of the main building to host new labs, automated ECHO platform, new AT compatible store. Storage zone is an access-controlled area, equipped with flood and fire detection systems

Collection management KPIs	% Gain
Compounds consumption	65%
Turnaround time for compound reception	20%
Consumables	95%
FTE	25%

Table 1. Estimated percentage of gain of collection manage-ment Key Performance Indicators (KPIs) after switching Evotec collections to AT. Based on a comparison of a standard process of ARP-HC and ARP-HP from 2D-microtubes and HC and HP done on projects of a customer for which collection compounds are in AT.

Evotec library switching to AcoustiX tubes ®

Methods

New ChemBridge library preparation

- ✓ **2 copies** Evotec Toulouse and Hamburg; back-up sites allowing risks mitigation , reduced cherry pick time and costs of shipments
- ✓ **3 labware types** Echo Qualified 1536-wells Low Dead Volume plates, Echo Qualified 384 wells Low Dead Volume plates and AcoustiX ® tubes

ChemBridge library process design

- ✓ **Source plates** 384-well source plates with around 70µL at 8mM
- ✓ **Equipment** fully enclosed automated platform containing a *Beckman Biomek i7* workstation and *Azenta IntelliXcap™ Automated Decapper Recapper*
- ✓ **3 steps** by run of 16*384-well source plates to limit freeze-thaw cycles

Figure 3. ChemBridge library process design.

Direct "serialization" from AcoustiX® tubes

Process set-up for Evotec compounds, Dose-Response from AcoustiX tubes®

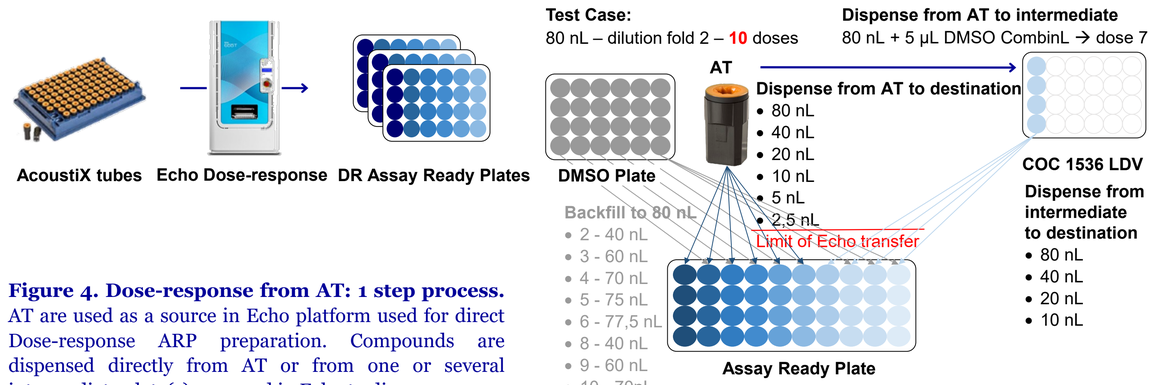

Figure 4. Dose-response from AT: 1 step process. AT are used as a source in Echo platform used for direct Dose-response ARP preparation. Compounds are dispensed directly from AT or from one or several intermediate plate(s) prepared in Echo to dispense one or several doses of a dilution series. DMSO plate is also used as a source to add DMSO during the dilution process.

Dose-response process for Evotec collections from AT set up

1. **Compounds consumption minimized**

✓ **Standard DR specifications**

→ 15nL<Volume<500nL/ up to 8 copies/ 11 doses/semi-log or demi/8mM (up to 6 intermediate microplates)

✓ **DR out of specifications**

→ adapt parameters (Echo software - EDR)

2. **Standard layouts** definition
3. **Reference (REF)** distribution **as regular compounds** (up to 2 in lines)
4. **Control and reference improved traceability**
5. **Transition period :** 2 serial dilutions methods

Dose-response from AT limitations & workarounds

Request	Limitation	Workaround
Multiple assays & different REF	Mosaic	REF in all destination ARP
REF and compounds in DR ≠ top concentration	Mosaic	• Script to update Mosaic import file • Mosaic upgrade
Consolidation of ARP for compounds from µplates and AT	Echo software (EDR)	2 sets of ARP (up to 1 additional plate per copy)

Biological validation: serialization "multi-step" vs "from AcoustiX tubes®"

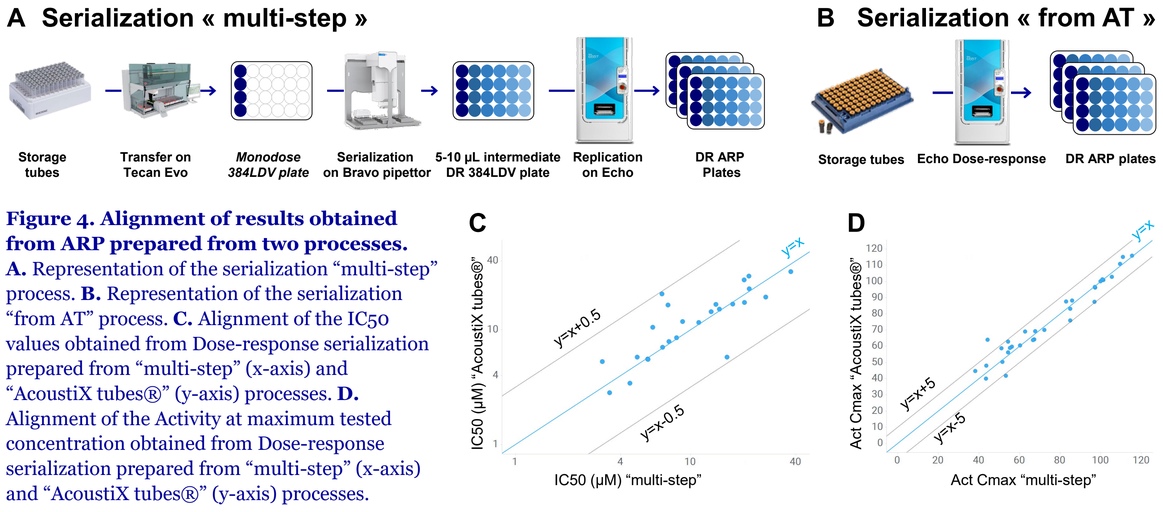

Figure 4. Alignment of results obtained from ARP prepared from two processes. **A.** Representation of the serialization "multi-step" process. **B.** Representation of the serialization "from AT" process. **C.** Alignment of the IC50 values obtained from Dose-response serialization prepared from "multi-step" (x-axis) and "AcoustiX tubes®" (y-axis) processes. **D.** Alignment of the Activity at maximum tested concentration obtained from Dose-response serialization prepared from "multi-step" (x-axis) and "AcoustiX tubes®" (y-axis) processes.

During the transition to AT period two methods of Dose-response preparation will be used *(Figure 4)*. A comparison of biological activities obtained with both process have been made on a group of compounds. A selection of 30 compounds have been tested in a cellular assay based on Nanoluciferase reporter system. 384-well format ARPs containing the compounds in 11-point Dose-response, with 2-fold dilution steps and 40µM top concentration, coming from both processes have been handled in the same assay run. The compounds have been chosen to reflect different ranges of activities and potencies and represent a high diversity of chemical structures. The results showed a great alignment, both in the activity and potencies, obtained from Dose-response analysis coming from the two processes, implying the biological validation of the Direct "serialization" from AcoustiX® tubes.

Example of benefits of AcoustiX tubes® for specific partners' workflows

	Implemented	Current process consumption (µL)	AT process consumption (µL)
Proteo./Transcripto. screens	YES (MD)	7	1.3
HT-HiBiT	YES (MD)	8	3.3
CRBN dep	Ongoing (DR)	8.5	0.7
nanoBRET	Ongoing (DR)	7	1.1
TOTAL		**30.5**	**6.4**

Table 2. Estimated compounds consumption for several assays before and after AT implementation. Current process is based on compounds being received, stored and processed from 2D-microtubes to prepare Dose-Response (DR) or Monodose (MD) ARP. Evotec decided to transfer the library into AT. This strategic move enabled up to 12-fold compounds consumption reduction depending on the assay.

Conclusion

This strategic investment in the AcoustiX tubes® technology enables Evotec to provide its partners the expected significant decrease in compound consumption, increased productivity, and a decrease in turnaround time to deliver ARP to the biology teams, increasing the speed data creation. The decrease in consumables usage has also been observed improving both the cost or the projects and the Green impact for our partners and for us.