Leveraging Sample Management Strengths to Support Biologics Research

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Poster Number: 2

Background

With the growing interest in Biologics within the drug discovery field, the Evotec Sample Management teams have been asked to offer their platforms and expertise to enhance throughput and timelines by establishing robust, high-quality processes in aqueous solvents.

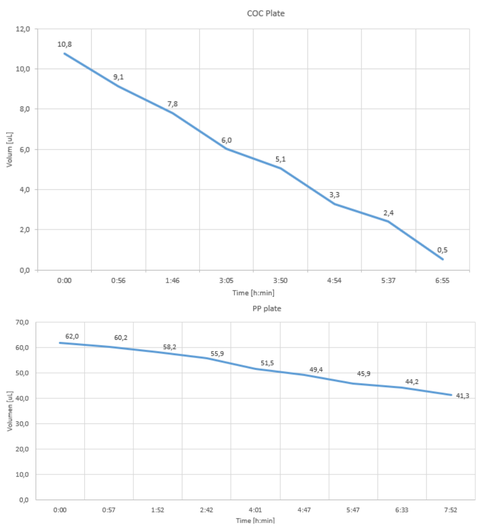
While extensive experience with DMSO-based solvents was leveraged, the significantly different properties of the water-based solvents raised new questions regarding the handling of source and destination plates at Sample Management.

To address these challenges, comprehensive tests were conducted, focusing particularly on evaporation and the establishment of a device verification process. This poster presents these processes and provides an overview of the results.

Replicate Aqueous Solutions with Echo

Based on a request to dispense more than 100 plates with sgRNA in water using the Echo 655T, questions arose regarding the stability of the concentration in the source solution due to evaporation during the process.

Our quality criteria: evaporation of solvent must not exceed 10%.



Water evaporation in Beckman COC Plate 001-12782 (low volume: max. 12µl)

- Loss of more than 15% solvent after ca. one hour (20% humidity in lab)
- Almost linear loss of solvent

→ Theoretical number of replicates in the range of our quality criteria: 6 (Assumption: 6 min/replicate)

Water evaporation in Beckman Polypropylene Plate 001-14555 (high volume: max. 65µl)

- Loss of 10% solvent after about 2.5h
- Almost linear loss of solvent

→ Theoretical number of replicates in the range of our quality criteria: 25 (Assumption: 6 min/replicate)

The limitation of working volumes in PP and especially COC plates, combined with evaporation, poses a significant constraint of the process.

Our recommendation:

- PP 001-14555 plates are favored for high number of replicates as the evaporation is not as quick, samples need to be available in high amount (dead volume of the plate in the Echo 15µl)
- COC 001-12782 plates should only be used for low number of replicates, suitable for costly samples (dead volume of the plate in the Echo 3µl)

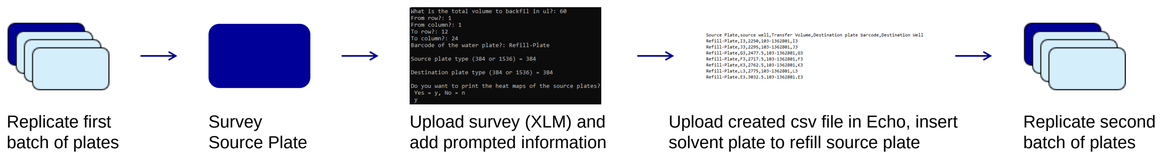
Water Refill Process

Since the issue of evaporation cannot be eliminated and a variability of the source solution's concentration is unacceptable, a process must be developed to counterbalance the effect.

A "Solvent Refill Process", where the plates are replenished to their theoretical volume has been developed for this purpose.

The core item of this process is a R script to calculate the volume of solvent to refill each well individually.

The process can be described as follow:




```
What is the total volume to backfil in ul?: 60
From row?: 1
From column?: 1
To row?: 12
To column?: 24
Barcode of the water plate?: Refill-Plate

Source plate type (384 or 1536) = 384

Destination plate type (384 or 1536) = 384

Do you want to print the heat maps of the source plates?
 Yes = y, No = n
 y
```

Figure 1: Input interface for the R script

- The process is only available for source plates with identical start - and dispensed- amounts.
- The script can refill full plates or selected areas
- The process is compatible with 384 or 1536 format

Example:

Starting Volume 65ul / Dispensed Volume 5ul

→ Targeted remaining volume to adjust to 60ul

- The output of the script is a CSV file which can be directly uploaded to the Tempo Software
- The solvent plate is tracked by barcode

```
Source Plate,source well,Transfer Volume,Destination plate barcode,Destination Well
Refill-Plate,I3,2250,103-1362801,I3
Refill-Plate,J3,2295,103-1362801,J3
Refill-Plate,G3,2477.5,103-1362801,G3
Refill-Plate,F3,2717.5,103-1362801,F3
Refill-Plate,K3,2762.5,103-1362801,K3
Refill-Plate,L3,2775,103-1362801,L3
Refill-Plate,E3,3032.5,103-1362801,E3
```

Figure 2: CSV file generated by the R script, detailing the transfer volumes and well locations

- We had to find a compromise between the efficiency and quality for water-based bulk replication with the Echo
- For this type of request, we worked in batches, the size of the batch being dependent on the plate type. The process has to be stopped after each batch to re-adjust the concentration of the source plate.
- This process is successfully in place at Evotec since 2023

Established Device Verification Process for Aqueous Solutions with the Echo

To establish an appropriate device verification process, a suitable dye is required. Tests were conducted to find the best possible dye. After a preliminary selection, the following three dyes were tested more intensively.

All Tests are done with a Infinite M200 Pro (Tecan) reader and the dye was solved in DPBS (1x), pH 7-7.3

Sulforhodamine 101:

- Not recommended to store aqueous solution more than 24h
- Linearity is not reproduceable and emission very close to the buffer

Not used further

Tartrazine:

- Absorbance detection is not compatible with our standard verification processes and not as sensitive.
- Harmful, toxic, allergenic and irritating

Not used further

Fluorescein sodium salt:

- pH-dependent fluorescence, ideal pH 7.4
- Low CV
- Working concentration with linear range 10µM +/- 50%

Our candidate

- A linear range of the dye is important because it allows for accurate and consistent measurements at different concentrations. This is crucial for obtaining reliable and comparable results in the QC process.
- Based on these tests, a QC process for the Echo was developed and established with Fluorescein

Establishment and implementation of the test

- Requirements for Device Verification Process
  - Test volume at Echo: 20nL (Minimum volume to reach the linear range with the stock solution)
  - Deviation accepted for accuracy is up to 10% and precision up to 5%
  - Frequency: Monthly and after major repair on the device
- Handling of the QC Process

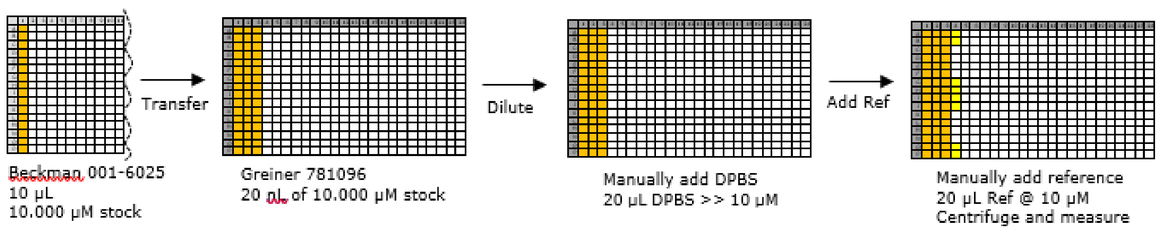


Figure 3: Practical Setup of the QC process

The Echo clearly exceeds expectations, and the process is easy to implement

	accuracy (deviation)	precision (CV)	outlier		acceptance criteria accuracy	acceptance criteria precision	acceptance criteria outlier
reference value for 0,02 µl		1,1 %				3,0 %	
result for test volume 0,02 µl	1,0 %	2,0 %	0 (0 fail)		± 10,0 %	5,0 %	max. 0

Figure 4: Results of the Echo QC

Case study : Gene knock-out efficiency analysis

For an KO efficiency (In/Del analysis) of selected genes in sgRNA/Cas9 reverse transfected podocytes, assay ready plates containing 250nl of sgRNA were prepared. The knockout of the selected genes was consistent across triplicates wells and across the sgRNAs tested.

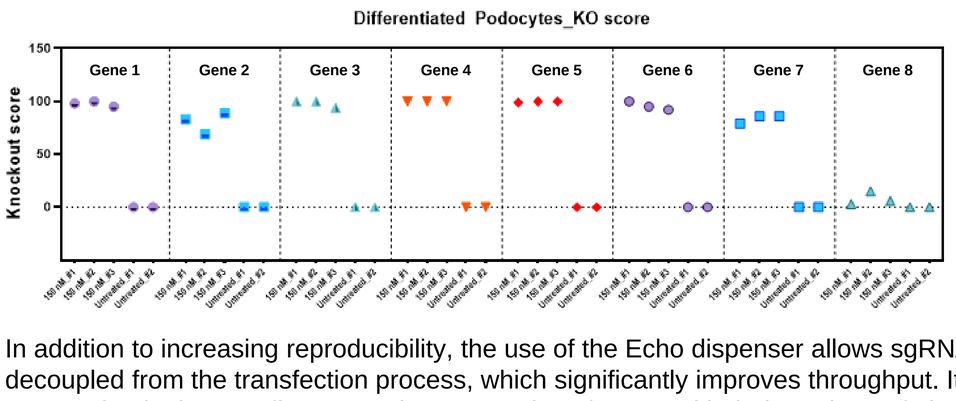


Figure 5: Knockout scores measured in triplicate (including 2 controls)

In addition to increasing reproducibility, the use of the Echo dispenser allows sgRNA spotting to be decoupled from the transfection process, which significantly improves throughput. It also enables preparation in the nanoliter range (as opposed to µl range with tip-based transfer), reducing dead volume and thus library usage by approximately 40%. Furthermore, the Echo dispenser offers the flexibility to spot combinations of sgRNAs from any source well to any destination well, facilitating custom sgRNA pooling, a task that is much more complex with traditional tip-based liquid handling systems.

Conclusion

- In this study, we highlighted the importance of minimizing solvent evaporation to maintain the integrity and concentration of samples. By implementing a refill process, we were able to significantly reduce evaporation-related issues
- The selection of Fluorescein as the optimal dye for device validation was based on its stability, low toxicity and reliable linear range. This choice ensures accurate and consistent process validation, which are essential for high-quality results in research.
- Overall, our approach enhances the efficiency and reliability of sample management in Biologics research, providing a scalable solution that meets the requirements of modern drug discovery
- Finally, we would like to thank Kamran Honarnejad and the CRISPR team to embark in this journey with us and share data