Understanding DRUG-seq multiplexing: when do you need a UDI Expansion Module?

Tracking samples with DRUG-seq

For laboratories new to DRUG-seq or Total DRUG-seq, a common practical question is how many samples can be processed together and how many independently pooled libraries need to be sequenced in the same run.

Depending on the kit format, these workflows can support pooling of 96 or 384 samples into a single tube. Larger projects can scale to up to 1,536 samples by processing multiple 384-sample pools. This high level of multiplexing is possible because these workflows use two barcode layers with different roles.

Internal sample barcodes and UMIs are introduced during reverse transcription. The sample barcode identifies the original well or sample, while the UMI tags individual transcript molecules before amplification. Together, these elements allow reads to be assigned back to the correct sample and support accurate gene expression quantification (Figure 1).

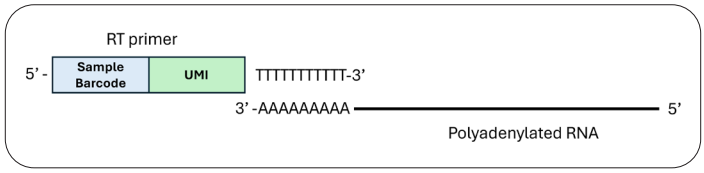

Figure 1: Incorporation of sample barcode and UMI during reverse transcription. The RT primer introduces a 14 nt barcode for sample identification and a 14 nt UMI for molecule counting and PCR duplicate correction. The RT primer structure is the same for DRUG-seq and Total DRUG-seq workflows.

Unique Dual Indexes, or UDIs, are added later during library preparation. At this point, individual wells have already been pooled, so each UDI pair labels the pooled library rather than the individual samples. In simple terms, internal barcodes identify samples within a pool, while UDIs identify the pool during sequencing (Figure 2).

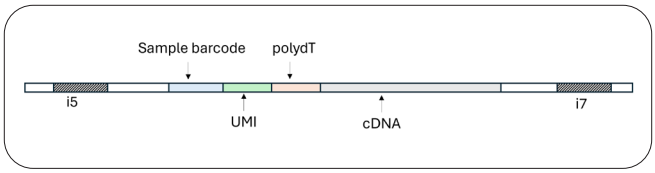

Figure 2: Schematic representation of the final DRUG-seq library molecule. Individual transcripts are labeled early in the workflow during in-well reverse transcription. Following sample pooling and cDNA tagmentation, terminal i5 and i7 UDIs are appended via PCR to the ends of the final molecule flanking the cDNA insert. Library structure is the same for DRUG-seq and Total DRUG-seq.

How pooling works

A pool is the group of samples that is combined into a single tube before downstream library preparation. Depending on the kit format and project setup, one pool may contain up to 96 or 384 samples. Within that pool, individual samples are distinguished by the internal sample barcodes introduced during reverse transcription.

Because each pool becomes one final library, each pool that will be sequenced together with other pools must receive its own UDI pair. If one full 384-well plate is processed as a single 384-sample pool, only one UDI pair is required. A 384-well plate can also be split into multiple smaller pools; for example, four independent 96-sample pools. In that case, the four pools can be combined in the same sequencing run or lane, only if each pool receives a different UDI pair (Figure 3).

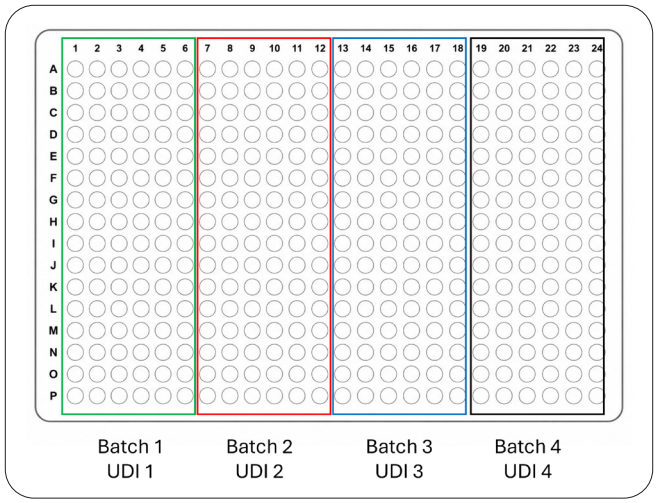

Figure 3: Use of UDI pairs to sequence multiple DRUG-seq batches together. In this example, one 384-well plate is split into four independent 96-sample pools. Each pool receives a different UDI pair during library preparation, allowing the four pools to be combined and sequenced simultaneously while keeping each pool distinguishable during demultiplexing.

When the expansion module is needed

The standard kit configuration provides 4 UDI pairs. Therefore, no expansion module is required when up to four pools are sequenced together.

If five or more pools need to be combined in the same sequencing run, each pool still requires a distinct UDI pair. In that situation, use the appropriate expansion module:

For DRUG-seq, use the MERCURIUS™ UDI Expansion Module.

For Total DRUG-seq, use the MERCURIUS™ F UDI Expansion Module.

The expansion module increases the number of pooled libraries that can be uniquely demultiplexed in one run. It does not increase the number of samples that can be identified within each pool.

Importantly, generating more than four pools across an entire project does not necessarily mean that an expansion module is required. If those pools are sequenced in separate runs, or in batches of four pools or fewer, the same UDI pairs can generally be reused because each run is demultiplexed independently.

Examples

Scenario	Samples	Pools sequenced together	UDI pairs required	Expansion module needed?
One 384-well plate split into 4 x 96-sample pools	384	4	4	No
One 384-well plate split into eight 48-sample pools	384	8	8	Yes
One 384-well plate split into eight 48-sample pools	384	≤4	≤4	No

Practical considerations for partial plate use

When processing only part of a plate, indexing is not the only consideration. Laboratories should also consider plate handling and the availability of other workflow components.

Unused wells should be protected from repeated handling and exposure to moisture. If only part of a plate is used, the remaining wells should be resealed and stored according to the kit instructions.

Partial plate processing can also increase reagent consumption and reagent loss. Although the number of UDI pairs may be sufficient, other kit components such as enzymes, buffers, lysis reagents, cleanup reagents, plates, seals, and consumables may not scale linearly when a workflow is divided into many small batches.

Repeated master mix preparation can increase dead volume losses from reservoirs, pipette tips, and tubes, and may reduce the number of practical reactions that can be completed from a kit.

For this reason, splitting plates into well-defined sections, such as four 96-sample pools, is generally more practical than creating many very small pools. If a plate is divided into more than four pools, laboratories should contact our tech support team to confirm not only that the indexing strategy is appropriate, but also that sufficient reagent volume and consumables are available to complete all planned batches.