

## READ ME

We are so excited that you are interested in adopting the mini-MEndR/mini-IDLE culture systems! Below is a quick summary of published works and resources located in the Gilbert Lab Github that will ensure you reach expert level as efficiently as possible.

### Resources

1. [mini-MEndR publication](#) - Contains detailed instructions for preparing the miniaturized myotube templates. For those with prior experience culturing myoblasts it typically takes ~ 3 rounds of practice to achieve expert level (i.e. maximized and even myotube coverage). We encourage new users to pay special attention to SI Figure 2 which describes our in-house benchmarking approach that we use to determine expert level. For those interested in recreating our myotube template benchmarking, our image analysis code and sample images (to practice using the code) is on our Github site in the MEndR Image Analysis folder. In our experience, it is necessary to optimize cell seeding density for each new cell line you use in the platform to produce the myotube templates. We do not recommend conducting studies with added MuSCs until you achieve 'expert level' in producing the myotube template component.
2. [cryo-MEndR publication](#) - Contains detailed instructions for selecting an alternative cellulose scaffold source and includes ordering information about the new paper scaffold we use in-house.
3. mini-MEndR / mini-IDLE in-house protocols
4. Myoblast Dissociation Practice protocol - If you are struggling to reach expert level when making myotube templates, it is possible that preparation of a monodisperse solution of myoblasts in the ECM precursor solution is to blame. This QC protocol walks you through methods to rule out or remedy this issue.
5. Myoblast Distribution Practice protocol - If you are still struggling to reach expert level when making myotube templates after completing the myoblast dissociation practice protocol, it is possible that uneven distribution of myoblasts within the cellulose paper scaffold after seeding is to blame. This QC protocol walks you through methods to rule out or remedy this issue.

As noted in 1, you will want to start by achieving expert level in myotube template fabrication. So far there has been excellent success by more than a dozen labs around the globe (that we know of!) in getting the myotube template component to work in their lab by using our in-house protocols.

The MuSC seeding step of the mini-MEndR (regeneration)/mini-IDLE (quiescence) assays require a bit more practice to reach expert level and so we added substantial detail on this to the mini-MEndR manuscript. In-house we always have new users start by seeding labeled myoblasts (GFP, CellTracker, etc) onto the myotube templates as a sanity check. Most new users need 2x rounds of practice w/ the GFP+ myoblasts before moving to MuSCs. The goal is the day after seeding to (a) be able to see GFP+ cells and (b) for those cells to be evenly distributed across the surface. From there, our new users are expected to recreate Figure 2D of the [mini-IDLE paper \(500 MuSCs vs 2500 MuSCs\)](#), before moving on with their studies. These benchmarks serve as ground truths to ensure new users have reached expert level on the skills.

Have fun! We can't wait to see what you use these culture assays to discover!