

Assisted SeqWell: What to expect?

SeqWell is currently offered as an Assisted Service through the Koch Institute Nanowell Cytometry Platform (Flow Cytometry Core). The Platform works in collaboration with the Koch Institute Integrated Genomics & Bioinformatics Core/MIT BioMicro Center to process samples from SeqWell for amplification, sequencing and data analysis. Before you begin, you will need to [set up a Consultation](#) with Core staff (Stuart & Shanu), in which the following questions will be addressed:

- What cell type are you planning to use?
- What is the source, viability & availability of your cells?
- If using dissociated cells, do you have a protocol in place for tissue dissociation?
- If using flow cytometry-sorted cells, do you know the impact to your cells?
 - Ensure that cell viability does not decrease within 1-2h of dissociation/sorting

Before the first run

- Optimize your sample preparation protocol. This can take a long time, and will have a huge impact the quality of single cell data. Therefore, it is critical to ensure the highest quality starting material possible.
- Minimize presence of dead cells, degrading nuclear material or anything that can impact reverse transcription in your sample preparation. Keeping cell prep time to a minimal will avoid decreased cell viability and increased cell clumping.
- We recommend a quick viability check using Trypan Blue to estimate cell viability in the samples you bring.
- Core staff will enter your project request in iLab; the user must have an iLab account and must review and approve the charges before work can begin.

Important information

- Plan to bring 50,000 cells in 200µl of media with serum
- Before transferring cells to tubes for transfer to core, coat tubes in BSA/FBS overnight to prevent cell loss
- Cut off time for receiving SeqWell samples: 11 AM
- Up to 4-6 samples may be run at once
- Approximate turnaround time: 3-4 weeks (from sample receipt to completion of basic data analysis)

Getting ready for the run

A few days before the scheduled run, check in with Core staff to review the experimental details (cell number, how to deliver the cells) and to confirm the schedule.

On the day before the run, Core staff will prepare the arrays and membranes.

What to bring:

1. Cells in complete media

SeqWell Guidelines

- a. Minimize time between preparing, transporting and loading cells
- b. If the cells have been dissociated, coordinate with Core staff in advance to ensure that there is minimal wait time between dissociation and array loading
- c. If you are performing FACS prior to SeqWell, coordinate with Core staff to minimize wait time

SeqWell Training: What to expect?

Consider getting trained for SeqWell only if one or more of the following conditions apply:

1. You anticipate running 5 or more rounds of SeqWell samples (20 samples or more)
2. Your samples are BSL2+/3
3. Your samples cannot be processed under the the Core's current scheduling constraints

SeqWell Training involves two stages:

1. **Beginner Level** – The user becomes familiar with the hands-on process of SeqWell from bead loading to bead collection. Samples are processed up to (i.e., not including) the Reverse Transcription step. Experiments are run with "dummy" cells. Typically, 3-4 Beginner Level trainings are required before moving onto experimental samples, especially those that are limited or "precious". Two arrays will be processed during each training session.
2. **Independent Level** – The user will move to this level at the discretion of Core staff. The user runs a SeqWell experiment with Core Staff supervision. The user may use real/experimental samples during this training. Costs related to library preparation and sequencing (either through the MIT BioMicro Center or elsewhere) are not included. Two arrays will be processed during each training session.