Sample requirements and submission guidelines for Smart-seq3

Plate types and purpose

Smart-seq3 lysis plates are 384-well plates containing Smart-seq3 lysis buffer and ERCC. There are 2 types of plates:

- **Sample plates**: Sample plates have lysis buffer in all wells. These are the plates from which libraries for sequencing are produced.
- **Validation plates**: The validation plates contain lysis buffer in only column 1-2 (A1-P1 and A2-P2). These plates are used for QC of sample quality, FACS-sorting efficiency, and optimization of PCR cycle numbers for the sample plates.

We recommend submitting 1 validation plate per sample type (cell type/timepoint/treatment etc.). The validation plate should be from the same sample prep and sorting occasion as the corresponding sample plate.

See below for details on plate layout and how to sort into sample plates and validation plates.

Delivery of Smart-seq3 lysis plates

Smart-seq3 lysis plates are delivered to the user in a plastic bag. The plates are labeled both on the front and the side and are supplied with additional aluminum foil sheets to be used for sealing after FACS sorting has been completed.

Shipping costs

If plates are shipped, ESCG will order transport after coordinating with the recipient. The shipment costs will be added to the iLAB project. The cost is typically **1000-1700 Sek** within Sweden but may vary, and is directly forwarded from the courier's invoice. No extra fees are added.

Plates containing lysis buffer should be handled according to the following protocol

Storage and transport of plates before and after sorting

- **Store the plates at -20°C until the day of sorting.** The ESCG facility will make sure the plates never thaw during delivery by packing and shipping the plates with a sufficient amount of dry ice. ESCG ships plates at the beginning of the week with a reliable courier to avoid the risk of packages getting stuck in transport over the weekend.

- **Store the plates at -80°C after sorting**.

- **Always transport plates in dry ice.** Plates should never thaw during transport. Place plates in small plastic bags to avoid contamination before placing the plates in a box of dry ice. Add a sufficient amount of dry ice both in the bottom of the box and on top of plates to ensure plates do not thaw. Seal the box properly. ESCG advice users to avoid piling multiple plates on top of each other in boxes containing dry ice just in the bottom as the top plates might thaw. Make sure the box contains plenty of dry ice in case of unforeseen delays in shipment.

Sorting and sealing of plates

1. **Just before sorting, thaw the plates on ice or in the fridge**. It is very important that the lysis buffer in all the wells has thawed completely before the sorting. If cells are deposited on frozen lysis buffer the RNA will degrade and become unusable.

2. **After plates are thawed quickly spin down and keep on ice or at 4°C until use**. The plate should not stand thawed on ice or in fridge for too long (a couple of hours is fine, a whole day probably not).

3. **Perform FACS sorting according to plate layout suggested by ESCG** (see attached Single-cell FACS layout).
    a. It is important to document what will be sorted in each plate. The ESCG facility will use the plate ID throughout processing. If different conditions/cells/time points etc. are sorted to different columns/rows in the same plate, please include such information to facilitate the evaluation of QC steps at the ESCG facility. Enter all information into the Smart-seq_Plate_Information form in iLAB.

    b. Always include negative controls. As a standard we expect the wells P1 and P2 of validation plates, or P23 and P24 on sample plates to contain negative controls, i.e., only lysis buffer without cells (see attached Single-cell FACS layout). Negative controls are useful both for the user and the ESCG facility in checking for cross-contamination since the process is long and samples undergo extensive manipulation.

    c. Include two cell-pools on validation plates. As a control for the sorting and/or cell isolation protocol we recommend sorting 20 cells in wells A1 and A2 of the validation plate only. Please do not sort multiple cells on the sample plate.

    d. Even if there are not enough cells for an entire sample plate, the facility will process it as a full plate because of the robotic automated setup.

4. **After sorting, seal the plate with the new aluminum foil sealers provided by ESCG.** Don't use any other sealer or foil since it might detach and fall of during prolonged storage at -80°C.

    a. It is important to seal the plate with the aluminum foil sealers when it is still at room temperature, immediately after sorting. Do not put the plate on ice or dry ice and then seal it, since this will cause the seal not to stick properly.

    b. Perform a quick spin if the lysis buffer is not at the bottom of the wells. Otherwise, a quick spin is generally not necessary and would normally not affect the results since cells that did not reach the lysis buffer directly (those that end up on the walls for example) are dead and the RNA degraded by the time the centrifugation is performed

    c. Keep sorted and sealed plates on dry ice while any remaining plates are sorted

5. **Store the plate a -80°C until shipping or until you bring it to ESCG**.

Shipping of plates after sorting

6. Contact the ESCG facility to arrange for delivery of plates. Plates can be delivered in person or sent by courier. **Only deliver or ship plates after confirming with the project coordinators in advance that they or other facility staff will be on site.**
   Shipping Address:
   Att: Mattias Ormestad
   NGI-SciLifeLab
   Tomtebodavägen 23B,
   17165 Solna, Sweden

   Mark shipment as COLD DELIVERY -80°C

   Opening hours for samples shipped via courier at SciLifeLab (campus Solna):
   Monday – Thursday, 8 am – 4 pm
   Packages are normally collected by Project coordinators before 4 pm Monday-Thursday

   If the courier company requests a telephone number state: +46 8 790 9861

7. Please remember to update the *Smart-seq_Plate_Information* in iLAB (or otherwise submit the information to the facility).

Good luck with your experiment!

Checklist for Smart-seq3 plate handling

- □ Bring enough dry ice to transport the plates without risk to thaw. Store unused plates at -20°C until the day of sorting.
- □ Thaw the plates on ice or in a +4°C fridge and keep them there until ready to sort. Once thawed, spin down the plate(s) before FACS sorting.
- □ Perform the FACS sorting for the validation plate. The plate is labeled with "SS3_YY_NNN validation" and contains lysis buffer only in the first 2 columns on the left-hand side (1-2). All the other wells are empty, do not sort in them. We recommend sorting 20 cells to wells A1 and A2 and no cells (neg. control) to wells P1 and P2 of the validation plate only (see the Single-cell FACS layout).
- □ Perform the FACS sorting for the sample plate(s). The plate(s) is labeled "SS3_YY_NNN sample" and contains lysis buffer in all the wells. Sort single cells into all wells, except P23 and P24 (neg. controls) (see the Single-cell FACS layout).
- □ Keep records of which plate ID corresponds to each sorted condition and if there are any exceptions to the suggested sort layout. ESCG will use only the plate ID for sample tracking.
- □ Seal the plate(s) with the aluminum covers provided by ESCG. Seal the plate while the plate is still at room temperature.
- □ Put the sealed plate(s) on dry ice while any remaining plates are sorted. Store the sorted plates in a -80°C freezer until delivered to the ESCG facility.
- □ Fill in the *Smart-seq_Plate_Information* form in iLAB (or send it to ESCG) and arrange for the delivery of plates.

Single Cell FACS sort layout

VALIDATION PLATE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	20	20																						
B	1	1																						
C	1	1																						
D	1	1																						
E	1	1																						
F	1	1																						
G	1	1																						
H	1	1																						
I	1	1																						
J	1	1																						
K	1	1																						
L	1	1																						
M	1	1																						
N	1	1																						
O	1	1																						
P	0	0																						

EXPERIMENT PLATE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
J	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
K	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
O	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0