Requirements for QC and Shipment of Ultra-Low Input RNA

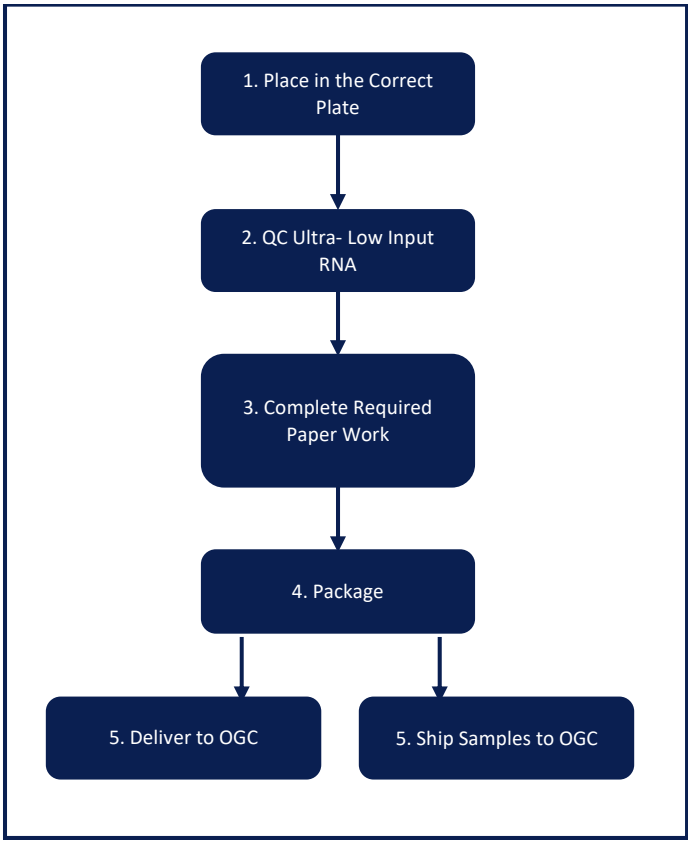
OGC receives thousands of samples every month. Help us to keep your samples safe and to return good quality data in a timely manner by taking the time to read and follow these instructions. Failure to follow these guidelines and incorrect submission of samples could:

1. Delay the initiation of your project
2. Put your samples at risk
3. Lead to poorer quality data
4. Result in additional charges being applied to your project

Obviously, as we've written this document for you, we think it is all important but there are some really key points, which are highlighted with the symbol below. If using a screen reader, these will be marked up as the "key points" style.

If in doubt at any point then please consult your project manager.

The schematic below is designed to give you an overview of the steps that you are required to take, to ensure the necessary amount of material is provided in the correct container, at the right temperature and with all the required paperwork. Further details are provided below the schematic. We have outlined the steps to take in order. However, we recommend reading the whole document through prior to sample extraction and collection.



As technologies improve, the boundaries between low input and high input RNA start to blur, however, the only prep in our portfolio that this document includes is the RNA-Seq ultra low prep. If your quote states any other library type, then this is not the document for you, please return to the main page or contact your project manager if you are unsure.

How should I plate my samples?

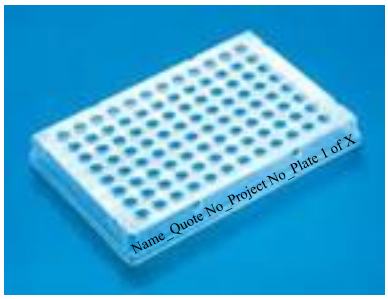
With the number of samples likely to be submitted for this type of prep, it's likely that you'll be preparing samples directly onto the plate. The details below are the same regardless of whether you're submitting RNA or cells in lysis buffer.

1. **Correct plates**: The 384-well 4titude Framestar skirted PCR plate is essential for automation compatibility, all wells must be colourless and transparent.
   (catalogue number 4ti-0384/C https://www.brookslifesciences.com/products/framestar-384-well-skirted-pcr-plate#ordering)
2. **Correct seals**: The plate should be sealed with Thermo Scientific Adhesive PCR Seals (catalogue number #AB-0558).
3. **Fill by quadrants**: 384-well plates should be filled one quadrant at a time, starting with Quadrant (Q)1 and progressing to Q2, Q3, Q4 in that order. Quadrants should be filled by column from top to bottom, see plate map and well orders below but in summary Q1 covers rows A, C, E etc and columns 1, 3, 5 etc. Q2 covers the same columns but the rows are B, D, F etc.

	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	193	9	201	17	209	25	217	33	225	41	233	49	241	57	249	65	257	73	265	81	273	89	281
B	97	289	105	297	113	305	121	313	129	321	137	329	145	337	153	345	161	353	169	361	177	369	185	377
C	2	194	10	202	18	210	26	218	34	226	42	234	50	242	58	250	66	258	74	266	82	274	90	282
D	98	290	106	298	114	306	122	314	130	322	138	330	146	338	154	346	162	354	170	362	178	370	186	378
E	3	195	11	203	19	211	27	219	35	227	43	235	51	243	59	251	67	259	75	267	83	275	91	283
F	99	291	107	299	115	307	123	315	131	323	139	331	147	339	155	347	163	355	171	363	179	371	187	379
G	4	196	12	204	20	212	28	220	36	228	44	236	52	244	60	252	68	260	76	268	84	276	92	284
H	100	292	108	300	116	308	124	316	132	324	140	332	148	340	156	348	164	356	172	364	180	372	188	380
I	5	197	13	205	21	213	29	221	37	229	45	237	53	245	61	253	69	261	77	269	85	277	93	285
J	101	293	109	301	117	309	125	317	133	325	141	333	149	341	157	349	165	357	173	365	181	373	189	381
K	6	198	14	206	22	214	30	222	38	230	46	238	54	246	62	254	70	262	78	270	86	278	94	286
L	102	294	110	302	118	310	126	318	134	326	142	334	150	342	158	350	166	358	174	366	182	374	190	382
M	7	199	15	207	23	215	31	223	39	231	47	239	55	247	63	255	71	263	79	271	87	279	95	287
N	103	295	111	303	119	311	127	319	135	327	143	335	151	343	159	351	167	359	175	367	183	375	191	383
O	8	200	16	208	24	216	32	224	40	232	48	240	56	248	64	256	72	264	80	272	88	280	96	288
P	104	296	112	304	120	312	128	320	136	328	144	336	152	344	160	352	168	360	176	368	184	376	192	384

Quadrant	First well	Quadrant Position
Q1	A:01	Upper Left
Q2	B:01	Lower Left
Q3	A:02	Upper Right
Q4	B:02	Lower Right

4. Include at least **2 blanks in each quadrant**: One of the blanks in each quadrant will be used to add our internal control, so take this into account if you require more than 1 blank for your own analysis.
5. Use an **appropriate layout for your samples**: we recommend that you organise your samples on each plate so that:
   - the distribution of your different experimental groups (WT vs. Mut, Control vs. Treatment…) is even within and between plates and quadrants, where multiple quadrants are used.
   - if you submit more than one plate, place the blanks at different positions, so the identity of each plate can be confirmed during data analysis, as the overall pattern of each plate will be asymmetrical (position of blanks and potentially bulks), while the orientation of the plate can also be confirmed during data analysis
6. **Send back-ups**: It is strongly recommended that regardless of the types of plates or samples being sent that you send additional back-up plates. This/these backup plate(s) could be used as replacement if, for any reason, the library preparation failed and having them in-house means that we can proceed immediately and without delay. N.B. backup plates will be disposed of if unused at the end of the project, unless you arrange to collect them.
7. **Seal the plate thoroughly**: Please make sure the plates are sealed properly to minimize contamination, we recommend using a pen lid or credit card to apply pressure between each line of wells. Note that applying a seal to a cold surface will result in the seal lifting.
8. **Label appropriately**: The sealed sample plate must be labeled with your name and quote number, once you receive the project number, we ask for you to add that too, like in the image below, on the skirt along the long edge of the plate. If submitting multiple plates, please include Plate 1 of X on the label.



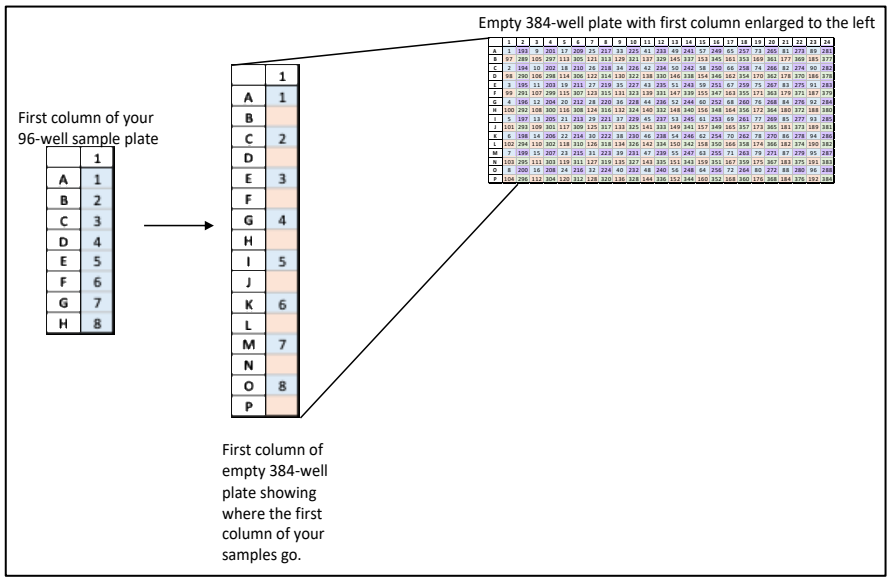
9. **Protect from extreme cold**: Plates should be placed within a plastic bag and the bag sealed with tape (to prevent dislodging in transit) before putting on dry ice or into the freezer. This stops the plate seal from becoming brittle and lifting. Ideally, the bagged plate would be placed in a small box before putting into a larger polystyrene box with dry ice.

What should I do if I used different plates?

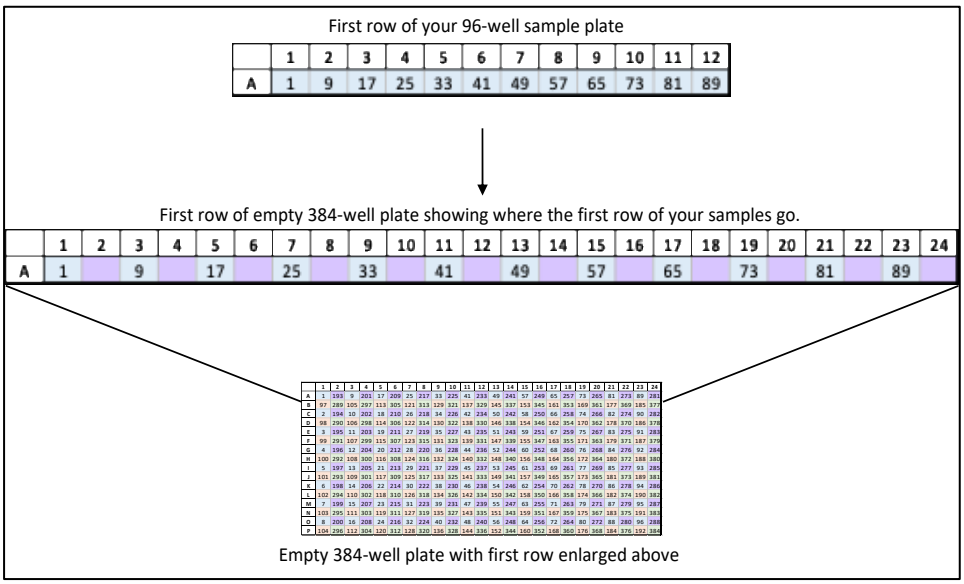
If sorting has been carried out into other types of plate, we recommend resorting using the above guidelines. If resorting is not possible then you must transfer samples to these 384-well plates, this introduces the risk that material will not be transferred- this will be at your own risk.

Samples plated in non-compatible plates will be refused and returned to you at your cost.

To transfer samples from a 96-well to a 384- well plate, use a multi-channel pipette to transfer the first plate into Q1 in the diagram above (and so on). E.g. if pipetting using an 8-channel pipette, your first column needs to go in as below, with samples going into A:1, C:1, E:1 etc.



It is important that you plate each quadrant in columns and not in rows. However, if you have full quadrants and only a 12-channel pipette then your first row needs to go in as below, with samples going into A:1, A:3, A:5 etc.


First row of your 96-well sample plate
1 2 3 4 5 6 7 8 9 10 11 12
A 1 9 17 25 33 41 49 57 65 73 81 89
First row of empty 384-well plate showing where the first row of your samples go.
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 9 17 25 33 41 49 57 65 73 81 89
Empty 384-well plate with first row enlarged above

RNA is susceptible to degradation: things to do

Whether you are submitting cells in lysis buffer or extracted RNA, it is important to protect the RNA.

- **Take all precautions suitable for RNA work**, including the use of RNase-free plasticware throughout.
- **DNase treat with a PCR-grade DNase**. If submitting RNA (rather than cells in lysis buffer), DNase treatment of the samples is essential. Preferably this would be done as part of the extraction process (e.g. on-column DNAse step); but if samples must be DNase treated subsequently, then the enzyme should be inactivated afterwards if necessary for the kit being used and purification should be carried out using columns or phenol/chloroform.

I'm submitting cells, what should they be in?

Cells can be submitted in either 0.8% (vol/vol) Triton X-100 or in NEB 1x lysis mix, with NEB lysis mix being the preferance. **Lysis efficiency in either buffer should be checked** prior to the final sort using a visual check under a microscope/cell counter. We know from other users that certain cell types may not lyse properly, such as keratinocytes. NK cells and potentially macrophages will contain a lot of native RNases which may require harsher conditions. **We will not check lysis efficiency**.

Triton-X can be made by combining 0.8% (vol/vol) Triton X-100 and 2 U/µl RNase inhibitor. This buffer can be stored at 4 degrees for 6 months. **Cells in this buffer should be in a final volume of 2µl.**

NEB lysis mix is available to purchase from NEB and can be used as detailed in the table below, using 0.2µl of 10X buffer, 0.1µl of RNase Inhibitor and topped up with nuclease free water. Again, the **final volume should be 2µl**. The catalogue number for the lysis mix is E5530.

Reagent	**Input Type**	
	Single cell	**<100 cells**
10x Lysis buffer	0.2µl	0.2µl
Murine RNase Inhibitor	0.1µl	0.1µl
Nuclease free water	1.7µl	<1.7µl (to final volume 2µl)[1]
Total volume	2.0µl	2.0µl

[1] It is our observation that with 100 cells, the overall volume of cells, lysis buffer and RNase inhibitor is already 2µl, so no nuclease-free water is required. This will depend on the chip used in the cell sorter, but if the volume is lower and no nuclease-free water is added, it won't affect the prep.

Once cells are lysed, they should be briefly centrifuged then stored at -80C until they are being shipped to us.

I'm submitting RNA, what is needed?

Quantification should be done using a low-range bionanalyzer or tapestation. These systems will allow you to quantify the samples and to check their quality. **These samples will not be QC'd in-house.**

The concentration and volume required is given in the table below, RNA samples must be submitted in **ultrapure water**. We require a maximum of 1ng RNA in a volume of 2µl, samples to be at a concentration of 5pg-0.5ng/µl.

All RNA samples must be normalised as the PCR cycling conditions in this protocol are determined based on input amount. We will therefore increase PCR cycling to match the information you give for your lowest sample.

Type of Sample	Amount required	Concentration	Volume	OD 260/280 and 260/230
RNA-Seq ultra low	<1ng	5pg-0.5ng/µl	2 µl	~2

Before delivering samples, complete the paperwork

These steps should be carried out at least two days before you wish to ship your samples, in order to give your project manager time to log the details in to our database.

Step 1
Sign your quotation and return via email along with a PO number. If outside the University of Oxford, please provide a PDF copy of the PO.

Step 2
Download the latest "**sample submission form RNA-Seq ultra low**", which has the 384-well plate layout from our website. It is important to always use the most recent version because we make improvements and old versions will not work with our login process.

Complete the sample submission form ensuring that the label on your plate(s) exactly matches the entry in the submission form.

To ensure there are no delays in initiating your project, please confirm that all the requested information is provided on the submission excel form. There are more details on the form itself but required minimum information is:

- sample name
- reference genome
- sample concentration (for RNA, except for the occasional sample where quantification is impossible)
- volume
- pooling (if applicable, e.g. specify multiplex groupings by mp1 for all samples in the first multiplex, mp2 etc).

Please extend the table as appropriate and ensure that there are **no duplicate sample names**. The container ID should also be provided, this is what your plate is labelled with your name, quote number and plate number (e.g. plate 1 of 4). Once you have the project number from your project manager, we ask for you to add the project number to the plate also.

In 'additional comments', please note if you want your samples returned and if there is a priority order (1, 2, 3 etc) within the project, we will try to accommodate this where possible.

Email the completed form to your project manager.

Step 3
Wait for confirmation of receipt before shipping samples. Your project manager will assign you a project number. Your plates are already labelled but need to have this project number added. This can be done without defrosting, by wiping the edge with a tissue prior to labelling. Please also include the project number on the packaging and then ship accordingly following the instructions below.


To ensure the safety of your samples, please do not send them to us prior to receiving notification by email from your project manager that it is OK to do so. Samples received unexpectedly, poorly labeled or without correct paperwork will delay the initiation of your project, risk the safety of your samples and incur additional charges.

How should I package and ship my samples?

All samples should be on dry ice, with care taken to protect the plates and seals as detailed above. The amount of dry ice that you require will depend on the size and quality of your container as well as the number of days that the parcel will be in transit. Please include extra dry ice if your parcel needs to go through customs as there can be unexpected delays. If in doubt, please speak with your courier for advice.

For delivery details please see:
https://www.well.ox.ac.uk/ogc/sample-delivery/

The essential steps of this process are summarized as a checklist in Appendix 1.

Appendix 1

Checklist for Ultra- Low Input RNA

After reading the full text, this checklist can be used to ensure all the steps are carried out prior sending any samples to OGC. These are only brief summaries of each step and this checklist should not be used as a standalone document.

No.	Processes	Tick
	Plating	
1.	Fully skirted, clear plastic, 384 well plates (NB we will only accept samples in the plates detailed above)	
2.	Sealed with our recommended adhesive seal	
3.	Correct layout (Fill by quadrants, with sufficient blanks)	
4.	Correct label on the side of plate (name, quote, project number, plate number)	
	QC if cells	
5	Lysis efficiency check	
	QC if RNA	
6.	DNase Treatment	
7.	Bioanalyzer/ TapeStation	
	Paperwork PRIOR sending	
7.	Quotation signed and returned	
8.	Emailed purchase order to project manager	
9.	Completed sample submission form	
10.	Emailed sample submission form to project manager	
11.	Received ‘go- ahead’ from project manager	
	Packaging & Shipping	
12.	Suitable box with dry ice	
13.	Delivery	