Guidelines for Shipping DNA to the Wellcome Trust Sanger Institute.

1. Sample Manifest and Barcodes.

Prior to sending samples to the Sanger Institute an electronic manifest and adhesive barcodes will be provided so that sample information can be collected and corresponding plates/tubes correctly labelled.

1.1 Manifest

Plate Manifest:

Within the manifest, the first three columns will be pre-populated with the following;

Sanger Plate ID: (DNXXXXX – will correspond to the barcodes provided)

Well Address:

Sanger Sample ID:

Study:	XXXXSTDY
Supplier:	Sanger Centre
No. Plates Sent:	2

SANGER PLATE ID	WELL	SANGER SAMPLE ID
DN290834E	A1	XXXXSTDY5573566
DN290834E	B1	XXXXSTDY5573567
DN290834E	C1	XXXXSTDY5573568

Tube Manifest:

Within the manifest, the first two columns will be pre-populated with the following:

- Sanger Tube ID: (NTXXXXXXX – will correspond to the barcodes provided)
- Sanger Sample ID

Study:	XXXXSTDY
Supplier:	Sanger Centre
No. Tubes Sent:	9

SANGER TUBE ID	SANGER SAMPLE ID
NT600361B	XXXXSTDY6050207
NT600362C	XXXXSTDY6050208
NT600363D	XXXXSTDY6050209

The minimum information required to process samples through the QC pipeline is listed below:

Supplier Sample Name:

Gender (if applicable):

Volume:

Concentration:

Taxon ID:

Common Name:

Phenotype (if applicable):

If any of the above fields are blank this may result in a processing delay.

For your information, Taxon ID can be sourced by using the following link:

http://www.ncbi.nlm.nih.gov/taxonomy

The taxon ID is submitted automatically to the EBI and may, in the absence of a sample or study genome reference, associate the sample with a genome reference which is then used for QC and alignment of the data provided. Please ensure the correct Taxon IDs are used: Incorrect taxon IDs have some reputational risk to the Institute, and when corrected require personnel and compute time to fix alignments which in turn delays the provision of the final data product. In using a spreadsheet program to complete a manifest it is very easy to incorrectly complete the taxon ID field - any series of regular increments of taxon ID e.g. 9606, 9607, 9608, 9609..., are likely to be wrong.

The Phenotype column is used for downstream analysis and is compulsory for the EGA, but not the ENA. Please find example annotations for completion of this column below:

Control (which would be a 'normal' phenotype)

Disease/population (e.g Anorexia Nervosa or HIV or Uganda or Finland)

Customers submitting **RNA** and **ChIP** samples should always complete the following fields (whenever possible):

- Sample description – A brief description of characteristics of each sample.
- Cell type
- Organism part
- Immunoprecipitate (ChIP)

Please note: that although some fields are optional and not required for sample processing, it is always useful to provide as much information as possible. In particular Ethnicity, and Geographical information are required for genotype calling; there may be a delay in data release while this information is gathered if not provided upfront.

Once completed the manifest should be returned by replying to the initial request email. It is important to note that samples will not enter the QC pipeline without the corresponding manifest.

1.2 Barcodes.

Adhesive barcodes will be sent by Royal Mail for domestic recipients, overseas suppliers will receive barcodes via FedEx. Included with the barcodes will be an adhesive address label for shipping purposes and a laboratory guide for preparing samples.

Internal customers will have details of the collection point of their barcodes provided to them in the request email.

2. Plate Type and Barcodes

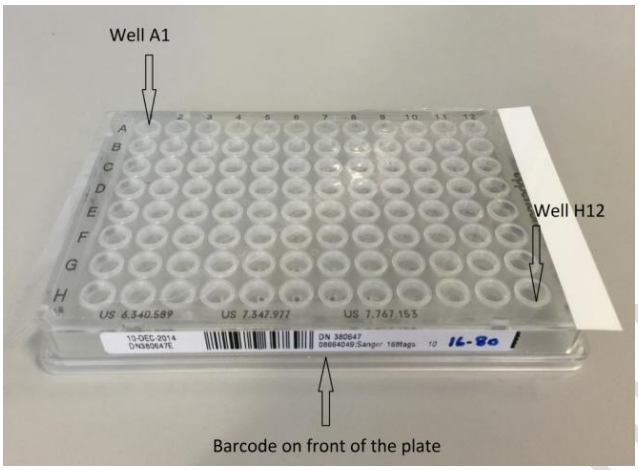
We require samples to be provided in 96 well formats using the following specific, validated plate types.

ABgene AB_0800 0.2ml full skirted clear/colourless 96 well plates (volumes <100µl)

FluidX 0.3ml externally threaded 2D barcoded tube rack (volumes <200µl)

Please note: If samples are received in any other format, customers will incur a transfer cost of £55.10 in order to bring this in line with our automation.

Appropriate plates can be sent upon request.



For human samples, a maximum number of 95 samples should be supplied in any one plate; H12 should be left empty for a Fluidigm processing control. For non-human samples, all wells can be utilised.

If less than 95 samples are being submitted the plate should be filled in column order.

Each plate should be identified by attaching the adhesive barcode to the middle of the front side of the plate, orientated so that well A1 is at the top left hand corner when viewed from above (See diagram above).

2.2 Tube type and Barcodes

qPCR-only and Pre-quality controlled samples should be delivered ONLY in 1.5ml microtubes and labelled as illustrated below with the barcodes provided.

Figure 2: Labelled 1.5ml microtube

Please note if SM receive samples that are <u>not</u> in the specified tube types the samples will either be returned to the customer or transferred by us into tube types which are compatible. The latter will incur a consumable cost charge. Appropriate tubes can be sent upon request.

3. DNA and RNA Requirements

3.1 DNA extracted from Pathogen sources.

Any DNA being shipped to the WTSI originating from a Schedule 5 micro-organism must be flagged to us at the earliest possible opportunity. Prior to shipping samples in this category, certification validating that the sample is inert and that no live material is present in the sample must be forwarded to sample-management@sanger.ac.uk along with the lysis method used for obtaining the DNA. Without this paperwork samples will not be received on site.

3.2 Quantity of DNA/RNA required.

Quantity of DNA is dependent upon processing requirements. Tabulated below are the quantities required for Illumina Genotyping, Agena Genotyping and Illumina Sequencing.

DNA Requirements	Total µg Required	Ideal Volume (µl)	Ideal Concentration (ng/µl)	Minimum volume (µl)
Illumina Genotyping	1.75	25	70	20
Agena Genotyping (up to 3 plexes)	0.2	20	10	10
ISC WES (Multiplex Capture) Whole Genome Sequencing	0.5	25	20	20
PCRfree X10	2	25	80	20
PacBio Sequencing	10	50	200	25
TraDIS	1			≤ 100

Please note: Samples destined for Agena genotyping will not undergo QC and therefore should not be merged on the same plate with samples destined for any other process.

The above table shows ideal volume and concentrations; the minimum volume must be strictly adhered to, to allow automated processing of samples. Although a wide range in concentrations (up to 300ng/µl) can be processed, it is important to note that if a normalisation step is required, an additional quantification cost will be applied. QC utilizes 2µl of sample (1µl for WGS and ISC samples) therefore higher concentration supplied will result in higher amounts used during the QC process.

Please note: The PCRfree X10 line has stringent submission requirements. Samples falling outside of these parameters will be highlighted as such in the QC progress report post QC and will not be able to proceed.

Please note: As we often receive ChIP samples of very low quantity, we do not request a specific amount for ChIP DNA. Please provide as much as possible and we will perform an initial QC step prior to library prep.

Total RNA	Total µg Required	Concentration (ng/µl)	Volume (µl)	RIN (RNA integrity number)
RNAseq (manual prep <48 samples)	≥ 1		≤ 50	≥ 8
RNAseq (automated prep 48+ samples)	≥0.5	10≥100	≥50	≥ 8
RNAseq (automated prep 48+ samples) with ribozero	≥1	36≥100	≥28	≥ 8
Small RNA	≥ 1		≤ 6	≥ 8
DAFT-seq	≥ 2		≤ 50	≥ 8

Please note: We request that all Total RNA is free from high molecular weight material before submitting samples for library prep as we do not perform DNase treatment.

Ready-made libraries	Ideal concentration (nM)	Ideal Volume (µl)
qPCR-only	4 – 20 *	20
Pre-quality controlled	4 *	20

Please note: We ideally only like to QC a maximum of 24 individual samples per submission. Any more than 24 and we request that premade pools are made and submitted for QC.

*We are aware that it can be difficult to provide final libraries at 4nM; therefore we will accept lower concentrations although this could increase the volume we require for loading on to our sequencing machines.

4. Plate Sealing and Shipping

4.1 Plate Sealing

Plates should be sealed using either a removable heat seal or adhesive seal capable of withstanding dry ice conditions. For RNA samples we recommend the use of the Applied Biosystems MicroAmp™ Optical Adhesive Film seals part number 4311971, as these remain in place at -80 °C. Appropriate seals can be sent on request.

4.2 Shipping

Internal customers:

Details of sample drop off will be provided in the request email.

External customers:

Shipping on dry ice is the optimum method of sample transportation; plates should be protected if dry ice blocks are used as plates can become brittle and break during transit. Samples and dry ice should be placed in a polystyrene igloo and outer cardboard packaging. The address label received with the barcodes should be placed clearly on the outer packaging along with dry ice labelling and sender details.

Samples must arrive at the Sanger Institute no later than 16:00 on a Friday, if you have arranged courier transportation during the latter part of the week please confirm expected delivery times with your chosen courier company. Please note the sample management facility is unmanned over the weekend and any samples arriving during this time will not be accepted.

5. Sample Storage

Any remaining stock DNA samples not required for processing will be stored by Sample Management at -20oc for the duration of 5 years unless specified otherwise. Any retention beyond this is reviewed in discussion with the sample custodian.

Any daughter samples generated internally from stock to support the service provision will be stored in Sample Management for 3 months and then destroyed. **If an alternative approach to these samples is required this must be proactively flagged to Sample Management on submission**.

Please refer to the Sample Retention and Destruction Policy for more details.

6. Contact Details.

Shipping Address:

Sample Management

Wellcome Trust Genome Campus

Wellcome Trust Sanger Institute

Hinxton

Cambridge, UK

CB10 1SA

Email:

For any queries please reply to the request ticket, alternatively please contact the most appropriate programme specific RT alias below:

humgen-dnap@sanger.ac.uk - *Human Genetics Programme*

casm-dnap@sanger.ac.uk – *CASM Programme*

ig-dnap@sanger.ac.uk – *Infection Genomics Programme*

malaria-dnap@sanger.ac.uk – *Malaria Programme*

cellgen-dnap@sanger.ac.uk – *Cellular Genomics Programme*