# How to submit NGS sequencing data to the European Nucleotide Archive (ENA)

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#### **Overview of required information**

You need to provide information on the

- 1) **Study** = title and abstract for the study
- 2) **Samples** = information on the biological samples
- 3) **Experiment** = details on how the sequencing was done

Steps 2 and 3 can either be done manually for each sample or you can download spreadsheets which you can complete and upload. This tutorial explains only this second option which is much easier as soon as you have multiple samples.

## 1) Create an account and log in

## https://www.ebi.ac.uk/ena/submit/sra/#home

MBL-EBI	Services Research Training About us
WWENA	
European Nucleotide Archive	
NA Home Search & Browse Submit & Update About ENA Contact FAQ	
	Contact Helpdesk
Welcome to the Webin submission service. You can use this service to submit sequence reads, genome assemblies, targeted a please use <u>EGA Webin</u> . To register umbrella studies, used to group together sequence New to Webin? Please register using the button below. * Register C Reset Password	I assembled and annotated sequences and to register studies (projects) and samples. To submit human data requiring controlled access ncing studies, please contact <u>datasubs@ebi.ac.uk</u> .
Login	
* Username: Webin-40910 🔘 🔕	
* Password:	
Login >	home-view

#### 2) Select «New Submission» tab

## 2) Pre-register the study

Please select the type of submission         Submit sequence reads and exprise         Image: Submit other assembled and and submit sequence	eriments ata submission to ENA. w to submit a Study to ENA.	Complete the required fields and press «Submit»	
	Please specify the release date of your study:         This is when your study will be made public.         23-Dec-2020         Please provide a short name for the study:         Please provide a short descriptive title for the study: (*)         Please provide a short descriptive title for the study: (*)         Please provide an abstract to describe the study in detail: (*)	Please provide attributes to add a deeper description of the study:          Tag       FieldType         Add         Please provide PubMed IDs of publications you want to associate with the study: (numeric value)	otation? (*) xciated to
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		Nestal Submission	

## 3a) Register the samples - Download template

This page explains how to download a template for the sample spreadsheet. You can skip this step if you already have a template, e.g. from a previous submission

Back on the «New Submission» page:

Please select the type of submission you would like to make:								
O Submit sequence reads and experiments								
O Register study (project)								
O Taxonomy Check/Request	🛋 Select Ch	ecklist >						
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Register Samples to give context to your data.								
Read here for information on how to submit your Samples to ENA.								
O Submit other assembled and annotated sequences [formerly EMBL-Bank]	Home	New Submission	Studies	Samples	Runs	Analyses		
	Start 🛩	>>		Sample	>>	Finish		
	Please select the most app	propriate checklist from the list below I	hen click the Next >> button					
	Environmental Checklists This group currently includes Gen	omic Standards Consortium (GSC) MixS sam	ple checklists					
	Marine Checklists     This group currently includes Micr	o B3 and Tara Oceans sample checklists						
	Pathogens Checklists This group currently includes several prokaryote and virus pathogen sample checklists							
	Other Checklists This group currently includes the ENA default sample checklist and a few project specific checklists This group currently includes the ENA default sample checklist and a few project specific checklists ENA Global Microbial Identifier Proficiency Test (GMI PT) checklist Minimum information to standardise metadata and can be used for submission of sample metadata derived from Campylobacter coli, Campylobacter jejuni, Listeria monocytogenes, Klebisella pneumoniae, Sathonella enterica, Escherichia coli and Staphylococcus areaus. ENA mutagenesis by carcinogen treatment checklist Minimum information to standardise metadata derived from carcinogen induced animal tumours. This minimum metadata standard was developed in collaboration with Duncan Odom lab for the Mouse Liver Cancer Evolution Project. ENA plan Sample Checklist ENA signification of plant specimen contextual information associated with molecular data. The checklist has been developed in collaboration with the NCBI-GenBark and iPlant data resources under the umbrells of the Genomic Standards Consortium.							
	ENA default sample chec     Minimum information required	klist for the sample						
	ENA Crop Plant sample e The ENA Crop sample enhan systematic transcriptomic real	nhanced annotation checklist ed checklist has been developed in collaboratio ignment-based analyses.	n with a number of EMBL-EBI teams to	capture enriched annotation of published crop	plant samples that lack sufficient reported met	tadata and are typically associated with		
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(May vary between projects)

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### 3a) Register the samples - Download template

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Here, you can specify various optional fields that you could provide if available, e.g. sex, tissue type etc.	<ul> <li>Please select any additional</li> <li>Filter fields</li> <li>Add your own custom field</li> <li>Filter fields</li> <li>Add your own custom field</li> <li>Part and developmental stage of</li> <li>cell_type - optional</li> <li>cell_type - optional</li> <li>if the sample was obtained from</li> <li>germline - optional</li> <li>the sample described presented</li> <li>tissue_tib - optional</li> <li>tissue type from which the sample</li> <li>tissue_tib - optional</li> <li>tissue_tib - optional</li> <li>tissue_tib - optional</li> <li>tissue tibrary from which the sample</li> <li>tissue type from which the sample</li> <li>to of 30 fields selected</li> <li>+ Expand</li> <li>Collapse</li> <li>When you have selected the have filled the spreadsheet</li> <li>&gt; Download Template Spread</li> <li>&lt;</li> </ul>	optional fields. Mandatory and recommendations of the second state	it is specified with this qualifier: it is specified with this qualifier: ic rearrangement as part of an adaptive immur agin entering your data. Alternatively and upload the spreadsheet using the Comparison of the spreadsheet using the comparison of the spreadsheet using the spin entering your data.	t. You may add any of the option t. You may add any of the option te response; it is the unrearranged molecu te response; it is the unrearranged molecu ty, download a template spreadsh te Upload Completed Spreadsh the upload Completed Spreadsh th	al fields. You may also add custo le that was inherited from the parental ge set using the Download Templ heet button.	emline ate Spreadsheet button. Once you Next >>

You may want to go to the next page to look up the taxonomic ID for your species. You will need this in the sample description file (next slide)

#### 3b) Register the samples - Fill in sample spreadsheet

#unique_name_prefix	
sample_alias tax_id scientific_name common_name sample_title tissue_type	
T1 10090 Mus musculus house mouse Triaka ESRP1 mutant mouse (Esrp1 <m1btlr> or Esrp1<triaka;triaka>) intestinal ep</triaka;triaka></m1btlr>	ithelial cells
T2 10090 Mus musculus house mouse Triaka ESRP1 mutant mouse (Esrp1 <m1btlr> or Esrp1<triaka;triaka>) intestinal ep</triaka;triaka></m1btlr>	ithelial cells
T3 10090 Mus musculus house mouse Triaka ESRP1 mutant mouse (Esrp1 <m1btlr> or Esrp1<triaka;triaka>) intestinal ep</triaka;triaka></m1btlr>	ithelial cells
T4 10090 Mus musculus house mouse Triaka ESRP1 mutant mouse (Esrp1 <m1btlr> or Esrp1<triaka;triaka>) intestinal ep</triaka;triaka></m1btlr>	ithelial cells
WT5 10090 Mus musculus house mouse wildtype (C57BL/6J ) intestinal ep	ithelial cells
WT6 10090 Mus musculus house mouse wildtype (C57BL/6J ) intestinal ep	ithelial cells
WT7 10090 Mus musculus house mouse wildtype (C57BL/6J ) intestinal ep	ithelial cells
WT8 10090 Mus musculus house mouse wildtype (C57BL/6J ) intestinal ep	ithelial cells

- The sample alias must match the fastq filename. For example, if your fastq file is called T1\_R1.fastq.gz, the correct sample alias would be T1.
- Save file as .tsv or .txt

### 4a) Register the experiments - Download template

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Data

type

This page explains how to download a template for the experiment spreadsheet. You can skip this step if you already have a template, e.g. from a previous submission

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+					,	download template

#### 4b) Register the experiments - Fill in spreadsheet

sample_alias	instrument_model	library_source	library_selection	library_strategy	library_construction_protocol	insert_size	forward_file_name	forward_file_md5	reverse_file_name	reverse_file_md5
T1	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	350	T1_R1.fastq.gz	a122c9eafb484e97073aae05fedf5620	T1_R2.fastq.gz	dce8368de0ca1ddab8fa49283ad213d7
T2	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	350	T2_R1.fastq.gz	d8f9e1e3fc417a4c9a44152c99ebbed8	T2_R2.fastq.gz	ff138de09b6f94f3f0c4c497bab43aa3
Т3	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	340	T3_R1.fastq.gz	826ad86910dee4108dfc1bb8b14e6938	T3_R2.fastq.gz	f35f238559e74bda181a17aa832e644e
T4	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	340	T4_R1.fastq.gz	b66152260d7fd7c3a5481f58c43e5573	T4_R2.fastq.gz	7e4a97c04286e81ab9a5adc7149ac318
WT5	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	370	WT5_R1.fastq.gz	a319acfd4410e1f00b35bb3f84dea12b	WT5_R2.fastq.gz	93b9078910be3075ad6f6ec78b9f29dd
WT6	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	360	WT6_R1.fastq.gz	a7133b26d8cb5466e2464501e2fe3f12	WT6_R2.fastq.gz	3cf40b077a1b0b7ca5883cdd9a2d09e5
WT7	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	350	WT7_R1.fastq.gz	750bbb87b9fbc23bf73f10901e098a97	WT7_R2.fastq.gz	0435b510d3435440e33abfefe51dbf3c
WT8	Illumina HiSeq 2500	TRANSCRIPTOMIC	Oligo-dT	RNA-Seq	TruSeq Stranded mRNA	360	WT8_R1.fastq.gz	66b467abe1cea822ada15756249a8a57	WT8_R2.fastq.gz	bf1feac97431d3755222c6ace9c14335

#### Files and associated md5sums

Important: The file names must match the sample\_alias in the samples spreadsheet (step 3b)

#### For sequencing data produced by NGSP Bern and analysed by IBU:

Columns in brown: This information is available in the LIMS. Columns in red: Contact IBU for this information. 5) Upload the fastq files

Instructions are here: https://ena-docs.readthedocs.io/en/latest/submit/fileprep/upload.html

Wait until the sample upload is complete before going to the next step!

**For sequencing data analysed by IBU**: Contact IBU to do the file upload for you. We will let you know as soon as it is complete and you can continue with the final step of the submission.

#### 6) Complete the submission

Submit

