Operating rules of the UAT Sanger Sequencing Service

1. APROVACIÓ:

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This document describes the **operating rules of the Sanger Sequencing Service** managed by UAT and outsourced to the external Provider **Macrogen**, as well as the **sample requirements for Macrogen sequencing.**

1. OPERATING RULES OF THE SEQUENCING SERVICE

- The service must be requested through the UAT application in the VHIR webpage, as done for internal services (Service Request Menu -> Genomics Platform-> Sequencing Service->Service Type). The appropriate template for each service type must be filled in along with the service request.

- **Both samples and primers must fulfill the conditions specified below,** otherwise neither Macrogen nor the UAT will be held responsible for the results and the samples will be invoiced independently of the results.

- Samples should be left in the **fridge located at the Metabolomics lab**, in the box labeled "Macrogen."

- Samples will be sent to Macrogen every **Monday and Wednesday,** so must be delivered to the UAT **before 12 a.m.** of each one of these days. In the case it is an urgent sample, it could be sent any day of the week

- The results will be placed inside each research group/user folder in the Q: server. The **estimated delivery time** for individual sequences is 48 hours and 4-6 working days for plates. Results from fragment analysis take 1 week approximately.

- Sequences will be invoiced as internal charges for VHIR users, as any other UAT service.

- In addition to standard sequencing and fragment analysis, Macrogen offers **additional services** (i.e. "primer walking", sequencing of difficult templates, plasmid and PCR products purification) that we can manage from the UAT. Users interested in performing any of these services should contact the Technician in charge.

- **Standard services fees** are specified in the official rates list published in the UAT webpage.

- UAT is committed to monitor the proper functioning of the service supplied by the external Provider, to check that the results accomplish the expected quality and to periodically re-evaluate the Provider. If any incidence with the service occurs, UAT staff will be in charge of handling it.

2. SAMPLE CONDITIONS FOR SEQUENCING

It is extremely important that samples meet the below indicated criteria, otherwise the quality of the sequencing results cannot be guaranteed neither by Macrogen nor by the UAT.

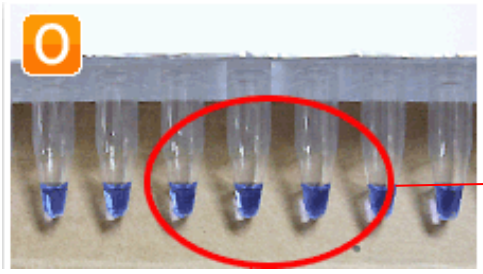
Two sample formats are available:

- ✓ **1.5 ml tubes** (for single samples).

- ✓ **96-well plates.** Plates should be **"out-skirted"** to prevent physical damage during shipping and tightly sealed using an **adhesive cover** after primer addition to avoid cross-contamination and sample evaporation. Samples should be prepared to avoid any well-to-well concentration or size difference to obtain quality results.

  Plates will be invoiced as a single unit independently of the number of samples included.

Out-skirted well should be used to avoid any physical damage during shipment



Enough volume, constant concentration, tight sealing, are important to obtain good results

Tubes or plates containing samples must be **clearly labeled** and match the data introduced in the sequencing form of the service request.

2.1 EZ-sequencing (tube or 96-plate)

DNA samples and primers have to be premixed before shipping to Macrogen

- **Sequencing with users' own primers:** please bring to the UAT your premixed DNA plus primer as indicated below.

- **Sequencing with universal primers:** we will manage them at the UAT, so users are only asked to bring the DNA samples. The universal primer specific for each sample must be indicated in the "Primer name" column of the template included in the service request.

- In particular cases, e.g. for those users that most frequently use a specific primer, the Technician in charge can store and handle it at the UAT.

- **Each sample must have a final volume of 10 µl (5 µl of DNA+5 µl of primer)**, as indicated below.

- **Sample requirements**

Starting material	Concentration	Volum	Solvent
Purified PCR product	**Over 700 bp:** higher than 50 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)
	300 – 700 bp: 25-50 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)
	Less than 300 bp: 10-15 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)
Plasmid	**100 ng/µl** (plasmids 2-4kb in length. If plasmids are larger, please increase concentration accordingly)	5 µl	Distilled H_2O or 10 mM Tris (no TE)

Sequencing primers: 5-10 pmole/uL

The success of automated sequencing critically depends on having high purity template in the correct concentration. **Both plasmid and amplicons must be purified:**

- For **plasmids**, available commercial methods are strongly recommended (Qiagen Mini and MidiPrep-like kits).

- **PCR products** must be free of contaminants, unused primers or dNTPs. PCR templates that do not undergo any kind of post PCR clean up are not suitable for sequencing and are prone to yield unusable sequencing data. Commercial cleaning kits such as Qiagen Gel Extraction Kit or PCR-Clean Up Kit usually produce good results. It is highly recommended to check the PCR template in a gel to confirm the presence of a specific product with the right size.

Sequencers are able to handle a wide range of DNA concentrations; however, scarce DNA input will affect negatively data quality. Using UV absorbance-based methods to quantify DNA solutions often produces widely inaccurate results. Alternatively, a good way to quantify DNA is to run an aliquot in a minigel and compare its intensity with a known concentration control (e.g. a DNA ladder).

2.2 STD sequencing

The DNA sample and the sequencing primer must go separately, each in one 1.5 mL tube correctly identified.

Only the DNA sample tube have to be labelled with the UAT code.

- **Sample requirements**

Starting material	Concentration	Volum	Solvent
Purified PCR product	**Over 700 bp:** higher than 50 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)
	300 – 700 bp: 25-50 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)
	Less than 300 bp: 10-15 ng/uL	5uL	Distilled H_2O or 10 mM Tris (no TE)

Plasmid	**100 ng/µl** (plasmids 2-4kb in length. If plasmids are larger, please increase concentration accordingly)	5 µl	Distilled H_2O or 10 mM Tris (no TE)

Sequencing primers: 5-10 pmole/uL

Please be advised that quantification by gel electrophoresis rather than Nanodrop is strongly recommended.

- **Primer requirements.**

5 µl of a 5 pmol/µl primer solution are required per sample. Each primer must be premixed with its matching DNA sample in the same tube/well **(10 µl final volume).**

The following **universal primers** are **available** at the UAT **without any additional cost:**

Primer Name	Primer Sequence (5'-->3')	Nº Bases
AOX1 For	GAC TGG TTC CAA TTG ACA AGC	21
pBAD FP	ATG CCA TAG CAT TTT TAT CCA	21
pBAD-R	GAT TTA ATC TGT ATC AGG	18
BGH-R	TAG AAG GCA CAG TCG AGG	18
Bluescript_KS	TCG AGG TCG ACG GTA TC	17
CMV-F	CGC AAA TGG GCG GTA GGC GTG	21
CYC1 Rev	GCG TGA ATG TAA GCG TGA C	19
DsRed1-N	GTA CTG GAA CTG GGG GGA CAG	21
DsRed1-C	AGC TGG ACA TCA CCT CCC ACA ACG	24
pET-RP	CTA GTT ATT GCT CAG CGG	18
EBV RP	GTG GTT TGT CCA AAC TCA TC	20
EGFP C	CAT GGT CCT GCT GGA GTT CGT G	22
EGFP CR	CGT CCA TGC CGA GAG TG	17
EGFP N	CGT CGC CGT CCA GCT CGA CCA G	22
p-EGFP FP	TTT AGT GAA CCG TCA GAT C	19
p-Fast Bac-Rev	CAA ATG TGG TAT GGC TGA TT	20
p-Fast-Bac-F	GGA TTA TTC ATA CCG TCC CA	20
Gal4AD	TACCACTACAATGGATG	17
GL Primer 1	TGT ATC TTA TGG TAC TGT AAC TG	23
GL primer 2	CTT TAT GTT TTT GGC GTC TTC CA	23
pGEX3	GGA GCT GCA TGT GTC AGA GG	20
PGEX5	GGC AAG CCA CGT TTG GTG	18
ITS 1	TCC GTA GGT GAA CCT GCG G	19

ITS 2	GCT GCG TTC TTC ATC GAT GC	20
ITS3	GCA TCG ATG AAG AAC GCA GC	20
ITS4	TCC TCC GCT TAT TGA TAT GC	20
pJET 1-2 Fwd	CGA CTC ACT ATA GGG AGA GCG GC	23
pJET 1-2 Rev	AAG AAC ATC GAT TTT CCA TGG CAG	24
LR7	TAC TAC CAC CAA GAT CT	17
LR0R	ACC CGC TGA ACT TAA GC	17
LCO 1490	GGTCAA CAA ATC ATA AAG ATA TTG G	25
M13 F	GTA AAA CGA CGG CCA GT	17
M13 R	GCG GAT AAC AAT TTC ACA CAG G	22
M13 F Puc	GTT TTC CCA GTC ACG AC	17
MT for	CAT CTC AGT GCA ACT AAA	18
pMaIE 5 p	TCA GAC TGT CGA TGA AGC	18
NS1	GTA GTC ATA TGC TTG TCTC	18
pQE-F	CCC GAA AAG TGC CAC CTG	18
pQE-R	GTT CTG AGG TCA TTA CTG G	19
pRH Fwd	CTG TCT CTA TAC TCC CCT ATA G	22
RV Primer 4	GAC GAT AGT CAT GCC CCG CG	20
RV Primer 3	CTA GCA AAA TAG GCT GTC CC	20
pRH-F	CTGTCTCTATACTCCCCTATAG	22
SP6	ATT TAG GTG ACA CTA TAG	18
SV40 PRO	GCC CCT AAC TCC GCC CAT CC	20
SV40-pArev	CCTCTACAAATGTGGTATGG	20
T3	AAT TAA CCC TCA CTA AAG GG	20
T7	AAT ACG ACT CAC TAT AG	17
T7 Terminator	GCT AGT TAT TGC TCA GCG G	19
T7 PRO	TAA TAC GAC TCA CTA TAG GG	20
T7 EEV	ATG TCG TAA TAA CCC CGC CCC G	22
27F	AGA GTT TGA TCM TGG CTC AG	20
800R	TAC CAG GGT ATC TAA TCC	18
907R	CCG TCA ATT CMT TTR AGT TT	20

Macrogen offers more universal primers at no extra charge, so this list is under continous update. The complete list can be checked at the Macrogen website. Users interested in sequencing with universal primers not included in this list should contact UAT staff before bringing samples.

Primers must be diluted in distilled H_2O at the stated concentration and meet the following **requirements:**

- High purity (free of salts, EDTA or other contaminants). Always avoid TE buffer.
- No secondary priming sites
- No mismatches
- A length of 18-25 bases
- GC content between 40% and 60%
- Tm between 55°C and 60°C
- No significant hairpins (>3bp)

2.1 Fragment Analysis

- **Sample requirements:** 20 µl of a 50 ng/µl DNA solution are required per sample.

- **Sample format:** 1.5 ml tubes or 96-well plates ("out-skirted").

- The **Internal Standard Size Marker** must be selected in the service request and indicated in the template, according to the following table:

Dye Set	DS-30	DS-33	DS-33	
Filter Set	D	G5	G5	
Blue	6-FAM	6-FAM	6-FAM	Sample labeling dyes
Green	HEX	VIC	VIC	
Yellow	NED	NED	NED	
Red	ROX	PET	PET	
Orange	-	LIZ	LIZ	
Internal standard size marker (Maximum detection size)	400HD (400bp)	500LIZ (500bp)	1200LIZ	