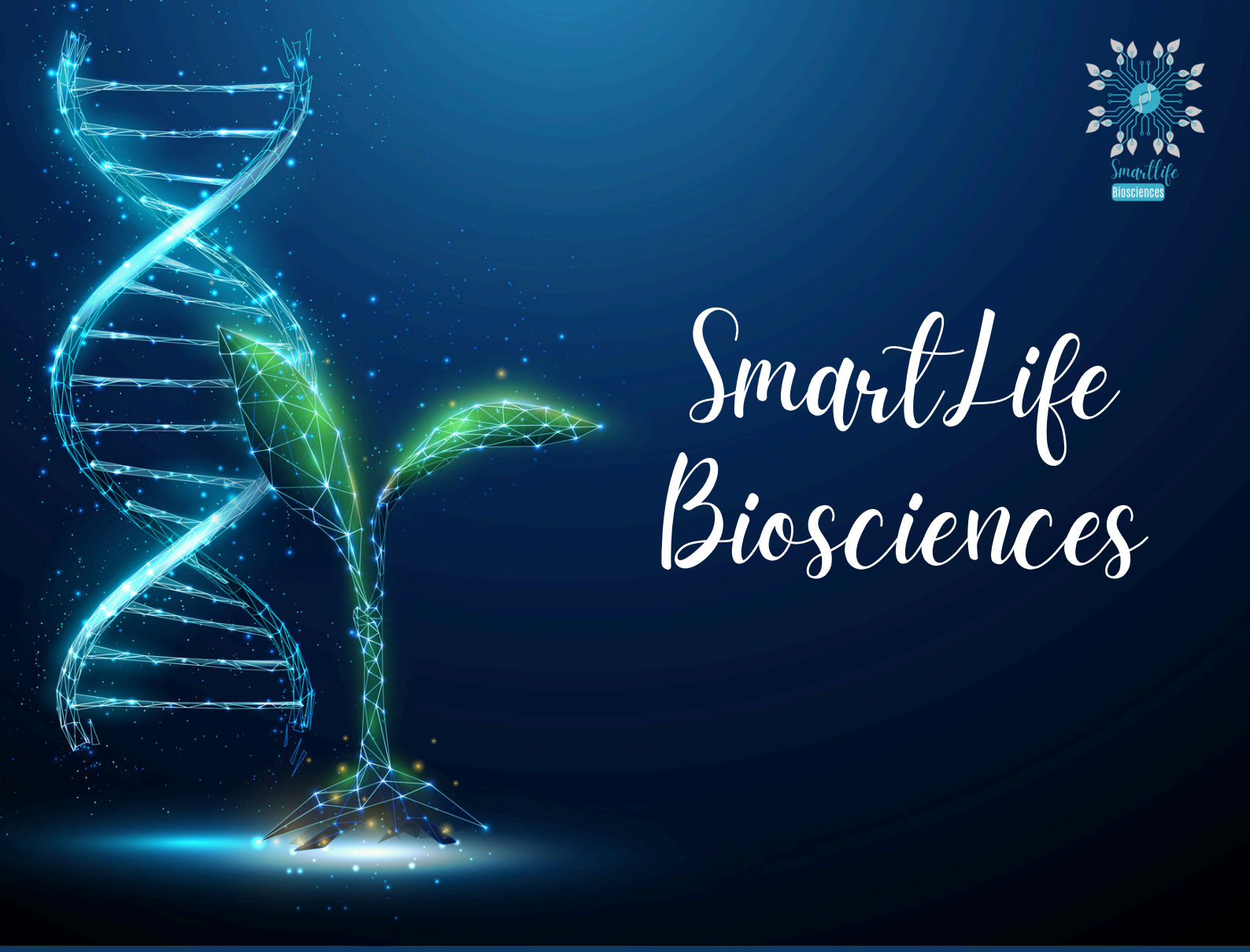

SmartLife Biosciences

LONG READ SMART SEQUENCING

User Guide

SMARTLIFE BIOSCIENCES

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SUBMISSION INSTRUCTIONS

ORDER ONLINE

01. Submission Instructions

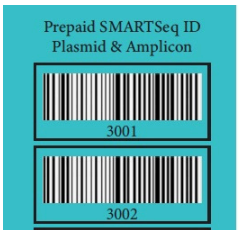
1. Create your SMART account

2. Place your order online

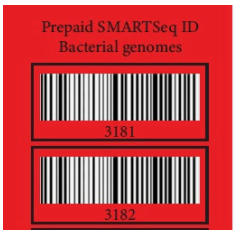
3. Fill in the sample sheet

4. Label your samples

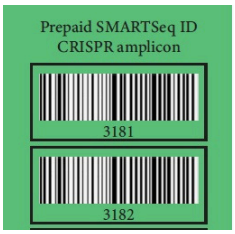
Blue label for Plasmids and Amplicons



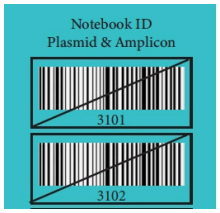
Red label for Bacterial Genomes



Green label for CRIPR plasmid and amplicon



5. Keep the duplicate crossed out label for the lab notebook



6. Place labelled samples in your SMART box and specify your order number and date defined in the order confirmation.

7. Request easySmart pick up by SMS

Marseille

Sarah Durand

07-84-38-59-40

Montpellier

Lukas Brichet

06-08-52-57-10

02. Submission Recommendations

- You can place an order for a **single sample** or **multiple samples**.
- Samples should be sent in 1.5 mL Eppendorf tubes or 96 well plate (full or half plate).
- DO NOT includ any extra sample that is not listed in your order details.
- If you need to change the number of samples, please contact us at support@smartlifebiosciences.com. We'll cancel your existing order and ask you to submit a new order with an order comment.
- DO NOT include any tubes that contain just water (no sample).
- DO NOT put samples from multiple orders within the same box. Use one box per order and sepcify the order # on each box. In case you're placing two orders at the same time, please use two boxes.

SAMPLE PREP RECOMMENDATIONS

PREPARE YOUR SAMPLES

A. Plasmid

The whole plasmid sequencing service is intended for the full-length sequencing and annotation of clonal circular plasmid DNA between 2.5 kb and 300 kb in length. This service is performed using the newest long-read sequencing technology from Oxford Nanopore Technologies (ONT) based on the most accurate R10.4.1 flow cells (raw data is > 99% accurate).

Our **SMART sequencing** service requires 600 ng (standard plasmid), 1000 ng (big plasmid), or 2000 ng (huge plasmid) of circular, double-stranded DNA, normalized to the specific concentration listed in the table below :

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
STANDARD	2,5 to 25 kb	30 ng/µL	⩾ 20 µL
BIG	25 to 125 kb	50 ng/µL	⩾ 20 µL
HUGE	125 to 300 kb	50 ng/µL	⩾ 40 µL

B. Amplicon & Linear DNA

The linear DNA/amplicon sequencing service is intended for the sequencing of clonal linear DNA from 600 bp to 125 kb in length. This service is performed using the newest long-read sequencing technology from Oxford Nanopore Technologies (ONT) based on the most accurate R10.4.1 flow cells (raw data is >99% accurate).

Our **SMART sequencing service** requires 600 ng (standard linear DNA / amplicon between 600 bp and 25 kb), or 1000 ng (big linear DNA / amplicon between 25 and 75 bp and huge linear DNA / amplicon between 75 and 125 kb) of linear double-stranded DNA, normalized to the specific concentration listed in the table below :.

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
SMALL	450 bp to 25 kb	30 ng/µL	⩾ 20 µL
BIG	25 to 75 kb	50 ng/µL	⩾ 20 µL
HUGE	75 to 125 kb	50 ng/µL	⩾ 20 µL

C. Bacterial / viral and YeastGenome

The genome sequencing service is intended for the full-length sequencing of a whole bacterial, viral or yeast genome up to 10 000 kb in length. This service is performed using the newest long-read sequencing technology from Oxford Nanopore Technologies (ONT) based on the most accurate R10.4.1 flow cells (raw data is > 99% accurate).

Our **SMART sequencing** service requires double-stranded DNA, purified and normalized to the specific concentration listed in the table below :

BACTERIAL

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
SMALL	Up to 7 Mb	100 ng/µL	⩾ 20 µL
BIG	7 Mb to 10 Mb	100 ng/µL	⩾ 20 µL
HUGE	10 Mb to 102Mb	110 ng/µL	⩾ 20 µL

VIRAL

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
SMALL	2,5 to 25 kb	30 ng/µL	⩾ 20 µL
BIG	25 to 125 kb	50 ng/µL	⩾ 20 µL
HUGE	125 to 300 kb	50 ng/µL	⩾ 40 µL

YEAST

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
SMALL	Up to 7 Mb	100 ng/µL	⩾ 20 µL
BIG	7 Mb to 10 Mb	100 ng/µL	⩾ 20 µL
HUGE	10 Mb to 102Mb	110 ng/µL	⩾ 20 µL

D. CRISPR / Cas9

The CRISPR/ Cas9 long read sequencing service is intended to the analysis of complete sequencing of circular construct to check sgRNA vectors library and/or long read deep (100X) sequencing of amplicon of the CRISPR targeted region. You can choose between two services depending on your priorities :

- Analyze predicted target and off-target loci
- Analyse a library of plasmids containing sgRNAs to determine which sgRNAs are present in your constructs and what their frequency is.

This service is performed using the newest long-read sequencing technology from Oxford Nanopore Technologies (ONT) based on the most accurate R10.4.1 flow cells (raw data is >99% accurate).

Our **SMART sequencing** service requires double-stranded DNA, purified and normalized to the specific concentration listed in the table below :

CRISPR

CATEGORY	SIZE	CONCENTRATION	MINIMUM VOLUME
CRISPR Vector with sgRNAs	2,5 to 25 kb	30 ng/µL	⩾ 20 µL
CRISPR Amplicon REGULAR (30x)	600 bp to 25 kb	30 ng/µL	⩾ 20 µL
CRISPR Amplicon DEEP (100x)	600 bp to 25 kb	80 ng/µL	⩾ 40 µL

04. General Recommendations

1 Do not mix any primer into your sample

DNA samples are sequenced WITHOUT primers.

2 Submit purified DNA

DNA should be eluted in elution buffer (10 mM Tris, pH 8.5) or nuclease-free water.
Avoid buffers containing EDTA (e.g. TE or AE buffer) whenever possible.
Avoid buffers containing EDTA (e.g. TE or AE buffer) whenever possible

3 Check DNA concentration

Sending samples at too high or too low concentration may adversely affect the library prep and/or sequencing reactions, possibly resulting in sequencing failure.

Quantify your samples with **Qubit** or equivalent fluorometric method.
Do not quantify samples using Nanodrop.
Nanodrop and equivalent spectrophotometric methods are not appropriate to quantify DNA.

4 Verify the integrity of your DNA sample

Degraded or fragmented DNA are likely to result in sequencing failure by yielding no consensus due to lack of full-length sequencing reads.
DNA fragment integrity check can be done by full length plasmid size verification via gel electrophoresis.

5 Verify the purity

Purity of your samples can be measured with spectrophotometric methods like Nanodrop (260/280 above 1.8 and 260/230 between 2.0-2.2).

Do not refer to the DNA concentration reported by Nanodrop to evaluate DNA quantification).

Samples should only contain copies of a single clonal plasmid molecule. Sending mixtures of molecular species will give mixed results and is at your own risk.

TO REMEMBER

For best results, samples should NOT contain any of the following:

- RNA (RNase treatment is recommended during extraction)

- Denaturants (guanidinium salts, phenol, etc.) or detergents (SDS, Triton-X100, etc.)

- Residual contaminants from the organism (heme, humic acid, polyphenols, etc.)

- Insoluble material, colors, or cloudiness

05. Our Failure Policy

Definition of failure

When we fail to produce a consensus sequence with at least 10x coverage, we consider that the sample failed. This definition is valid for long read SMARTSeq routine sequencing services of plasmids & amplicons PCR . This definition doesn't apply to bacterial genomes & customized projects with establishment phase protocol. During the establishment phase protocol, the feasibility of the project is evaluated during a first milestone. If the feasibility of the project is confirmed by our experts team or if the customer is willing to proceed and accept the risk of failure, the project will be accepted by SMARTlife Biosciences and performed. For bacterial genomes sequencing & customized projects, failure policy doesn't apply.

Reasons of failure

It's rare that sample fails, but as we're working with biological material, it can happen. Most common reasons for failure are :

1. **Samples not prepared at required DNA concentration.**
You can identify it by low read counts reported in the raw read length histogram. We do not recommend to blast such consensus as noise may exceed true sequencing signal and would lead to aberrant blast. We strongly recommend using a Qubit or equivalent to quantify your DNA. Please do not use a Nanodrop as it leads to biased quantification.

2. **Samples contain a mixture of plasmid species** and/or fragmented genomic DNA or fragmented plasmids.
If you face such casen you'll vizualise a wide range of read lengths reported in the raw read length histogram.

FAILURE POLICY

Our Policy

In case of failure, our Technical Support Team will be in touch with you in one business day to discuss what we could recommend you to improve your result. As resequencing the same sample at same conditions do not lead to better result, we offer you to re-prepare fresh starting material to re-sequence it.
In case you can't prepare fresh sample or in case the sample fails a second time, we would consider that the biological nature of your material makes it unsequenceable. We unfortunately have to charge for failed samples, since we invest 2x more time and resources on them than we do on successes.

SMART LIFE
BIOSCIENCES

We warmly thank you for your trust in our scientific expertise, for your loyalty to our services and for your Smart contribution to the reduction of carbone footprints by outsourcing routine sequencing to our Smart local laboratory established in your city.

With our very best regards,

SmartLife Biosciences

A TECHNICAL QUESTION ?

support@smartlifebiosciences.com

www.smartlifebiosciences.com

10 GOOD Reasons
to place a smart Order

1. The combination of SMART sequencing technology with mobile technology eliminates the need for sample transportation.

2. Results are delivery very fast, enabling you to rapidly identify pathogens, monitor microbial communities, detect genetic variants. It can be used for pathogens surveillance, viral genome sequencing, metagenomics studies, biodiversity monitoring, and more.

3. In case of sequencing failure, we can react quickly, enabling prompt decision making and action.

4. You receive ultra long reads which provide you a significant advantage in analyzing complex genomes, structural variations or repetitive regions.

5. SMART sequencing is a technology that generates multiple passes for each DNA template allowing error correction and consensus generation, improving the accuracy of base calls, including those within repetitive regions. This increased accuracy helps in resolving the true sequence in repetitive or homopolymeric regions and reduces potential ambiguities.

6. SMART sequencing improves the accuracy and resolution of genomic analyses and increases the chance of capturing critical genetic information.

7. When placing a Smart order, your local order contributes to reduce carbon emissions associated with transportation. You minimize the environmental impact of shipping samples over long distances. This aligns with sustainable practices and demonstrates your committment to minimizing your carbon footprint.

8. Proximity to your Smart local technical Team facilitates direct and frequent communication, enabling better collaboration and understanding of your specific requirements. You secure more efficient interactions and the potential for tailored solutions to meet your needs.

9. You are supporting local economy, promotes local businesses and job creation in your region.

10. When investing in local Smart teams and Smart services, you're contributing to local job opportunities of PhD students. and to the sustainability of your local research community, fostering a mutual beneficial relationship.