 **SEQUENCING SERVICE REQUEST FORM**

**DNA/RNA SEQUENCING - *GridION/PromethION***

High throughput sequencing facility

Find us on our website <https://www.i2bc.paris-saclay.fr/sequencing/ng-sequencing/addon-ng-sequencing/#request>

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| **Contact info** |
| Date : First name, last name :  Institution :  Laboratory :  Team :  Phone number :  E-mail address : |
| **Request for agreement** (Agreement required) |
| The quality of sequences generated using Oxford Nanopore Technology (ONT) are directly correlated with the quality of samples. For this reason, the sequencing service cannot take responsability for obtaines results either in terms of throughput or read length.  By ticking this box, I confirm having read the above information and accept the following condition : the sequencing service cannot be held responsable for obtained sequencing results. |
| **Detailed description of your project** (Any information may be useful) |
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| **Source of funding (Purchase order)** |
| Please specify the type of purchase order that will be supplied. This information is required to prepare the quotation.  **Purchase order :**  CNRS  Université Paris-Saclay/CEA Paris-Saclay/INRAE Jouy-en-Josas    Other French Academic  Private or Foreign Academic (please specify) : |
| **Informations about the samples** (indicate differences among samples, if any. Provide information for each sample separately in this case) |
| Organism :  Number of samples (number of libraries to prepare) :  Number of libraries to multiplex together on one run (DNA only) : |
| **Samples** |
| Declaration of harmlessness (required):  You acknowledge that your samples can be handled safely.  Sample preparation :  **You can prepare your samples following the ‘Guidelines for sample preparation’ below.**  In this document you will find the quantities and quality of samples needed for each type of library.  Upon their arrival, we will check your samples and we will contact you if there is a problem.  Sample conservation (required):  Once deposited at our facility, your samples/libraries will be kept at 4°C, unless you ask otherwise.  After the library preparation, what should we do with your samples?  we keep them *(by default our facility keeps the samples for 3 years, then discard them.)*  we discard them  you come and take them back |
| **Type of sequencing** |
| **GridION (GridION/MinION Flowcell)** *(Around 4-10 Gbases, 250k-3000k reads)*  DNA Sequencing and Basecalling (10.4.1 Flowcell)  RNA Sequencing and Basecalling (RNA Flowcell)  **P2 solo (PromethION Flowcell)** *(Around 10-80 Gbases, 106 – 107 reads)*  DNA Sequencing and Basecalling (10.4.1 Flowcell)  RNA Sequencing and Basecalling (RNA Flowcell)  **Enrichment by Adaptive Sampling**  *(for this option, please contact us beforehand to configure the .fasta and .bed files)* |
| **Number of Flowcells** (1FC = 1 run) |
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| **Estimated date when your samples are ready** |
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| **Bioinformatics (required)** |
| Default service: we provide FastQ files  Raw electrical signal files (pod5 files)  *Please note: these files are very large (up to 1 To / run). Due to their size, we cannot send them or keep them for longer than a week. Please provide us with an external hard drive to recover these files (sending/returning at the user's expense).*  Basecall files for modified bases. Modification to identify (5mC on CpG, 5mC any context, 6mA any context) :  *Please note: these files are very large. We cannot send them due to their size. Please provide us with an external hard drive to retrieve these files (sending/returning at the user's expense).*  Additional analyses:  Mapping on reference genome. **Please specify all necessary informations on reference genome:**   * Version : * Download URL (UCSC, Specific site) :   D*e novo* genome assembly  Please note: In case of publication of this assembly, the facility should be associated as an author.  Annotation  Mapping of identified modifications on reference genome.   * Version : * Download site (UCSC, specific site):   Others: |

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| **ADDITIONAL INFORMATION** |
| **VALORIZATION**  The technical contribution of the platform must be recognized and mentioned clearly in the publications or reports in the following way: “We acknowledge the sequencing and bioinformatics expertise of the I2BC High-throughput sequencing facility, supported by France Génomique (funded by the French National Program "Investissement d’Avenir" ANR-10-INBS-09)”. A copy of the communication/publication will have to be sent (PDF) to the staff of the platform (i2bc-sequencage@i2bc.paris-saclay.fr).  The intellectual property of the inventors will be protected in agreement with the general rules in force at CNRS.  Know-how and knowledge implemented by the Platform(s) I2BC to carry out the performances of service remain the property of CNRS; consequently any improvement of know-how will remain the property of CNRS.  The property of the results of the services belongs to the customer.  **INVOICING**  Following the reception of this form, an estimate will be sent to you by e-mail. Thanks for forwarding to us a copy of the estimate signed for agreement as well as the purchase order corresponding to this estimate before the beginning of your service (no service will be able to start without preliminary reception of the purchase order). An invoice will be emitted after the complete realization of the ordered services (average time 3 months after returned results).  **PRIVACY**  Each part begins not to publish nor to reveal some way that it either the scientific information, technical or commercial of the other Part, and in particular former knowledge belonging to the other Part of which it could be aware at the time of the execution of the services and this, as long as this information is not accessible to the public. This commitment will remain in force during five years (5 years) as from the date of emission of the invoice, notwithstanding the resolution of the order or the implementation of the avoidance clause of present the TERMS AND CONDITIONS (TERMS AND CONDITIONS detailed in the quotation).  Any publication or communication of the bearing Customer on the results of the services must indicate that these results were got by the Platform(s) I2BC. It should not in no case to reveal the know-how implemented by the Platform(s) I2BC. |

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| **Possible modifications following exchange with the user**  (to be completed by the platform only) |
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* + 1. **Guidelines for sample preparation**

1. **Before preparing your material for sequencing, please carefully read these guidelines**
2. Samples must be in **1.5 or** **2mL tubes**.
3. Distinctly write the sample codes received with your quote **on the lid** of your tubes.
4. Please avoid using stickers.
5. Feel free to add any useful information on the side of the tube.
6. **We exclusively rely on the Qubit fluorometric quantitation system** (Thermo Fisher) for any nucleic acid quantification. Shortly after reception a Qubit quantification of your samples will be performed. Note that spectrometric methods (such as Nanodrop) often provide an overestimation of the concentration and are very sensitive to contaminants (chemicals or other nucleic acids).
7. **If your material does not follow the described criteria, please contact us before sending your samples.**
8. **Genomic DNA libraries:**
9. **High molecular weight genomic DNA extraction kits** are highly recommended.
10. Please provide us with **3 µg of genomic DNA**. The concentration should be at least of 20 ng/ µL (measured with Qubit), in Tris 10mM buffer (pH 7.5 – 8) or H2O.
11. **Please contact us if you can’t respect those conditions.**
12. **Do not use TE buffer or any buffer containing EDTA,** as it can inhibit some enzymatic reactions.
13. DNA **must be in a clear solution** without any precipitate.
14. DNA should be checked by running an aliquot on agarose gel.
15. If you have a **Field Inversion Gel Electrophoresis System**, or any system who permit checking the high molecular weight of samples, please send us the results.
16. There should be no significant degradation or small size material visible on the gel.
17. **RNA Libraries:** 300 ng of polyA+ RNAs in H20. RIN should be >8