1. Investigator / Client Information

Name: ______________________ Date: ________________

PI/Lab: ______________________ Phone Number: ________________

Mailing address: ______________________

Email address: ______________________ @______________

Account to charge: ______________________

PI Signature: ______________________ (required)

You must either have the ticket signed to authorize the billing charges, or the PI must send an authorization email. Samples without authorization will not move forward.

2. Sample Information: Complete one ticket per sample OR one ticket per sample set to be multiplexed and run in one lane. Sample name(s) on the ticket should match name(s) on sample tube(s). When multiplexing a sample set with Illumina or your own-designed barcodes, each sample should be submitted in a separate tube. Please indicate the desired mixing ratio of the samples in the sample set. For example: 1:1, 2:1, etc.: ______________________. (Note: Default mixing is equimolar.)

Lane/Mix Name: ______________________ If a mix, choose a name for the sample set. Median Insert Size: __________

Approximate Library Concentration: ______________________ Volume Submitted: ______________________

Sample Set Names: Please attach an Excel sheet with each sample name, its index, its median insert size, the approximate library concentration, and the volume submitted. Only if you are sending a mix or multiple samples. You just need ONE ticket per LANE. The pipeline will only accept names <= 20 characters.

Sample Classification: Is the sample(s) infectious or pathogenic to humans? YES or NO. If yes, please describe the material(s) and any potential biohazards. ______________________

*Recommended Library Concentration and Volume for Submission: 20 microliters of a 10 - 20nM solution. Please note: if you submit less than 10 microliters of your sample, there may be insufficient volume for subsequent analysis runs!

3. Library Adaptors Used:

Please indicate the linker/adapter set used for library construction:

If you aren't sure, list the kit name and part# used to build the library. If you used a custom sequence, please say so.

No Index in Adapters ______________________

Illumina Index in Adapters ______________________

Other Vendor Kit/Index Set ______________________

___(old) Illumina PE ______________________

___TruSeq DNA/RNA/Exome/PE ______________________

___TruSeq Small RNA ______________________

___TruSeq Stranded RNA ______________________

___TruSeq Targeted Capture ______________________

___Nextera ______________________

___Chromium 10X Genomics* Version ______________________

___Takara/Clontech (Name, P/N:__________________)

___NEB (Name, P/N:__________________)

___Other (Name, P/N:__________________)

___Custom (Describe:__________________)

* 10X Genomics samples contain 4 indices per P7 adapter/plate well. Please do not mix 10X Gen. samples unless all four index sequences are different between samples. If you do not know which indices are in the mix, please list the ID#/adapter wells with your index information.

4. Multiplexed/Indexed/Barcoded Run: (Please answer all that apply.) Index read analysis is required for Illumina-type indexing, even if you intend to perform sorting as part of your own analysis. You will only be delivered index sequencing reads for the indexing that was requested here. Note: There is an additional charge for the index read.

Please see the Example Ticket if you need an explanation of the different types. See final page.

MULTIPLEX, i7 End - Does your sample have an Illumina-style index at the i7 (downstream) position? Length:________bp

MULTIPLEX, i5 End - Does your sample have an Illumina-style index at the i5 (upstream) position? Length:________bp

* In order to sort your indices, we need sample names and corresponding index sequences. Please list on the back, attach, or email. If you aren't sure, list the kit name and part# used to build the library. If you used a custom sequence, please say so.

UNSORTED - Do you want your results to NOT be demultiplexed, even though they contain Illumina-style indices?

MERGED - Results will be sorted (unless you checked 'unsorted' above), and then merged back into a single fastq file.

RANDOM - There are random bases in your index (in GuideSeq). The Core's pipeline can read these but not sort them.

INLINE BARCODES - Does your sample have in-line (part of the insert) barcodes or indices? (We cannot sort these for you.) If yes, are they base-balanced? YES or NO Please give location and length or attach the information:

INLINE LINKERS - Does your sample have linkers, primers, or any other non-random sequences in the insert? If yes, please give location and length or attach a sheet with the information:

Adding an i5 index is what is meant by Dual Indexing. It is unrelated to Single-Read and Paired-End run types.

Request for Library Construction Information: To facilitate processing and workflow, please submit a library design schematic, reference, results from topo cloning/sequencing (when available), and/or other QC analysis performed prior to library submission. If you made any modification to the library construction design (e.g. added linkers, cloned out of a vector, etc.) you must submit a schematic. If using a custom primer for side 1, you must submit a schematic and topo cloning results. Please note that the use of a custom primer on side 2 is only feasible on the MiSeq instrument. If you did not perform any pre-run QC analysis such as sequencing topo clones, MiSeq pre-check, or library profiling, you will be ineligible for a re-run should your library(s) fail during cluster formation or the actual sequence analysis run.
5. Selection of Sequence Analysis Run Type:
Single Read (SR) is sequencing from one end of the library insert (e.g., a SR100 is 100 bases read on side 1). Paired End (PE) Reads are sequenced from both ends of the library fragment (e.g., a PE50 is 50 bases read on side 1 + 50 bases read on side 2).

- **HiSeq 4000**
  - Single Read 50 bases
  - Single Read 100 bases
  - Paired End Read 50 bases
  - Paired End 50 x 100 bases
    (Trim for Chromium 10X pipeline to
    26 x 98 bases
    28 x 98 bases
    x bases)
  - Paired End Read 100 bases
  - Paired End Read 150 bases

- **MiSeq**
  - Single Read 50 bases
  - Paired End Read 25 bases
  - Single Read 100 bases
  - Paired End Read 100 bases
  - Single Read 150 bases
  - Paired End Read 150 bases
  - Paired End Read 250 bases
  - Paired End Read 300 bases
  - Asymmetric Read x bases

**FIRST AVAILABLE**
If pressed for time, check all run types that would be appropriate. Your libraries will be assigned to the first run available. The cost of the type of run used will be charged accordingly.

Ex: 100x0 or 25x125. R1 length must be $\geq$ 25, and R2 length must be 0 or $\geq$ 25.

Do you want the *PhiX* DNA control added to your sample? Yes, circle one: 5%, 10%, 15%, or 20%. This addition is required for libraries with low sequence diversity/complexity (such as Chromium 10X) to ensure the base balance needed for optimal imaging.

**Please Note:** Based on the information you provide, should we deem it necessary, we will automatically add the appropriate % of *PhiX* DNA to your sample(s).

6. Data Delivery Information
The resulting data files can be quite large in size; the DSCL delivers the entire data set generated. Please make arrangements for the mode of data transfer before sample submission. Data should be retrieved within five business days of notification, unless other arrangements are made in advance. We do not routinely archive analysis run data. However, we offer a data archive option and data recovery service at an hourly fee. If data archiving is required, you must notify the DSCL within the same five business days of notification. For UMass investigators, the default mode for data delivery is to the pick-up area on the Green High Performance Cluster. For all non-UMass clients, your data can be uploaded to an outside server (using an SFTP) or transferred to an external drive and shipped overnight.

Please contact DeepSequencingCoreLabs@umassmed.edu to arrange for archiving or retrieval.

7. Whom should the DSCL contact to arrange the transfer of data?
   Name: ___________________________ Email Address: ___________________________

8. Whom should we notify when the data is ready?
   Name: ___________________________ Email Address: ___________________________
   Name: ___________________________ Email Address: ___________________________

9. Payment Policy
Sample processing requires time and reagents. Clients withdrawing samples that fail the QC process or prior to the analysis run will be charged a fee to recover the assay costs. For the return of samples post-run analysis, the client will be charged a fee per sample. In the event of a reagent or equipment failure, samples will be re-run at no additional charge. Payment for services rendered should occur in a timely fashion.

**Questions? Contact us at** [DeepSequencingCoreLabs@umassmed.edu](mailto:DeepSequencingCoreLabs@umassmed.edu)

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**DSCL Notes:**

Samples should be shipped overnight for delivery on Monday through Thursday.

**Ship to:**

Drs. E. Kittler / M. L. Zapp
UMass Medical School, DSCL
222 Maple Avenue
Reed Rose Gordon Building, Room 141
Shrewsbury MA 01545 (508-856-4787)
The i5 index is read AFTER Read 1 and the i7 index. Reading an i5 index in addition to the i7 index is what we call Dual Indexing. It does not matter if you want a Single-End or Paired-End run; you can read both indices anyway. The i5 index read may or may not require a sequencing primer, depending on the instrument used, chemistry version, and flowcell type. See Illumina document #15057455 for details.

Anything in this section between the sequencing primer sites will be part of your sequencing read(s). If you place "inline" barcodes here, the Core can read them, but not sort them for you. If you have any sequence here that is non-random or low-complexity, such as a linker, please note that on the ticket, because a base-balancer (i.e. phiX) may need to be added.

The i7 index is the standard single-index. If you only have one index, it should be this one. We will need to know the length of your index even if you don't want us to sort for you. Otherwise, there may not be sufficient number of chemistry cycles programmed for the run to read your indices through to the end. If you are planning a custom build that only has an index in the i5 position, please discuss it with the Core prior to submitting libraries.

This adapter is needed for both the i7 index read and Read 2.

The i5 index read may or may not require a sequencing primer, depending on the instrument used, chemistry version, and flowcell type. See Illumina document #15057455 for details.