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Core Lab Use Only v11.20

Sample ID# ____________________

BA File1 # ____________________

BA File2 # ____________________

Deep Sequencing Core Labs @ UMass Medical School

Sample Submission Ticket

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.

Sample preparation is key to optimal performance. The presence of carrier, partial PCR products, modified bases, *etc.* will adversely affect run performance. Please contact us if you have related questions.

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	_____Chromium 10X Genomics‡ Version ____________
	_____TruSeq Small RNA	_____Takara/Clontech (Name, P/N:_____________________)
	_____TruSeq Stranded RNA	_____NEB (Name, P/N:______________________________)
	_____TruSeq Targeted Capture	_____Other (Name, P/N:_____________________________)
	_____Nextera_______________	_____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.

Only apply if i7 / i5 above is checked:

_____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 (as in GuideSeq). The Core's pipeline can read these but not sort them.

_____INLINE BARCODES - Does your sample have in-line (between adapter and insert) barcodes or indices? (We cannot sort these.) If yes, are they base-balanced? **YES** or **NO** Please give location and length or attach thnformation:____________________

_____INLINE LINKERS or HOMOGENEITY - Does your sample have linkers, primers, or any other non-random sequences in the insert, or homogenous sequence such as a normalization control (ex: Active Motif)? Enter or attach info: ______________

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* (*4 lane min.)

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_____

R1 length must be >= 25, and R2 length must be 0 or >= 25.

_______ **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. You can number them in order of preference in case more than one option is available.

**Sample insert sizes >800bp are not guaranteed!*

Do you want the *PhiX* DNA control added to your sample?_________. If 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

DSCL Notes:

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

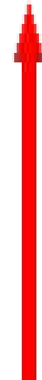
Ship to:

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)

5' P5 linker---i5 index---Read 1 adapter/seq primer site---inline barcodes/INSERT/non-random sequence---Read 2 and i7-index adapter/seq primer site---i7 index---P7 linker 3'

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.

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



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.

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.*