

HTSF Recommended Submission DNA and RNA and QAQC Values

Welcome! Below are guidelines on sample requirements when submitting to the HTSF.

You will find library input requirements for both RNA and DNA. Please follow the key below for information on our material type abbreviations.

Material Type Abbreviation Key

| Input material Types: | Abbreviation |
|-----------------------|--|
| TRN | Total RNA |
| RN PE | Total RNA made from Formalin fixed Paraffin Embedded tissues |
| RN FF | Total RNA made from Fresh Frozen Tosses |
| RN LMD | Total RNA from Laser Microdissection |
| WG_DN | Whole Genomic DNA |

RNA Library Preparation Input

| Total RNA Seq Protocols | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Ribosomal Depletion for: Organisms Compatible | Special notes |
|---|---------------------|----------------------------------|----------------------------------|---|----------------------|---|--|
| Truseq Total RNA Seq with RZG | TRN | 60 | 1750 | 29 | RIN > 3, DV200 > 30% | HMR | Stranded |
| | RN PE | | | | | | |
| | RN FF | | | | | | |
| | RN LMD | | | | | | |
| Roche Seq (Kapa) Total RNA Seq with RiboErase | TRN | 60 | 1750 | 29 | RIN > 3, DV200 > 30% | HMR | Stranded |
| | RN PE | | | | | | |
| | RN FF | | | | | | |
| Tecan Genomics Universal RNA Seq with NuQuant | TRN | 20 | 700 | 35 | RIN > 3, DV200 > 30% | **Inquire (Human, Mouse, arabidopsis thaliana, drosophila melanogaster) | Total RNA Assay, Non-Stranded, must be DNase Treated prior to prep (we can do this for an additional fee -- concentration will be lost). |

| | | | | | | | |
|-----------------------|-------|----|-----|------|----------------------|---|---|
| mRNA Seq with NuQuant | RN PE | | | | 30% | | |
| | RN FF | | | | | | |
| Tecan Genomics Solo | TRN | 25 | 10 | 0.40 | RIN > 3, DV200 > 30% | **Inquire (Human, Mouse, arabidopsis thaliana, drosophila melanogaster) | **not able to be automated Works down to 0.01ng Must be DNase Treated |
| | RN PE | | | | | | |
| | RN FF | | | | | | |
| Tecan Genomics Trio | TRN | 25 | 500 | 20 | RIN > 3, DV200 > 30% | **Inquire (Human, Mouse, arabidopsis thaliana, drosophila melanogaster) | **preferred low-input option for large number of samples Works down to 0.5 ng input Must be DNase Treated |
| | RN PE | | | | | | |
| | RN FF | | | | | | |

| <u>mRNA Seq Protocols</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Ribosomal Depletion for: Organisms Compatible | Special notes |
|--|---------------------|----------------------------------|----------------------------------|---|---------|---|---|
| Kapa mRNA | TRN | 60 | 1750 | 29 | RIN > 7 | | Stranded |
| | RN FF | | | | | | |
| Kapa Hyper mRNA | TRN | 25 | 250 | 10 | RIN > 7 | | Non-Stranded |
| | RN FF | | | | | | |
| SMART-HT + NexteraXT | TRN | 7 | 5 | 1 | RIN > 7 | | Non-Stranded |
| Tecan Genomics Universal mRNA Seq with NuQuant | TRN | 60 | 1750 | 29 | RIN > 7 | | Non-Stranded **not recommended** Many clients complain about results |
| Lexogen QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina | TRN | 15 | 500 | 33 | RIN > 4 | | 3' bias **not recommended due to high failure rate |
| | RN PE | | 10 | 0.7 | | | Works down to 0.1 ng |

| <u>RNA Exome Protocols</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Ribosomal Depletion for: Organisms Compatible | Special notes |
|----------------------------|---------------------|----------------------------------|----------------------------------|---|------------------|---|---------------|
| TruSeq RNA Exome | TRN | 25 | 250 | 10 | RIN > 3, DV200 > | | |
| | RN PE | | | | | | |

| | | | | | | | |
|------------------|--------|----|-----|----|----------------------|--|-----------------|
| TruSeq RNA Exome | RN FF | 25 | 250 | 10 | 30% | | |
| | RN LMD | | | | | | |
| SureSelect XT | TRN | 25 | 600 | 24 | RIN > 3, DV200 > 30% | | special request |
| | RN PE | | | | | | |
| | RN FF | | | | | | |

| <u>miRNA/small RNA Protocols</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Ribosomal Depletion for: Organisms Compatible | Special notes |
|------------------------------------|---------------------|----------------------------------|----------------------------------|---|---------|---|---|
| BioO Scientific Nextflex small RNA | TRN | 30 | 2000 | 67 | NA | | ** works down to 1 ng, so submission concentration can be very low. |
| | RN PE | | | 0.0 | | | |
| | RN FF | | | | | | |
| | RN LMD | | | | | | |
| HTG miRNA | TRN | 60 | 88 | 1.5 | NA | | **only detects KNOWN miRNA's |
| | RN PE | | | | | | |
| | RN FF | | | | | | |

| <u>Two Library Preps with Single Aliquot Submission</u> | Input Material Type | Min Submission Vol (ul) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Special notes |
|--|---------------------|----------------------------------|----------------------------------|---|----------------------|---------------|
| Total RNA (TruSeq Ribo Zero Gold) + Small RNA (BioO Next Flex) | TRN | 25 | 1.5ng | 2ug | RIN > 3, DV200 > 30% | |
| mRNA (Kapa) + Small RNA (BioO Next Flex) | TRN | 25 | 1.5ng | 2ug | RIN > 3, DV200 > 30% | |

DNA Library Preparation Input

| <u>DNA Protocols</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Special notes |
|----------------------|---------------------|----------------------------------|----------------------------------|---|--|--|
| Kapa Hyper (DNA) | Whole Genome DNA | 60 | 2000 | 33.33 | Optimal with full sized genome fragments | *Covaris Sonication Preferred prep for most applications |

| | | | | | | |
|--|------------------|----|------|-------|---|--|
| Tecan Genomics (NUGEN) Celero | Whole Genome DNA | 30 | 1500 | 50.00 | Optimal with full sized genome fragments | *Enzymatic Fragmentation Preferred for PCR-Free Assays |
| Thruplex DNA Seq | Whole Genome DNA | 25 | 30 | 1.20 | Optimal with full sized genome fragments | *Optimal for low-input for materials such as PCR Products, ChIP DNA, FAIRE-Seq DNA, Cut-and-Run, etc *works down to 0.050ng |
| Nextera XT | Whole Genome DNA | 15 | 3 | 0.20 | Optimal for small genomes, plasmids, cDNA | *fragmenting via tagmentation |
| NexteraFlex | Whole Genome DNA | 90 | 1500 | 16.67 | Optimal with full sized genome fragments | *fragmenting via tagmentation |

| <u>DNA Exome</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Special notes |
|--|----------------------------|---|---|--|--|---|
| Kapa Hyper + IDT xGEN Lockdown Probes | Whole Genome DNA | 60 | 2000 | 33.33 | Optimal with full sized genome fragments | Human and Mouse |
| Kapa Target Enrichment **replaces Roche SeqCap | Whole Genome DNA | 60 | 1500 | 25.00 | >3 DIN | Human and Mouse |
| SureSelectXT Low Input (enzymatic fragmentation) | Whole Genome DNA | 20 | 500 | 25.00 | >3 DIN | Custom Human, Human, and Mouse |
| SureSelectXT Low Input (sonication fragmentation) | Whole Genome DNA | 60 | 500 | 8.33 | >3 DIN | Custom Human, Human, and Mouse |
| SureSelectXT v10 | Whole Genome DNA | 30 | 850 | 28.33 | na | Custom Human, can work with as little as 75ng in 25ul |

| <u>16S, 18S, ITS</u> | Input Material Type | Min Submission Vol (uL) required | Optimal Submission ng (total ng) | Optimal Submission ng/uL (Optimal Conc) | Quality | Special notes |
|-----------------------------|----------------------------|---|---|--|----------------|---|
| 16S | Whole Genome DNA | 15 | 30 | 2.00 | >3 DIN | In-house custom primers for amplicon sequencing |

| | | | | | | |
|------------|------------------|----|----|------|--------|--|
| 18S | Whole Genome DNA | 15 | 30 | 2.00 | >3 DIN | Customer Supplied custom primers for amplicon sequencing |
| ITS | Whole Genome DNA | 15 | 30 | 2.00 | >3 DIN | In-house custom primers for amplicon sequencing |