Reagent list for RNA-seq, PAT-seq

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| --- | --- | --- | --- |
| Item | Supplier | cost | $ per sample |
| Reagents for sample preparation | | | |
| Oligo-dT beads | NEB | $241 (enough for ca. 300 samples) | 0.80 |
| RNA Fragmentation kit | NEB | $45 (200 reactions) | 0.23 |
| RT primers (NOTE that I have included costs for 20 different primers, to allow extensive bar coding; this may be reduced by a factor of two if each group is OK using the same sets of bar codes) | Various vendors | ca. $10 each (>1000 reactions) | 0.10 |
| SMARTSCRIBE | Clontech | $68 (20 reactions) | 3.40 |
| RNAse Inhibitor | NEB | $76 (40 reactions) | 1.90 |
| Agencourt AMPure Beads | Beckman Coulter | $305 (100 reactions) | 3.00 |
| SWITCH7.5 (this has a locked nucleic acid linkage at its 3’ end) | Exiqon | $60 (>200 reactions) | 0.25 |
| PCR primers | Various vendors | ca. $20 (>1000 reactions) | 0.02 |
| Phire Hot Start II Taq DNA polymerase | Thermo | $141 (400 25 µL reactions) | 2.00 |
| Totals: | | $1056 | 11.70 |
| Reagents for nucleic acid purification | | | |
| RLT | Qiagen | $142 (300 samples) | 0.48 |
| RPE | Qiagen | $35 (360 samples) | 0.10 |
| PB | Qiagen | $79 (>1000 samples) | 0.08 |
| PE | Qiagen | $79 (>1000 samples) | 0.08 |
| QG (gel extraction buffer) | Qiagen | $58 (>250 samples) | 0.02 |
| RNA columns | Enzymax | $59 (100 samples) | 0.59 |
| DNA columns | Enzymax | $36 (100 samples) | 0.36 |
| Totals: | | $488 | 1.63 |
| TOTAL OUTLAY: | | $1544 | 13.33 |
| Ribosomal RNA removal kits (for prokaryotic samples) | Various vendors | $80 x # of samples | 80.00 |
| Disposables – pipette tips (various sizes), microcentrifuge tubes (1.5 ml, 0.5 ml, 0.2 ml) |  |  |  |
| Agarose gel supplies (agarose, suitable dye for visualization, scalpels and blades in case we need to be excising bands from gels) |  |  |  |

Primers:

>PE-PCR1

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

>PE-PCR2

CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

>SMART7.5

CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGG+G

(We should have plenty of the above)

(The following replace the RT-PE4 series we used last year. They have an additional “N” before the bar code – this was added because we have had two instances, one from a HiSeq and one from a MiSeq, where the first base calls for an entire lane were “N”’s, which defeats the bar-coding in the RTPE4 series. This doesn't happen very often, but when it does it ruins the experiment. In my experience, sequencing centers wont repeat a run when this happens.)

>RT-PE6a

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAGGNNNNNN

>RT-PE6b

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCGGNNNNNN

>RT-PE6c

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAACNNNNNN

>RT-PE6d

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAGCNNNNNN

>RT-PE6e

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNACGNNNNNN

>RT-PE6f

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAGANNNNNN

>RT-PE6g

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCCGNNNNNN

>RT-PE6h

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCAANNNNNN

>RT-PE6j

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCAGNNNNNN

>RT-PE6k

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGGANNNNNN

>RT-PE6l

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCCANNNNNN

>RT-PE6m

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCACNNNNNN

>RT-PE6n

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGAGNNNNNN

>RT-PE6o

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGCANNNNNN

>RT-PE6p

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGAANNNNNN

>RT-PE6q

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGGCNNNNNN

>RT-PE6r

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNACCNNNNNN

>RT-PE6s

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGACNNNNNN

>RT-PE6t

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCCCNNNNNN

>RT-PE6u

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCGANNNNNN

>RT-PE6v

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNCGCNNNNNN

>RT-PE6w

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGCGNNNNNN

>RT-PE6x

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNGCCNNNNNN

>RT-PE6y

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAAGNNNNNN

>RT-PE6z

ACACTCTTTCCCTACACGACGCTCTTCCGATCTNAAANNNNNN