A

```
samtools view accepted_hits.bam | htseq-count -m
intersection-strict --stranded=no -
/home/ken/BioSoft/GeneGTF/mm9_genes.gtf > Example1.counts.txt
```

B

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | geneid | Sample1_rep1 | Sample1_rep2 | Sample2_rep1 | Sample1_rep2 |
| 2 | Xkr4 | 0 | 0 | 0 | 0 |
| 3 | Rp1 | 181 | 31 | 11 | 67 |
| 4 | Sox17 | 1657 | 1349 | 546 | 969 |
| 5 | Mrpl15 | 1612 | 1899 | 887 | 1476 |
| 6 | Lyplal | 5694 | 4186 | 5965 | 4757 |
| 7 | Tceal | 3083 | 3617 | 3152 | 2884 |
| 8 | Rgs20 | 0 | 2 | 1 | 0 |
| 9 | Atp6v1h | 1833 | 2768 | 3629 | 3812 |
| 10 | Oprk1 | 0 | 0 | 0 | 0 |
| 11 | Npbwr1 | 0 | 0 | 0 | 0 |
| 12 | Rblcc1 | 162 | 2720 | 912 | 733 |
| 13 | Fam150a | 0 | 0 | 0 | 0 |
| 14 | St18 | 0 | 0 | 5 | 0 |
| 15 | Pcmtd1 | 13712 | 7767 | 9451 | 5110 |
| 16 | Sntg1 | 0 | 0 | 2 | 0 |
| 17 | Rrs1 | 96 | 2 | 24 | 87 |
| 18 | Adhfe1 | 0 | 0 | 5 | 0 |
| 19 | 2610203C22Rik | 16 | 0 | 3 | 3 |
| 20 | 3110035E14Rik | 3 | 0 | 22 | 15 |
| 21 | Mybl1 | 1 | 0 | 64 | 32 |
| 22 | Vcpip1 | 676 | 1241 | 667 | 357 |

| | A | B | C |
|---|---|---|---|
| 1 | sample.Name | Condition | libType |
| 2 | Sample1_rep1 | Sample1 | paired-end |
| 3 | Sample1_rep2 | Sample1 | paired-end |
| 4 | Sample2_rep1 | Sample2 | paired-end |
| 5 | Sample1_rep2 | Sample2 | paired-end |
| 6 | | | |
| 7 | | | |

C

```
ken@seqboy:~$ R

R version 3.0.1 (2013-05-16) -- "Good Sport"
Copyright (C) 2013 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)

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  Natural language support but running in an English locale

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'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Previously saved workspace restored]

>
```

D

```
countTable  = read.table("Raw_Count_Table.txt", header=TRUE, row.names=1)
designTable = read.table("ExperimentDesign.txt", header=TRUE, row.names=1)
```

E

```
library(DESeq)
```

F

```
conditions.Sample1.vs.Sample2 = factor(countTable)
```

G

```
cds = newCountDataSet(countTable, conditions.Sample1.vs.Sample2)

cds = estimateSizeFactors(cds)

result = nbinomTest(cds, "Sample1", "Sample2")
```

H

```
result_Sig0.05 = subset(result, !is.na(padj) & padj < 0.05)

write.csv( result_Sig0.05, file =
"./Sample1.vs.Sample2.DE.sig0.05.csv")
```