

Pipeline RNASeq Analysis - Gene Expression Profiling in the Axillary Bud Outgrowth of Enery Cane and Sugarcane: A Comparative Transcriptome Analysis

Project: Enegy/Sugar cane RNASeq
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0 - DATA SUMMARY

#Libraries that were not sequenced due to low quality

ADV050-150_Vertix_CTR_48h ---> 4 files
ADV058-158_CTC_CTR_48h ---> 4 files

#Number of reads in each library

---> Vertex_ET_0h

ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L002_R1_001.fastq:10149532
ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L002_R2_001.fastq:10149532
ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L003_R1_001.fastq:9929319
ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L003_R2_001.fastq:9929319
ADV002-102_Vertix_ET_0h_AATCGGCG-AATATTGA_S2_L002_R1_001.fastq:8823652
ADV002-102_Vertix_ET_0h_AATCGGCG-AATATTGA_S2_L002_R2_001.fastq:8823652
ADV002-102_Vertix_ET_0h_AATCGGCG-AATATTGA_S2_L003_R1_001.fastq:8617698
ADV002-102_Vertix_ET_0h_AATCGGCG-AATATTGA_S2_L003_R2_001.fastq:8617698
ADV003-103_Vertix_ET_0h_AATCCGTT-AATATGCT_S3_L002_R1_001.fastq:13157512
ADV003-103_Vertix_ET_0h_AATCCGTT-AATATGCT_S3_L002_R2_001.fastq:13157512
ADV003-103_Vertix_ET_0h_AATCCGTT-AATATGCT_S3_L003_R1_001.fastq:12855031
ADV003-103_Vertix_ET_0h_AATCCGTT-AATATGCT_S3_L003_R2_001.fastq:12855031
ADV004-104_Vertix_ET_0h_AAGATACA-AATATCTG_S4_L002_R1_001.fastq:8744927
ADV004-104_Vertix_ET_0h_AAGATACA-AATATCTG_S4_L002_R2_001.fastq:8744927
ADV004-104_Vertix_ET_0h_AAGATACA-AATATCTG_S4_L003_R1_001.fastq:8552264
ADV004-104_Vertix_ET_0h_AAGATACA-AATATCTG_S4_L003_R2_001.fastq:8552264
ADV005-105_Vertix_ET_0h_AAGACGAA-AATAGATT_S5_L002_R1_001.fastq:9622476
ADV005-105_Vertix_ET_0h_AAGACGAA-AATAGATT_S5_L002_R2_001.fastq:9622476
ADV005-105_Vertix_ET_0h_AAGACGAA-AATAGATT_S5_L003_R1_001.fastq:9434985
ADV005-105_Vertix_ET_0h_AAGACGAA-AATAGATT_S5_L003_R2_001.fastq:9434985

---> Vertex_CTR_0h

ADV006-106_Vertix_CTR_0h_AAGTAAGT-AATAGTCC_S6_L002_R1_001.fastq:13116791
ADV006-106_Vertix_CTR_0h_AAGTAAGT-AATAGTCC_S6_L002_R2_001.fastq:13116791
ADV006-106_Vertix_CTR_0h_AAGTAAGT-AATAGTCC_S6_L003_R1_001.fastq:12815046
ADV006-106_Vertix_CTR_0h_AAGTAAGT-AATAGTCC_S6_L003_R2_001.fastq:12815046
ADV007-107_Vertix_CTR_0h_AAGTTATC-AATAGCAA_S7_L002_R1_001.fastq:7514692
ADV007-107_Vertix_CTR_0h_AAGTTATC-AATAGCAA_S7_L002_R2_001.fastq:7514692
ADV007-107_Vertix_CTR_0h_AAGTTATC-AATAGCAA_S7_L003_R1_001.fastq:7351425
ADV007-107_Vertix_CTR_0h_AAGTTATC-AATAGCAA_S7_L003_R2_001.fastq:7351425
ADV008-108_Vertix_CTR_0h_AAGTTGGA-AATACAGG_S8_L002_R1_001.fastq:10767241
ADV008-108_Vertix_CTR_0h_AAGTTGGA-AATACAGG_S8_L002_R2_001.fastq:10767241
ADV008-108_Vertix_CTR_0h_AAGTTGGA-AATACAGG_S8_L003_R1_001.fastq:10520582
ADV008-108_Vertix_CTR_0h_AAGTTGGA-AATACAGG_S8_L003_R2_001.fastq:10520582
ADV009-109_Vertix_CTR_0h_GTCTACAT-TTCTTGAA_S9_L002_R1_001.fastq:12293790
ADV009-109_Vertix_CTR_0h_GTCTACAT-TTCTTGAA_S9_L002_R2_001.fastq:12293790
ADV009-109_Vertix_CTR_0h_GTCTACAT-TTCTTGAA_S9_L003_R1_001.fastq:12074681
ADV009-109_Vertix_CTR_0h_GTCTACAT-TTCTTGAA_S9_L003_R2_001.fastq:12074681
ADV010-110_Vertix_CTR_0h_TTCGCCGA-GTATACCG_S10_L002_R1_001.fastq:11475701
ADV010-110_Vertix_CTR_0h_TTCGCCGA-GTATACCG_S10_L002_R2_001.fastq:11475701
ADV010-110_Vertix_CTR_0h_TTCGCCGA-GTATACCG_S10_L003_R1_001.fastq:11221173
ADV010-110_Vertix_CTR_0h_TTCGCCGA-GTATACCG_S10_L003_R2_001.fastq:11221173

---> CTC_ET_0h

ADV011-111_CTC_ET_0h_TGCGTACA-TTCTCATA_S11_L002_R1_001.fastq:8596659
ADV011-111_CTC_ET_0h_TGCGTACA-TTCTCATA_S11_L002_R2_001.fastq:8596659
ADV011-111_CTC_ET_0h_TGCGTACA-TTCTCATA_S11_L003_R1_001.fastq:8433696
ADV011-111_CTC_ET_0h_TGCGTACA-TTCTCATA_S11_L003_R2_001.fastq:8433696
ADV012-112_CTC_ET_0h_GTCGCTGT-TTATATCA_S12_L002_R1_001.fastq:8343570
ADV012-112_CTC_ET_0h_GTCGCTGT-TTATATCA_S12_L002_R2_001.fastq:8343570
ADV012-112_CTC_ET_0h_GTCGCTGT-TTATATCA_S12_L003_R1_001.fastq:8150566
ADV012-112_CTC_ET_0h_GTCGCTGT-TTATATCA_S12_L003_R2_001.fastq:8150566
ADV013-113_CTC_ET_0h_TTATTATG-TTAGCGCA_S13_L002_R1_001.fastq:8497379
ADV013-113_CTC_ET_0h_TTATTATG-TTAGCGCA_S13_L002_R2_001.fastq:8497379
ADV013-113_CTC_ET_0h_TTATTATG-TTAGCGCA_S13_L003_R1_001.fastq:8337622
ADV013-113_CTC_ET_0h_TTATTATG-TTAGCGCA_S13_L003_R2_001.fastq:8337622
ADV014-114_CTC_ET_0h_TGACTGAA-TTAGACGT_S14_L002_R1_001.fastq:10134242
ADV014-114_CTC_ET_0h_TGACTGAA-TTAGACGT_S14_L002_R2_001.fastq:10134242
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ADV014-114_CTC_ET_0h_TGACTGAA-TTAGACGT_S14_L003_R2_001.fastq:9968471
ADV015-115_CTC_ET_0h_GTACAGCT-TGATCGGT_S15_L002_R1_001.fastq:10662455
ADV015-115_CTC_ET_0h_GTACAGCT-TGATCGGT_S15_L002_R2_001.fastq:10662455
ADV015-115_CTC_ET_0h_GTACAGCT-TGATCGGT_S15_L003_R1_001.fastq:10468320
ADV015-115_CTC_ET_0h_GTACAGCT-TGATCGGT_S15_L003_R2_001.fastq:10468320

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ADV016-116_CTC_CTR_0h_GGACAACG-TGATGGCC_S16_L002_R2_001.fastq:10202480
ADV016-116_CTC_CTR_0h_GGACAACG-TGATGGCC_S16_L003_R1_001.fastq:9993871
ADV016-116_CTC_CTR_0h_GGACAACG-TGATGGCC_S16_L003_R2_001.fastq:9993871
ADV017-117_CTC_CTR_0h_CGCTGCTC-GGCAGATC_S17_L002_R1_001.fastq:10601817
ADV017-117_CTC_CTR_0h_CGCTGCTC-GGCAGATC_S17_L002_R2_001.fastq:10601817
ADV017-117_CTC_CTR_0h_CGCTGCTC-GGCAGATC_S17_L003_R1_001.fastq:10434892
ADV017-117_CTC_CTR_0h_CGCTGCTC-GGCAGATC_S17_L003_R2_001.fastq:10434892
ADV018-118_CTC_CTR_0h_CTGGCCTC-GATCCAAC_S18_L002_R1_001.fastq:10646710
ADV018-118_CTC_CTR_0h_CTGGCCTC-GATCCAAC_S18_L002_R2_001.fastq:10646710
ADV018-118_CTC_CTR_0h_CTGGCCTC-GATCCAAC_S18_L003_R1_001.fastq:10436373
ADV018-118_CTC_CTR_0h_CTGGCCTC-GATCCAAC_S18_L003_R2_001.fastq:10436373
ADV019-119_CTC_CTR_0h_GAATCAAT-TCTGTGAT_S19_L002_R1_001.fastq:8295643
ADV019-119_CTC_CTR_0h_GAATCAAT-TCTGTGAT_S19_L002_R2_001.fastq:8295643
ADV019-119_CTC_CTR_0h_GAATCAAT-TCTGTGAT_S19_L003_R1_001.fastq:8128996
ADV019-119_CTC_CTR_0h_GAATCAAT-TCTGTGAT_S19_L003_R2_001.fastq:8128996
ADV020-120_CTC_CTR_0h_TCGGATGT-CTGCGGAT_S20_L002_R1_001.fastq:8344549
ADV020-120_CTC_CTR_0h_TCGGATGT-CTGCGGAT_S20_L002_R2_001.fastq:8344549
ADV020-120_CTC_CTR_0h_TCGGATGT-CTGCGGAT_S20_L003_R1_001.fastq:8179437
ADV020-120_CTC_CTR_0h_TCGGATGT-CTGCGGAT_S20_L003_R2_001.fastq:8179437

---> Vertex_ET_24h

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ADV021-121_Vertix_ET_24h_CGCTATTA-GCGGCCGT_S21_L003_R1_001.fastq:7080943
ADV021-121_Vertix_ET_24h_CGCTATTA-GCGGCCGT_S21_L003_R2_001.fastq:7080943
ADV022-122_Vertix_ET_24h_AAGACTGT-GTGGACTA_S22_L002_R1_001.fastq:11347336
ADV022-122_Vertix_ET_24h_AAGACTGT-GTGGACTA_S22_L002_R2_001.fastq:11347336
ADV022-122_Vertix_ET_24h_AAGACTGT-GTGGACTA_S22_L003_R1_001.fastq:11173600
ADV022-122_Vertix_ET_24h_AAGACTGT-GTGGACTA_S22_L003_R2_001.fastq:11173600
ADV023-123_Vertix_ET_24h_CAACTGCT-AGTAGTAT_S23_L002_R1_001.fastq:10112982
ADV023-123_Vertix_ET_24h_CAACTGCT-AGTAGTAT_S23_L002_R2_001.fastq:10112982
ADV023-123_Vertix_ET_24h_CAACTGCT-AGTAGTAT_S23_L003_R1_001.fastq:9960856
ADV023-123_Vertix_ET_24h_CAACTGCT-AGTAGTAT_S23_L003_R2_001.fastq:9960856
ADV024-124_Vertix_ET_24h_TTCGAACC-TGTCACCT_S24_L002_R1_001.fastq:12074094
ADV024-124_Vertix_ET_24h_TTCGAACC-TGTCACCT_S24_L002_R2_001.fastq:12074094
ADV024-124_Vertix_ET_24h_TTCGAACC-TGTCACCT_S24_L003_R1_001.fastq:11863118
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ADV025-125_Vertix_ET_24h_GATCAACA-CTATGTTA_S25_L002_R1_001.fastq:9853888
ADV025-125_Vertix_ET_24h_GATCAACA-CTATGTTA_S25_L002_R2_001.fastq:9853888
ADV025-125_Vertix_ET_24h_GATCAACA-CTATGTTA_S25_L003_R1_001.fastq:9686747
ADV025-125_Vertix_ET_24h_GATCAACA-CTATGTTA_S25_L003_R2_001.fastq:9686747

---> Vertex_CTR_24h

ADV026-126_Vertix_CTR_24h_GAACTTAT-AGATACGC_S26_L002_R1_001.fastq:10235318
ADV026-126_Vertix_CTR_24h_GAACTTAT-AGATACGC_S26_L002_R2_001.fastq:10235318
ADV026-126_Vertix_CTR_24h_GAACTTAT-AGATACGC_S26_L003_R1_001.fastq:10064899
ADV026-126_Vertix_CTR_24h_GAACTTAT-AGATACGC_S26_L003_R2_001.fastq:10064899
ADV027-127_Vertix_CTR_24h_TGAGTCAG-CCGAACCT_S27_L002_R1_001.fastq:8086089
ADV027-127_Vertix_CTR_24h_TGAGTCAG-CCGAACCT_S27_L002_R2_001.fastq:8086089
ADV027-127_Vertix_CTR_24h_TGAGTCAG-CCGAACCT_S27_L003_R1_001.fastq:7940818
ADV027-127_Vertix_CTR_24h_TGAGTCAG-CCGAACCT_S27_L003_R2_001.fastq:7940818
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ADV028-128_Vertix_CTR_24h_CGAGCCGG-GCGGCTTG_S28_L003_R1_001.fastq:7606417
ADV028-128_Vertix_CTR_24h_CGAGCCGG-GCGGCTTG_S28_L003_R2_001.fastq:7606417
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ADV030-130_Vertix_CTR_24h_CAATGATG-CACGGACG_S30_L002_R2_001.fastq:9359339
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---> CTC_ET_24h

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ADV031-131_CTC_ET_24h_CATGATGA-GTTAGAGG_S31_L003_R1_001.fastq:7570950
ADV031-131_CTC_ET_24h_CATGATGA-GTTAGAGG_S31_L003_R2_001.fastq:7570950
ADV032-132_CTC_ET_24h_CAGACCAC-GCTTCGGC_S32_L002_R1_001.fastq:9338485
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ADV035-135_CTC_ET_24h_GAATGCTC-GTCTAACA_S35_L003_R1_001.fastq:8755362
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---> CTC_CTR_24h

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ADV036-136_CTC_CTR_24h_CGATTATC-GAGTTGAT_S36_L003_R2_001.fastq:10508977
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ADV039-139_CTC_CTR_24h_TGACTACT-CCTTACAG_S39_L002_R2_001.fastq:11082790
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ADV039-139_CTC_CTR_24h_TGACTACT-CCTTACAG_S39_L003_R2_001.fastq:10892559
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---> Vertex_ET_48h

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ADV042-142_Vertix_ET_48h_GAAGCACA-CCTCTGGC_S42_L002_R1_001.fastq:7541711
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ADV043-143_Vertix_ET_48h_GAATATCC-GACGCCAT_S43_L003_R2_001.fastq:6787814
ADV044-144_Vertix_ET_48h_TCGAAGCT-GCACTGAG_S44_L002_R1_001.fastq:8467551
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ADV046-146_Vertix_CTR_48h_TGGTCATT-GCAGATGG_S46_L002_R2_001.fastq:8827033
ADV046-146_Vertix_CTR_48h_TGGTCATT-GCAGATGG_S46_L003_R1_001.fastq:8634641
ADV046-146_Vertix_CTR_48h_TGGTCATT-GCAGATGG_S46_L003_R2_001.fastq:8634641
ADV047-147_Vertix_CTR_48h_CAGAAGAT-GTACATTG_S47_L002_R1_001.fastq:9336430
ADV047-147_Vertix_CTR_48h_CAGAAGAT-GTACATTG_S47_L002_R2_001.fastq:9336430
ADV047-147_Vertix_CTR_48h_CAGAAGAT-GTACATTG_S47_L003_R1_001.fastq:9090597
ADV047-147_Vertix_CTR_48h_CAGAAGAT-GTACATTG_S47_L003_R2_001.fastq:9090597
ADV048-148_Vertix_CTR_48h_CAATCGAA-GCACACGC_S48_L002_R1_001.fastq:7289169
ADV048-148_Vertix_CTR_48h_CAATCGAA-GCACACGC_S48_L002_R2_001.fastq:7289169
ADV048-148_Vertix_CTR_48h_CAATCGAA-GCACACGC_S48_L003_R1_001.fastq:7110207
ADV048-148_Vertix_CTR_48h_CAATCGAA-GCACACGC_S48_L003_R2_001.fastq:7110207
ADV049-149_Vertix_CTR_48h_CTACGAAG-CTCGACAG_S49_L002_R1_001.fastq:12031356
ADV049-149_Vertix_CTR_48h_CTACGAAG-CTCGACAG_S49_L002_R2_001.fastq:12031356
ADV049-149_Vertix_CTR_48h_CTACGAAG-CTCGACAG_S49_L003_R1_001.fastq:11753656
ADV049-149_Vertix_CTR_48h_CTACGAAG-CTCGACAG_S49_L003_R2_001.fastq:11753656

---> CTC_ET_48h

ADV051-151_CTC_ET_48h_CTTATGAA-GCCAATGT_S50_L002_R1_001.fastq:8322200

ADV051-151_CTC_ET_48h_CTTATGAA-GCCAATGT_S50_L002_R2_001.fastq:8322200

1 - READS QUALITY ANALYSIS AND TRIMMING

FastQC ANALYSIS:

```
./fastqc -t 15 ./*.fastq -o ./
```

MultiQC: QUALITY CONTROL ANALYSIS OF SEQUENCING DATA

```
#Run MultiQC
```

```
./multiqc .
```

```
#Result:
```

 multiqc_report_rnaseq_gui.html

TRIMMOMATIC: REMOVAL OF ADAPTERS (Trimming and Filtering)

```
./trimmomatic PE -phred33 -threads 10 ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L002_R1_001.fastq.gz  
ADV001-101_Vertix_ET_0h_AATCGTTA-AATAACGT_S1_L002_R2_001.fastq.gz ADV001-101_Vertix_ET_0h_AATCGTTA-  
AATAACGT_S1_L002_R1_001.Ptrim.fastq.gz ADV001-101_Vertix_ET_0h_AATCGTTA-  
AATAACGT_S1_L002_R1_001.Utrim.fastq.gz ADV001-101_Vertix_ET_0h_AATCGTTA-  
AATAACGT_S1_L002_R2_001.Ptrim.fastq.gz ADV001-101_Vertix_ET_0h_AATCGTTA-  
AATAACGT_S1_L002_R2_001.Utrim.fastq.gz ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 SLIDINGWINDOW:5:30 LEADING:5  
TRAILING:5 MINLEN:100 AVGQUAL:30
```

FastQC ANALYSIS AFTER RUN TRIMMOMATIC IN PAIRED FILES:

```
./fastqc -t 15 *.fastq.gz -o  
/opt_hd/nicholas/energy_cane/2.QUALITY_CONTROL_AND_TRIMMING/2.1.FASTQC_MULTQC/2.1.2.AFTER_TRIM
```

MultiQC ANALYSIS AFTER RUN TRIMMOMATIC:

```
#rodar MultiQC
```

```
./multiqc .
```

```
#resultado Geral
```

Energy cane (VERTIX) and Sugar cane (CA) after trimming:

 multiqc_report_after_trim.html

2 - REMOVAL OF RIBOSOMAL READS

SortMeRNA: EVALUATE THE PRESENCE OF RIBOSOMAL SEQUENCES (28S, 18S, 16Sbac+arc) IN LIBRARIES

```
#Editar o perl script "script_verifica_ribossomal.pl"
```

```
#28S,18S,16Sbac+arc
```

```
sortmerna-2.0-linux-64/sortmerna --ref /usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-euk-18s-id95.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-euk-18s-id95.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-euk-28s-id98.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-euk-28s-id98.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-arc-16s-id95.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-arc-16s-id95.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-bac-16s-id90.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-bac-16s-id90.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/rfam-5.8s-database-id98.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/rfam-5.8s-database-id98.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/rfam-5s-database-id98.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/rfam-5s-database-id98.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-arc-23s-id98.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-arc-23s-id98.fasta:/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-bac-23s-id98.fasta,/usr/local/genome/shortsequenceassemblers/sortmerna-2.0-linux-64/rRNA_databases/silva-bac-23s-id98.fasta --reads $fastqc --aligned Sortmerna.$nome.fq --fastx --log -v -a 5
```

3 - DE NOVO TRANSCRIPTOME ASSEMBLY

```
# Run Trinity
```

```
# CE - Cana energia | PAIRED AND UNPAIRED FILES
```

```
Trinity --seqType fq --min_kmer_cov 2 --max_memory 20G --left ADV001-
101_Vertix_ET_0h_R1.Ptrim.cleaned_final.fq,ADV002-102_Vertix_ET_0h_R1.Ptrim.cleaned_final.fq,ADV003-
103_Vertix_ET_0h_R1.Ptrim.cleaned_final.fq,ADV004-104_Vertix_ET_0h_R1.Ptrim.cleaned_final.fq,ADV005-
105_Vertix_ET_0h_R1.Ptrim.cleaned_final.fq,ADV006-106_Vertix_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV007-
107_Vertix_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV008-108_Vertix_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV009-
109_Vertix_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV010-110_Vertix_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV021-
121_Vertix_ET_24h_R1.Ptrim.cleaned_final.fq,ADV022-122_Vertix_ET_24h_R1.Ptrim.cleaned_final.fq,ADV023-
123_Vertix_ET_24h_R1.Ptrim.cleaned_final.fq,ADV024-124_Vertix_ET_24h_R1.Ptrim.cleaned_final.fq,ADV025-
125_Vertix_ET_24h_R1.Ptrim.cleaned_final.fq,ADV026-126_Vertix_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV027-
127_Vertix_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV028-128_Vertix_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV029-
129_Vertix_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV030-130_Vertix_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV041-
141_Vertix_ET_48h_R1.Ptrim.cleaned_final.fq,ADV042-142_Vertix_ET_48h_R1.Ptrim.cleaned_final.fq,ADV043-
143_Vertix_ET_48h_R1.Ptrim.cleaned_final.fq,ADV044-144_Vertix_ET_48h_R1.Ptrim.cleaned_final.fq,ADV045-
145_Vertix_ET_48h_R1.Ptrim.cleaned_final.fq,ADV046-146_Vertix_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV047-
147_Vertix_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV048-148_Vertix_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV049-
149_Vertix_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV001-101_Vertix_ET_0h_R1.Utrim.cleaned.fq,ADV002-
102_Vertix_ET_0h_R1.Utrim.cleaned.fq,ADV003-103_Vertix_ET_0h_R1.Utrim.cleaned.fq,ADV004-
104_Vertix_ET_0h_R1.Utrim.cleaned.fq,ADV005-105_Vertix_ET_0h_R1.Utrim.cleaned.fq,ADV006-
106_Vertix_CTR_0h_R1.Utrim.cleaned.fq,ADV007-107_Vertix_CTR_0h_R1.Utrim.cleaned.fq,ADV008-
108_Vertix_CTR_0h_R1.Utrim.cleaned.fq,ADV009-109_Vertix_CTR_0h_R1.Utrim.cleaned.fq,ADV010-
110_Vertix_CTR_0h_R1.Utrim.cleaned.fq,ADV021-121_Vertix_ET_24h_R1.Utrim.cleaned.fq,ADV022-
122_Vertix_ET_24h_R1.Utrim.cleaned.fq,ADV023-123_Vertix_ET_24h_R1.Utrim.cleaned.fq,ADV024-
124_Vertix_ET_24h_R1.Utrim.cleaned.fq,ADV025-125_Vertix_ET_24h_R1.Utrim.cleaned.fq,ADV026-
126_Vertix_CTR_24h_R1.Utrim.cleaned.fq,ADV027-127_Vertix_CTR_24h_R1.Utrim.cleaned.fq,ADV028-
128_Vertix_CTR_24h_R1.Utrim.cleaned.fq,ADV029-129_Vertix_CTR_24h_R1.Utrim.cleaned.fq,ADV030-
130_Vertix_CTR_24h_R1.Utrim.cleaned.fq,ADV041-141_Vertix_ET_48h_R1.Utrim.cleaned.fq,ADV042-
142_Vertix_ET_48h_R1.Utrim.cleaned.fq,ADV043-143_Vertix_ET_48h_R1.Utrim.cleaned.fq,ADV044-
144_Vertix_ET_48h_R1.Utrim.cleaned.fq,ADV045-145_Vertix_ET_48h_R1.Utrim.cleaned.fq,ADV046-
146_Vertix_CTR_48h_R1.Utrim.cleaned.fq,ADV047-147_Vertix_CTR_48h_R1.Utrim.cleaned.fq,ADV048-
148_Vertix_CTR_48h_R1.Utrim.cleaned.fq,ADV049-149_Vertix_CTR_48h_R1.Utrim.cleaned.fq --right ADV001-
101_Vertix_ET_0h_R2.Ptrim.cleaned_final.fq,ADV002-102_Vertix_ET_0h_R2.Ptrim.cleaned_final.fq,ADV003-
103_Vertix_ET_0h_R2.Ptrim.cleaned_final.fq,ADV004-104_Vertix_ET_0h_R2.Ptrim.cleaned_final.fq,ADV005-
105_Vertix_ET_0h_R2.Ptrim.cleaned_final.fq,ADV006-106_Vertix_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV007-
107_Vertix_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV008-108_Vertix_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV009-
109_Vertix_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV010-110_Vertix_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV021-
121_Vertix_ET_24h_R2.Ptrim.cleaned_final.fq,ADV022-122_Vertix_ET_24h_R2.Ptrim.cleaned_final.fq,ADV023-
123_Vertix_ET_24h_R2.Ptrim.cleaned_final.fq,ADV024-124_Vertix_ET_24h_R2.Ptrim.cleaned_final.fq,ADV025-
125_Vertix_ET_24h_R2.Ptrim.cleaned_final.fq,ADV026-126_Vertix_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV027-
127_Vertix_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV028-128_Vertix_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV029-
129_Vertix_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV030-130_Vertix_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV041-
141_Vertix_ET_48h_R2.Ptrim.cleaned_final.fq,ADV042-142_Vertix_ET_48h_R2.Ptrim.cleaned_final.fq,ADV043-
143_Vertix_ET_48h_R2.Ptrim.cleaned_final.fq,ADV044-144_Vertix_ET_48h_R2.Ptrim.cleaned_final.fq,ADV045-
145_Vertix_ET_48h_R2.Ptrim.cleaned_final.fq,ADV046-146_Vertix_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV047-
```

147_Vertix_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV048-148_Vertix_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV049-
149_Vertix_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV001-101_Vertix_ET_0h_R2.Utrim.cleaned.fq,ADV002-
102_Vertix_ET_0h_R2.Utrim.cleaned.fq,ADV003-103_Vertix_ET_0h_R2.Utrim.cleaned.fq,ADV004-
104_Vertix_ET_0h_R2.Utrim.cleaned.fq,ADV005-105_Vertix_ET_0h_R2.Utrim.cleaned.fq,ADV006-
106_Vertix_CTR_0h_R2.Utrim.cleaned.fq,ADV007-107_Vertix_CTR_0h_R2.Utrim.cleaned.fq,ADV008-
108_Vertix_CTR_0h_R2.Utrim.cleaned.fq,ADV009-109_Vertix_CTR_0h_R2.Utrim.cleaned.fq,ADV010-
110_Vertix_CTR_0h_R2.Utrim.cleaned.fq,ADV021-121_Vertix_ET_24h_R2.Utrim.cleaned.fq,ADV022-
122_Vertix_ET_24h_R2.Utrim.cleaned.fq,ADV023-123_Vertix_ET_24h_R2.Utrim.cleaned.fq,ADV024-
124_Vertix_ET_24h_R2.Utrim.cleaned.fq,ADV025-125_Vertix_ET_24h_R2.Utrim.cleaned.fq,ADV026-
126_Vertix_CTR_24h_R2.Utrim.cleaned.fq,ADV027-127_Vertix_CTR_24h_R2.Utrim.cleaned.fq,ADV028-
128_Vertix_CTR_24h_R2.Utrim.cleaned.fq,ADV029-129_Vertix_CTR_24h_R2.Utrim.cleaned.fq,ADV030-
130_Vertix_CTR_24h_R2.Utrim.cleaned.fq,ADV041-141_Vertix_ET_48h_R2.Utrim.cleaned.fq,ADV042-
142_Vertix_ET_48h_R2.Utrim.cleaned.fq,ADV043-143_Vertix_ET_48h_R2.Utrim.cleaned.fq,ADV044-
144_Vertix_ET_48h_R2.Utrim.cleaned.fq,ADV045-145_Vertix_ET_48h_R2.Utrim.cleaned.fq,ADV046-
146_Vertix_CTR_48h_R2.Utrim.cleaned.fq,ADV047-147_Vertix_CTR_48h_R2.Utrim.cleaned.fq,ADV048-
148_Vertix_CTR_48h_R2.Utrim.cleaned.fq,ADV049-149_Vertix_CTR_48h_R2.Utrim.cleaned.fq --CPU 15 --
min_contig_length 200 --full_cleanup

CA - Cana-de-açúcar | PAIRED AND UNPAIRED FILES)

```
Trinity --seqType fq --min_kmer_cov 2 --max_memory 20G --left ADV011-
111_CTC_ET_0h_R1.Ptrim.cleaned_final.fq,ADV011-111_CTC_ET_0h_R1.Utrim.cleaned.fq,ADV012-
112_CTC_ET_0h_R1.Ptrim.cleaned_final.fq,ADV012-112_CTC_ET_0h_R1.Utrim.cleaned.fq,ADV013-
113_CTC_ET_0h_R1.Ptrim.cleaned_final.fq,ADV013-113_CTC_ET_0h_R1.Utrim.cleaned.fq,ADV014-
114_CTC_ET_0h_R1.Ptrim.cleaned_final.fq,ADV014-114_CTC_ET_0h_R1.Utrim.cleaned.fq,ADV015-
115_CTC_ET_0h_R1.Ptrim.cleaned_final.fq,ADV015-115_CTC_ET_0h_R1.Utrim.cleaned.fq,ADV016-
116_CTC_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV016-116_CTC_CTR_0h_R1.Utrim.cleaned.fq,ADV017-
117_CTC_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV017-117_CTC_CTR_0h_R1.Utrim.cleaned.fq,ADV018-
118_CTC_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV018-118_CTC_CTR_0h_R1.Utrim.cleaned.fq,ADV019-
119_CTC_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV019-119_CTC_CTR_0h_R1.Utrim.cleaned.fq,ADV020-
120_CTC_CTR_0h_R1.Ptrim.cleaned_final.fq,ADV020-120_CTC_CTR_0h_R1.Utrim.cleaned.fq,ADV031-
131_CTC_ET_24h_R1.Ptrim.cleaned_final.fq,ADV031-131_CTC_ET_24h_R1.Utrim.cleaned.fq,ADV032-
132_CTC_ET_24h_R1.Ptrim.cleaned_final.fq,ADV032-132_CTC_ET_24h_R1.Utrim.cleaned.fq,ADV033-
133_CTC_ET_24h_R1.Ptrim.cleaned_final.fq,ADV033-133_CTC_ET_24h_R1.Utrim.cleaned.fq,ADV034-
134_CTC_ET_24h_R1.Ptrim.cleaned_final.fq,ADV034-134_CTC_ET_24h_R1.Utrim.cleaned.fq,ADV035-
135_CTC_ET_24h_R1.Ptrim.cleaned_final.fq,ADV035-135_CTC_ET_24h_R1.Utrim.cleaned.fq,ADV036-
136_CTC_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV036-136_CTC_CTR_24h_R1.Utrim.cleaned.fq,ADV037-
137_CTC_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV037-137_CTC_CTR_24h_R1.Utrim.cleaned.fq,ADV038-
138_CTC_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV038-138_CTC_CTR_24h_R1.Utrim.cleaned.fq,ADV039-
139_CTC_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV039-139_CTC_CTR_24h_R1.Utrim.cleaned.fq,ADV040-
140_CTC_CTR_24h_R1.Ptrim.cleaned_final.fq,ADV040-140_CTC_CTR_24h_R1.Utrim.cleaned.fq,ADV051-
151_CTC_ET_48h_R1.Ptrim.cleaned_final.fq,ADV051-151_CTC_ET_48h_R1.Utrim.cleaned.fq,ADV052-
152_CTC_ET_48h_R1.Ptrim.cleaned_final.fq,ADV052-152_CTC_ET_48h_R1.Utrim.cleaned.fq,ADV053-
153_CTC_ET_48h_R1.Ptrim.cleaned_final.fq,ADV053-153_CTC_ET_48h_R1.Utrim.cleaned.fq,ADV054-
154_CTC_ET_48h_R1.Ptrim.cleaned_final.fq,ADV054-154_CTC_ET_48h_R1.Utrim.cleaned.fq,ADV055-
155_CTC_ET_48h_R1.Ptrim.cleaned_final.fq,ADV055-155_CTC_ET_48h_R1.Utrim.cleaned.fq,ADV056-
156_CTC_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV056-156_CTC_CTR_48h_R1.Utrim.cleaned.fq,ADV057-
157_CTC_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV057-157_CTC_CTR_48h_R1.Utrim.cleaned.fq,ADV059-
159_CTC_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV059-159_CTC_CTR_48h_R1.Utrim.cleaned.fq,ADV060-
160_CTC_CTR_48h_R1.Ptrim.cleaned_final.fq,ADV060-160_CTC_CTR_48h_R1.Utrim.cleaned.fq, --right ADV011-
111_CTC_ET_0h_R2.Ptrim.cleaned_final.fq,ADV011-111_CTC_ET_0h_R2.Utrim.cleaned.fq,ADV012-
112_CTC_ET_0h_R2.Ptrim.cleaned_final.fq,ADV012-112_CTC_ET_0h_R2.Utrim.cleaned.fq,ADV013-
113_CTC_ET_0h_R2.Ptrim.cleaned_final.fq,ADV013-113_CTC_ET_0h_R2.Utrim.cleaned.fq,ADV014-
114_CTC_ET_0h_R2.Ptrim.cleaned_final.fq,ADV014-114_CTC_ET_0h_R2.Utrim.cleaned.fq,ADV015-
115_CTC_ET_0h_R2.Ptrim.cleaned_final.fq,ADV015-115_CTC_ET_0h_R2.Utrim.cleaned.fq,ADV016-
116_CTC_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV016-116_CTC_CTR_0h_R2.Utrim.cleaned.fq,ADV017-
117_CTC_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV017-117_CTC_CTR_0h_R2.Utrim.cleaned.fq,ADV018-
118_CTC_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV018-118_CTC_CTR_0h_R2.Utrim.cleaned.fq,ADV019-
119_CTC_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV019-119_CTC_CTR_0h_R2.Utrim.cleaned.fq,ADV020-
120_CTC_CTR_0h_R2.Ptrim.cleaned_final.fq,ADV020-120_CTC_CTR_0h_R2.Utrim.cleaned.fq,ADV031-
131_CTC_ET_24h_R2.Ptrim.cleaned_final.fq,ADV031-131_CTC_ET_24h_R2.Utrim.cleaned.fq,ADV032-
132_CTC_ET_24h_R2.Ptrim.cleaned_final.fq,ADV032-132_CTC_ET_24h_R2.Utrim.cleaned.fq,ADV033-
133_CTC_ET_24h_R2.Ptrim.cleaned_final.fq,ADV033-133_CTC_ET_24h_R2.Utrim.cleaned.fq,ADV034-
```

```
134_CTC_ET_24h_R2.Ptrim.cleaned_final.fq,ADV034-134_CTC_ET_24h_R2.Utrim.cleaned.fq,ADV035-
135_CTC_ET_24h_R2.Ptrim.cleaned_final.fq,ADV035-135_CTC_ET_24h_R2.Utrim.cleaned.fq,ADV036-
136_CTC_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV036-136_CTC_CTR_24h_R2.Utrim.cleaned.fq,ADV037-
137_CTC_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV037-137_CTC_CTR_24h_R2.Utrim.cleaned.fq,ADV038-
138_CTC_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV038-138_CTC_CTR_24h_R2.Utrim.cleaned.fq,ADV039-
139_CTC_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV039-139_CTC_CTR_24h_R2.Utrim.cleaned.fq,ADV040-
140_CTC_CTR_24h_R2.Ptrim.cleaned_final.fq,ADV040-140_CTC_CTR_24h_R2.Utrim.cleaned.fq,ADV051-
151_CTC_ET_48h_R2.Ptrim.cleaned_final.fq,ADV051-151_CTC_ET_48h_R2.Utrim.cleaned.fq,ADV052-
152_CTC_ET_48h_R2.Ptrim.cleaned_final.fq,ADV052-152_CTC_ET_48h_R2.Utrim.cleaned.fq,ADV053-
153_CTC_ET_48h_R2.Ptrim.cleaned_final.fq,ADV053-153_CTC_ET_48h_R2.Utrim.cleaned.fq,ADV054-
154_CTC_ET_48h_R2.Ptrim.cleaned_final.fq,ADV054-154_CTC_ET_48h_R2.Utrim.cleaned.fq,ADV055-
155_CTC_ET_48h_R2.Ptrim.cleaned_final.fq,ADV055-155_CTC_ET_48h_R2.Utrim.cleaned.fq,ADV056-
156_CTC_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV056-156_CTC_CTR_48h_R2.Utrim.cleaned.fq,ADV057-
157_CTC_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV057-157_CTC_CTR_48h_R2.Utrim.cleaned.fq,ADV059-
159_CTC_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV059-159_CTC_CTR_48h_R2.Utrim.cleaned.fq,ADV060-
160_CTC_CTR_48h_R2.Ptrim.cleaned_final.fq,ADV060-160_CTC_CTR_48h_R2.Utrim.cleaned.fq, --CPU 15 --
min_contig_length 200 --full_cleanup
```

 **Result:**

Sugarcane

FILE result_assembly_trinity_ca.fa (contigs >= 0 bp)

#Clusters: 168038

Total length: 165328481

Total length w/o "N"s: 165328481

Mean cluster size: 983.87555790952

N50: 1515 (35362 contigs)

N90: 431 (111060 contigs)

N95: 299 (134074 contigs)

N99: 217 (160116 contigs)

CLUSTER LENGTHS (descending order)

TRINITY_DN11209_c0_g1_i9 13172

TRINITY_DN25585_c0_g1_i5 174

Energy cane

#Clusters: 211136

Total length: 202917353

Total length w/o "N"s: 202917353

Mean cluster size: 961.074155994241

N50: 1503 (43139 contigs)

N90: 412 (139524 contigs)

N95: 290 (168886 contigs)

N99: 216 (201389 contigs)

CLUSTER LENGTHS (descending order)

TRINITY_DN12908_c1_g1_i5 13584

TRINITY_DN86917_c0_g1_i20 179

3.1 Busco analysis Trinity De Novo assembly

 CE - Cana energia

```
busco -i trinity_out_dir.Trinity.fasta -o busco-trinity -l poales_odb10 -m transcriptome -cpu 15
```

---> FINAL VERSION (PAIRED + UNPAIRED)

C:87.5%[S:21.9%,D:65.6%],F:3.3%,M:9.2%,n:4896

4286	Complete BUSCOs (C)
1072	Complete and single-copy BUSCOs (S)
3214	Complete and duplicated BUSCOs (D)
161	Fragmented BUSCOs (F)
449	Missing BUSCOs (M)
4896	Total BUSCO groups searched

CA - Cana-de-açúcar

```
busco -i trinity_out_dir.Trinity.fasta -o busco_trinity_ca -l poales_odb10 -m transcriptome --cpu 15
```

---> FINAL VERSION (PAIRED + UNPAIRED)

C:81.6%[S:26.9%,D:54.7%],F:4.6%,M:13.8%,n:4896

3995	Complete BUSCOs (C)
1319	Complete and single-copy BUSCOs (S)
2676	Complete and duplicated BUSCOs (D)
225	Fragmented BUSCOs (F)
676	Missing BUSCOs (M)
4896	Total BUSCO groups searched

4. TRANSDECODER: PREDICTING CODING REGIONS FROM A TRANSCRIPT FASTA FILE (CONVERSION NUC TO PT):

OBS:

-m <int> minimum protein length (default: 100aa = 300 bp)

-m 50 --> 50aa = 150bp

CE - Energycane

Step 1: extract the long open reading frames

```
TransDecoder.LongOrfs -t result_assembly_trinity_ce.fa -m 50 -O result_transdecoder_longorfs_ce
```

Step 2: predict the likely coding regions

```
TransDecoder.Predict -t result_assembly_trinity_ce.fa --single_best_only -O result_transdecoder_longorfs_ce
```

CA - Sugarcane

Step 1: extract the long open reading frames

```
TransDecoder.LongOrfs -t result_assembly_trinity_ca.fa -m 50 -O result_transdecoder_longorfs_ca
```

Step 2: predict the likely coding regions

```
TransDecoder.Predict -t result_assembly_trinity_ca.fa --single_best_only -O result_transdecoder_longorfs_ca
```

5 - TRINITY TRANSCRIPT QUANTIFICATION ABUNDANCES OF TRANSCRIPTS FROM RNA-SEQ WITH KALLISTO (v0.46.2):

#Estimating transcript abundance

```
$TRINITY_HOME/util/align_and_estimate_abundance.pl
```

```
/trinity-2.11.0/util/align_and_estimate_abundance.pl --transcripts result_assembly_trinity_ce.fa --est_method kallisto --prep_reference --trinity_mode --samples_file samples.txt --seqType fq --thread_count 18
```

```
/trinity-2.11.0/util/align_and_estimate_abundance.pl --transcripts result_assembly_trinity_ca.fa --est_method kallisto --prep_reference --trinity_mode --samples_file samples.txt --seqType fq --thread_count 18
```

#Build Transcript and Gene Expression Matrices

```
/trinity-2.11.0/util/abundance_estimates_to_matrix.pl --gene_trans_map result_assembly_trinity_ce.fa.gene_trans_map --name_sample_by_basedir --est_method kallisto --quant_files target_files.txt
```

```
/trinity-2.11.0/util/abundance_estimates_to_matrix.pl --gene_trans_map result_assembly_trinity_ca.fa.gene_trans_map --name_sample_by_basedir --est_method kallisto --quant_files target_files.txt
```

#Filtering Transcripts Based on Expression Values

```
/trinity-2.11.0/util/filter_low_expr_transcripts.pl --matrix kallisto.gene.TPM.not_cross_norm --transcripts result_assembly_trinity_ce.fa --min_expr_any 1 --gene_to_trans_map result_assembly_trinity_ca.fa.gene_trans_map
```