

Digital Gene Expression – Small RNA FAQ

Q: What are the sizes of the reads/tags?

A: For small RNA the end user can choose the lengths of smRNA they want to study. Small RNAs are typically 18-26 bp long. We recommend sequencing as much as 35 bp and then trimming back the sequencing information to subtract out the adapters to leave the small RNA insert.

Q: What programs do you use to analyze the data from a digital gene expression run?

A: We will be releasing a short description on how to analyze Small RNA data with kit release. For small RNA we essentially use the standard pipeline to determine the sequence and then count the number of times each unique sequence appears. For annotation we suggest end users BLAST against any publicly available miRNA data base and look for similarities to what they have sequenced

Q: Would the PCR step introduce RNA subpopulation biases?

A: For small RNA the amplified fragment ranges from roughly 88-100 bp where the only unique section is the 18-30 bp small RNA itself. Again we only do 15 cycles of PCR to limit PCR bias.

Q: What are the chances that mutations can be introduced by the PCR step?

A: We do very limited amplification over very short amplicons to limit this.

Q: What is the recommended depth of coverage?

A: You do not really recommend depth of coverage with Small RNA Discovery and Analysis. We recover 4 million tags per flow cell lane. This should be enough to quantify and call things as not present with confidence.

Q: Can this technology distinguish different transcript isoforms?

A: With our small RNA method we are actually sequencing the entire small RNA so we can detect all isoforms.

Q: Do you have any publications using this technology for gene expression analysis?

A: Data has been presented at meeting by early adopters but there are no pubs yet.

Q: How much total RNA input is required?

A: For small RNA we suggest 10 ug.

Q: Are the adapters for small RNA the same as the sequencing adapters?

A: The sequencing adapters for gene expression and small RNA are not identical to each other or the oligos from genomic DNA sample prep. Also note that they are added

sequentially so we can control the orientation and position of the adapter. For genomic DNA sample prep, we add the adapters in one step and need them to be specially modified to get them in the right orientation and position.

Q: What is the accuracy of the small RNA measurements? How is accuracy measured?

A: We are measuring the actual number of times the sequence is present. With a pool of 4 million tags this is a very confident count down to saying with confidence the transcript is not present .

Q: Do we need to run a control DNA lane?

A: At this point, all Gex and smRNA SBS runs need a lane of control DNA be it BAC or PhiX. This is mandatory to generate the sequencing matrix. We are working on a spike in control so a lane will not have to be used for the control but do not have that yet and have NO data for its release.

Q: Is there a special protocol/recommendation for using smaller total RNA input? What is the lower limit? Can this technology be used to profile samples from laser dissection?

A: For small RNA we suggest starting with 10 ug.

Q: Can I analyze transcripts from organisms for which the genome has not been fully assembled/sequenced?

A: You can absolutely get smRNA data from organisms that are not sequenced. This is one of the main features of our system compared to miRNA TaqMan assays or miRNA arrays. This will just make annotation more difficult but you get the data. Comparisons to similar databases can help identify novel smRNA molecules. Small RNAs are fairly well conserved across species.

Q: Do you have a tool for mapping the tags to the reference sequence?

A: We provide clear instructions for collecting the sequencing data and then comparing it against smRNA databases for annotation

Q: If I were to run total RNA from different treatments, how would I normalize the results?

A: You do not need to normalize for Small RNA analysis. 4 million tags is very deep coverage and will not be saturated by excessive transcripts.

Q: Do you provide technical support for small RNA data analysis?

A: We train customers upon pipeline training

Q: Does the end user need to provide any reagents for small RNA analysis?

A: Yes. The kit covers 20 items required but we do require the end user purchase an Invitrogen Superscript II Reverse Transcription kit and supply typical lab elements like PAGE gels and buffers, Ethidium bromide, phenol, chloroform, isoamyl alcohol, NaOAc, and ethanol.