International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **6(8),** 44-46, August **(2017)** *Int. Res. J. Biological Sci.*

Short Communication

MicroRNAs Specific Primer Design using miRNA Design Tool

Maulik Rachh*and Piyush Desai
Veer Narmad South Gujarat University, Surat, Gujarat, India
mrachh@gmail.com

Available online at: www.isca.in, www.isca.me
Received 27th May 2017, revised 2nd August 2017, accepted 9th August 2017

Abstract

MicroRNAs (miRNAs) are tiny (only 18-24 nucleotide long) non-coding RNAs which involved in post-transcriptional regulation of gene expression in multi-cellular organisms by affecting both translation of mRNAs and the stability. There are several different methods used for quantification of miRNAs like Northern Blotting, quantitative RT-PCR, Microarray etc. but, quantitative RT-PCR is used as the standard that is used to validate & confirmation of the results of various methods. The design of primers for miRNA qRT PCR is very much difficult because of short length of miRNA, which size is the very much near to the length of normal PCR primers. The miRNA Design Tool is based on the Universal Probe Library (ULP) probes to design primer(s) for miRNA detection. The tool is a software based and easy method for design of working primers for target specific miRNA for qRT-PCR. The application is available as online service by AstridBio.

Keywords: miRNAs, UPL, Primer Design, qRT PCR, AstridBio.

Introduction

MiRNAs are short length RNAs molecule, not a part of protein coding sequences which are involved in between the stage of transcription and the translation of gene expression in multi-cellular organisms by disturbing the stability as well as the translation of mRNAs[1]. The biogenesis of miRNAs is transcribed by enzyme RNA polymerase II as part of capped and pri-miRNAs which may be converted into either protein-coding gene or protein non-coding region. The initial transcript split by the class 2 ribonuclease III enzyme Drosha to produce an about 70 nucleotide long stem loop pre-miRNA (precursor miRNA), which is additionally split by the cytoplasmic endoribonuclease Dicer ribonuclease (helicase RNase motif) to generate the mature miRNA and antisense miRNA Oligonucleotides products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which is multi ribonucleo protein complex, recognizes target mRNAs through inadequate complimentary base pairing with miRNA and in translational destabilization or inhibition of the target mRNA[1].

MiRNA primer design is difficult task as the average size of miRNA is only 22-24 nucleotides long only. On the other hand, several techniques have been found out to reduce these inconveniences. All the methods are depending on increase the length of miRNA which create a long sequence template sufficient to the design a set of primers[2]. Several methods use only one target specific stem-loop RT-PCR with a particular Probe and primers[2] and another the miRNA-specific Quantitative - reverse transcription PCR (RT-qPCR) with specific set of primers[2] have the benefit that these methods use a set of specific primers forwards and reverse, which is good for additional flexibility for primer design and better specificity.

It is possible to design miRNA Primers with the limitation of designing one forward primer 12 – 18 nucleotides sequence long and with 3 – 8 specific nucleotides long reverse primer at 3' end which further extended which is complementary sequence to the universal sequence tag, which will add the additional base pair at a time of cDNA synthesis. The famous method is LNA specific set of primers but it's possible to achieve same specificity with DNA primers with melting temperatures (Tm) optimization[2]. Furthermore, the DNA primers are very easy to design and amplification capability of miRNA-specific DNA primers is more compare to Locked Nucleic Acids (LNA) primers.

The miRNA Design Tool is based on the UPL probes to design primer(s) for miRNA detection. It gave output by following two Tm calculation methods. The tool designs the miRNA specific stem-loop RT primer. It allowed user to design several primer sets simultaneously by separating two sequences by each others.

UPL works based on 165 short sequences specific probes alternate with LNA, which allows you to design real-time qPCR assays in seconds and investigate over more than five million transcripts of virtually represent any sequenced organism[3]. UPL assays are validated on all the kind of real-time PCR machines, make it competent of detecting fluorescein (an organic compound) dye, Fluorescein isothiocyanate (FITC), SYBR Green, and FAM, and also pursue standard cycling protocols of PCR for hydrolysis probe assays which make it very easy to use.

Here we took one example of mir-145 for designing primers using miRNA Primer Design Tool. In the field of molecular biology, mir-145 miRNA is a short sequence of RNA molecule. Mir-145 role is to regulate the expression levels of related genes by a several mechanisms like its involved in down-regulation in many cancers cells and a diversity of target genes have shown that over-expression of mir-145 will down-regulates the junctional cell adhesion molecule which can be show through experiments. Mir-145 is hypothesised to be tumour suppressor genes, which are normal genes that slow down regulation of cell division, also repair the mistakes of DNA, also control the process of apoptosis and control the cell death mechanism[4]. MiR-145 has been exposed to be down-regulated in breast cancer, also involved in colon cancer and acute myeloid leukaemia[5-8].

Materials and methods

Sequence Input for miRNA Design Tool: The input for miRNA Design Tool is in the form of miRNA names and in normal plain format sequence as a list. We can enter more than one miRNA sequence at a time for the primer design. Sequence should be in plain format because tool cannot accept Fasta single-line description format of Sequence. The sequence can be in any case either Uppercase format or Lowercase format but it should be written as RNA sequence not DNA sequence.

Selection of desired sequence: miRNA Sequence you can derive from the database; miRBase. The miRBase is a collection of searchable database of miRNA sequences which are published. Each and every entry in the database represents miRNA transcript, a predicted hairpin portion, known as "miR" in the database, also represent the information on mature miRNA sequence and the location, known as "miR". Sequence of human miRNA 145 is hsa-mir-145 (MI0000461) - CACCUUGUCCUCACGGUCCAGUU UUCCCAGGAAUCCCUUAGAUGCUAAGAUGGGGAUUC CUGGAAAUACUGUUCUUGAGGUCAUGGUU.

Primer Design for miRNAs: Designing primers for miRNAs using miRNA Primer Design Tool, required free registration. Registration can be completed through particular email address. Once registration is completed, after that you can login to your account for further primer design process. You need to put your MiRNA sequence to sequence window. One more is option available for the selection of UPL Probe. You can select your desired probe or tool itself select one probe based on input sequence.

Results and Discussion

Primer design for miRNA PCR having many challenges like it is difficult to design a set of two primers forward and reverse on a short miRNA sequence which can be solved by increasing a length of sequence by adding a tail to the miRNA will be useful solution for miRNA-specific RT-qPCR design. The one more challenge is short sequence template of miRNA did not allowed a freedom for selecting the sequence of the primer that specifically bind to the 3′-end of the primer which is important parameter for the performance of PCR reactions[9]. Thus, a technically deep move towards to achieve high-quality PCR results is to concentrate on best possible primer designing at 3′-end[9]. This methodology can be accepted only in to DNA primers[10].

Output of miRNA Design Tool contains 3 different parts, first (Figue-1) its stem loop representation which also included the prediction of cDNA sequence. cDNA sequence will be complementary sequence of mature miRNA, which will lead through miRNA specific sequence. Second, (Figure-2) each fragments of stem loop structure including universal probe, which will be a part of stem loop sequence, and third (Figure-3) universal reverse primer and two forward primers with two different Tm calculation methods. We can choose any one of the according to our requirement and based on enzyme which we have selected. Universal reverse primer will be a part of stem loop structure which go forward through universal probe to cDNA sequence. Forward primer will be complimentary to cDNA sequence.

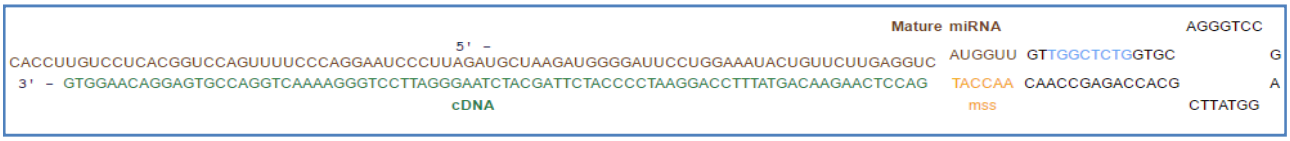

Figure-1: Output of miRNA Design Tool - Stem Loop Representation of hsa mir-145.

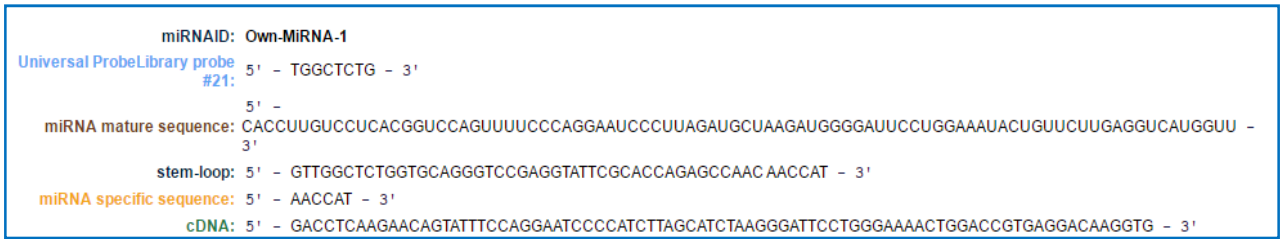

Figure-2: Output of miRNA Design Tool - Each fragments of Stem loop structure includes universal probe 21, miRNA mature Sequence, Stem-loop sequence, miRNA specific sequence and cDNA sequence.

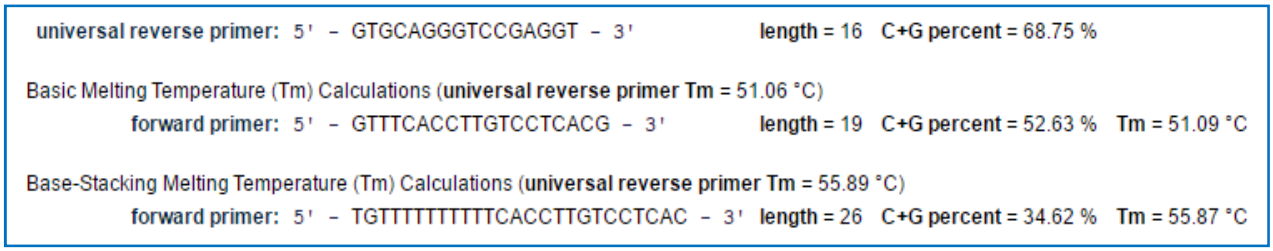

Figure-3: Output of miRNA Design Tool - Two forward primers with 2 different Tm calculation methods and one universal reverse primer.

The main advantages of using online tools for primer design is the calculation of the effectiveness of secondary structure of the primer on the quality of PCR compared to manual design of primer. Self complimentary of the primer and primer dimer formation are two main parameters to be considered based on the calculation of secondary structures while designing primers which increase the possibility of successful assay design.

Conclusion

The miRNA Design Tool designs primers and probes for the amplification of microRNAs using PCR; it's very easy to use. The primers designed are useful for the protocol for microRNAs specific Real Time PCR. MicroRNA-specific RT-qPCR is a very simple and particular method to design self primers which minimize the cost for the procurement of ready to use primers. In a less cost it's possible to perform the RT PCR for MiRNA with own designed primer and probes.

References

1. E.G. (2015). Entrez Gene: MicroRNA 145. Retrieved 2015-01-26.

2. Götte M., Mohr C., Koo C.Y., Stock C., Vaske A.K., Viola M., Ibrahim S.A., Peddibhotla S., Teng Y.H., Low J.Y., Ebnet K., Kiesel L. and Yip G.W. (2010). miR-145-dependent targeting of junctional adhesion molecule A and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness. Oncogene, 29(50),6569-6580.doi:10.1038/onc.2010.386.PMID 20818426.

3. UPL (2017). Universal Probe Library. https://lifescience. roche.com/en_in/brands/universal-probe-library.html. 05/06/2017

4. Larsson E., Fredlund Fuchs P., Heldin J., Barkefors I., Bondjers C., Genové G., Arrondel C., Gerwins P., Kurschat C., Schermer B., Benzing T., Harvey S.J., Kreuger J. and Lindahl P. (2009). Discovery of microvascular miRNAs using public gene expression data: miR-145 is expressed in pericytes and is a regulator of Fli1. Genome Medicine, 1(11), 108. doi:10.1186/gm108.PMC 2808743. PMID19917099.

5. Zhang J., Guo H., Zhang H., Wang H., Qian G., Fan X., Hoffman A.R., Hu J.F. and Ge S. (2011). Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. Cancer, 117(1),86-95. doi:10.1002/cncr.25522.PMC 2995010.PMID 20737575.

6. Sachdeva M., Zhu S., Wu F., Wu H., Walia V., Kumar S., Elble R., Watabe K. and Mo Y.Y. (2009). p53 represses c-Myc through induction of the tumor suppressor miR-145. Proceedings of the National Academy of Sciences of the United States of America, 106(9), 3207-12. Doi:10.1073/pnas.0808042106. PMC 2651330. PMID 1920 2062.

7. Slaby O., Svoboda M., Fabian P., Smerdova T., Knoflickova D., Bednarikova M., Nenutil R. and Vyzula R. (2007). Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology, 72(5-6), 397-402. doi:10.1159/000113489.PMID 18196926.

8. Starczynowski D.T., Morin R., McPherson A., Lam J., Chari R., Wegrzyn J., Kuchenbauer F., Hirst M., Tohyama K., Humphries R.K., Lam W.L., Marra M. and Karsan A. (2011). Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. Blood, 117(2),595-607. doi:10.1182/blood-2010-03-277012.PMID 20962326.

9. Zsolt C., Julianna H., Zoltan S., Eva V., Zoltan H., Erzsebet S., Attila V., Balazs D., Maria B., Attila H., Balint D., Zsolt T., Laszlo N. and Balint A. (2013). A Versatile Method to Design Stem-Loop Primer-Based Quantitative PCR Assays for Detecting Small Regulatory RNA Molecules. PLoS One, 8(1), e55168. doi: 10.1371/journal.pone.0055168. PMCID: PMC3561390

10. Busk P.K. (2014). A tool for design of primers for microRNA-specific quantitative RT-qPCR. BMC Bioinformatics, 15, 29. doi: 10.1186/1471-2105-15-29.