Question 1:

Identification of a novel lncRNA may be performed by _____.

❍ Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

❍ RNA fluorescence in situ hybridization (RNA FISH)

❍ Chromatin isolation by RNA purification followed by deep sequencing (ChIRP-seq)

● RNA sequencing (RNA-seq)

Explanation:

RNA-seq allows the detection of all RNAs expressed by cells or tissues, including lncRNAs, whereas the other techniques are useful when the RNA has already been identified.

Question 2:

Validation of novel identified lncRNAs may be determined by _____.

● Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Explanation:

qRT-PCR is the most effective technique to validate and quantify the expression of RNAs, including lncRNAs

❍ RNA interactome analysis followed by deep sequencing (RIA-seq)

❍ Chromatin isolation by RNA purification followed by deep sequencing (ChIRP-seq)

❍ lncRNA interacting protein analysis

Question 3:

RNA fluorescence in situ hybridization (RNA FISH) is capable of _____.

❍ identifying RNA-interacting proteins

❍ identifying the RNA interactome

● determining the subcellular localization of RNA

Explanation:

RNA FISH is a cytogenetic technique that uses fluorescent probes with a high degree of sequence complementarity to the RNA of interest. Probes are designed to detect the subcellular localization of RNA and their relative abundance by direct visualization of the RNA molecules inside the cells, whereas the other techniques are more useful for determining the RNA function.

❍ identifying the functional role of RNA

Question 4:

Identification of lncRNA-binding proteins can be achieved by _____.

❍ RNA interactome analysis followed by deep sequencing (RIA-seq)

❍ Chromatin isolation by RNA purification followed by deep sequencing (ChIRP-seq)

● RNA pulldown followed by Mass Spectrometry

Explanation:

The pulldown assay uses a RNA probe labeled with a high-affinity biotin tag which allows the probe to be recovered. The lncRNA-protein complex is purified using magnetic streptavidin beads and the proteins are then eluted from the RNA and detected by mass spectrometry.

❍ Specific knockdown of lncRNA

Question 5:

RNA interactome analysis followed by deep sequencing (RIA-seq) is capable of _____.

❍ localizing the cellular compartment in which the lncRNA is expressed

● identifying the RNAs that interact with lncRNA

Explanation:

RIA-Seq consists in the use of biotinylated DNA probes to pull-down the endogenous lncRNA and its associated RNAs via streptavidin-associated magnetic beads. After purification, the RNAs are identified by RNA-seq.

❍ identifying novel transcribed regions and alternative spliced forms of annotated genes

❍ identifying the RNA interacting proteins