RNase PROTECTION ASSAY & REPORTER ASSAY

Submitted BY

Dr. Namrata Kahar (Guest Faculty)

Department of Biotechnology

Govt. Digvijay Autonomous College Rajnandgaon

(C.G.) 491441, INDIA

RNase prOtection assay and reporter assay

CONTENTS

CONTENTS OF RNASE PROTECTION ASSAY

- WHAT IS RNASE PROTECTION ASSAY?
- WHAT IS RNA PROBE?
- PROTOCOL FOR RNASE PROTECTION ASSAY
- HYBRIDIZATION AND PROCEDURE
- RNASE DIGESTION OF HYBRIDIZED PROBE AND SAMPLE RNA
- ELECTROPHORESES AND IMAGE ANALYSES OF SAMPLES
- ADVANTAGES
- DISADVANTAGES
- APPLICATIONS

RNase prOtection assay and reporter assay

CONTENTS

CONTENTS OF REPORTER ASSSAY

- WHAT IS REPORTER GENE
- WHAT IS REPORTER GENE ASSAY
- PROTOCOL FROM LUCIFERASE ASSAY
- ADVANTAGES
- DISADVANTAGES
- APPLICATIONS
- SUMMARY
- CONCLUSION
- REFERENCES

RNase prOtection assay and reporter assay

INTRODUCTION

➢ WHAT IS RNase PROTECTION ASSAY?

- ❖ RNase protection assay is a specific, sensitive and qualitative method for detection, mapping and quantitation of specific mRNAs transcription start-site localization.
- ❖ It provides more information that cannot be obtained reliable by other methods.
- ❖ It can be performed with either total RNA or poly A+RNA and the results are not depended upon purified or non-degraded RNA.

RNase prOtection assay and reporter assay

ABOUT RNA PROBE

- RNA probe is synthesized by bacteriophage RNA polymerase which initiates transcription from specific phage promoters that have been engineered into a number of common plasmid vectors.
- RNA probes are sequences of a variable length that are used to detect the presence of complementary nucleotide sequences in a sample.

RNase prOtection assay and reporter assay

PROTOCOL

Prepare a linearized template DNA which produces the sense strand RNA of a target gene.

The template should be purified with phenol at least twice and chloroform/isoamyl alcohol

RNase prOtection assay and reporter assay

PROTOCOL

Incubate at 37°C (T3 or T7), or 40°C (SP6) for 1hr.

Add 1ul DNase I (2U/ul). Incubate at 37°C for 15 min.

Add 180ul distilled water, vortex, and add 200ul of phenol (pH 8.0). Vortex 10 sec and centrifuge (15 k rpm) at RT for 5 min.

Repeat the P-OH treatment, and then do a chloroform/isoamyl treatment.

RNase prOtection assay and reporter assay

PROTOCOL

Add 15ul of 10M NH_4OAc and 375ul EtOH. 15min at –80C, and centrifuge.

Repeat the NH4OAc/EtOH precipitation to remove free NTPs.

Suspend precipitate with 50ul distilled water, and measure OD at 260.

Dilute the solution to desired range of concentration with 100% formamide, and store the diluted solutions at –20C.

RNase prOtection assay and reporter assay

PROTOCOL

A. HYBRIDIZATION PROCEDURE

EtOH-precipitate 10 to 30ug of RNA samples, and resuspends them in 10ul of Hybridization III buffer (Ambion)

Incubate the samples at 55C for 30min or longer to resuspend the ppts completely

Add 0.5ul of the radio labeled probes (2000cpm/ul) to the samples (Final 100cpm/ul). Vortex briefly and heat them at 95C for 10min. Vortex again after the incubation

Incubate tubes at 55C for 16 to 18 hrs.

RNase prOtection assay and Reporter assay

PROTOCOL

B. RNASE DIGESTION OF HYBRIDIZED PROBE AND SAMPLE RNA

Prepare fresh master mix of RNase I and RNase digestion buffer. Each sample requires 70 units of RNase I and 140ul of the mix. Put this on ice.

After hybridization, transfer the sample tubes to another tube rack in RT, for cooling purpose, and add 140ul of the ice-cold mix. Vortex immediately for a few sec

RNase prOtection assay and reporter assay

PROTOCOL

RNASE DIGESTION OF HYBRIDIZED PROBE AND SAMPLE RNA

Incubate at 30C for 45min. (Use water bath instead of heat block.)

Prepare fresh master mix of EtOH, NH_4OAc and yeast RNA. Leave this mix at RT, and vortex very well before use.

Stop digestion by adding 4ul of 10% SDS. Vortex 5sec. Leave them for 1min

RNase prOtection assay and reporter assay

PROTOCOL

RNASE DIGESTION OF HYBRIDIZED PROBE AND SAMPLE RNA

Add 354.5ul of the precipitation mix per tube, and vortex briefly

Cool tubes at –80C for 30min

Centrifuge at 15k rpm for 30min.

Pipette off supernatant very carefully. The residual EtOH inhibit electrophoreses later. Do not dry the ppts.

Resuspend the ppts with 2ul of 1x loading buffer. Vortex briefly.

RNase prOtection assay and reporter assay

PROTOCOL

C. <u>ELECTROPHORESES AND IMAGE ANALYSES OF SAMPLES</u>

Heat samples to 95°C 10min before loading.

Run the samples on a 1x TBE, 0.35mm thick 5% PAGE/6M Urea. Pre-run gel 200V 30min before loading.

Run the gel at 200V for 30min, followed by 350V for 90min.

RNase prOtection assay and reporter assay

PROTOCOL

ELECTROPHORESES AND IMAGE ANALYSES OF SAMPLES

After EP, transfer the gel onto Whatman 3mm chromatograph paper.

Dry the gel by a vacuuming gel drier for 0.5 to 1hr.

RNase prOtection assay and reporter assay

DIA GRA MAT IC

VIE W

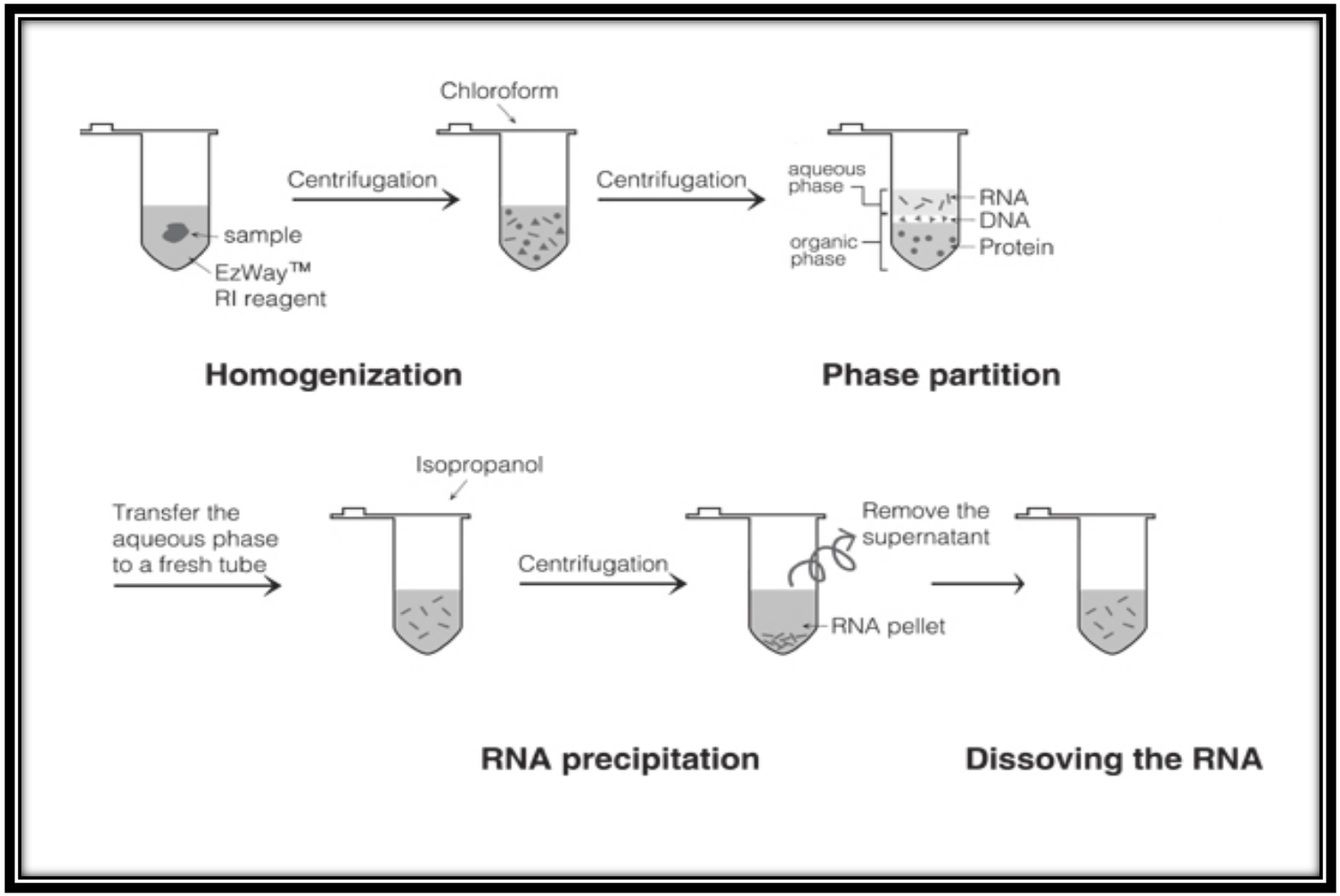

fig no. 1Protcol for RNase protection assay

RNase prOtection assay and reporter assay

DIA GRA MAT IC

VIE W

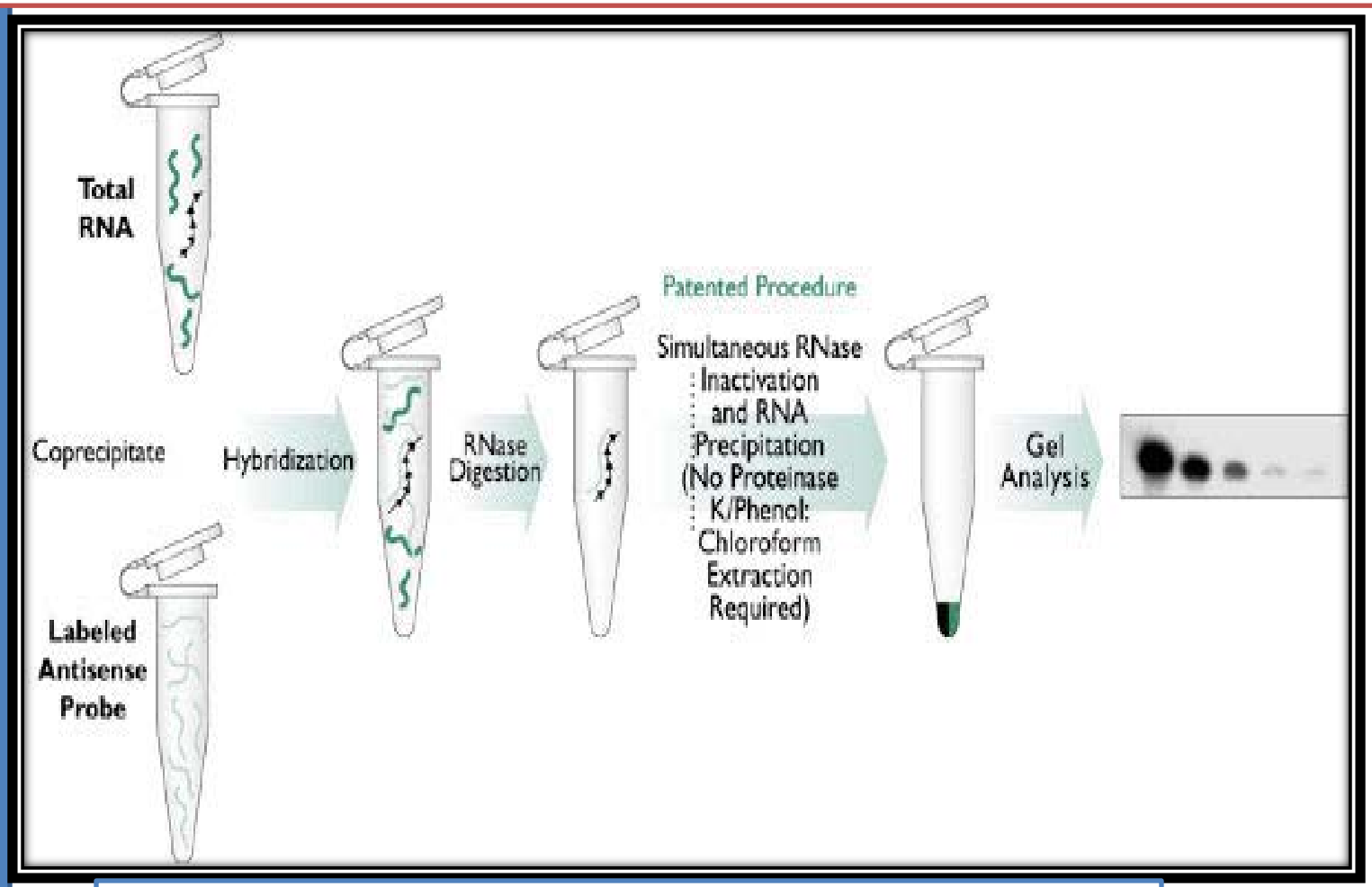

Fig no. 2 diagrammatic view of RNase protection assay

RNase prOtection assay and reporter assay

ADVANTAGES

- It is extremely sensitive.
- Low level transcripts can be easily detectable by this.
- Probes can be synthesized with high levels of radioactivity to increase signals.
- It is cost effective.

RNase prOtection assay and reporter assay

DISADVANTAGES

- Keep one tube RNase free.
- Use a sense RNase probe.
- One assay takes several days to complete, lengthy exposure times.

RNase prOtection assay and reporter assay

APPLICATIONS

- Different types of tissues or tumors in different tissues.
- Drug treatment versus control tissues (i.e. antidepressant)

RNase prOtection assay and reporter assay

INTRODUCTION

WHAT IS REPORTER GENE?

- They are also called as marker gene which produces a phenotype, which permits either an easy selection or quick identification of the cells in which it is present.

- They are either selectable or scorable marker.

RNase prOtection assay and reporter assay

INTRODUCTION

WHAT IS REPORTER GENE?

- They are also called as marker gene which produces a phenotype, which permits either an easy selection or quick identification of the cells in which it is present.

- They are either selectable or scorable marker.

RNase prOtection assay and reporter assay

INTRODUCTION

- A selectable marker is that marker which is defined as it enables only those cells that possess it to survive under the selective conditions. For ex :- an antibiotic like **kanamycin**

- A scorable marker produces distinct phenotypes which allow an easy identification of the cells having them from those that do not contain them. For ex: - **lux** (**luciferase**; produces phosphorescence) etc

RNase prOtection assay and reporter assay

INTRODUCTION

WHAT IS REPORTER GENE ASSAY?

They are the versatile and sensitive methods of assaying numerous targets, variety of reporter genes allow us a signal that can be used to the required sensitivity.

RNase prOtection assay and reporter assay

PROTCOL

Distribute 2 ml of cells into each well of 6-well culture plate

Incubate the cells for 24 h in CO_2 incubator

Prepare plasmid DNA (10 mg of target LUC plasmid [Firefly luciferase] + 0.5 mg of pRL-SV40 control vector [*Renilla* luciferase] / 0.5 ml Opti-MEM I) and transfection reagent (Lipofectin, 1 mg/ml, InVitrogen, 84 mg [84 mL] / 0.5 ml Opti-MEM I) in separate tubes, and allow to stand at room temperature for 30 – 45 min.

RNase prOtection assay and reporter assay

PROTCOL

Combine two solutions, mix gently, and incubate at room temperature for 10 – 15 min → "Transfection solution"

Wash cells once with serum-free growth media

Add 1 ml of transfection solution prepared above to each well

Incubation for 1.5 h at 37 °C → "Transfection"

Remove transfection solution from each well, add 2 ml of complete growth media (w/ serum) into each well and incubate the cells for 24 h in CO_2 incubator → "Recovery of cells"

RNase prOtection assay and reporter assay

PROTOCOL

The cells are washed with HANKS and experimental growth media with chemical treatments are replenished

Incubation for additional 16 h in CO_2 incubator

RNase prOtection assay and reporter assay

PROTOCOL

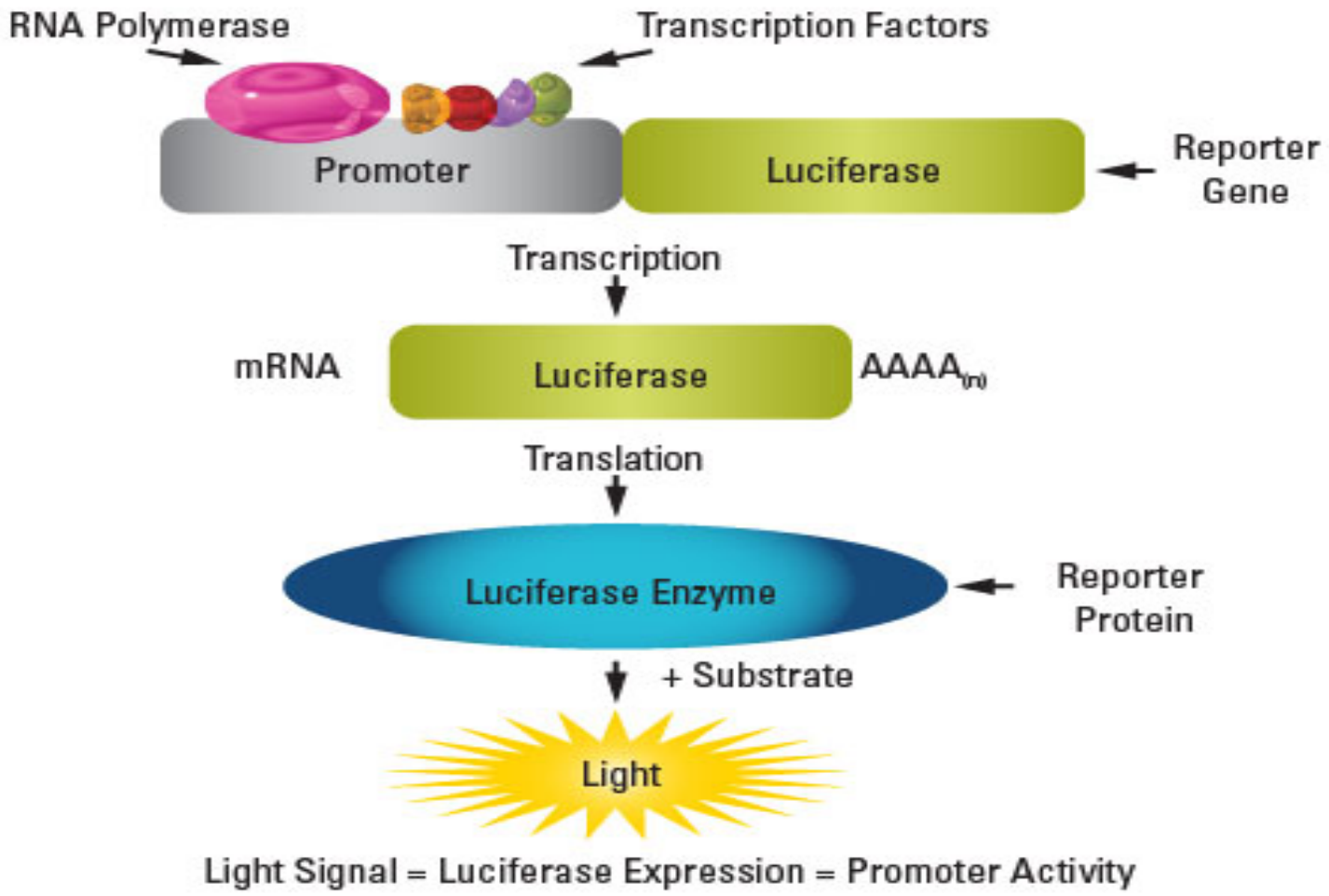

Fig no. 3 reporter gene assay using luciferase marker

RNase prOtection assay and reporter assay

ADVANTAGES

- They have high sensitivity and broad linear range.
- The absence of luciferase enzyme in most of the cell types.
- Rapidity and low costs.

RNase prOtection assay and reporter assay

DISADVANTAGES

- They are very costly as compared to other assays.

RNase prOtection assay and reporter assay

APPLICATIONS

- Studying protein interactions with luciferase reporter.
- Analyzing nuclear receptor signaling with luciferase receptors.

RNase prOtection assay and reporter assay

SUMMARY

- RNase protection assay is a specific, sensitive and qualitative method for detection, mapping and quantitation of specific mRNAs transcription start-site localization.
- Protocol for RNase performed were tedious and easy.

- Reporter gene were the versatile and sensitive methods of assaying numerous targets, variety of reporter genes allow us a signal that can be used to the required sensitivity.

RNase prOtection assay and reporter assay

CONCLUSION

- They were easy to perform and are very useful.
- Reporter assay and RNase assay are important assay to be performed.

RNase prOtection assay and reporter assay

REFERENCES

P.S. VERMA N
A. K. AGARWAL 2009 GENETICS S.CHAND

B.D. SINGH 2010 BIOTECHNOLOGY EXPANDING HORIZONS

SATYANARAYANA U. 2010 BIOTECHNOLOGY

INTERNET *www.ncbi.nlm.nih.gov/pubmed*