RNA detection

RNA denature more easily than DNA, temperature sensitive

Phenol-chlorofoam extraction:

- RNA dissolve in phenol chlorofoam, precipitated in isopropanol.
- Quick, low cost,high yield
- Not safe, not pure

Column Based:

- Lysed solution passed through a silicone column, RNA will adhere to the column while DNA and impurities gets washed off.
- Higher purity
- Lower yield, more expensive

Bead based:

- Magnetic beads extract RNA
- High yield, quick
- High cost

DIfferent tissues yield various amounts of RNA:

- Metabolically active tissue such as muscle or liver yield more RNA
- In contrast, bones have relatively smaller amount of RNA

Northern blotting:

- Electrophoresis in 0.5% agarose gel with 0.8% denaturing chemical (Urea/formaldehyde), disrupt the secondary structure.
- When labelled with P32 and transferred onto a blotting paper, a autoradiograph can be generated.
- mRNA is shown as clean and bulky lines, while a smear indicates degraded mRNA
- tRNA with ~100nucleotide should show faint signal at the end.
- DNA is shown as a blot near the loading well

Bioanalyser

- Uses capillary electrophoresis and fluorescence
- Give a RNA integrity number (RIN), reflect the proportion of RNA above a certain size
- 1-10 with 10 being the highest scale of intactness
- PCR only require quality of 3~4

Nuclease Protection Assay

- Single stranded, P32/fluorescent labelled probe is added to the RNA extract solution
- RNA nuclease/S1 nuclease is also added into the solution to digest single strand RNA/probe
- The probe is then removed to analyse the remaining RNA sequence
- The assay can be used to assess presence of a particular transcript / map the position of a splice site (by assessing where the probe was cut), level of fluorescence can reflect RNA quantity.
- However the assay is restricted to probe size (20-30bp)

Reverse transcriptase: RNA dependent DNA polymerase

- RNA is fragile, degrade easily, card to use for analysis, cDNA is used

- Contains the same components as a DNA polymerase (Finger, thumb, palm) + RNA nuclease
- Primer used to start reverse transcription:
  tRNA in vivo/artificial primer (OligT targeting poly A) to provide 3'-hydroxyl group for DNA synthesis
- Single stranded cDNA is synthesised as the RNA is degraded by RNase
- DNA polymerase used to synthesise dscDNA

RT-PCR/qRT-PCR

- RT-PCR semi quantitative, compare with control, qRT-PCR measure real time fluorescence level
- Double stranded stable cDNA can be used to conduct Real time quantitative PCR\

Sequencing of RNA molecule uses cDNA molecule

- Fragmentation, adaptor, sequencing similar to illumina or Sanger
- Short sequences can be generated, as it derives from mRNA, it is exon only, can be compared to database
- Allow sequencing of: mRNA, rRNA, sncRNA, exons.
- Allow functions such as: cell type identification, diagnostic/prognostic, detect expression changes, personalised medicine

Deep sequencing: sequence the tissue for thousands of times:

- Complete transcriptome representation
- Detection of rare RNA species
- Single cell resolution
- Expensive, takes long, sample degradation, more starting material

Regulatory non-coding RNAs:

- Small interference RNA (siRNA): derived from dsRNA, maturation aided by dicer, mature in cytoplasm
- Micro RNA (miRNA), derived from long primary precursor RNA, maturation aided by Drosha, mature in cytoplasm
- Piwi-interacting RNA (piRNA): derived from long repetitive clusters, cleaved by PIWI, mature in male germline cytoplasm
- Short hairpin RNA (shRNA): its DNA can be introduced into cells via vector, induce expression, mimick miRNA, cleaved by Drosha, mature in nucleus

The sncs bind with their argonaute protein, form RISC(RNA -induced silencing complex)

RISC interact with complementart RNA sequence induce degradation or inhibition of RNA

SncRNA possible action positions:

- Antisense: binding prevent protein binding
- Cis-encoded: binding next to the target, disrupt structure
- Trans-encoded: binding away from target, influence target gene expression.

RNAi therapies:

- HIV
- Hepatitis B and C
- Fragile X
- Cancer
- Neurodegenerative diseases

Difficulties: Delivery specificity, influence endogenous genes, off target, clinical side effects.

RNA extraction pro + con

Tissue yield

Northern blot

RNA quality bioanalyser

NPA

Reverse transcriptase

RNA sequencing method targets functions

Deep sequencing

SncRNA 4 type, protein, mechanism

Therapeutics