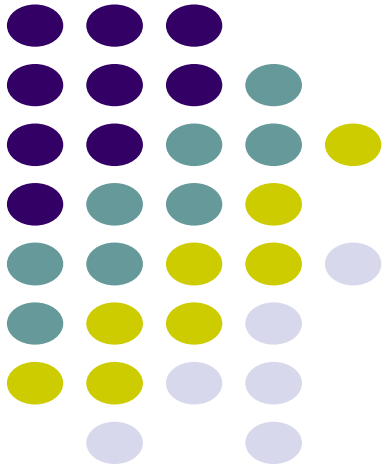
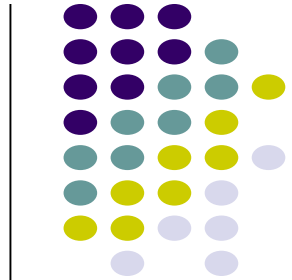
siRNA based techniques

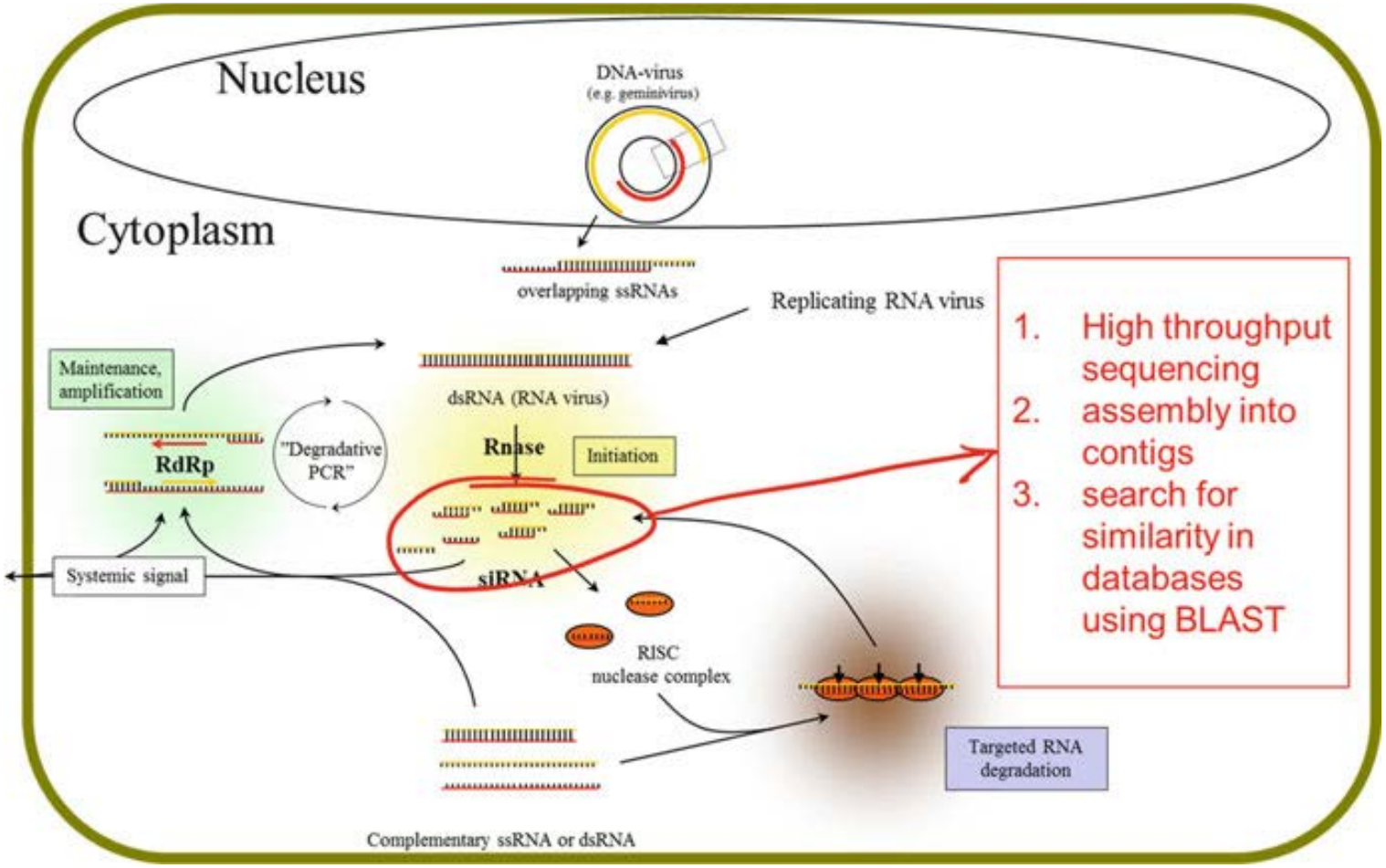


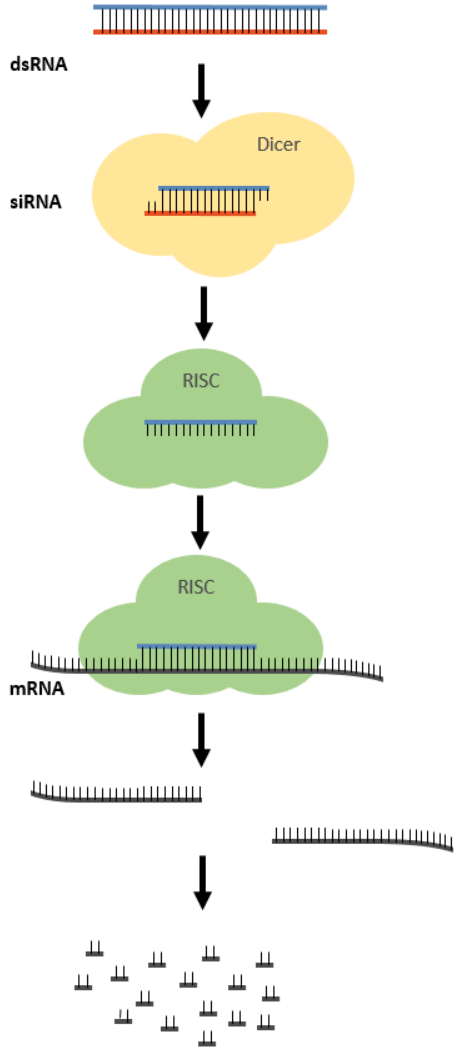


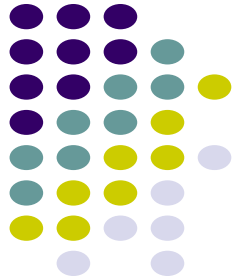
- RNA silencing (RNAi) is a cytoplasmic cell surveillance system to recognize double standard RNA and specifically destroy single and double stranded RNA molecules homologous to the inducer, using small interfering RNAs (siRNA) as a guide.
- Viruses are both inducers and targets of RNAi that constitutes a fundamental antiviral defence mechanism in eukaryotic organisms. It is particularly important in plants that use RNAi to recover from virus disease. The use of high-throughput sequencing of small RNAs (sRNA) from plants can successfully identify the viruses infecting them, including previously unknown viruses, even in extremely low titre symptomless infections.

- RNA silencing constitutes a fundamental antiviral defense mechanism in plants in which host enzymes cut viral RNA into pieces of 20–24 nt. When isolated, sequenced en-mass and properly assembled these virus-derived small RNA (sRNA) sequences can reconstitute genomic sequence information of the viruses being targeted in the plant.
- This approach is independent of the ability to culture or purify the virus and does not require any specific amplification or enrichment of viral nucleic acids. Using this technique known and novel DNA and RNA viruses as well as viroids have been identified at sensitivity levels comparable to PCR.

Antiviral RNA silencing leads to the accumulation of virus derived siRNAs, which can be isolated, sequenced and assembled to posteriorly identify viruses by homology to known virus sequences

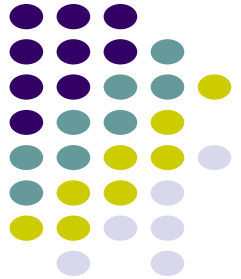






- RNA silencing recognizes double-stranded RNA (dsRNA) and eliminates RNAs homologous to the inducer RNA by cleavage using RNase III endonucleases called dicers.
- Plants encode several Dicer-like enzymes that recognize and cleave long dsRNA molecules to 21-, 22-, and 24-bp fragments that act as siRNAs.
- siRNAs bind to ribonuclease H–like proteins in the RNA induced silencing complex (RISC) and are used to detect homologous single-stranded RNA (ssRNA) molecules for cleavage, producing more siRNAs.
- In plants, RNAi becomes amplified when the cleaved RNA recruits an RNA-directed RNA polymerase to generate more dsRNA, which is again cleaved by a dicer protein to produce secondary siRNAs, that are once again able to detect and cleave homologous RNA in a type of ‘degradative PCR’ cycle. This leads to the accumulation of large amounts of siRNAs with homology to the invading virus.

Application of Next Generation Sequencing (NGS) for siRNA detection



- NGS platforms such as Illumina (Solexa) and SOLiD generates massive amounts but rather short reads of nucleotide sequences. With them a set of bioinformatics tools are used for de novo assembly of such short reads. Deep sequencing of siRNAs could thus lead to detection and diagnosis of plant viruses.
- NGS platforms has been used to detect
  - Reverse Transcribing Viruses
  - Known viruses
  - New viruses
  - Mixed Infections, Defective RNA/DNAs and Contamination