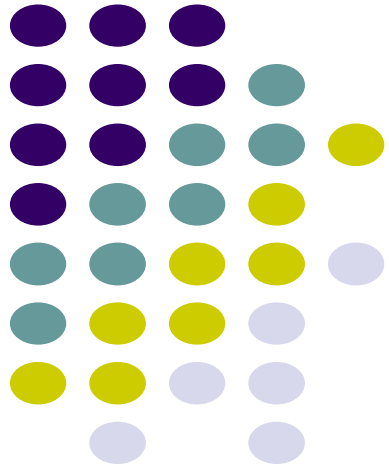
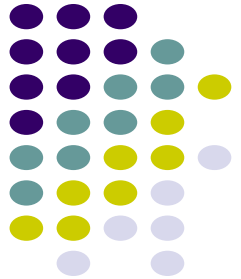
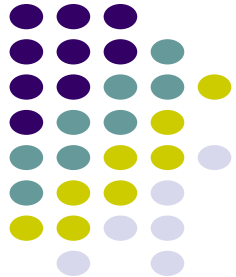
Genomics based diagnosis



Genomics-Based Detection

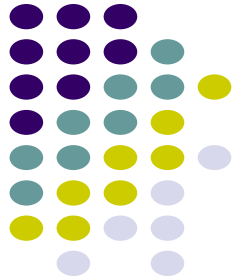


- Next-generation sequencing (NGS) technology, also known as pyrosequencing or high-throughput sequencing, revolutionizing in the field of detection of pathogens in a variety of plant samples.
- Unlike other molecular methods, which require prior knowledge of sequence information on the pathogens, the NGS approach is unlimited, making it possible to detect any known and novel pathogens in a single assay



- This approach is not a rapid test but important for identification of unknown bacteria, viruses and viroids, as no previous sequence data of the organism is needed, and there is also no need to culture the organism (only around 10 % of bacteria are culturable)
- Discovery of new viruses/viroids and new hosts has increased rapidly after the introduction of NGS.

Application of NGS in diagnosis of plant pathogen: case study



- The advent of high throughput sequencing has made it feasible to determine the genome sequence for the phytopathogenic bacteria *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of emerging pathogen bacterial canker of kiwifruit in New Zealand, in a short time.
- The original outbreak strain was sequenced using Roche 454 GS Junior sequencing platform and within the first weeks of the outbreak a draft genome sequence was assembled and the causal agent was diagnosed and the biovar was established.
- Proper management schedule could be adopted due to rapid diagnosis of the pathogen in New Zealand

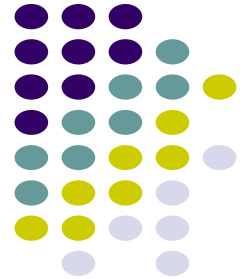
Detection NGS

454	Illumina HiSeq2000	Pacbio RS	Illumina MiSeq
2008	2011	2012	2014
			
<900 < 1 Gb/run	100 < 300 Gb/run	>3000 < 500 Mb/run	100 < 8.5 Gb/run
De-novo Amplicon Metagenome	De-novo RNA seq genome reseq Metagenome Epigenome Bisulphite Chip-Seq	De-novo Amplicon Epigenome	genome reseq Amplicon Metagenome

Platform Features

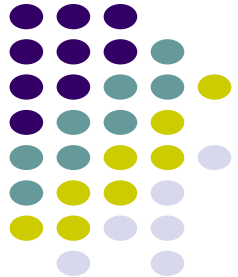
Feature	HiSeq2500 - Highoutput	HiSeq2500 – Rapid mode	MiSeq	PacBio RSII
Number of reads	150-180M/lane	100-150M/lane	12-15M (v2) 20-25M (v3)	50-80K/SMRT cell
Read length	2 x 100 bp	2 x 150 bp	2 x 300 bp (v3)	~ 10-20 kb
Yield per lane (PF data)	up to 35 Gb	up to 45Gb	up to 15 Gb	up to 0.4 Gb
Instrument Time	~12-14 days	~2 days	~2 days	~2 hours
Pricing per Gb	$59 (PE100)	$53 (PE150)	$108 (PE300)	$697

NGS: known and unknown plant diseases identified within a day



- With NGS, one can find out within a day whether or not a plant material is infected with pathogens.
- As this technique captures both the usual and unknown suspects, it is a true breakthrough in genetic plant diagnostics.
- PCR technique detects pathogens like fungi, viruses and bacteria by multiplying specific parts of their DNA. The disadvantage of this is that the search is highly selective: one base his/her assessment of which pathogen is likely to be present on certain symptoms, and then one adapt the analysis accordingly. NGS eliminates the need to make that determination in advance, as all of the possible pathogens can be directly identified

Billions of building blocks per sample



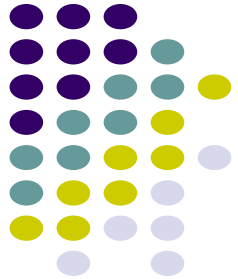
- Unlike the old sequencing techniques, NGS maps billions of genetic sequences for an entire plant sample. One can scan billions of nucleotides, the building blocks of DNA, and the order of these nucleotides determines to which type they belong.
- This reveals the plant's own sequences and allows us to see which divergent sequences are present in the sample. One conduct his/her analysis using advanced software that have 'fed' with genetic information on plants and pathogens, which enable us to quickly issue a reliable and definitive answer. Depending on the quality of the DNA sample, that answer can even be provided within a day.

- This speed is a major advantage in a sector where time is money. For instance, if a quarantine organism is discovered in a shipment, all other biological products in the shipment is detained. This leads to a significant loss of value.
- The same principle applies to cultivation: the longer it takes to acquire results that are certain, the longer one has to wait to combat a disease. All parties in the chain — the producers, importers and exporters — benefit from rapid diagnostics because it can give them considerable cost savings.

Tracking & Tracing

- The NGS technology is also suitable for tracking and tracing. Many bacteria and viruses are transmitted via plant material like seeds. Because this material is transported on such a large scale, however, it is sometimes difficult to trace the origin of an infection.
- By ascertaining the sequence of a pathogen with NGS, we can figure out information such as "this occurs naturally in a country or not." This allows us to promptly take targeted measures in the country of origin.

Steps involved in NGS based diagnosis



- Collection of Specimens
- Conventional testing
- DNA/RNA extraction and sequencing
- Pathogen identification