



WAGENINGENUR  
For quality of life

# NGS Special

March 2014

## Welcome!

Our next generation sequencing facility has been improved by the PacBio *RS II* upgrade and a new MiSeq. This creates new possibilities for sequencing much longer read lengths and provides much more flexibility! The possibilities of the improved PacBio and the key advantages of using extra long sequence read lengths will be demonstrated during a seminar on 26 March 2014. Learn more about the seminar and these exciting new sequencing technologies in this NGS Special.

We are looking forward to working with you on your sequencing projects!

*Andries Koops – Manager Business Unit Bioscience, Wageningen UR*  
*Petra Caessens – Manager Operations CAT-AgroFood*

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## Learn more about the applications of the PacBio *RS II*

### PacBio Seminar

**Date** 26 March 2014

**Time** 9 – 16 hours

**Venue** Hof van Wageningen

Lawickse Allee 9

6701 AN Wageningen

The Netherlands

**Registration and more information**

[www.wageningenUR.nl/pacbioseminar](http://www.wageningenUR.nl/pacbioseminar)

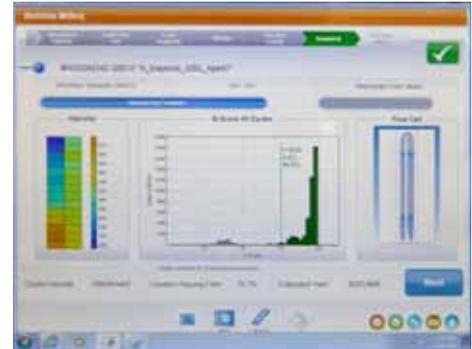


Join the seminar on  
**26 March**

# ILLUMINA MiSeq added to Next Generation Sequencing facility

**The MiSeq Desktop Sequencer was added to CAT-AgroFood's Next Generation Sequencing facility in February 2014. This purchase allows for sequencing 300 base pair long read lengths and gives maximum flexibility using nano, micro or normal scalable runs. The MiSeq's short nano run takes less than six hours. The longest run is 65 hours, during which it sequences up to 15 gigabases.**

The MiSeq is particularly suited for projects that benefit from a larger read, like any de-novo genome sequencing, metagenomics or amplicon sequencing as well as for QC purposes to streamline large HiSeq sequencing projects.



## MiSeq is faster, HiSeq is cheaper

"The MiSeq is a great addition to the HiSeq", said Gabino Sanchez Perez, genomics & bioinformatics specialist at Wageningen UR, who ran his first samples soon after the machine was installed. The most important advantage, according to Sanchez Perez, is that by making use of the 300 bp paired end reads of the MiSeq we can create merged reads that are five times longer than the reads of the HiSeq: up to 550 base pairs long. The HiSeq, on the other hand, has a better "price per base".

## Throughput or targeted application?

The HiSeq sequences up to 300 gigabases in one run. This run, however, usually takes twelve days. Sanchez Perez: "So what do you do in the meantime? You use the MiSeq for targeted applications." And he sees other good possibilities to combine the HiSeq and MiSeq. It can happen that samples are contaminated. This will only become apparent after they have been sequenced. So before starting a twelve day run in the HiSeq, it would be wise to first check the samples for contamination in the MiSeq, Sanchez Perez suggests. This check is done in a matter of hours.





# PacBio machine in Wageningen 'among the best performing in the world'

**CAT-AgroFood's third generation sequencer PacBio *RS II* is performing better than ever before. Since it was upgraded from *RS I* to *RS II* and supplied with a new chemistry kit in the summer of 2013, the throughput has doubled and the read length has massively increased. "It is now one of the best PacBio's in the world, delivering the longest read lengths amongst the existing service providers", says Gabino Sanchez Perez. In Wageningen the PacBio is used to sequence tomato, melon, animal, fungal and microbial genomes.**

## **Reads of 30,000 base pairs**

Where the Illumina HiSeq sequences fragments of up to 100 base pairs, the PacBio *RS II* produces reads with an average of 8,000 base pairs, and can even produce reads of up to 30,000 base pairs. Hence, it becomes much easier to locate the position of base pairs and piece the genome puzzle together.

The PacBio is known to have an error rate of 15 per cent. "But the good thing is that the errors are absolutely random, so if you read a sample three times you can eliminate almost all errors", explains Sanchez Perez.



## **Blue Pippin makes PacBio even more useful**

**To get the most out of the PacBio *RS II*, CAT-AgroFood also purchased a Blue Pippin for preparing higher quality libraries. This instrument selects genome fragments according to size. To make sure that only the long fragments will be subsequently sequenced by the PacBio, which gives much more useful results.**

## **Longer reads at lower costs**

**Besides the fact that we have upgraded our PacBio, we have also reduced our costs. This means longer reads at lower prices. To get a quote for your sequencing project, making use of the PacBio *RS II* and the expertise of Wageningen UR Bioscience, please contact:**

### **Elio Schijlen**

E elio.schijlen@wur.nl  
T +31 (0)317 48 12 09

### **Gabino Sanchez Perez**

E gabino.sanchezperez@wur.nl  
T +31 (0) 317 48 17 42



# The Hybrid Approach

**To find out what a gene does, high throughput is required. To understand how genes come to expression it is necessary to know the location of a gene. Knowledge of gene function and location is best acquired using 'The Hybrid Approach'.**

## Finding traits

The Illumina second generation sequencers of CAT-AgroFood are great tools to get detailed information about genes. By comparing large numbers of genomes, it becomes possible to find out which genes are responsible for what traits.

## Finding a gene's location

Locating where on the genome which gene is found, however, is much easier with the PacBio. The long reads of the PacBio are particularly useful for breeding purposes. Once it's known where wanted or unwanted genes are located, they can be targeted accurately.

## Combining trait and location

The cheapest and fastest way to get a better understanding of genomes is to use the Hybrid Approach. For high throughput the HiSeq is the best option, the MiSeq is very useful for targeted applications and the PacBio helps to generate complete large and complex genome assemblies.



## About CAT-AgroFood and Bioscience research

**CAT-AgroFood's** shared research facilities provide researchers the opportunity to use the advanced research equipment of Wageningen UR. Facilities are available for:

- Next generation sequencing & biomarker identification
- Microscopy & imaging
- Advanced mass spectroscopy
- Separation & fractionation

### Contact

info.cat-agrofood@wur.nl  
www.wageningenUR.nl/cat-agrofood

**Wageningen UR Bioscience** combines biochemical, bioinformatics and biostatistics expertise with extensive knowledge of DNA sequencing, proteomics and metabolomics.

### Contact

bioscience@wur.nl  
www.wageningenUR.nl/pri/bioscience

