



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

Faculty of Natural and  
Agricultural Sciences

Fakulteit Natuur- en Landbouwetenskappe  
Lefapha la Disaense tša Tlhago le Temo

# The Forest Molecular Genetics (FMG) Programme

[www.up.ac.za](http://www.up.ac.za)



**The Forest Molecular Genetics (FMG) Programme** focuses on the genetic control of growth and development in fast-growing plantation trees with a view to enhance biomass production and improve wood properties for timber, pulp, paper, and biomaterials production. Concomitant with this, we also aim to understand molecular pest and disease resistance mechanisms in trees for woody biomass protection. The programme is hosted in the **Department of Genetics** and affiliated with the **Forestry and Agricultural Biotechnology Institute (FABI)**, as well as the **Genomics Research Institute (GRI)** at the University of Pretoria (UP). We work in close collaboration with South African forestry companies through the **FMG Consortium (FMGC)** to develop capacity and resources for the application of tree biotechnology in operational tree improvement programmes and the development of woody biomass feedstocks supporting the bioeconomy of South Africa.

**Director:** Prof Zander Myburg

#### **Forest Molecular Genetics Programme**

Department of Genetics  
Private Bag X20  
Hatfield  
0028

#### **Contact details:**

**Tel** +27 (0) 12 420 4945  
**Email** zander.myburg@fabi.up.ac.za  
**URL** <http://www.fabinet.up.ac.za/index.php/research-groups/forest-molecular-genetics>  
or <http://www.fabinet.up.ac.za/zmyburg>



**Follow FMG on Twitter:** [https://twitter.com/FMG\\_UP](https://twitter.com/FMG_UP)  
**@FMG\_UP**



**Prof Zander Myburg**  
Director: Forest Molecular  
Genetics Programme

## About the Director

Zander Myburg is a professor in the Department of Genetics in the Faculty of Natural and Agricultural Sciences at the University of Pretoria (UP). He holds the Chair in Forest Genomics and Biotechnology and directs the Forest Molecular Genetics (FMG) Programme, a successful research venture that he started with UP, Sappi and Mondi in 2003 and expanded with co-funding from THRIP, the NRF and DST (over R60 million over the past 13 years) and with several new industry partners (e.g. Hans Merensky and York Timbers) joining the Programme in 2015.

Together with his research team, Prof Myburg's work has focused on the genomics and molecular genetics of wood development in forest trees and, in particular, the genetic regulation of cellulose biosynthesis in *Eucalyptus* trees. He also leads the development and application of DNA marker

technology for fingerprinting, parentage analysis and molecular breeding of *Eucalyptus* and pine tree species grown in South Africa.

Prof Myburg was the lead investigator of the US Department of Energy (DOE) *Eucalyptus* Genome Project which resulted in the publication of the reference *E. grandis* genome sequence (Myburg *et al* 2014, *Nature*) and a special issue of the journal *New Phytologist* (April 2015) expanding on the genome biology of eucalypts. In 2015, Prof Myburg received the National Science and Technology Forum (NSTF)-BHP Billiton-W Kambule Award for his contribution to research and its outcomes in South Africa in the last 5 to 10 years. He has supervised 40 postgraduate (MSc and PhD) students and is author of 68 peer-reviewed papers and book chapters in the field of plant molecular genetics and genomics.

**Under his leadership, the FMG research team has pioneered the use of population genomics and systems genetics approaches to unravel the genetic control of wood formation in *Eucalyptus*.**

# New Phytologist

*International Journal of Plant Science*



## Special Issue of New Phytologist on *Eucalyptus* Genomics

Following the publication of the *Eucalyptus grandis* genome sequence (Myburg *et al.* 2014, *Nature*) the journal *New Phytologist* published a special issue celebrating the completion of the *Eucalyptus* genome and featuring genome companion papers further unfolding insights into the unique biology of *Eucalyptus* trees gained from the analysis of the genome sequence.



Together with our international collaborators, FMG researchers and students contributed to eight articles in the special issue covering diverse topics such as genome diversity, comparative genomics, protein evolution, carbon allocation, reproductive biology and woody biomass production. This set of papers represents a significant advance in the understanding of the biology of the most widely planted hardwood fibre crop in the world. Together with the completed genome of *Populus*, the *E. grandis* genome resource will serve as a model and reference for the study of fast-growing woody plants that are used as renewable feedstocks for a growing number of bio-based products such as timber, pulp, paper, cellulose, textiles, pharmaceuticals and bioenergy.

# Deputy Director General of DST visits FMG

On 27 July 2015, the Forest Molecular Genetics (FMG) Programme hosted a visit by a delegation from several government departments and industry associations. The delegates included the Deputy Director General: Technology Innovation of the Department of Science and

Technology (DST), Mr Mmboneni Muofhe, as well as senior representatives from the Department of Agriculture, Forestry and Fisheries (DAFF), Department of Public Enterprises (DPE), the University of Pretoria, Forestry South Africa (FSA), Sappi, the Agricultural Research Council (ARC) and GrainSA.



# NRF Science for Society Lecture Series

Prof Zander Myburg and Prof Bernard Slippers (Professor in Genetics at the University of Pretoria and member of the Tree Protection Co-operative Programme in FABI) delivered talks on 'Biotechnology: Productive forest plantations as a possible answer to our energy and material needs' on 13 August 2015 at the University of Pretoria. The talks, part of the NRF Science for Society Lecture Series, were aired on SAfm 104-107 *The Talkshop*. In their talks, they explored key issues on woody biomass crops as a sustainable resource and discussed threats to this resource in the form of pathogens and pests,

development of genetically modified plants and creating high-value tree varieties that are fast growing and resistant to pests and disease. The presentations were followed by a panel discussion involving Prof Slippers, Prof Myburg, Dr Sanushka Naidoo, Dr Steven Hussey and Dr Eshchar Mizrahi. Questions were posed by the audience and listeners at home. Discussions covered a range of topics including advances in developing GM trees, use of indigenous forest tree species to increase productivity, protection of biodiversity and environmental impact of forestry.



**From Top Left Clockwise:** The Vice-Chancellor and Principal of UP, Prof Cheryl de la Rey introduces the speakers, Prof Myburg gives a talk on tree biotechnology, Mr Ike Phaahla of SAfm with panellists.



## The FMG Team

In 2015, Dr Steven Hussey joined the FMG Leadership Team with a contract appointment as Junior Lecturer. Dr Nanette Christie continues to lead the Bioinformatics Team in her capacity as Postdoctoral Fellow. We welcomed back our former colleague, Mrs L'Zanne Jansen van Rensburg, who re-joined the team as manager of the Wood Chemistry Technology Platform.

The Wood Chemistry and Bioinformatics teams also welcomed new interns, Ms Maureen Tladi and Mr Dirkie Boshoff. Sadly, we have had to bid farewell to our group Administrative Assistant, Ms Resego Moje, as she dedicated herself to further her tertiary education full-time. To fill her position as Administrative Assistant, we are pleased to welcome Ms Lauren Moonsamy.

### Management Team

Prof Zander Myburg	(Programme Director and Population Genomics Team Leader)
Dr Sanushka Naidoo	(Tree Disease Resistance Team Leader)
Dr Eshchar Mizrahi	(Modelling Wood Formation Team Leader)
Dr Steven Hussey	(Transcriptional Regulation of Wood Development Team Leader)
Dr Nanette Christie	(Bioinformatics Team Leader)
Mrs Marja O'Neill	(Project Coordinator)
Mrs Elna Cowley	(Laboratory Manager)
Ms Melissa Reynolds	(DNA Fingerprinting Platform Manager)
Mrs Adri Veale	(Plant Tissue Culture Platform Manager)
Mrs L'Zanne Jansen van Rensburg	(Wood Chemistry Platform Manager)
Ms Jane Bredenkamp	(QuantStudio™12K Flex Platform Manager)

# Principal Investigators



**Prof Zander Myburg**  
Population Genomics and Molecular Breeding

“Genomics technologies are providing powerful new avenues to manage genetic variation in breeding populations and predict breeding value of trees at an early age.”

My primary research interest is the genetic control of growth and development in trees with a focus on wood formation in fast-growing plantation trees such as *Eucalyptus* and tropical pine species.

Most commercially important traits are quantitative in nature and affected by hundreds to thousands of genes throughout the genome. DNA marker technology has proven to be a powerful tool to tag this genetic variation and develop predictive models of the genetic or breeding values of individual trees. By adding molecular traits such as gene expression and metabolite variation, we can gain further biological insight into the molecular basis of quantitative variation in tree growth and development. This forms the foundation of systems genetics



approaches combining the power of population genetics and systems biology to understand the nature of genetic variation affecting biological processes such as wood formation. We have successfully used this approach in an interspecific backcross population of *E. grandis* x *E. urophylla* to map key genomic regions affecting gene expression and metabolic profiles associated with variation in growth and wood chemistry.

Over the past two years we have successfully implemented the use of a single nucleotide polymorphism (SNP) chip with over 60,000 DNA markers to genotype several thousand *Eucalyptus* tree genomes from *E. grandis*, *E. dunnii* and *E. grandis* x *E. urophylla* hybrids. This has provided us with unprecedented resolution to rapidly dissect complex traits in *Eucalyptus* and





Population Genomics and Molecular Breeding Team 2015

develop predictive models for genomic selection of growth and wood properties. In 2015, we received funding from the Forestry Sector Innovation Fund (FSIF) to expand genotyping to other *Eucalyptus* and pine tree species. This project will generate a Genome Diversity Atlas for *Eucalyptus* and pine species grown in South Africa and will expand the development of experimental tree populations for genetic dissection and genomic breeding. The project will also form the foundation for the development of the emerging field of landscape genomics which combines population genomics with analysis of environmental phenotypes such as cold, drought and disease to predict tree genotypes that are adapted to these environments, or that can be deployed to combat new biotic challenges such as pests and diseases.

“Over the past two years we have successfully implemented the use of a single nucleotide polymorphism (SNP) chip with over 60,000 DNA markers to genotype several thousand *Eucalyptus* tree genomes from *E. grandis*, *E. dunnii* and *E. grandis* x *E. urophylla* hybrids.”



EPPI "Plant Defenders" Team 2015

“What makes a tree sick? This question intrigues the *Eucalyptus* and Pine Pathogens Interactions group in FMG. When a plant is challenged by pathogens or pests, a cascade of defence responses ensues in the plant. The timing of this response and the activation of the correct gene pathways determine the outcome of the interaction. The pathogen is also known to actively manipulate the plant defence responses.”



NRF Acting CEO, Dr Beverley Damonse (left) presenting Dr Sanushka Naidoo (right) with the NRF Y2 Rating Award at the UP Achiever Awards 2015.

## Dr Sanushka Naidoo

### *Eucalyptus* and Pine Pathogens Interaction Group: Plant Defenders

A successful defence response results in resistance while an unsuccessful response results in susceptibility. We are interested in the differences in such susceptible and resistant interactions in forest trees. Comparison of these responses allows us to identify potential factors within the host-pathogen/pest interaction that are driving susceptibility within the host. With genomic resources available for both host and pathogen, dissecting the interaction between the two role players is now possible.

Using this approach we are currently investigating *Eucalyptus* defence responses against four different organisms, each representing a different type of biological threat. These four biological threats are: the fungal stem canker pathogen *Chrysosporthe austroafricana*, the root rot oomycete *Phytophthora cinnamomi*, the fungal leaf rust *Puccinia psidii*, and the insect pest *Leptocybe invasa*. The *Eucalyptus* work is in collaboration with Dr Carsten Külheim (Australian National University), an expert on *Eucalyptus* terpenoid metabolism and their effects, Dr Geoff Pegg, a forest pathologist from Australia (Queensland Department of Agriculture and Fisheries) and Prof Katherine Denby (University of Warwick, UK), who specialises in producing interaction networks to produce systems biology models of complex plant interactions.

Pine is the other half of our focus. *Pinus patula* is highly susceptible to the fungal pathogen *Fusarium circinatum* and manifests as a

wilting disease in seedlings. An understanding of the interaction would facilitate the identification of factors contributing to susceptibility. This is now possible due to the completion of the *P. patula* juvenile shoot transcriptome. This resource will provide the means to determine responses in the host during infection, and can be applied to other *Pinus* species. Once again, we will compare these responses with a resistant interaction to determine which genes are contributing to susceptibility or resistance. Collaborator Prof Jill Wegrzyn (University of Connecticut) continues to support the bioinformatics aspects of this research.

To improve our capacity to study defence response genes and genetic modification, we have embarked on a project employing the powerful genome editing CRISPR-Cas9 technology in *Arabidopsis*. We have also increased tissue culture capacity and have initiated a collaborative project with Prof Steve Strauss (Oregon State University), on transgenic *Eucalyptus* for research purposes.

Taken together, these advancements will culminate in improved understanding of “what makes a tree sick?” and prompts active research to address the next question i.e. “how do we prevent a tree from becoming sick?”





## Dr Nanette Christie

Bioinformatics and Statistical Genomics

Understanding the regulatory gene networks underlying traits in *Eucalyptus* trees will enable molecular breeding and genetic engineering to improve wood quality and disease resistance in the long term. Achieving this goal involves the application and development of advanced data analysis tools for large genomics datasets across many individuals in breeding populations.

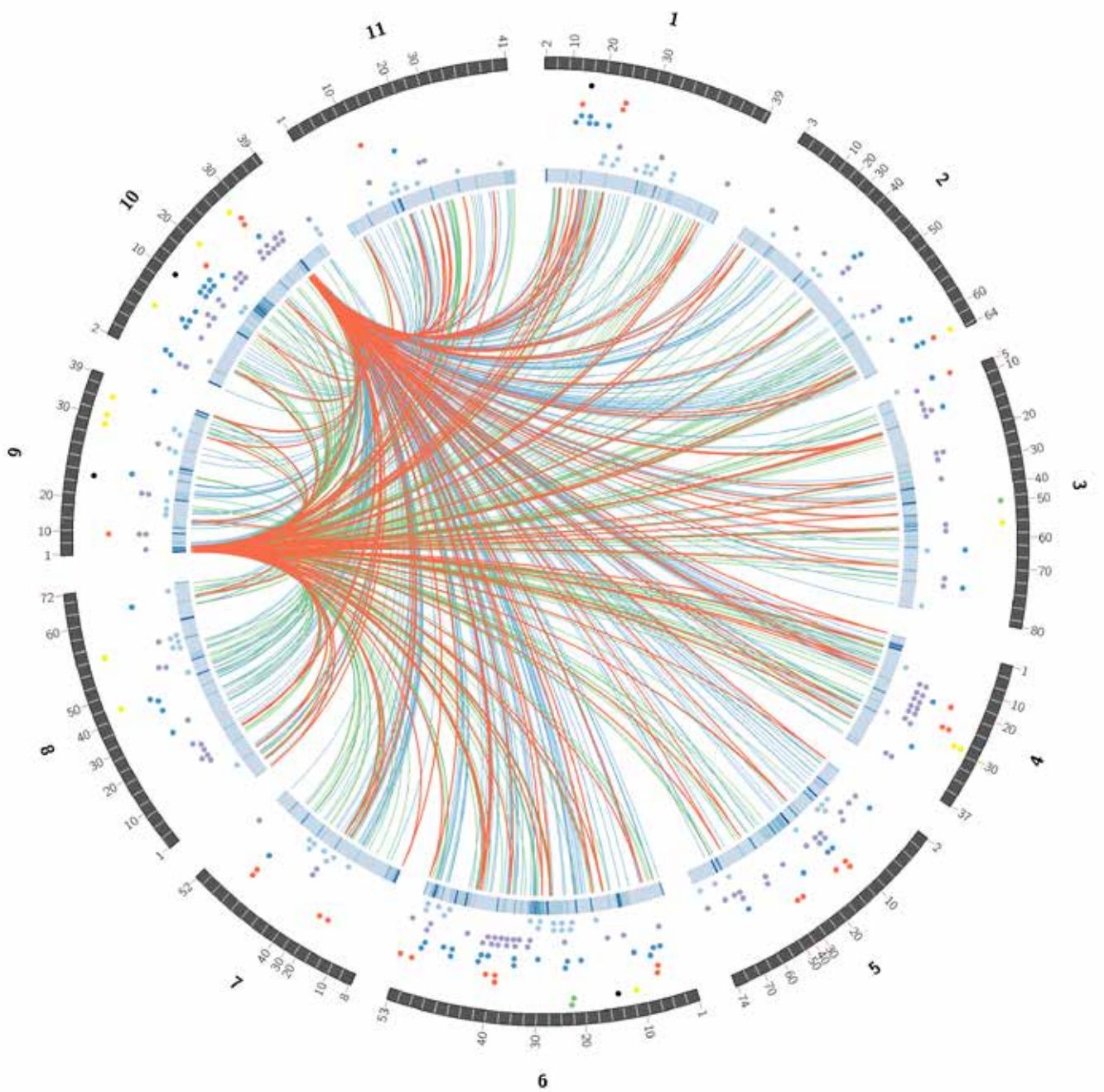
The first branch of my research includes the development of systems genetics data analysis pipelines for *Eucalyptus* data from our breeding populations. This involves the identification of quantitative trait loci (QTLs), i.e. genomic regions containing genetic variation, for phenotypic, gene expression (eQTLs) and metabolite (mQTLs) traits, as well as the visualisation and co-localisation of the different types of QTLs. This platform, together with gene co-expression and correlation analysis, provides an excellent basis for the study of molecular networks underlying our phenotypic traits of interest.

I am also interested in studying the extent and complexity of alternative mRNA splicing, an important level of genetic regulation that is completely under-studied in *Eucalyptus*. During gene expression, alternative splicing results in a single gene coding for multiple mRNA transcripts with regulatory functions or encoding multiple protein variants with different

biological functions. We are using RNA sequencing to measure and quantify gene expression, and to identify the full repertoire of alternatively spliced isoforms in the xylem transcriptome of *Eucalyptus*. Ultimately, we would like to identify the DNA variants that cause these splice events and investigate the contribution of splice variation to wood quality and pest and disease resistance traits in *Eucalyptus* trees.

Finally, the identification of novel and functional resistance (R)-genes, which provide specific resistance to biotic stress, is a key objective for strategies to enhance resilience. I am involved in characterising the large family of R-genes, with nucleotide binding sites and leucine-rich repeat domains (NBS-LRR), in *Eucalyptus*. The genomic arrangement of R-genes in clusters and superclusters suggest recent evolution via tandem duplication.





Overview of wood property trait QTLs and trans-eQTL hotspots mapped in the *Eucalyptus urophylla* backcross population.





From Left to Right: Mr Matt Laubscher, Dr Steven Hussey and Ms Katrien Brown working with transgenic *Arabidopsis* plants



“Transcriptional networks and chromatin architecture comprise the “hardware” that reads and interprets the genome, or the DNA “software”, of plants.”

## Dr Steven Hussey

Understanding Transcriptional and Epigenomic Regulation of Wood Formation

All organisms develop and respond to stimuli through complex regulatory interactions between genes. Vast transcriptional networks underlie wood development in plants, strictly regulating the deposition of secondary cell wall biopolymers and directing the differentiation of xylem. Additionally, the chromatin environment in which genes are housed is dynamically regulated at the epigenetic level. Together, transcriptional networks and chromatin architecture comprise the “hardware” that reads and interprets the genome, or the DNA “software”, of plants.

Our group aims to decipher the regulatory functions and evolution of transcription factors implicated in wood formation in *Eucalyptus*, and to understand the role of chromatin in the epigenomic regulation of transcription in woody tissues. We are using a number of reverse genetics systems to study *Eucalyptus* transcription factor candidates. For example, we have used *Arabidopsis* mesophyll protoplasts as a chassis to overexpress *Eucalyptus* fibre and vessel regulators for the rapid identification of their targets (hours post-transfection) using RNA-seq.

*Arabidopsis* is also used for reporter analysis of the promoter regions of these regulators. We use *Populus* as a model tree to study the phenotypic effects of *Eucalyptus* transcription factor overexpression on growth and wood development over the timescale of months and years. Chromatin immunoprecipitation sequencing (ChIP-seq) experiments of these transcription factors in the developing wood of field-grown *Eucalyptus* trees allow us to identify their direct gene targets.

In the field of epigenomics, we have generated the first genome-wide profiles of histone modifications (e.g. H3K4me3, H3K27me3) in developing xylem, using ChIP-seq analysis. These experiments have allowed us to quantify the contributions of these marks to gene expression and associate distinct chromatin modifications with different gene classes, such as tandem duplications. Future projects will aim to understand the differences in chromatin architecture between different tissues and build a comprehensive chromatin map for several modifications.



## Dr Eshchar Mizrachi

Modelling Wood Formation

**Understanding the evolution of these components in other vascular plant species provides an additional layer of information with regards to which components are essential, unique, redundant, or auxiliary, which further guides understanding and strategic intervention for biomass engineering.**



During wood formation, several processes occur concurrently on multiple levels of a tree. Leaves fix carbon from CO<sub>2</sub> in the air and water from the ground in the form of sugars and complex carbohydrates. Secondary phloem (transport tissue underlying the tree bark) transports these sugars and nutrients to dividing cells all along the circumference of the tree trunk. These cells differentiate from stem cells into fibre and vessel cells, which make up the majority of the cells in wood. Following coordinated cell division towards the inside of the tree, fibre cells elongate along the tree's axis of growth and fulfil their primary function — to synthesise large quantities of secondary cell wall polysaccharide and phenolic biopolymers and deposit them in a complex matrix that provides mechanical strength.

Taken together, trillions of fibre cells provide enough mechanical support to maintain a crown weighing thousands of kilograms, while maintaining flexibility in the face of stresses from multiple directions. Xylogenesis (this division of cambial stem cells and their development to ultimately form wood in a tree) is a tightly regulated and coordinated process. Within each fibre cell, a single copy of the genome expresses millions of copies of thousands of genes that are translated into proteins such as structural proteins, regulators, transporters and enzymes, which interact with and act upon several hundred metabolites (intermediate small molecules such as sugars or phenolics) in specific compartments within the cell to synthesise and deposit the cell wall components.





Wood Formation Team 2015

Modelling wood formation involves understanding how the multitude of potential interactions between these components (genes, proteins, metabolites) is organised in space and time, at all levels from single proteins to the whole tree. This is done by applying reverse engineering and systems reconstruction approaches to biological systems. We do this by obtaining quantitative data of components such as genes, proteins and metabolites from various tissues, organs, conditions, or individuals within a population, and integrating this data computationally, taking into account other information about the system (for example, previous knowledge about protein function, protein-protein or protein-DNA interaction or location within a cell). These models can be integrated for unique insights into fundamental biology and biotechnology of trees, particularly when co-analysing these components

with complex wood traits such as tree growth, wood properties and processability.

In addition, understanding the evolution of these components in other vascular plant species provides an additional layer of information with regards to which components are essential, unique, redundant, or auxiliary, which further guides understanding and strategic intervention for biomass engineering.

# Platforms

The FMG programme has developed five technology platforms which support student training and industry research projects.



## DNA Fingerprinting Platform

The DNA fingerprinting platform is currently supported by a team of four technical support staff. We work alongside our industry partners with the aim of complementing tree improvement research by providing a high-throughput DNA fingerprinting service for commercially grown eucalypt and pine species in South Africa. Our processing pipeline begins with DNA isolation from a range of plant tissues. The DNA is genotyped using either single nucleotide polymorphism (SNP, outsourced to a genomics service provider) or microsatellite DNA markers for applications such as clonal verification, parentage analysis and species discrimination. We have implemented a published panel of microsatellite markers to discriminate among seven commercially important *Eucalyptus* species; however, no comparable tool was available for *Pinus* species.

This was identified as a key research area, which led to a collaboration with Camcore and several industry partners in South Africa and Colombia over the last two years to develop a method to discriminate commercially important *Pinus* species such as *Pinus patula* and *P. tecunumanii*. In 2015, FMG received funding from the Forestry Sector Innovation Fund for several student projects which will focus on expanding these applications through DNA analysis of larger reference populations and additional eucalypt and pine species. This funding has also enabled the purchase of a liquid handling robot which will increase the accuracy and sample throughput of the platform.

## The Team:

### Ms Melissa Reynolds

I am the manager of the platform and my main roles are to oversee the team, liaise with our industry partners and assist in interpreting DNA analysis results within the biological context of each DNA fingerprinting project. Over the last two years I have enjoyed spearheading the development of the *Pinus* species discrimination tool, and more recently working alongside students towards expanding this application to include additional pine species and develop a new SNP marker based fingerprinting resource for *Eucalyptus* species.

### Mrs Patience Ralikonyana

First in the fingerprinting pipeline, I receive and process samples from our industry partners, which may be in the form of leaves, needles or bark from either eucalypts or pine trees. Thereafter, I isolate DNA from the tissue and perform quality control. This is one of the most important elements of my job and I take great care to ensure the quality and purity of the extracted DNA and tracking the identity of each sample through the process.

### Ms Maleshoane Selaocoe

Over the last year I have been involved in various aspects of laboratory work from sample processing to DNA isolation; however, my primary role in the team involves preparing samples for DNA fragment analysis. This requires preparation of samples for multiplex polymerase chain reaction (PCR) amplification with different panels of microsatellite markers. Following this, samples are submitted for fragment size based genotyping at the UP DNA Sequencing Facility. I have recently started taking responsibility for DNA marker data analysis and quality control.

### Mrs Christy Marais

My main roles in the team are to oversee the laboratory processing pipeline, perform data analysis and assist in interpretation and reporting of results. I have also enjoyed my involvement in new student projects such as the development of a SNP marker panel for *Eucalyptus*. I look forward to being part of the growth of our team in the coming year through mentoring the younger students working alongside our platform.

## QuantStudio™12K Flex Platform

The QuantStudio™ 12K Flex Real-Time PCR platform aims to promote large-scale quantitative real-time PCR-based functional research at the University of Pretoria and at a national level. Funding for the facility was obtained from the NRF National Equipment Platform (NEP) Grant and the QuantStudio™ 12K Flex service is therefore available to researchers throughout South Africa. The QuantStudio™ 12K Flex instrument together with high-throughput OpenArray® technology supports two important functional applications. Large-scale gene expression analysis of whole biological pathways under varying conditions and treatments will help piece together the roles that genes play in specific biological states or processes. Additionally, high-throughput SNP genotyping of several hundred variants in hundreds of samples will enable researchers to identify variants important for biological processes such as disease resistance and wood development.

### For more information please visit:

The FMG website service page at [www.fabinet.up.ac.za/index.php/fm-facilities-services](http://www.fabinet.up.ac.za/index.php/fm-facilities-services)



### Ms Jane Bredenkamp

I am a research assistant and manager of the QuantStudio™ 12K Flex Real-Time PCR facility. My responsibilities include maintaining and operating the instrument, managing user accounts, ordering and management of consumables associated with the QuantStudio™ facility, organising training sessions for new users and assisting users in designing their experiments and interpreting their results. Also, I am working to improve the visibility and market the applications and technologies of the QuantStudio™ 12K Flex system.



**Large-scale gene expression analysis of whole biological pathways under varying conditions and treatments will help piece together the roles that genes play in specific biological states or processes.**



## Plant Transformation Platform

The introduction of genes into plants by genetic engineering has become a routine technique in plant genetic improvement. Plant genetic engineering is the process by which useful genes are isolated from donor organisms and incorporated into a plant genome, or by which the expression of genes in the plant genome can be altered. Genes of agricultural importance, for example viral, fungal or insect resistance genes have been inserted into the genomes of several crops. These advances have been due to the development of efficient gene vector systems for plant transformation and regeneration. Production of transgenic plants has been perceived as a possible approach for the control of diseases, and improvement of plant quality.

The genetic engineering of tree species is still not as routine as transformation procedures in crops such as maize, potato, tobacco and tomato. Transformation efficiency for *Eucalyptus* trees has been very low and a breakthrough is required to make routine genetic manipulation feasible in *Eucalyptus*. *Populus* species have become the model tree for genetic transformation due to their high efficiency of *Agrobacterium*-mediated transformation, making the expression and characterisation of foreign genes in transgenic trees possible. The plant transformation platform in the FMG group has established capacity for genetic transformation and gene testing in *Arabidopsis* and *Populus* plants. We have also developed capacity for tissue culture and *in vitro* propagation of different *Eucalyptus* genotypes.

### The Team:

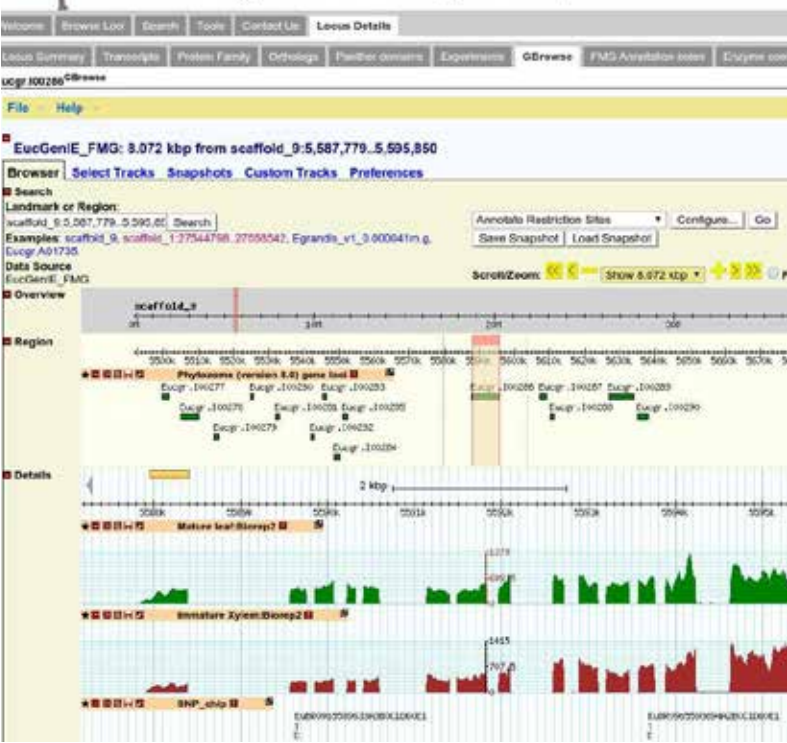
#### Adri Veale

I am the manager of the tissue culture and growth facilities of the FMG Programme. I am also involved in the development of transgenic *Populus* plants. This involves *Agrobacterium tumefaciens*-mediated transformation of poplar plantlets with secondary cell wall related genes, the growth of transformed lines in greenhouse trials and the use of molecular biology techniques to confirm the presence and expression of transgene constructs.

#### Grieta Mahlangu

I am responsible for the maintenance and multiplication of *Eucalyptus* and *Populus* tissue culture plants. This involves implementation of optimised techniques for plant sub-culturing and routine inspection for signs of contamination. I take great care to maintain the tissue culture plants in excellent condition prior to hardening-off and phenotyping trials.

**“The first GM *Eucalyptus* trees were approved for commercial use in 2015 — we are expanding our capacity to become GM ready.”**



EucGenIE showing a GBrowse instance of the Eucgr.H02200 gene, characterised as a cellulose synthase gene (CESA9). The first track shows the position of the gene in the genome on chromosome 8. The second track shows SNPs (Single nucleotide polymorphisms) identified in the gene that can cause changes in structure and expression, as well as help with genotyping of trees in a population. The last two tracks show the difference in this gene's expression between mature leaf and immature xylem tissues

## Bioinformatics Platform

The FMG Bioinformatics Platform is hosted in the Centre for Bioinformatics and Computational Biology at the University of Pretoria. Our team currently consists of a full-time bioinformatics programmer, Ms Karen van der Merwe, a postdoctoral fellow, Dr Nanette Christie, and the recent addition of a final year BIS Multimedia student, Mr Dirkie Boshoff. Working alongside biologists and geneticists in the FMG Programme, we are able to support and build capacity for the analysis, management and interpretation of large amounts of genomics and transcriptomics data for hundreds to thousands of individual trees. Web services, novel and commercial software, as well as a Galaxy workflow environment have been implemented to facilitate modular development of data processing and visualization pipelines. These pipelines can be accessed remotely by group members for high-throughput genomics analysis and enable quality control, processing of raw data and downstream analyses such as gene and genome annotation, gene expression analysis, detection of genomic variants, and discovery of genetics associations with phenotypic traits. Currently, a major focus is the development of the *Eucalyptus* Genome Integrative Explorer (EucGenIE), which is an online resource for *Eucalyptus* genomics and transcriptomics data.

## The Team:

### Dr Nanette Christie

Platform manager and Bioinformatics Team Leader, see page 8.



### Ms Karen van der Merwe

My main project has been the development and maintenance of The *Eucalyptus* Genome Integrative Explorer (EucGenIE: [www.eucgenie.org](http://www.eucgenie.org)). My duties also include student and staff support in the form of writing data analysis scripts, generating complex graphs and handling and maintaining data on the servers as well as on the Galaxy data analysis platform.



### Mr Dirkie Boshoff

My role is assisting Ms Karen van der Merwe in the development of EucGenIE by keeping the database up to date and contributing to the functionality and appearance of new and existing EucGenIE tools.



## Wood Chemistry Platform

The wood biochemistry platform was founded as a collaborative initiative between Sappi, the University of Pretoria and the University of British Columbia to support research in the FMG Programme and tree improvement in industry for commercially grown eucalypt and pine species in South Africa.

The platform is currently hosted in the Sappi Technology Centre (STC) in Pretoria where employees from FMG and STC are working closely together to develop new, and use established analytical protocols, to chemically characterise the major constituents of wood (cellulose, hemicellulose and lignin). The technology is not only used to generate phenotypic data for student projects in functional genetics and for breeding programmes, but also provides valuable data to generate and update near infra-red analysis (NIRA) calibration models for prediction of wood chemistry traits.

### The Team:

#### **Dr Berdine Coetzee**

My main role is to provide academic and operational oversight for the FMG Wood Chemistry Platform and to coordinate interaction with the STC. Over the last three years I have worked on applying existing analytical protocols in the bioanalytical lab as well as developing additional analytical protocols to expand the platform. I have a joint appointment in Sappi and the University of Pretoria as an extraordinary lecturer in the Chemical Engineering Department.

#### **Mrs L'Zanne Jansen van Rensburg**

I am managing the FMG Wood Chemistry Platform and my main roles are to oversee staff in sample preparation, processing and analysis, data processing and running and maintenance of analytical equipment.

#### **Ms Maureen Tladi**

As intern in the FMG Wood Chemistry Platform, my responsibilities are to provide technical assistance for diverse analytical techniques such as determination of lignin S:G ratios, Klason lignin, alpha-cellulose, and monosaccharides analysis in wood samples. These analyses involve the operation of equipment for HPLC, Gas-Chromatography and UV-VIS spectrophotometry.

#### **Ms Sharon Masipa**

As a research assistant for wood chemistry analysis my responsibility is to execute protocols that include determination of cell wall sugar content, Klason lignin, alpha-cellulose and lignin S:G monomer ratios in wood samples from *Eucalyptus* and *Populus*, and stem tissues from *Arabidopsis*. Quality control is one of the most important elements of my job and I take great care to ensure the quality and purity of results.

# Support Staff



## Project Coordinator:

### Mrs Marja O'Neill

We want to use molecular marker profiles to predict if a tree will be a good candidate for breeding. In order to achieve this, we draw associations between commercially relevant traits and molecular marker profiles. This process of Marker Assisted Breeding (MAB) generates large data sets from different sources (in-house, industry, academic collaborators, and international service providers) that require collation and continuous management. I am responsible for coordinating communication between the different contributors (staff, students and industry collaborators), data management and data analyses towards MAB projects. My personal interest lie in identifying and profiling formerly uncharted molecular variants, such as genome structural variants, to better understand the different aspects that make an individual unique.



## Laboratory Manager:

### Mrs Elna Cowley

As Laboratory Manager, I am responsible for the day to day activities in the FMG laboratories and equipment facilities. These duties include organisation of the laboratory, ordering consumables necessary for the daily research activities and importantly, I conduct safety training of all students and staff within the group. I also supervise the engagement of several undergraduate assistants and mentorship students who are associated with senior students in the laboratory. I work closely with the FMG Director to manage administrative activities for the FMG group.



## Administrative Assistant:

### Ms Lauren Moonsamy

My role is to provide administrative assistance to the Programme Director and academic staff of the FMG programme. This involves, in addition to others, preparation of research applications and technical/ financial reports, management of FMG student and personnel databases, coordination of group activities (e.g. field trips, visiting scientists, symposium) and marketing and communication functions (e.g. FMG website and brochure).

# Student Projects

## Postdoctoral Researchers



### Dr Gaby Nkouaya Mbanjo

My research focuses on the population genomics of growth and wood properties in *Eucalyptus*. More specifically, I am exploiting the latest high-throughput genotyping technology to generate high density genetic maps for *Eucalyptus* that are subsequently used for identification of genomic regions influencing economically and ecologically important traits including growth, wood properties and disease resistance. The new technologies have been used to generate the first SNP-based genetic maps of *Eucalyptus* using the recently developed EuchIP60K.Br SNP chip, and the map was used to find key genomic regions related to the expression of traits. This is the first step toward understanding of the genetic architecture of these complex traits and the precise characterisation of the polymorphisms involved in trait variation in the most planted tree genus worldwide. In terms of breeding implications, integrating molecular tools in forest tree breeding programs through selection of associated DNA markers is expected to result in early selection of progeny with desirable genetic variability for the targeted traits.

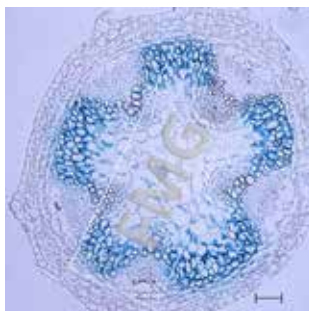


### Dr Louise Shuey

Plant pathogens are a constant threat to the productivity of the forestry industry. New diseases emerge and overcome current host resistance in a never ending arms race. The development of durable, broad spectrum resistance to pathogens in plants requires identification of key resistance genes and pathways. Identification of factors that contribute to host susceptibility allows for a greater understanding of plant-pathogen interactions. My postdoctoral work focuses on identification of plant defence responses in both resistant and susceptible hosts. Another factor of importance to disease resistance is the identification of genes and gene pathways used by a pathogen for disease development. The genes expressed by a pathogen and a host in the infection process can now be determined simultaneously by highly sensitive RNA sequencing technologies. This new technique, known as Dual RNAseq, provides information on the gene mechanisms used by the pathogen, and genes induced in a resistance response by the host. The eucalypt diseases I am currently working on include Myrtle rust, (causal agent *Puccinia psidii*), Quambalaria shoot blight (*Quambalaria eucalypti*), Chrysoporthe canker disease (*Chrysoporthe austroafricana*) and Phytophthora root rot (*Phytophthora cinnamomi*). The interactions of these fungal pathogens with each other will be the focus of future experiments.







### Dr Libert Brice Tonfack

Secondary cell walls have gained increased economical and biological importance, as their ultrastructure and composition are one of the major factors that significantly impact wood fibre quality and plant health. However, genetic modification of secondary cell walls in woody plants suffers from limitations due either to i) non-specific cell or tissue targeting causing undesirable phenotypes (drastic reduction of plant strength, destruction of vessels and significant reduction of hydro-mineral and photosynthate flows) or ii) compensatory effects by other genes in the same gene family or pathway. One of the best solutions is to design and construct a molecular tool that can improve predictability and specificity of gene expression or pathway modification. As part of my postdoctoral research I have constructed tissue-specific expression vectors using specific promoter sequences from *Arabidopsis thaliana* and from woody plants *Eucalyptus grandis* and *Populus* (*Populus alba* x *P. grandidentata*). We hypothesised that these promoters can drive gene expression specifically in the xylem fibres, or the vessel elements of woody plants. The vectors have been tested, using marker genes in the model plant *A. thaliana* with success and are being tested in *Populus* transgenics. These molecular tools could be used in wood biotechnology for functional studies related to specific tissue modification woody plants. This will help to better understand the processes of cell wall biopolymer biosynthesis and facilitate the bioengineering of modifications that may reduce recalcitrance of the cell wall to bioprocessing, or introduce novel properties to the extracted biopolymers.



### Mr Mmoledi Mphahlele

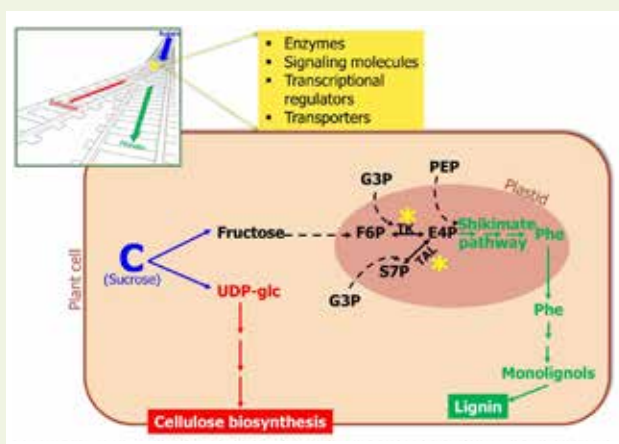
The development of the *Eucalyptus* EuchIP60K.Br single nucleotide polymorphism (SNP) DNA marker chip is propelling the development and implementation of genomic-wide selection (GS) technology in the forestry industry, in a similar manner as observed in the dairy cattle industry. Genomic selection involves the aggregation of genome-wide SNP marker effects on traits of interest to predict genomic estimated breeding value (GEBV) of individuals, for whom only genomic information is available without phenotypic information. As a Forest Biotechnology Specialist working in the Mondi Forest Biotechnology programme, I am involved in the operational implementation of practical marker assisted breeding (MAB) tools within *Eucalyptus* species to address improvements in wood properties, growth potential, and pest and disease resistance. My PhD study, as part of the FMG Programme, focuses on the adoption of genome-wide SNP information using best linear unbiased prediction (BLUP) and GS models to estimate and predict GEBV of wood properties and growth traits in *Eucalyptus grandis*. The study interrogates the use of pedigree and genomic relationship matrices, random regression (RR) BLUP and Bayesian GS models as MAB tools.

### Ms Elodie Ekoka

Wood is made of economically important biopolymers which include polysaccharides (e.g. cellulose and xylan) or lignin. These can be used to produce or add to a wide variety of high-value products, or can serve as a source of energy. Based on the economic importance of these biopolymers, effort has been put into characterising the genes which play a role (directly or indirectly) into carbon partitioning between sugars and lignin during the deposition of secondary cell wall in wood forming tissue (Figure). For example, the plastid enzymes Transaldolase (TAL) and Transketolase (TK) have been hypothesised to exclusively control the flux of Erythrose-4-phosphate (E4P) sugar towards the shikimate pathway producing lignin.



Although several other genes remain to be characterised, progress is now facilitated by the availability of a *Eucalyptus* genome and transcriptome, in addition to proteomics data from other model genera such as *Populus* or *Arabidopsis*. The long-term focus of my doctoral project is to elucidate the proteomics, metabolomics and the role of plastids in wood formation. Specific research questions to be addressed will include: How do the proteome profiles of the secondary xylem, phloem and leaves in *Eucalyptus grandis* compared to each other? What are the specific function(s) of transaldolase and transketolase in carbon-partitioning during xylogenesis? What are other transporters or signalling molecules which influence this carbon partitioning? What are their function(s)? The knowledge gained from this study can be exploited in future to produce transgenic trees with desirable wood properties.



By identifying and characterising the molecules (\*) which play a controlling role in carbon-partitioning between lignin (phenolic biopolymer route) and sugars (cellulosic biopolymer route), we can in future engineer trees with altered cellulose or lignin content.



### Ms Danielle Roodt

The focus of my PhD research is on the evolution and development of xylem in non-coniferous gymnosperms. As most research has focused on angiosperms, I will attempt to fill the gap in the knowledge of the evolution of xylogenesis by specifically focusing on the three lineages where the least amount of emphasis has been in terms of genomic resources: Cycadophyta, Ginkgophyta and Gnetophyta. Studying the evolution of xylogenesis is of interest to a range of scientific fields, including evolutionary and developmental biology, systematics biology, botany and biotechnology. The comparative genomics approach I will be applying to non-coniferous gymnosperms will not only shed light on the evolution of wood, but will also be pioneering work in these ancient organisms and can result in discovery of novel genes and pathways. I hope to lay the groundwork for future scientists from a diversity of backgrounds to add their own knowledge to what we are discovering of the evolution and development of wood.



### Ms Nare Ngoepe

The availability of a fully sequenced genome of *Eucalyptus* has opened a whole new window for forest tree genomics research. I am interested in genome-wide studies of genetic architecture of growth and wood properties in *Eucalyptus grandis* elite breeding trials. The main interest will be to find out which genes and genome-wide haplotypes are associated with differences in growth, fibre development, cellulose, xylan, and lignin biosynthesis in *E. grandis*. Genome-wide association studies (GWAS) have become one of the state-of-the-art biotechnological methods for detection of DNA markers associated with trait variation in plant and animal populations. My PhD study will contribute significantly to understanding of the genetic architecture of complex traits in *E. grandis* breeding trials and accelerating tree breeding by increasing precision and selection intensity for late expressing traits or characteristics that display low heritability in *Eucalyptus* species and *E. grandis* in particular.

### Mr Erik Visser

My work focuses on developing resources to study the interaction between pine species and the pitch canker fungus *Fusarium circinatum*. During my MSc I assembled the transcriptome of *Pinus patula*, the predominantly cultivated pine in South Africa, in collaboration with Prof Jill Wegrzyn from the University of Connecticut. For my PhD I will assemble the transcriptome for low elevation *Pinus tecunumanii* and use the assembled transcriptomes to compare defence responses between *P. tecunumanii*, which is resistant to *F. circinatum* infection, and *P. patula*, which is susceptible. In this manner, a model of defence in conifers can be developed, facilitating future biotechnological applications to enhance defence in these economically and ecologically important forest trees.



### Mr Jonathan Botha

It is becoming increasingly important to find environmentally friendly and sustainable solutions to current global challenges. Biomaterials (materials derived from a biological source) are a promising alternative to current petrochemical-derived products, but require appropriate feedstocks to be truly viable. Hardwood plantations hold great potential as second generation lignocellulosic biomass feedstocks, but the secondary cell walls (SCWs) of wood fibre cells are highly recalcitrant to enzymatic digestion, requiring extreme conditions and expensive enzyme cocktails to access and extract the cell wall polymers. Standard biotechnology techniques such as enzyme addition or *in planta* expression of lignocellulosic degrading enzymes from microbes hold some promise in addressing this problem. My PhD study aims to advance these approaches, by mining the genomes of sequenced and published microorganisms for novel SCW modifying enzymes using bioinformatic approaches, as well as designing and testing enzymes that may be expressed *in planta* that will reduce the recalcitrance of SCWs without negatively affecting the growth of the plant. In this way, the cost of processing lignocellulosic feedstocks may be greatly reduced, allowing for a sustainable and economically viable alternative to petroleum derived products and contributing to the growing bioeconomy in South Africa.

### Mrs Ronishree Mangwanda

The aim of my study is to investigate the transcriptomic changes that occur in two *Eucalyptus grandis* clones following infection by the fungal pathogen *Chrysosporthe austroafricana* as well as to understand how the pathogen is able to cause disease. This year, I have been involved in optimising the open array expression profiling system on the QuantStudio™12K Flex Platform. Using this platform we were able to elucidate transcriptional changes pertaining to phytohormone defence signalling that occurs in *E. grandis* in response to the fungus over a time series. Exploring the transcriptional changes of *C. austroafricana* revealed putative pathogenicity factors employed by the fungus. These included the expression of various cell wall degrading enzymes and the possible manipulation of phytohormone signalling. The fungus was found to express genes that potentially alter the salicylic acid and gibberellic acid pathways in the host. These pathways were identified to be involved in defence in *E. grandis*. This suggests the pathogen may actively target these hormone pathways for its own proliferation. Investigating both the hosts defence responses and the pathogenicity mechanisms of the pathogen, we were able to generate a hypothetical model for fungal defence in *E. grandis* and aim to extend this investigation to a *E. grandis* x *E. urophylla* backcross population in future.





### Ms Caryn Oates

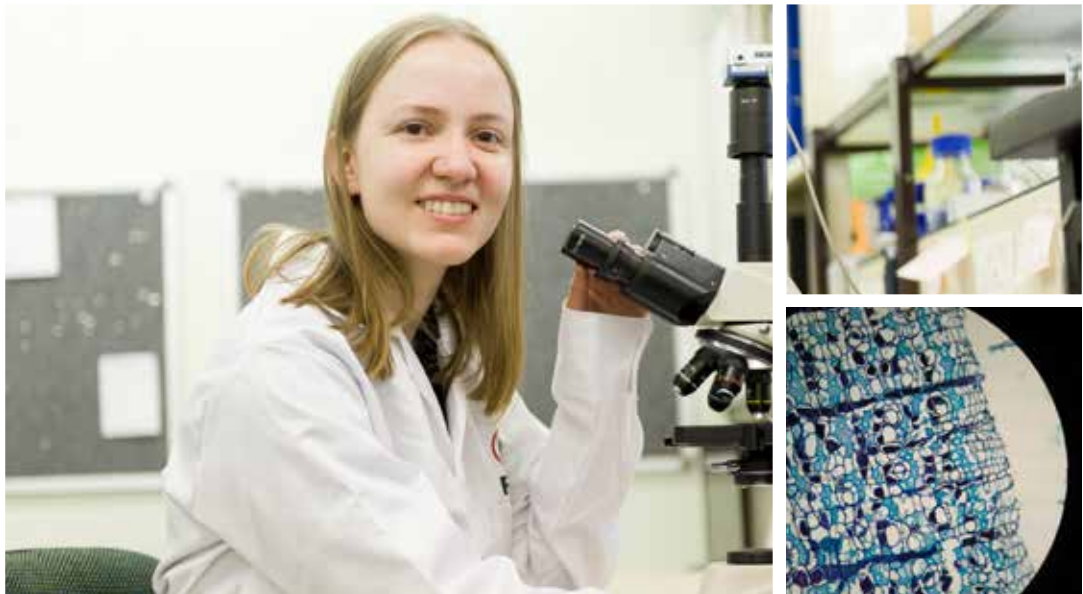
My research focuses on understanding the interaction between *Eucalyptus* and the gall-inducing wasp, *Leptocybe invasa*. This wasp is a devastating pest of *Eucalyptus* that can cause stunted growth, die-back and death in severe cases leading to the failure of some commercially important clones. Biological control is currently the most effective way of dealing with an infestation. By improving our understanding of the interaction we will be able to build on our current management practises and create an effective and integrated control system. I am interested in studying the transcriptional and cellular reprogramming that occurs to initiate gall development. I have produced transcriptome datasets of the earliest interaction between the wasp and its host (immediately following oviposition) over a time series from 24 to 72 hours post-oviposition. Analyses of these datasets will allow me to develop a network model of the *Eucalyptus* response to *Leptocybe* infestation, thereby identifying key genes in the interaction which will be of interest in biotechnological control strategies. I am also using light microscopy to track the induction and development of the *Leptocybe*-induced gall over the wasp's lifecycle to better understand the interaction between the organisms.



### Ms Desre Pinard

How is carbon metabolism regulated spatially within the cell? My primary research interest is the compartmentation and regulation of carbon metabolism in secondary cell wall formation. The role of plastids in carbon flux and partitioning to cellulosic and phenolic biopolymers is of particular interest to me. We plan to construct and use xylem co-expression networks from *Eucalyptus urophylla* and *E. grandis* hybrid backcross populations to identify regulatory modules that are responsible for carbon metabolism in plastids and mitochondria, and use this information to identify metabolite transporters and transcription factors that regulate carbon partitioning to different cellular compartments. The spatial aspect of carbon allocation in secondary cell wall formation towards wood has largely been ignored thus far. The underlying biology behind the compartmentation of metabolites and enzymes into even smaller metabolons within organelles is an unknown that has implications for carbon partitioning and cell biology in a broader context. Identification of metabolite transporters, signalling molecules, *cis*-elements and transcription factors that influence the partitioning of carbon metabolism within the cell and its allocation to cellulosic or phenolic biopolymers is a goal we aim to achieve within this PhD project.





### Ms Lizahn Zwart

My PhD research is focused on the interaction between *Eucalyptus* and the fungal stem canker pathogen *Chrysosporthe austroafricana*. I am investigating the physical interaction and host responses by means of histochemistry and microscopy, and have described the location of the fungus within this host and identified several putative host responses. The pathogen occurs in several cell types throughout the stem and appears to move through cell wall pits, while the host appears to respond by blocking vessel elements with tyloses and producing gum-like substances in the xylem. One of the significant findings to

date was that the fungus appears to interact directly with living cells in the xylem. This is a significant finding as the pathogen has been described as a necrotroph and thus we expected to find the fungus predominantly associated with dead cells. We decided to investigate the molecular responses of the living cells to infection by studying quantitative changes in the proteome of a partially resistant *Eucalyptus grandis* clone using iTRAQ (isobaric tags for relative and absolute quantitation). Genes that are differentially expressed in response to infection may provide clues about the processes that determine the outcome of infection.



### Ms Lorraine Mhoswa

The gall wasp, *Leptocybe invasa*, has emerged as a significant threat to production of *Eucalyptus* hybrids and clones in South Africa forestry. Therefore, my PhD research aims to define and validate a genome-wide association profile for *L. invasa* resistance in *E. grandis* with the potential to develop

a *Leptocybe* screening marker panel. This research aims to improve breeding efficiency for resistance to *L. invasa*. Also, this research aims to detect resistance associated leaf chemical signatures (chemotypes) in *E. grandis* as this could lead to identification of new signalling secondary metabolites involved in resistance to *L. invasa*.



### Mr Yves Du Toit

My MSc Bioinformatics degree involves comparative studies between three plant pathogen/pest interactions. These three, well established, pathosystems involve the interaction between *Eucalyptus spp.* and *Chrysosporthe austroafricana* (cause of stem cankers), *Leptocybe invasa* (a gall wasp) and *Phytophthora cinnamomi* (cause of root rot and stem cankers). Gene and transcript datasets have been produced for these three interactions, and I will perform functional analysis, by first defining gene and pathway function and then finding the over represented functions within the clusters produced. Novel insights will be generated for *Eucalyptus* defence for the first time, by using comparative analysis to ascertain the common and unique defense responses to an insect pest, an oomycete and a fungal pathogen. This project will contribute to our knowledge of why certain genotypes are resistant and others are susceptible to pathogen and pest attack, which defence genes and transcripts are in common among the pathosystems and could be a potential target for added resistance and defence using biotechnology applications.



### Mr Junior Tii-Kuzu

Genetic variants (alleles) undergo frequency changes within and among species or populations due to evolutionary and demographic factors. Understanding the frequency distribution of these alleles can allow us to discriminate different species, populations and even individuals and this knowledge can be used to manage gene conservation and tree improvement. Tropical pine tree species have been introduced into South Africa from Central America and Mexico. Natural populations in these regions are under threat due to forest logging and human development and, as a consequence, gene conservation programs are needed. Further, pine plantation forestry in South Africa is threatened by pitch canker disease and pine hybrid breeding has shown some promise to introduce resistance. Using DNA markers, I aim to explore and characterise the genetic diversity and structure of pine tree species grown in South Africa and utilise this knowledge to improve pine conservation and breeding.



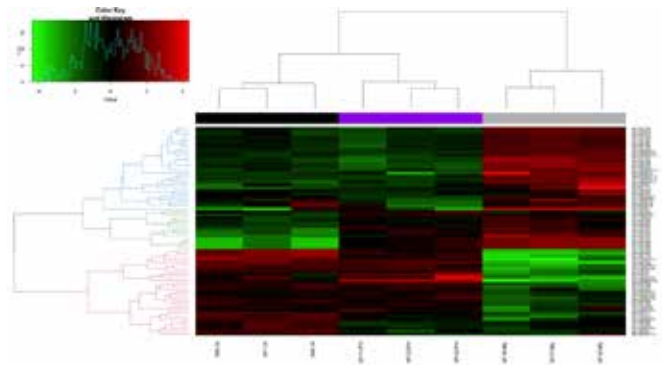
### Mr Riaan Swanepoel

As an alternative to genome sequencing and assembly, *de novo* assembly of transcriptome (expressed gene) sequence offers a large amount of data for gene and pathway discovery, as well as comparative genomics to understand the evolution of organisms. *De novo* assembly of transcriptome data can also identify novel structural variants of transcripts that may not be annotated in the genome. My project will focus on the *de novo* assembly of transcriptome data from *Eucalyptus grandis*, testing a variety of assembly algorithms and parameters. By doing this we can i) identify novel transcript structure such as alternative splice variants, and ii) optimise assembly parameters for other ongoing *de novo* transcriptome assembly projects.

### Mr Andrew Behrens

Research in xylem formation in vascular plants has revealed complex transcriptional networks regulating genes at all stages in this tissue's development. Additionally, finely-tuned coordination of carbohydrate partitioning and phytohormone release is crucial for the spatiotemporal progression of xylem development. As we gain an understanding of the regulation of protein coding genes necessary for wood formation, knowledge regarding non-coding genes is lacking, particularly in *Eucalyptus*. These genes produce RNA that do not encode proteins, but rather interact with the DNA, RNA and proteins of other genes to coordinate their expression. Among these are the microRNAs (miRNA), small interfering RNAs (siRNA) and long non-coding RNAs (lncRNA). These acts as pervasive gene regulators in most eukaryotic organisms that show intense tissue and lineage specificity, owing to their power to affect cell fate and

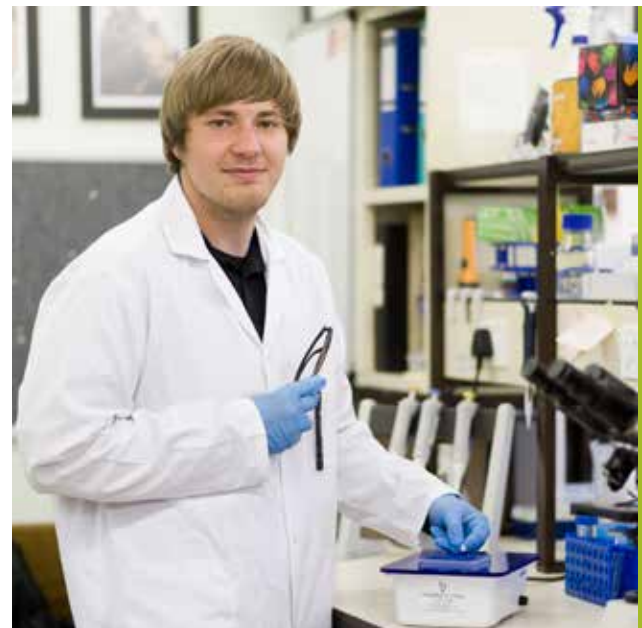
specialisation, which are integral aspects of xylogenesis. In my MSc, I aim to characterise the catalogue of miRNA, siRNA and lncRNA genes in three *Eucalyptus grandis* tissues; immature xylem, secondary phloem and mature leaf. Subsequently, I will determine their tissue specificity and interactions with each other and their coding RNA targets. Presently, I have discovered 135 *E. grandis* miRNA genes. Of these, 90 belonging to 25 families show conservation with other known plant miRNA genes. Additionally, 45 belonging to 34 families are potentially novel, uncharacterised miRNA genes. The expression profiles of these miRNAs divide them into two distinct sets; one that is specific to xylem and phloem, and another larger set specifically expressed in leaf. This implies that they play a role in the regulation of tissue-specific processes, and presents a novel opportunity to further resolve the complex molecular interactions and progression of xylem formation.



Heatmap showing tissue specificity of microRNA genes between immature xylem (IX), phloem (PH) and mature leaf (ML) tissues in *E. grandis*. Dendrogram colours indicate tissue specific expression clusters; blue = ML specific miRNAs, green = IX down-regulated miRNAs, red = vascular tissue specific miRNAs

### Mr Martin Wierzbicki

Wood is a complex bio-composite that could be compared to well styled hair with cellulose representing the hair, hemicellulose functioning as styling mousse and lignin being the hairspray that stabilises and hardens the entire product. For biorefinery applications it would be desirable to cleanly separate the three components into parallel processing streams, each with distinct bio-based products. In woody dicots like *Eucalyptus*, the predominant hemicellulose is glucuronoxylan (xylan). It is a short, highly decorated polysaccharide that aids in organising and stabilising the cellulose microfibrils along with providing a surface for lignin deposition through interaction with the xylan decorations. Much remains unknown about the biosynthesis, distinct roles, natural variation and genetic regulation of these decorations. A novel approach to provide insight into the biological process of xylan modification would entail examining genetic variation for xylan modifications in tree populations. By making use of genome-wide genotyping in an interspecific backcross population of *Eucalyptus grandis* x *E. urophylla* and analysing the variation in xylan decorations in hundreds of individuals, we can identify areas of the genome that contribute to trait variation. Additionally, we can employ transcriptome profiling of the same individuals to dissect the gene expression network regulating xylan modification genes in the backcross progeny. The intersection of these two sets of data will allow us to identify novel genes and regulatory mechanisms contributing to variation in xylan modification in *Eucalyptus* trees — information that will be valuable for molecular breeding and genetic engineering of trees with novel xylan modification and biomass processing traits.







### Mr Colan Balkwill

The circadian clock, an innate biological timer, regulates numerous agriculturally important traits, such as yield and stress tolerance, and has been shown to play an important role in the allocation of carbon to various metabolic processes and the regulation of development. Exploring its role during the maturation of xylem, a major carbon sink and agriculturally important tissue in trees, is therefore of great importance. Current research, however, has not to a large extent focussed specifically on the clocks of woody plant species, despite their economic importance.

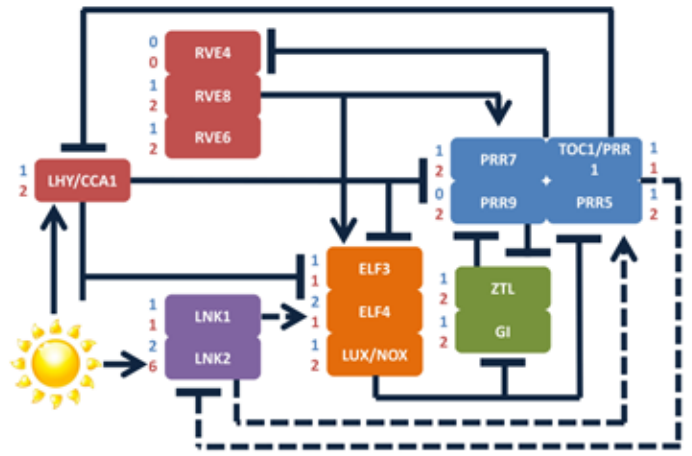
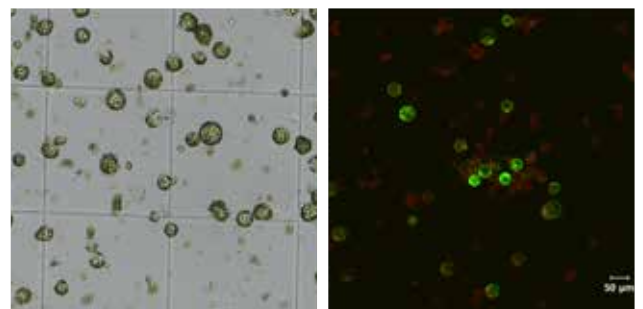


Diagram of the circadian clock

My MSc focuses on identifying circadian rhythm associated genes in both *Populus* and *Eucalyptus*, as well as exploring their expression dynamics across time, with a specific focus on their functionality in xylem. Resultantly, I have had to develop a thorough understanding of phylogenetics, systems, and genome biology, with concentration on the function of regulatory networks. To date, I have fully annotated the clock components in these two species and shown that the clock appears to be intact and acting during xylogenesis, thereby opening the doors for study of chronobiology in this important tissue. I will complete the project before the end of 2015 and continue with the research at PhD level.

### Mr Marius Laubscher

My research interests are to understand fibre and vessel development during wood formation in *Eucalyptus*. Understanding transcriptional regulation of wood formation in *Eucalyptus* using reverse genetics approaches is challenged by its recalcitrance to stable transformation. Systems based on transient expression in protoplast cells lacking cell walls bypass stable transformation limitations and have been successfully developed in model plants, such as those of *Arabidopsis* mesophyll tissue. The work I am currently doing in my MSc is to understand the involvement of two key *Eucalyptus* transcription factors in secondary cell wall (SCW) regulation, EgrNAC26 and EgrNAC61. We overexpressed these two transcription factors (TFs) in *Arabidopsis* leaf mesophyll protoplasts. Through RNA sequencing we have confirmed that these TFs play an important role in SCW formation as important genes were up-regulated through overexpression of these TFs. Genes upregulated by these TFs are highly enriched for secondary cell wall candidates. This work will enhance our understanding of how the SCW is formed in the economically important tree species *Eucalyptus*.



*Arabidopsis* leaf mesophyll protoplasts expressing green fluorescent protein (left) and bright field image and fluorescence micrograph (right)

## BSc Honours Students:



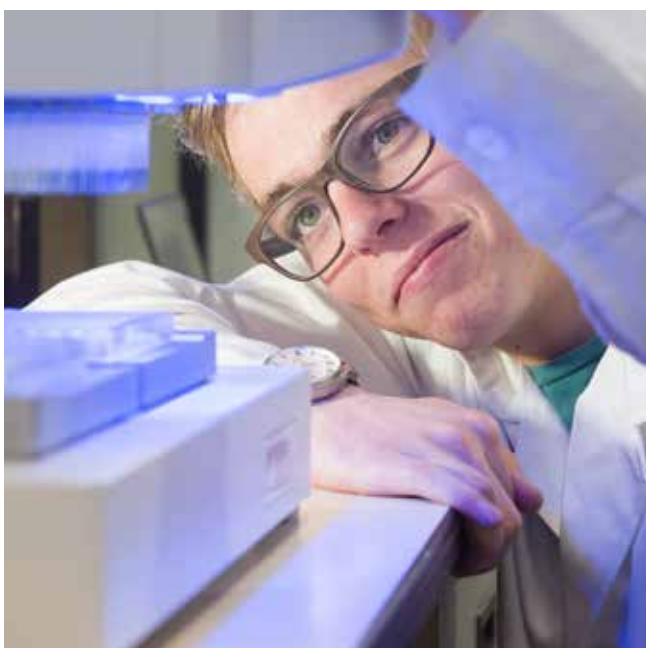
### Mr Donovan Coles

Previous studies at the *Eucalyptus* and Pine Pathogen Interactions group identified 61 core defence response genes in *Eucalyptus* interactions with *Leptocybe invasa*, *Chrysosporthe austroafricana* and *Phytophthora cinnamomi*. Of these genes, Nitrate Transporter 2.5 (NRT2.5) was consistently up-regulated in the susceptible *Eucalyptus* genotype ZG14 and down-regulated in the tolerant and resistant *Eucalyptus* genotypes of TAG5. To date, the function of NRT2.5 in plant defence is unknown. To determine the function of NRT2.5 in plant defence, the current and very exciting CRISPR-Cas9 system will be utilised. The key components of this system are the Cas9 protein and the guide RNA (gRNA). Together, an active molecule is produced which can edit any position in the genome. By designing the gRNA to target NRT2.5, the CRISPR-Cas9 system can cause this gene to be knocked-out. My study aims to develop a CRISPR-Cas9 construct for functional characterisation of NRT2.5 in *Arabidopsis thaliana* as a proof of concept. I have developed the CRISPR-Cas9 construct and have verified that there are no off-target sites in the genome. In future, this system can be utilised to determine the function of genes including NRT2.5 in *Eucalyptus*, something which has been difficult in the past due to its recalcitrance to stable transformation.



### Ms Katrien Brown

For my Honours research project I am using a variety of molecular techniques to investigate the expression of a gene thought to be involved in vessel differentiation in *Eucalyptus grandis*, *EgrNAC26*. The transgenic *Arabidopsis thaliana* plants I am currently growing are expressing fluorescent protein under the control of its promoter. When viewing the seedling root and stem tissue with a fluorescent microscope I will be able to see the localisation of *EgrNAC26*-promoter driven gene expression. This is like flipping a switch and seeing where in the house a light goes on. Through continued research and development, we may one day be able to artificially control wood development. By being part of this research, I believe I am contributing not only to our current genetic understanding of tree development, but also to the future of sustainable forestry.



### Mr Stephan Engelbrecht

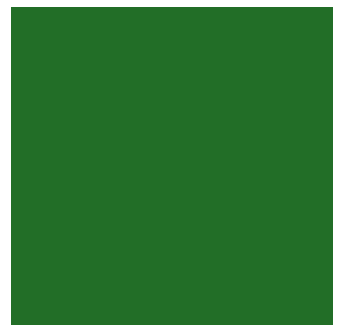
My honours project focuses on the development of a new fingerprinting platform that can be used to characterise the genetic make-up of commercially important *Eucalyptus* trees. DNA fingerprinting can help tree breeders to identify which species a particular tree belongs to and its most likely set of parents. Tree breeders use this information to manage tree stands and seed orchards, to ensure that the best trees are propagated. Currently, routine forestry tree fingerprinting is conducted with a small set of DNA markers (microsatellites) that are laborious and often difficult to analyse, which can lead to errors in downstream applications. By utilising an alternative fingerprint, single nucleotide polymorphisms, a larger set of DNA markers can be obtained which is amenable to accurate, automated analysis. SNP markers are more evenly and densely distributed throughout the genome than microsatellites, and therefore provides a more accurate and detailed description of the genomic composition of any particular tree. Ultimately, the aim of the project is to develop a platform which possesses the potential to make forest tree genotyping faster, cheaper and more accurate.

## Undergraduate Assistant and Mentorship Students

This year we hosted seven undergraduate assistant and mentorship students in our research programme. Exposure to postgraduate research plays an important role in attracting these students into postgraduate studies and we have a good track record of retaining many of the top performing students into our programme.



**From Left to Right:** Ms Tanya Welgemoed, Ms Nomakula Zim, Ms Modjadji Makwela, Ms Loandi Richter, Mrs Elna Cowley, Ms Cara Botha, Ms Katlego Sebothoma and Ms Cornelia Hansen



# FMG Outreach

## Investing in Synthetic Biology Innovation



FMG has entered the first undergraduate team from UP to participate in the International Genetically Engineered Machines (iGEM) competition, a prestigious synthetic biology tournament with over 250 competing teams. Pretoria\_UP, the team of eight, is instructed by Dr Steven Hussey and advised by Prof Zander Myburg and Dr Eshchar Mizrahi. Representatives of the team presented their “Switch-coli” project ([http://2015.igem.org/Team:Pretoria\\_UP](http://2015.igem.org/Team:Pretoria_UP)) at the 2015 Giant Jamboree in Boston, MA from 24 to 28 Sep 2015, where they were awarded a bronze medal. Their project involved using genetic switches to re-program bacterial chemotaxis in a synchronous and conditional manner.

In addition to documenting new DNA “BioBricks” which have been submitted to the iGEM Registry of Standard Biological Parts, FMG’s Pretoria\_UP iGEM team also reached out to High School science learners in Mamelodi to raise awareness of biology, synthetic biology and genetic engineering.

They conducted a survey comparing results of schools in previously disadvantaged areas (Lehlabile Secondary School) and historically favoured areas (Pretoria Boys’ High School) as part of their Human Practices portfolio of the iGEM competition.

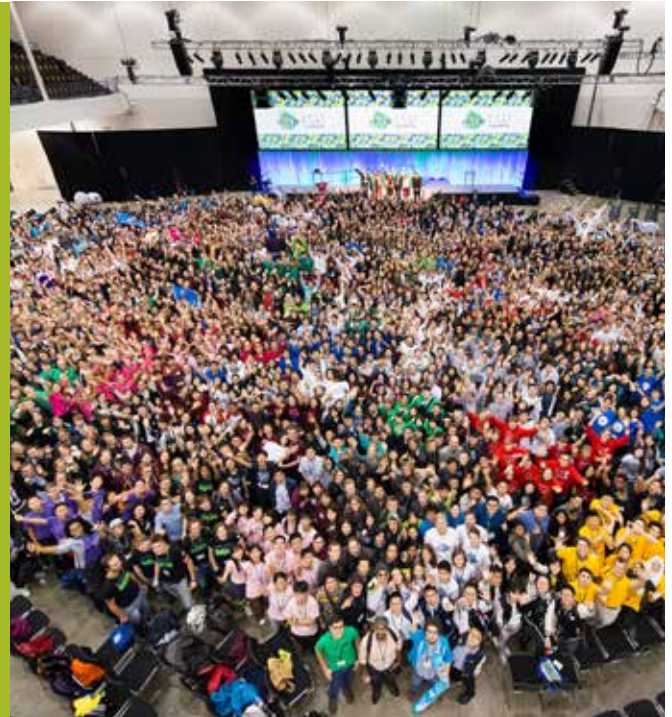


**From Left to Right:** Mr Gert Pietersen, Dr Eshchar Mizrahi, Ms Modjadji Makwela, Mr Axel Ind, Ms Thabang Msimango, Mr Vaughn Barendsen, Ms Nomakula Zim, Mr Ricu Claassens, Dr Steven Hussey, Prof Zander Myburg and Mr Brad Querl

**FMG’s Pretoria\_UP iGEM team also reached out to High School science learners in Mamelodi to raise awareness of biology, synthetic biology and genetic engineering.**



**Community Outreach:** The Pretoria\_UP iGEM team addressed Grade 11 learners at Lehlabile Secondary School about synthetic biology and asked them questions about their knowledge and acceptance of genetic engineering.



iGEM 2015 Giant Jamboree, Boston, Ma, 24 – 28 September 2015



## NSTF Share'n Dare Programme

As part of his NSTF Award, Prof Myburg is participating in the NSTF's Share 'n Dare Programme which aims to raise the awareness of science careers to high school learners. On 6 August 2015, Prof Myburg participated as a guest speaker of a National Science Week event hosted in the ArcelorMittal Science Centre in Sebokeng. More than 300 learners and interested persons from the Sebokeng community attended the event.



# Awards

## National Science and Technology Forum (NSTF)-BHP Billiton-W Kambule Award

Prof Myburg was nominated for and received the National Science and Technology Forum (NSTF)-BHP Billiton-W Kambule Award for his contribution to research and its outcomes in South Africa in the last 5 to 10 years. The award was presented at the annual NSTF Awards Ceremony by Minister Naledi Pandor (Science and Technology).

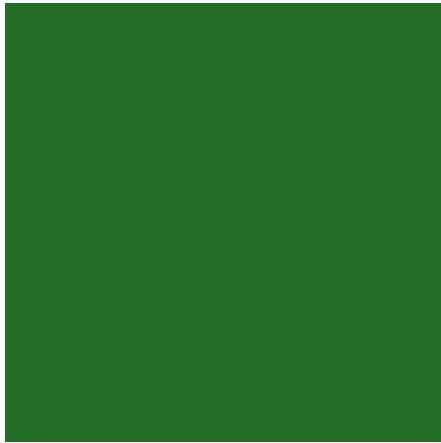


**From Left to Right:** Minister of Science and Technology, Naledi Pandor, Prof Alexander Myburg and NSTF Chairperson, Prof Ali Dhansay.

## International Conferences and Workshops

FMG students and staff have had the privilege of presenting their research at several international conferences and symposia in 2015 including the International Plant & Animal Genome XXIII Conference (10-14 January, San Diego, CA), the 2015 Plant Cell Wall Research Symposium (20-21 March, Nara, Japan), the Tree Genomics Symposium (30 March, Helsinki, Finland), the IUFRO Tree Biotechnology 2015 Meeting

(8-12 June, Florence, Italy), the IUFRO Tree Parasite Interactions Workshop (23-23 August, Orleans, France), the Australasian Plant Pathology Meeting (14-16 September, Perth, Australia), the Plant Genome Evolution 2015 Conference (6-8 September, Amsterdam, The Netherlands) and the International Plant Molecular Biology (IPMB) 2015 Conference (25-30 October, Iguazu Falls, Brazil).



# Collaborators

Local Academic Collaborators	
Dr Albe van der Merwe	Department of Genetics, FABI, UP
Dr Noëlani van den Berg	Department of Genetics, FABI, UP
Prof Dave Berger	Department of Plant Sciences, FABI, UP
Prof Bernard Slippers	Department of Genetics, FABI, UP
Prof Fourie Joubert	Bioinformatics and Computational Biology Unit, UP
Prof Brenda Wingfield	Deputy Dean Natural & Agricultural Sciences, FABI, UP
Prof Mike Wingfield	FABI, UP
Prof Paulette Bloomer	Department of Genetics, FABI, UP
Prof Don Cowan	Genomics Research Institute, CMEG Laboratory, UP
Dr Charles Hefer	Biotechnology Platform, Agricultural Research Council, Onderstepoort, Pretoria
Industry Collaborators and Contacts	
Dr Nicky Jones	Sappi Forests, Shaw Research Centre, Howick, KZN
Dr Arnulf Kanzler	Sappi Forests, Shaw Research Centre, Howick, KZN
Dr Sharmane Naidoo	Sappi Forests, Shaw Research Centre, Howick, KZN
Dr Arlene Bayley	Sappi Technology Centre, Innovation Hub, Pretoria
Dr Marius du Plessis	Mondi Ltd, Tree Improvement Research, Pietermaritzburg, KZN
Mr Ben Pienaar	Mondi Ltd, Tree Improvement Research, Pietermaritzburg, KZN
Dr Kitt Payn	Mondi Ltd, Tree Improvement Research, Hilton, KZN
Mr Mmoledi Mphahlele	Mondi Ltd, Tree Improvement Research, Hilton, KZN
Mr Eric Droomer	York Timbers
Mr John Crawford-Brunt	York Timbers
Mr Chris Pienaar	Hans Merensky Foundation
Dr Kevin Mossie	Hans Merensky Foundation
Dr Steven Verryn	Hans Merensky Foundation
Dr Andrew Morris	Institute for Commercial Forestry Research (ICFR), Howick, KZN
Dr Tammy Swain	Institute for Commercial Forestry Research (ICFR), Howick, KZN
Mr Craig Norris	NCT Forestry Co-operative Limited
Mr André van der Hoef	Cape Pine (MTO)
Mr Nico Olivier	Komatiland Forests (KLF)
International Collaborators	
Prof Shawn Mansfield	Department of Wood Science, University of British Columbia, Vancouver, BC
Prof Carl Douglas	Department of Botany, University of British Columbia, Vancouver, BC
Prof Dario Grattapaglia	EMBRAPA, Brasilia, Brazil
Prof Rene Vaillancourt	School of Plant Science, University of Tasmania, Hobart, Australia
Prof Brad Potts	Plant Science, University of Tasmania, Hobart, Australia
Prof Steven Strauss	Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR, USA
Dr Gary Hodge	CAMCORE, Department of Forestry, North Carolina State University, Raleigh, NC, USA



Dr Jerry Tuskan	Oak Ridge National Laboratory (ORNL), Oak Ridge, TN, USA
Dr Jacqueline Grima-Pettenati	Centre National de la Recherche Scientifique (CNRS), Université Paul Sabatier, Tolosan, France
Prof Yves van de Peer	Bioinformatics & Systems Biology, Ghent University, Ghent, Belgium
Dr Carsten Külheim	Eucalyptus Terpenes, Australian National University (ANU), Canberra, Australia
Prof Katherine Denby	Plant Systems Biology, University of Warwick HRI, Coventry, UK
Prof Jill Wegrzyn	Pine Genomics, University of Connecticut, CT, USA
Dr Kathleen Marchal	Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium
Dr Fikrit Isik	Department of Forestry, North Carolina State University, Raleigh, NC, USA
Prof Andrew Groover	USDA Forest Service, Davis, CA, USA
Prof Taku Demura	Nara Institute of Science and Technology, Nara, Japan
Prof Eric Schranz	Biosystematics, Plant Science Group, Wageningen University, Wageningen, Netherlands
Dr Frederic Lens	Institute of Biology Leiden, Leiden University, Leiden, Netherlands



# Publications

1. Külheim C, Padovan A, Hefer CA, Krause ST, Köllner TG, Myburg AA, Degenhardt J and Foley W. 2015. The *Eucalyptus* terpene synthase gene family. *BMC Genomics* 16:450. (Published online: 22 May 2015, doi: 10.1186/s12864-015-1598-x). IF 3.98
2. Pinard D, Mizrachi E, Hefer C, Kersting A, Joubert F, Douglas CG, Mansfield SD and Myburg AA. 2015. Comparative analysis of Plant Carbohydrate Active enzymes and their role in xylogenesis. *BMC Genomics* 16:402. (Published online: 22 May 2015, doi: 10.1186/s12864-015-1571-8). IF 3.98
3. Vanneste K, Sterck L, Myburg AA, Van de Peer Y and Mizrachi E. 2015. Horsetails Are Ancient Polyploids: Evidence from *Equisetum giganteum*. *The Plant Cell* 27: 1567-1578. (Published online: 22 May 2015, doi: http://dx.doi.org/10.1105/tpc.15.00157). IF 9.33
4. Hussey SG, Mizrachi E, Groover A, Berger DK and Myburg AA. 2015. Genome-wide mapping of histone H3 lysine 4 trimethylation in *Eucalyptus* developing xylem using nano-ChIP-seq. *BMC Plant Biology*. 15:117. (Published online: 10 May 2015, doi: 10.1186/s12870-015-0499-0). IF 3.81
5. Strauss SH and Myburg AA. 2015. Plant scientists celebrate new woody plant genome. *New Phytologist*. 204:6, 1185-1187. (Published online: 8 May 2015, doi: 10.1111/nph.13443). IF 7.67\*
6. Oates CN, Külheim C, Slippers B, Myburg AA and Naidoo S. 2015. The transcriptome of *Eucalyptus grandis* reveals mechanisms of defence against the insect pest, *Leptocybe invasa*. *Plant and Cell Physiology*. pii: pcv064. (Epub ahead of print, Published online 5 May 2015). IF 4.93
7. Mangwanda R, Myburg AA and Naidoo S. 2015. Transcriptome and hormone profiling reveals *Eucalyptus grandis* defence responses against *Chrysoporthe austroafricana*. *BMC Genomics* 16:319. (Published online: 18 April 2015, doi: 10.1186/s12864-015-1529-x). IF 3.98
8. Visser EA, Mangwanda R, Becker, JW, Külheim C, Foley WJ, Myburg AA and Naidoo S. 2015. Foliar terpenoid levels and corresponding gene expression are systemically and differentially induced in *Eucalyptus grandis* clonal genotypes in response to *Chrysoporthe austroafricana* challenge. *Plant Pathology*. (Epub ahead of print, Published online: 14 March 2015, doi: 10.1111/ppa.12368). IF 2.12
9. Li Q, Yu H, Cao PB, Fawal N, Mathe C, Azar S, Wang H, Myburg AA et al. 2015. Explosive tandem and segmental duplications of multigenic families in *Eucalyptus grandis*. *Genome Biology and Evolution*. 7(4):1068-81. (Published online: 13 March 2015, doi: 10.1093/gbe/evv048). IF 4.22
10. Carocha V, Soler M, Hefer CA, Cassan-Wang H, Myburg AA, Fevreiro P, Paiva Jap and Grima-Pettenati J. 2015. Genome-wide analysis of the lignin toolbox of *Eucalyptus grandis*. *New Phytologist*. 206:4, 1397-1312. (Published online: 12 February 2015, doi: 10.1111/nph.13313). IF 7.67\*
11. Hudson CJ, Freeman JS, Myburg AA, Potts BM and Vaillancourt RE. 2015. Genomic patterns of species diversity and divergence in *Eucalyptus*. *New Phytologist*. 204:6, 1378-1390. (Published online: 10 February 2015, doi: 10.1111/nph.13316). IF 7.67\*
12. Hefer C, Mizrachi E, Myburg AA, Douglas CJ and Mansfield SD. 2015. Comparative interrogation of the developing xylem transcriptomes of two large perennial wood-forming plants: *Populus trichocarpa* and *Eucalyptus grandis*. *New Phytologist*. 204:6, 1391-1405. (Published online: 6 February 2015, doi: 10.1111/nph.13277). IF 7.67\*
13. Yu H, Soler M, Clemente HS, Mila I, Paiva JA, Myburg AA, Bouzayen M, Grima-Pettenati J and Cassan-Wang H. 2015. Comprehensive genome-wide analysis of the Aux/IAA gene family in *Eucalyptus*: evidence for the role of Egr1AA4 in wood formation. *Plant and Cell Physiology*. (Published online: 9 January 2015, doi: 10.1093/pcp/pcu215). IF 4.93
14. Kersting AR, Mizrachi E, Bornberg-Bauer E and Myburg AA. 2015. Protein domain evolution is associated with reproductive diversification and adaptive radiation in the genus *Eucalyptus*. *New Phytologist*. 204:6, 1328-1336. (Published online: 11 December 2014, doi: 10.1111/nph.13211). IF 7.67\*
15. Hussey SG, Saïdi MN, Hefer CA, Myburg AA and Grima-Pettenati J. 2015. Structural, evolutionary and expression analysis of the NAC domain protein family in *Eucalyptus*. *New Phytologist*. 206:4, 1337-1350. (Published online: 10 November 2014, doi: 10.1111/nph.13139). IF 7.67\*
16. Mizrachi E, Malonie V, Silberbauer J, Hefer C, Berger DK, Mansfield SD and Myburg AA. 2015. Investigating the molecular underpinnings underlying morphology and changes in carbon partitioning during tension wood formation in *Eucalyptus*. *New Phytologist*. 204:6, 1351-1363. (Published online: 12 November 2014, doi: 10.1111/nph.13152). IF 7.67\*
17. Vining K, Elisson R, Jones R, Klocko A, Alves-Ferreira M, Hefer CA, Amarasinghe V, Dharmawardhana P, Naithani S, Ranik M, Wesley-Smith J, Jaiswal P, Myburg AA, Solomon L and Strauss S. 2015. Floral transcriptome of *Eucalyptus grandis*. *New Phytologist*. 206:4, 1406-1422. (Published online: 13 August 2014, doi: 10.1111/nph.13077). IF 7.67\*
18. Soler M, Camargo ELO, Carocha V, Cassan-Wang H, Clemente SH, Savelli B, Hefer CA, Myburg AA, Paiva JP and Grima-Pettenati J. 2015. The *Eucalyptus grandis* R2R3-MYB transcription factor family: evidence for woody growth related evolution and function. *New Phytologist*. 206:4, 1364-1377. (Published online: 5 August 2014, doi: 10.1111/nph.13039). IF 7.67\*

\* These papers are associated with the special feature section

# Research and Postgraduate Degree Positions

The Forest Molecular Genetics (FMG) Programme offers exciting opportunities for postdoctoral and postgraduate research and training in Plant Genomics, Bioinformatics, Molecular Genetics, Molecular Biology and Biotechnology. NRF, THRIP, DST and industry-supported bursaries are available for postgraduate (BSc Hons, MSc and PhD) studies. The programme also hosts UP and Vice-Chancellor's postdoctoral fellowships. Finally, the FMG Programme annually offers several undergraduate mentorship and assistant positions to second- and third-year BSc and BSc Agric students.

**Interested students can contact our Administrative Assistant:**

Ms Lauren Moonsamy (lauren.moonsamy@up.ac.za)

## Funding

FMG gratefully acknowledge research funding from the following institutions and companies that are members of the FMG Consortium:

University of Pretoria

National Research Foundation (NRF)

Department of Science and Technology (DST)

Technology and Human Resources for Industry Programme (THRIP)

Sappi

Mondi

Hans Merensky Foundation

York Timbers





University of Pretoria  
Private Bag X20,  
Hatfield, 0028  
Republic of South Africa

[www.up.ac.za](http://www.up.ac.za)