Benchpress

The official newsletter of the
Australian Institute of Medical Scientists (Victorian Branch)
A.C.N 010 985 403

July 2018 Edition

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In the July edition:

Branch chair report 2018

Dear Colleagues,

Welcome to another edition of Benchpress for 2018. **Geoff Magrin** is our guest editor for the July edition and I would like to give him a warm welcome.

Again, the Morphology workshop in May was a huge success with all places taken. This is a hugely popular event and both workshops have been well received. Please turn to page 13 for a summary of the day events. Many thanks to **Cindy O'Malley** and her team of dedicated scientists (Gaby Roche, Lidia de Rosa, Ray Dauer, Sonia Tencic and Peter Fayle) for holding this event and all their hard work. Morphology is an essential tool in the armoury of haematology scientists and this workshop is designed to increase the skills of both beginners and more experienced scientists. You can read about the success of this workshop in Tina Pham's article on Page 14.

We would like to hold this workshop as an annual event but that depends on the needs of the workforce and the kindness of volunteers to give their time as trainers and demonstrators. The feedback from the May workshop was terrific and indicated that there is a real need for this training in the diagnostic community. If you are interested in volunteering your time and becoming a morphology demonstrator for the next workshop please contact Cindy O'Malley or myself. Also it would be great if you could register your interest in attending a session next year so that we have an idea of demand.

I am very happy to announce that the next **AIMS workshop** will be held in conjunction with the **Victorian Immunohaematology Discussion Group (VIDG)** on the 16th March 2019. We are really excited about this joint workshop called **"It's in the Blood"** as it will canvas different aspects of topics such as haemophilia, transplantation, blood safety and much more. This should be a very informative workshop so we are looking forward to seeing you there.

Again, as every year, the AIMS Victorian Branch will be honouring the top graduates from RMIT. These students have studies hard over their 4 years at RMIT and I will like to congratulate them for being such excellent students.

This year the awards will be presented at the **Haematology Discussion Group (HDG) meeting being held at RMIT on Tuesday 17th July**. The HDG meeting is open to all medical scientists, so come along and listen to the new crop of scientists giving their presentations. An added bonus will be to see the top students from 2017 being honoured with their AIMS award. I wish the awardees every success as they enter their chosen field in medical science.

The AIMS Victorian Branch will soon be holding their **2017-2018 Annual General Meeting (AGM)** so watch this space for details. We are calling for nominations for the **George Milsom Memorial Award** which will presented on the night, in this newsletter.

The George Milsom Memorial Award is provided to a Victorian AIMS member for significant achievement or service in the field of Medical Laboratory Science in Victoria.

So think about nominating someone you know who has contributed to the profession by way of teaching, a paper or just because you have found them to be an inspirational medical scientist. Nominations close on Friday August 10th. You will find the nomination form on page 16.

The George Swanson Christie Memorial Award is offered to an inspirational medical scientist who has shown ongoing excellence in the field and will be nominated by the AIMS Victorian Branch. This prestigious award will also be presented at the AGM.

At the National level, all scientists should be interested in how the formation of **National Certification Scheme for Medical Laboratory Scientists** is progressing.

The objective is to define an agreed and sustainable certification model for Australian medical scientists. AIMS and the Australasian Association of Clinical Biochemists (AACB) with funding from the Commonwealth Department of Health through its Quality use of pathology program (QUPP) have set up a Project Coordinating Group to prepare a position paper.

A draft of the Medical Laboratory Scientific Workforce Certification discussion paper can be seen at http://humancapitalalliance.com.au/projects-and-publications/projects/cert-med-science/

What has happened to date? The draft was published in November 2017 after two workshops were held. An updated draft position paper was developed in April and in May the first round of the Delphi conference was held. In this conference stakeholders were consulted for contributions and feedback on specific elements of the proposed certification scheme. These have now been incorporated into the position paper and a further Delphi conference round is being held for follow up. If you have been approached to participate in the second Delphi round please set aside some time to think about how you would like a certification scheme to work and contribute to this round.

Go to https://www.surveymonkey.com/r/RND2_DELPHI_MEDSCI

The questions are exacting and require a lot of thought but through your participation a certification scheme should be developed that fits the requirements of medical scientists. I encourage you to be involved in the shaping of your profession.

Don't forget to use the travelling orator to come to your next seminar if you are looking for a guest speaker. This year it is Ms Fleur Francis, Pathology Queensland's Supervising Scientist at the Molecular Microbiology and Serology departments at Townsville Hospital. Fleur has agreed to share her knowledge experience and expertise about the introduction and incorporation of molecular testing in the micro/virology world eg GeneXpert, MaldiToffs etc. and how these new technologies will impact on laboratories and requesting clinicians. Her bio is on page 15

We have a number of contributors to this edition of Benchpress and I would like to thank them all for giving up their time to writing a few words; Tina, Jaelyne, Leah, David and Geoff – you will find their articles over the next few pages. Enjoy!

Kerryn Weekes

Chair AIMS Victorian Branch

Guest Editorial

I would like to thank Kerryn and the Victorian branch for the opportunity to assist with this letter and write a brief editorial. For those who remember me in my time at the Alfred it was always said that I would never say in three words what I could state in 3000. I hope to keep this editorial short.

In this edition we have published a short paper on CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats. This is hot technology and you know it is topical when MARVEL comics use it to describe how their superheroes have the DNA genetically modified. It even gets a mention in the latest Dan Brown book “ORIGIN”. For those who haven’t yet heard of CRISPR I have put together a brief history of what it is, where it came from and the potential uses and ethical questions it raises. The article isn’t in a format for publishing in our journal and is a short communication. I encourage members who have a short review article or subject of interest or even something from a special interest group that is worth writing about to write and send it to us for the newsletter.

Kerryn has mentioned the certification project in her column and I encourage everyone to read with interest how this is going and if you haven’t done so already register for regular updates.

In my recent role as the AIMS Education Consultant I was able to look at a lot of the content presented at the various special interest groups. I think the quality and content is always great but only gets delivered to a select few who attend in person. I am aware that it is becoming increasingly difficult to get to some of the regular monthly meetings due to work and home pressure. The challenge in this day and age of electronic data is, how do we get this content to a larger audience and those who cannot attend as well as do justice to the speakers who put time into the preparation. This newsletter was often the vehicle to deliver SIG reports, but this has waned, and I understand that everyone is busy and time poor. It is important that every effort is made to deliver not only a report but educative content that can be distributed to all the membership who seek APAACE point and continuous profession development (maybe to get Certification in the future).

For those who are unaware all recent versions of PowerPoint have a function that allows you to record the talk as a video file. The easiest solution is use a laptop, as most have a microphone inbuilt for conference calls. The mike will record the dialogue, as long as the speaker stays in close proximity and stores it in PowerPoint along with the timing of the slide duration. I used this successfully in the production of numerous talks given around the country which are posted on the AIMS website. I recorded the talk as the presenter gave it or if this failed asked the presenter to redo the talk on the computer to themselves, save it and send it to me. You can even use this method at work to save having to give the same talk over and over again just record it and put it on YouTube…..

Methods for all recent versions of PowerPoint are available from national head office and the branch.

I hope you enjoy this newsletter and please pass it on to other members and non-members alike. Give us some feedback on what content you want and enjoy the read

Geoff Magrin (F.A.I.M.S.)

P.S. In the events section at the end I have reached out to anyone from the RMIT class of 73-74. Such an auspicious group and some of us catch up on a regular basis for lunch. We would love to know who else is out there whether still working in laboratories, semi-retired, retired or in another industry. Contact details in the events section so pass it on if you are from that era or know others who we went to college with my vintage.

For the interest of all scientists and individuals

Self-collection for cervical screening comes to Australia

On December 1st 2017, Australia began its renewed National Cervical Screening Program (NCSP) which utilises primary HPV screening with reflex cytology. Australia has one of the lowest rates of cervical cancer in the world, primarily due to the highly effective pap screening program that began in the early 1990's. Over the last ten years the rate of cervical cancer has plateaued and a number of new approaches to cervical screening were investigated to determine which would likely lead to further decreases in disease.

One of the biggest risks factors for cervical cancer is being under- or never-screened. Over 80% of women diagnosed with cervical cancer are under- or never-screened. With the traditional pap test, cells needed to be collected from the cervix with the assistance of a speculum, however many women will not undergo a speculum exam. The advantage of the HPV-based screening approach is that samples do not need to be taken from the cervix. In fact a sample taken from the (high) vagina can produce a satisfactory sample for HPV testing for the assessment of risk of cervical disease.

In order to extend the benefits of cervical screening, women can now be offered the opportunity to collect their own sample for HPV testing using a PCR-based assay. The renewed (NCSP) encourages under- and never-screened women to utilise self-collection if they are over thirty years of age, are more than two years overdue for screening, and have refused a clinician collection. There are a large number of studies which demonstrate that PCR-based HPV testing of self-collected samples are as sensitive as clinical collected specimens including a meta-analysis by Marc Arbyn and colleagues published in 2014.

In January 2018, VCS Pathology received accreditation from NATA to begin offering self-collection for HPV-based cervical screening for eligible women. In 2017 VCS Pathology and the Royal Woman's Hospital Dysplasia Clinic in Melbourne undertook a clinical trial which recruited over 300 women and compared self-collection using a flocked swab, with clinician-collected samples, using five different PCR-based commercial HPV assays.

Since January, nearly 500 women from all states and territories who have previously refused screening have participated in the renewed NCSP. The ability of women who have previously declined to participate in cervical screening to take their own sample is hoped to contribute to a reduction in cervical cancer.

More information on how to access self-collection for HPV-based cervical screening can be found at http://www.vcspathology.org.au/practitioners/self-collection-resources

Dr David Hawkes (PhD)

Director of Molecular Biology & Biochemistry
Victorian Cytology Service

Newsletter review article (new feature)

WHAT IS CRISPR?

CRISPR is a family of DNA sequences in bacteria. These sequences contain snippets of DNA from viruses that have attacked the bacterium. These snippets are used by the bacterium to detect and destroy DNA from similar viruses during subsequent attacks. These sequences play a key role in a bacterial defence system, (1) and form the basis of a technology known as **CRISPR/Cas9** that effectively and specifically changes genes within organisms. (2).

CRISPR is an acronym for Clustered Regularly Interspaced Short Palindromic Repeat. In popular usage, "CRISPR" (pronounced "crisper") is shorthand for "CRISPR-Cas9." CRISPRs are specialized stretches of DNA and the protein and Cas9 (or "CRISPR-associated") is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA.

The name was minted at a time when the origin and use of the interspacing sub sequences were not known. At that time the CRISPRs were described as segments of prokaryotic DNA containing short, repetitive base sequences. In a palindromic repeat, the sequence of nucleotides is the same in both directions. Each repetition is followed by short segments of spacer DNA. CRISPRs are separated by theses short spacer sequences that match bacteriophage or plasmid sequences and specify the targets of interference. Upon phage infection, CRISPR arrays can acquire new repeat-spacer units that match the challenging phage. Cells with this extended CRISPR locus will survive phage infection and thrive. Therefore, the spacer content of CRISPR arrays reflects the many different phage's and plasmids that have been encountered by the host, and these spacers can be expanded rapidly in response to new invasions. Accordingly, CRISPRs constitute a 'genetic memory' that ensures the rejection of new, returning and ever-present invading DNA molecules, which put simply serve as a bank of memories, which enables bacteria to recognize the viruses and fight off future attacks. (3)

CRISPR technology is a simple yet powerful tool for editing genomes. Having learnt its function in bacteria researchers have found a way to easily alter DNA sequences and modify gene function. Its many potential applications include correcting genetic defects, treating and preventing the spread of diseases and improving crops. Genetic editing has been possible for over 40 years (the first GMO bacteria and mice were created in 1973 and 1974) but the process has remained expensive, time consuming and relatively limited in its scope. CRISPR is changing all of that.

Uses of CRISPR (4)

- Gene editing
- Slowing growth of cancer cells
- Create semi-synthetic organisms
- Create cancer busting genes
- Create viruses that force superbugs to kill themselves
- Limit spread of mosquitos by hacking their fertility gene thus reducing mosquito borne diseases
- Gene edit algae that produce biofuel
- Cure or fix inherited diseases i.e. Haemoglobinopathies, Diabetes

The potential uses for CRISPER and its effect of the environment is game changing for many researchers and the ethical problems posed by CRISPER are only starting to be discussed.(21)

CRISPRs use as a gene editing technology was adapted from the natural defence mechanisms of bacteria and archaea (the domain of single-celled microorganisms). These organisms use CRISPR-derived RNA and various Cas proteins, including Cas9, to foil attacks by viruses and other foreign bodies. They do so primarily by chopping up and destroying the DNA of a foreign invader. When these components are transferred into other, more complex, organisms, it allows for the manipulation of genes, or "editing." CRISPR technology is a simple yet powerful tool for editing genomes which allows researchers to easily alter DNA sequences and modify gene function. Its many potential applications include correcting genetic defects, treating and preventing the spread of diseases and improving crops.

ORIGINS (5)

The discovery of clustered DNA repeats began independently in three parts of the world. One of the first discoveries was in 1987 at Osaka University in Japan. Researcher Yoshizumi Ishino and colleagues published their findings on the sequence of a gene called "iap" and its relation to E. coli. Technological advances in the 1990s allowed them to continue their research and speed up their sequencing with a technique called metagenomics.

1993 - 2005 Francisco Mojica, University of Alicante, Spain, discovered CRISPRs in archaea (and later in bacteria). He was the first researcher to characterize what is now called a CRISPR locus, reported in 1993. He worked on them throughout the 1990s, and in 2000, he recognized that what had been reported as disparate repeat sequences actually shared a common set of features, now known to be hallmarks of CRISPR sequences (he coined the term CRISPR through correspondence with Ruud Jansen, who first used the term in print in 2002). In 2005 he reported that these sequences matched snippets from the genomes of bacteriophage (6). He hypothesized correctly that CRISPRs serve as part of the bacterial immune system, defending against invading viruses and is an adaptive immune system. Another group, working independently, published similar findings around this same time (7)

May 2005 — Alexander Bolotin, French National Institute for Agricultural Research (INRA). **Discovery of Cas9 and PAM:** Bolotin was studying the bacteria, Streptococcus thermophilus, which had just been sequenced, revealing an unusual CRISPR locus (8). Although the CRISPR array was similar to previously reported systems, it lacked some of the known Cas genes and instead contained novel Cas genes, including one encoding a large protein they predicted to have nuclease activity, which is now known as **Cas9.** Furthermore, they noted that the spacers, which have homology to viral genes, all share a common sequence at one end. This sequence, the **Protospacer Adjacent Motif (PAM),** is required for target recognition.

March 2007 — Philippe Horvath, Danisco France SAS Researching S. thermophilus which is widely used in the dairy industry to make yogurt and cheese wanted to explore how it responds to phage attack, a common problem in industrial yogurt making. Horvath and colleagues showed experimentally that CRISPR systems are indeed an adaptive immune system: They integrated new phage DNA into the CRISPR array, which allows them to fight off the next wave of attacking phage (9). Furthermore, they showed that Cas9 is likely the only protein required for interference, the process by which the CRISPR system inactivates invading phage, details of which were not yet known

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August 2008 — John van der Oost, University of Wageningen, Netherlands. Scientists soon began to fill in some of the details on exactly how CRISPR-Cas systems “interfere” with invading phage. The first piece of critical information came from John van der Oost and colleagues who showed that in *E-Escherichia coli*, spacer sequences, which are derived from phage, are transcribed into small RNAs, termed **CRISPR RNAs (crRNAs)**. These spacer sequences guide Cas proteins to the target DNA (10) **transcribed into guide RNAs.**

December 2008 — Luciano Marraffini and Erik Sontheimer, North-Western University, Illinois. Marraffini and Sontheimer elegantly demonstrated that the target molecule is DNA, not RNA (11). This was somewhat surprising, as many people had considered CRISPR to be a parallel to eukaryotic RNAi silencing mechanisms, which target RNA. Marraffini and Sontheimer explicitly noted in their paper that this system could be a powerful tool if it could be transferred to non-bacterial systems. (It should be noted, however, that a different type of CRISPR system can target RNA (12).

March 2011 — Emmanuelle Charpentier, Umea University, Sweden and University of Vienna, Austria.

The final piece to the puzzle in the mechanism of natural CRISPR-Cas9-guided interference came from the group of Emmanuelle Charpentier. They performed small RNA sequencing on Streptococcus pyogenes, which has a Cas9-containing CRISPR-Cas system. They discovered that in addition to the crRNA, a second small RNA exists, which they called trans-activating **CRISPR RNA (tracrRNA)** (13). They showed that tracrRNA forms a duplex with crRNA, and that it is this duplex that guides Cas9 to its targets.

July 2011 — Virginijus Siksnys, Vilnius University, Lithuania. Siksnys and colleagues cloned the entire CRISPR-Cas locus from S. thermophilus (a Type II system) and expressed it in E. coli (which does not contain a Type II system), where they demonstrated that it was capable of providing plasmid resistance (14). This suggested that CRISPR systems are self-contained units and verified that all of the required components of the Type II system were known. Going further in *September, 2012* Siksnys and his team purified Cas9 in complex with crRNA from the E. coli strain engineered to carry the S. thermophilus CRISPR locus and undertook a series of biochemical experiments to mechanistically characterize Cas9's mode of action (15).They verified the cleavage site and the requirement for the PAM, and using point mutations, they showed that the RuvC domain cleaves the non-complementary strand while the HNH domain cleaves the complementary site. They also noted that the crRNA could be trimmed down to a 20-nt stretch sufficient for efficient cleavage. Most impressively, they showed that they could reprogram Cas9 to target a site of their choosing by changing the sequence of the crRNA.

June 2012 — Charpentier and Jennifer Doudna, University of California, Berkeley. Similar findings as those in Gasiunas et al (15). were reported at almost the same time by Emmanuelle Charpentier in collaboration with Jennifer Doudna at the University of California, Berkeley (16). Charpentier and Doudna also reported that the crRNA and the tracrRNA could be fused together to create a single, synthetic guide, further simplifying the system. (Although published in June 2012, this paper was submitted after Gasiunas et al (15).)

January 2013 — Feng Zhang, Broad Institute of MIT and Harvard, McGovern Institute for Brain Research at MIT, Massachusetts. Zhang, who had previously worked on other genome editing systems such as TALENs, was first to successfully adapt CRISPR-Cas9 for genome editing in eukaryotic cells (17). Zhang and his team engineered two different Cas9 orthologs (from S. thermophilus and S. pyogenes) and demonstrated targeted genome cleavage in human and mouse cells. They also showed that the system
(I) could be programmed to target multiple genomic loci, and
(ii) could drive homology-directed repair. Researchers from George Church's lab at Harvard University reported similar findings in the same issue of Science (18).

In January 2013, the Zhang lab published the first method to engineer CRISPR to edit the genome in mouse and human cells.

How Does It Work?

Due to its comparative simplicity and adaptability, the CRISPR system has rapidly become the most popular genome engineering approach. At their most basic level, CRISPR/Cas9 genome editing systems use a non-specific **endonuclease** (An **endonuclease** is an enzyme that breaks down a nucleotide chain into two or more shorter chains by cleaving the internal covalent bonds linking nucleotides). They cleave the phosphodiester bond within a polynucleotide chain to cut the genome and a small guide RNA (**gRNA**) is used to guide this nuclease to a user-defined cut site.

CRISPR was originally employed to knock out target genes in various cell types and organisms, but modifications to various Cas enzymes have extended CRISPR to selectively activate/repress target genes, purify specific regions of DNA, image DNA in live cells, and precisely edit DNA and RNA. Furthermore, the ease of generating **gRNAs** makes CRISPR one of the most scalable genome editing technologies. This advantage makes CRISPR perfect for genome-wide screens.

So what makes up the CRISPR/Cas9 system?

CRISPRs are specialised structures of DNA. They consist of repeating palindromic sequences of genetic code, interrupted by "spacer" sequences found in bacteria. The spacers are remnants of genetic code from past invaders.

Spacer: The spacer sequences in CRISPR are derived from previous phage attacks and are transcribed into small RNAs. These short RNA sequences ("CRISPR RNAs" or "**crRNAs**") are the capable of guiding the system to matching sequences of DNA if the bacteria is attacked again.

As stated CRISPR arrays in bacterial genomes consist of repeated elements separated by unique sequences. When researchers first discovered these arrays, they did not know their functions and simply called the repeated elements "direct repeats" and the unique stretches of DNA between them "spacers" (Figure 1 previous page).

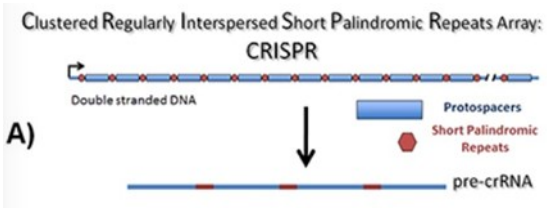

Figure 1

After years of research, we now know that each direct repeat, combined with its adjacent spacer, ultimately encodes a single **crRNA.** The direct repeat regions contain sequences required for processing pre-crRNA into mature crRNA and tracrRNA binding. The spacer regions, are the unique, foreign DNA target sequences specific to each individual crRNA.
Cas 9: The protein Cas9 is an enzyme that acts like a pair of molecular scissors, capable of cutting strands of DNA. The crRNAs confer target specificity to Cas9, but they can't bind to the Cas9 alone.

In the native Type II CRISPR/Cas system, Cas9 is guided to its target sites with the aid of two RNAs: the crRNA which defines the genomic target for Cas9, and the tracrRNA which acts as a scaffold linking the crRNA to Cas9. That is the "direct repeat region" combined with the tracrRNA forms the scaffold portion of a gRNA.

In most systems used for CRISPR-mediated genome editing, the two small RNAs have been condensed into one RNA sequence known as the guide RNA (**gRNA**) or single guide RNA (**sgRNA**). The **crRNA** region (shown in red at right) is a 20-nucleotide sequence that is homologous to a region in your gene of interest and will direct Cas9 nuclease activity.

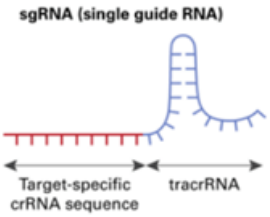

Figure 2

Synthetic Guide RNA or gRNA (Sometimes sgRNA)

Researchers have learnt to make tailor-made “guide” RNA (**gRNA**) sequences designed to lead them to their DNA targets. Tens of thousands of such gRNA sequences have already been created and are available to the research community.

Once expressed, the **Cas9** protein and the **gRNA** form a ribonucleoprotein complex through interactions between the gRNA scaffold and surface-exposed positively-charged grooves on Cas9. Cas9 undergoes a conformational change upon gRNA binding that shifts the molecule from an inactive, non-DNA binding conformation into an active DNA-binding conformation. Importantly, the spacer region of the gRNA remains free to interact with target DNA.

What happens next is formation of a double-stranded RNA-DNA hybrid between the guide RNA loaded into Cas9 and one of the two strands of the genome to be edited. This presents a substantial difficulty, because of the strength of the rock-hard network of hydrogen bonding that holds the two strands of genomic DNA together. (Genomic DNA doesn't meaningfully dissociate until temperatures approaching boiling, to give you an idea of how tight the interaction between complementary DNA strands is.) Thus, something has to intervene to separate DNA strands to allow the Cas9-gRNA to "search" for targets. This is what the **PAM** (or protospacer adjacent motif) is used for. The PAM is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease. The **PAM** sequence is located on the non-complementary strand. That is, it is on the strand of DNA that contains the same DNA sequence as the target crRNA (19). The PAM sequence should not be included in the design of the crRNA. The most commonly used Cas9 nuclease, derived from S. pyogenes, recognizes a PAM sequence of NGG that is found directly downstream of the target sequence in the genomic DNA, on the non -target strand.

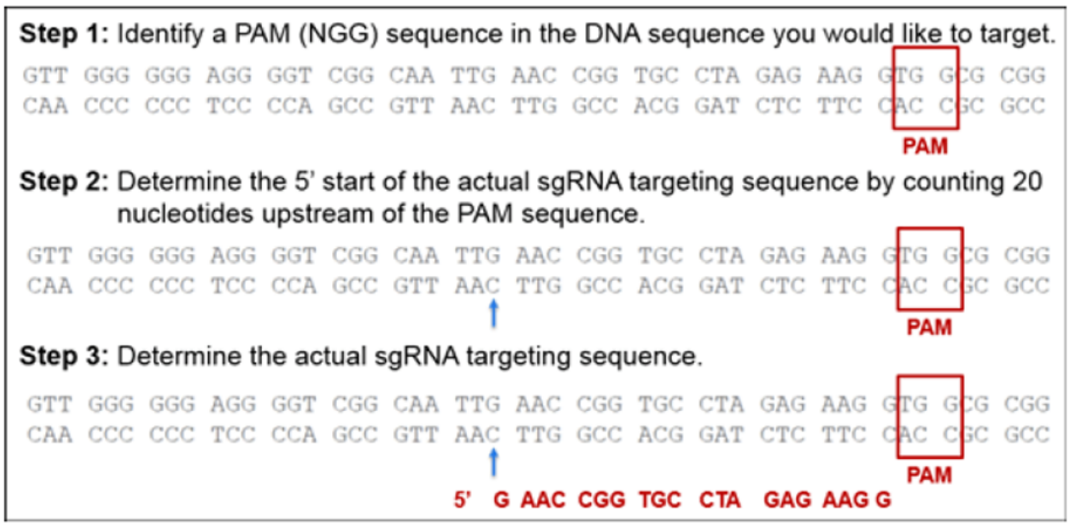

Figure 3

Figure 3 Is a typical map of the DNA strand to edit or modify. Depending on the source of the Cas 9 the PAM sequence is determined (see red box). The 20 nucleotide bases prior to the PAM are determined and used to create the sgRNA. This slight destabilization of the DNA double helix is enough to promote formation of the necessary DNA-RNA hybrid, if the sequence immediately upstream of the PAM matches that of the associated guide RNA

Cas9 Species/Variants and PAM Sequences

Species/Variant of Cas9	PAM Sequence
Streptococcus pyogenes (SP); SpCas9	NGG
SpCas9 D1135E variant	NGG (reduced NAG binding)
SpCas9 VRER variant	NGCG
SpCas9 EQR variant	NGAG
SpCas9 VQR variant	NGAN or NGNG
Staphylococcus aureus (SA); SaCas9	NNGRRT or NNGRR(N)
Neisseria meningitidis (NM)	NNNNGATT
Streptococcus thermophilus (ST)	NNAGAAW
Treponema denticola (TD)	NAAAAC
Cpf1 (from Various Species)	TTN
Additional Cas9s from various species	PAM sequence may not be characterized

Figure 4 The PAM sequence to look for in each species (20) http://www.clontech.com/US/Products/Cell_Biology_and_Epigenetics/ Genome_Modification/CRISPR_Cas9/ sgRNA_Selection

In summary this individual protein has all the components necessary to:

1. **Bind to a guide RNA:** The guide RNA, enables Cas9 to cut a specific genomic locus of many possible loci. Without binding to the guide RNA, Cas9 cannot cut.
2. **Bind to target DNA** in the presence of a Guide RNA provided that Target is Upstream (5') of a Protospacer Adjacent Motif (PAM). Cas9 endonuclease binding to the target genomic locus is mediated both by the target sequence contained within the guide RNA and a 3-base pair sequence known as the Protospacer Adjacent Motif or PAM. In order for dsDNA to be cut by Cas9, it must contain a PAM sequence immediately downstream (3') of the site targeted by the guide RNA. In the absence of either the guide RNA or a PAM sequence, Cas9 will neither bind nor cut the target.
3. **Cleave Target DNA Resulting in a Double-Strand Break (DSB)**
   Cas9 and its variants have two endonuclease domains: Upon target binding, Cas9 undergoes a conformational change that positions the nuclease domains to cleave opposite strands of the target DNA. Thus, the end result of Cas9-mediated DNA damage is a DSB within the target DNA ~3-4 nucleotides upstream of the PAM sequence

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Additional reading: Doudna J.A. and Sternberg S.H. A Crack in Creation: Gene editing and the Unthinkable Power to Control Evolution. 2015

AIMS Victorian Morphology Workshop 2018

The AIMS Victorian Branch Morphology Workshop was held on the 19th and 20th of May 2018. Around 30 delegates convened at Melbourne Pathology each day to advance their morphology skills.

Delegates were allocated a microscope each and viewed roughly 50 films throughout the day. Time was allocated to view each film and to discuss the features and diagnoses. A range of conditions were presented including RBC, WBC and platelets artefacts, microcytic anemias, macrocytic anemias, haemoglobinopathies, membranopathies, enzymopathies, parasitology, non-malignant/malignant disorders and paediatric morphology. The day concluded with a Kahoot quiz. It was closely contested with not only knowledge but speed critical for success.

ESL Biosciences and Cellavision, the wonderful sponsors, treated the delegates to delicious food and beverages throughout the day. In addition, delegates received complimentary USBs, Rubik's cubes and reusable coffee cups.

Whether the morphology workshop assisted with studies, was a refresher, upskilled, for interest only or to network, the feedback from delegates was overwhelmingly positive.

A huge thank-you to Melbourne Pathology for providing the outstanding venue and to the demonstrators Cindy O'Malley (RMIT), Gaby Roche (Alfred Hospital), Lidia Derosa (Royal Children's Hospital), Peter Fayle (Angliss Hospital), Raymond Dauer (Box Hill Hospital) and Sonia Tencic (Cabrini Hospital) for sharing their knowledge to the budding morphologists of the future.

Tina Pham
Vice Chair
AIMS Victorian Branch

The Australian Society for Microbiology
bringing Microbiologists together
VIC BRANCH

Southern News & VIC Branch AGM

Event Report

Proudly sponsored by:
ThermoFisher SCIENTIFIC

Southern News was presented by the Australian Society for Microbiology (ASM) Victorian Branch at the Alfred Hospital, Melbourne, on Tuesday 22nd May 2018. The evening began with the VIC Branch Annual General Meeting (AGM) conducted by Victorian Chair Karena Waller. The AGM was followed by seven presentations chaired by Dr Denis Spelman, Head of Microbiology at Alfred Health. Speakers ranged from Alfred Health, Royal Children's Hospital, Monash Health, Cabrini Pathology and La Trobe University. Presentations featured a range of fascinating case studies encountered in the medical microbiology laboratory highlighting the pathogenic role of diverse and rare bacteria and fungi in human infections, and current microbiology research relating to human health and the effect of antidepressants on gut microbiota. Over 100 attendees came to enjoy the very interesting talks which were preceded by finger food, drinks and networking.

Congratulations to Kylie Hui from the Royal Children's Hospital who was the winner of the ASM membership prize for a medical scientist up to Grade 1 Year 4 for her case study presentation on zoonotic pathogen *Bergeyella zoohelcum.*

A special thank you to Dr Spelman for chairing the event and to all the speakers for their informative talks which informed, delighted and challenged the audience. A warm thank you is extended to ThermoFisher for their generous sponsorship of the evening, to Alfred Hospital for use of their lecture theatre, and to the organisers of the event.

Report contributed by: Jaelyne Birrell (ASM Committee – Clinical/Diagnostic)

News from RMIT

3rd year Placement Students

It is with much excitement that the third year Laboratory Medicine students wrap up 1st semester 2018 and anticipate their 40 week placements in laboratory's around Victoria commencing in July. Having completed two majors in either Haematology, Transfusion, Biochemistry, Microbiology, Histology or Cytology, its finally time to put theory into action through full time work in various disciplines. After a long process of immunisations, police checks, applications and seminars, thanks to the hard and dedicated work of those such as Genia Burchall, Michelle Kovak and Rhonda Greeves, placements were allocated, and majority of students were eagerly ready to contact and meet their laboratory supervisors. Some of the placements students received include hospitals such as Royal Melbourne Pathology, The Children's, Monash Hospital, The Alfred, Austin, and the more far reached rural areas such as Shepparton. Several students elected to take the opportunity to experience laboratories overseas in countries such as Sweden, Ireland or England. This is a privileged opportunity that will broaden RMITs students and equip them thoroughly for work in the world upon graduation.
RMIT's placement program is an opportunity that few other universities offer and enables students to put into practice the skills they have been learning for the last three years. It enables students to create those vital links of understanding between theory and practical in a laboratory. Students have the privilege of learning under senior scientists and stay in regular contact with carers at the university throughout the placement. It is with great anticipation and some nervousness that students look forward to mid-July and the commencement of the beginning of their careers as Medical Scientists.

Leah Smeaton
Student committee member

National news

Travelling Orator for 2019 " Fleur Francis"

Fleur Francis is Pathology Queensland's Supervising Scientist of the Molecular Microbiology and Serology departments at The Townsville Hospital. Fleur gained her degree in medical laboratory science with distinction from RMIT, was awarded the AIMS Victorian Branch top 3rd year Med Lab Science student in the course, the Medical Microbiology prize, and the ASM scholarship award for best 3rd year student in Microbiology. Fleur subsequently completed her Masters of Applied Biology in Microbiology and Biotechnology, also from RMIT.

Fleur commenced her career working as a trainee scientist at Fairfield Hospital in the Rubella/CMV laboratory and then in the Microbiology and Serology laboratories. When she graduated she commenced work in the Microbiology laboratory at Monash Medical Centre. In 1997 Fleur relocated to Townsville and joined Queensland Health Pathology Services, tasked with establishing a Virology and Mycobacteriology service for the local area.

Embracing many advances in the detection of viruses and bacteria, over time the laboratory has developed into a comprehensive molecular microbiology service. It is now a busy diagnostic molecular microbiology laboratory servicing a large area of Queensland, from Mackay in the south, to Mt Isa in the west and north to the Torres Strait. Fleur strives to achieve the highest quality patient-centric diagnostic pathology services to a wide range of clients throughout this region. Her passions lie with a special focus on indigenous health outcomes associated with 'Closing the Gap' initiatives in Tropical North Queensland.

Fleur has been an active member of AIMS and the ASM for over 25 years and currently sits on the Advisory Committee for the RCPA Molecular Infectious Diseases Quality Assurance Program. She is also an adjunct lecturer in the medical laboratory science and medicine courses at James Cook University.

When planning events for 2019 keep Fleur in mind as our Travelling Orator and provide advance notice of dates and times you would like to book her to present

THE MILSOM MEMORIAL AWARD

History: The Milsom Memorial Award was established at the end of 1982 upon the dissolution of the Medical Laboratory Scientists' Association of Victoria (MLSAV). An initial sum of $2,500 was donated by the MLSAV to set up an annual award in honour of the late Mr. George Milsom who had given an outstanding contribution of service to Medical Laboratory Service in Victoria.

Eligibility for Award: The award is available to any Victorian resident with the exception of the Executive members of the AIMS Victorian Branch.

Purpose of the Award: The following purpose and example will help members decide what type of person should be nominated for the award.

About the award:

The **"George Milsom Memorial Award"** is to be given for significant achievement or service in the field of Medical Laboratory Science in Victoria.
Examples of nominees for this award would be:

- A scientist involved in a major scientific breakthrough or achieving National recognition or International recognition for his or her work.
- Any person who has given significant service at either Committee level or outside Committee.

Nominations can be put forward by financial members of the AIMS Victorian Branch. Nomination form on the following page.

THE GEORGE SWANSON CHRISTIE MEMORIAL AWARD

This award is nominated & offered by the Vic Branch committee. It has been presented annually since 2004 & is offered to an inspirational medical scientist who has shown ongoing excellence in the field.

AIMS VICTORIAN BRANCH MILSOM MEMORIAL AWARD
NOMINATION FORM

We, the undersigned, being financial members with voting rights of the Australian Institute of Medical Scientists, nominate:

_____

(Name of nominee)

for the Milson Memorial Award to be presented at the 2018 Annual General Meeting of the AIMS Victorian Branch

Proposed by: _____

Signature: _____ **Date: ___ /___ / 2018**

Seconded by: _____

Signature: _____ **Date: ___ /___ / 2018**

Supported by: _____

Signature: _____ **Date: ___ /___ / 2018**

The nomination must be accompanied by a citation outlining the merits of the particular nominee for the Award.

Nomination forms should be returned to branch secretary Patricia Szczurek (patriciaszczurek@gmail.com)

No later than 4 pm (AEST) Friday 10th August 2018

Upcoming Events

HDG and presentation of student awards

Haematology Discussion Group

When: Tuesday July 17th 2018
18:00 for light refreshments
18.30 - 20:00pm for presentations
Gold coin donation for trivia night please

Where: RMIT CITY CAMPUS
Building 80, Level 11, Room 10

Address: Swanston St, Melbourne

OUR STUDENT SPEAKERS AND TOPICS...

Arina Kushnir	APML
Tegan Seitz	Novel therapies Haemophilia A
Muna Al Balushi	To be confirmed
LinlinChen	To be confirmed

Also included: Presentation of AIMS Student Awards

Sponsor : Ortho Clinical Diagnostics

Ortho
Clinical Diagnostics

HDG Convenor: s.schischka@alfred.org.au
Mob 0406 382 764

Apace

Directions

RMIT HDG Building 80 (Swanston Academic Building, Level 11, Room 10

Public Transport is best – Melbourne Central is less than a block away.

Parking in the City is always difficult, but in the side streets over Victoria Parade, there should be some spots available by 6pm.

Upcoming Events

Save the Date!

Saturday 16th March

Level 7
Peter MacCallum Cancer center

AIMS & VIDG present:

It's in the Blood!

Discussion Group Convenors

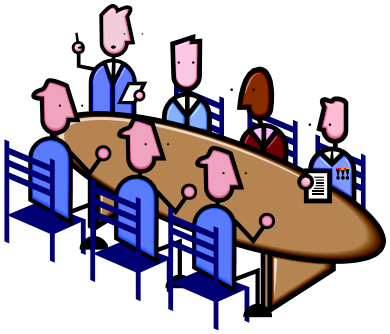
Clinical Flow Cytometry Users Group (CFUG)
Peter Gambell
email: peter.gambell@petermac.org

Haematology (HDG)
Steve Schischka
email: : Steven.Schischka@svhm.org.au

Histology (HGV)
Adrian Warmington
email: adrian.warmington@sjog.org.au

Immunohaematology (VIDG)
Helen Bardsley
email: Helen.Bardsley@austin.org.au

Immunology (DIDG)
Marilyn Clark
email: Marilyn.Clark@rch.org.au



Anybody requiring information concerning these discussion groups should

Upcoming Events 2018

HDG meeting and Student awards July 17th R.M.I.T. City campus

AIMS/AACB Combined NSM 2018 3-5 September 2018

Travelling Orator announced, book now for events in 2019

Sat 16th of March AIMS & VIDG, “Its in the blood Symposium”

Next 2018 Deadline for Benchpress

1st October 2018

Call to all scientists still working, semi-retired or doing something different who started the course in 1973-74.

A small group from this era still keeps in contact and have the occasional social lunch. We would like to see how many from that auspicious time we can get together and see where the last 40 years have gone. Names such as Geoff Magrin, John Allen, Damian McVeigh, Allan Balloch and Michael Taylor to name a few

If you know someone from this era who doesn't have access to this newsletter please pass on the details and ask them to reach out

Contact: John Allen email: allenrj@primus.com.au or on his mobile 0419303790 alternatively

Geoff Magrin email: bldbnkr@gmail.com or mobile 0418598571

This is a photo was taken in 1974 I think and it is the full time Medical Technology class at a Physics lecture. Do you recognise anyone?

Some of this group are still meeting and want to find others.

I believe I can still name everybody in that picture. You cannot see me as I took the photo. Geoff

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Published by
AIMS. (Victorian Branch)
P.O.Box 2100
Royal Melbourne Hospital
Parkville VIC. 3052
Benchpress will be sent by email each month

All copy and advertisements should be sent to :
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