MONASH University

MONASH MEDICINE, NURSING AND HEALTH SCIENCES

FLOWCORE-CLAYTON
PLATFORM USER GROUP MEETING
AGENDA

Meeting 2/2022 was held on Thursday 29 September at 11:30am via Zoom

	Attendees: Andy Fryga, Nicole Mifsud, Ellen Mann, Keith Niven, Adam Dinsdale, Kathryn Flanagan, Angela Nguyen, Justin Zhang, Nicole La Gruta, Chloe Shepherd, Michael Thomson, Patricia Illing, Daniela Moreno Vicencio, Olaf Perdijk, Alfie Baker, Jessie O'Hara, Daniel Thiele, Pirooz Zareie, Martin Stone, Claerwen Jones, Rose Huang, Jiahui Yu, Natalia Rosas, Ee Shan Pang, Svenja Loering, Anouk von Borstel, Kelvin Yip, Parikshit Banerjee, Yen Tran, Azadeh Anbarlou, Tao Zhang	**Due Date/ Assigned/ Notes**

	Business Arising	
1.	Welcome and introductions (Nicole Mifsud)	
2.	**Approval of Minutes 1/2022** Accepted by Keith Niven and Olaf Perdijk.	**Approved**
3.	**Platform Update (Andy Fryga)** • This half of the year has followed with the same pattern as the previous half with the Analysers being exceptionally busy and the Sorters very patchy. • It looks like this will continue for the rest of the year. • The analyser room is very busy with a lot of people working on analysis.	
4.	**Open Forum** <u>Flowjo licences</u> • Anyone on a 6 month Flowjo subscription were advised that Friday 30 September was the last day of that current subscription period and if they wanted to renew the licence to contact Kathryn. • Invoices will be sent shortly after the current subscription period has finished. <u>Cytek Aurora</u> • A seminar took place several weeks ago. • It is hoped that more people will start to use the machine in the coming weeks. • One new training session was requested that will take place in the next week. • Usage of the Cytek will keep being pushed throughout the rest of this year and into 2023. • At the moment there are a couple of main groups who are frequent users but there is still a lot of time available on the machine. • It is not a machine that can be used without first having training, so users are advised to plan ahead and contact Keith or Kathryn if they want to start to use it. • The slides from the seminar are available on the FlowCore website. <u>Bigfoot Spectral Cell Sorter – ThermoFisher</u>	

- FlowCore have had the Bigfoot on trial for 1 month. A slight extension has been given on the trial period until Friday 7 October.
- Trying to do an assessment on such a complex machine and actually learning how to use it at the same time has been very challenging.
- The team are getting on top of the use of the machine but it is still throwing up lots of challenges. This has caused Andy and team to have a slight change in mindset around the machine in the sense they have realised that it won't be a like for like with the Influx machines as it is too complicated for that.
- The Influx machines are very good at the 'volume' side of the business. Users can turn up and be sorting in 5 minutes which is not the case with the Bigfoot.
- The Bigfoot has many layers, controls and set up time.
- If they do decide to go ahead with the purchase, it will be aimed in the short to medium term as a high end machine that users bring high parameter panels.
- It won't be a machine to do GFP sorts as the workflow doesn't really suit the lower end sorting capacity.
- Some final testing will be completed this week with a big spectral panel to test the following week.
- Andy thanked everyone who provided material in order to test the machine, which was a great help. Without this help it would have been impossible to evaluate it.
- The traditional flow cytometry style workflow on the Bigfoot is one of it's good points.
- The issues the FlowCore team are having with the machine are a combination of learning how to use it and problems with the actual machine. This is version 1.2 as it has only been in the field for approximately 2 years, so it is still very early days in the development and Thermo and Propel Labs are continually working making improvements in real time.
- There have been some major hardware and software upgrades within the last 6 months.
- This is a machine that is not an out of the box solution sorter. It is a machine that has very high specs and is being developed in real time.

<u>BD Rhapsody</u>

- Nicole Mifsud gave a presentation on the BD Rhapsody. The full slides are attached.
- For some time FlowCore Users have been interested in acquiring an instrument, as an alternative to the 10X system, for sorted cells library preparation and sequencing.
- A demonstration unit was obtained in Feb this year and some pilot experiments were set up in order to evaluate the machine.
- Four groups were invited as BD provided free kits:
    - Nicole (Mifsud Group)
    - Pirooz (La Gruta Lab)
    - Chris (Degli- Esposti Lab)
    - Priyanka (Davey Group)
- The four groups looked at a number of different kits that are available:
    - Nicole and Priyanka: Human targeted VDJ/T cell panel for gene expression
    - Pirooz: Mouse targeted VDJ/T cell panel for gene expression
    - Chris: Human Whole Transcriptome Analysis (WTA)
- BD Rhapsody enables you to multiplex samples.
- Once the QC was performed and you have your single cell with a bead in every micro well, you can divide up what you want to do with your samples at that point.

	• Currently BD uses SeqGeq for data analysis, which is very similar to a Flowjo workstation. • It is very important to complete your sorting, sample tag, multiplexing and cDNA synthesis on the same day to ensure the viability of the cells is as high as possible. • For Nicole, the most important part is the QC viability markers which allow you to make decisions very early on as to the quality of your cells and whether you want to continue to sequence and spend that extra money. • Rather than using a single viability dye, they use 2 - Calcein AM to target live cells and DRAQ7 to target dead cells. You get a very good overview of the quality of the cells before moving on to the cDNA synthesis stage. • It is critical to have a single bead with a single cell in each of the cartridge wells. • Once the cDNA synthesis is complete, this can be put in the fridge/freezer as it is quite stable by this point. • An article has been published looking at the BD Rhapsody and Nicole encouraged anyone who is interested in this technology to read it: Optimized workflow for single-cell transcriptomics on infectious diseases including COVID-19 - ScienceDirect. • At the end of the sequencing run you end up with a couple of output files which need to be put into a system called Seven Bridges that de-convolutes the files. At the moment, BD are supporting the cost of this, so no payment is necessary, you just need to be given access. • For Nicole's samples, it took an overnight run in order to acquire the relevant files. • A huge number of files come out and there will be several that are the input files that you use for their associated SeqGeq software. • SeqGeq has been designed on Flowjo workflows. Nicole was able to get a 60 day free trial in order to evaluate the software. It has a lot to give you in terms of the type of analysis it can perform although there are still some bugs in the system that are yet to be sorted. • The files can also be put into R for those who are competent with that. • Nicole's total experiment cost $5964.50 (full details are on the slide). This works out at about 30 cents per cell. She felt that this was a good price for a very detailed experiment. • The purchase of this instrument has been raised with the BDI infrastructure committee and it is on the list to be funded. Should know within the next couple of weeks whether it will be funded to purchase this year. • This is an application that Nicole La Gruta and Mariapia Degli-Esposti are also supporting. Do need to show that this isn't just for a single user but can be used by multiple people right across BDI. • Anyone who is interested in the machine are asked to contact Nicole. • It was asked how the cost would change if you are looking at untargeted rather than targeted gene expression. Nicole wasn't sure but advised that they do have the full pricelist for all the reagents. Pirooz thought it would add roughly $2500 to the cost, which is still cheaper than 10X. • The BD Rhapsody is a very small footprint and if a machine is purchased it would probably be housed in Nicole Mifsud's lab, which is located across from FlowCore. It would be available for use by everyone and Nicole would be happy to assist people to get access to it and help with any training needed.	
5.	**Other Business** • Nicole La Gruta advised that she recently went to the Adelaide Immunology Retreat where she met Kate Pilkington who is very keen to help people with spectral flow cytometry. If anyone wants help transitioning to spectral flow, they are encouraged	

	to contact Kate. Contact Nicole La Gruta, Nicole Mifsud or Andy Fryga and they can pass on Kate's details. • Kathryn will also put Kate's details on the FlowCore website. • Nicole Mifsud added that if anyone comes across a technology or something that is of general interest to FlowCore, to please raise it at these meetings or raise in between the meetings by contacting Nicole, Andy or the team.	
6.	**ACTION ITEMS**	
6.1	Kathryn to put Kate Pilkington's contact details on the FlowCore website	**2022/KF**
	Next Meeting To be scheduled for March/April 2023	

Distribution

Chair: Nicole Mifsud (Biochemistry & Molecular Biology, BDI)	NM	Kathryn Flanagan (Platform Senior Research Officer)	KF
Platform Manager: Andrew Fryga	AF	Adam Dinsdale (Platform Senior Research Officer)	AD
Ellen Mann	EM	Keith Niven (Platform Senior Research Officer)	KN
Angela Nguyen	AN	Martin Stone	MS
Justin Zhang	JZ	Claerwen Jones	CJ
Nicole La Gruta	NLG	Rose Huang	RH
Chloe Shepherd	CS	Jiahui Yu	JY
Michael Thomson	MT	Natalia Rosas	NR
Patricia Illing	PI	Ee Shan Pang	ESP
Daniela Moreno Vicencio	DMV	Svenja Loering	SL
Olaf Perdijk	OP	Anouk von Borstel	AVB
Alfie Baker	AB	Kelvin Yip	KY
Jessie O'Hara	JO	Parikshit Banerjee	PB
Daniel Thiele	DT	Yen Tran	YT
Pirooz Zareie	PZ	Azadeh Anbarlou	AA
		Faculty Research Office	FRO

Secretariat: Melissa Lipscombe (FRO)