EAGMST - December 2020 Meeting

- Agenda point 4
- Omics: Updates on TRF/MRF and ongoing activities (50 mins)
  - Case studies: update on trialling the TRF/MRF (15 mins)
  - New OECD Omics website (10 mins)
  - EAGMST-WPHA project on omics regulatory applications, including the Joint session with WPHA in June 2021 (25 mins)

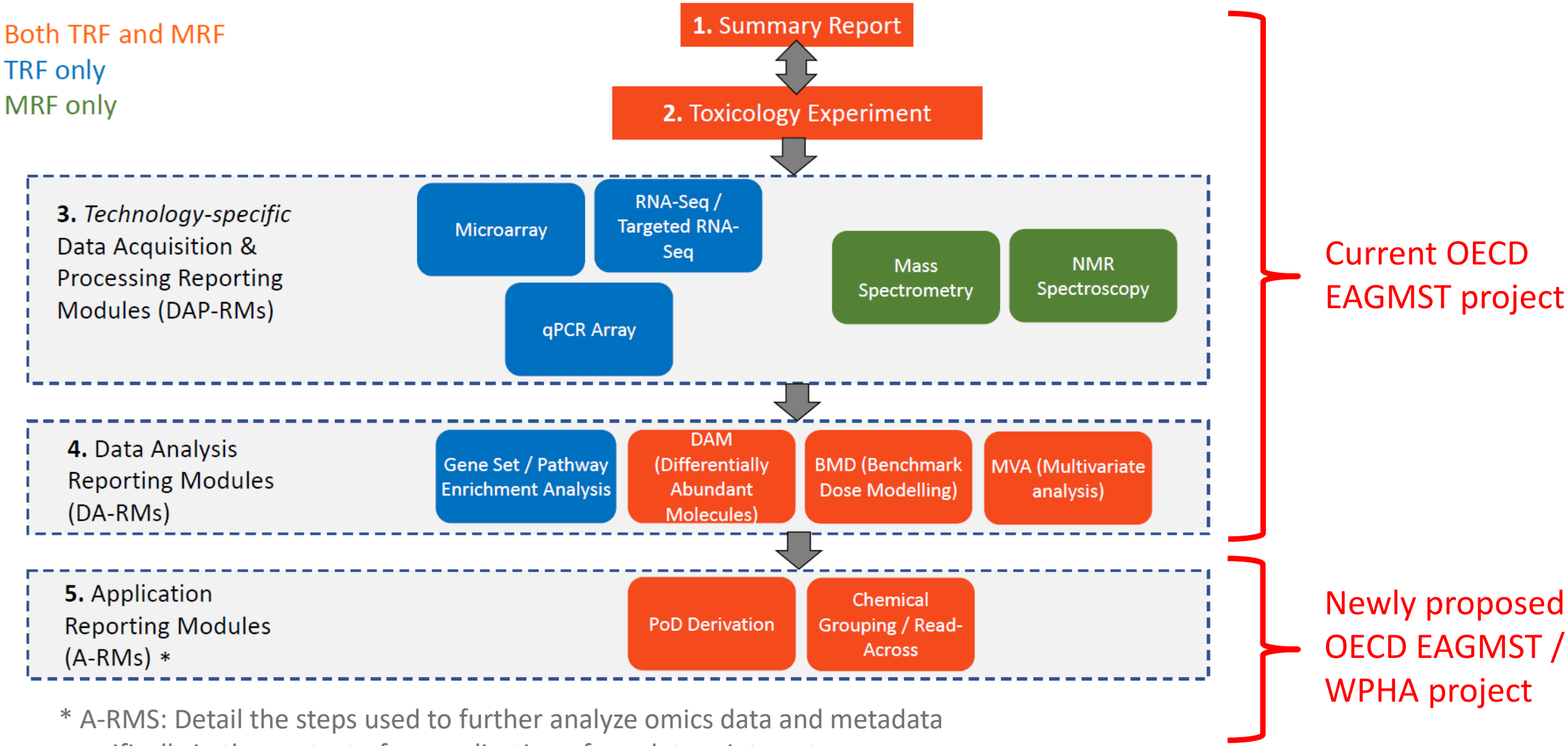
TRANSCRIPTOMICS REPORTING FRAMEWORK (TRF)

Joshua Harrill (US EPA)
Carole Yauk (University of Ottawa)

Disclaimer

- *The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency, nor does mention of trade names or products represent endorsement for use.*

Modular Structure of Omics Reporting Frameworks Harmonization of TRF and MRF



* A-RMS: Detail the steps used to further analyze omics data and metadata specifically in the context of an application of regulatory interest

TRF Module Status (Dec 2020)

Module Name	Module Development Lead	Status
Introduction	Joshua Harrill (US EPA) Carole Yauk (U Ottawa)	Complete
Study Summary	Carole Yauk (U Ottawa)	Complete
Toxicology Experiment Module (TEM)	Raffaella Corvi (JRC)	Complete
Technology Specific Data Acquisition and Processing Reporting Modules (DAP-RM)		
Microarray	Vikrant Vijay (NCTR)	Complete
RNA-Seq / Targeted RNA-Seq	Florian Caiment (U Maastricht)	Complete
qPCR Array	Jason O'Brien (ECCC)	In Process
Data Analysis Reporting Modules (DA-RM)		
Differentially Abundant Molecules (DAM)	Lyle Burgoon (ERDC)	Complete
Benchmark Dose Modeling (BMD)	Scott Auerbach (NIH DNTP)	In Process
Gene Set / Pathway Enrichment	TBD	Pending
Multivariate Analysis (MVA)	TBD	Pending

TRF Case Studies

Submitter:

1) Identify dataset
2) Compute DEGs
3) Fill in TRF
   a) Technology specific DAP-RM
   b) DAM DA-RM
4) Ease-of-use commentary

At the discretion of the Submitter:

- Transcriptomics technology
- Specification of meaningful contrasts
- Computing environment
- Method for DEG determination

Referee:

Provide to End User:
1) Completed TRF
2) Accessory files
3) Blinded / truncated data output

Review the contents of the submission and generate truncated data outputs with blinded gene IDs to pass to End User.

End User:

1) Review completed TRF
2) Reproduce DEG analysis
3) Ease-of-use commentary

End User communicates with referee if difficulties are encountered reproducing the analysis.

Referee:

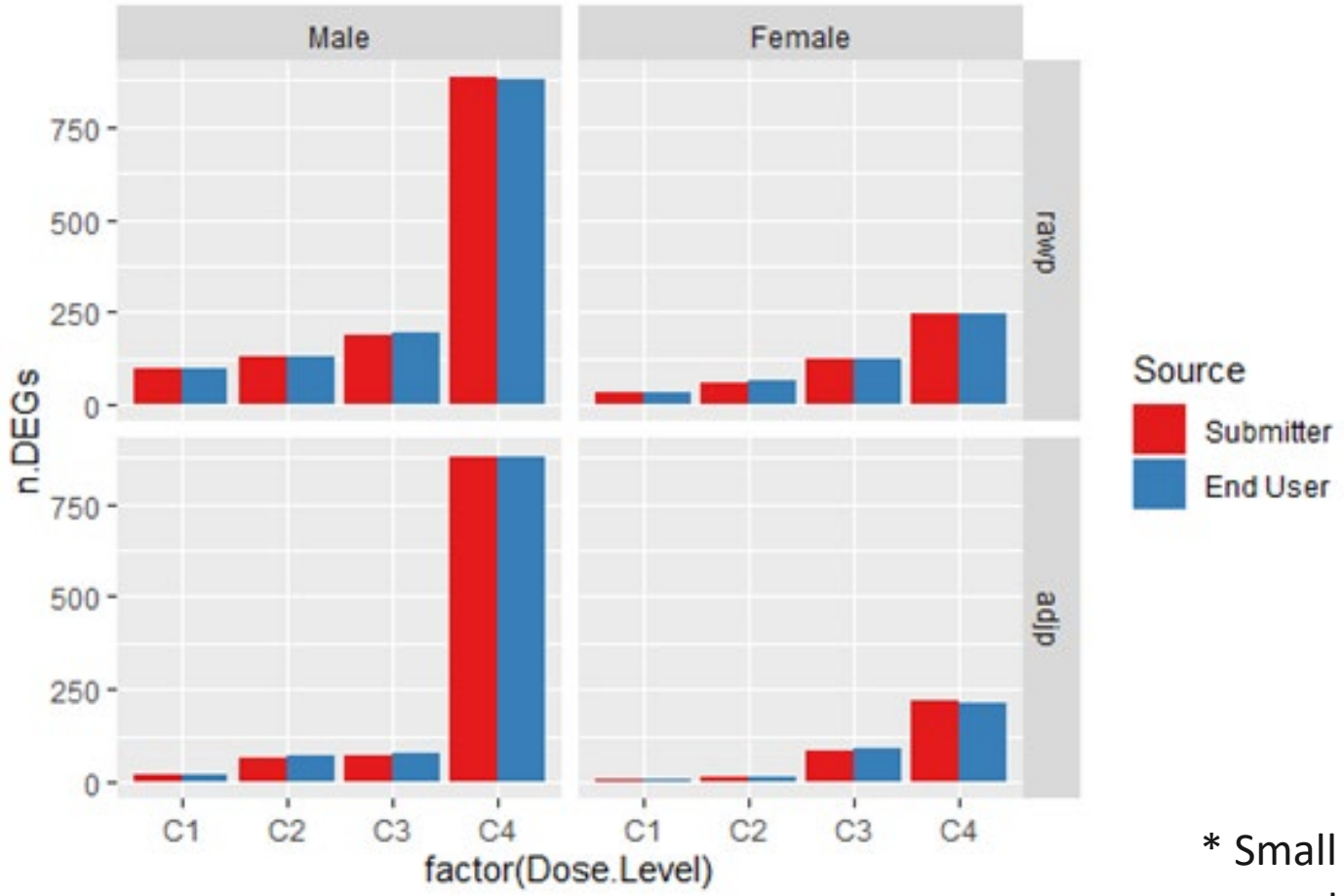
1) Concordance analysis
2) Summarize ease-of-use commentaries

TRF Case Study Descriptions

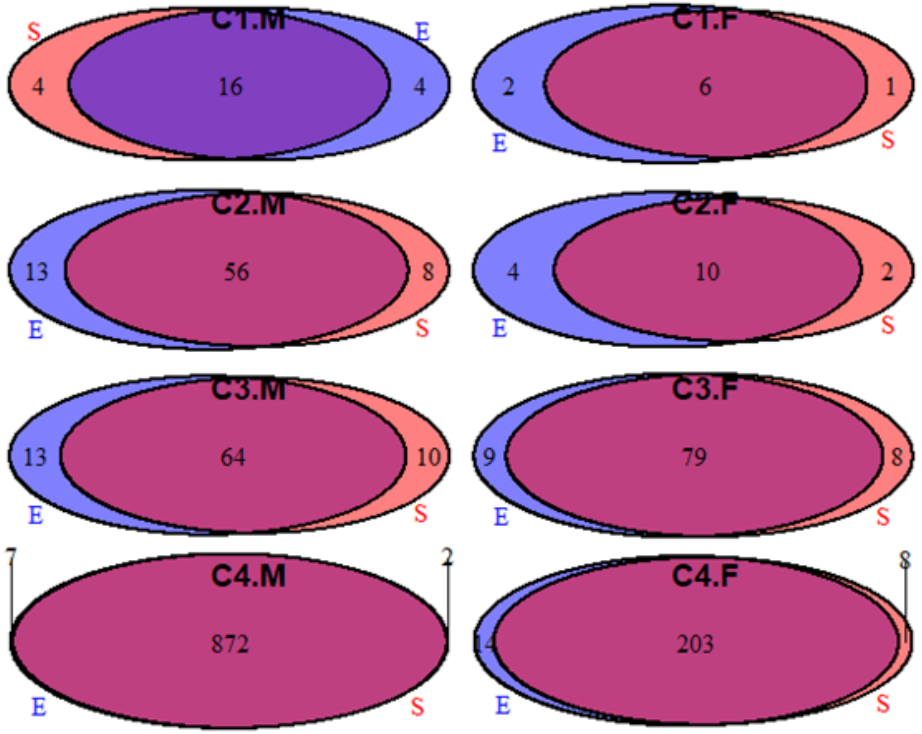
Platform	Study Description	DAM Method	Computing Environment	Submitter	End User	Status
Agilent Microarray	Four point concentration-response of furan in male and female Fisher rat liver (GEO GSE62805)	Submitter's Choice	R	Andrew Williams (Health Canada)	Leah Wehmas (US EPA)	Complete
Affymetrix Microarray	Comparison of PFOA responses in livers of 129S1/SvImJ wild-type and PPAR-alpha null mice (GEO GSE9786)	Submitter's Choice	Partek Flow	Beena Vallanat (US EPA)	Alison Harrill (HHS DNTP	In Process
RNA-Seq	Three point concentration-response of hexabromocyclododecane in male and female Fisher rat liver (PRJNA395549)	ODAF	R	Matt Meier (Health Canada)	Brian Chorley (US EPA)	In Process
RNA-Seq	TBD	Submitter's Choice	R	Natalia Garcia-Reyero (MS State IGBB)	Andrew Williams (Health Canada)	In Process

Case Study #1 Results

Number of Differentially Expressed Genes



Overlap of Differentially Expressed Genes



Comparison of DEGs identified by Submitter and End User (adjp)

* Small differences are expected because the analysis applied permutation-based *p*-value calculations

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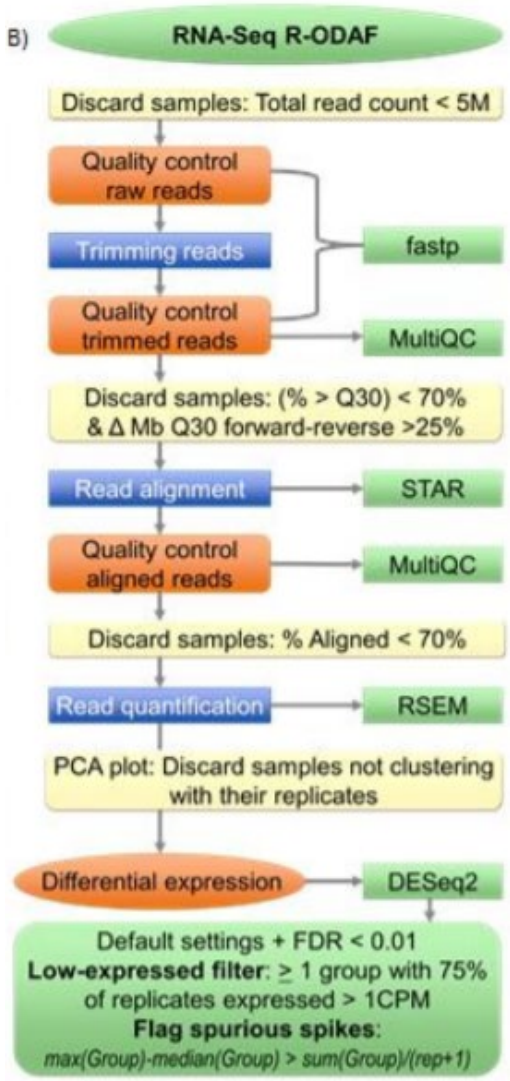
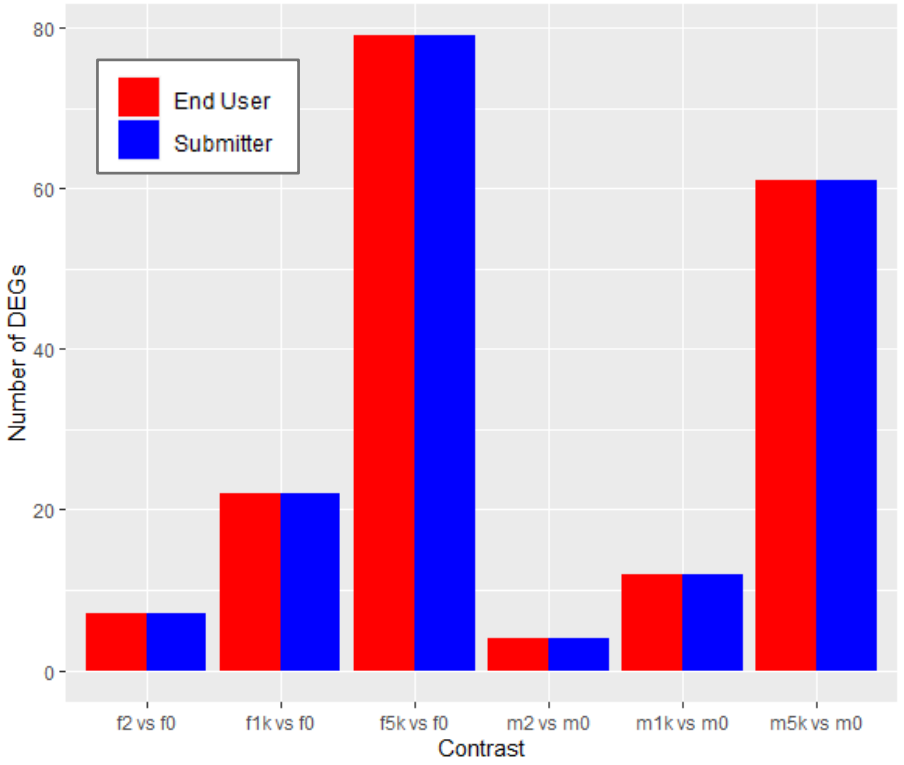
RNA-Seq	TBD	Submitter's Choice	R	Natalia Garcia-Reyero (MS State IGBB)	Andrew Williams (Health Canada)	In Process

Case Study #3 Results

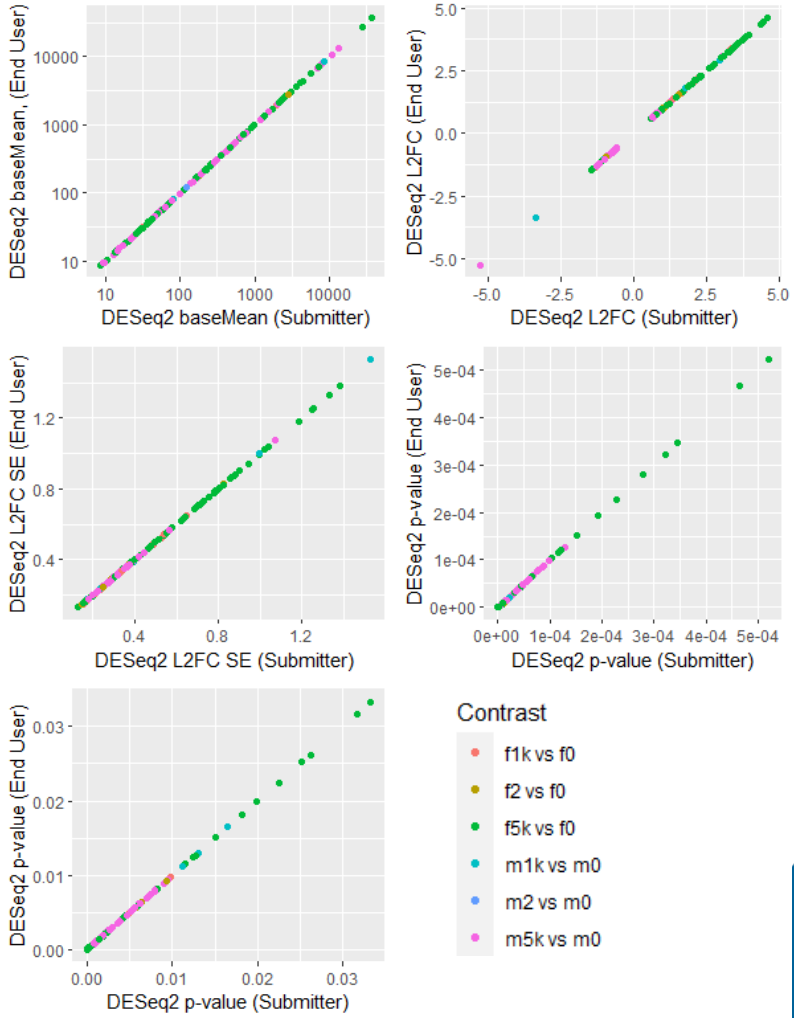


Number of Differentially Expressed Genes



- Highly comparable results from Submitter and End User

Correlation of DESeq2 Outputs



Case Study Lessons Learned

General findings relating to ability to reproduce analyses:

1. Analyses in **open source computing environments** (R, Python, etc.)
a) Much easier for an end user knowledgeable in coding languages to reproduce because they come with an "instruction manual" (i.e. the analysis script or notebook)
b) Details in the reporting fields become somewhat less critical for reproducing the analysis secondary to the scripts
c) There are also no financial or licensing barriers with regards to accessing the tools
d) Issue: users may not have sufficient expertise with open source computing environments

2. Analyses using **freeware analysis softwares or web applications** (BMDExpress; iDep)
a) These types of software are more user friendly and require less technical or statistical expertise to use
b) No "pay wall" barrier that would prevent an end user from accessing such tools.
c) Reproducibility depends on clear and precise reporting in the TRF documentation as well as provision of a configuration file or some other configuration snapshot that the end user could follow. ***NEEDS TO BE TESTED***

3. Analyses using **proprietary software** (Partek, Ingenuity, etc.):
a) End user needs access to the same software (and maybe even version)
b) "pay wall" issues.
c) Reproducibility depends on precise reporting in the TRF documentation as well as provision of a configuration or workflow that the end user could follow.

Case Studies Next Steps

Additional case studies:

- More developer and user feedback and participation.

- Testing different analytical platforms:
  - Open computing environments versus
  - Web applications

- “Test Driving” reporting modules in development
  - qPCR case studies
  - BMD case studies

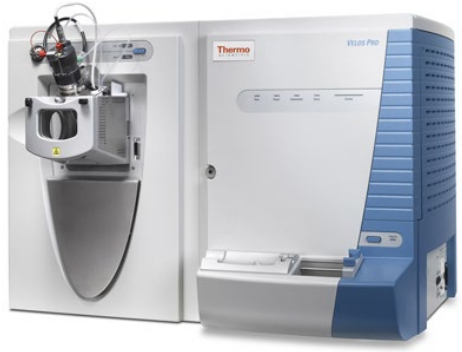
METABOLOMICS REPORTING FRAMEWORK (MRF)

Mark Viant (University of Birmingham, UK)

MRF Module Status (Dec 2020)

Module Name	Module Development Lead	Status
Summary Report (SR)	Mark Viant (U Birm)	Complete
Toxicology Experiment Module (TEM)	All MRF	Complete
Technology Specific Data Acquisition and Processing Reporting Modules (DAP-RM)		
Mass Spectrometry	All MRF	Complete
NMR Spectroscopy	All MRF	Complete
Data Analysis Reporting Modules (DA-RM)		
Differentially Abundant Molecules (DAM)	Tim Ebbels (Imperial College)	Complete
Multivariate Analysis (MVA)	Tim Ebbels (Imperial College)	Complete
Benchmark Dose Modeling (BMD)	David Crizer (NIH DNTP), Mark Viant (U Birm)	In Process

Trialling the MRF - Case Studies



Mass spectrometry metabolomics trial - Underway

Data submitter: David Crizer (National Toxicology Program, US)

- 5-day rodent assay, plasma samples, thujone exposure

- **MRF referees:** Oliver Schmitz (BASF, DE), Pim Leonards (VU University, NL), Aniko Kende (Syngenta, UK)
- **End user:** Tom Lawson (Michabo Health Science, UK)

NMR spectroscopy metabolomics trial - Underway

- **Data submitter:** Fabien Jourdan, Nicolas Cabaton, Cécile Canlet (INRA, FR)
  - Mouse study, brain tissue, bisphenol A exposure
- **MRF referees:** Drew Ekman (EPA, US), Mark Viant (University of Birmingham, UK)
- **End user:** Tracey Schock (NIST, US)

To be concluded by 30 April 2021

TRF and MRF Project Timeline

- Submit TRF and MRF to OECD EAGMST for formal review: June 2021

DISSEMINATION ACTIVITIES

Dissemination: TRF/MRF presentations

1. Cosmetics Europe Toxicogenomics Meeting, October 30th 2020

2. European Cluster to Improve Identification of Endocrine Disruptors (EURION), November 13th 2020

TRF/MRF publication plans

1. Towards OECD Reporting Frameworks for Transcriptomics and Metabolomics in Regulatory Toxicology
   - 95% drafted, for *Regulatory Toxicology and Pharmacology*
   - *Target date to submit January 2021*

2. Trialling the MRF using 2 metabolomics studies
   - Showcase the application of the MRF to two metabolomics case studies
   - Demonstrate its value and rigor via these case studies
   - Present all data using MRF reporting templates
   - Report data to EBI MetaboLights repository – “complete data path”; attempt to package up data for the regulator – “regulatory compliance path”
   - *Target date to submit June 2021*

3. Trialling the TRF using 4 transcriptomics studies?
   - Conceptually as above

New OECD Omics website

- Motivated by need to:
  - Increase dissemination of TRF/MRF
  - Make available version-controlled (draft & final) Guidance Documents and reporting templates (to encourage further use)
  - Facilitate the research publications by enabling the GDs to be cited
- To be modelled on, e.g., *OECD Work Related to Bees/Pollinators* website https://www.oecd.org/chemicalsafety/testing/work-related-beespollinators.htm

EAGMST-WPHA project: *progress to date*

- Agreement between EAGMST, WPHA Chairs and Secretariat to launch a new project focused on regulatory applications of omics

- WPHA members have been consulted
  - 8 members from Canada (3), US (2), UK (2) and ECHA (1) have stepped forward to represent the regulatory perspective

- (deleted organised by Magda) First meeting (14th January 2021)
  - *Development of project proposal*
  - *Development of the Joint Session between WPHA and EAGMST in June 2021 on 'omics*

Further information

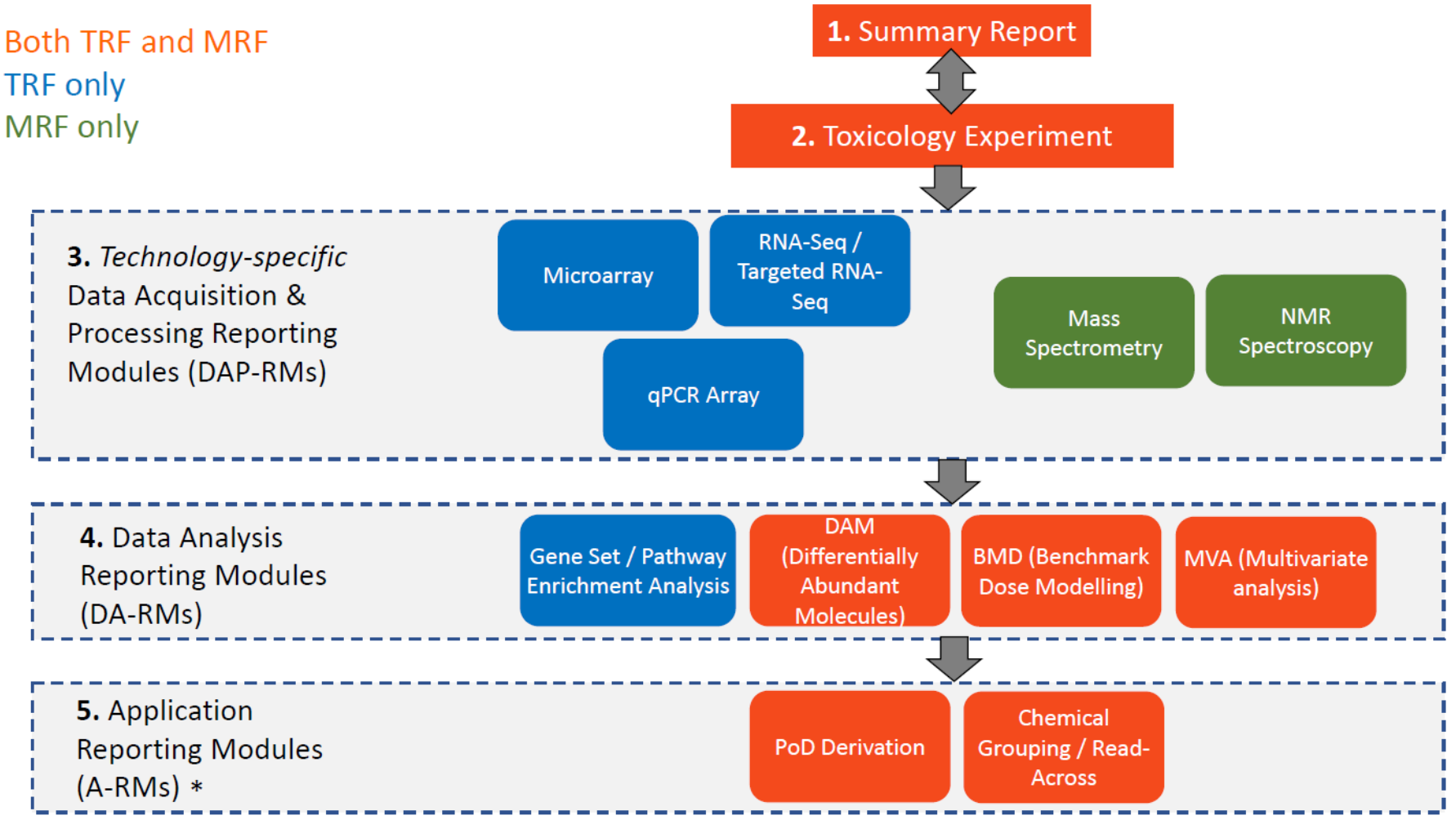
- TRF: Josh Harrill (Harrill.Joshua@epa.gov),
  Carole Yauk (Carole.Yauk@uottawa.ca)
- MRF: Mark Viant (M.Viant@bham.ac.uk)
- OECD: Magda Sachana (Magdalini.Sachana@oecd.org)

New OECD Omics website: *proposed contents*

- What are Omics approaches and why are they relevant to toxicology?
- Why is the OECD working on Omics?
  - Introduce OECD Chemicals Programme
  - Introduce EAGMST and its remit
  - List the topics that we are working on, and plan to work on
- Current and planned OECD activities
  - Omics Reporting Frameworks
    - Introduce TRF and MRF (who, what, where, when, why, how…, multi-omics, modular structure…)
    - Links to latest version-controlled (draft or final) GDs and reporting templates
    - Links to publication(s), recorded presentation(s)
  - ODAF?
  - New EAGMST-WPHA project?

EAGMST-WPHA project on omics regulatory applications

Both TRF and MRF
TRF only
MRF only



New project (SPSF)

* A-RMS: Detail the steps used to further analyze omics data and metadata *specifically in the context of an application of regulatory interest*

EAGMST-WPHA project

- What are we trying to achieve?
  - Describe omics best practice? (too early)
  - Describe how to report, expanding the TRF/MRF? (yes, but this is only a component of what we should try to deliver)
  - Broader goals, i.e., a framework articulated through case studies…

- Possible case studies (*to discuss at EAGMST-WPHA 1st meeting*):
  - PoD to help derive health-based guidance values
  - Omics-based grouping/read-across

EAGMST-WPHA project: *possible approach*

- **Case study** focused:
  - Identify and define the specific regulatory need and/or opportunity
  - Describe the input data required (both traditional and omics)
  - Describe the data interpretation procedure (‘algorithms’)
  - Ensure that findings of regulatory relevance are produced
  - Describe quality metrics, describe the uncertainty in the findings
  - Report the methods, data, results, uncertainties… (add Application Reporting Modules to the TRF/MRF, add how to report (OHTs))
  - Ultimately write a framework and guidance for the consistent application of an omics technology to contribute to… [*specific regulatory application*]… resulting in an output which is suitable for regulatory decision making

EAGMST-WPHA project: *next steps?*

- First meeting, 14th January 2021, clarify objectives and case studies
- Write SPSF by end of Q1 2021?
- More detailed scoping of project by the leads, in Q2 2021?
- Review plans at joint EAGMST/WPHA meeting, identify further experts, etc…