EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

EURL
European Union Reference Laboratory
for GM Food & Feed

REPORT

on the TRAINING WORKSHOP on DIGITAL PCR and NEXT GENERATION SEQUENCING for NATIONAL REFERENCE LABORATORIES

8-10 December 2015, Ljubljana, Slovenia

The training was organised upon request of the National Reference Laboratories assigned under Regulation (EC) No 882/2004 (NRLs/882) who had indicated these topics as the main training topics of interest during the NRL/882 workshops in 2014 and 2015. The EURL GMFF decided to co-organise this training together with experts of the National Institute of Biology in Ljubljana, Slovenia, and to hold the training at the NIB facilities, given their excellent experience in the application of digital PCR to GMO analysis and the availability of several dPCR instruments to be used during the training. A similar training was previously organised by the EURL GMFF in Ispra at the end of 2014.

The training took place 8-10 December 2015. All NRLs/882 were invited to the training on a first-come-first-served basis, with a maximum capacity of 20 registered participants. A total of 13 NRL/882 participants, from the same number of NRLs, registered to the training. In addition, representatives from two companies selling digital PCR instruments were invited and accepted to give a presentation on their platforms during the first day of the training. Two experts were invited to present their experiences in dPCR and Next Generation Sequencing (NGS), one from RIKILT/Wageningen UR, NL, the other from NVI, Norway. Four colleagues from the MBG Unit, including the Head of Unit, participated as experts and also supported the management of the training, and seven NIB staff members were involved in the event. While all administrative tasks related to the invitation and registration of the event, and re-imbursement of participants (flights, accommodation, daily allowance), were carried out by the MBG secretariat, the practical arrangements at the venue (transport and catering, facilities for meeting and laboratory activities) were taken care of by local NIB staff. The NIB was reimbursed for the consumables used during the training.

The programme of the training included the following general topics: -
day 1: introduction to digital PCR and platforms used
- day 2: hands-on demonstration in the laboratory and user experiences
- day 3: applications of NGS in GMO detection

Further details on the programme can be found in Annex 1.

The list of participants can be found in Annex 2.

At the end of the training, a feedback form was filled in by the participants. The results of this feedback are shown in Annex 3.

Summary of the training

The digital PCR and NGS Training was organised by the National Institute of Biology (NIB, SL) and the EURL-GMFF in order to inform National Reference Laboratories (NRLs) about the state of the art of digital PCR (dPCR) and Next Generation Sequencing (NGS) and their applications to GMO testing. Representatives of several laboratories were present, representing the wide interest that these technologies have raised among NRLs.

Day 1: General presentations on digital PCR

During the morning session, company representatives presented two platforms for digital PCR: the QuantStudio 3D (ABI), a chamber based platform with about 20.000 partitions, and the Naica System (Stilla), a novel droplet-based system with a short-time to results and 25.000-30.000 partitions.

The afternoon session consisted of presentations on the basic principles, statistics, performance parameters, and optimization of digital PCR assays.

Day 2: Laboratory Demonstration & Applications of dPCR

During the morning session, two digital PCR platforms were demonstrated in the lab: the Biorad QX200 platform and dedicated liquid handler, and the ABI QuantStudio 3D. The QX200 platform requires a more complex and delicate setup, but has a higher throughput and flexibility compared to the QuantStudio 3D.

The afternoon session consisted of presentations on current applications of digital PCR with regard to GMO and food analysis. The quantitative power of digital PCR and the multiplexing capacity of the droplet digital system were presented in great detail.

Day 3: Presentations on Next Generation Sequencing

The morning session consisted of presentations on the basic principles of NGS technology and its potentialities and experimental issues.

The afternoon session consisted of examples on current applications of NGS with regard to GMO and food analysis, followed by a final presentation on the main difficulties when applying Bioinformatics to NGS.

A final discussion on the future of dPCR and NGS applied to GMOs ended the workshop.

General conclusion:

Many GMO testing laboratories are looking to new technologies in order to help them cope with the increasing number of GM events on the market. The direct quantification of digital PCR and its multiplexing capabilities makes this an interesting option. NGS on the other hand, currently requires a steep investment to acquire the technology and represents additional analysis complexity. However, it offers a complete solution for both known and unknown GM detection (and possibly, quantification).

ANNEX 1. Detailed programme of the training

TRAINING WORKSHOP on DIGITAL PCR and NEXT GENERATION SEQUENCING

for NATIONAL REFERENCE LABORATORIES

8-10 December 2015, Ljubljana, Slovenia

AGENDA

DAY 1 - 8 December 2015 (M hotel)

Chairperson: David Dobnik

TIME	TOPIC	STAFF INVOLVED
09:30	Welcome by organizers and Introduction to the Workshop	J. Žel (NIB), J. Kreysa (JRC)
10:00	Naica System for Crystal Digital PCR	Magali Droniou (Stilla Technologies representative)
11:00	*Coffee Break*	
11:30	Digital PCR with QuantStudio 3D	Nataša Toplak (Omega d.o.o. representative)
12:30	*Lunch*	
13:30	Basics of Droplet digital PCR	David Dobnik (NIB)
14:30	Uncertanties in ddPCR and assesment of performance parameters	Antoon Lievens (JRC)
15:30	*Coffee Break*	
16:00	Introduction to experimental part for Day 2	David Dobnik (NIB)
16:30	Discussion	
17:00	***End of day 1***	
19:00- 21:30	***Social Dinner at Domačija pri Vodniku***	

DAY 2 - 9 December 2015 (NIB and M hotel)

Chairperson: David Dobnik

TIME	TOPIC	STAFF INVOLVED
09:00	Hands-on demonstration in the laboratory in two groups: setting up a droplet dPCR and Quantstudio 3D experiment	Dejan Štebih (NIB) Tina Demšar (NIB) Nataša Toplak (Omega d.o.o.)
09:15	First lab session	Dejan Štebih (NIB) Tina Demšar (NIB) Nataša Toplak (Omega d.o.o.)
10:45	*Coffee Break*	

11:15	Second lab session	Dejan Štebih (NIB) Tina Demšar (NIB) Nataša Toplak (Omega d.o.o.)
12:45	*Transfer to M Hotel*	
13:00	*Lunch*	
14:00	Experiences with restriction, inhibition and multiplexing in GMO detection	David Dobnik (NIB)
14:30	dPCR data in support to GMO method validation	Antoon Lievens (JRC)
15:00	*Coffee Break*	
15:30	Data analysis	David Dobnik (NIB) Dejan Štebih (NIB) Nataša Toplak (Omega d.o.o.)
17:00	Discussion	
17:30	***End of day 2***	
19:00– 21:30	***Optional dinner at M Hotel***	

DAY 3 - 10 December 2015 (M hotel)

NGS in GMO detection

Chairperson: Mauro Petrillo (JRC)

TIME	TOPIC	STAFF INVOLVED
09:00	NGS: an introduction	Valentina Paracchini (JRC)
10:00	NGS: a magic box?	Mauro Petrillo (JRC)
11:00	*Coffee Break*	
11:30	Case studies of GMO and NGS at the JRC	Valentina Paracchini and Mauro Petrillo (JRC)
13:00	*Lunch*	
14:00	Prospect on the use of high throughput whole genome sequencing for GMO detection	Arne Holst-Jensen (NVI)
14:30	Amplicon resequencing and enrichment of flanking regions of known GMO elements	Martijn Staats (RIKILT)
15:00	NGS and bioinformatics: tips and tricks	Mauro Petrillo (JRC)
15:30	*Coffee Break*	

Round table discussion

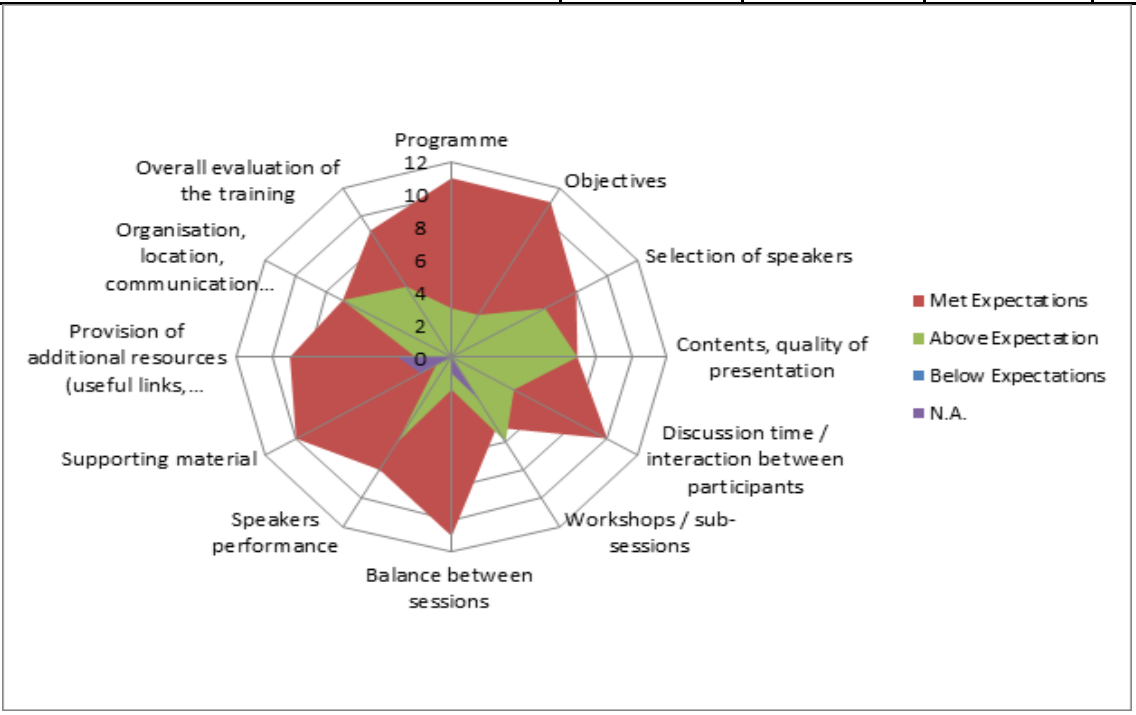
Chairperson: Joachim Kreysa (JRC)

	Round table discussion: New approaches for GMO detection and the regulatory implications	Joachim Kreysa (JRC) Jana Žel (NIB) Mojca Milavec (NIB)
17:30	***End of day 3***	

ANNEX 2. List of participants

ANNEX 3. Feedback results

2015-12-08 - 2015-12-10 Participants feedback_Workshop on digital PCR and Next Generation Sequencing	Below Expectations	Met Expectations	Above Expectation	N.A.
Programme		11	3	
Objectives		11	3	
Selection of speakers		8	6	
Contents, quality of presentation		7	7	
Discussion time / interaction between participants		10	4	
Workshops / sub-sessions		5	6	3
Balance between sessions		11	2	1
Speakers performance		8	6	
Supporting material	1	10	1	2
Provision of additional resources (useful links, downloads, contacts)		9	2	3
Organisation, location, communication with the participants, side events		7	7	
Overall evaluation of the training		9	5	



Remarks from participants:

- Excellent workshop. Demonstrates rapid, immediate and efficient response from EURL/NIB to provide a workshop for two of the key grow areas (dPCR/NGS) identified as priorities by NRLs in the 11th NRL workshop in Sept. 2015.
- It would be helpful to have a copy of the slides during the presentation (easier to make notes). Workshop on NIB was very good organised.
- Thank you for organizing very interacting and informative workshop.
- Thank you for all presentations, it was very interesting.