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Towards harmonised quality control of diagnostic nucleic acids

Key findings from the 2025 Euphresco-IVENAD interlaboratory comparison

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EPPO Conference on Diagnostics of Plant Pests
Recent developments and future trends

The Euphresco project consortium
2021-A-378

& ILC participants

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Euphresco IVENAD project | 2022 - 2026

Inventory / Consensus-building

Interlaboratory Comparison (ILC)

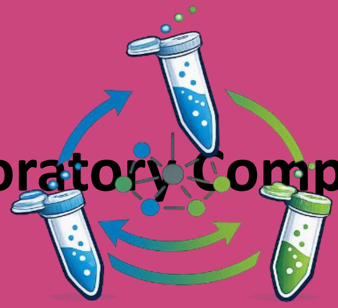
General agreement

- Need for harmonized procedures
- Strong variability in extraction quality & procedures to monitor
- Priority should be given to constitutive genes and qPCR/RT-qPCR

Euphresco IVENAD project | 2022 - 2026

Inventory / Consensus-building

Interlaboratory Comparison (ILC)



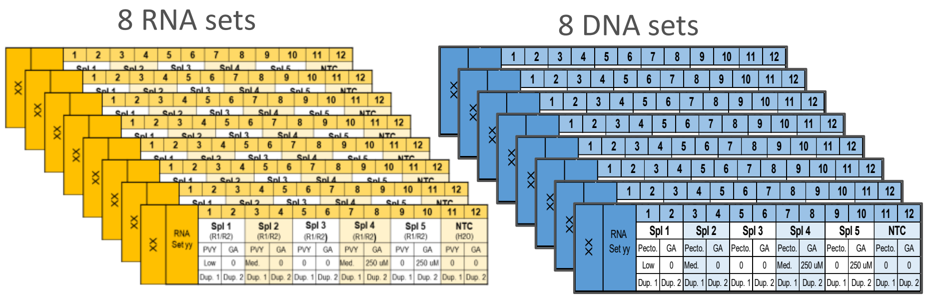
Purpose of the ILC

- Build a common evaluation framework
- Challenge selected control procedures across expert laboratories

Design of the test

- Matrices ➔ representative diagnostic sample types
- Targets ➔ representative DNA/RNA targets
- Extraction methods ➔ different purification strategies
- Primer sets ➔ selected IAC assays
- PCR inhibitor ➔ inhibitory pressure on performance

	RNA Set 1		1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1 (R1/R2)		Spl 2 (R1/R2)		Spl 3 (R1/R2)		Spl 4 (R1/R2)		Spl 5 (R1/R2)		NTC (H2O)	
			PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
Tomato seeds	A	IC1												
	B	IC2												
	C	IC3												
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	PVY												



Design of the test

Lab ID	Sets																																							
	RNA																				DNA																			
	Matrix 1					Matrix 2					Matrix 3					Matrix 4					Matrix 5					Matrix 6					Matrix 7					Matrix 8				
	Tomato seeds					Prunus leaves					Potato tubers					Aphids					Vitis leaves					Prunus leaves					Potato tubers					Leaf hopper				
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20
L1																					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
L2	X	X	X	X	X																X	X	X	X	X															
L3						X	X	X	X	X	X	X	X	X	X											X	X	X	X	X	X	X	X	X	X					
L4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X																				
L5	X	X	X	X	X											X	X	X	X	X	X	X	X	X	X											X	X	X	X	X
L6						X	X	X	X	X																X	X	X	X	X										
L7	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X																				
L8											X	X	X	X	X	X	X	X	X	X																				
L9	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X																				
L10	X	X	X	X	X	X	X	X	X	X											X	X	X	X	X	X	X	X	X	X										
L11																					X	X	X	X	X	X	X	X	X	X										
L12											X	X	X	X	X																X	X	X	X	X					
L13																					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
L14																																								
L15	X	X	X	X	X	X	X	X	X	X											X	X	X	X	X	X	X	X	X	X										
L16																															X	X	X	X	X	X	X	X	X	X
L17																															X	X	X	X	X	X	X	X	X	X
L18											X	X	X	X	X																X	X	X	X	X					
Total	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	5	5	5	5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	5	5	5	5

Description of the data received

	qPCR (Pecto)			RT-qPCR (PVY)		
	False positive	False negative	Signal in NTC	False positive	False negative	Signal in NTC
Lab1						
Lab2						
Lab3						
Lab4						
Lab5						
Lab6						
Lab7						
Lab8						
Lab9						
Lab10						
Lab11						
Lab12						
Lab13						
Lab14						
Lab15						
Lab16						
Lab17						
Lab18						

	DNA Set D1		1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
			Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
Vitis leaves	A	IC1												
	B	IC2												
	C	IC3												
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	Pecto.												

	RNA Set R1		1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
			PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
Tomato seeds	A	IC1												
	B	IC2												
	C	IC3												
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	PVY												

All the data except those from Lab 14 were used

Statistical model for the target Cq values

	DNA Set D1		1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
			Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
Vitis leaves	A	IC1												
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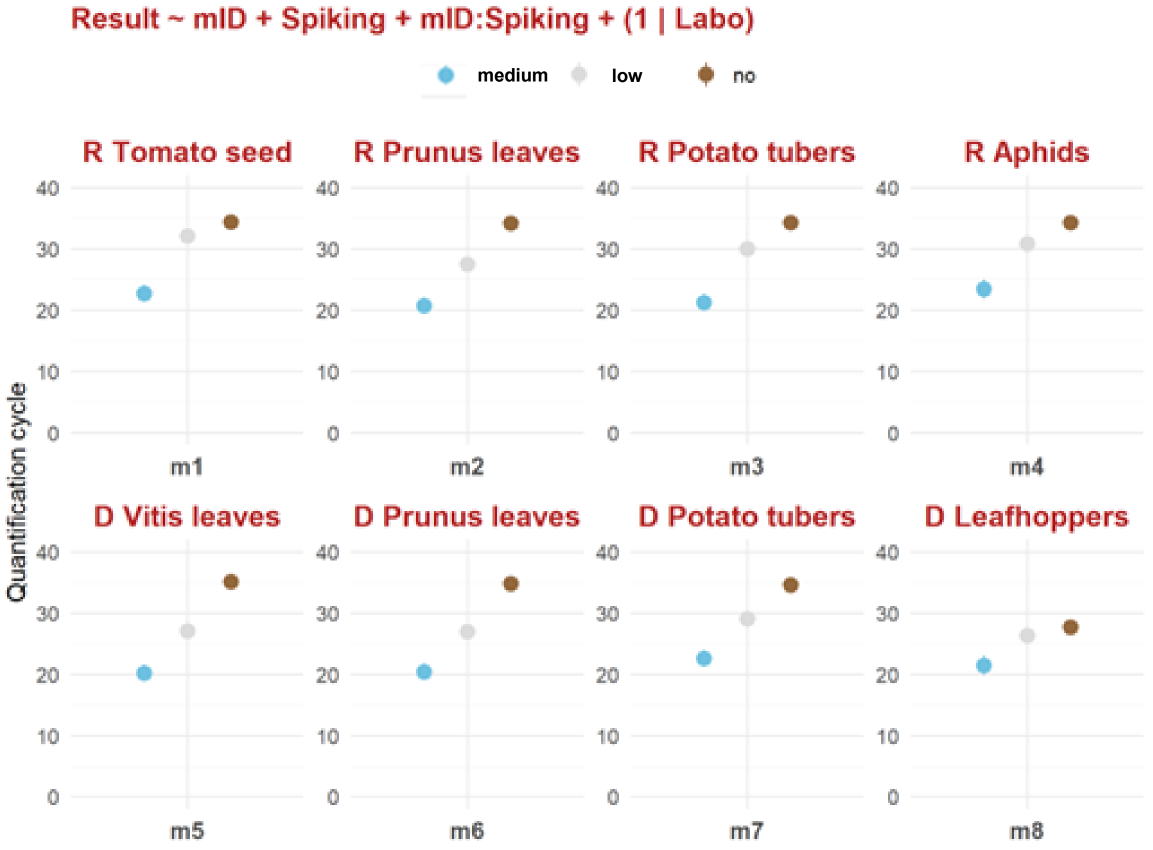
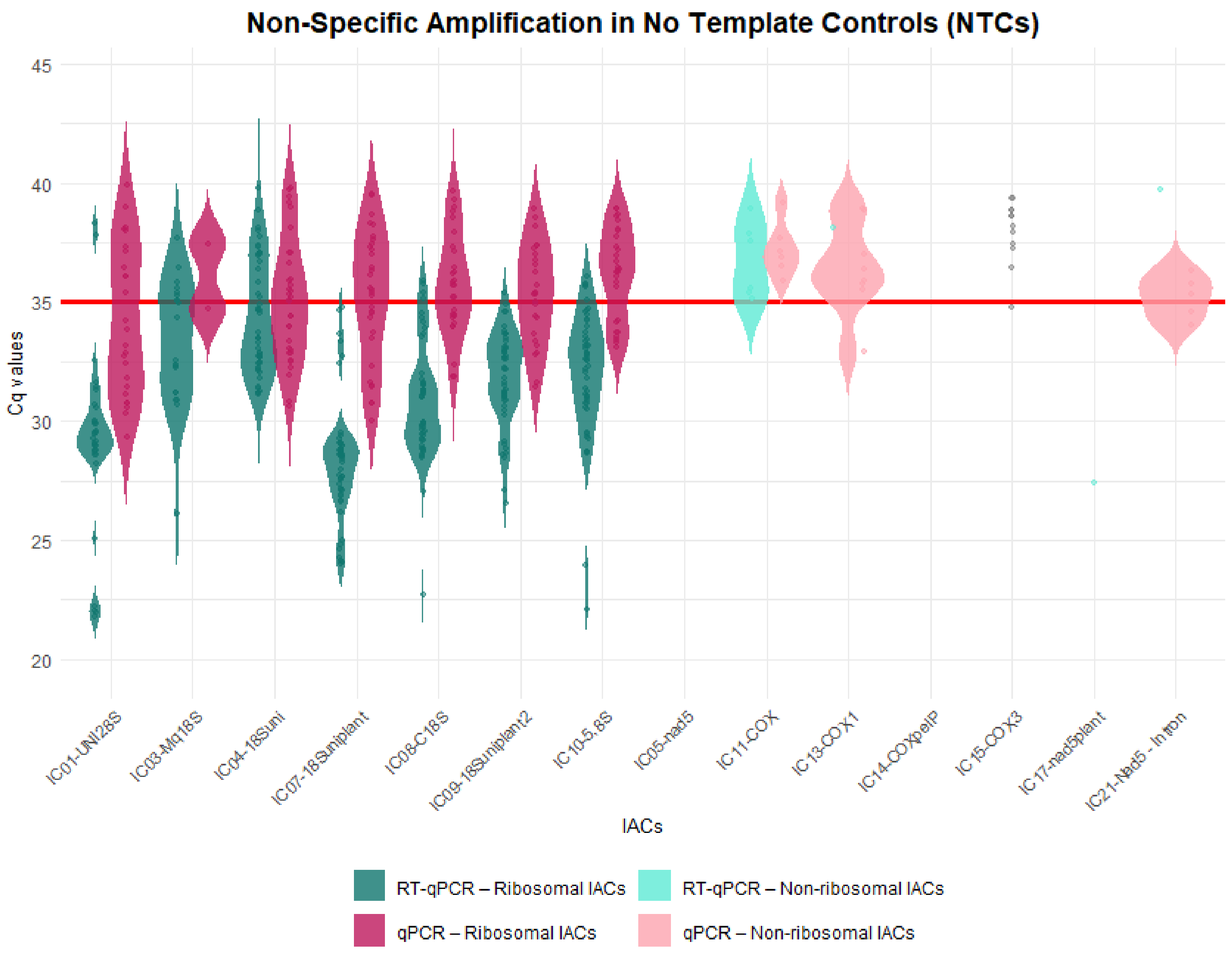
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	Pecto.												



- Marginal matrix effect
  - Interlaboratory variability of around 30% is observed for the detection of target microorganisms

How IACs behave on NTCs



Vitis leaves		DNA Set D1	1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
			Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
	A	IC1												
	B	IC2												
	C	IC3												
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	Pecto.												

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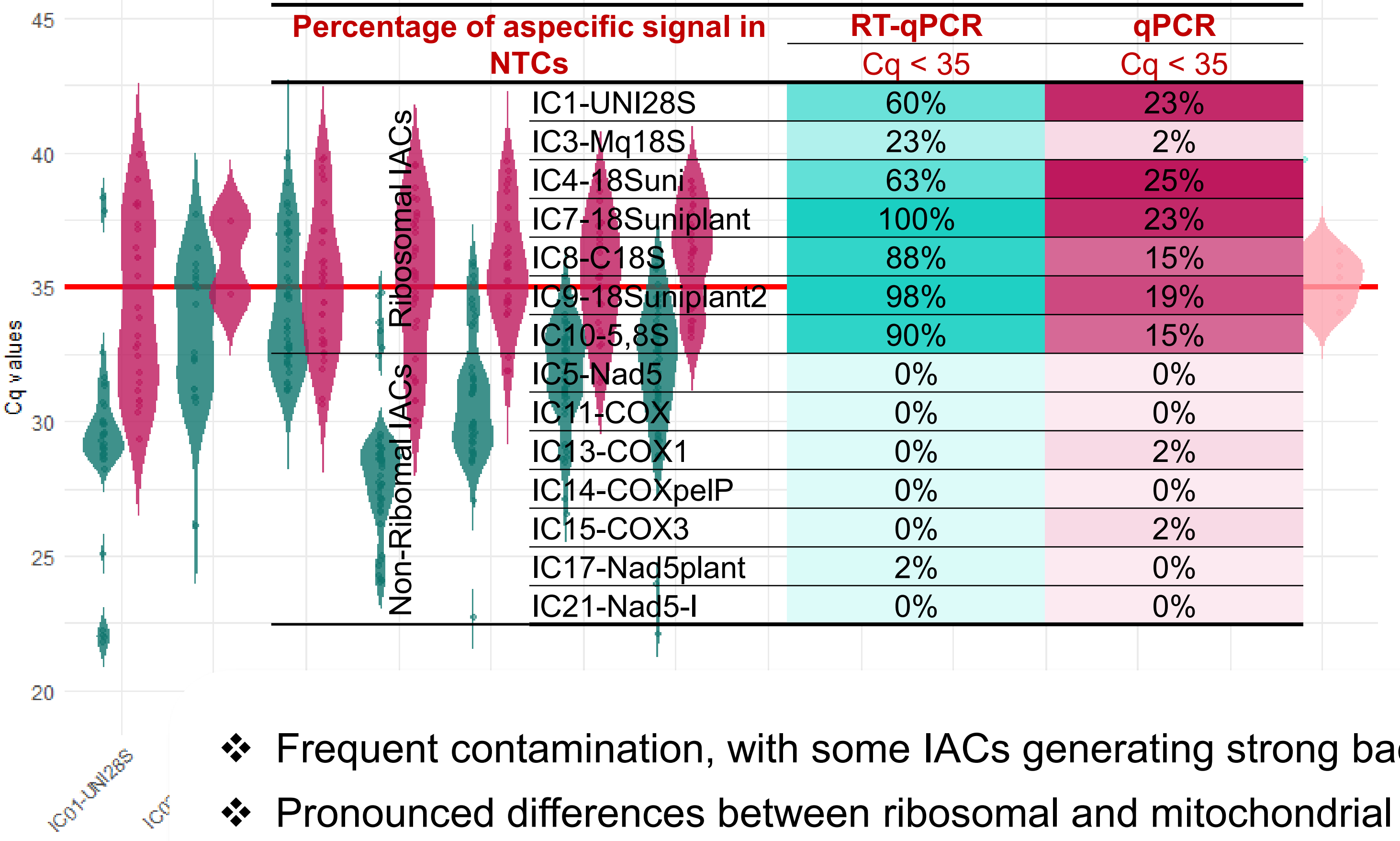
How IACs behave on NTCs



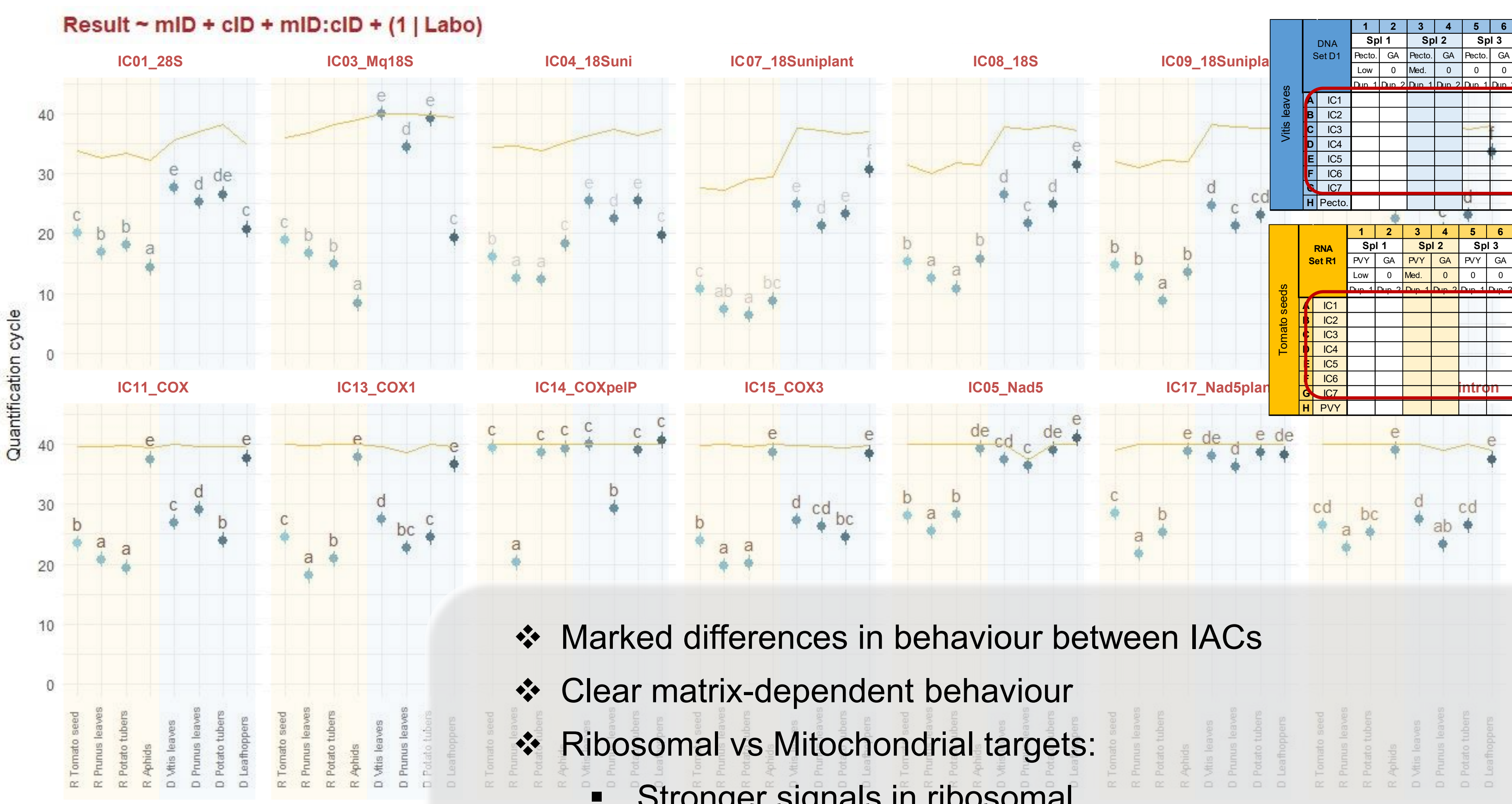
Non-Specific Amplification in No Template Controls (NTCs)

Percentage of aspecific signal in NTCs		RT-qPCR Cq < 35	qPCR Cq < 35
Ribosomal IACs	IC1-UNI28S	60%	23%
	IC3-Mq18S	23%	2%
	IC4-18Suni	63%	25%
	IC7-18Suniplant	100%	23%
	IC8-C18S	88%	15%
	IC9-18Suniplant2	98%	19%
	IC10-5,8S	90%	15%
Non-Ribomal IACs	IC5-Nad5	0%	0%
	IC11-COX	0%	0%
	IC13-COX1	0%	2%
	IC14-COXpelP	0%	0%
	IC15-COX3	0%	2%
	IC17-Nad5plant	2%	0%
	IC21-Nad5-I	0%	0%

Vitis leaves	DNA Set D1		1	2	3	4	5	6	7	8	9	10	11	12
			Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
			Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA
			Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
			Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
	A	IC1												
	B	IC2												
	C	IC3												
	D	IC4												
	E	IC5												
	F	IC6												
	G	IC7												
	H	Pecto.												

- ❖ Frequent contamination, with some IACs generating strong background signals
- ❖ Pronounced differences between ribosomal and mitochondrial targets
- ❖ Marked contrasts between qPCR and RT-qPCR
  - ► Ribosomal targets may carry a higher inherent risk of overestimating RNA quality

Statistical model for the IACs Cq



Vitis leaves	DNA Set D1	1	2	3	4	5	6	7	8	9	10	11	12
		Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
		Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA	Pecto.	GA
		Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
		Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
A	IC1												
B	IC2												
C	IC3												
D	IC4												
E	IC5												
F	IC6												
G	IC7												
H	Pecto.												

Tomato seeds	RNA Set R1	1	2	3	4	5	6	7	8	9	10	11	12
		Spl 1		Spl 2		Spl 3		Spl 4		Spl 5		NTC	
		PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA	PVY	GA
		Low	0	Med.	0	0	0	Med.	250 uM	0	250 uM	0	0
		Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2	Dup. 1	Dup. 2
A	IC1												
B	IC2												
C	IC3												
D	IC4												
E	IC5												
F	IC6												
G	IC7												
H	PVY												

- ❖ Marked differences in behaviour between IACs
- ❖ Clear matrix-dependent behaviour
- ❖ Ribosomal vs Mitochondrial targets:
  - ▪ Stronger signals in ribosomal
  - ▪ More robust profiles of Mitochondrial targets
- ► Minor interlaboratory variability of around 4% for IACs

Key Findings

1. Nucleic acid quality control is critical

- Monitoring is achievable using appropriate internal controls (IACs)

2. No single extraction method performs reliably across all matrices

- Nucleic acid yield and residual RNA/DNA contamination vary strongly by protocol

3. Interlaboratory consistency

- high for IAC performances (more variable for target detection)

4. IAC performance

- Plant matrices: IACs of Mitochondrial origin perform well
  - COX ==> DNA & RNA
  - Nad5 ==> RNA
- Insect matrices: IC01 (28S) and IC03 (Mq18S) perform best

5. Ribosomal IACs pose risks

- Frequent non-specific amplification and contamination, especially in RT-qPCR, including amplification in NTCs

6. Nucleic acid nature matters

- IAC behaviour differs markedly between qPCR and RT-qPCR (expression related ?)
- Residual DNA can bias RT-qPCR result interpretation

Path Toward a Horizontal Standard

- **Harmonization gaps persist**
  - lack of shared guidelines, interpretation frameworks, threshold ranges...
- **Path forward**
  - Develop horizontal guidelines (with all project partners and panel members)
  - Expand IAC gene repertoire for diverse matrices
  - Establish shared interpretation criteria and performance thresholds, building on IVENAD outcomes

Thank you for your attention

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