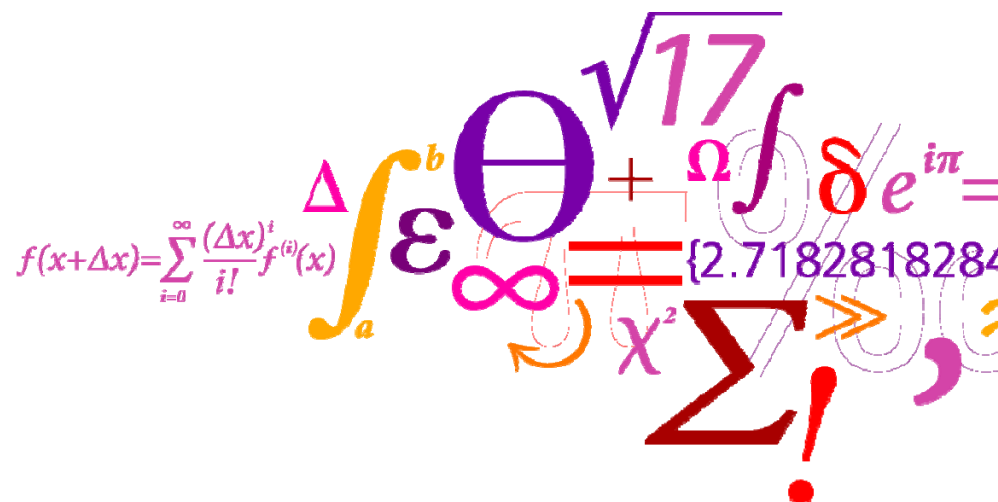


# EURL-AR training course on genotypic characterization of antimicrobial-resistant bacteria

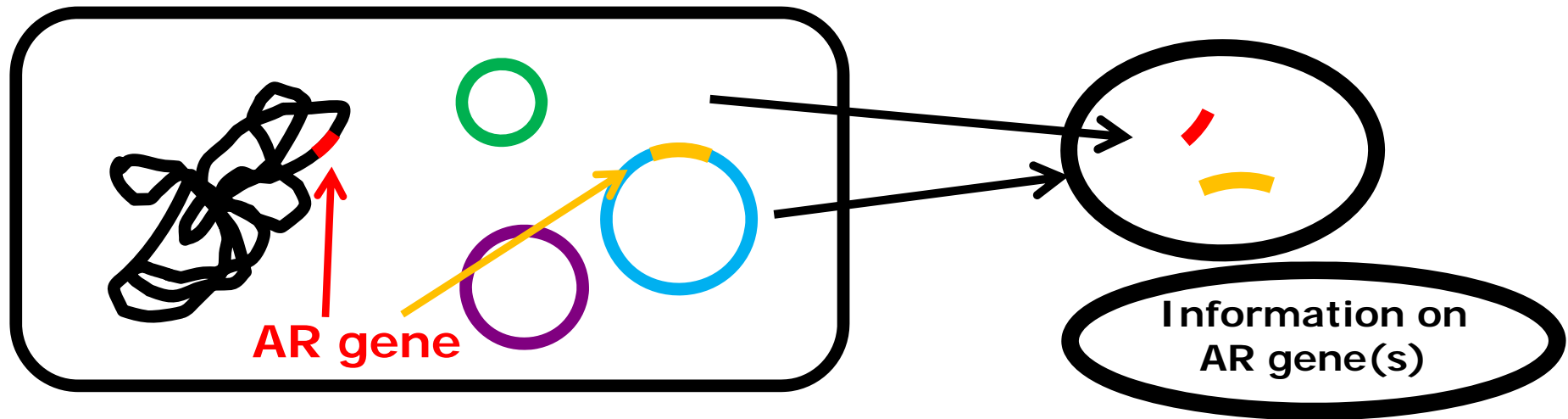
9 November 2011  
Kgs. Lyngby, Denmark

DTU Food  
National Food Institute

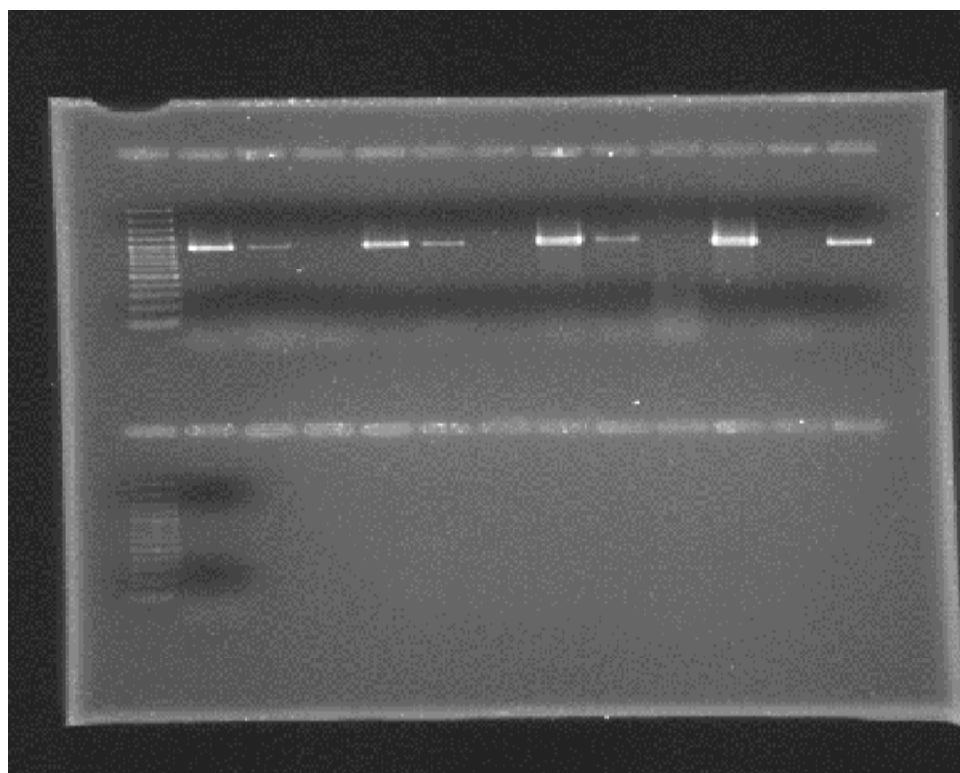
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# Yesterday (theme 1) ...



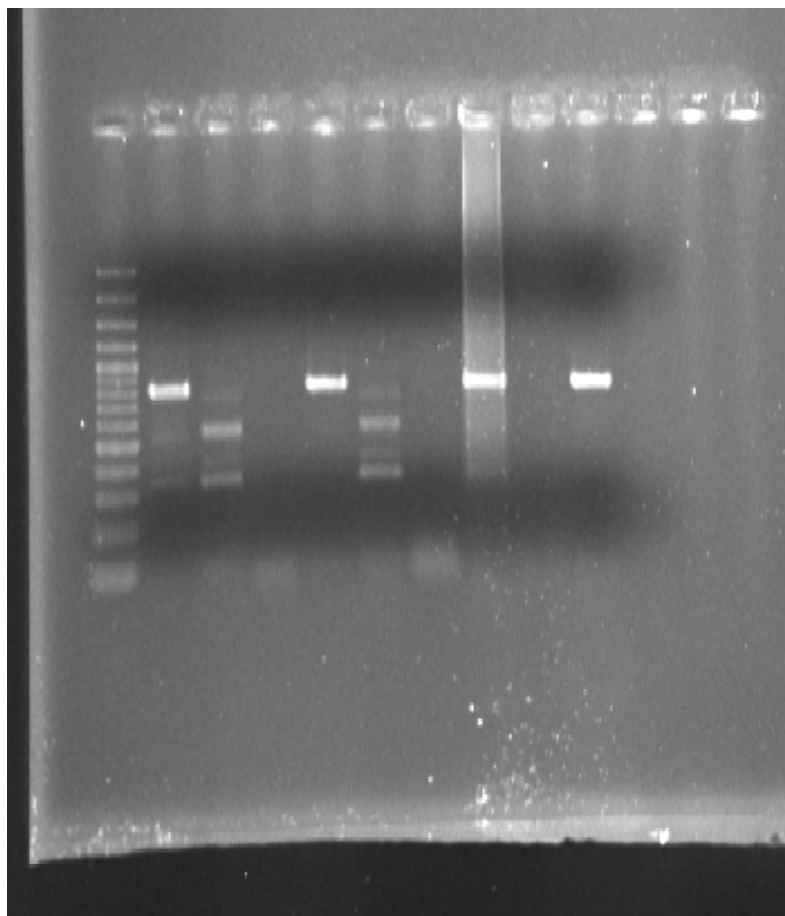
# *bla*<sub>TEM</sub> (963 bp)



M:	
1	1 B
2	Pos control B
3	Negative B
4	1 E
5	Pos control E
6	Negative E
7	1 G
8	Pos control G
9	Negative G
10	9 J
11	Negative J
12	1 L

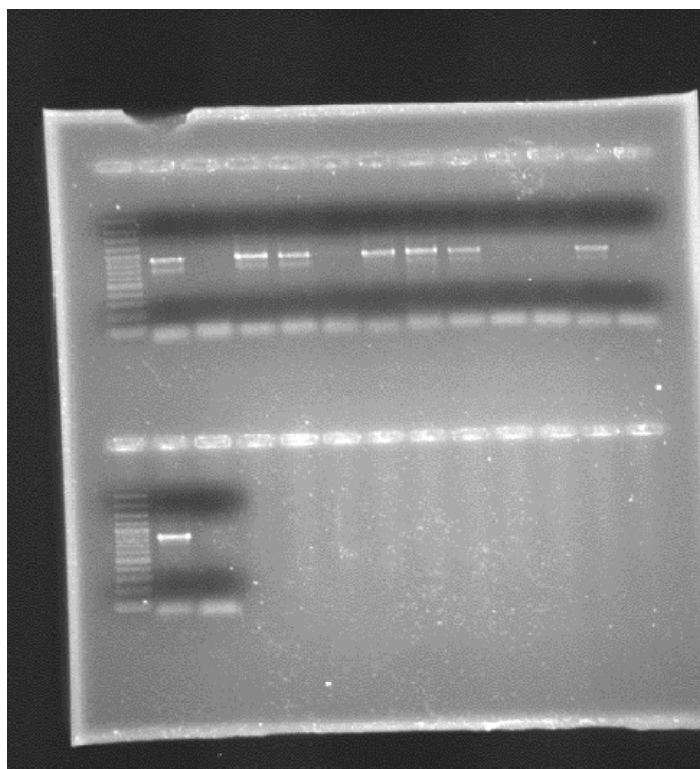
M:	
1	Negative L
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

# *bla*<sub>CTX-M-1</sub> group (754 bp)



M:	
1	2 A
2	Pos control A
3	Negative A
4	6 C
5	Pos control C
6	Negative C
7	2 G
8	Negative G
9	6 I
10	Negative I
11	
12	

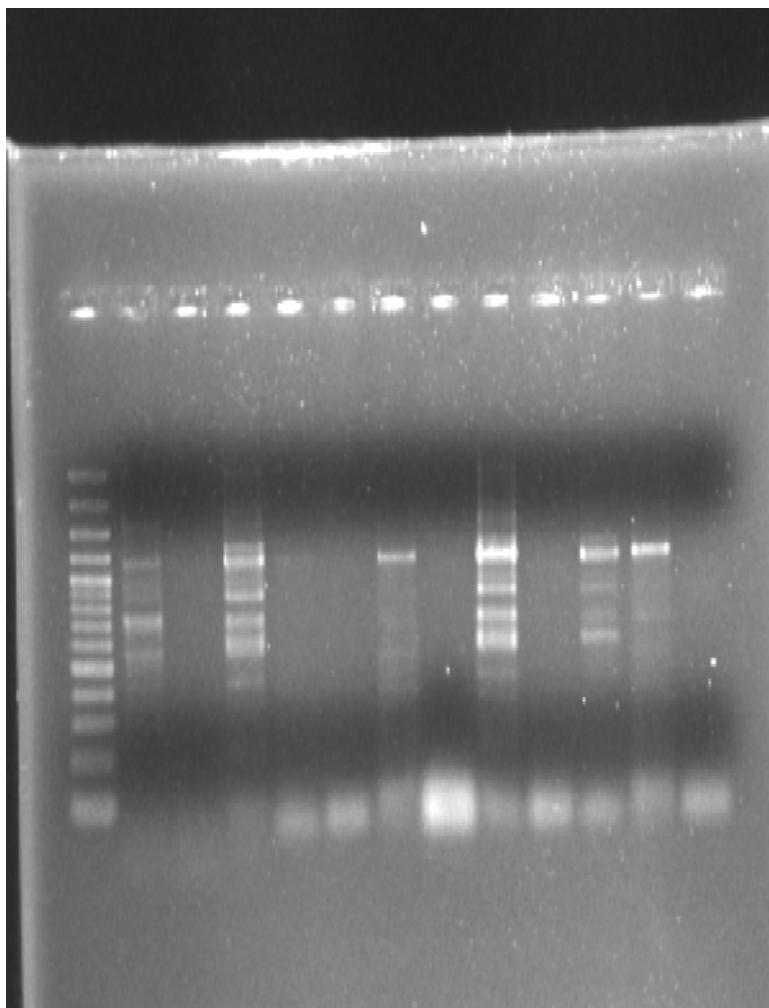
# *bla*<sub>CTX-M-9</sub> group (857 bp)



M:	
1	3 B
2	Negative B
3	3 D
4	Pos control D
5	Negative D
6	9 E
7	Negative E
8	3 H
9	Negative H
10	Negative H
11	9 K
12	Negative K

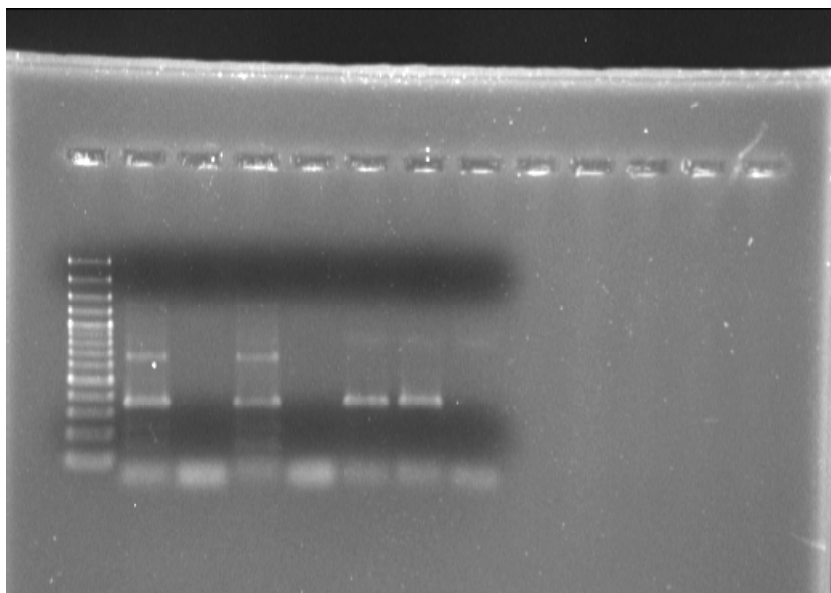
M:	
1	9 L
2	Negative L
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

# *bla*<sub>CMY-2</sub> (1138 bp)



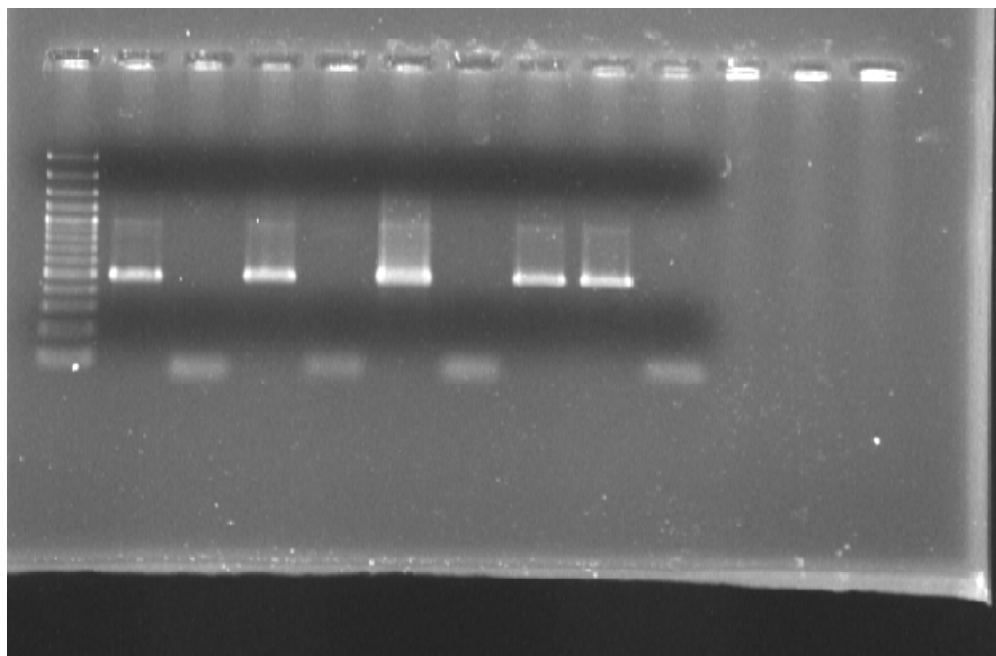
M:	
1	4 C
2	Negative C
3	4 F
4	Pos control F
5	Negative F
6	4 H
7	Negative H
8	4 J
9	Negative J
10	4 K
11	Pos control K
12	Negative K

# *ampC* (chrom.) (343 bp)



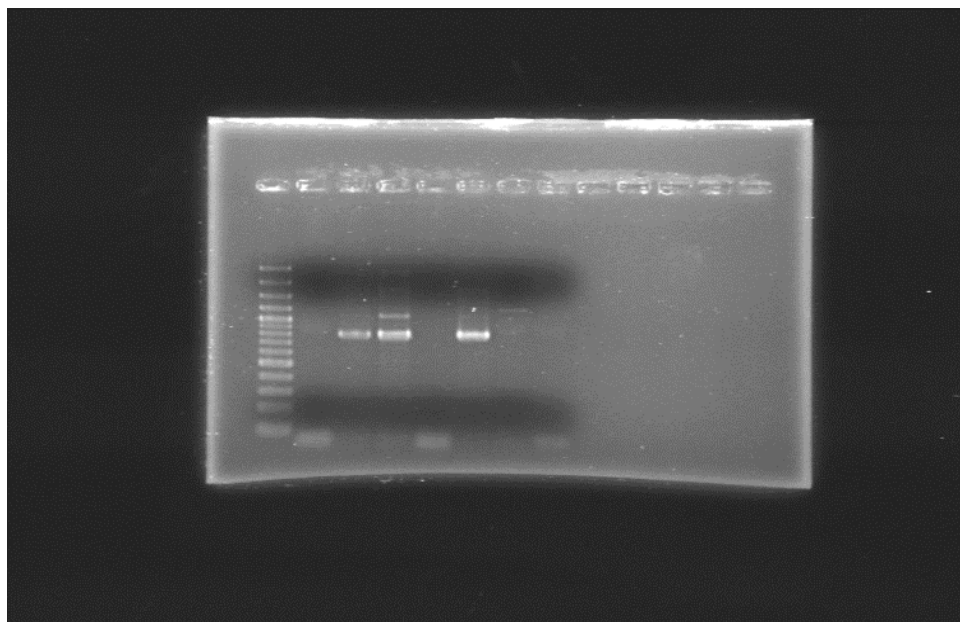
M:	
1	5 A
2	Negative
3	5 D
4	Negative
5	5 I
6	Pos control I
7	Negative I
8	
9	
10	
11	
12	

# *aac(6')-Ib* (482 bp)



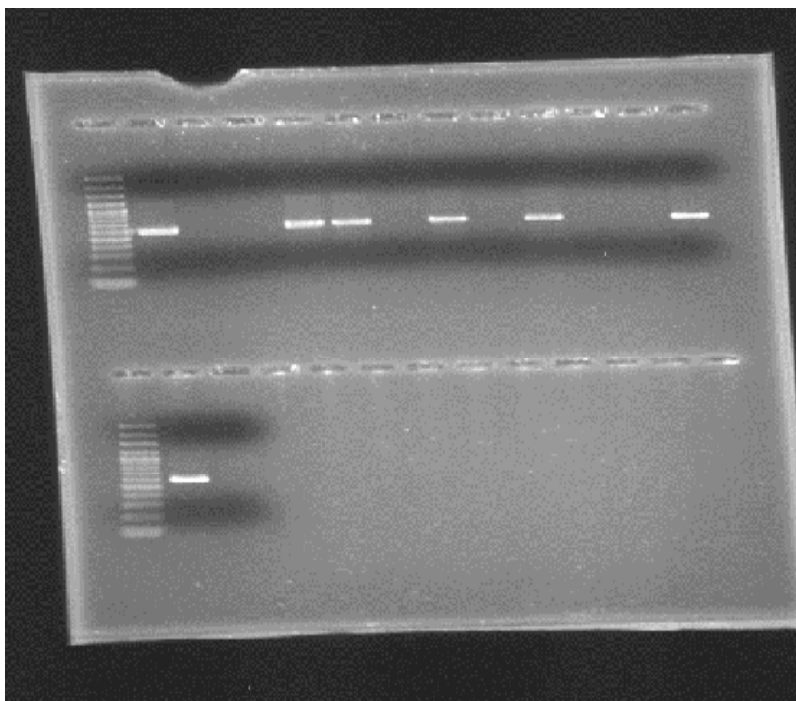
M:	
1	7 B
2	Negative B
3	7 F
4	Negative F
5	7 J
6	Negative J
7	7 K
8	Pos control K
9	Negative K
10	
11	
12	

# *qnrS* (802 bp)



M:	
1	9 C
2	Negative C
3	9 G
4	Negative G
5	9 I
6	Negative I
7	Negative I
8	
9	
10	
11	
12	

# *qnrD* (447 bp)

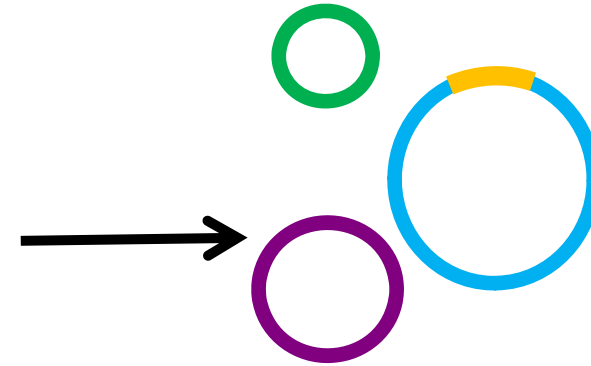
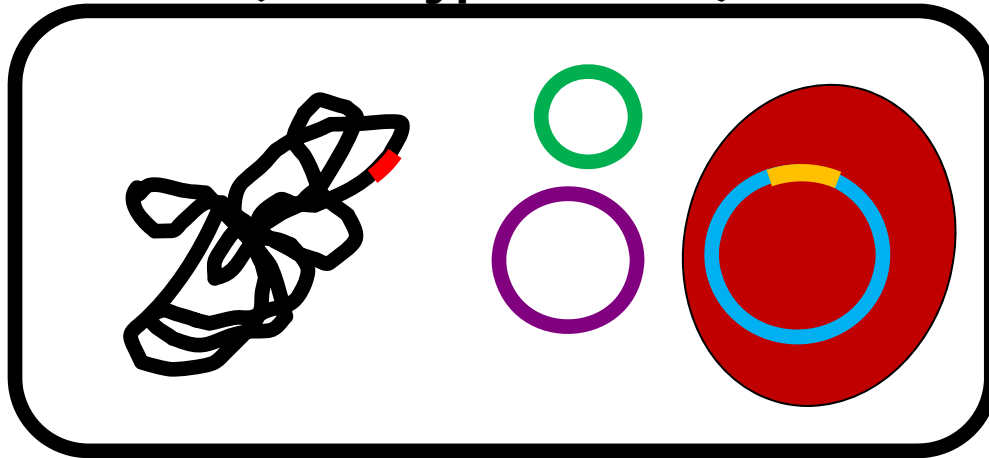


M:	
1	10 A
2	Negative A
3	10 D
4	Negative D
5	10 E
6	Negative E
7	10 F
8	Negative F
9	10 H
10	Pos control L
11	Negative H
12	10 L

M:	
1	Pos control L
2	Negative L
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

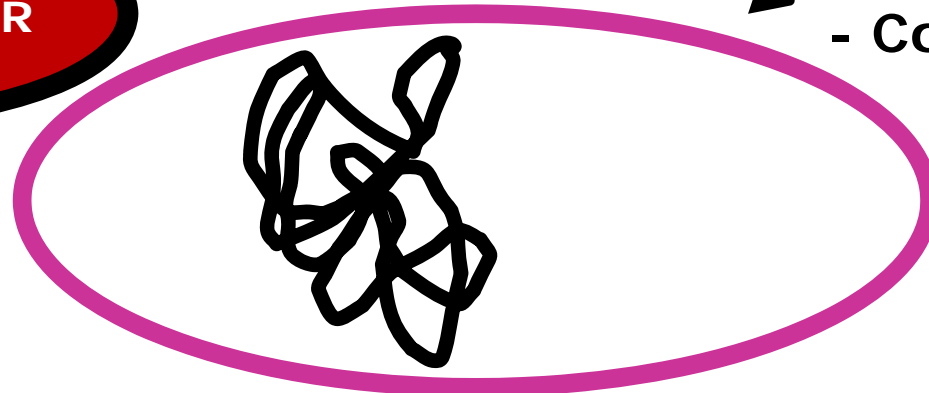
# Today (theme 1)...

DONOR (wild-type isolate)



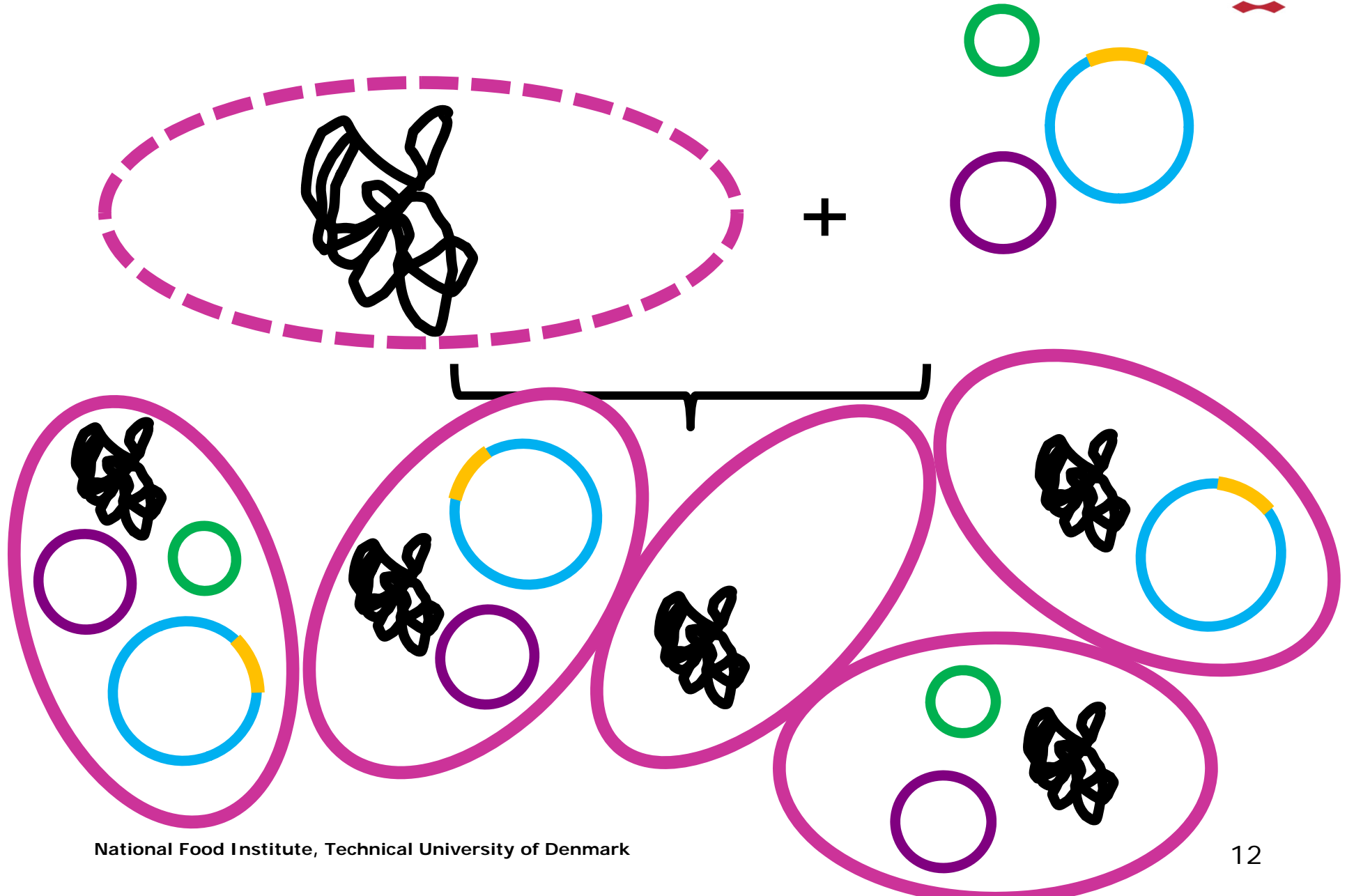
- TRANSFORMATION  
- Conjugation

Information on the vector of AR gene(s)

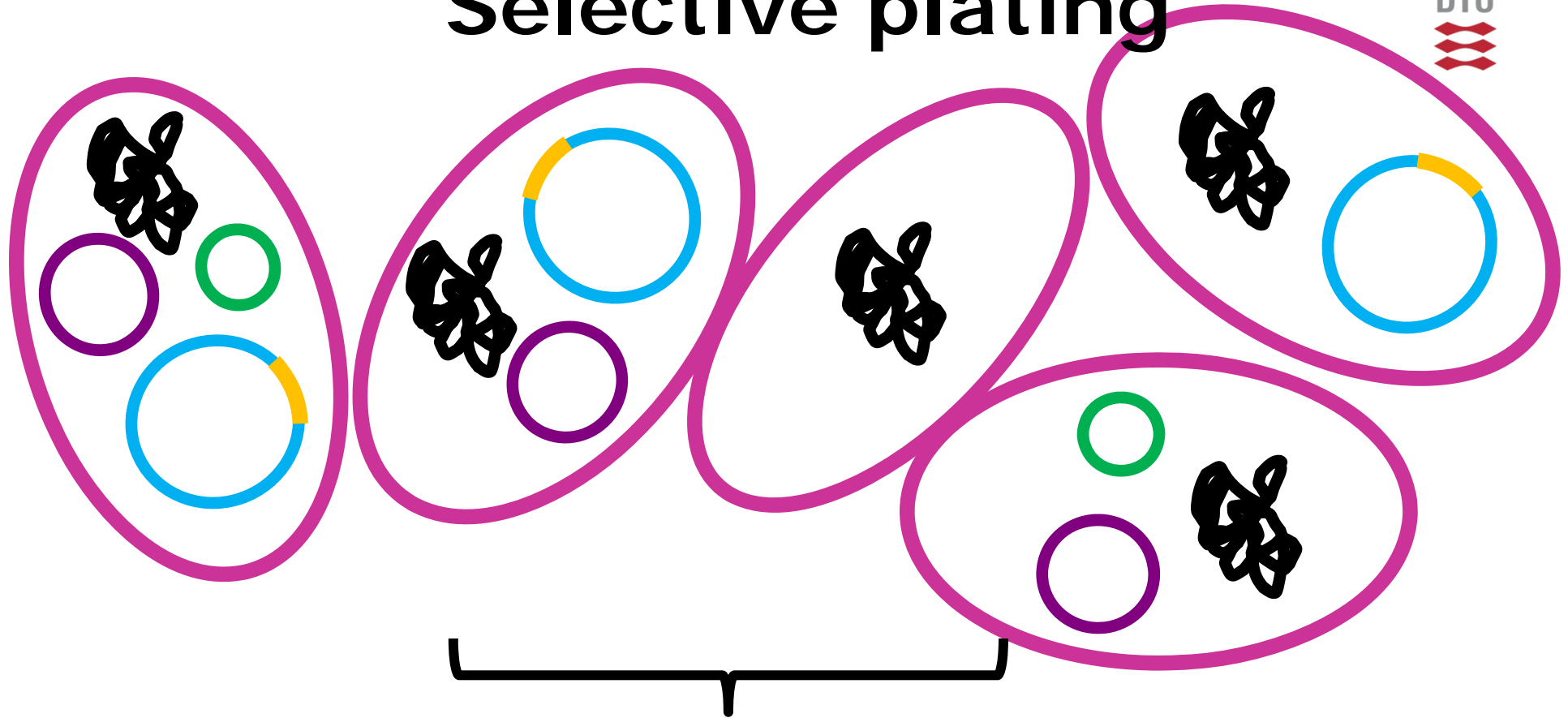


RECIPIENT (plasmid-free strain!)

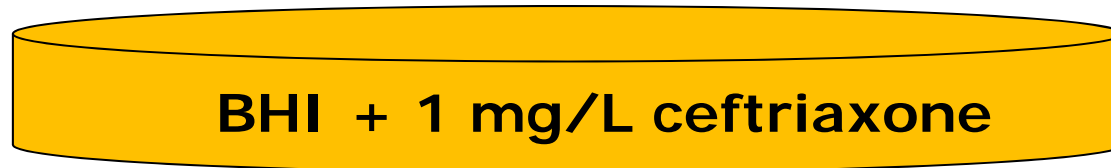
# Transformation



# Selective plating

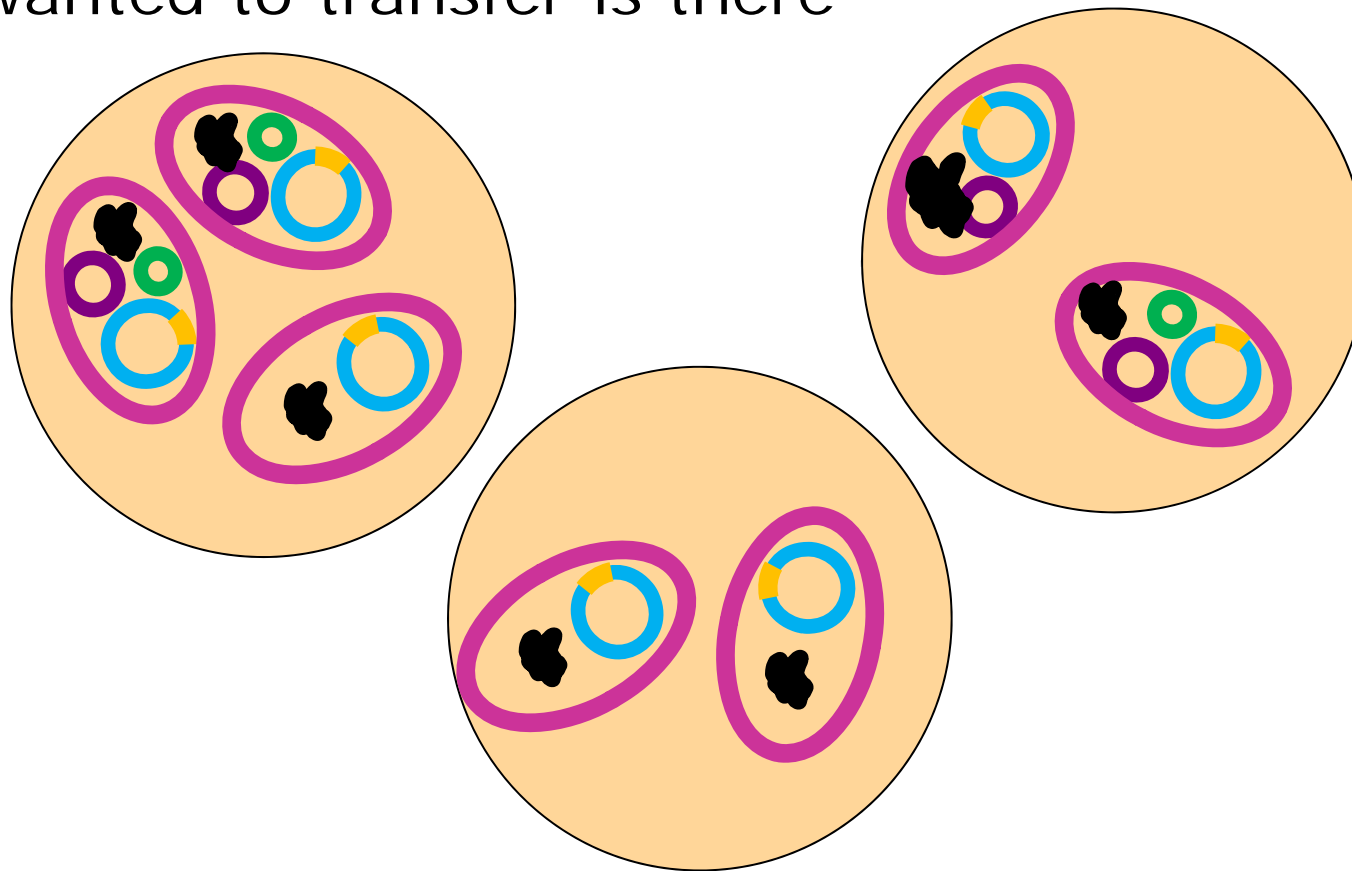


**SELECTIVE  
PLATE:**



# Shortcuts

- 1) Wait ON for growth on plate
- 2) PCR (e.g. colony PCR) to ensure the gene you wanted to transfer is there



and...



...if everything went fine, there is still something to do:

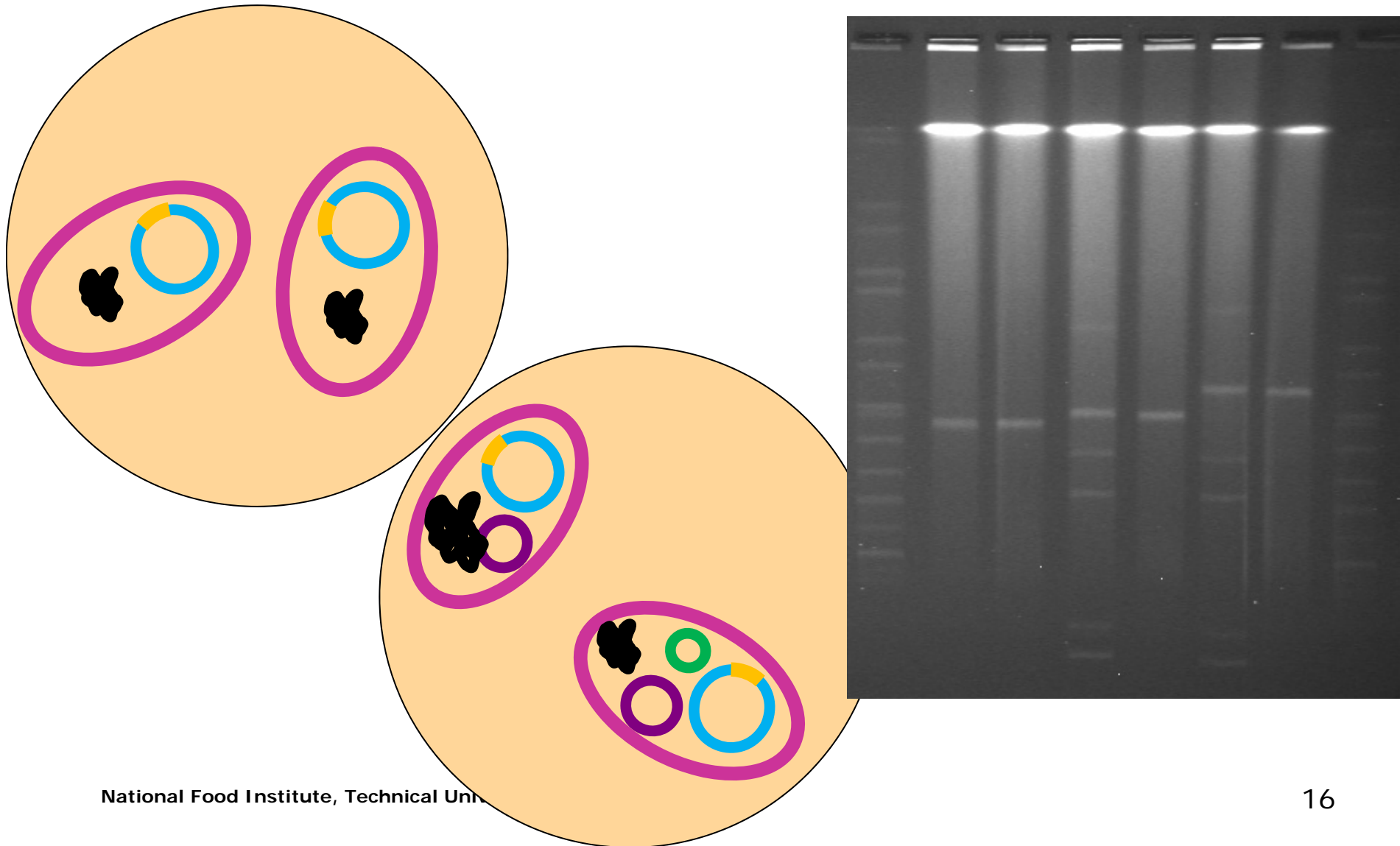
**BE SURE THAT THERE IS ONLY**

**THE RIGHT PLASMID IN YOUR**

**RECIPIENT**

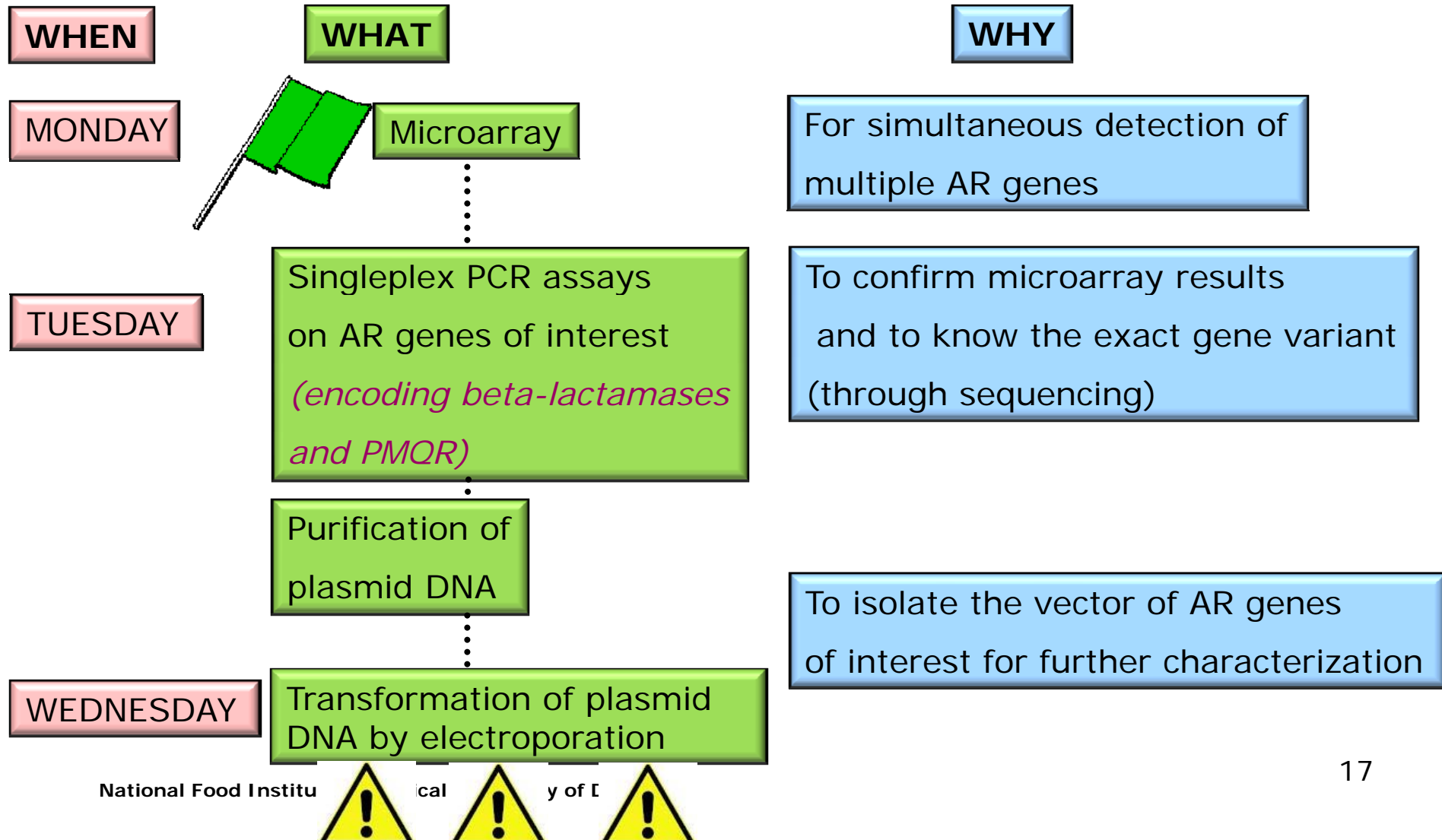
# S1 nuclease PFGE analysis

S1 nuclease: enzyme that cuts circular DNA

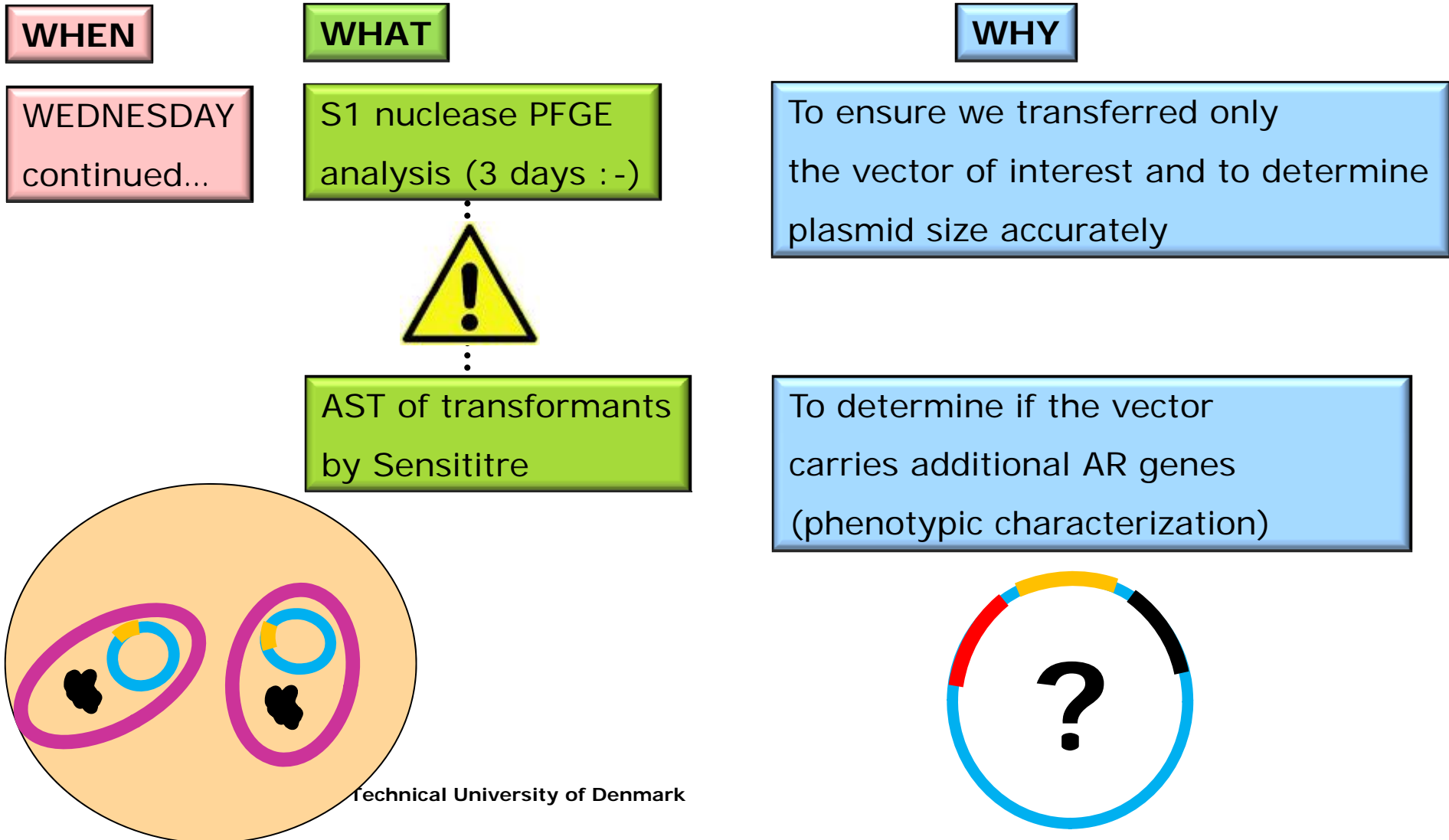


## Lab theme 1:

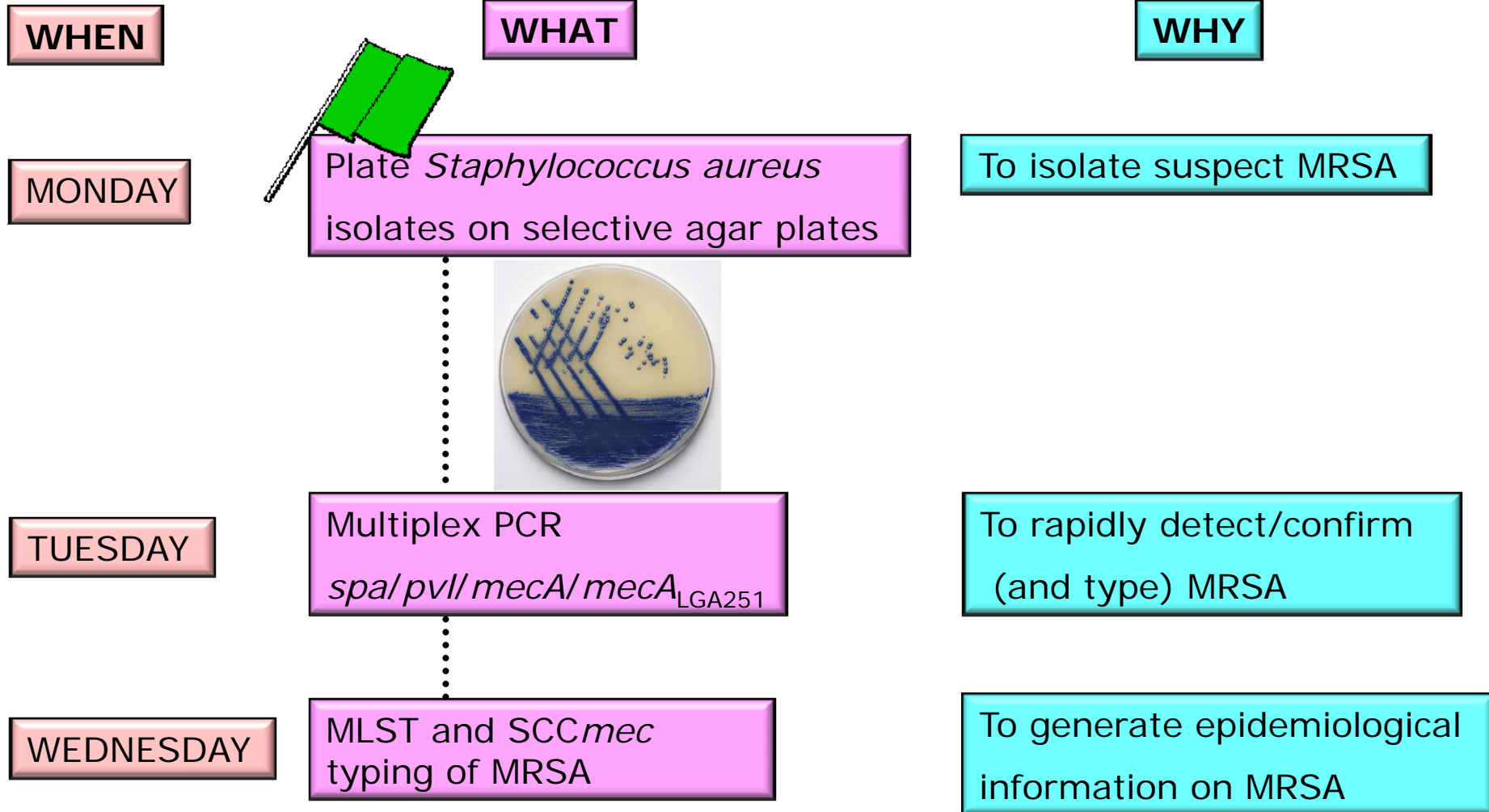
# Detection and characterization of AR determinants of public health relevance (beta-lactamase-encoding genes & PMQR)



# Lab theme 1: Detection and characterization of AR determinants of public health relevance (beta-lactamase-encoding genes & PMQR)

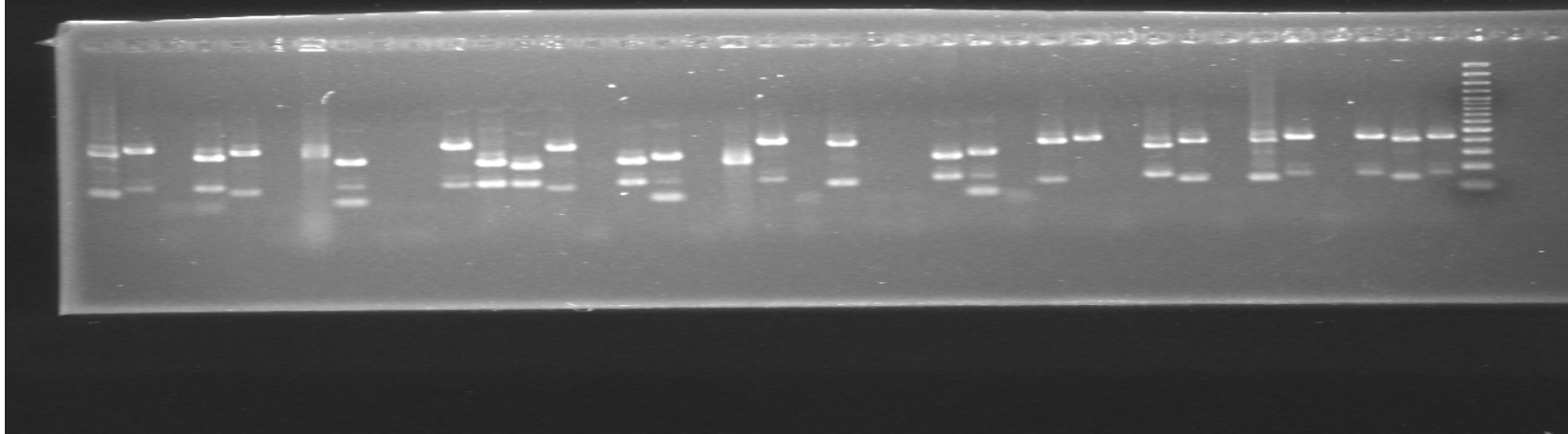


# Lab theme 2: MRSA surveillance



# Multiplex PCR

*spa*(variable:200-600bp); *mecA* (162 bp);  
*mecA*<sub>LGA251</sub> (138bp); *pvl* (~85bp)



M:	
1	19 A
2	MecA pos.
3	Negative A
4	14 B
5	Mec A <sub>LGA251</sub> Pos
6	Negative B
7	18 C
8	PVL Pos.
9	Negative C
10	16 D
11	MecA pos.
12	Negative D

M:	
1	13 E
2	Mec A <sub>LGA251</sub> Pos
3	Negative E
4	17 F
5	pos
6	Negative F
7	13 G
8	MecA pos.
9	Negative G
10	18 H
11	Mec A <sub>LGA251</sub> Pos
12	Negative H

M:	
1	17 I
2	PVL Pos.
3	Negative I
4	19 J
5	MecA pos.
6	Negative J
7	14 K
8	Mec A <sub>LGA251</sub> Pos
9	Negative K
10	19 L
11	PVL Pos.
12	Negative L

M:	
1	Mec A pos.
2	Mec A <sub>LGA251</sub> Pos
3	PVL Pos.
4	

# Lab theme 2: MRSA surveillance



WHEN

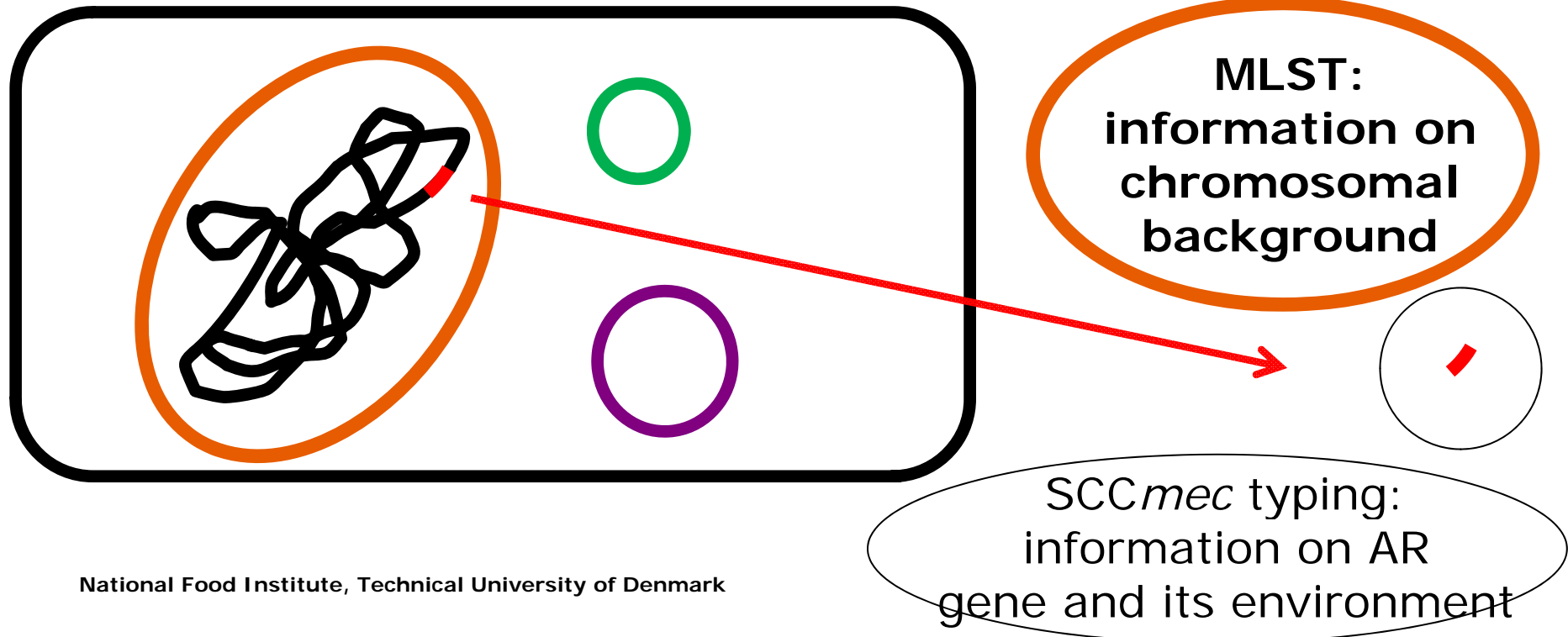
WHAT

WHY

WEDNESDAY

MLST and *SCCmec*  
typing of MRSA

To generate epidemiological  
information on MRSA



# Wednesday



TRANSFORMATION

S1 PFGE

Lunch...



SCCmec  
typing  
(PCR)

MLST  
(PCR)

AST

S1 PFGE

Jesper  
Larsen



Nation

Dinner in Copenhagen  
(bus at 18:30 from the hotel)

**Enjoy your day!**