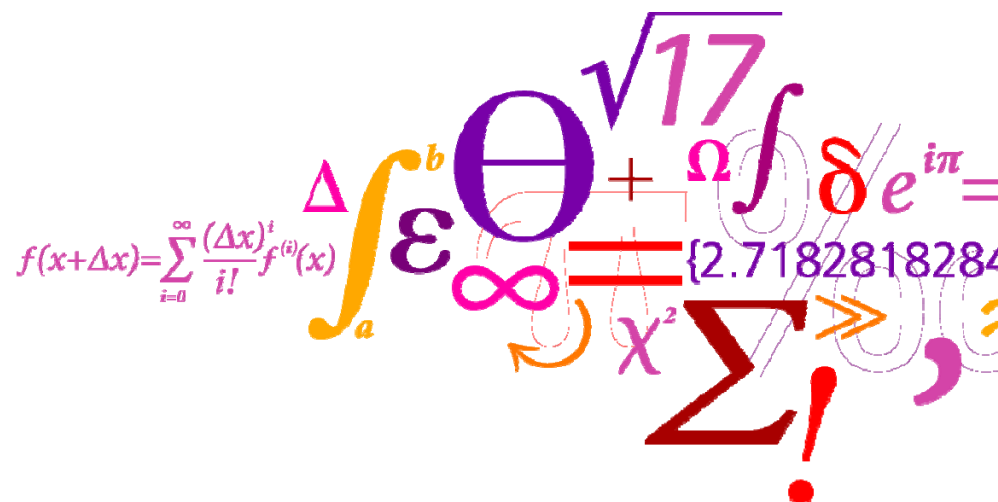


# EURL-AR training course on genotypic characterization of antimicrobial-resistant bacteria

11 November 2011  
Kgs. Lyngby, Denmark



# Lab theme 1

What is the genetic background of bacteria showing antimicrobial resistance phenotypes of public health interest within European countries?

Chosen phenotypes: resistance to beta-lactams and to fluoroquinolones (WHO: critically important antimicrobials). Focus on beta-lactamase-encoding genes including ESBL and AmpC, and on PMQR genes.

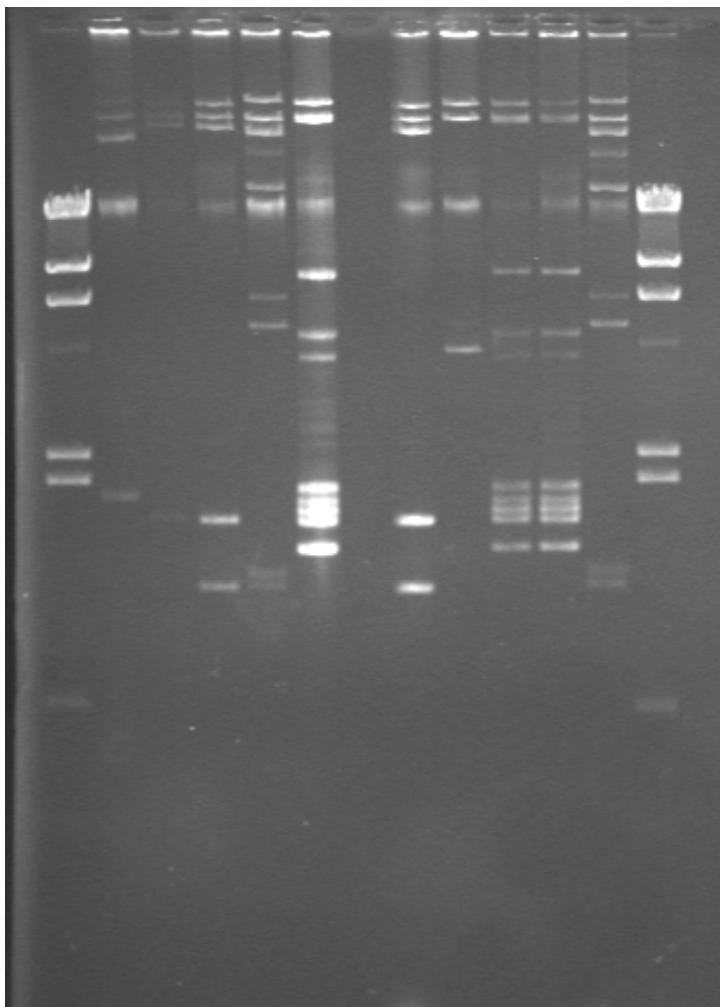
# Results

Strain code	Antimicrobial resistance phenotype	Microarray: agreement with phenotype?	Relevant gene(s): Microarray + PCR & sequencing	S1 PFGE	Transformant AR phenotype	RFLP	Inc group	pMLST
1	AMP, AUG, FOT, XNL, NAL, CIP, SPE, STR, TET, TMP, SMX	(L)	TEM-52 (B, F, G, L)		AMP, FOT, XNL, STR, SMX (E)		IncI1	
2	AMP, FOT, XNL, SPE, STR, TET, SMX	(A, B)	CTX-M-1/61 (A, G)		AMP, FOT, XNL (A)		IncN	
3	AMP, AUG, FOT, XNL, SPE, TET, SMX	(L)	CTX-M-14 (D, H) & TEM-52 (B, F, G, L)		AMP, FOT, XNL (A)			
4	AMP, AUG, FOT, XNL, SPE, TET, SMX	(L)	CTX-M-14 (D, H) & TEM-52 (B, F, G, L)		AMP, FOT, XNL (A)			
6	AMP, AUG, FOT, XNL, SPE, TET, SMX	(L)	CTX-M-14 (D, H) & TEM-52 (B, F, G, L)		AMP, FOT, XNL (A)			
7	AMP, AUG, FOT, XNL, SPE, TET, SMX	(L)	CTX-M-14 (D, H) & TEM-52 (B, F, G, L)		AMP, FOT, XNL (A)			
9	AMP, AUG, FOT, XNL, SPE, TET, SMX, TET	(L)	CTX-M-14 (D, H) & TEM-52 (B, F, G, L)		AMP, FOT, XNL (A)			
10	CIP, SMX	(L)	qnrD (A, E, F, H, L)					

**To avoid treatment failures!**

**To identify control measures to limit the spread of AR genes!**

# Plasmid DNA from donor isolates



M	$\lambda$ -HindIII
1	A-2
2	B-3
3	D-3
4	E-1
5	F-4
6	G-2
7	H-3
8	I-6
9	J-4
10	K-4
11	L-1
M	$\lambda$ -HindIII

## Lab theme 2

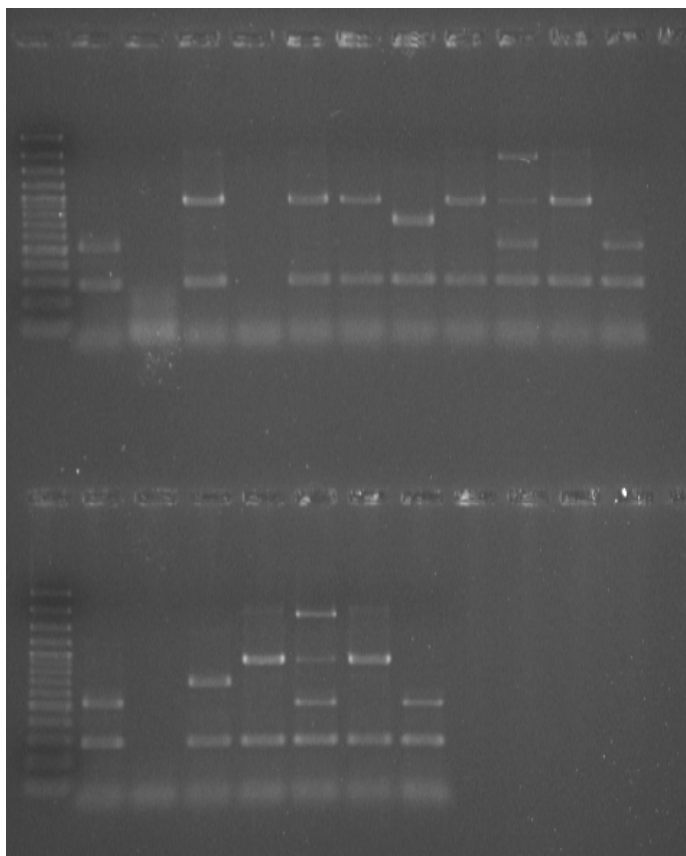
MRSA surveillance: is MRSA present in your country? If so, could you provide epidemiological information?

- Detection (e.g. selective plating + multiplex PCR)
- Epidemiological information (MLST, SCCmec typing)

# Results

Strain code	OXOID MRSA2	multiplex PCR	SCCmec type?	MLST type
13	blue	<i>mecA</i>	(E, F; G,H)	
14	blue	<i>mecA</i>	(K, B)	
16	blue	<i>mecA</i>		
17	blue	<i>mecA</i>		
18	blue	<i>mecA</i>	(C, D; I, J)	
19	blue	<i>mecA</i> <sub>Iga251</sub>		
20	pink			
21	pink			

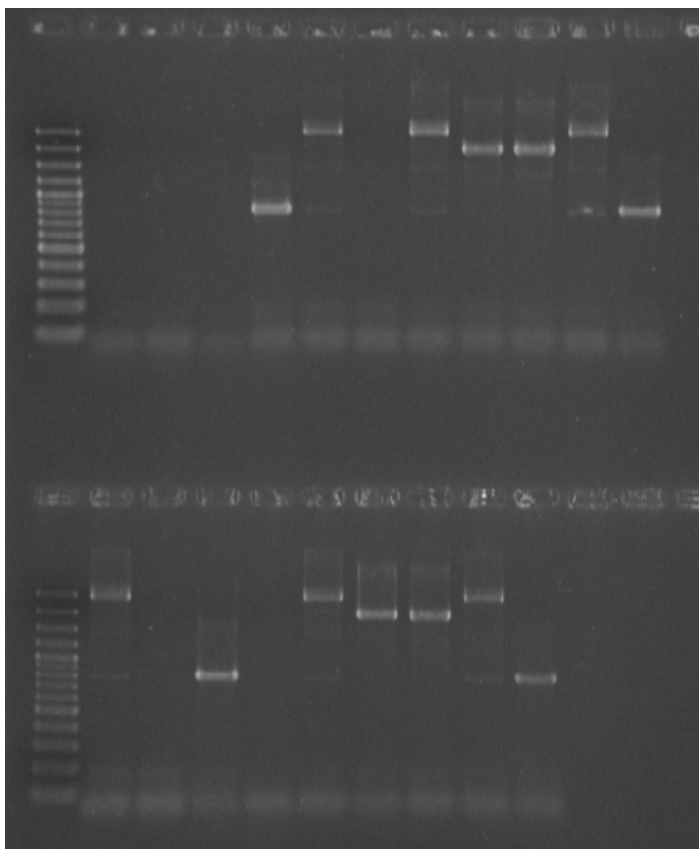
# SSCmec elements M-PCR1



:	100 bp Plus
1	Group C 18
2	Group C NTC
3	Group E 13
4	Group E NTC
5	Group G 13
6	Group K 14
7	Control SSCmec I <b>ccr1</b>
8	Control SSCmec II <b>ccr2</b>
9	Control SSCmec III <b>ccr3</b>
10	Control SSCmec IV <b>ccr2</b>
11	Control SSCmec V <b>ccr5</b>
12	

M:	100 bp Plus
1	Group I 18
2	Group I NTC
3	Control SSCmec I <b>ccr1</b>
4	Control SSCmec II <b>ccr2</b>
5	Control SSCmec III <b>ccr3</b>
6	Control SSCmec IV <b>ccr2</b>
7	Control SSCmec V <b>ccr5</b>
8	
9	
10	
11	
12	

# SCCmec elements M-PCR2

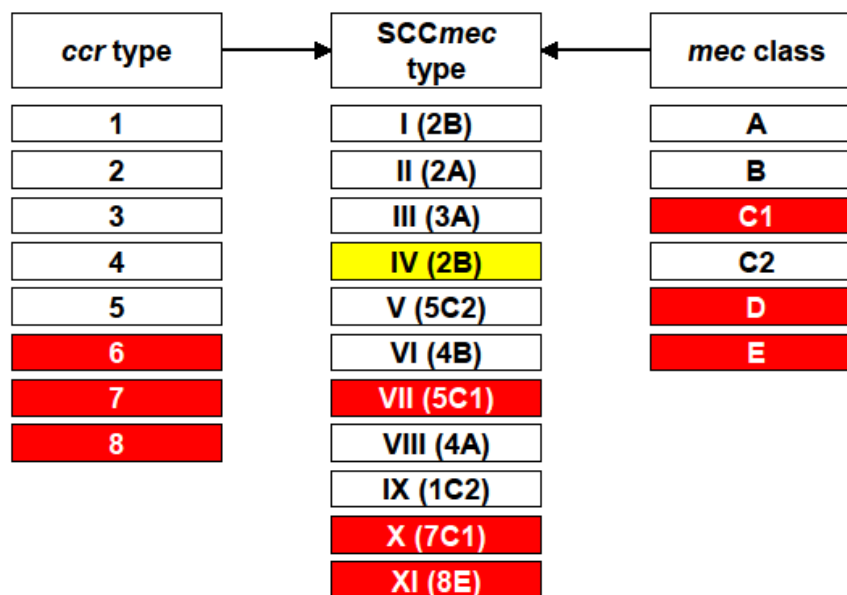


M	100 bp Plus
1	Group B 14
2	Group B NTC
3	Group D 18
4	Group D NTC
5	Group F 13
6	Group F NTC
7	Control SSCmec I <b>B</b>
8	Control SSCmec II <b>A</b>
9	Control SSCmec III <b>A</b>
10	Control SSCmec IV <b>B</b>
11	Control SSCmec V <b>C2</b>
12	

M:	100 bp Plus
1	Group H 13
2	Group H NTC
3	Group J 18
4	Group J NTC
5	Control SSCmec I <b>B</b>
6	Control SSCmec II <b>A</b>
7	Control SSCmec III <b>A</b>
8	Control SSCmec IV <b>B</b>
9	Control SSCmec V <b>C2</b>
10	
11	
12	



# Typing of SCCmec elements Kondo et al. (2007)

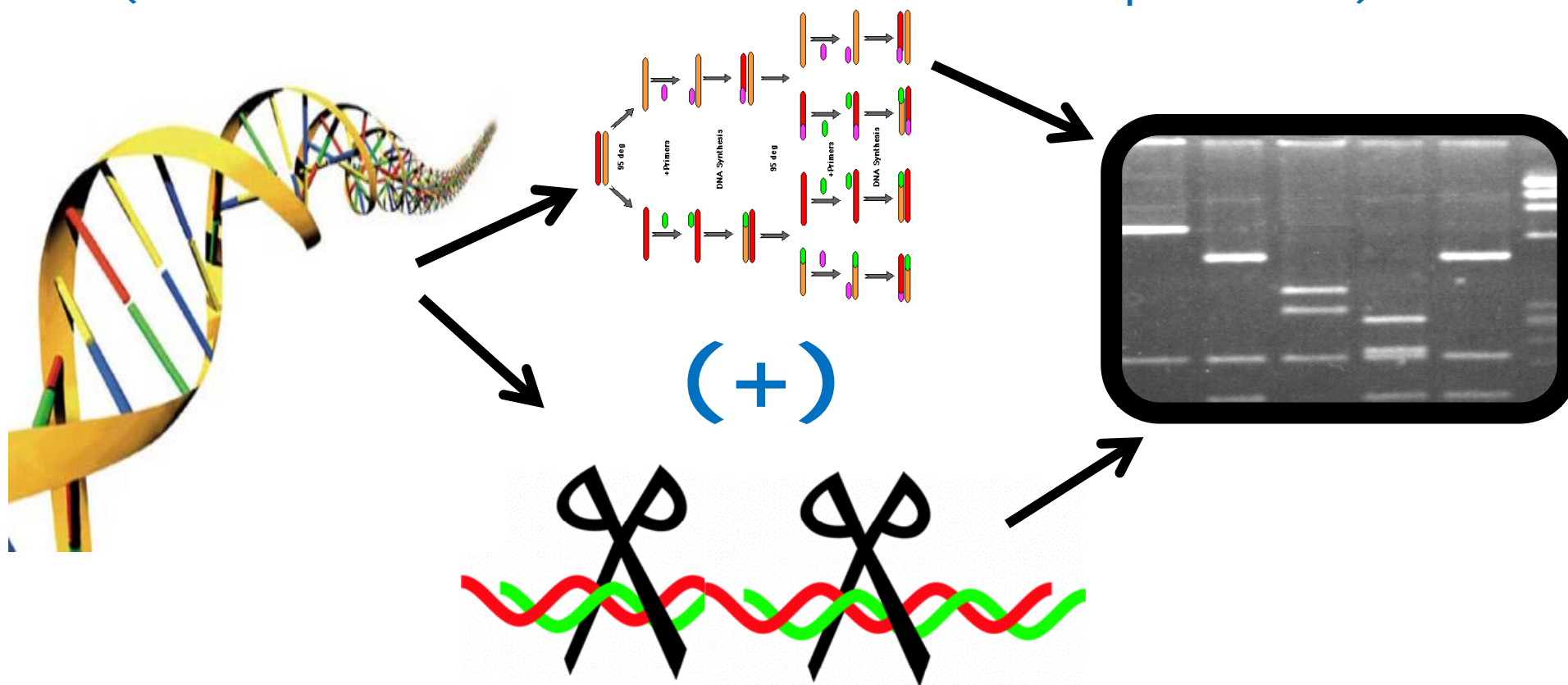


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# Genotyping methods: three main categories (1/3)

## DNA banding pattern

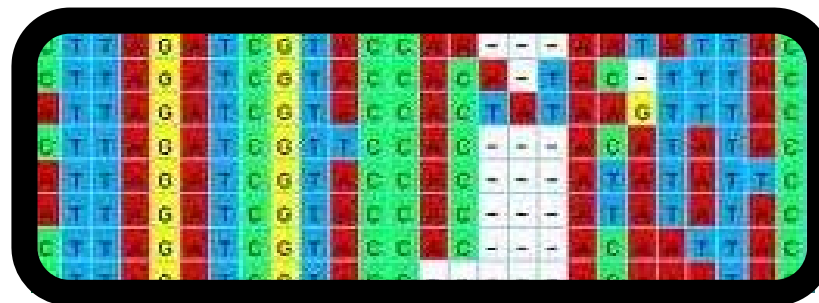
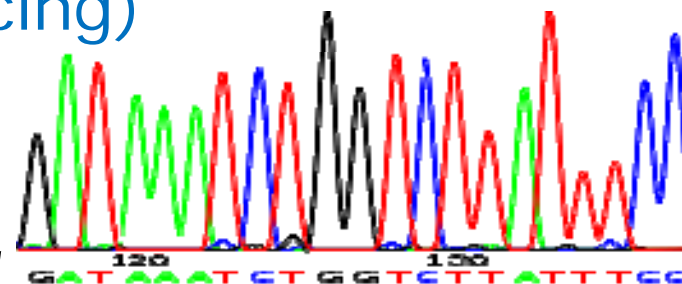
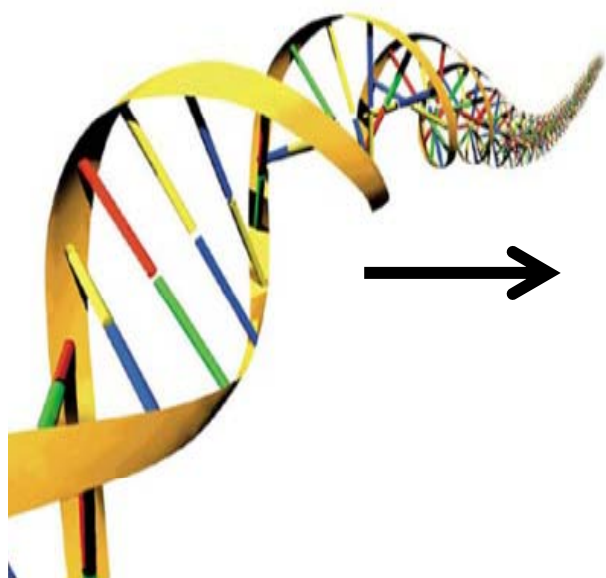
(in this course: S1 PFGE and RFLP on plasmids)



# Genotyping methods: three main categories (2/3)

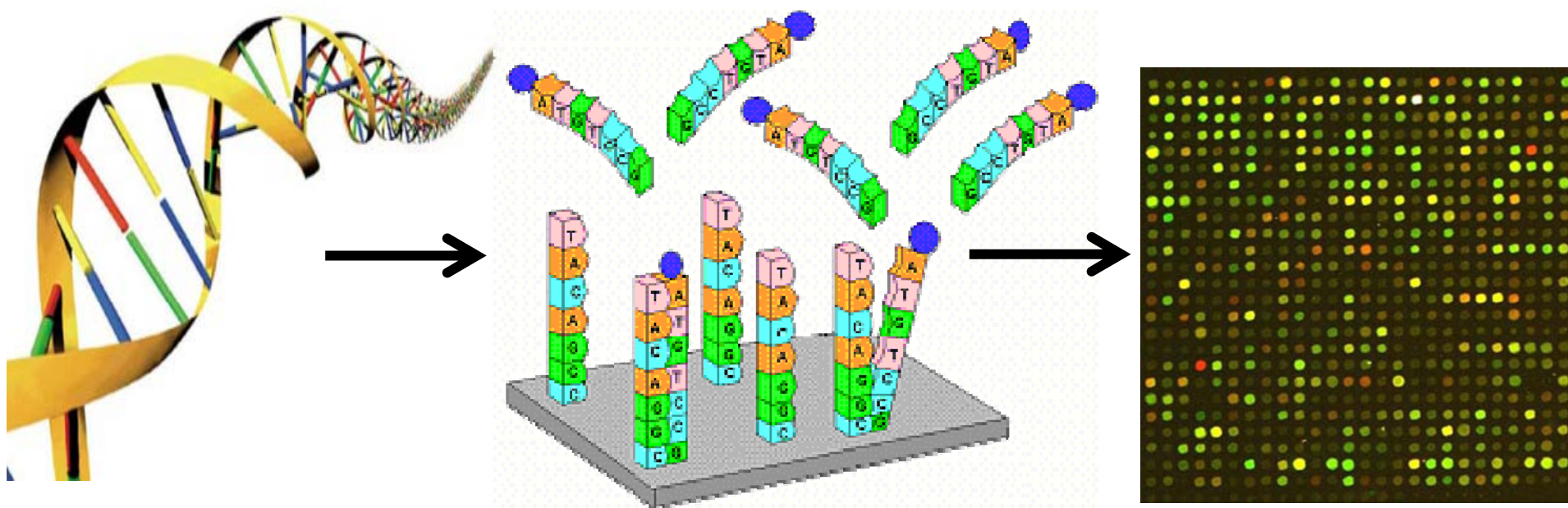
## DNA sequencing

(in this course: MLST of chromosomal and plasmid DNA, and gene sequencing)



# Genotyping methods: three main categories (3/3)

## DNA hybridization-based (in this course: microarray)



# Friday



**Computer Lab**

# Have a good day!

**NB: course material will be uploaded on**  
**[www.antimicrobialresistance.dk](http://www.antimicrobialresistance.dk)**