CLOSTRIDIUM PERFRINGENS NECROTIC ENTERITIS VIRULENCE PLASMIDS

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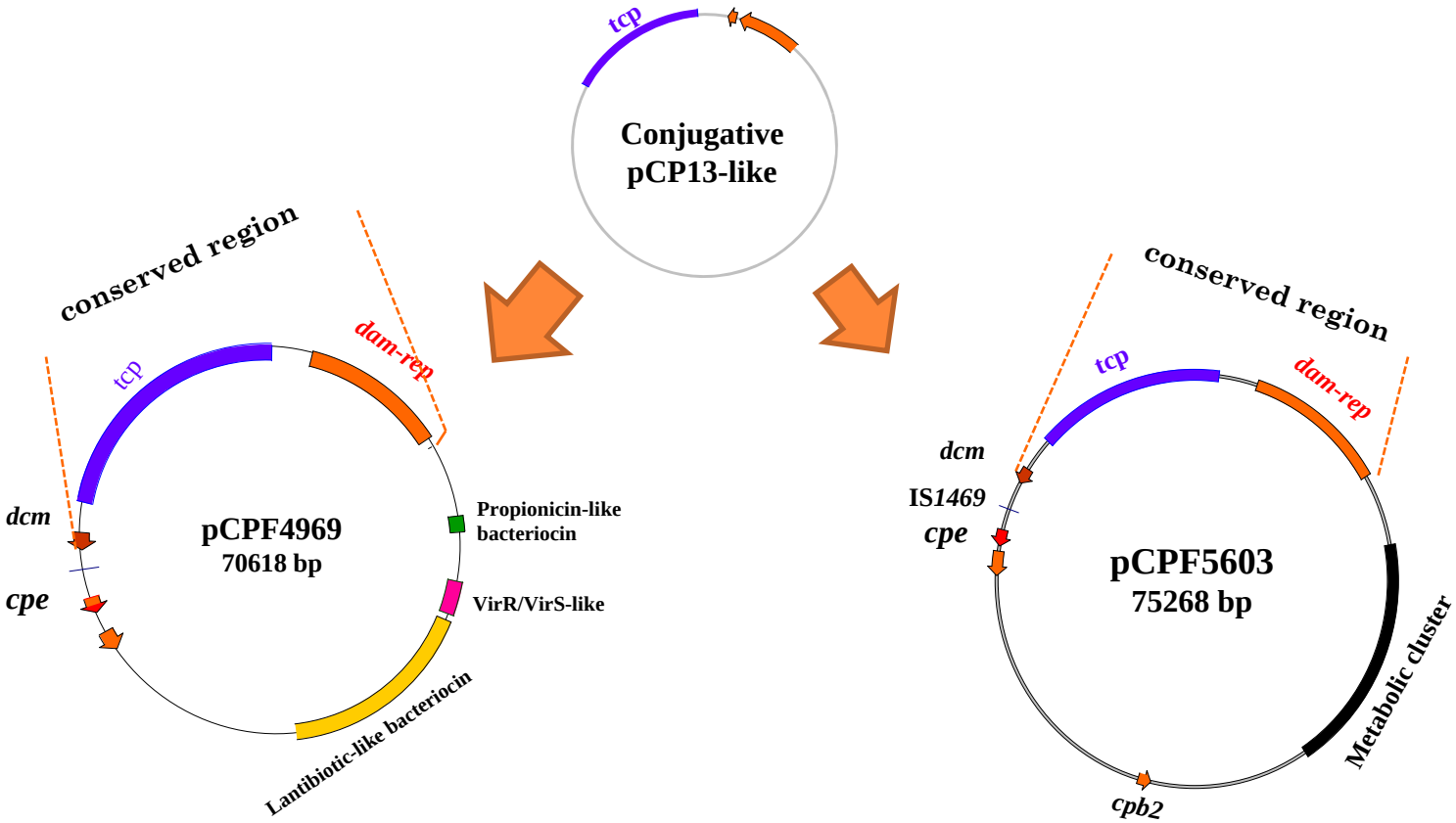
BACKGROUND

- *Clostridium perfringens* isolates classified into five toxinotypes (A to E)
- Most of the major toxin genes carried on large **plasmids**
- Necrotic enteritis (NE) caused mainly by type A strains
- Possess the recently described novel toxin, **NetB,** essential factor for disease
- We discovered that NetB, a novel pore-forming toxin, is present in an 80 kb **plasmid**, in one of the three novel pathogenicity loci (NELoc-1)

BACKGROUND

- Feature of *C. perfringens* **plasmids** is that many are conjugative.
- Diversity of toxinotypes results from plasmid exchange
- Plasmids of types A - E strains form two families, pCPF5603 and pCPF4969
- Share conserved backbone of 35-kb, includes transfer of a clostridial plasmid (*tcp*) locus for conjugative transfer, and a 20– 40 kb variable region that encodes toxins
- *C. perfringens* **plasmids** contribute to both virulence and antibiotic resistance
- Toxin-encoding plasmids of *C. perfringens* now under intensive study

C. PERFRINGENS TOXIN PLASMID FAMILIES

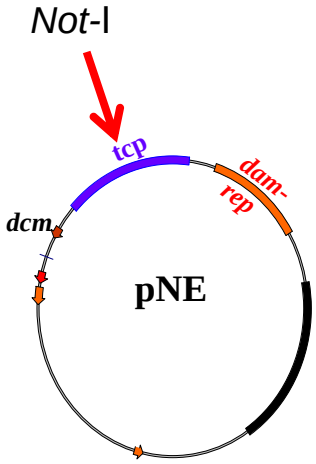


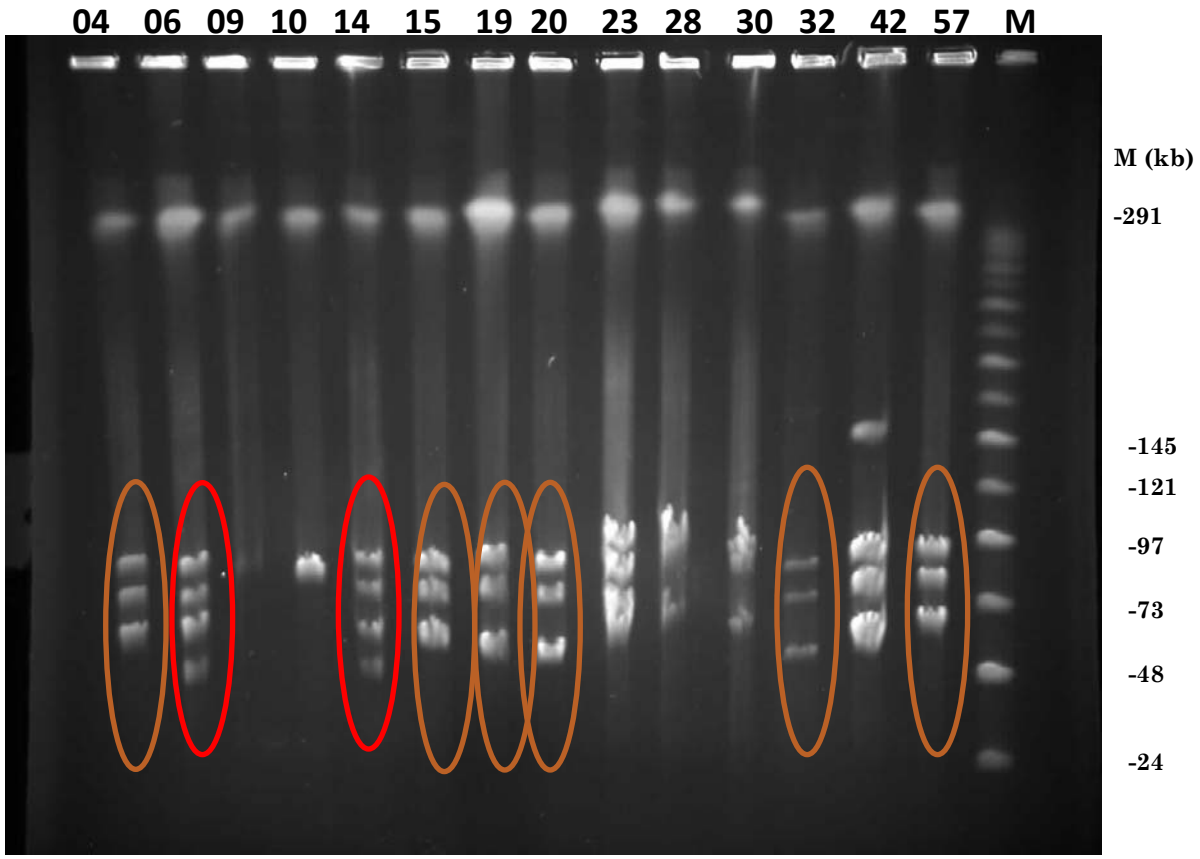
McClane B. Personal communication

OBJECTIVES

- Build a plasmid profile using PFGE for 14 *C. perfringens* isolates* from NE and healthy isolates (5 *netB*+ and 5 *netB*-)
- Characterize location of ***netB***, and ***cpb2*** genes in NE isolates and avirulent isolates
- Mark plasmids with antibiotic cassette using Clostron technology
- Show plasmids are conjugative

PLASMID PROFILE OF NE *C. PERFRINGES* TYPE A STRAINS





OBJECTIVES

-
- Characterize of the location of *netB*, and *cpb2* genes, in NE isolates and avirulent isolates
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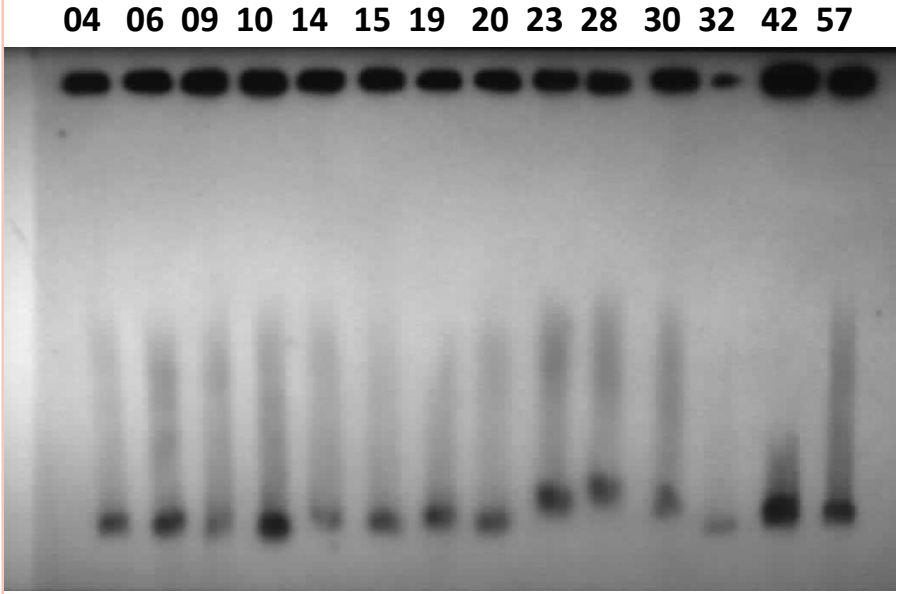
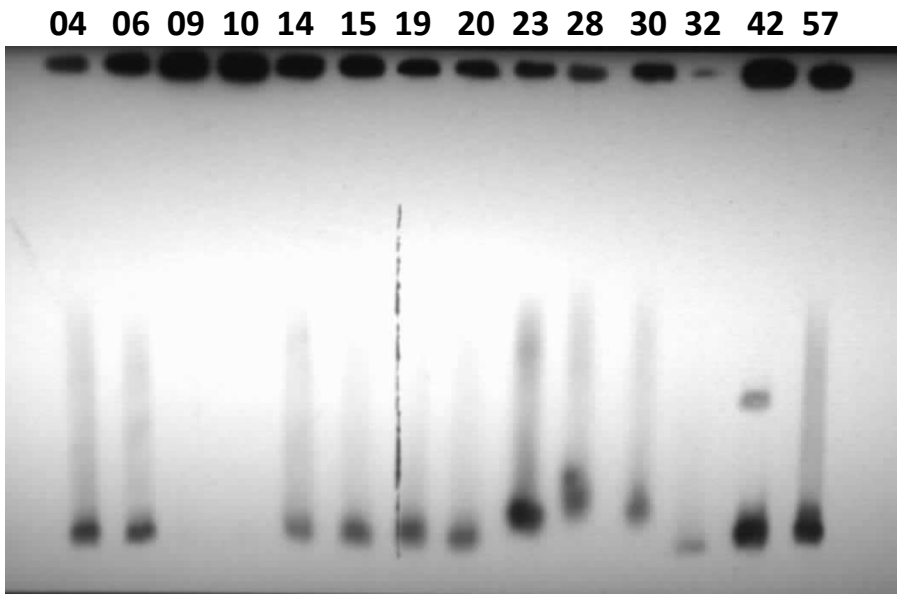
SOUTHERN BLOT OF NE *C. PERFRINGENS* TYPE A STRAINS

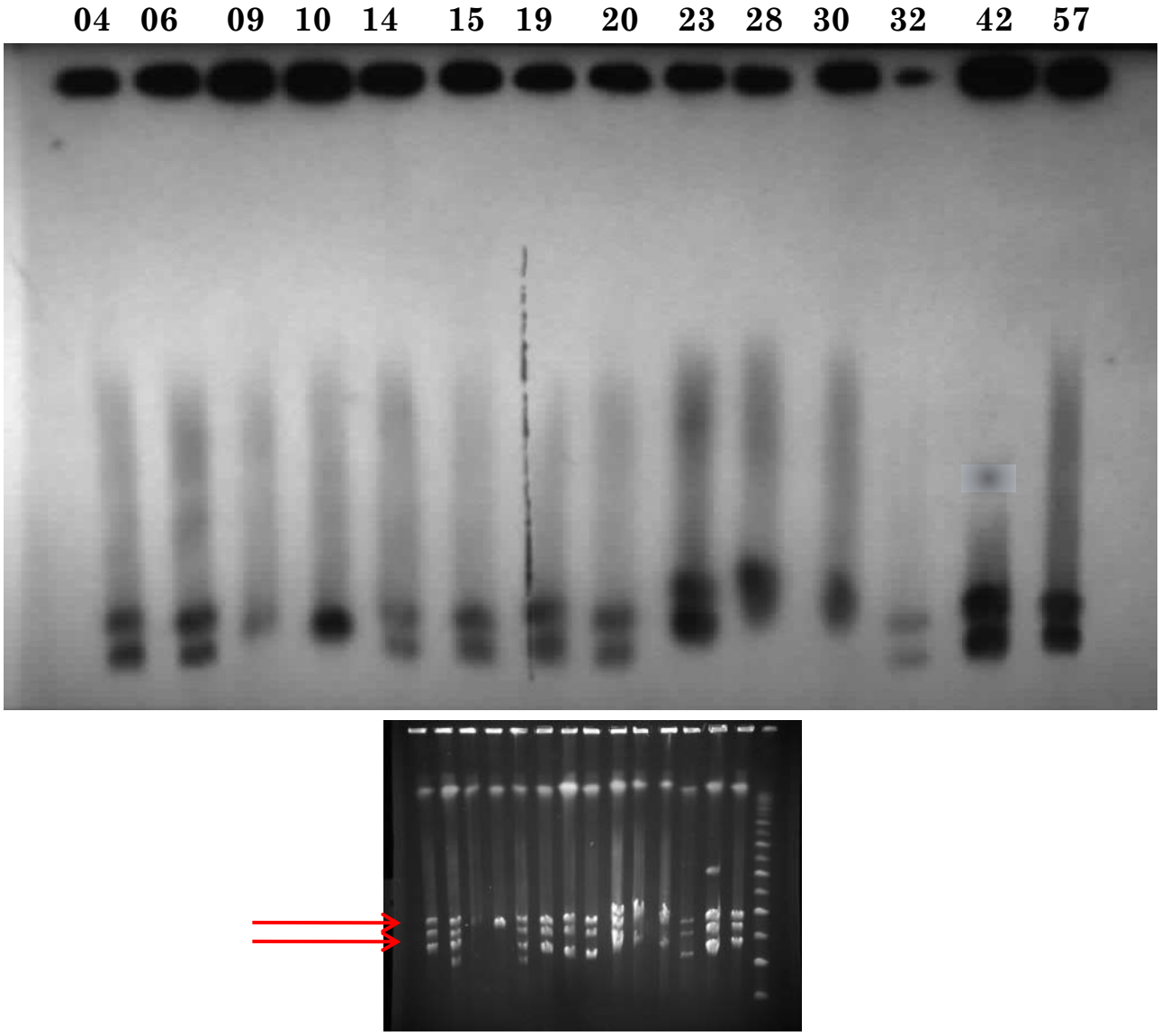
***netB* probe**



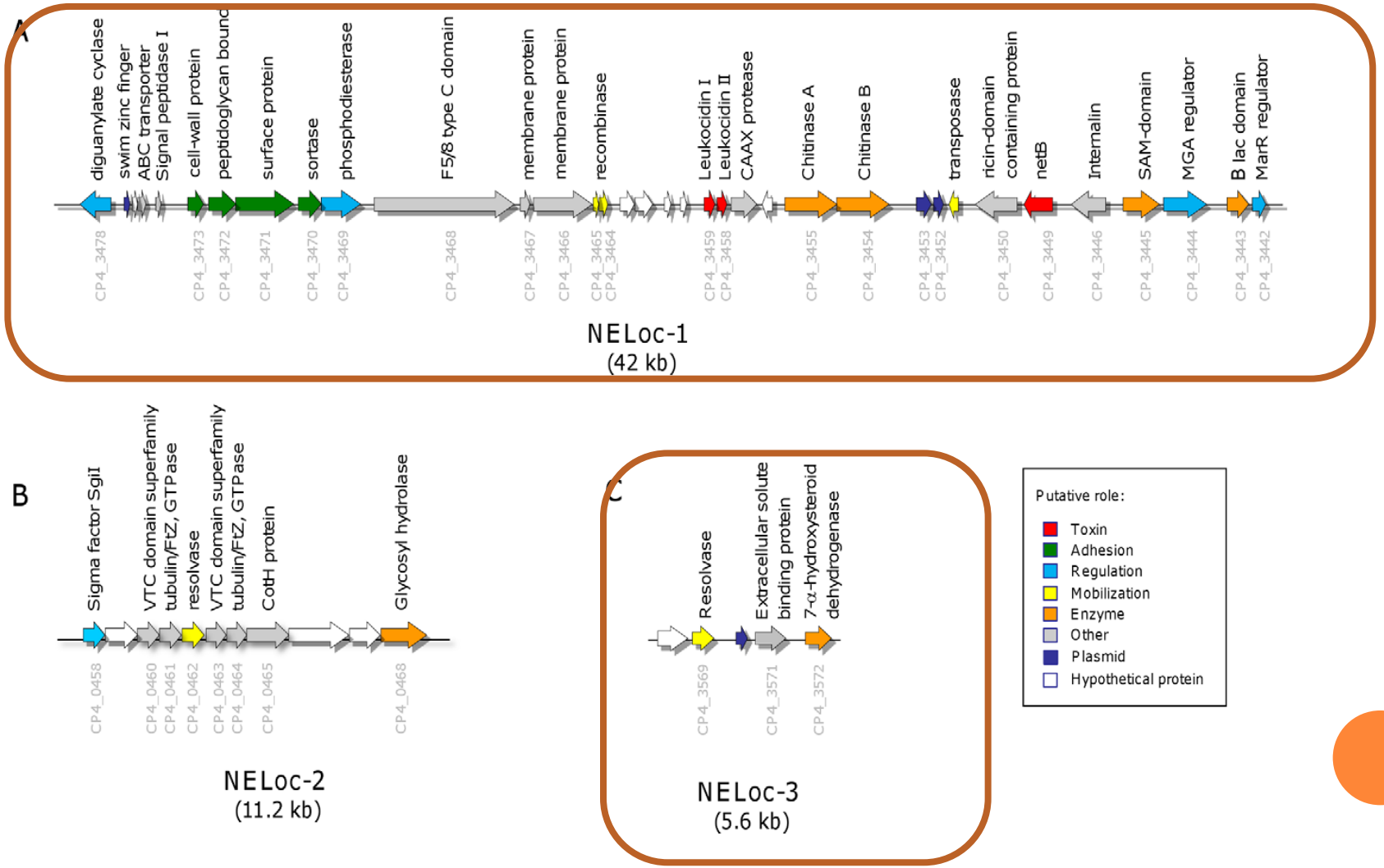
***cpb2* probe**



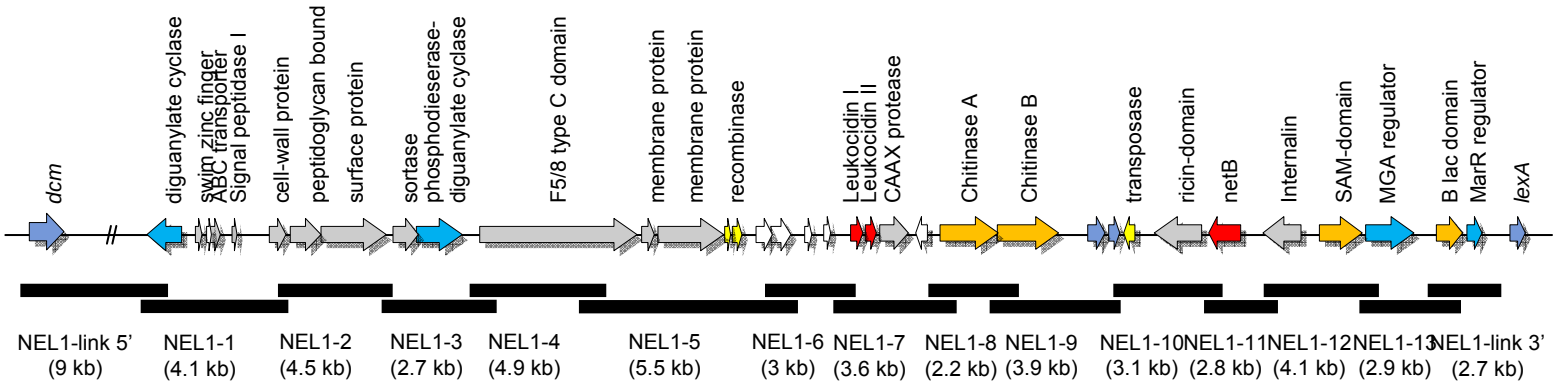
Overlapping SB of *netB* and *cpb2* probes

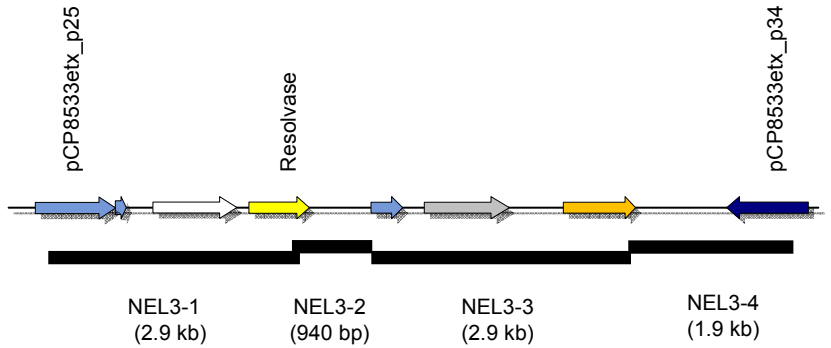


PATHOGENICITY LOCI



OVERLAPPING PCR ANALYSIS OF NE LOCUS 1 AND 3.





SUMMARY OF PLASMID SIZES IN *C. PERFRINGENS* TYPE A ISOLATES

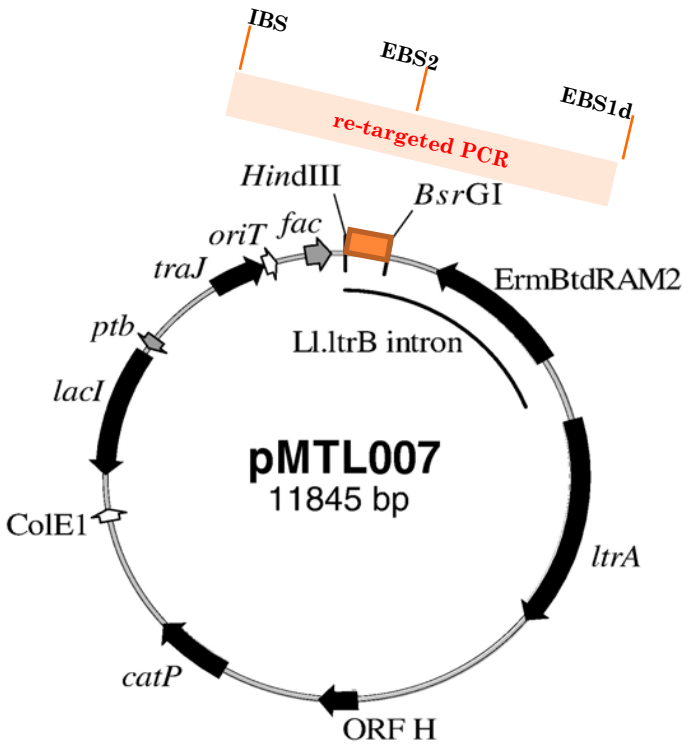
	Strains	Plasmids[2]	Southern blot			
			netB	*hdhA*	*cpb2*	*tcpF*
NE isolates	NE04	3	87	75	75	87, 75, 62
	NE06	4	87	75	75	87, 75, 64
	NE09	1	87	-[2]	-	-
	NE10	1	85	-	-	-
	NE14	4	88	77	77	88, 77, 62
	NE15	3	87	77	77	87, 77, 60
	NE19	3	90	77	77	90, 77, 58
	NE20	3	87	75	75	87, 75, 54
	NE23	4	100	87	87	100, 87, 73
	NE28	2	105	105	105, 69	105, 69
	NE30	3	99	94	94, 67	99, 94, 67
	NE32	3	85	74	74	85, 74
	NE42	4	103,94	150, 81	81	94, 81, 64
	NE57	3	93	82	82	82, 67

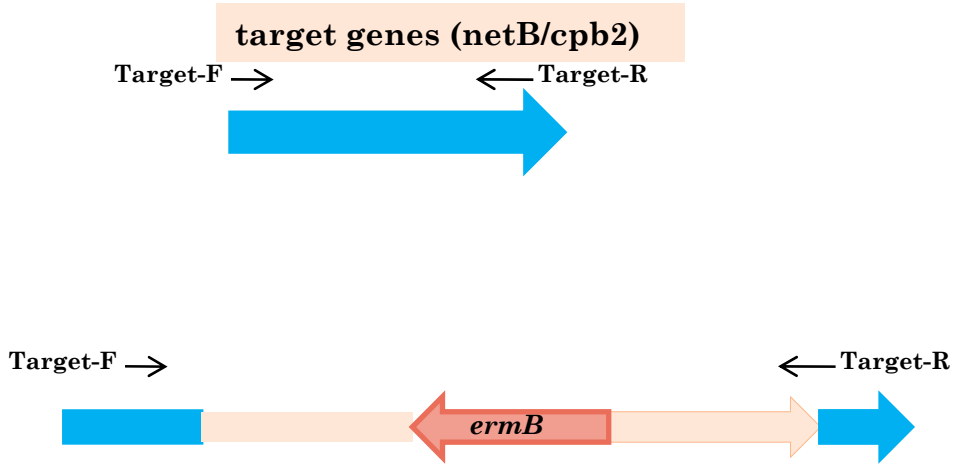
OBJECTIVES

- Mark plasmids with antibiotic cassette using Clostron technology.
- Show plasmids are conjugative.

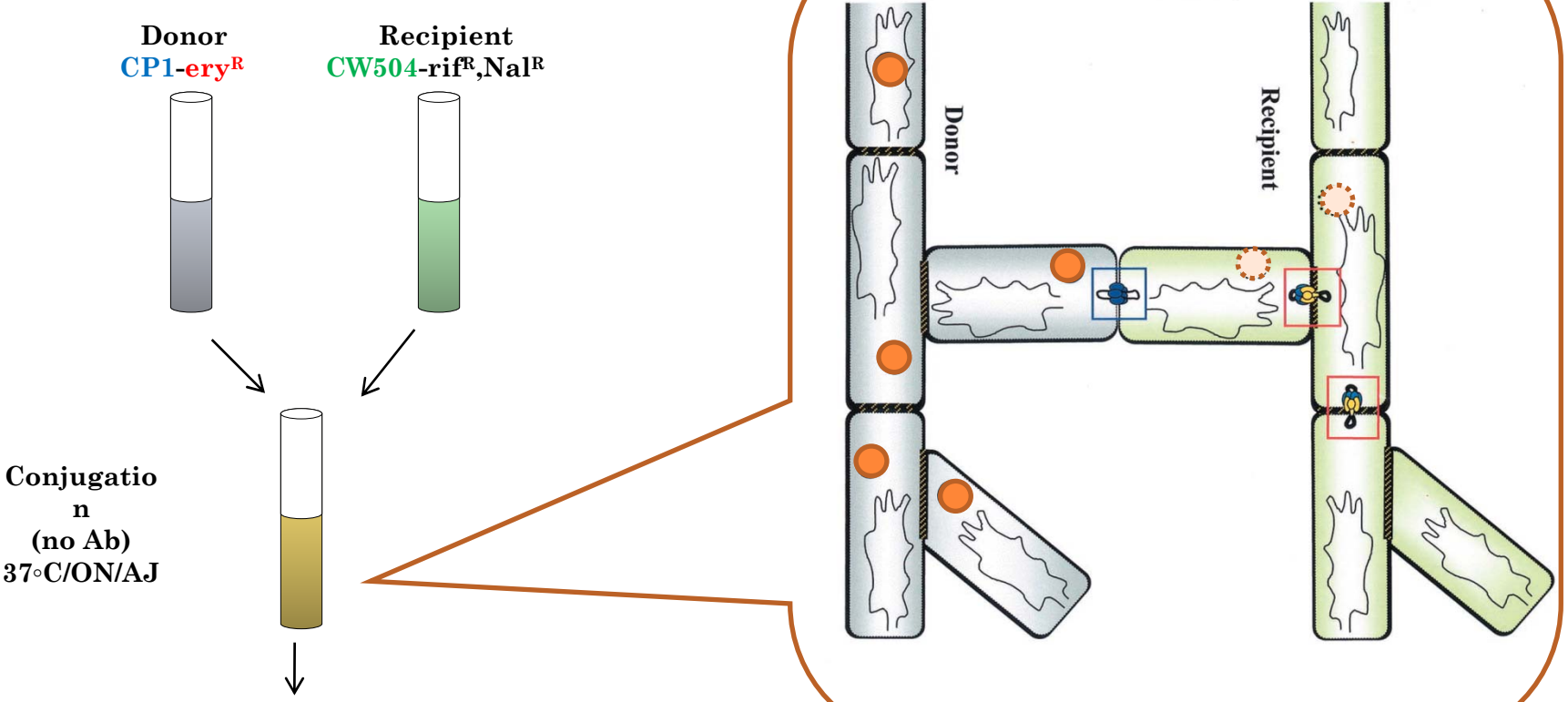
CLOSTRON: DIRECTED MUTAGENESIS

- Directed mutagenesis system : bacterial group II intron for *Clostridium* sp.,
- Target specificity is determined largely by base-pairing between intron RNA and target site DNA
- Re-targeted by altering the sequence of an intron RNA-encoding plasmid Retargeting group II intron from *Lactobacilus lactis* by subcloning 350 bp PCR product





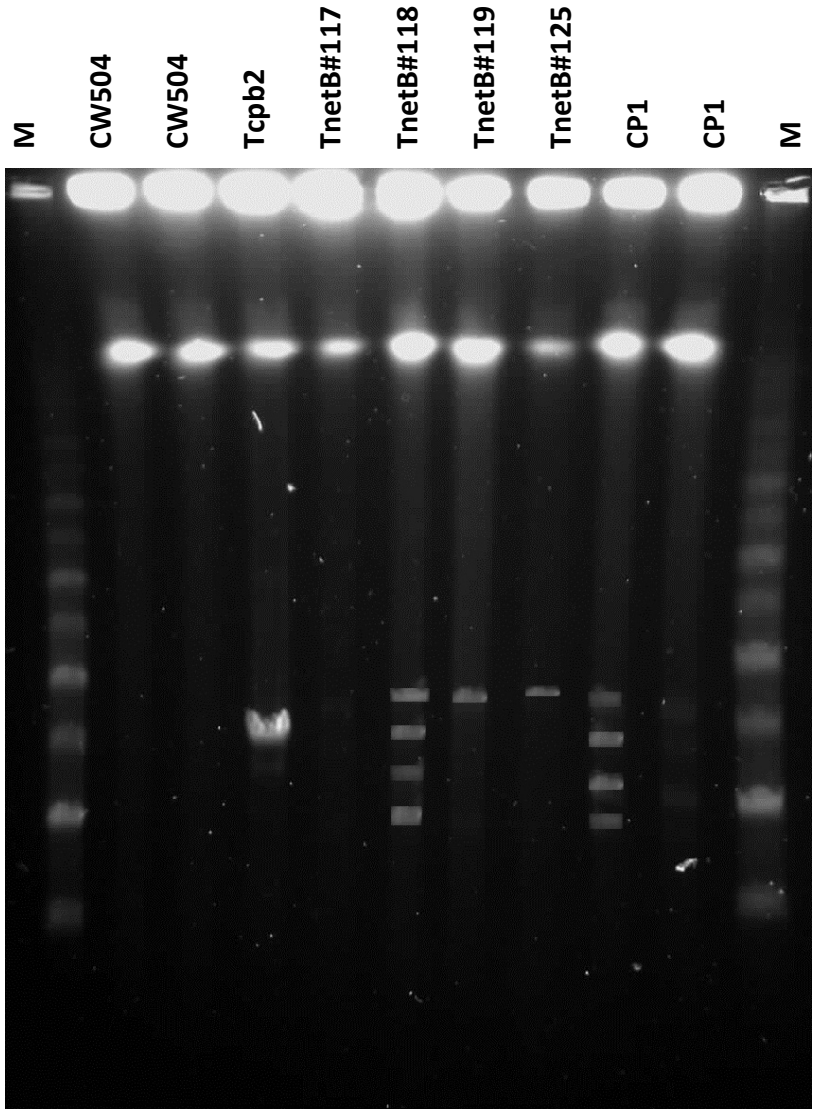
CONJUGATION ASSAY



Transconjugants
(CW504, rif^R, Nal^R, ery^R)
37◦C/ON/AJ

GROHMANN *et al.*, 2003

CONJUGATION –PFGE OF TRANSCONJUGANTS



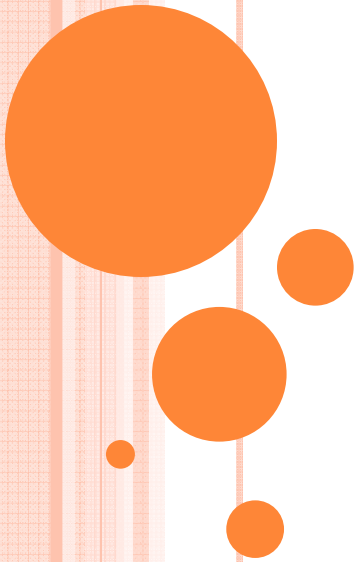
CONCLUSIONS

- 14 NE isolates harboured 3 – 5 plasmids with considerable variation in size
- Virulence plasmids of NE isolates are dynamic but NELoc-1 and 3 conserved
- *netB* gene can be present in two different plasmids in the same strain and/or also in combination with *cpb2*
- In most isolates *netB* in a single larger plasmid (~90-kb), located in NELoc-1
- The *cpb2* gene always linked to NELoc-3
- All pNetB and pCpb2 plasmids isolates carry the *tcp* conjugation locus and all large plasmids are conjugative

ACKNOWLEDGMENTS

- Dr. John Prescott
- Case Pope for the conjugation experiments
- All my lab mates for the creative and nice environment
- Financial support by Canadian Poultry Research Council - Agriculture and Agri-Food Canada; OMAFRA

THANK YOU



HOW DO POULTRY VETERINARIANS ENVISAGE THESE DATA AS BEING USEFUL IN CONTROLLING NECROTIC ENTERITIS?

OVERLAPPING PCR ANALYSIS OF NE LOCUS 1.

Strains	NEL-1 RX#												
	1 4.1Kb	2 4.5Kb	3 2.7Kb	4 4.9Kb	5 5.5Kb	6 3Kb	7 3.6Kb	8 2.2Kb	9 3.9Kb	10 3.1Kb	11 2.8Kb	12 4.1Kb	13 2.9Kb
NE04	+	+	+	+	+	+	+	+	+	+	+	+	+
NE09	+	+	+	+	+	+	+	+	+	+	+	+	+
NE10	+	+	+	+	+	+	+	+	+	+	+	+	+
NE14	+	+	+	+	+	+	+	+	+	+	+	+	+
NE20	+	+	+	+[1]	+	+	+	+	+	+	+	+	+
NE23	+	+	+	+	+	+	+	+	+	+	+	+	+
NE28	+	+	+	+	+	+	+	+	+	+	+	+	+
NE30	+	+	+	+	+	+	+	+	+	+	+	+	+
NE42	+	+[3]	+	+	+	+	+	+	+	+	+	+	+

OVERLAPPING PCR ANALYSIS OF NE LOCUS 3

Strains	NEL-3 RX#s			
	1 (2.9Kb)	2 (0.9Kb)	3 (2.9Kb)	4 (1.9Kb)
NE04	+	+	+	+
NE09	+	+	+	+
NE10	+	+	+	+
NE14	+	+	+	+
NE20	+	+	+	+
NE23	+	+	+	+
NE28	-	-	-	+
NE30	-	-	-	+
NE42	-	+	+	-