Better lives through livestock

Identification of virulence factors of contagious bovine pleuropneumonia causing bovine epithelial cell death by whole genome mutagenesis

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Scientific Seminar

Supervisors

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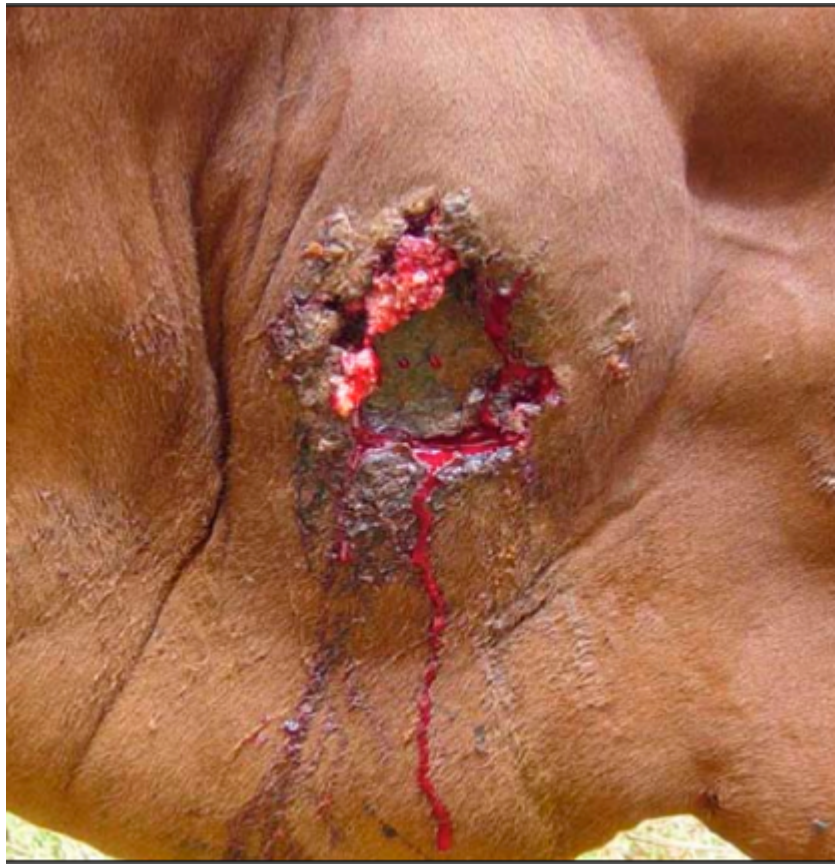
Introducing CBPP

- CBPP, caused by *Mycoplasma mycoides* subsp. *mycoides (Mmm*) is a highly contagious disease that affect cattle in many countries of SSA
- CBPP are among the most serious livestock diseases in Africa.
- Imposes an estimated minimal cost of >100.000.000 €/year in Africa and restricts trade
- Clinical signs include fever, coughing, respiratory distress and anorexia with unilateral lung lesions and pleural fluid -acute, subacute or chronic disease
- Control Methods are vaccines, antibiotics, movement control and slaughter methods.

Introducing CBPP – Available vaccines

- Available and OIE recommended vaccines:
  - Live attenuated vaccine (mostly T1/44)
  - Low efficacy
  - Short duration of protection
  - Remaining virulence causing occasional post-vaccination reactions (Willem's reactions) at site of injection
  - Continued attenuation: better safety profile, lower protection
  - Inactivated vaccines not working so far



Introduction

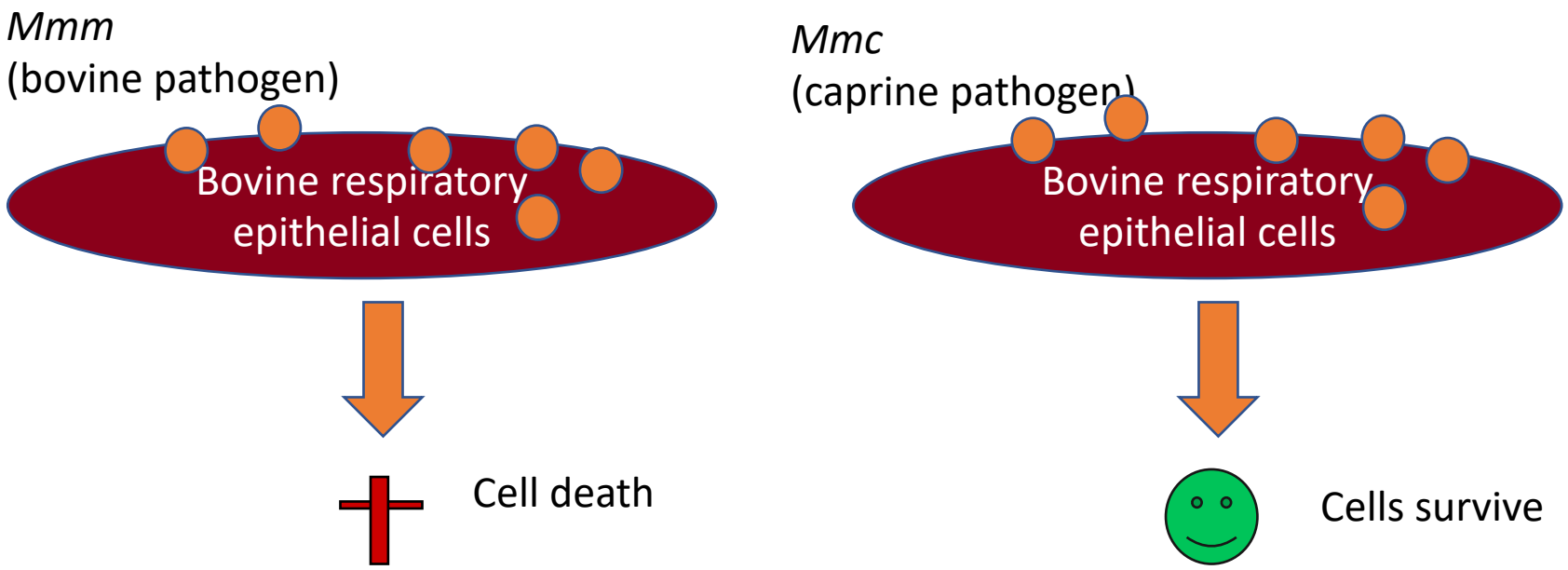
Inadequate knowledge of the host protective immune responses

CBPP, like most other mycoplasma diseases, is characterized by immunopathology.

Why? What goes wrong? When does the host make the wrong "immunodecision".

General Objective

To identify virulence factors of Contagious Bovine Pleuropneumonia causing bovine epithelial cell death by whole genome mutagenesis



Transcriptomics: "Intracellular viral infection" response

Transcriptomics: "Extracellular bacterial infection" response

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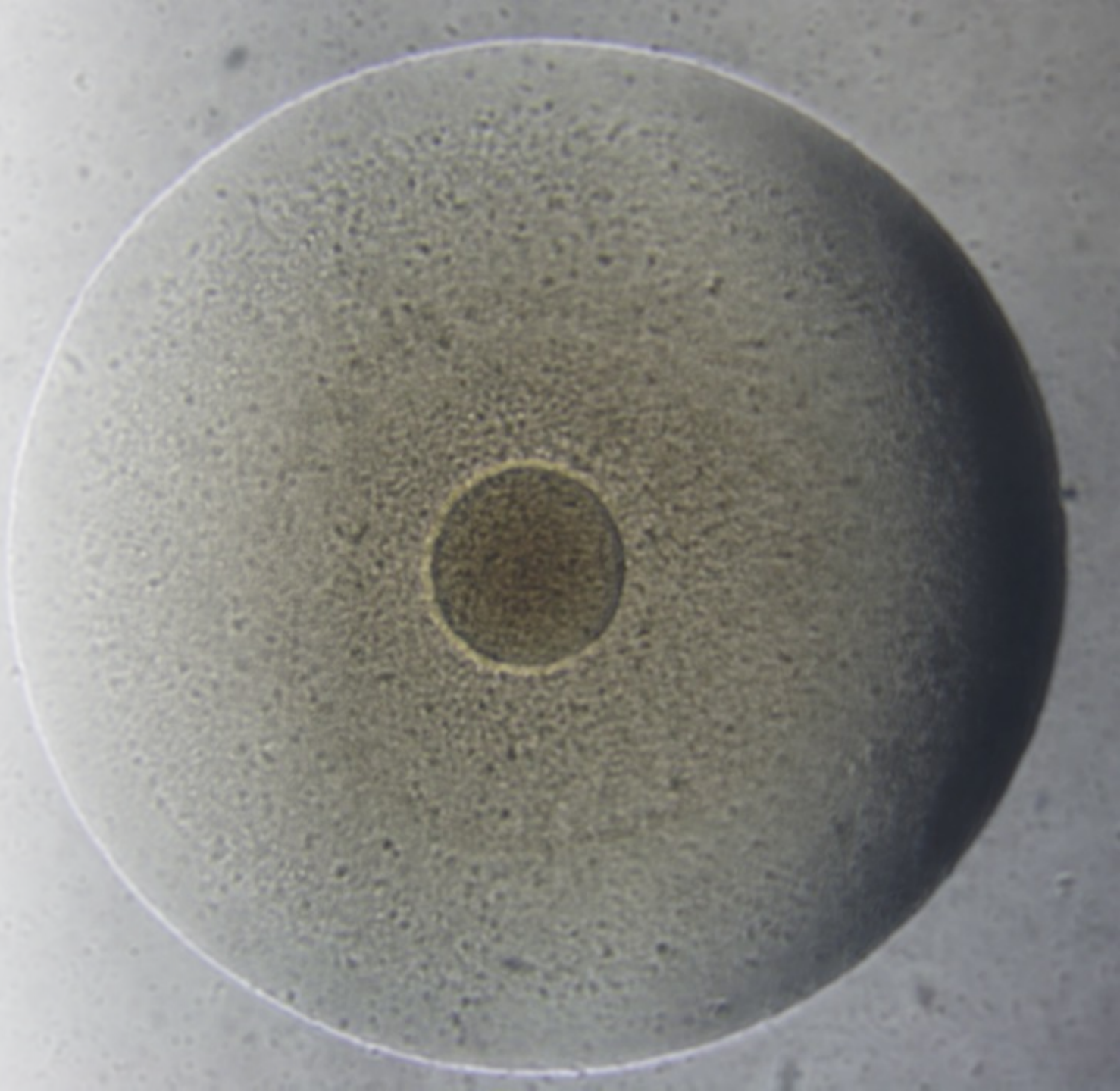
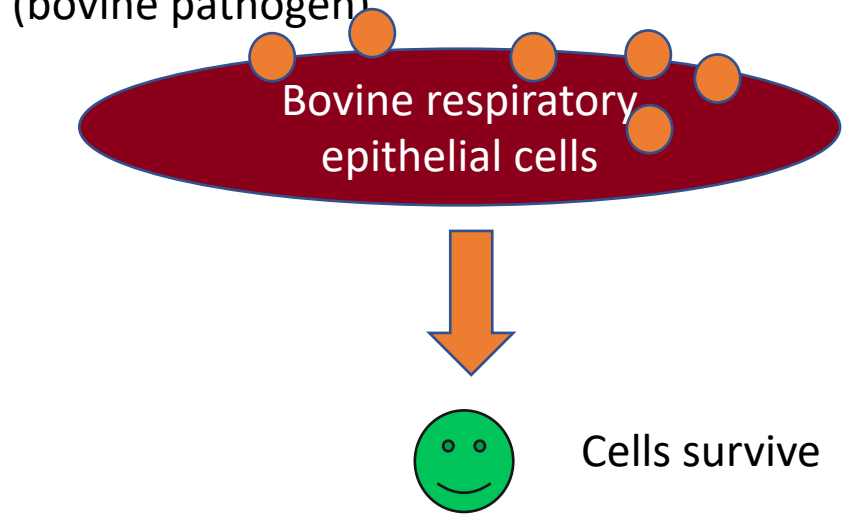
Specific Objectives

1. To generate *Mycoplasma mycoides subsp. mycoides* transposon mutant library
2. To determine the quality of *Mmm* mutant libraries
3. To screen transposon library for *Mmm* mutants that do not kill epithelial cells



Expected Output

Virulence factors that cause Epithelial cell death as novel vaccine targets.



Methodology

Molecular cloning of E. *coli* for Plasmid propagation

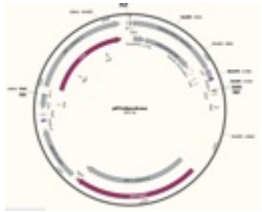
Transformation of *Mmm to get mutants*

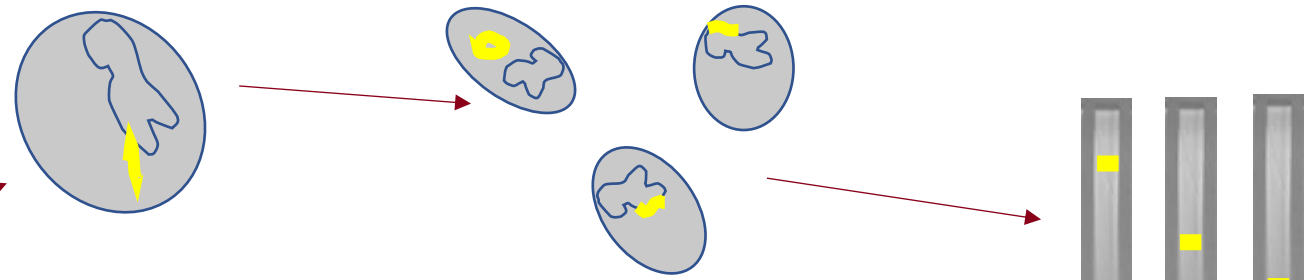
Mini-Sequencing of *Mmm Mutants* for quality checking

Embryonic Bovine Lungs cell assays

Mutant Sequencing

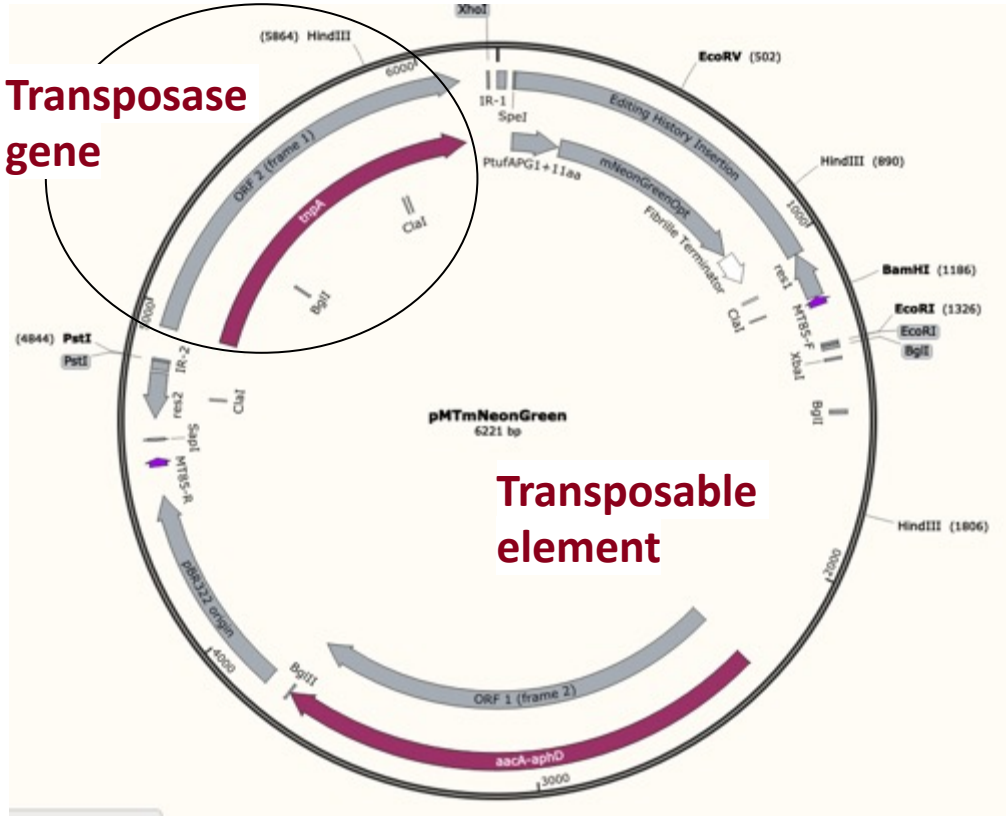
Cell assay Data and Sequence analysis



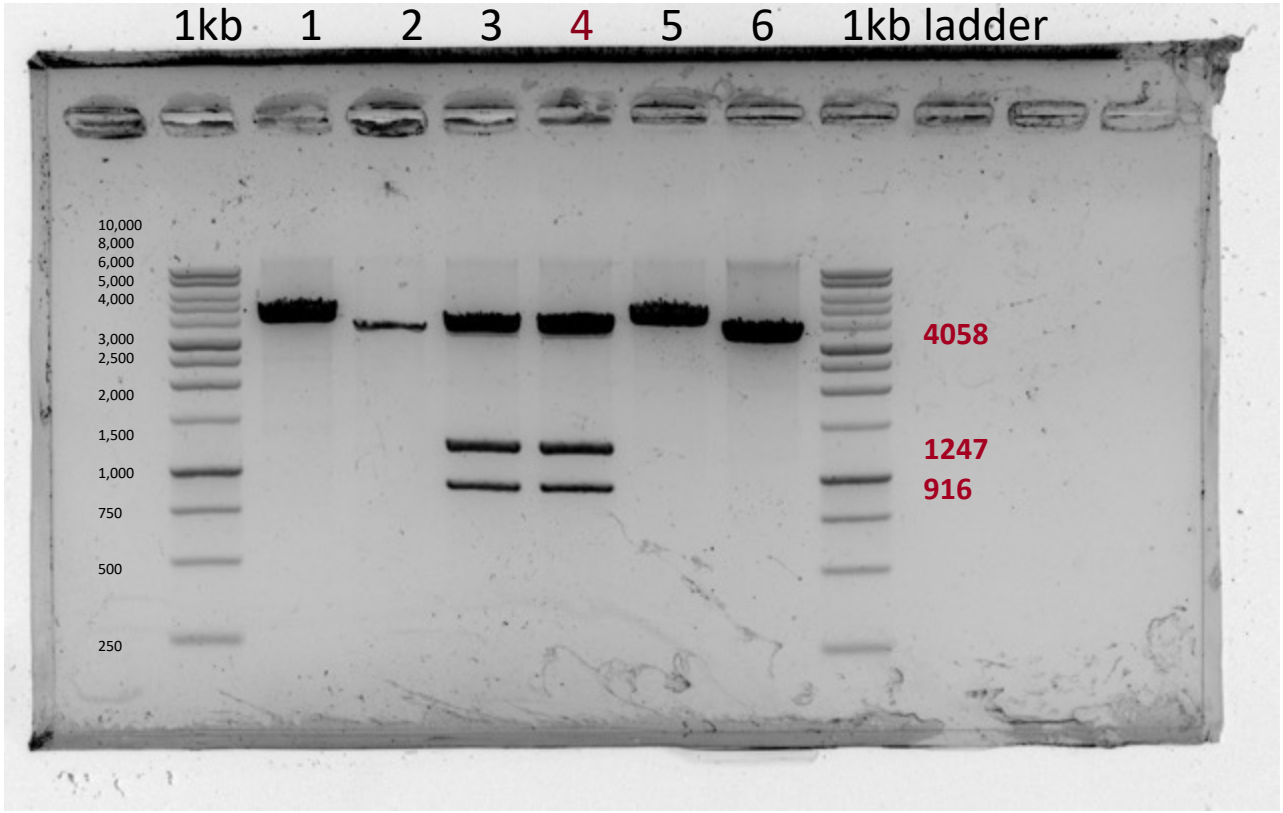


PEG-Transformation of *Mmm(Afade)*

- Transformation of E-coli cells and using Kanamycin to select Transformed cells.
- Miniprep done of 6 colonies and RFLP analysis using Hind111 restriction enzyme
- Maxiprep done to get the highest concentration of plasmid for better transformation.



Plasmid Extraction



Qiagen Maxiprep: Expired and not high enough concentration for better transformation.

Sambrook's Molecular Cloning Plasmid extraction protocol adapted had 3-5ug/ul concentration.

Mmm Transformation

Cells in Logarithmic phase obtained

↓

Calcium Chloride Treatment

↓

tRNA + Plasmid

↓

PEG 8000

↓

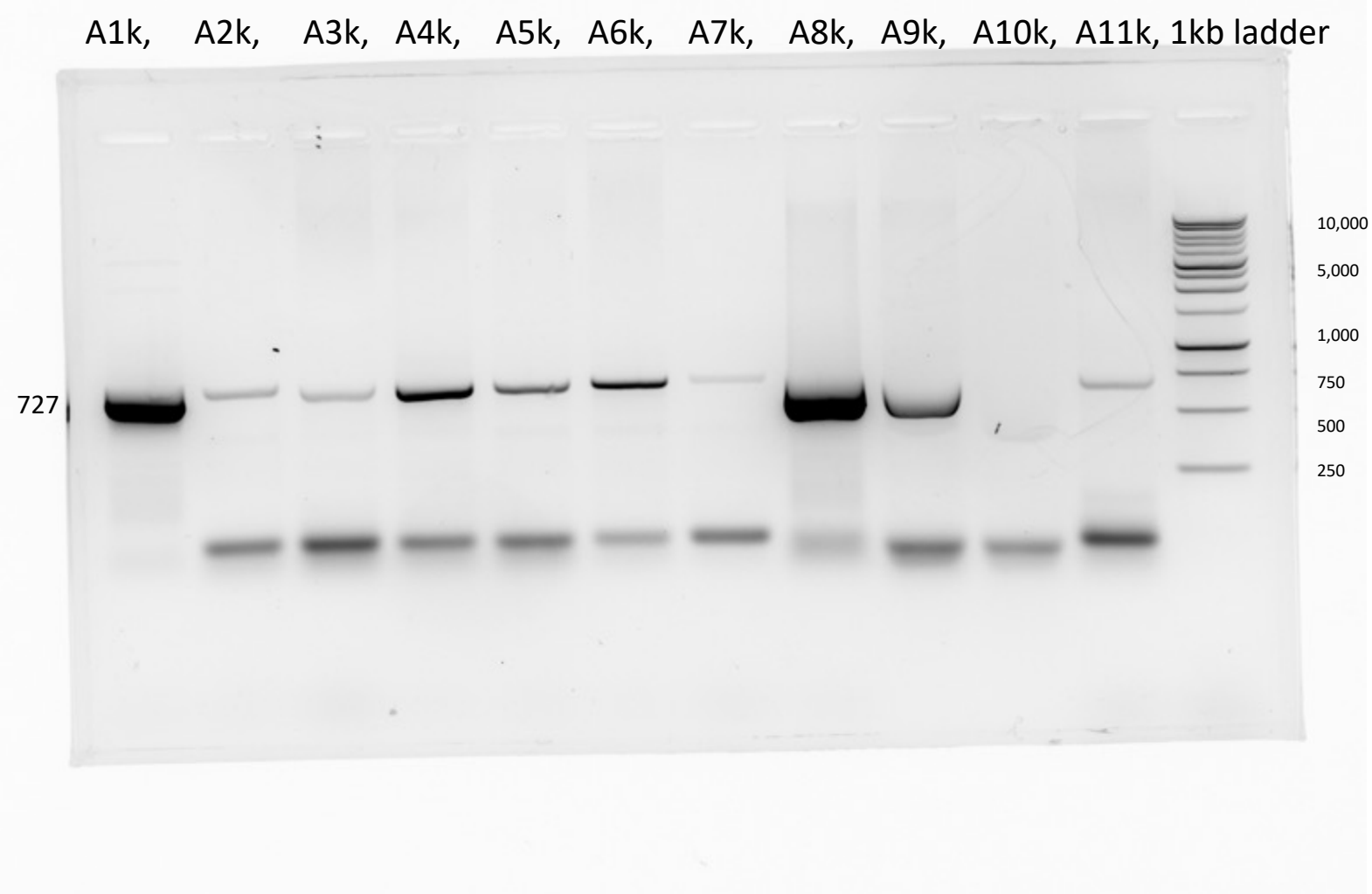
3-hour Incubation

↓

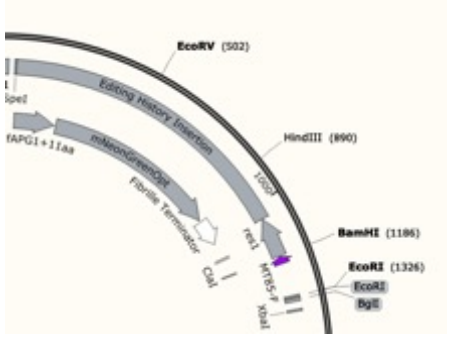
Inoculated on Selective Plates

Trans No.	No. of Mutants	Comments
1.	2	Low Transformation efficiency
2.	12	Afade: Low| Mmc: High
3.	400	Plasmid and Cell Concentration
4.	Too many	Hard to isolate
5.	Still…Too many	Still hard to isolate
6.	0	What Happened?
7.	0	Growing mutants are big and not *Mmm*
8.	?	Huge loss of cells

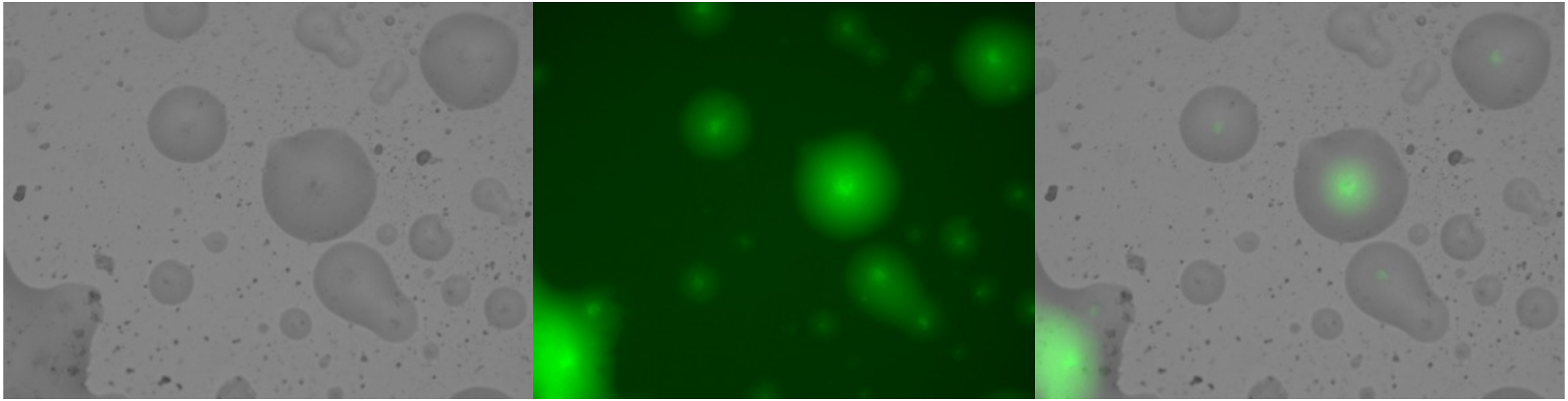
PCR Confirmation of Kanamycin gene



Green Fluorescence protein in Mutants



SNN 46 Colonies



Visible light

GFP

Overlay

Attempted: Identification of Transposon Insertion site

DNA extraction

↓

Bcl1 Digestion

↓

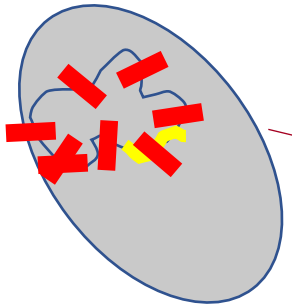
Adaptor ligation

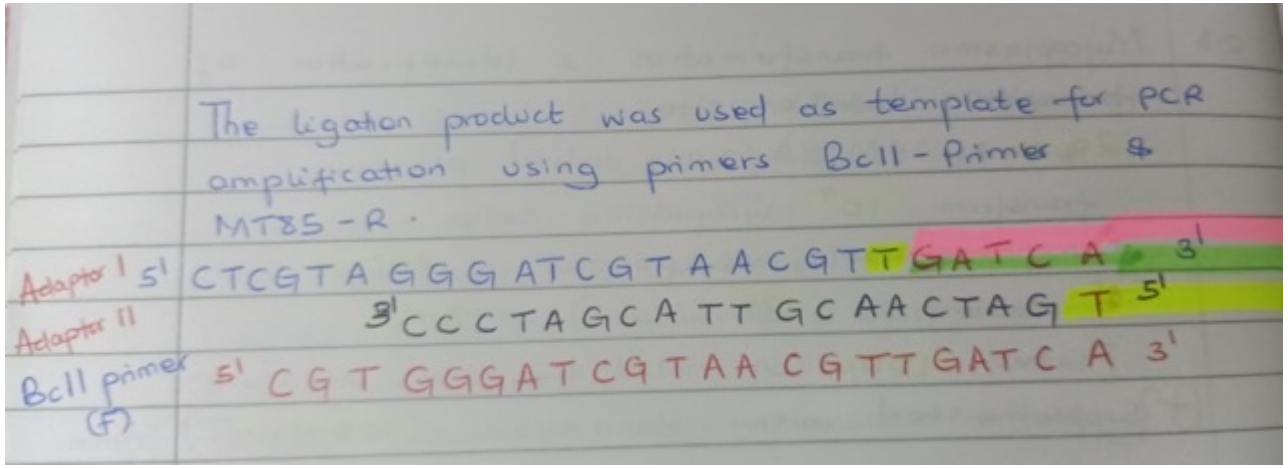
↓

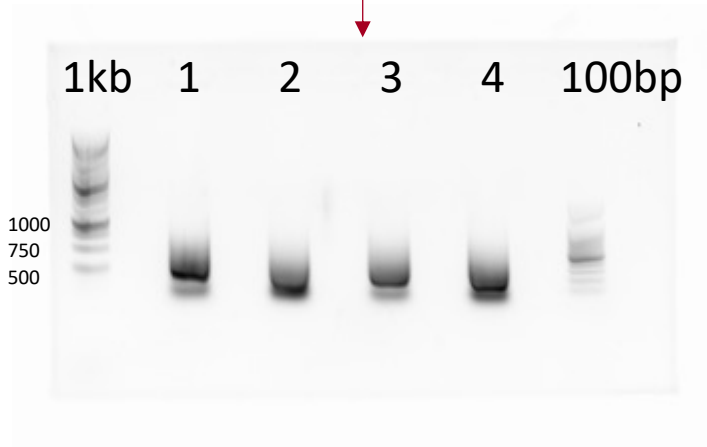
PCR: Adaptor+ Plasmid primers

↓

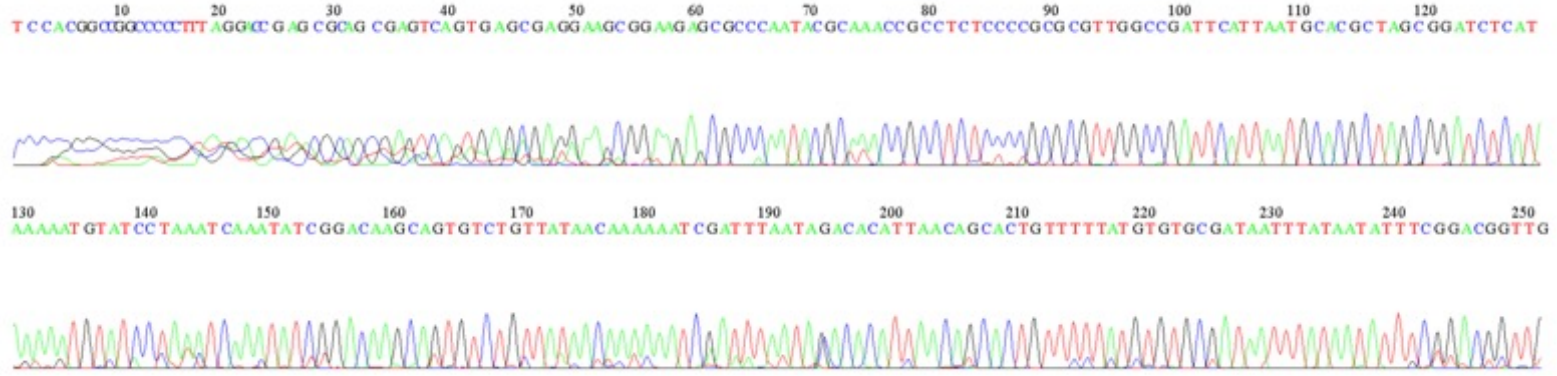
Sequence



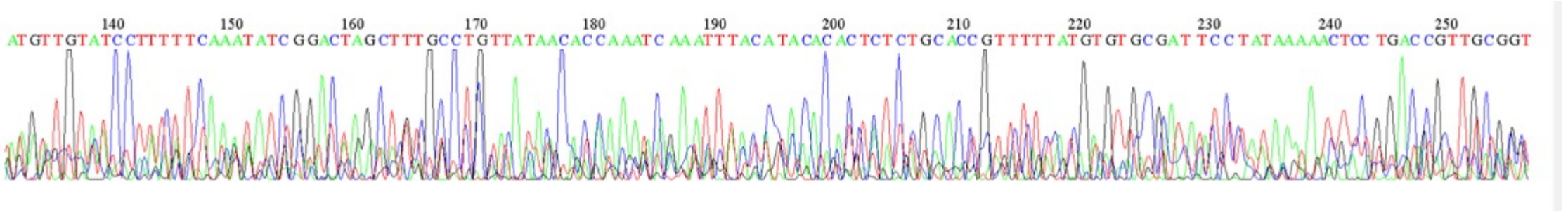




Done: Direct Sequencing



- Out of the 5 Samples, 2 had the transposon.
- Out of the 2 that have the transposon, one was a *Mmm* mutant.



Blast Results

1-304 Pmt85

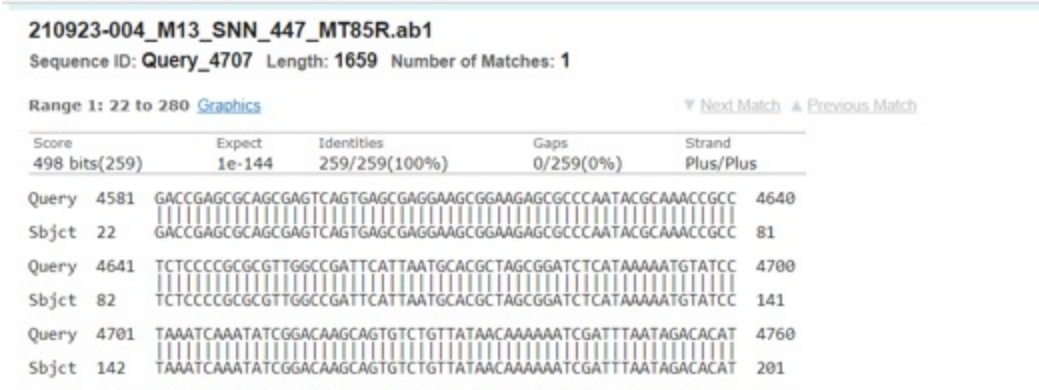
SNN 447: 100% Identity

SNN 443: 76% Identity

>304 | NCBI Blast

SNN 443: 86% Identity to *Mmm*

SNN 447: Scattered alignments to pmt85



210923-004_M13_SNN_447_MT85R.ab1

Sequence ID: Query_4707 Length: 1659 Number of Matches: 1

Range 1: 22 to 280 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
498 bits(259)	1e-144	259/259(100%)	0/259(0%)	Plus/Plus

```
Query  4581  GACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC  4640
Sbjct  22    GACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC  81
Query  4641  TCTCCCCGCGCGTTGGCCGATTCATTAATGCACGCTAGCGGATCTCATAAAAATGTATCC  4700
Sbjct  82    TCTCCCCGCGCGTTGGCCGATTCATTAATGCACGCTAGCGGATCTCATAAAAATGTATCC  141
Query  4701  TAAATCAAATATCGGACAAGCAGTGTCTGTTATAACAAAAAATCGATTTAATAGACACAT  4760
Sbjct  142   TAAATCAAATATCGGACAAGCAGTGTCTGTTATAACAAAAAATCGATTTAATAGACACAT  201
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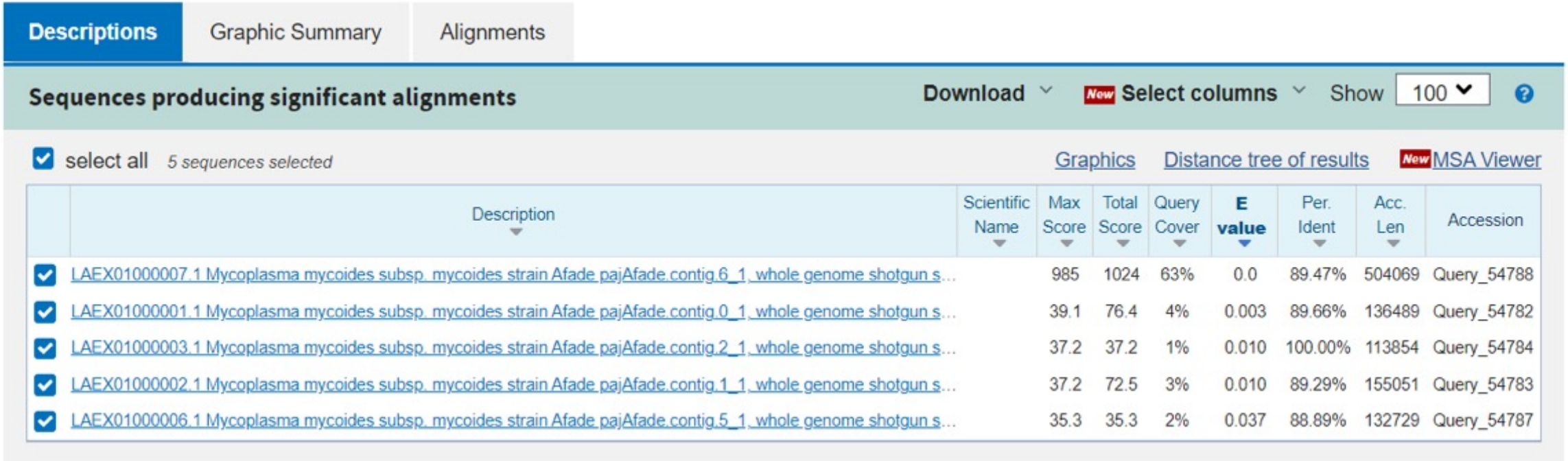


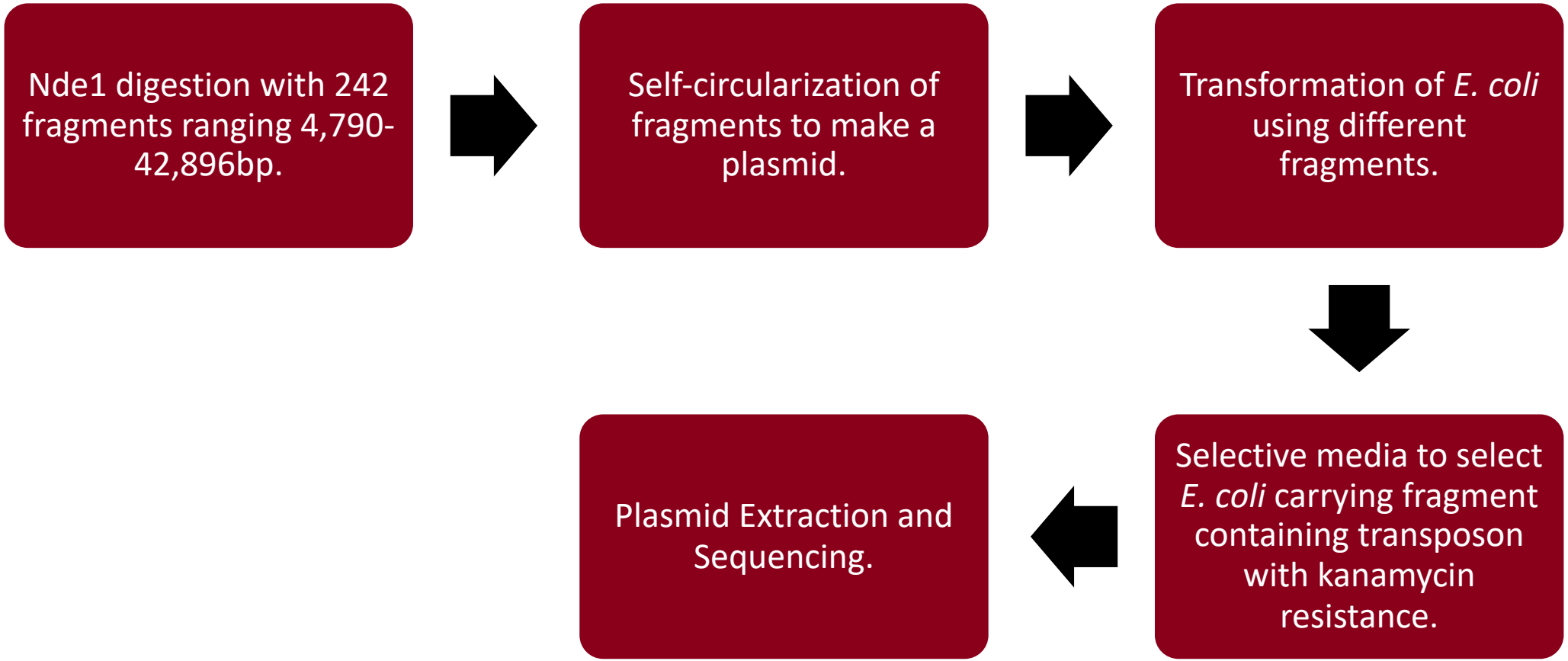
Descriptions | Graphic Summary | Alignments

Sequences producing significant alignments Download New Select columns Show 100

select all *5 sequences selected* Graphics Distance tree of results New MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
LAEX01000007.1 Mycoplasma mycoides subsp. mycoides strain Afade pajAfade.contig.6_1, whole genome shotgun s...		985	1024	63%	0.0	89.47%	504069	Query_54788
LAEX01000001.1 Mycoplasma mycoides subsp. mycoides strain Afade pajAfade.contig.0_1, whole genome shotgun s...		39.1	76.4	4%	0.003	89.66%	136489	Query_54782
LAEX01000003.1 Mycoplasma mycoides subsp. mycoides strain Afade pajAfade.contig.2_1, whole genome shotgun s...		37.2	37.2	1%	0.010	100.00%	113854	Query_54784
LAEX01000002.1 Mycoplasma mycoides subsp. mycoides strain Afade pajAfade.contig.1_1, whole genome shotgun s...		37.2	72.5	3%	0.010	89.29%	155051	Query_54783
LAEX01000006.1 Mycoplasma mycoides subsp. mycoides strain Afade pajAfade.contig.5_1, whole genome shotgun s...		35.3	35.3	2%	0.037	88.89%	132729	Query_54787

Proposed: Self-Circularization Fragments into plasmids



Acknowledgement

Capacity Development

Mycoplasma Lab Team:

1. Winnie Chebore
2. Rose Ojuok
3. Stephen Munyao

Supervisors:

1. Dr. Elise Schieck, PI, ILRI
2. Dr. Robert Kammerer, FLI
3. Dr. Hassan Musa, University of Edinburgh
4. Dr. Stephen Nyanjom, JKUAT

Dr. Lucia Manso-Silvan

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