

Background

Mumps virus is a single-stranded, non-segmented, negative-sense RNA virus belonging to the Paramyxoviridae family. Aseptic meningitis and orchitis are two complications from its infection¹. Despite high vaccine coverage, infections with the mumps virus are increasing within recent years in western countries². Due to the insufficient knowledge about the genetic basis of vaccines that contributes to attenuation, safety concerns are also rising. For example, Urabe mumps vaccine was removed from market due to its insufficient attenuation. Studies suggest that Urabe GW7, is more attenuated than Urabe 1004-10/2, a virus from a case of post-vaccination meningitis^{3,4,5}. One gene that is showing differences between two viruses is the viral RNA polymerase (L gene/protein). One focus of Dr. Wright's lab is to determine whether the difference of L gene between two viruses affect the functions of the polymerase, such as mRNA production and binding to other proteins.

Objectives

My project was to prepare plasmid DNA from the clones of the two L proteins, send for sequencing, and then analyze the results. Specifically, the objectives were to isolate the pCITE plasmid containing L gene in GW7 or 1004-10/2 virus from *Escherichia coli* (*E. coli*) *DH5a*, and to find out which nucleotides of L genes were mutated during cloning for both viruses by comparing them to published L gene sequences of GW7 and 1004-10/2 in GenBank.

Materials & Methods

Table 1 Sequencing for L genes of GW7 and 1004-10/2

| Primers | |
|---------|-----------|
| GW7 | 1004-10/2 |
| 15,105 | |
| EJ8- | |
| 14767+ | 14767+ |
| EJ10- | EJ10- |
| EJ12- | EJ12- |
| 13938R- | 13938R- |
| 13883+ | 13883+ |
| 11311+ | 11311+ |
| EJ11+ | EJ11+ |
| EJ9+ | EJ9+ |
| 8436+ | 8436+ |

The size of L gene is around 6800 nucleotides (nt), and primers had previously been designed to sequence the entire L gene. Since each sequencing reaction would yield between 700 and 750 nts of sequence we prepared. Thus, it was estimated that enough DNA for 10 sequencing reactions would be required.

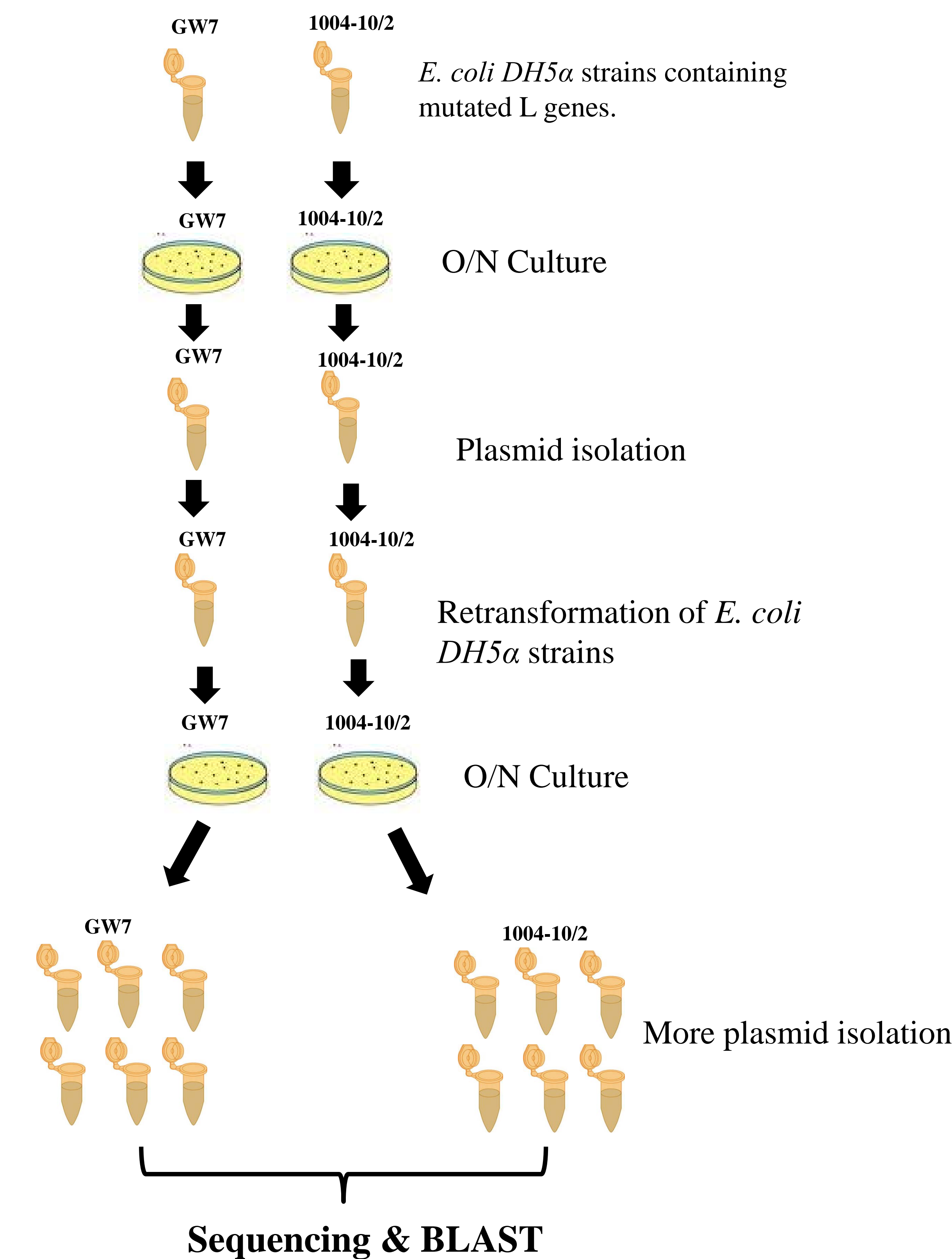


Table 2 Mutated Nucleotides of Gw7 L gene compared to published sequence

| GW7 | | | | | |
|--------------------|----------------|----------------|----------------|----------------|-----------------|
| nt 10112-10114 | nt 11954-11956 | nt 11897-11899 | nt 11891-11893 | nt 11663-11665 | nt 11669->11671 |
| gct->ggt | att->atg | tcc->tgc | aaa-aat | ggc->agc | aga->aaa |
| AA559 | AA1173 | AA1154 | AA 1152 | AA 1076 | AA 1078 |
| A->G | I->M | S->C | K->N | G->S | R->K |

Although 6 mutations had been identified as above, they are not mutations that were introduced during cloning process because they were from the same sequencing reaction. The sequencing reaction must be repeated to confirm the mutated nucleotides.

Table 3 Mutated Nucleotides of 1004-10/2 L gene compared to published sequence

| 1004-10/2 | | | | | |
|--------------------|-----------------|-----------------|-------------------------|---------------|----------------|
| nt 14162->14164 | nt 10889->10891 | nt 10895->10897 | nt 8957->8959 | nt 9491->9493 | nt 11171-11173 |
| gat->ggt | cat -> gat | att->gtt | gag->ggg | aag->aat | ttc->tac |
| AA 1909 | AA 818 | AA 820 | AA 174 | AA 352 | AA 912 |
| D->G | H->D | I->V | E->G | K->N | F->Y |

Only two nucleotides that are bold font are mutated nucleotides that were introduced during cloning process because they had been identified twice by the sequencing reaction. The rest of mutated nucleotides require repeated sequencing reaction to confirm their identity.

Discussion

Two mutations that had been identified in 1004/10-2 appear to be introduced by cloning process (table 3). Mutations in GW7 require repeated sequencing reaction to confirm their identity (table 2). So far, about 80% of gene in both GW7 and 1004-10/2 had been sequenced. More sequencing will be done to fill the gap and locate mutant nucleotides that were introduced during the cloning process. Every mutation identified will be corrected before functional assay.

Acknowledgement

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Reference

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- Wright KE, Dimock K, Brown EG. Biological characteristics of genetic variants of urabe AM9 mumps vaccine virus. *Virus Res* 2000 Mar;67(1):49-57.
- Afzal MA, Yates PJ, Minor PD. Nucleotide sequence at position 1081 of the hemagglutinin-neuraminidase gene in the mumps urabe vaccine strain. *J Infect Dis* 1998 Jan;177(1):265-6.

Results

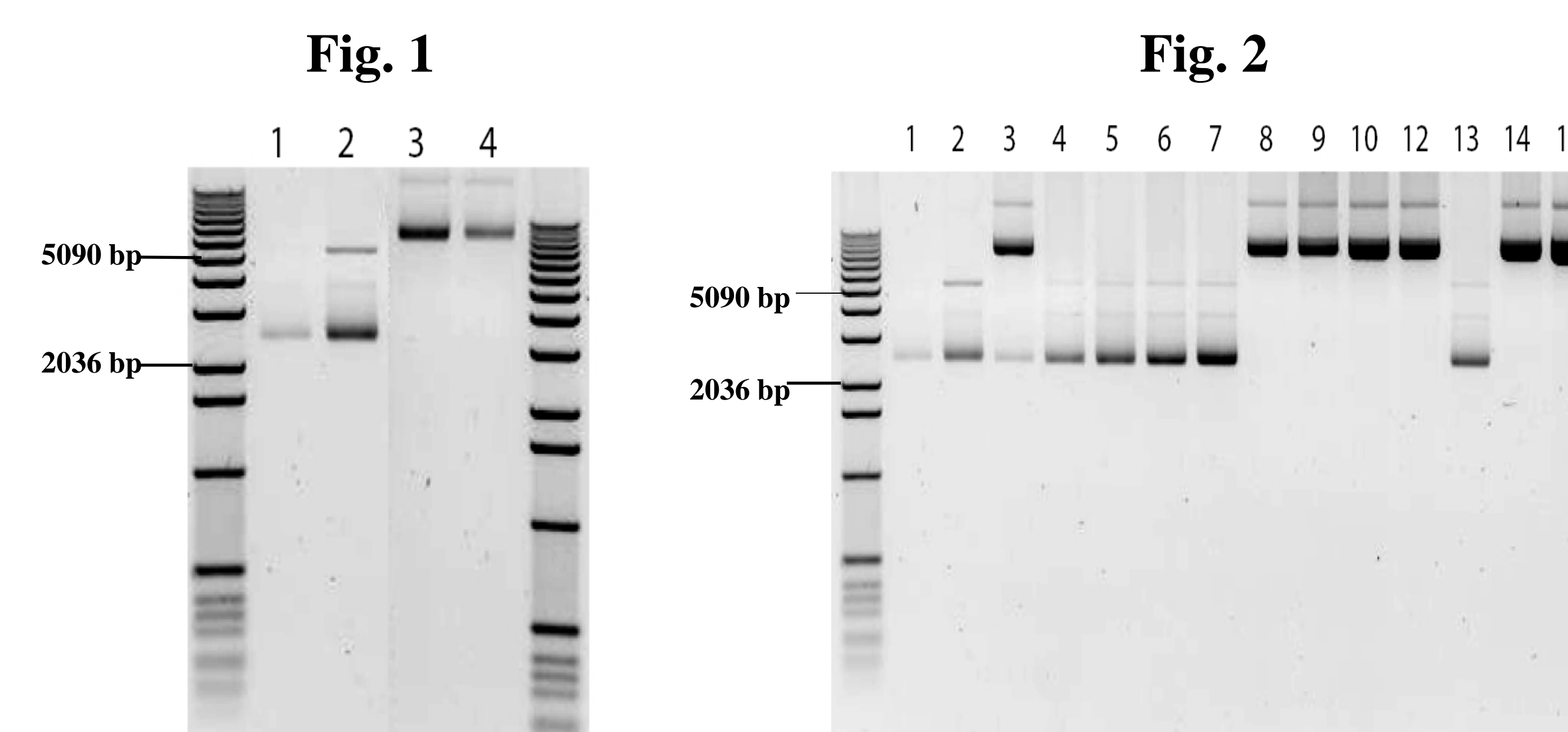


Fig. 1
Lane 1 & 2: empty vector (pCITE)
Lane 3: GW7 (colony #1+2 mix)
Lane 4: GW7 (colony #7)

Both GW7 (colony #1 +2 mix) and GW7 (colony #7) have coding sequence of L gene inserted into the pCITE.

Fig. 2
Lane 1 & 2: empty vector (pCITE)
Lane 3-15: 1004-10/2 colonies #1-12

Colony # 1 (Lane 3), Colony #6-9 (Lane 8 to 12), Colony #11 (Lane14), and Colony #12 (Lane 15) containing plasmid with L gene of 1004-10/2.