Review Article

International Journal of Forensic Research

Review of RNA sequence of pX330-U6

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Submitted: 27 Nov 2020; **Accepted**: 26 Dec 2020; **Published**: 25 Jan 2021

Citation: *Manu Mitra. (2021). Review of RNA sequence of pX330-U6. In j Fore Res, 2(1), 49-56.*

Abstract

RNA sequencing can be used for studying various factors such as mRNA including single cell gene expression. For more than 10 years RNA sequencing has become vital means for wide analysis of gene expression and differential splicing of mRNAs. Gene manipulation has become more important and sophisticated to study the gene structure of RNA especially when it comes to deadly viruses. In this review paper RNA sequence of pX330-U6 is elaborated based on plasmid structure, its RNA sequence is illustrated from 10 to 8500 bases. Index Terms — pX330-U6, RNA, RNA sequence

Introduction

Plasmids contain two expression containers, a human codon-optimized SpCas9 and the single guide RNA. Vector can be processed using Bbsl and a pair of annealed oligos can be cloned scarlessly into the vector before the sgRNA scaffold. The oligos are calculated based on the target site sequence (20bp) and essential to be trailed on 3' end by a 3bp NGG PAM arrangement.

One of the applications are cloning grade DNA is applicable for PCR, cloning reactions or transformation into E. coli. Important aspects of these are Genome Editing, expression vectors for cancer modeling, expression vectors for genome editing in the brain [1-3].

RNA SEQUENCE OF PX330-U6

Below is the RNA sequence of pX330-U6 for comprehension of how RNA sequence is distributed [2].

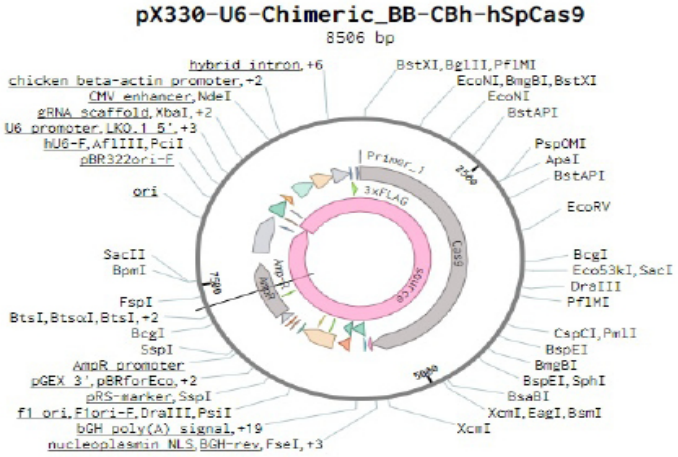

Figure 1: Plasmid structure of pX330-U6 [2, 3]

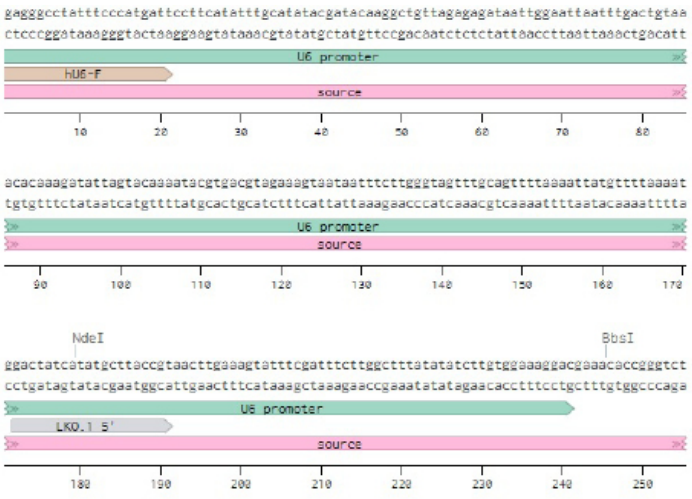

Figure 2: U6 Promotor (10 - 250)

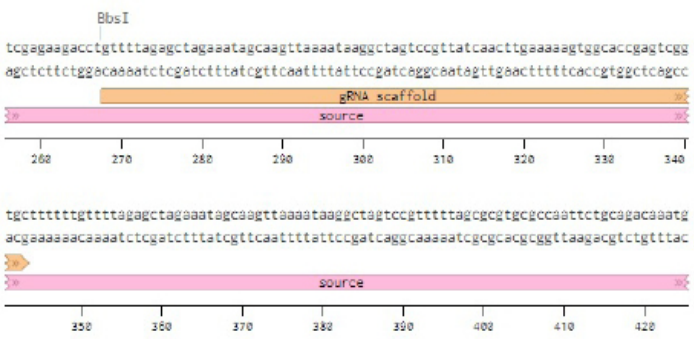

Figure 3: gRNA Scaffold (260 - 420)

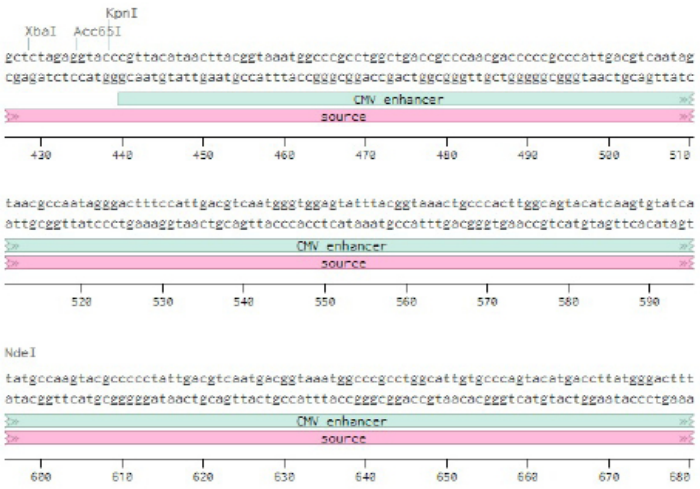

Figure 4: CMV Enhancer (430 - 680)

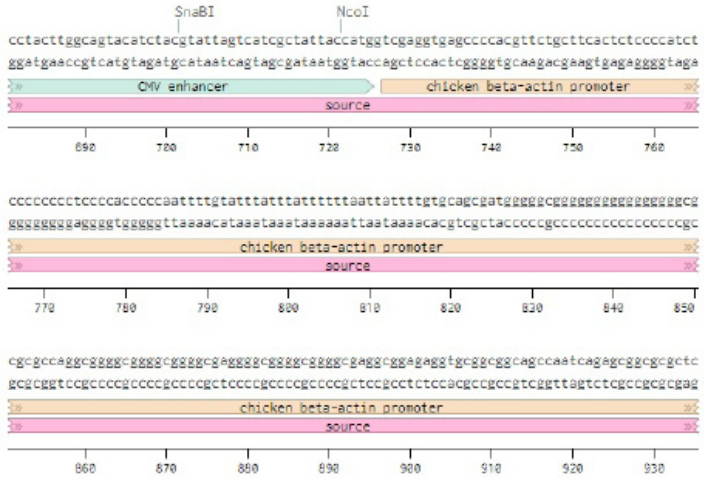

Figure 5: Chicken beta-actin promoter (690 - 930)

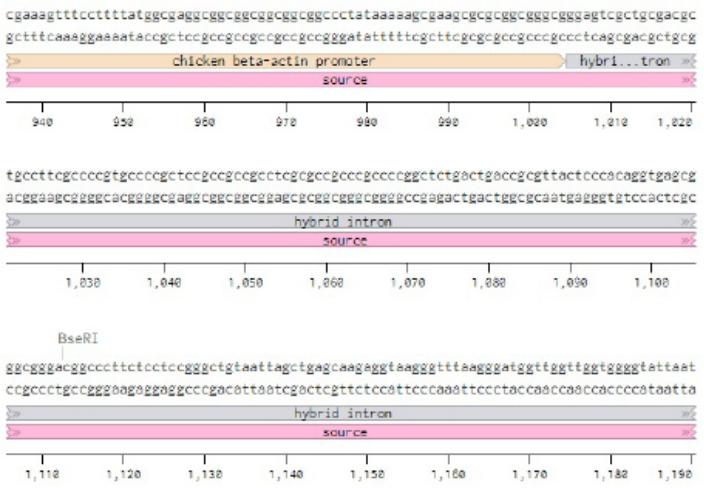

Figure 6: Hybrid intron (940 – 1,190)

Figure 7: 3xFLAG

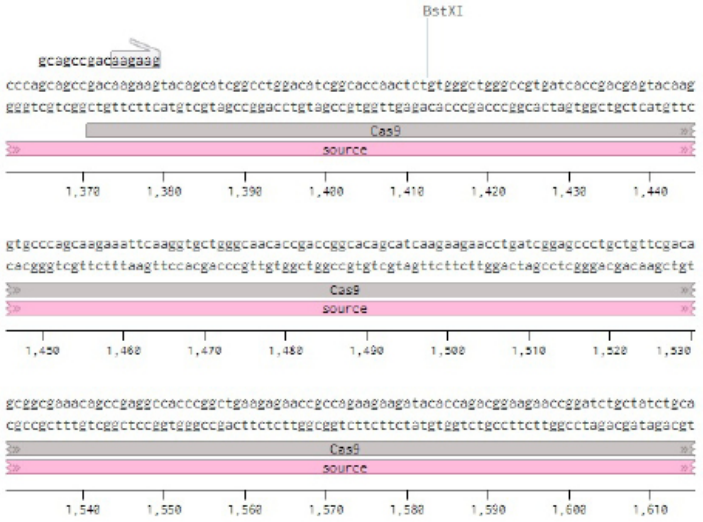

Figure 8: Cas9 (1,370 – 1,610)

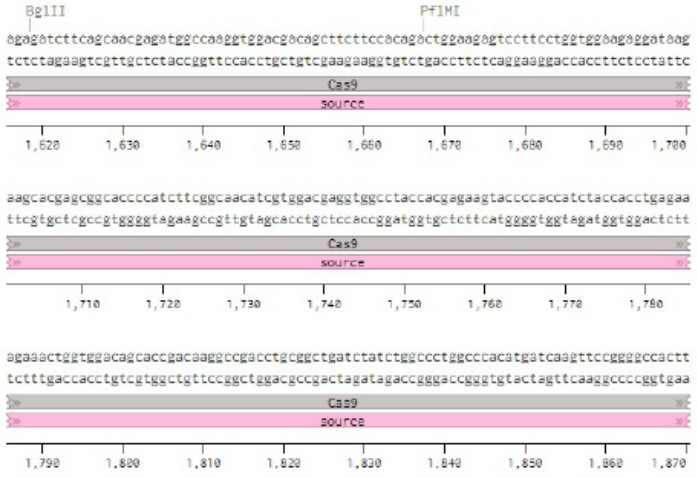

Figure 9: Cas9 (1,620 – 1,870) [BglII]

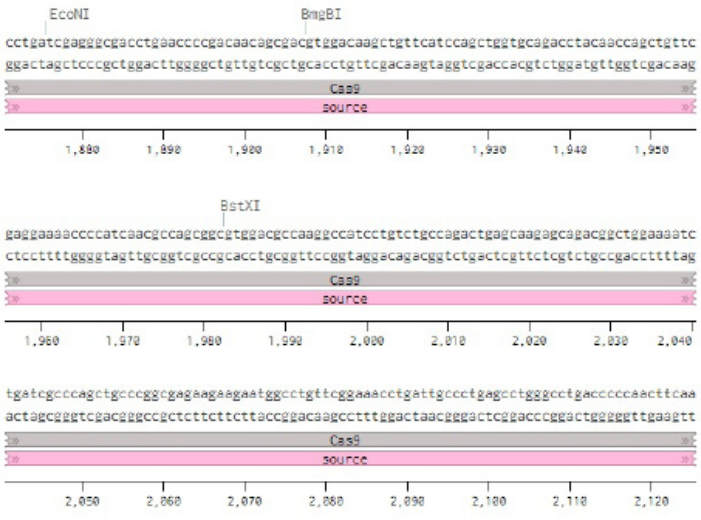

Figure 10: Cas9 (1,880 – 2,120) [EcoNI and BmgBI]

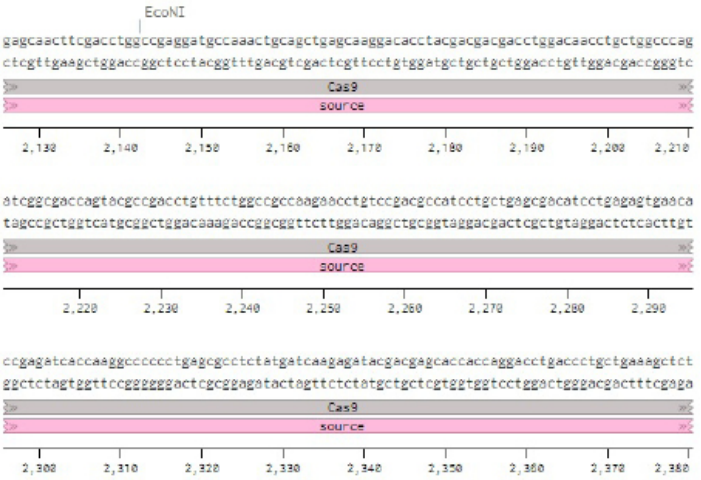

Figure 11: Cas9 (2,130- 2,380) [EcoNI]

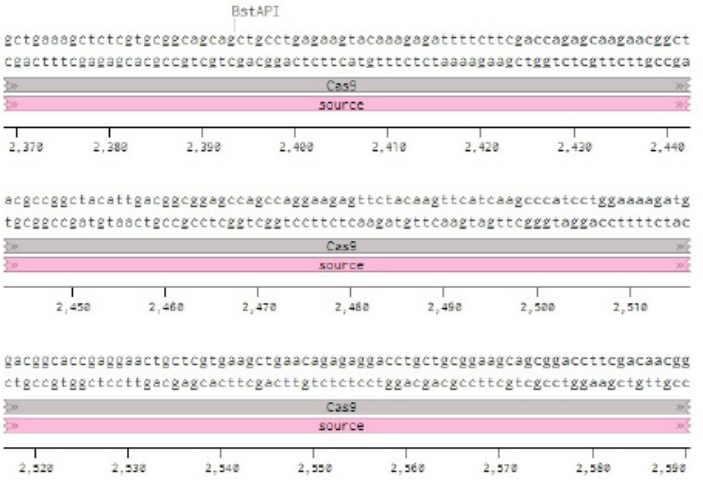

Figure 12: Cas9 (2,370 – 2,590) [BstAPI]

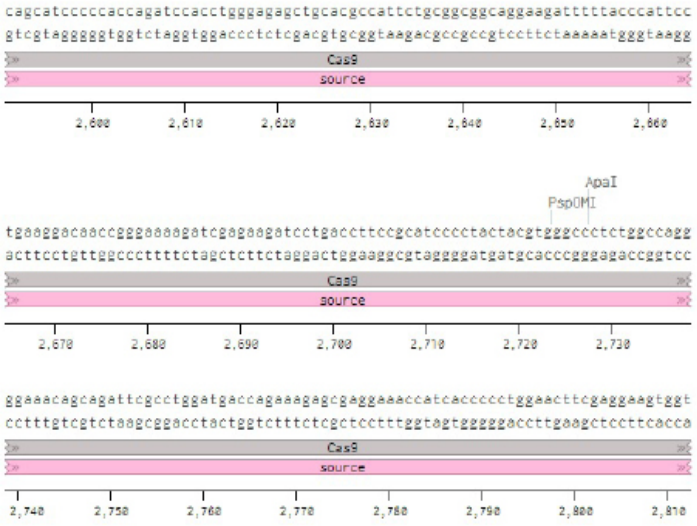

Figure 13: Cas9 (2,600 – 2,810) [ApaI]

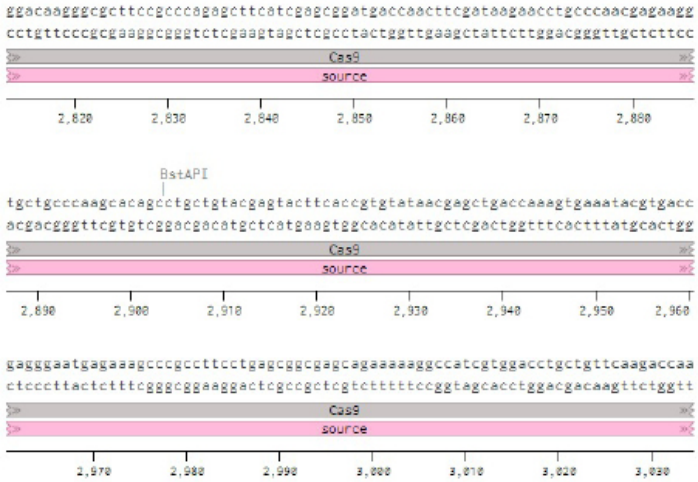

Figure 14: Cas9 (2,820 – 3,030)

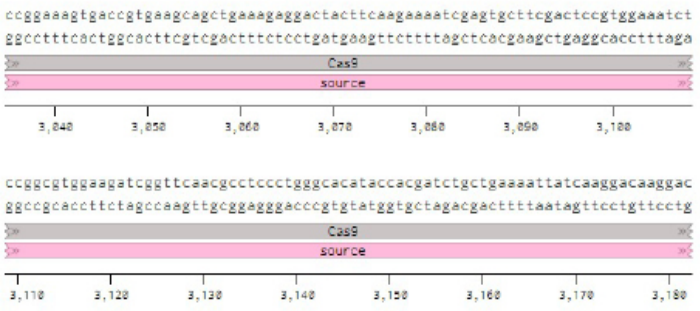

Figure 15: Cas9 (3,040 – 3,180)

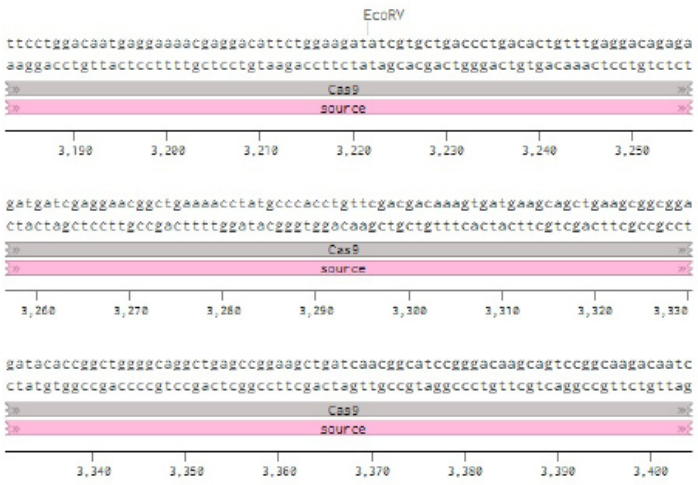

Figure 16: Cas9 (3,190 – 3,400) [EcoRV]

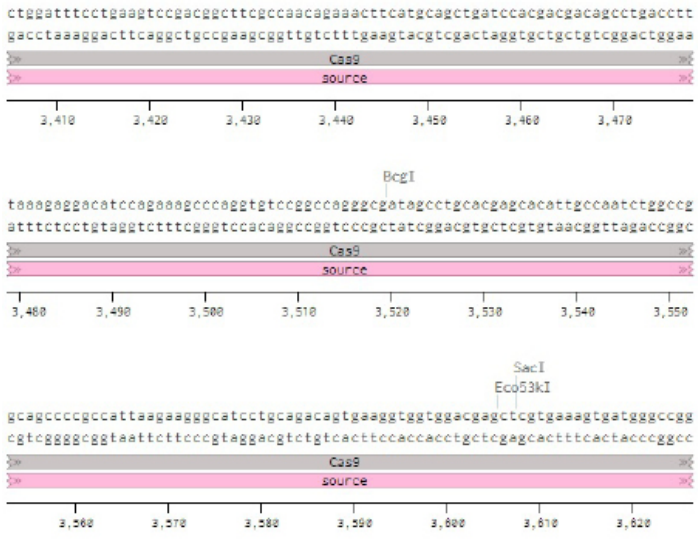

Figure 17: Cas9 (3,410 – 3,620)

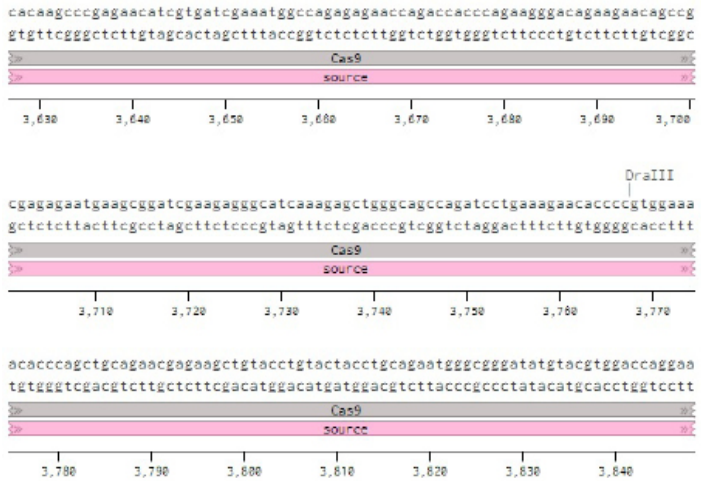

Figure 18: Cas9 (3,630 – 3,840)

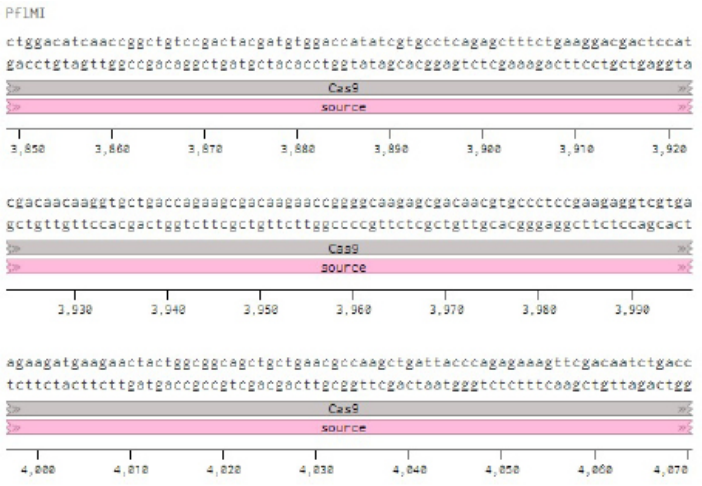

Figure 19: Cas9 (3,850 – 4,070) [pflMI]

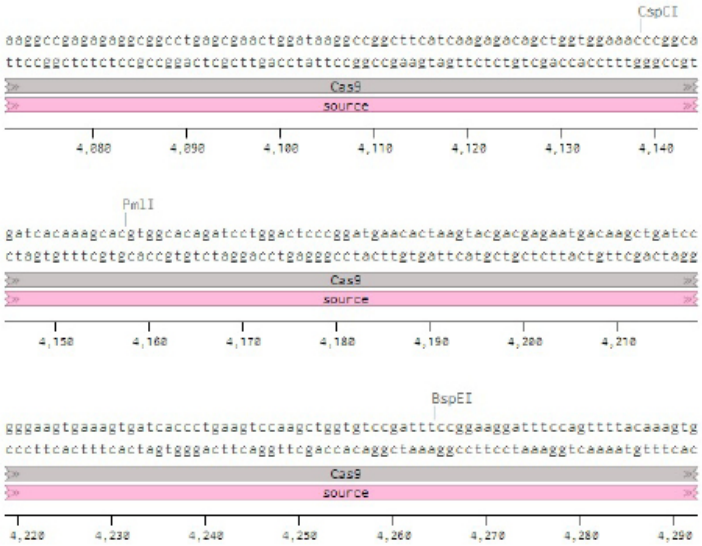

Figure 20: Cas9 (4,080 – 4,290) [PmlI]

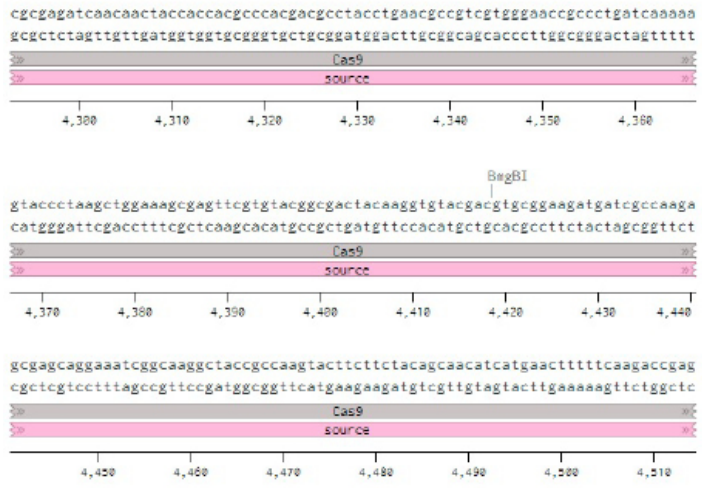

Figure 21: Cas9 (4,300 – 4,510)

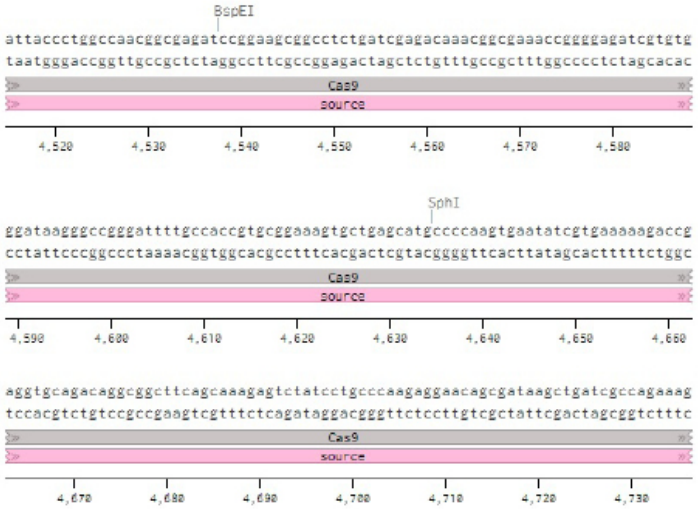

Figure 22: Cas9 (4,520 – 4,730)

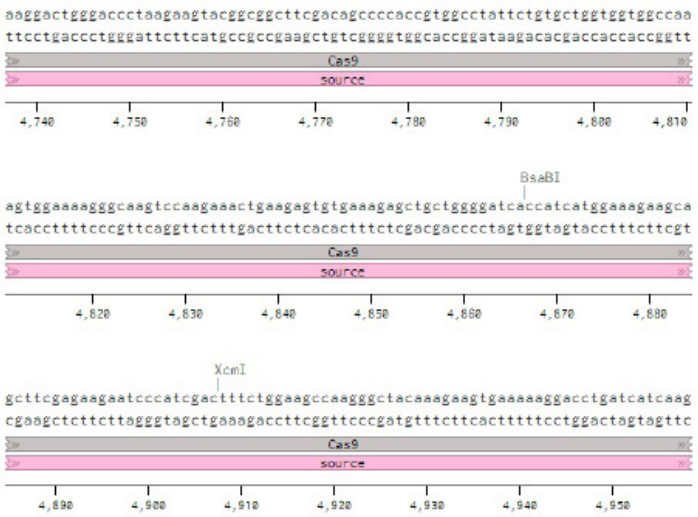

Figure 23: Cas9 (4,740 – 4,950)

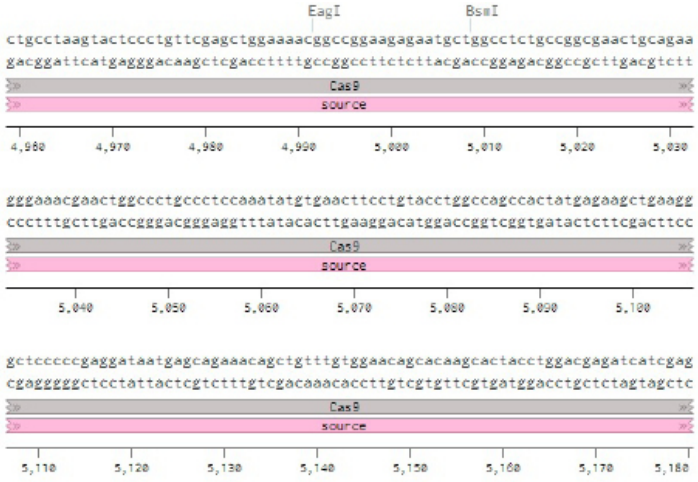

Figure 24: Cas9 (4,960 – 5,180)

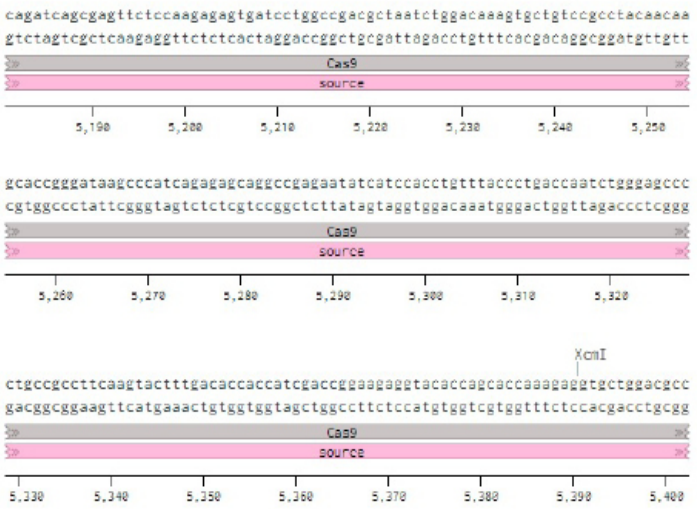

Figure 25: Cas9 (5,190 – 5,400)

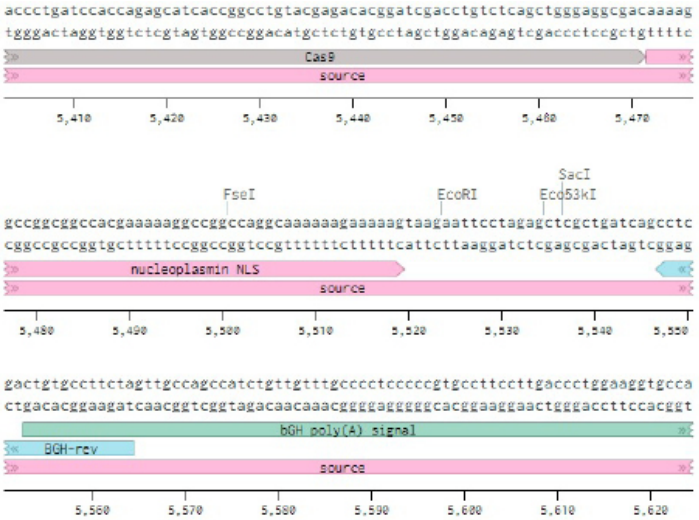

Figure 26: Nucleoplasmin NLS (5,410 – 5,620)

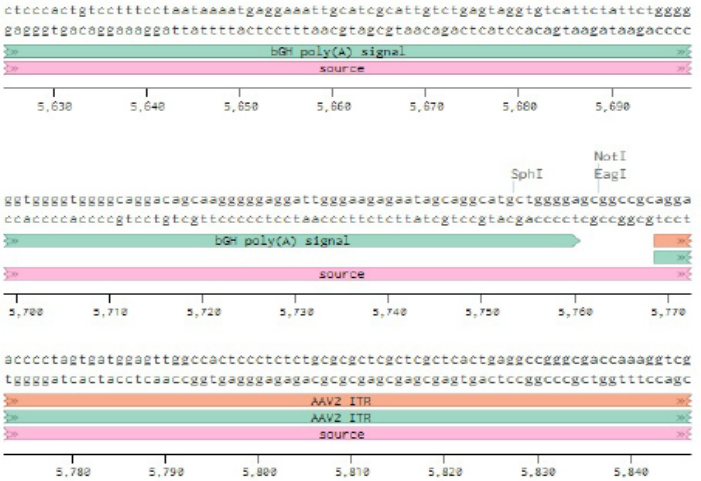

Figure 27: bGH poly(A) signal (5,630 – 5,840)

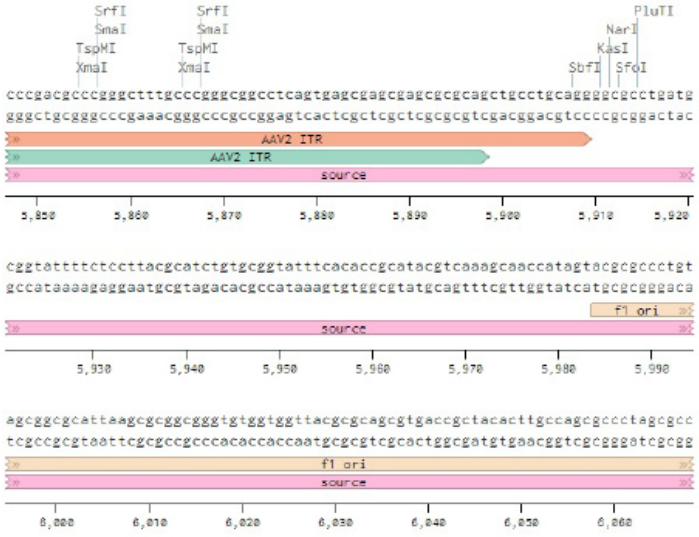

Figure 28: AAV2 ITR (5,850 – 6,060)

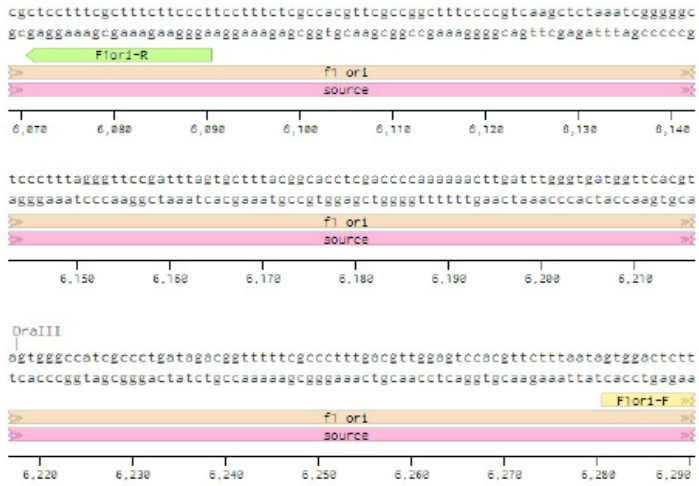

Figure 29: f1 ori (6,070 – 6,290)

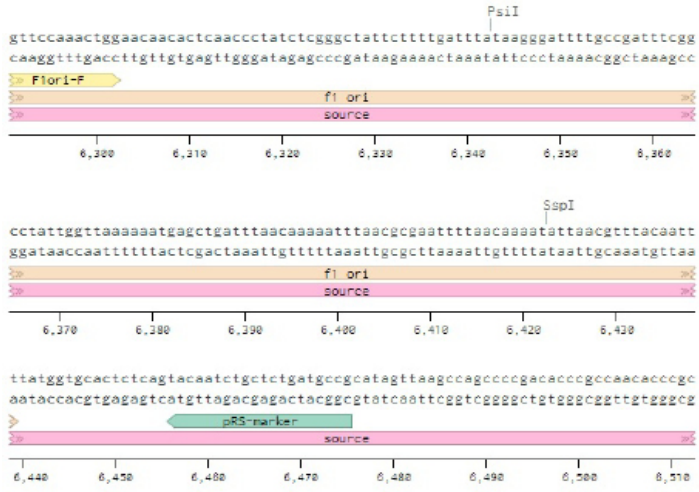

Figure 30: pRS-marker (6,300 – 6,510)

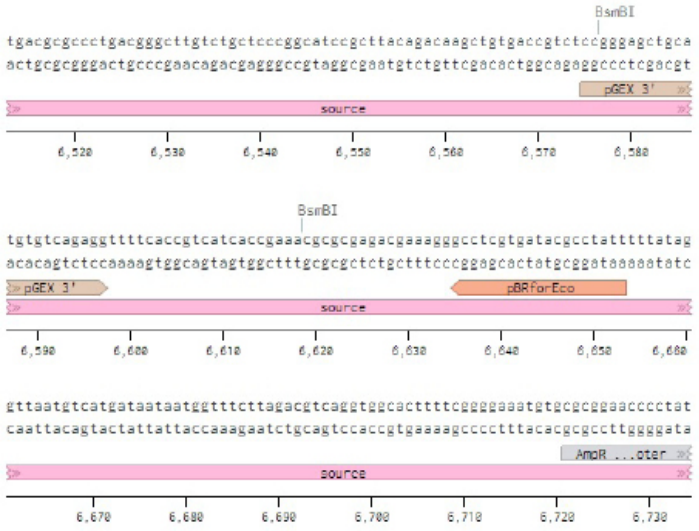

Figure 31: pGEX 3' (6,520 – 6,730)

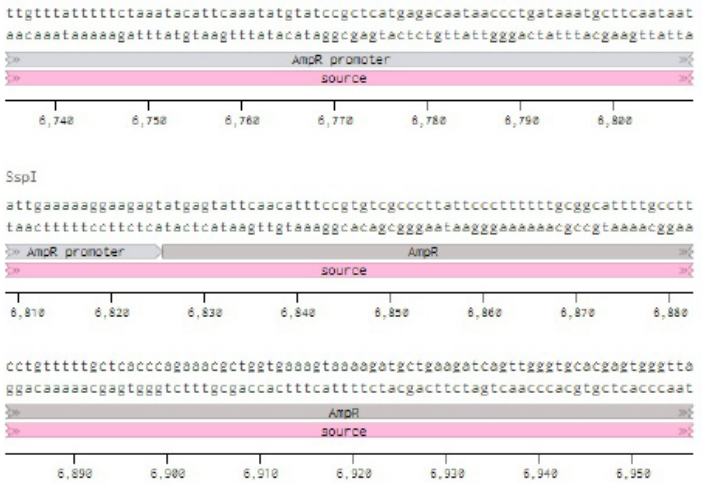

Figure 32: AmpR promotor (6,740 – 6,950)

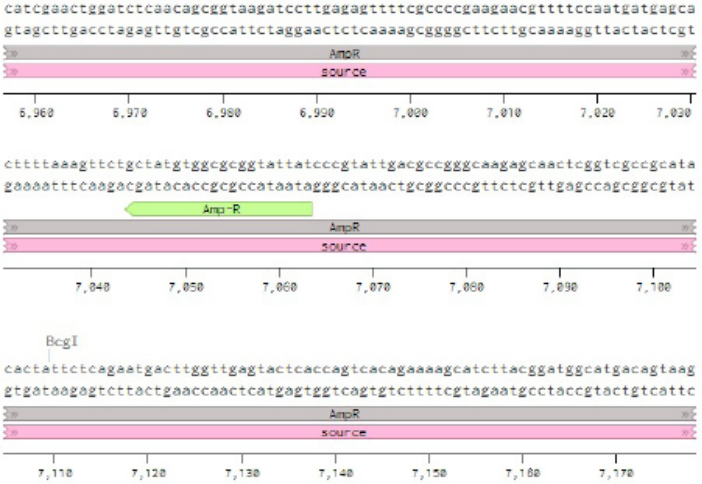

Figure 33: AmpR promotor (6,960 – 7,170)

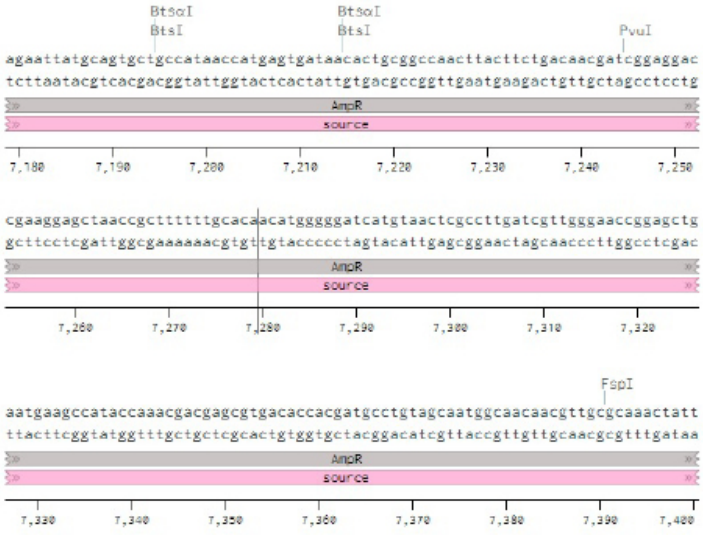

Figure 34: AmpR promotor (7,180 – 7,400) [BtsI]

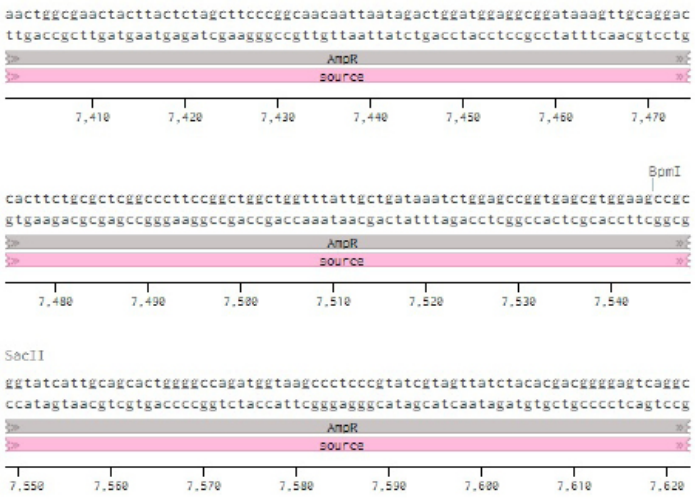

Figure 35: AmpR promotor (7,410 – 7,620)

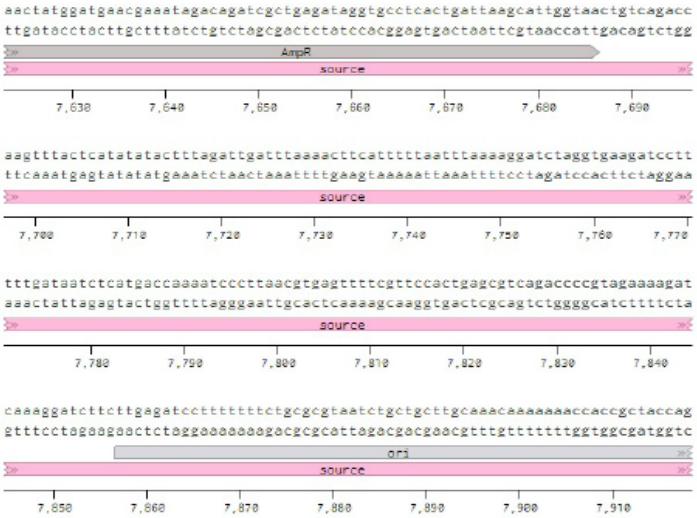

Figure 36: AmpR promotor (7,630 – 7,910)

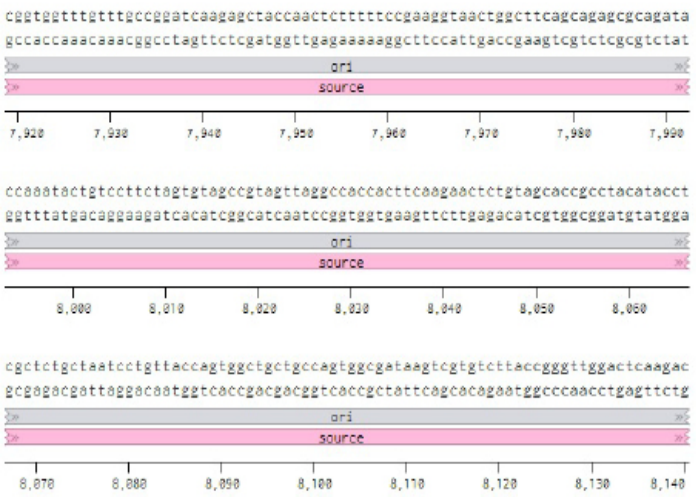

Figure 37: ori (7,920 – 8,140)

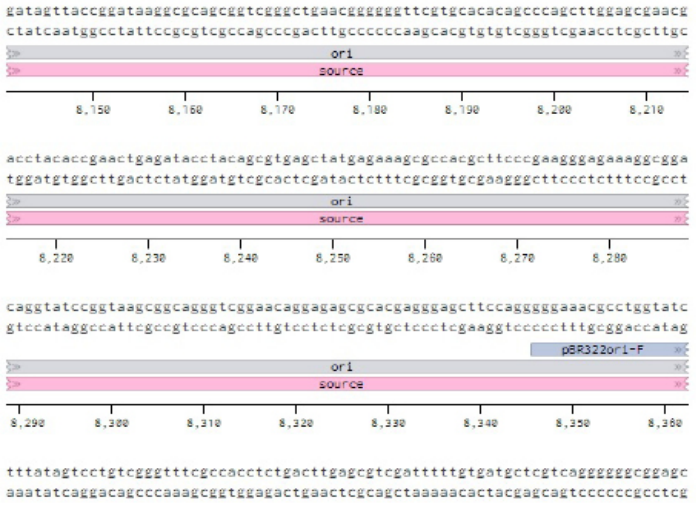

Figure 38: ori (8,150 – 8,360)

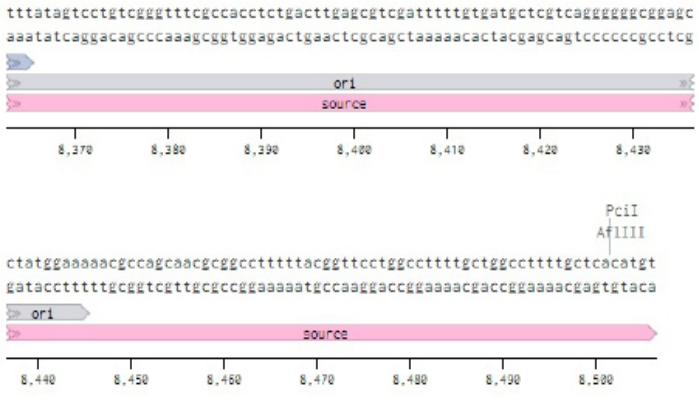

Figure 39: ori (8,370 – 8,500)

Conflicts of Interest

There is no conflict of interest as per Author's point of view.

Acknowledgements

Author would like to thank Prof. Navarun Gupta for their academic support. Author also thanks anonymous reviewers for their comments.

CONCLUSION

Below is the sequence discussed on high level of

1. BstXI, BglII, Pf1MI
2. EcoNI, BmgBI, BstXI
3. EcoNI
4. BstAPI
5. PspOMI
6. ApaI
7. BstAPI
8. EcoRV
9. BcgI
10. Eco53kI, SacI
11. DraIII
12. Pf1MI
13. CspCI, Pm1I
14. BspEI
15. BmgEI
16. BspEI, SphI
17. BsaBI
18. XcmI, EagI, BsmI
19. XcmI
20. nucleoplasmin NLS, BGH-rev, FseI, +3
21. bGH poly(A) signal, +19
22. fl ori, F1ori-F, DraIII, PsiI
23. pRS-marker, SspI
24. pGEX 3', pBRforEco, +2
25. AmpR promotor
26. SspI
27. BcgI
28. BtsI, BtsαI, BtsI, +2
29. FspI
30. BpmI
31. SacII
32. ori
33. pBR322ori-F
34. hU6-F, Af1III, PciI
35. U6 promotor, LK0.15', +3
36. gRNA scaffold, XbaI, +2
37. CMV enhancer, NdeI
38. chicken beta-actin promotor, +2
39. hybrid intron, +6

References

1. Addgene (2020) Addgene: Zhang lab CRISPR. https://www.addgene.org/crispr/zhang/
2. Benchling (2019) https://benchling.com/
3. pX330-U6-Chimeric_BB-CBh-hSpCas9 was a gift from Feng Zhang (Addgene plasmid # 42230; http://n2t.net/addgene:42230; RRID: Addgene_42230)