Investigating the Relationship between CARM1 and CBP within RL Human Lymphoma Cells

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Introduction

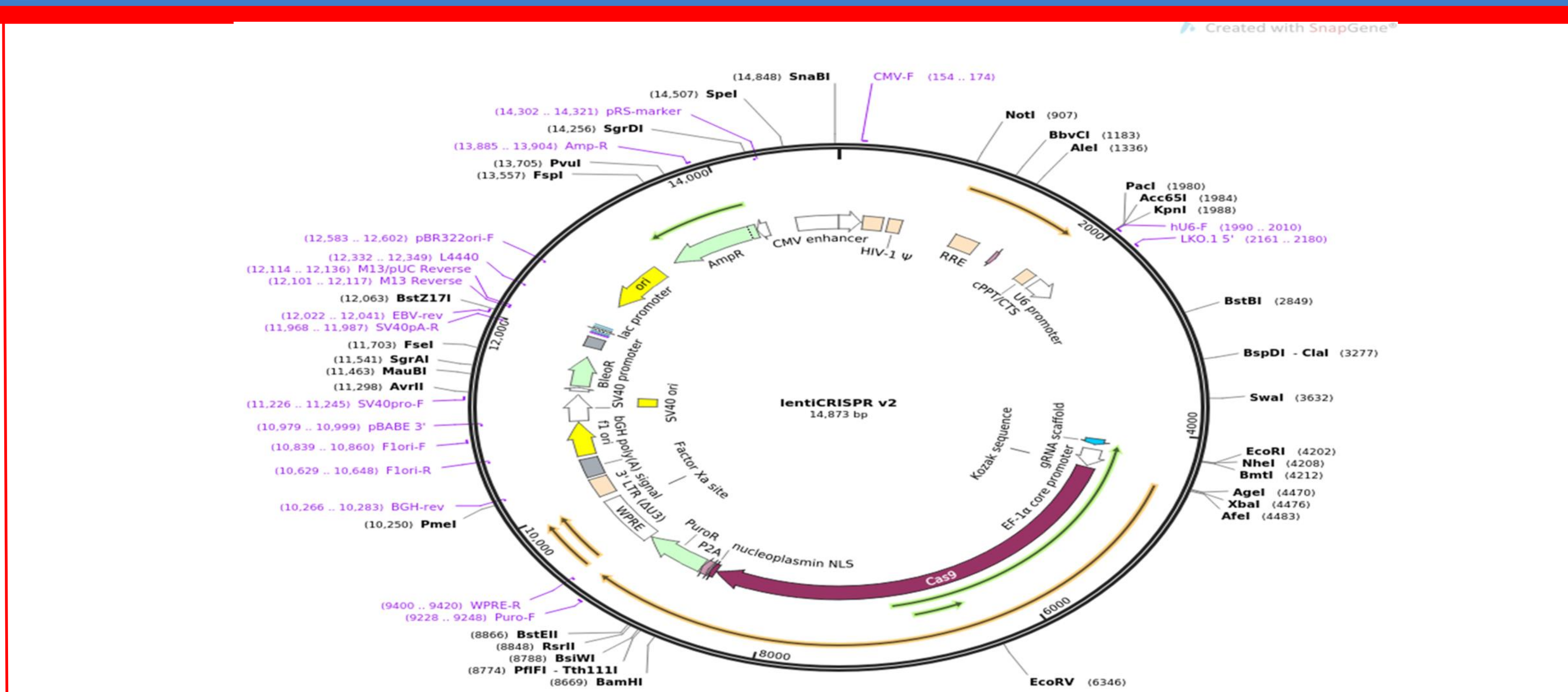
Human lymphoma cells show an increased mutation in the protein CBP. To understand the relationship between CBP and CARM1 they are both investigated by having their gene expression lowered. It has been noted that within lymphoma cells CBP is mutated and for the cell survival CARM1 methylates CBP. Within this experiment, RL WT and RL CBP KO cells are examined.

Objective

The goal behind this study is to further understand the relationship between CBP and CARM1 by creating CARM1 CRISPR KO cells.

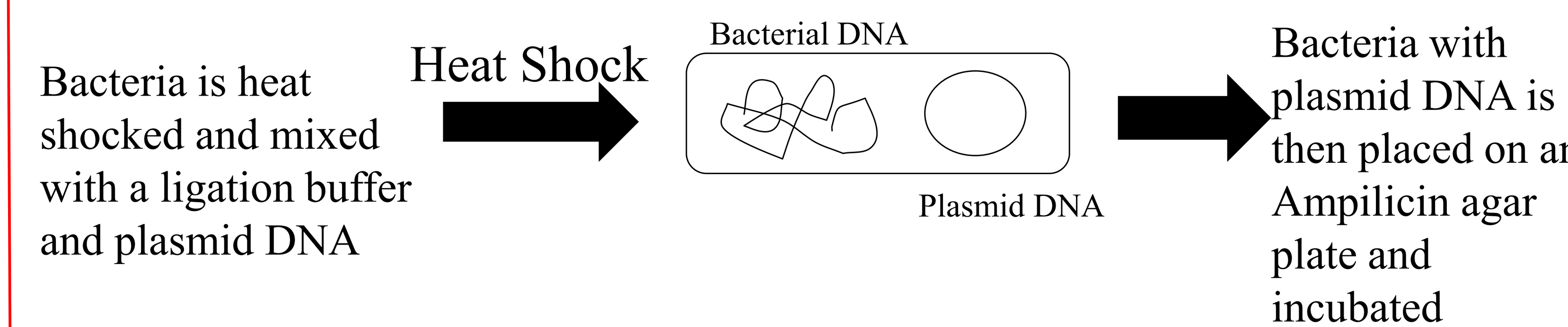
Methods

- E. Coli cells are harvested using LB Media. As their colony grows, they will be transferred into four conical tubes.
- Through these tubes, they will have their DNA mixed with four different kinds of RNA oligos
- Plasmid DNA is purified, with the filler extracted from an agarose gel
- The plasmid DNA is dephosphorylated and opened for receiving the oligo
- The oligo and plasmid DNA are annealed together
- The COS-1 cell and new oligo plasmid DNA are mixed, creating the start of the lentivirus
- The lentivirus is then incubated with RL human lymphoma cells along with puromycin for a week
- After puromycin selection, the cells can be tested using T7E1 Assay and Western blot to determine the efficiency of lentiCRISPRv2 and its guide RNA

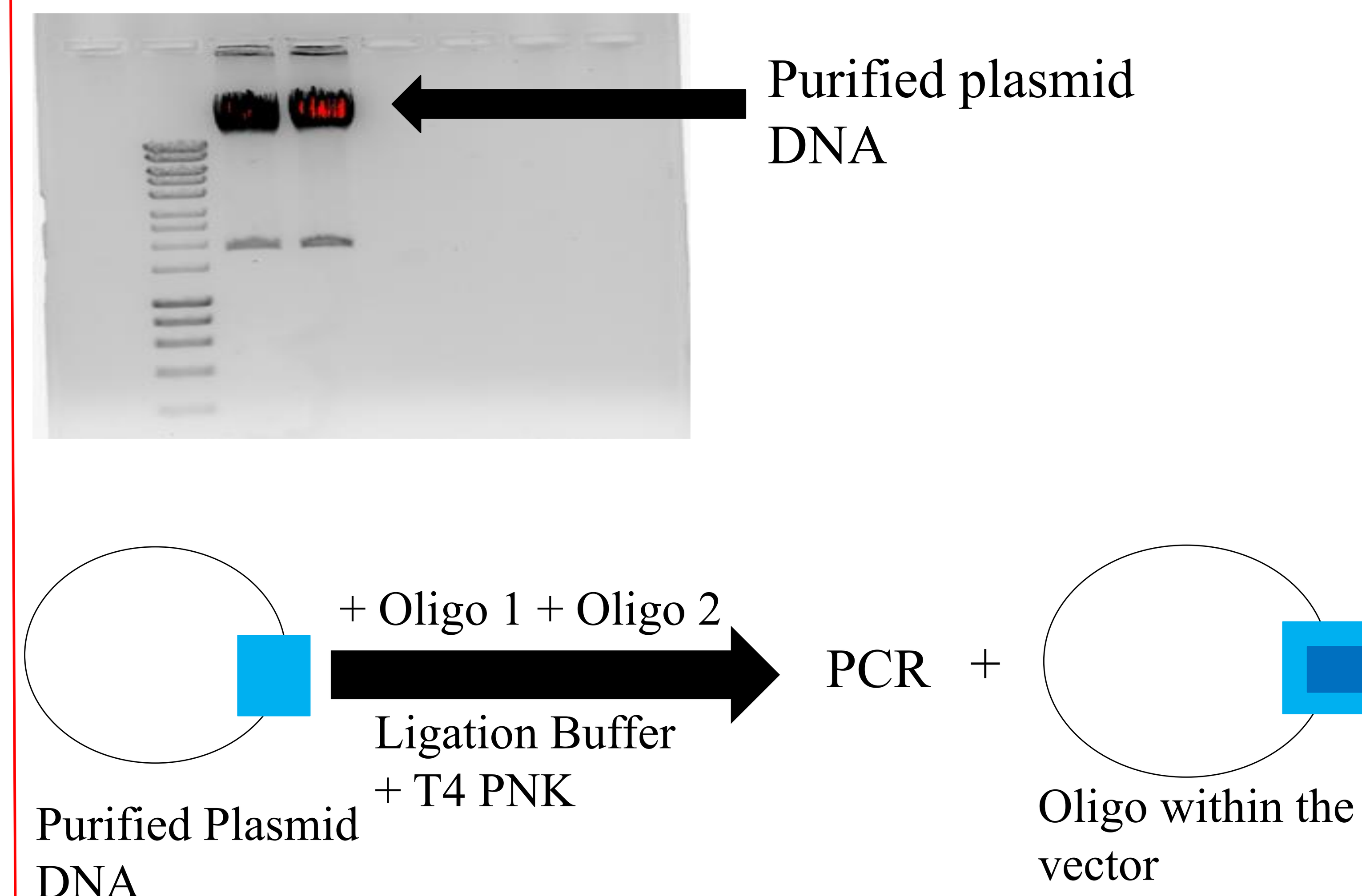


This is the lentiCRISPRv2 plasmid. Within the bottom right region, the Cas9 mechanism, Puromycin resistance gene and filler can be seen.

Bacteria Transformation



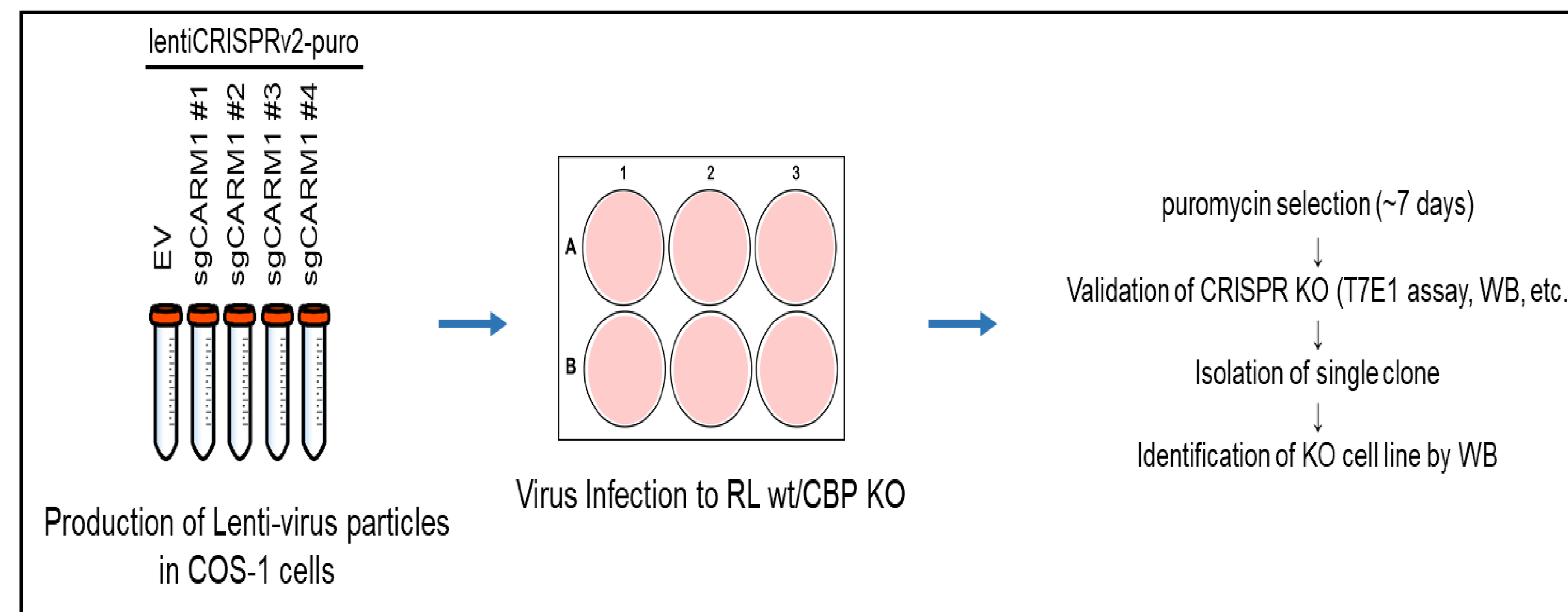
Annealing Oligos Inside Plasmid



DNA Sequencing of Annealed Oligo Inside Plasmid

Depicted are the four gCARM1 oligo sequencing. The U6 Promoter, gRNA scaffold and polIII terminator can be seen.

lentiVirus Protocol



After the creation of the lentivirus using Cos-1 cells, the media is extracted and placed in two six well plates. The first six well plate is RL WT cells and the second is RL CBP KO.

Conclusions

The puromycin selection went successful, with the negative control being eradicated after the first week. The surviving RL cells are the ones with the lentiCRISPRv2 plasmid, meaning the living cells have been correctly transfected with the puromycin resistance gene. To test the accuracy of the four guide RNA's, the cells will be placed in a T7E1 assay and Western Blot. T7E1 Assay is used to investigate the efficiency of the guide RNA's. This is done by amplifying the strands of DNA. It does this by recognizing strands of DNA that were not perfectly matched before cleaving them. These strands of DNA can be amplified by PCR. Each colony will be created from a single cell clone, which means there will be four colonies, but each colony comes from one single cell clone. These clones will be investigated using western blot. WB will test for CARM1 and CBP.

References

-Veazey KJ, Cheng D, Lin K, et al. CARM1 inhibition reduces histone acetyltransferase activity causing synthetic lethality in CREBBP/EP300-mutated lymphomas. Leukemia. 2020;34(12):3269-3285. doi:10.1038/s41375-020-0908-8

-Sanjana NE, Shalem O, Zhang F. Nat Methods. 2014 Aug;11(8):783-4. doi: 10.1038/nmeth.3047

-Drew, A.E., Moradei, O., Jacques, S.L. et al. Identification of a CARM1 Inhibitor with Potent In Vitro and In Vivo Activity in Preclinical Models of Multiple Myeloma. Sci Rep 7, 17993 (2017).

-Jee Won Hwang (2022),