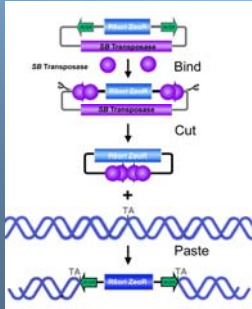


SB-Transposition: MAR & SV40 excision assay

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Introduction



Sleeping Beauty transposon (SB-Tn) is an effective system that incorporates genes into vertebrate (human) chromosomes without using viruses.

Matrix-attachment regions contribute to retention of the plasmid in the host nucleus by interacting with the host nuclear proteins.

SV40 enhancer promotes a high translocation rate of the SB-Tn vector into the host nucleus.

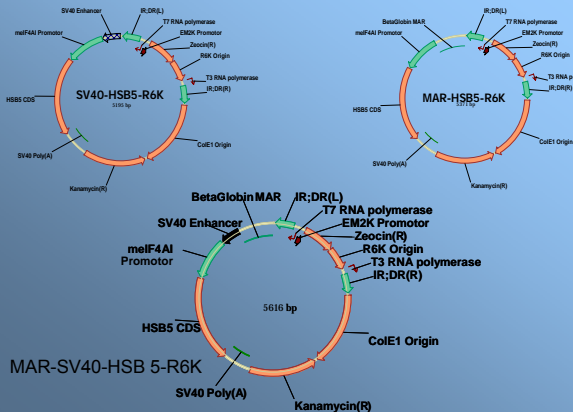
Hypothesis

- Excised and intact plasmid will only retain in host cells after several days of transfection
- SB transposon would not be expressed after all excised and intact plasmids are not present in cells.
- Higher concentration of intact plasmids in SV40 transfected cells compared to MAR
- Higher concentration of excised plasmids in MAR transfected cells after several days of transfection

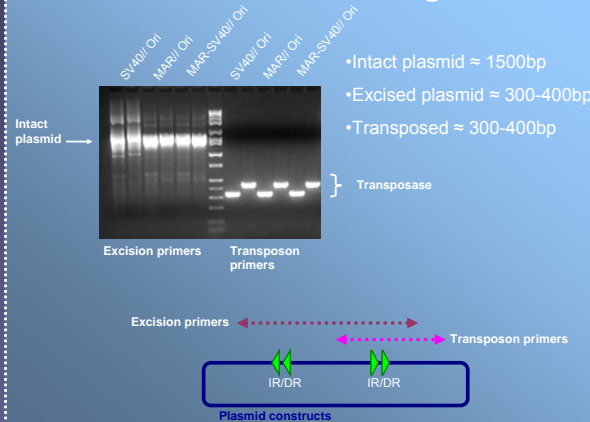
Purpose

- Addresses if vector backbone modifications influence persistence of the original vector and/or excision product.
- Want to determine the SB transposase activity by the host cell after the desired insertion of the SB-Tn carrying the therapeutic gene.
- Continued expression of the SB-transposase might potentially lead to harmful transposition activity remobilizing the SB-Tn integrated in the host genome.

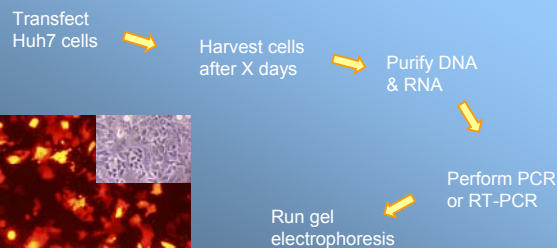
Plasmid Constructs



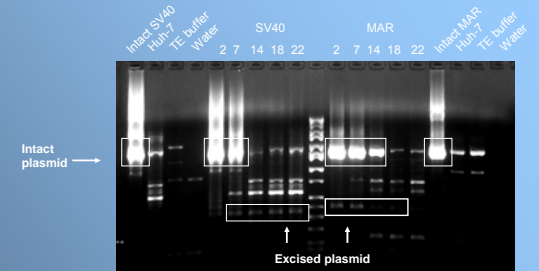
PCR Primer Design



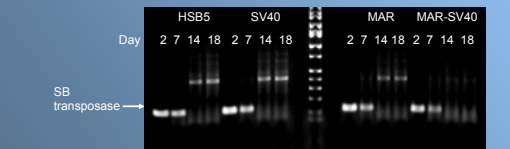
Methods



Results



Day	2	7	14	18	22
Excised SV40	--	✓	✓	✓	✓
Intact SV40	✓	✓	--	--	--
Excised MAR	✓	✓	--	--	--
Intact MAR	✓	✓	✓	--	--



• No SB transposase expression after 14th day of transfection in all plasmid vectors.

• β-actin (housekeeping gene) expression is constant in all cells, indicating mRNA expression is constant in all of the cells.

Discussion & Conclusion

- Intact and excised plasmids eventually secreted out from host cells, except for excised SV40 plasmid.
- No continued expression of SB-transposon after 2 weeks of transfection.
- Unable to determine ratio of intact SV40 to MAR concentration from visual interpretation of gel electrophoresis.
- Excised SV40 plasmid retains in host cell longer than excised MAR plasmid.
- Future steps: determine how does MAR-SV40 plasmid differ in intact and/or excised plasmid retention.

Acknowledgements

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