A plasmid-launched, highly tractable coronavirus reverse genetics system

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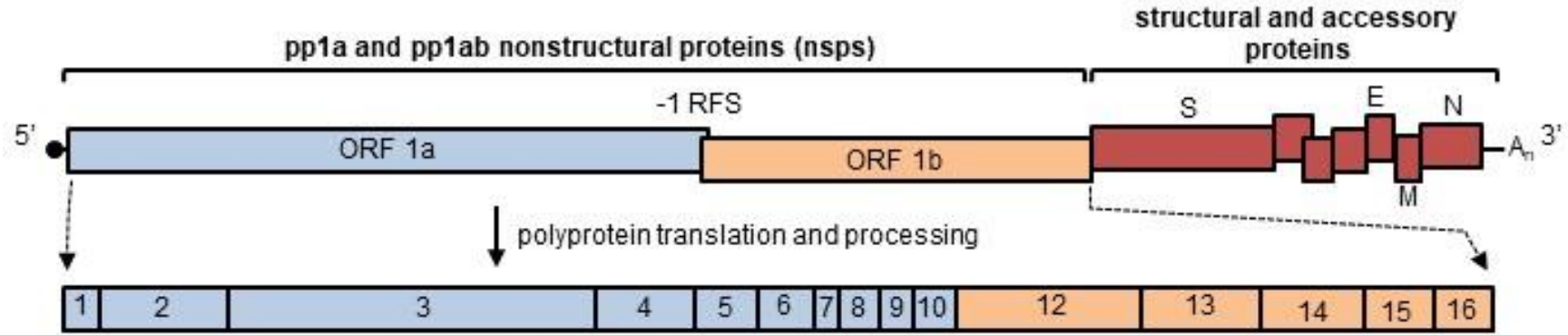
Future coronavirus zoonoses are almost certain

Endemic human coronaviruses	**Three severe CoVs in 20 years**	**Coronaviruses are everywhere**
HCoV-HKU1 (Unkwn)	SARS-CoV-2 (2019)	Birds and bats
HCoV-OC43 (~1890)	MERS-CoV (2012)	Cats and dogs
HCoV-229E (~1750)	SARS-CoV (2002)	Domestic swine
HCoV-NL63 (~1350)		Domestic cattle
		And more!

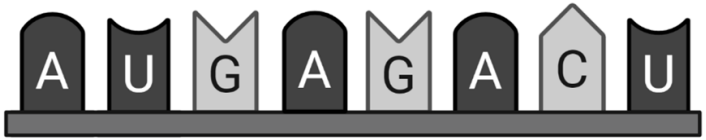
Graham et al. (2013). *Nature Reviews Microbiology*, 11(12), 836–848.

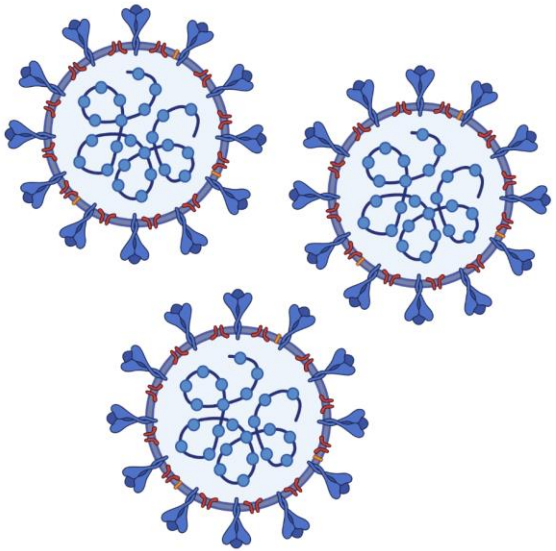
Coronaviruses have the largest-known positive-sense, single-stranded RNA non-segmented genome

+ssRNA is functionally mRNA

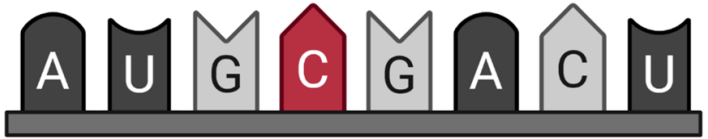


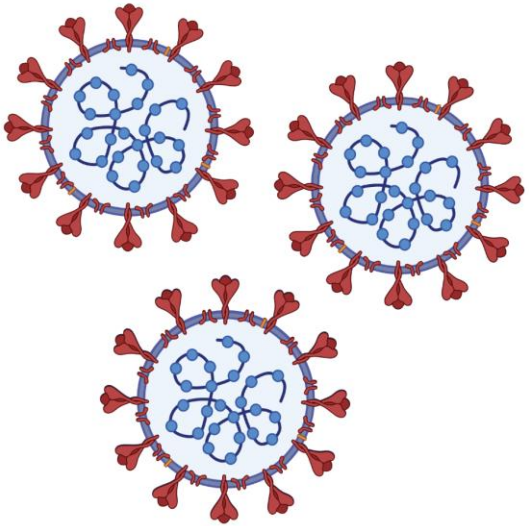
Reverse genetics allows us to go from genome to viral particles





Reverse genetics allows us to introduce mutations that change viral phenotypes



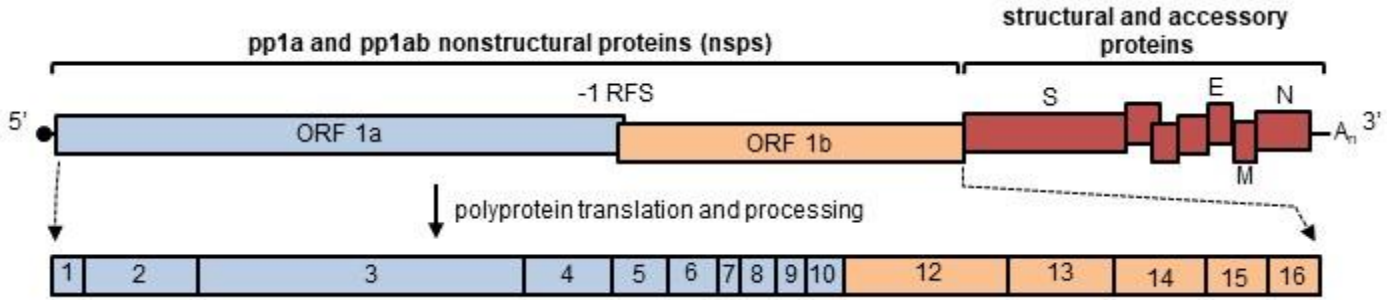


Central Dogma of biology

Coronavirus RNA genomes complicate reverse genetics

RNA ⟶ Protein

Coronavirus genome — positive-sense, single-stranded RNA (functionally mRNA)



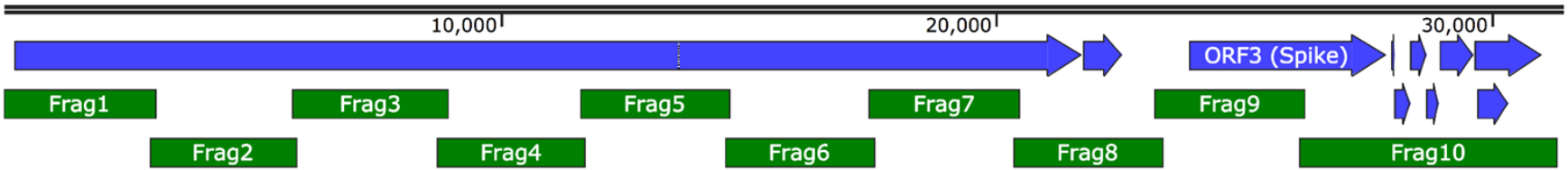
Reverse transcription generates DNA fragments

Viral

DNA ⟵ RNA ⟶ Protein

Fragments

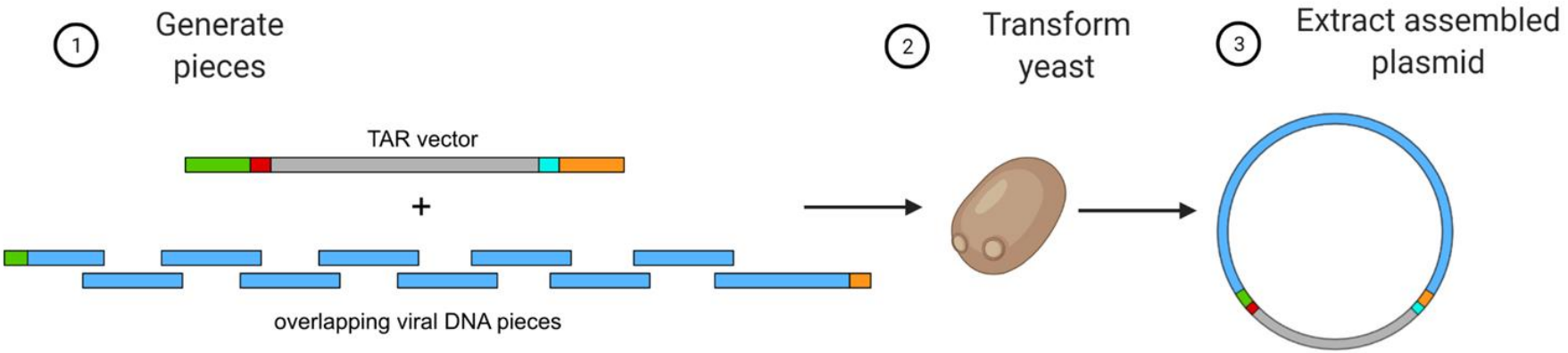
Coronavirus genome — positive-sense, single-stranded RNA (functionally mRNA)



Genome ORFs (blue)
Fragments (green)

MHV-A59 Genome with TAR Fragments
31,409 bp

Yeast quickly assemble plasmids via transformation-associated recombination (TAR)



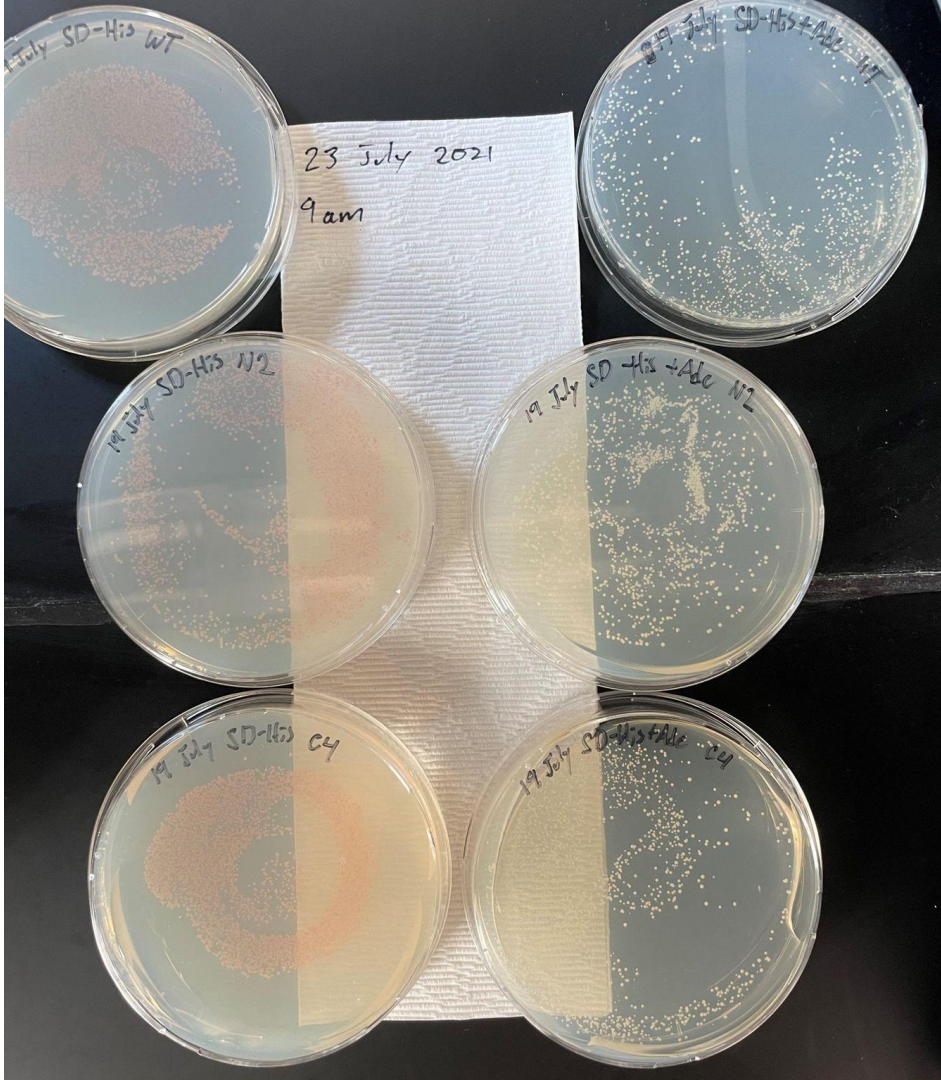
TAR vector with 5’ hook (green), 3’ hook (orange), poly(A) tail (cyan), promoter (red), and elements needed for replication and selection (grey).

MHV-A59 genome (blue) with 5’ hook overlap (green) and 3’ hook overlap (orange).

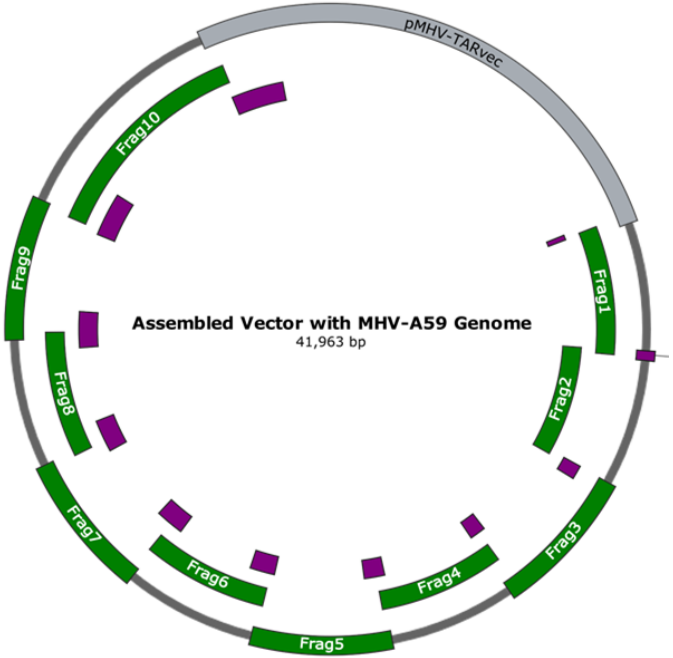
Yeast are selected post-transformation by histidine-deficient media

Our yeast are histidine auxotrophs

The TAR Vector contains the gene for histidine synthesis

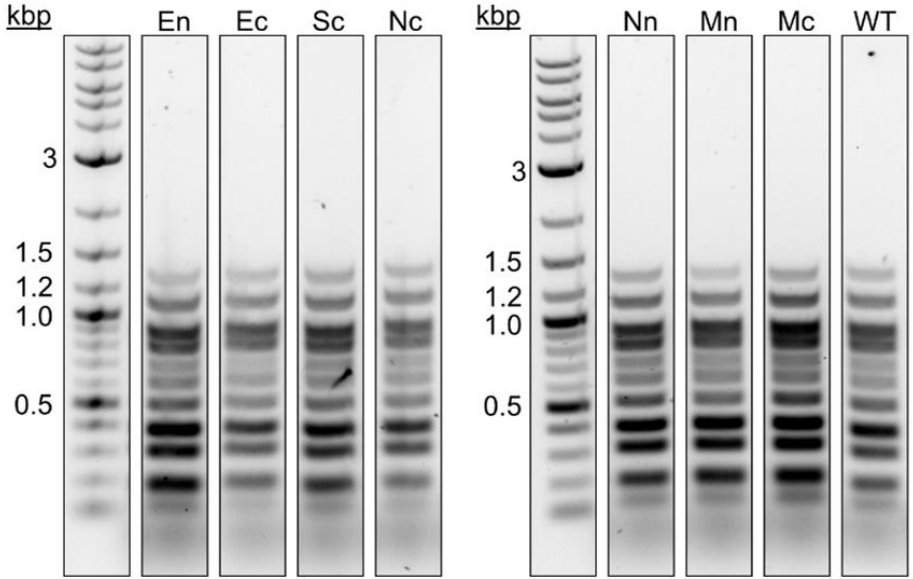


Junction amplicons suggest proper viral fragment assembly with the TAR vector



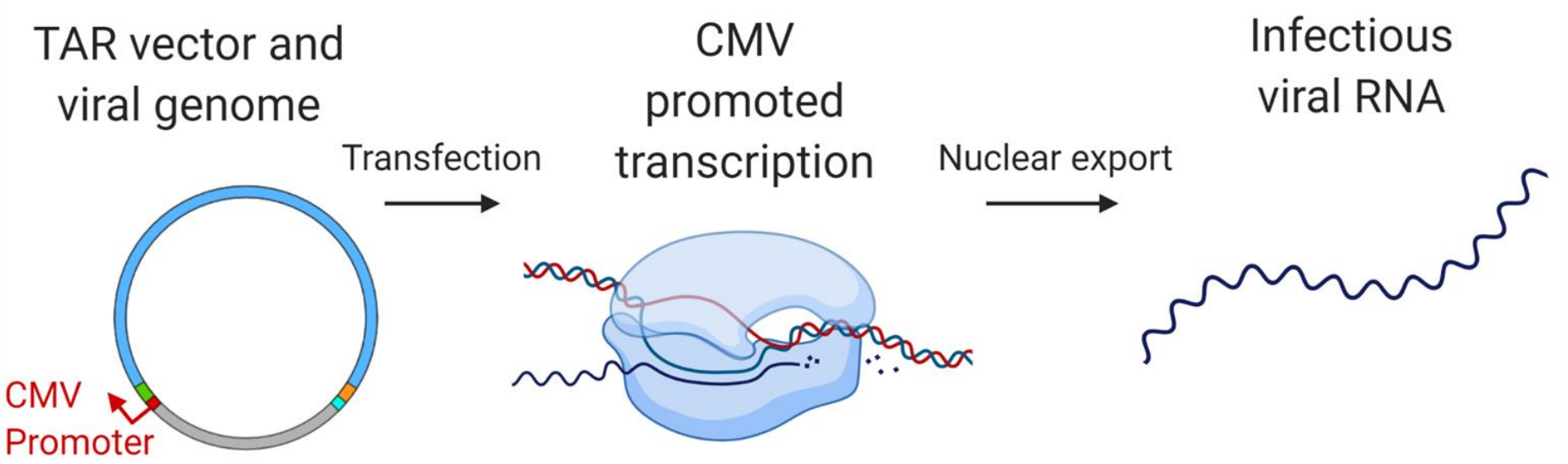
Genome fragments (green), TAR vector (grey), junction amplicons (purple)

Agarose gel of junction amplicons

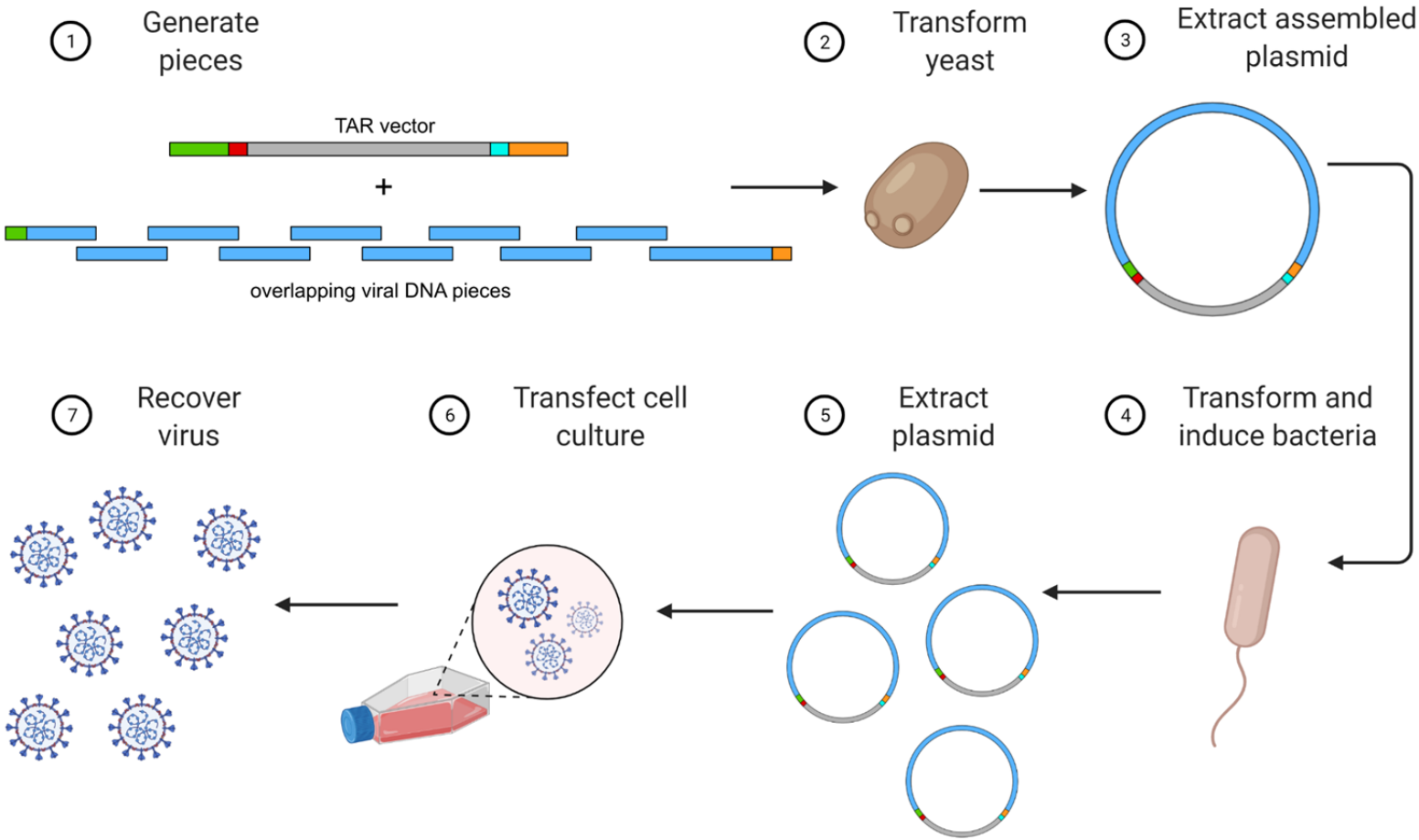


CMV promotion allows for direct transfection of the assembled plasmid to establish infection

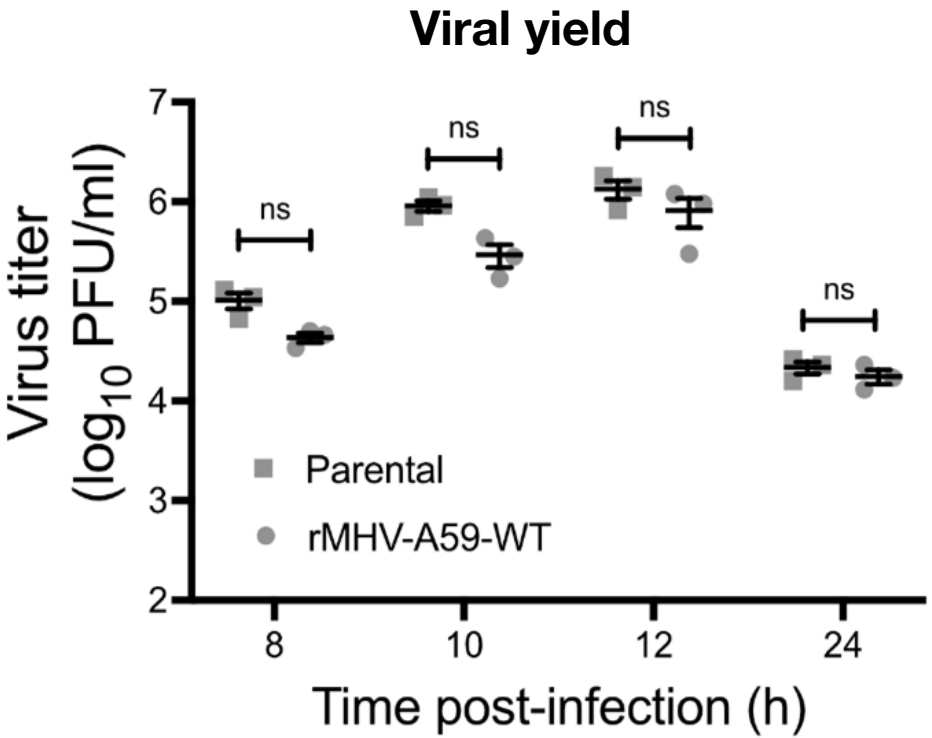
DNA → RNA → Protein



Overview of TAR viral recovery workflow



TAR-generated MHV-A59 has similar viral yield as the parental virus, suggesting biological equivalency



MOI = 1
Statistic: Kruskal–Wallis test

Acknowledgements

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