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# Rapid assembly of MERS-CoV genomes using a yeast-based reverse genetics system

## Inhalt

The Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic in Africa and the Middle East. While MERS-CoV phylogenetic clade C strains circulate among African dromedary camels, clade B strains continuously cause human spillover in the Arabian Peninsula. To enable functional comparison, we used the transformation-associated recombination (TAR)-cloning methodology to clone the full genome of distinct MERS-CoV lineages into yeast-artificial chromosomes. Briefly, 11 primer pairs were designed to bind conserved genomic regions of all MERS-CoV lineages (clades A, B and C), allowing amplification of viral RNA either from camel or human samples. PCR products were assembled into a pCC1-His vector using highly transformable *S. cerevisiae* VL6-48N. After successful yeast transformation, correctly cloned MERS-CoV genomes were amplified in *E. cloni* 10G electrocompetent bacteria and sequences were verified through Oxford Nanopore Sequencing. Plasmids containing MERS-CoV genomes of interest were purified, linearized, *in-vitro* transcribed, and transfected into BHK-J cells. Recombinant MERS-CoV isolates were rescued and purified for subsequent functional assays. Overall, this method allows to quickly generate recombinant MERS-CoVs within 2-3 weeks. Moreover, it faciliates complex site-directed mutagenesis in individual genomic regions, enabling rapid characterization of new MERS-CoV variants emerging in camels and humans.

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## Professional Status of the Speaker

PhD Student

## Junior Scientist Status

Yes, I am a Junior Scientist.

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