Virologie 2025, 29 (6) : 77-88

# Contribution of yeast to synthetic virology

## *Contribution de la levure à la virologie synthétique*

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*Article accepted on 15 October 2025*

**Abstract.** The yeast *Saccharomyces cerevisiae* has long been an essential model in molecular biology. Its exceptional recombination capacity and efficiency in propagating large DNA fragments surpass those of organisms such as Escherichia coli or *Bacillus subtilis*. These properties make *S. cerevisiae* the preferred choice for cloning and manipulating bacterial and viral genomes. Among the available methods, transformation-associated recombination (TAR) cloning has established itself as a reliable and effective approach for isolating and manipulating large DNA molecules. To date, numerous viral genomes have been successfully cloned in yeast. The ability to modify these cloned genomes and reconstitute infectious viral particles highlights the role of *S. cerevisiae* as a powerful bioengineering platform. Its versatility addresses various biomedical needs, including virus research, vaccine production, and gene therapy. Combining efficiency, reliability, and flexibility, *S. cerevisiae* stands out as an invaluable tool for advancing biomedical applications and tackling modern biological challenges.

**Key words:** synthetic virology, entire viral genomes, saccharomyces cerevisiae, recombination, "rescue"

**Résumé.** La levure *Saccharomyces cerevisiae* est depuis longtemps un modèle incontournable en biologie moléculaire. Sa capacité exceptionnelle de recombinaison et son efficacité à propager de grands fragments d'ADN surpassent celles d'organismes comme *Escherichia coli* ou *Bacillus subtilis*. Ces propriétés font de *S. cerevisiae* un choix privilégié pour le clonage et la manipulation de génomes bactériens et viraux. Parmi les méthodes disponibles, le clonage par recombinaison associée à la transformation (*transformation-associated recombination*, TAR) s'est imposé comme une méthode fiable et efficace pour isoler et manipuler de grandes molécules d'ADN. À ce jour, de nombreux génomes viraux ont été clonés avec succès en levure. La possibilité de modifier ces génomes clonés et de reconstituer des particules virales infectieuses illustre le rôle de *S. cerevisiae* comme plateforme puissante de bio-ingénierie. Sa polyvalence permet de répondre à divers besoins biomédicaux, notamment dans la recherche sur les virus, la production de vaccins et la thérapie génique. En combinant efficacité, fiabilité et flexibilité, *S. cerevisiae* se positionne comme un outil inestimable pour faire progresser les applications biomédicales et relever les défis de la biologie moderne.

**Mots clés :** virologie synthétique, génomes viraux entiers, *Saccharomyces cerevisiae*, recombinaisons, sauvetage

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Pour citer cet article : Rey C, Agaoua A, Erbs P. Contribution of yeast to synthetic virology. *Virologie* 2025 ; 29(6) : 77-88 doi:10.1684/vir.2025.1121

## Introduction

Synthetic virology is a rapidly evolving field that applies the principles and tools of synthetic biology to the study and engineering of viruses. These tools are enabling the design, synthesis and manipulation of viral genomes from scratch. The reconstruction of complete viral genome allows researchers to study viral biology with unprecedented precision and create novel tools for biotechnology, vaccine development and gene therapy.

Reverse genetics is a key approach in synthetic virology by the deliberate introduction of specific mutations into a viral genome to study their functional consequences while forward genetic is limited to the observation of natural variations. This approach has transformed our ability to dissect viral replication, pathogenesis, and host interactions. It has been used for a wide range of DNA and RNA viruses, understanding viral evolution, designing attenuated vaccine strains, constructing chimeric virus or facilitating the rescue of viruses from complementary DNA (cDNA) and synthetic genomes. Synthetic virology serves as a foundational tool for reverse genetics, enabling the functional testing and propagation of entirely synthetic or heavily modified viral genomes in host cells. It is reshaping our approach to virus research, offering powerful platforms for both fundamental discovery and applied innovation.

In synthetic virology, model organisms play a pivotal role in the development of new technologies. Laboratory essentials such as *Escherichia coli* and *Saccharomyces cerevisiae* have been largely used as hosts for genetic material propagation and modification. Despite a great interest, the complexity of manipulating large viral genomes had long been a barrier in virological research. However, gradually these organisms have been harnessed to enable the cloning and engineering of full-length viral genomes, advancing the field considerably.

Bacterial artificial chromosomes (BACs) are specially engineered DNA molecules used to clone and maintain large fragments of DNA in bacteria, typically *E. coli*. BACs are man-made plasmids designed to carry very large inserts of DNA up to about 300,000 base pairs [1]. BACs were employed to clone numerous viral genomes, such as herpesviruses (HSV), poxviruses and adenoviruses [2-5]. Still, cloning viral genomes presents significant challenges. In their study, Cao and colleagues suggested that coronaviruses are difficult to clone and manipulate in *E. coli* due to the large size of their genomes [6, 7]. Structural complexity and high G/C contents were also highlighted for the cloning inefficiency of herpesviruses [8, 9]. An additional limitation of cloning in bacteria is its inability to allow the simultaneous manipulation of multiple genome loci [10]. Multiple changes in the cloned genome greatly decrease the timeframe of making mutant viruses [11]. More frequently, the construction of cDNA clones have been hampered by the instability of some full-length viruses' sequences in *E. coli* such as flaviviruses, herpesviruses and coronaviruses [12-15]. This apparent instability can be in some cases related to the toxicity induced by full-size genomes during their propagation in bacteria. Some of produced viral proteins of Potyviruses and Flavivirus are unstable due to their toxicity [16, 17]. In the case of zika virus, cryptic bacterial promoters in the viral genome allow the expression of toxic viral proteins reaching a toxic concentration level with standard high-copy-number plasmids [18]. To overcome the toxicity and improve the stability of full-length cDNA clones several approaches have been developed such as low-copy number plasmids, reducing the activity of the cryptic prokaryotic promoters [19]. Another approach is to use the incorporation of introns in the genome to disrupt toxic regions [20]. While BACs remain a valuable tool for cloning and manipulating large DNA viral genomes, their use in synthetic virology is somewhat limited by stability issues, size constraints and the rise of more flexible synthetic genome assembly methods that are better suited for certain viruses and synthetic constructs.

Previous study of Amarilla and colleagues demonstrated that full-length severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cDNA could be assembled *in vitro* by a circular polymerase extension reaction (CPER) [21]. Nevertheless, the accuracy of the CPER approach have been proven to be lower than using the BAC approach and induce a risk of unwanted mutations [22]. Evans' team assembled a replicating synthetic-chimeric horsepox virus with vaccinia virus hairpins from Shope fibroma virus-infected cells using chemically synthesized DNA fragments [23]. However, this method is labour-intensive and does not overcome the limitations associated with BAC cloning.

Cloning in yeast offers substantial added value, allowing the current limitations in viral genome cloning to be overcome and presenting significant advantages for biological applications. This review highlights the expanding role of yeast as an efficient platform for propagating and editing viral genomes. It provides a detailed overview of the process, encompassing genome cloning, potential engineering, regeneration of infectious viral particles and applications, while also evaluating the associated benefits and risks.

## In-yeast cloning of complete viral genomes

Yeasts have been known to be living organisms able to take up and recombine several large DNA fragments by homologous recombination [24]. Thanks to their enhanced recombination abilities, fragments with limited sequence homology can be successfully cloned. Therefore, yeasts have long been used to propagate and edit genetic material. In 1978, Hinnen and colleagues reported the transformation of yeast *S. cerevisiae* using plasmid DNA [25]. Shortly afterward, Rothstein team showed that homologous recombination was responsible for DNA molecules integration into yeast chromosomes [26]. Linear DNA molecules up to 400 kbp in size were then cloned into yeast artificial chromosomes (YACs) in the late 1980s [27].

These pioneer works opened the possibility of cloning parts, whole genome or chromosomes from a wide range of organisms. The now called "transformation-associated recombination" (TAR) technique was used for the cloning of bacterial and viral genomes as well as for the selective isolation and maintenance of large eucaryotic human DNA regions in *S. cerevisiae* [28-30]. Thanks to their ability to propagate large DNA fragments more easily than organisms such as *Escherichia coli* or *Bacillus subtilis*, yeast appears as the preferred cellular host for cloning entire bacterial genomes [31, 32]. To construct and propagate an entire bacterial genome in a stable and manipulable form, the TAR technique was employed to assemble the complete synthetic genome of *Mycoplasma genitalium* (583 kbp) within yeast [24]. This pioneering work proved to be instrumental in establishing the foundations of synthetic biology.

### *TAR technology*

TAR cloning refers to all techniques allowing DNA cloning in yeast cells with at least two DNA molecules, the target sequence and the vector. Multiple approaches can thus be used to transform yeasts such as electroporation, lithium acetate transformation or spheroplast transformation. A spheroplast is a yeast cell whose cell wall has been enzymatically removed, commonly using enzymes such as zymolyase, while leaving the plasma membrane intact. These fragile, wall-less cells are more permeable, allowing them to take up foreign DNA. The spheroplast method, developed in the 1970s, was a pioneering technique for introducing large DNA molecules into yeast cells. It played a crucial role in early molecular biology and genetic engineering, particularly in yeast transformation experiments prior to the development of more advanced techniques like electroporation. In recent years, the method has regained importance with the rise of synthetic biology and genome engineering, which often involve manipulating very large DNA constructs that benefit from the capabilities of the spheroplast approach. Depending on the characteristics of the donor organism and genetic material quality and availability, TAR-cloning approach can be carried out with entire native genomes or multiple overlapping fragments, either PCR amplified, synthetized, fragmented genome or previously TAR-cloned. Complete genome cloning can also be performed combining several types of theses genetic input material.

In order to clone DNA segments using TAR approach in yeast, an adapted vector is necessary *(figure 1)*. The team of Larionov showed that total genomic DNA can be co-transformed into yeast cells along with a vector carrying hooks, homologous to the 5′ and 3′ ends of a target DNA region to be cloned [33,34]. *In vivo* recombination can thus occur resulting in the isolation of the DNA region of interest, which then propagates in yeast cells [33]. At least 60 base pairs of overlapping sequences are required to be readily recombined and ligated by homologous recombination in yeast [35]. Noskov and colleagues indeed demonstrated that cloning efficiency drops significantly when the hook size falls below this threshold [34]. Hooks should be unique sequences and cloned into TAR vector in the same orientation as they occur in the targeted genome. In addition to hooks, other common plasmid elements are required in the TAR vector, including a yeast centromere (CEN) of about 125 base pairs to ensure proper chromosome segregation during cell division, a yeast origin of replication (ARS) of usually 100-200 base pairs long, allowing autonomous replication of the vector in *S. cerevisiae* and an auxotrophic selection marker such as HIS3 or URA3 (1-1.5 kb) for yeast selection.

As the main limitation of yeast cloning is the low copy number, if possible, construct could be transferred into bacterial cells for better amplification. In this scenario, TAR vector should also contain a bacterial origin of replication and a bacterial selectable marker. To address the low copy number of TAR vectors in yeast, strategies such as incorporating a high-copy 2 µ origin of replication or using episomal amplification systems can increase yield, but these are generally not sufficient to fully replace bacterial systems like *E. coli* for large-scale plasmid amplification and storage [36, 37].

TAR cloning can assemble multiple overlapping DNA fragments [38]. However, the efficiency of TAR cloning decreases as the number of DNA fragments increases. While yeast can reliably recombine 2 to 5 fragments, the success rate drops significantly beyond 6 to 10 fragments

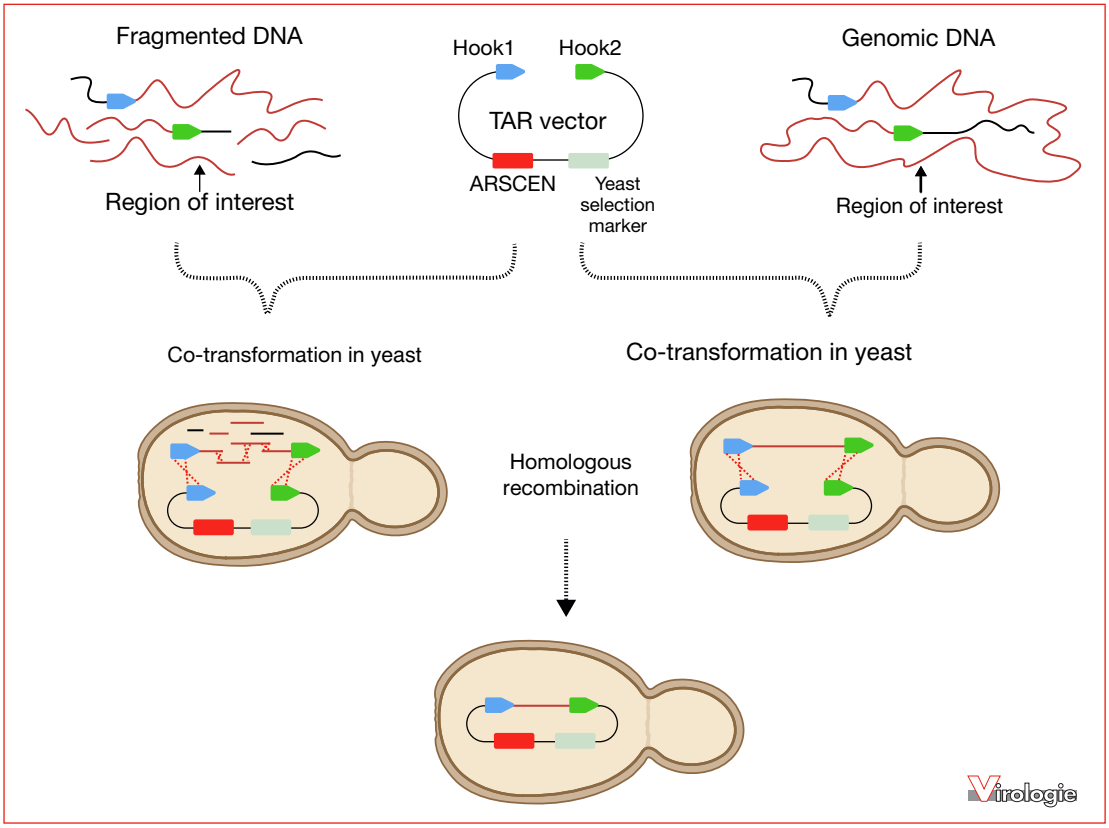



**Figure 1. TAR cloning principle.** TAR cloning consists of co-transformation of genomic or fragmented DNA, either native, PCR amplified, synthetized, or previously TAR-cloned and TAR vector in yeast. TAR vector contains an autonomously replicating sequence (ARS), a centromere (CEN) and a selection marker. Two unique targeting sequences, named hook 1 and 2, homologous to a target DNA region to be cloned are also added in construction. TAR vector is linearized previous transformation procedure, allowing for exposure of targeting hooks. Thanks to yeast high recombination abilities, recombination events can occur between DNA and hooks sequences leading to fragment or genome cloning in TAR vector (created with Biorender.com).

due to increased complexity and competition among recombination events. Additionally, the total size of the construct and the length of homologous overlaps – ideally 40 to 60 base pairs – are critical factors influencing recombination fidelity [38]. To overcome these limitations, a modular assembly strategy is often employed, where smaller subassemblies are constructed first and then combined into the final construct [24]. Despite these constraints, TAR cloning remains a versatile and essential tool for synthetic genomics and large-scale genome engineering.

### *TAR application to viral genomes cloning*

With TAR cloning, yeast has emerged as a major tool for manipulating large microbial genomes, including those of bacteria, viruses, and even eukaryotes [39]. At least 22 mammalian and several bacteriophages and plant viruses' genomes, both native and synthetic, have been cloned in yeast *(table 1)*. TAR cloning was indeed applied to a wide range of viruses, genomes and sizes. Cloning was performed on DNA viruses like the Herpes simplex virus type 1 (152 kbp), the human Cytomegalovirus (CMV) (235 kbp) or RNA viruses like the SARS-CoV-2 (30 kbp) [10, 11, 40]. All the viruses listed in *table 1* were cloned using the well-established model organism *S. cerevisiae*. Its extensive biological characterization has made it the system of choice for many years.

TAR cloning can be of great interest in the case of difficult-to-modify genomes in their infectious form. This technique can be applied to viruses with large genome difficult to manipulate like CMV [10]. It can be also useful for viruses that are difficult to cultivate or that require a host challenging to grow, such as the African swine fever virus (ASFV) [41]. ASFV vaccine production efficiency is also limited due to the primary swine macrophages used as host cells [42]. Moreover, ASFV replication in cell culture has been reported to result in adaptive

**Table 1. Viral genomes cloned through TAR-based methods.**

| Virus | Cloned genome size (kbp) | Genome type | Input viral genetic material | In-yeast transformation type | Rescue strategy | References |
|---|---|---|---|---|---|---|
| Adenovirus type 2 | 36 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - calcium phosphate transfection | [30] |
| Herpes simplex virus type 1 | 152 | DNA | Isolated viral DNA HSV-1 BAC cloned DNA | Lithium acetate Spheroplasts transformation | DNA transfection – reagent non-specified | [11] |
| Human cytomegalovirus | 235 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - electroporation | [10] |
| Autographa californica multiple nucleopolyhedrovirus | 146 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - lipofection | [58] |
| African swine fever virus | 190 | DNA | Isolated viral DNA | Spheroplasts transformation | Not done | [41] |
| Herpes simplex virus H129-G4 | 152 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - lipofection | [9] |
| Avian infectious laryngotracheitis virus | 136 | DNA | Isolated viral DNA | Lithium acetate | DNA transfection – nonliposomal reagent | [59] |
| Rat cytomegalovirus | 203 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - lipofection | [8] |
| Kaposi's sarcoma-associated herpesvirus | 137 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection – nonliposomal reagent | [8] |
| Herpes simplex virus type 1 F strain | 152 | DNA | Isolated viral DNA | Spheroplasts transformation | DNA transfection - lipofection | [60] |
| Bacteriophage øX174 | 5,38 | DNA | Isolated viral DNA Chemically synthetized DNA | Spheroplasts transformation | Bacterial transformation | [61] |
| Dengue virus type 2 | 11 | RNA | Isolated viral RNA | Lithium acetate | RNA transfection – lipofection or electroporation | [12] |
| Middle east respiratory syndrome coronavirus | 31 | RNA | Chemically synthetized cDNA | Lithium acetate | cDNA transfection – nonliposomal reagent | [44] |
| Severe acute respiratory syndrome coronavirus 2 | 30 | RNA | Isolated viral RNA Chemically synthetized cDNA | Lithium acetate | RNA transfection - electroporation | [40] |
| Mouse hepatitis virus | 31.9 | RNA | Isolated viral RNA | Lithium acetate | RNA transfection - electroporation | [40] |
| Middle east respiratory syndrome coronavirus | 30.1 | RNA | BAC DNA clone | Lithium acetate | RNA transfection - electroporation | [40] |
| Human coronavirus-229E | 27.3 | RNA | Isolated viral RNA | Lithium acetate | Not done | [40] |
| ZIKA virus | 10.8 | RNA | Isolated viral RNA | Lithium acetate | Not done | [40] |
| Human respiratory syncytial virus B | 15 | RNA | Isolated viral RNA | Lithium acetate | Not done | [40] |
| Porcine epidemic diarrhea virus | 28 | RNA | Isolated viral RNA | Lithium acetate | cDNA transfection - lipofection | [45] |
| Japanese encephalitis virus | 11 | RNA | Isolated viral RNA | Lithium acetate | RNA transfection - lipofection | [50] |
| Feline infectious peritonitis virus | 28 | RNA | Isolated viral RNA | Lithium acetate | cDNA transfection – nonliposomal reagent | [7] |
| Severe acute respiratory syndrome coronavirus 2 | 30 | RNA | Isolated viral RNA | Spheroplasts transformation | cDNA transfection - lipofection | [62] |
| Cucumber mosaic virus and Tomato aspermy virus | 3,4, 3,1 and 2,2 | RNA | Isolated viral RNA | Lithium acetate | cDNA transfection – particle bombardment | [63] |
| Apple chlorotic leaf spot trichovirus | 7.55 | RNA | Isolated viral RNA | Lithium acetate | cDNA transfection - Agroinfiltration | [64] |
| Watermelon mosaic virus | 10.0 | RNA | Isolated viral RNA | Polyethylene-glycol-induced transformation | cDNA transfection – particle bombardment | [16] |

mutations, genome deletions and rearrangements associated with phenotype changes [41, 43]. TAR cloning and genome manipulations in yeast allows to overcome all these limitations. Sequenced but not available as viral particles viruses can also benefit from TAR cloning. In 2020, the study of Thao reported SARS-CoV-2 genome reassembly in yeast using chemically synthetized cDNA based only on the released genomic sequence [40]. In-yeast cloning could therefore be applied to extinct viruses such as the Horsepox virus [23].

Over the last decade, the cost of DNA synthesis has drastically reduced. Therefore, the number of synthetic genomes designed with partially or fully synthetic DNA has increased. Genome editing could now be performed by the assembly of synthetic fragments in yeast on the model of works performed on Middle east respiratory syndrome coronavirus (MERS-CoV) or SARS-CoV-2 genomes [40, 44]. This approach remains nonetheless costly at the mega base scale, and may be excessive for small, localized editing tasks. Depending on the need, it may be more appropriate to use one of the many genome engineering tools already available in yeast to modify the native cloned genome. CRISPR-Cas9, the well-known and broadly used RNA-guided endonuclease, has been adapted to a wide array of organisms, including yeast. Examples of TAR-cloned viral genomes Cas-9 edited are now commonly found. The team of Li combined CRISPR-Cas9 technology and homologous recombination to generate a recombinant Porcine Epidemic Diarrhea virus (PEDV) in which the ORF3 gene was swapped with an EGFP gene [45]. On the same principle, with the purpose of generating fluorescent to structural HSV-1 proteins fusions, Oldfield and colleagues also used this technology [11].

Moreover, TAR cloning of entire viral genomes can be done including multiple types of genetic inputs combined. Indeed, for TAR cloning of the Mouse Hepatitis virus (MHV), Thao *et al*. included to transformed viral cDNA, PCR-amplified sequences comprising a T7 RNA polymerase promoter directly upstream of the MHV-GFP 5′ as a cleavage site after the poly(A) sequence at the MHV-GFP 3′ end [40]. Alternatively, once the entire genome is cloned in yeast, we can imagine that its modification can be easily performed by further transformation associated recombination in yeast.

## Rescue of cloned viral genomes

The cloned viral genome in yeast can be "rescued" using various approaches. In this review, "rescue" is defined as the process by which the cloned genome is converted into the biological entity it encodes.

In view of reforming infectious viral particles, most of the cloned genomes presented in table 1 were transfected into their host cells (*figure 2*). Transfection can be

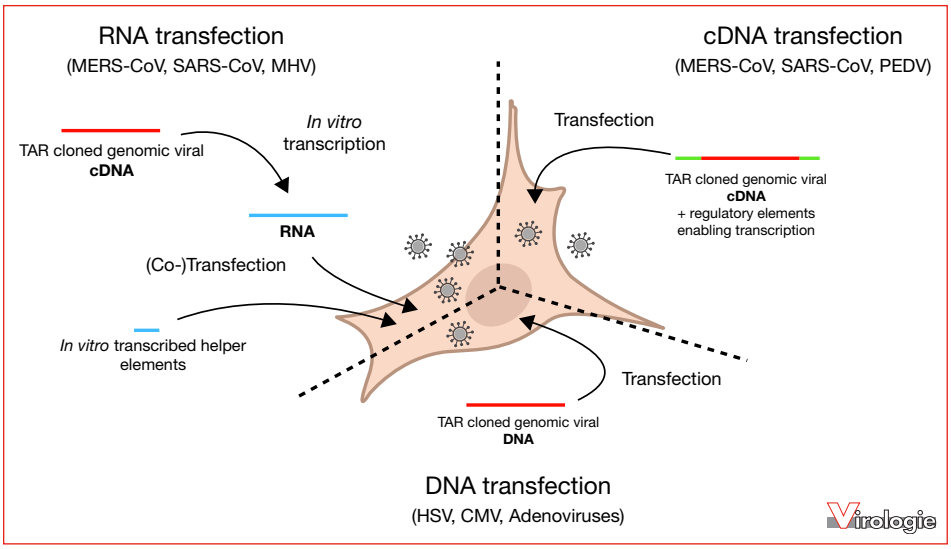



**Figure 2. Strategies for regeneration of infectious viral particles from TAR cloned genomes by viral rescue.** Strategies for rescuing mammalian viruses depend on the genetic material type. As most DNA viruses rely on the host cell's replication and transcription machinery, direct transfection of cloned viral DNA constructs can be performed. RNA viruses, however, require an additional transcription step. Their cDNA genomes can be *in vitro* transcribed, with the resulting RNA directly transfected. In some cases, helper elements, such as mRNA encoding viral nucleocapsids, may be needed to initiate replication. Alternatively, direct cDNA transfection can be performed with added regulatory elements, like a' promoter at the genome's 5′ end, to enable RNA transcription. Each transfection method is accompanied by examples of viruses for which it has been applied (created with Biorender.com).

performed using various techniques such as electroporation, lipofection or using non-liposomal reagents. In the case of viruses infectious for plants, techniques such as agroinfiltration or particle bombardment can be chosen. If focusing on viruses infectious for mammalian cells, rescue strategies differ depending on viruses' genetic material types. During infection, most DNA viruses use cell's replication and transcription machinery. Viral DNA genomes are generally infectious and, during rescue, do not require additional elements for replication. However, poxviruses and African swine fever virus (ASFV) are notable exceptions. Unlike most DNA viruses, they replicate entirely in the cytoplasm and encode all the proteins necessary for viral genome replication, transcription, and virion assembly [46,47]. For the reactivation of poxvirus and ASFV genomes, purified viral DNA is transfected into cells that have been previously infected with a helper virus, which supplies the essential trans-acting factors required for initiating replication and reactivation.

In the case of RNA viruses, an additional transcription step is necessary for viral rescue. The cDNA cloned genomes can be *in vitro* transcribed using purified RNA polymerase and the resulting RNAs transfected into host cells leading to infectious viral particles. In some cases, the use of helper elements is necessary to boot-up the recombinant genomes. To rescue MHV-GFP, Thao and colleagues transfected together *in vitro*-transcribed YAC with an *in vitro*-transcribed mRNA that encodes the MHV nucleocapsid [40]. In other cases, direct cDNA transfections are performed but with the prior addition, before the rescue step, of regulatory elements such as CMV promoter at the 5′ of inserted genome, enabling transcription of viral RNA and therefore the cDNA to be infectious by itself [7,44,45].

Nikiforuk and colleagues exploited this strategy for rescue of a cloned MERS-CoV genome [44]. Their work, with exclusive use of synthetic DNA, is a great example of the versatility of the TAR cloning system as of its potential benefits for rapid response to potentially threatening or non-available emerging pathogens. Their strategy, described in *figure 3* did not indeed involve the virus in its infectious form but implicated the complete chemical synthesis of thirty-one 1-kb fragments covering the entire genome length. Three large PCR amplicons were obtained from these synthetised fragments by their stepwise assembly by slice-to-overlap extension polymerase chain reaction. Regulatory elements such as cytomegalovirus promoter, beta globulin terminator and hepatitis delta virus ribozyme were added at this step. Once obtained, amplicons were further PCR amplified to add homologous fragments to their adjacent sequences or to the vector at their ends. Direct yeast transformation was then performed using lithium acetate for synthetic genome cloning into the vector. The positively screened yeast colonies containing the MERS-CoV genome inserted in the vector were then electroporated into bacteria, followed by rescue to produce infectious viral particles. The addition of regulatory elements in construct indeed allowed to bring back infectious ability of the cloned cDNA without the need of additional *in vitro* transcription step.

By their process Nikiforuk *et al.*, among others, shows that homologous recombination in yeast is a rapid and promising candidate methodology for assembly of large viral genomes [44]. In most of the articles cited in this review, rescue of viral genomes is not a major concern. An exception is the African swine fever virus, for which no viral particles have yet been successfully generated by rescue. Their genomes are non-infectious and no known helper genes or helper viruses are available to reconstitute live virus from recombinant genome [48]. Another challenge for viral rescue is the poor quality and limited quantity of DNA extracted from yeast cultures, which can prevent direct rescue from yeast-derived constructs. Indeed, when extracted from yeast, plasmids represent only a tiny fraction of the total DNA, which consists predominantly of yeast genomic DNA. As a result, a bacterial cloning step must be required before further viral rescue efforts can be made extending the handling time between cloning and reformation of infectious viral particles. In fact, as mentioned by Knickmann *et al.* it is extremely difficult to isolate useful quantities of yeast circular plasmids from yeast cultures. An additional cloning step in bacteria is therefore needed for further plasmids sequence analysis, manipulation and infectious viral particles formation by viral rescue [8]. However, this additional cloning step can be time consuming and laborious mostly due to the large size of the constructs [11].

## Basic research and biomedicine applications

TAR cloning ability to isolate and edit unperturbed native and synthetic genomic regions can provide a strong basis for a multitude of practical applications in biomedicine and biotechnology (*figure 4*). The main outcome of this procedure is thus to provide a fast and flexible genetic engineering platform for the modification of genomes derived from viruses.

Such model system provides great support for basic research and study of virus biology [49]. It can indeed

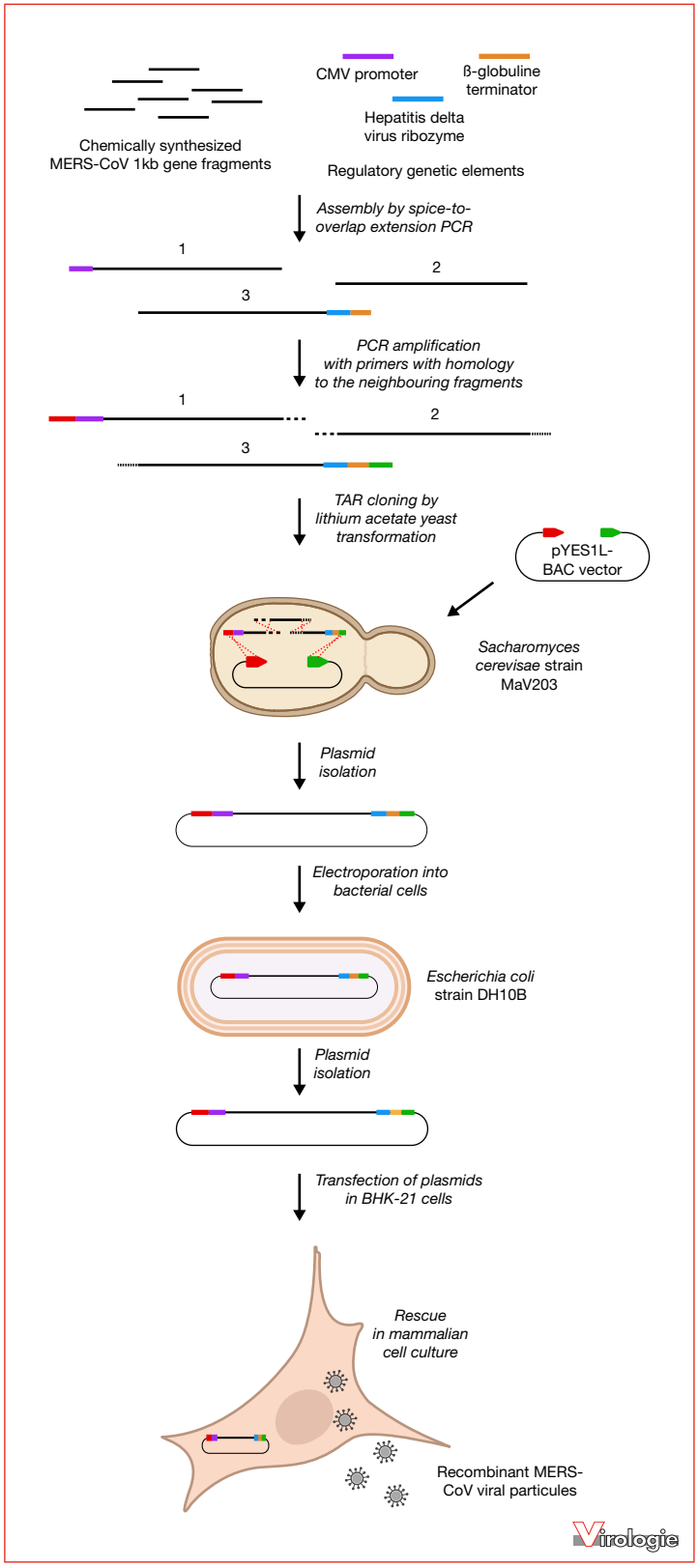



**Figure 3. Strategy used by Nikiforuk and colleagues for one-step construction of an infectious clone system of the Middle East Respiratory Syndrome coronavirus by homologous recombination in *S. cerevisiae*.** Thirty-one synthesized gene fragments of the MERS-CoV EMC/2012 genome were stepwise assembled with regulatory elements (cytomegalovirus promoter, beta globulin terminator and hepatitis delta virus ribozyme) by splice-to-overlap extension PCR resulting in three large PCR amplicons. Sequences homologous to the neighbouring fragments or pYES1L-BAC vector were then added at each border of the amplicons by PCR allowing homologous recombination during transformation. Homologous recombination was performed in *S. cerevisiae* MaV203 cells by lithium acetate transformation and correctly assembled MERS-CoV genomes in vector were extracted from yeast for electroporation into Max Efficiency DH10B cells. Functionality of the MERS-CoV infectious clone system was then examined by rescue on Baby Hamster Kidney cells (BHK-21), leading to replicative recombinant MERS-CoV viral particles (created with Biorender.com).

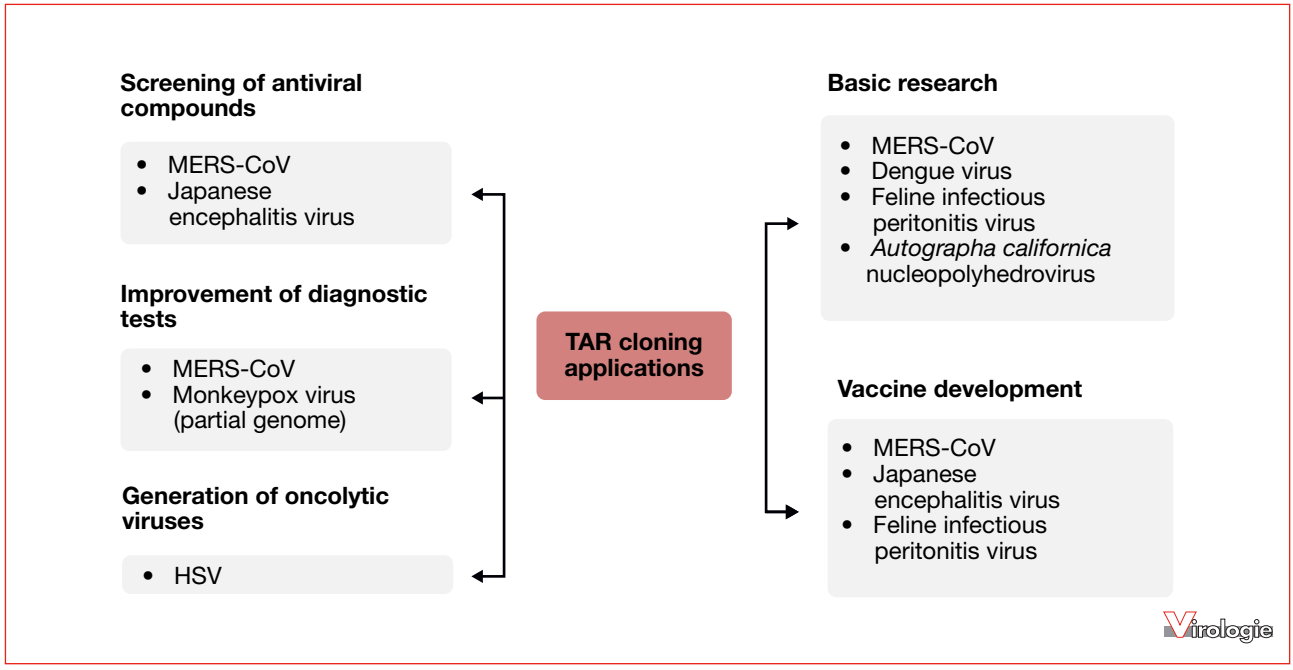



**Figure 4. TAR cloning technology applications.** Applications are illustrated with examples of viruses for which they have been implemented or mentioned as potential prospects (created with Biorender.com).

also be used for virus characterisation within gene-function studies. By this way, it can facilitate improvement of diagnostic tests, screening of antiviral compounds and vaccine production [50].

Oncolytic viruses are immunotherapies that exploit the innate ability of certain replication-competent viruses to preferentially infect and lyse tumour cells while leaving normal ones intact, making them of interest in a wide range of cancer's therapies [51]. Different viruses have been proven to show oncolytic properties. They include members of both RNA viruses such as measles virus, retroviruses or vesicular stomatitis virus and DNA viruses as adenoviruses, poxviruses or herpes simplex viruses [52]. By successful assembly and reconstitution into viral particles of the entire HSV-1 genome, Oldfield *et al.* suggests that potential benefits of this method could include efficient generation and test of engineered candidate viruses to improve virus-based therapeutics, such as oncolytic herpesviruses [11]. Given the variety of viral types listed in table 1, we hypothesize that this assumption could be broadly applicable to a large proportion of them. More importantly, oncolytic viruses can be used in combination with other treatments by modifying them to express therapeutic payloads. Alliances of this kind would lead to more efficient therapies or improved immune system activation. TAR cloning, in turn, could facilitate more efficient and rapid cloning of therapeutic genes.

Moreover, the ability to quickly synthesize or modify viral genomes might be a critical factor to respond to emerging pathogens. The use of TAR technology could shorten the time between emergence of a novel viral pathogen and construction of an infectious clone system for further medicines development [40,44]. It was indeed shown that only 30 days were necessary to go from a publicly released sequence of SARS-CoV-2 to a functional, rescued recombinant virus [40].

## Conclusion and perspectives: benefits and risks of such technology

The construction of infectious clone systems via homologous recombination in yeast, particularly through TAR cloning, holds significant promise as a standard approach for the assembly and engineering of large and complex viral genomes. TAR cloning has demonstrated broad utility in the selective isolation and manipulation of sizable DNA molecules, effectively circumventing limitations inherent to traditional cloning systems. The yeast-based platform thus represents a robust and

versatile foundation for a wide array of applications in virology and synthetic biology.

Despite these advantages, a key technical limitation remains: the relatively low copy number of constructs maintained in yeast compared to those in *E. coli*. Although multicopy vectors in yeast exist, they have yet to match the high yields typically achieved in bacterial systems, underscoring a critical area for further development.

Beyond its biotechnological potential, TAR cloning also offers a safer alternative for studying high-risk pathogens. The ability to manipulate complete viral genomes synthetically—without handling infectious particles—opens new avenues for the investigation of viruses typically restricted to high-containment laboratories. For example, synthetic genomics approaches have enabled the reconstruction of the Monkeypox virus genome [53] and could similarly support research on Ebola, yellow fever, dengue, and chikungunya viruses under safer laboratory conditions. Furthermore, TAR cloning may facilitate the resurrection and study of extinct or ancient viruses, as exemplified by the reconstruction of Horsepox virus [23]. However, these advances also introduce profound dual-use concerns [54]. The possibility of synthesizing pathogenic viruses raises biosecurity and ethical challenges, prompting urgent discussions within the scientific community. Regulatory frameworks and oversight mechanisms, such as sequence screening and design-stage controls, are thus essential to ensure that the benefits of this powerful technology are realized responsibly and safely [55]. In this sense, numerous committees and boards have addressed a series of issues related to synthetic biology, proposing measures to be taken and regulatory proposals [56,57].

**Links of interests:** Camille Rey declares that she was employed by Transgene at the time of writing this review. Aimeric Agaoua and Philippe Erbs disclose that they are current employees of Transgene and hold shares in the company.

## References

**1.** Monaco AP, Larin Z. YACs, BACs, PACs and MACs: Artificial chromosomes as research tools. *Trends in Biotechnology* 1994; 12: 280-6.

**2.** Tischer BK, Kaufer BB. Viral bacterial artificial chromosomes: generation, mutagenesis, and removal of mini-F sequences. *J Biomed Biotechnol* 2012; 2012: 472537.

**3.** Domi A, Moss B. Cloning the vaccinia virus genome as a bacterial artificial chromosome in Escherichia coli and recovery of infectious virus in mammalian cells. *Proc Natl Acad Sci U S A* 2002; 99: 12415-20.

**4.** Chiuppesi F, Salazar M d'Alincourt, Contreras H, Nguyen VH, Martinez J, Park Y, *et al*. Development of a multi-antigenic SARS-CoV-2 vaccine candidate using a synthetic poxvirus platform. *Nat Commun* 2020; 11: 6121.

**5.** Chartier C, Degryse E, Gantzer M, Dieterle A, Pavirani A, Mehtali M. Efficient generation of recombinant adenovirus vectors by homologous recombination in Escherichia coli. *J Virol* 1996; 70: 4805-10.

**6.** Hall RN, Meers J, Fowler E, Mahony T. Back to BAC: the use of infectious clone technologies for viral mutagenesis. *Viruses* 2012; 4: 211-35.

**7.** Cao H, Gu H, Kang H, Jia H. Development of a rapid reverse genetics system for feline coronavirus based on TAR cloning in yeast. *Front Microbiol* 2023; 14: 1141101.

**8.** Knickmann J, Staliunaite L, Puhach O, Ostermann E, Günther T, Nichols J, *et al*. A simple method for rapid cloning of complete herpesvirus genomes. *Cell Rep Methods* 2024; 4: 100696.

**9.** Xiao H, Hu H, Guo Y, Li J, Wen L, Zeng W-B, *et al*. Construction and characterization of a synthesized herpes simplex virus H129-Syn-G2. *Virol Sin* 2023; 38: 373-9.

**10.** Vashee S, Stockwell TB, Alperovich N, Denisova EA, Gibson DG, Cady KC, *et al*. Cloning, Assembly, and Modification of the Primary Human Cytomegalovirus Isolate Toledo by Yeast-Based Transformation-Associated Recombination. *mSphere* 2017; 2: e00331-17.

**11.** Oldfield LM, Grzesik P, Voorhies AA, Alperovich N, MacMath D, Najera CD, *et al*. Genome-wide engineering of an infectious clone of herpes simplex virus type 1 using synthetic genomics assembly methods. *Proc Natl Acad Sci U S A* 2017; 114: E8885-94.

**12.** Polo S, Ketner G, Levis R, Falgout B. Infectious RNA transcripts from full-length dengue virus type 2 cDNA clones made in yeast. *J Virol* 1997; 71: 5366-74.

**13.** Kanda T, Shibata S, Saito S, Murata T, Isomura H, Yoshiyama H, *et al*. Unexpected instability of family of repeats (FR), the critical cis-acting sequence required for EBV latent infection, in EBV-BAC systems. *PLoS One* 2011; 6: e27758.

**14.** Yakushko Y, Hackmann C, Günther T, Rückert J, Henke M, Koste L, *et al*. Kaposi's Sarcoma-Associated Herpesvirus Bacterial Artificial Chromosome Contains a Duplication of a Long Unique-Region Fragment within the Terminal Repeat Region. *J Virol* 2011; 85: 4612-7.

**15.** Almazán F, González JM, Pénzes Z, Izeta A, Calvo E, Plana-Durán J, *et al*. Engineering the largest RNA virus genome as an infectious bacterial artificial chromosome. *Proc Natl Acad Sci U S A* 2000; 97: 5516-21.

**16.** Desbiez C, Chandeysson C, Lecoq H, Moury B. A simple, rapid and efficient way to obtain infectious clones of potyviruses. *J Virol Methods* 2012; 183: 94-7.

**17.** Zheng X, Tong W, Liu F, Liang C, Gao F, Li G, *et al*. Genetic instability of Japanese encephalitis virus cDNA clones propagated in Escherichia coli. *Virus Genes* 2016; 52: 195-203.

**18.** Li D, Aaskov J, Lott WB. Identification of a cryptic prokaryotic promoter within the cDNA encoding the 5′ end of dengue virus RNA genome. *PLoS One* 2011; 6: e18197.

**19.** Mutso M, Saul S, Rausalu K, Susova O, Žusinaite E, Mahalingam S, *et al*. Reverse genetic system, genetically stable reporter viruses and packaged subgenomic replicon based on a Brazilian Zika virus isolate. *J Gen Virol* 2017; 98: 2712-24.

**20.** Liu Z-Y, Yu J-Y, Huang X-Y, Fan H, Li X-F, Deng Y-Q, *et al*. Characterization of cis-Acting RNA Elements of Zika Virus by Using a Self-Splicing Ribozyme-Dependent Infectious Clone. *J Virol* 2017; 91: e00484-17.

**21.** Amarilla AA, Sng JDJ, Parry R, Deerain JM, Potter JR, Setoh YX, *et al*. A versatile reverse genetics platform for SARS-CoV-2 and other positive-strand RNA viruses. *Nat Commun* 2021; 12: 3431.

**22.** Furusawa Y, Yamayoshi S, Kawaoka Y. The accuracy of reverse genetics systems for SARS-CoV-2: Circular polymerase extension reaction versus bacterial artificial chromosome. *Influenza Other Respir Viruses* 2023; 17: e13109.

**23.** Noyce RS, Lederman S, Evans DH. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. *PLoS One* 2018; 13: e0188453.

**24.** Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, *et al*. Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. *Science* 2008; 319: 1215-20.

**25.** Hinnen A, Hicks JB, Fink GR. Transformation of yeast. *Proc Natl Acad Sci U S A* 1978; 75: 1929–33.

**26.** Orr-Weaver TL, Szostak JW, Rothstein RJ. Yeast transformation: a model system for the study of recombination. *Proc Natl Acad Sci U S A* 1981; 78: 6354-8.

**27.** Burke DT, Carle GF, Olson MV. Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 1987; 236: 806-12.

**28.** Larionov V, Kouprina N, Graves J, Chen XN, Korenberg JR, Resnick MA. Specific cloning of human DNA as yeast artificial chromosomes by transformation-associated recombination. *Proc Natl Acad Sci U S A* 1996; 93: 491-6.

**29.** Kuspa A, Vollrath D, Cheng Y, Kaiser D. Physical mapping of the Myxococcus xanthus genome by random cloning in yeast artificial chromosomes. *Proc Natl Acad Sci U S A* 1989; 86: 8917-21.

**30.** Ketner G, Spencer F, Tugendreich S, Connelly C, Hieter P. Efficient manipulation of the human adenovirus genome as an infectious yeast artificial chromosome clone. *Proc Natl Acad Sci U S A* 1994; 91: 6186-90.

**31.** Lartigue C, Vashee S, Algire MA, Chuang R-Y, Benders GA, Ma L, *et al*. Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* 2009; 325: 1693-6.

**32.** Benders GA, Noskov VN, Denisova EA, Lartigue C, Gibson DG, Assad-Garcia N, *et al*. Cloning whole bacterial genomes in yeast. *Nucleic Acids Res* 2010; 38: 2558-69.

**33.** Kouprina N, Kim JH, Larionov V. Highly Selective, CRISPR/Cas9-Mediated Isolation of Genes and Genomic Loci from Complex Genomes by TAR Cloning in Yeast. *Curr Protoc* 2021; 1: e207.

**34.** Lee NCO, Larionov V, Kouprina N. Highly efficient CRISPR/Cas9-mediated TAR cloning of genes and chromosomal loci from complex genomes in yeast. *Nucleic Acids Res* 2015; 43: e55.

**35.** Noskov VN, Koriabine M, Solomon G, Randolph M, Barrett JC, Leem SH, *et al*. Defining the minimal length of sequence homology required for selective gene isolation by TAR cloning. *Nucleic Acids Res* 2001; 29: E32.

**36.** Murray AW, Szostak JW. Construction of artificial chromosomes in yeast. *Nature* 1983; 305: 189-93.

**37.** Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, *et al*. Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 1998; 14: 115-32.

**38.** Gibson DG, Benders GA, Axelrod KC, Zaveri J, Algire MA, Moodie M, *et al*. One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic Mycoplasma genitalium genome. *Proc Natl Acad Sci U S A* 2008; 105: 20404-9.

**39.** Vashee S, Arfi Y, Lartigue C. Budding yeast as a factory to engineer partial and complete microbial genomes. *Curr Opin Syst Biol* 2020; 24: 1-8.

**40.** Thi Nhu Thao T, Labroussaa F, Ebert N, V'kovski P, Stalder H, Portmann J, *et al*. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature* 2020; 582: 561-5.

**41.** Labroussaa F, Mehinagic K, Cippa V, Liniger M, Akarsu H, Ruggli N, *et al*. In-yeast reconstruction of the African swine fever virus genome isolated from clinical samples. *STAR Protoc* 2021; 2: 100803.

**42.** Álvarez B, Revilla C, Poderoso T, Ezquerra A, Domínguez J. Porcine Macrophage Markers and Populations: An Update. *Cells* 2023; 12: 2103.

**43.** Borca MV, Rai A, Ramirez-Medina E, Silva E, Velazquez-Salinas L, Vuono E, *et al*. A Cell Culture-Adapted Vaccine Virus against the Current African Swine Fever Virus Pandemic Strain. *J Virol* 2021; 95: e0012321.

**44.** Nikiforuk AM, Leung A, Cook BWM, Court DA, Kobasa D, Theriault SS. Rapid one-step construction of a Middle East Respiratory Syndrome (MERS-CoV) infectious clone system by homologous recombination. *J Virol Methods* 2016; 236: 178-83.

**45.** Zhou Y, Li C, Ren C, Hu J, Song C, Wang X, *et al*. One-Step Assembly of a Porcine Epidemic Diarrhea Virus Infectious cDNA Clone by Homologous Recombination in Yeast: Rapid Manipulation of Viral Genome With CRISPR/Cas9 Gene-Editing Technology. *Front Microbiol* 2022; 13: 787739.

**46.** Moss B. Poxvirus DNA replication. *Cold Spring Harb Perspect Biol* 2013; 5: a010199.

**47.** Dixon LK. Advances in African swine fever virus molecular biology and host interactions contributing to new tools for control. *J Virol* 2025; e0093224.

**48.** Revilla Y, Pérez-Núñez D, Richt JA. African Swine Fever Virus Biology and Vaccine Approaches. *Adv Virus Res* 2018; 100: 41-74.

**49.** Barajas D, Aponte-Ubillus JJ, Akeefe H, Cinek T, Peltier J, Gold D. Generation of infectious recombinant Adeno-associated virus in Saccharomyces cerevisiae. *PLoS One* 2017; 12: e0173010.

**50.** Li C, Chen X, Zhou Y, Hu J, Wang X, Li Y. Novel reverse genetics of genotype I and III Japanese encephalitis viruses assembled through transformation associated recombination in yeast: The reporter viruses expressing a green fluorescent protein for the antiviral screening assay. *Antiviral Res* 2022; 197: 105233.

**51.** Shalhout SZ, Miller DM, Emerick KS, Kaufman HL. Therapy with oncolytic viruses: progress and challenges. *Nat Rev Clin Oncol* 2023; 20: 160-77.

**52.** Rahman MM, McFadden G. Oncolytic Viruses: Newest Frontier for Cancer Immunotherapy. *Cancers (Basel)* 2021; 13: 5452.

**53.** Yang L, Tian L, Li L, Liu Q, Guo X, Zhou Y, *et al*. Efficient assembly of a large fragment of monkeypox virus genome as a qPCR template using dual-selection based transformation-associated recombination. *Virol Sin* 2022; 37: 341-7.

**54.** York A. Resurrection of a poxvirus causes alarm. *Nat Rev Microbiol* 2018; 16: 184.

**55.** Moe-Behrens GHG, Davis R, Haynes KA. Preparing synthetic biology for the world. *Front Microbiol* 2013; 4: 5.

**56.** Gronvall GK. HHS guidance on synthetic DNA is the right step. *Biosecur Bioterror* 2010; 8: 373-6.

**57.** Kobokovich A, West R, Montague M, Inglesby T, Gronvall GK. Strengthening Security for Gene Synthesis: Recommendations for Governance. *Health Secur* 2019; 17: 419-29.

**58.** Shang Y, Wang M, Xiao G, Wang X, Hou D, Pan K, *et al*. Construction and Rescue of a Functional Synthetic Baculovirus. *ACS Synth Biol* 2017; 6: 1393-402.

**59.** Spatz S, García M, Fuchs W, Loncoman C, Volkening J, Ross T, *et al*. Reconstitution and Mutagenesis of Avian Infectious Laryngotracheitis Virus from Cosmid and Yeast Centromeric Plasmid Clones. *J Virol* 2023; 97: e0140622.

**60.** Xiao H, Hu H, Guo Y, Li J, Zeng W-B, Luo M-H, *et al*. Efficient Strategy for Synthesizing Vector-Free and Oncolytic Herpes Simplex Type 1 Viruses. *ACS Synth Biol* 2024; 13: 3268-80.

**61.** Jaschke PR, Lieberman EK, Rodriguez J, Sierra A, Endy D. A fully decompressed synthetic bacteriophage øX174 genome assembled and archived in yeast. *Virology* 2012; 434: 278-84.

**62.** Khan D, Terenzi F, Liu G, Ghosh PK, Ye F, Nguyen K, *et al*. A viral pan-end RNA element and host complex define a SARS-CoV-2 regulon. *Nat Commun* 2023; 14: 3385.

**63.** Fernandez-Delmond I, Pierrugues O, de Wispelaere M, Guilbaud L, Gaubert S, Divéki Z, *et al*. A novel strategy for creating recombinant infectious RNA virus genomes. *J Virol Methods* 2004; 121: 247-57.

**64.** Youssef F, Marais A, Faure C, Gentit P, Candresse T. Strategies to facilitate the development of uncloned or cloned infectious full-length viral cDNAs: Apple chlorotic leaf spot virus as a case study. *Virol J* 2011; 8: 488.