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Chromosome Engineering: Technologies, Applications, and Challenges

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Abstract

Chromosome engineering is a transformative field at the cutting edge of biological science, offering unprecedented precision in manipulating large-scale genomic DNA within cells. This discipline is central to deciphering how the multifaceted roles of chromosomes—guarding genetic information, encoding sequence positional information, and influencing organismal traits—shape the genetic blueprint of life. This review comprehensively examines the technological advancements in chromosome engineering, which center on engineering chromosomal rearrangements, generating artificial chromosomes, de novo synthesizing chromosomes, and transferring chromosomes. Additionally, we introduce the application progress of chromosome engineering in basic and applied research fields, showcasing its capacity to deepen our knowledge of genetics and catalyze breakthroughs in therapeutic strategies. Finally, we conclude with a discussion of the challenges the field faces and highlight the profound implications that chromosome engineering holds for the future of modern biology and medical applications.

INTRODUCTION

Within the nucleus of a eukaryotic cell, chromosomes play an indispensable role in orchestrating the genetic blueprint of life through their intricate organization of DNA. These structures not only safeguard the complete array of genes and gene regulatory elements but also encode the positional information of these sequences, which directly participate in the encoding of an organism's traits. Chromosomes are essential for DNA replication and faithful transmission to daughter cells during mitosis and meiosis. Moreover, the dynamic positioning and architecture of chromatin are crucial for gene expression and cellular function, underscoring the multifaceted role of chromosomes that extends beyond their fundamental duty as genetic repositories.

In mammalian organisms, chromosomal rearrangements are intricately linked to evolutionary processes, developmental pathways, and immune responses. During evolution, chromosomal rearrangements, such as subchromosomal inversions, translocations, and entire chromosome fusions, are closely associated with phenotypic evolution, adaptation, and speciation (1). Developmentally, chromosomal territories and gene clusters, like the *homeobox* gene cluster, play a key role in shaping the body plan (2). The immune system relies on variable–diversity–joining recombination to generate a diverse antibody repertoire crucial for pathogen defense (3). Furthermore, abnormal chromosomal arrangements are also implicated in various human diseases, including cancers. For instance, the Philadelphia chromosome, resulting from a Robertsonian translocation between chromosomes 22 and 9, leads to the fusion of *BCR* (*breakpoint cluster region*) and *ABL* (*Abelson leukemia virus*) genes, causing chronic myeloid leukemia (4).

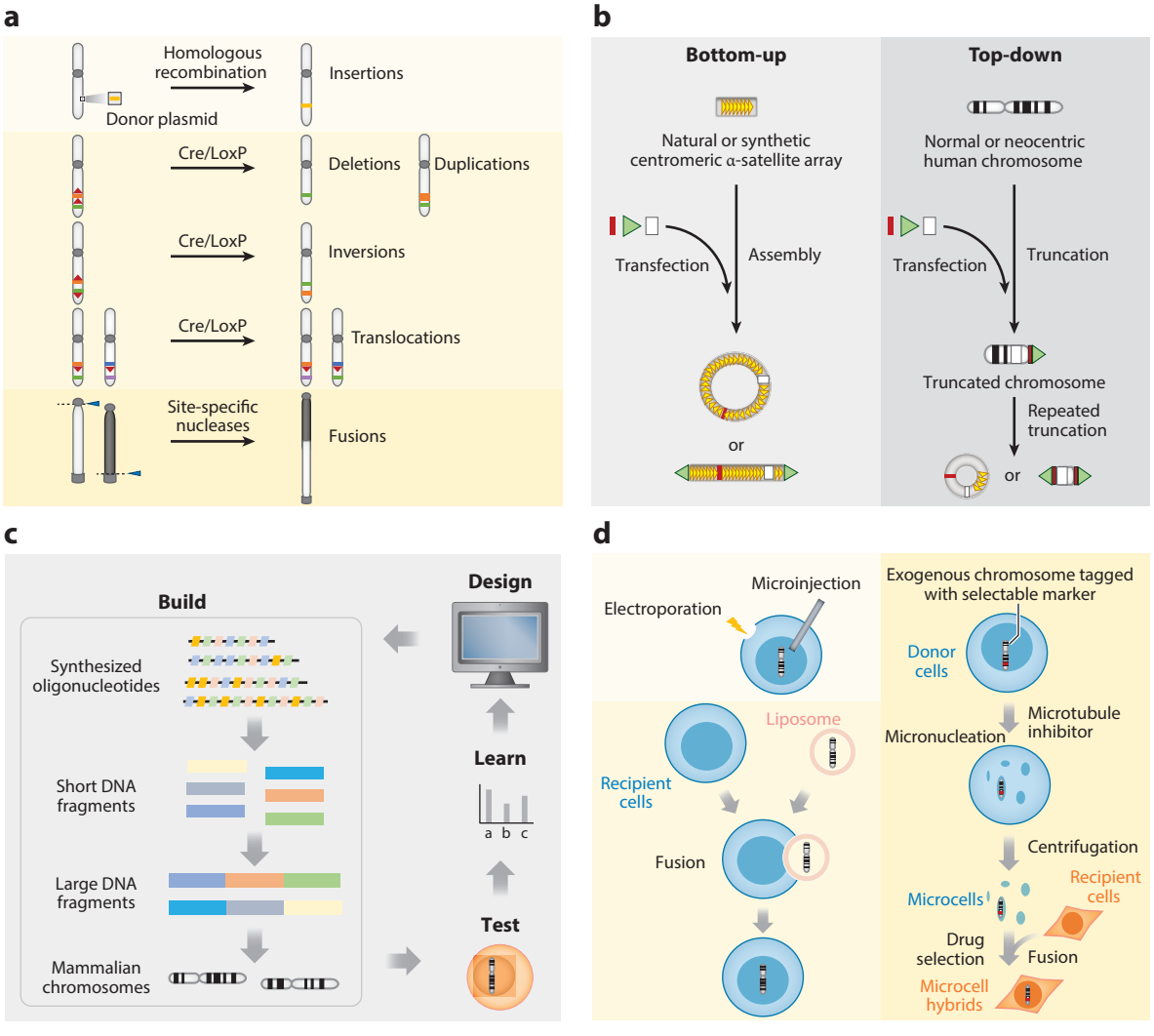
Chromosome engineering enables direct experimental investigations into these phenomena, offering insights into the mechanisms underlying chromosome function. Advances in technologies such as gene editing, chemical DNA synthesis, and stem cell techniques have revolutionized chromosome engineering, shifting the focus from isolated genetic elements to the broader genomic landscape (>100 kb). This includes engineering chromosomal rearrangements, creating artificial chromosomes (ACs), synthesizing chromosomes de novo, and transferring chromosomes in vivo and ex vivo.

These technological advancements empower researchers to create targeted chromosomal variations, providing a robust tool kit to explore the biological consequences of such alterations. The ability to engineer chromosomes has opened new avenues for understanding the complex relationship between genetic variations and phenotypic outcomes. Applications of chromosome engineering range from modeling trisomy 21 to constructing cancer models driven by chromosomal rearrangements and using chromosome fusion techniques to study karyotypic evolution (5). These applications deepen our understanding of fundamental biological processes and offer potential therapeutic advancements in genetic disorders. Precision manipulation of chromosomes prompts profound questions about genetic intervention boundaries and the essence of life itself. By delving into the intricate interplay between chromosomes and biological processes, scientists aim to illuminate the transformative potential of chromosome engineering in shaping modern biology and medicine. This review provides a comprehensive exploration of the technological advancements, applications, challenges, and future prospects of chromosome engineering.

PROGRESS OF MAMMALIAN CHROMOSOME ENGINEERING TECHNOLOGIES

In the field of genomics, the intricate manipulation of chromosomes is central to elucidating the origins of biological variation and the pathogenesis of genetic diseases. As we examine the

cutting-edge advances of mammalian chromosome engineering, we are introduced to a sophisticated field where nuanced genetic intervention is in harmony with a deep understanding of chromosomal structure and function. This area of study has seen remarkable progress, spanning from the engineering of chromosomal rearrangements to the generation of AC vectors and the de novo synthesis of chromosomes, culminating in the transfer of these engineered chromosomes into cells. The ensuing sections dissect the underpinning techniques that catalyze these breakthroughs and the hurdles that investigators face (**Figure 1** and **Table 1**).



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Chromosome engineering technologies. (*a*) Engineering chromosome rearrangements. (*top*) Homologous recombination technology involves the insertion of donor sequences at specific locations on the chromosome. (*middle*) Site-specific recombination enzyme technology, using LoxP sequences in various orientations, enables Cre to accomplish deletion, duplication, inversion, or translocation at a specific chromosomal site. (*bottom*) Site-specific nuclease technology, introducing DSBs at specific locations on the chromosome to achieve fusion of complete chromosomes. (*b*) Strategy for constructing artificial chromosomes. In the bottom-up approach, synthetic or natural α-satellite sequences are integrated with a selectable marker, genomic DNA, and artificial telomeres to construct an artificial chromosome. In the top-down approach, truncated natural chromosomes, in combination with selectable markers and genomic DNA, constitute the artificial chromosomes. (*c*) Strategies for the de novo synthesis of mammalian chromosomes involve a meticulous process of rational design and synthesis of oligonucleotide sequences. The approach is to create these sequences from scratch and gradually assemble them into a chromosomal structure, progressively refining the design strategies through iterative testing and feedback. (*d*) Chromosome transfer strategies, including electroporation, microinjection, liposome-mediated transfection, and MMCT. During liposome-mediated transfection, the target chromosome is encapsulated in liposomes, which subsequently fuse with recipient cells. During MMCT, the target chromosome of the donor cells is labeled. Then, the donor cells are treated with the microtubule inhibitor to induce micronucleation, forming microcells encapsulated in the cell membrane after centrifugation. The filtered small microcells are subsequently fused with recipient cells. After drug selection, those cells with target chromosomes can be enriched. Abbreviations: DSB, double-strand break; MMCT, microcell-mediated chromosome transfer.

Engineering of Chromosomal Rearrangements

Chromosomal rearrangements refer to a spectrum of alterations in chromosomal organization, including deletions, duplications, insertions, inversions, translocations, and fusions. The process is initiated by the induction of DNA double-strand breaks (DSBs), which trigger the cell's inherent repair mechanisms including non-allelic homologous recombination, non-homologous end-joining, fork stalling and template switching, and microhomology-mediated break-induced replication. Although chromosomal rearrangements can occur naturally, such as the *IGH* (*immunoglobulin heavy*) *locus* variable–diversity–joining rearrangement, their artificial induction through engineered chromosomal rearrangement technology has become a powerful tool in genetic research and biotechnology. The precise induction of DSBs within the chromosome is critical to the technique's success.

Chromosomal rearrangements can occur spontaneously, albeit at a significantly low rate. Exposure to chemical or physical mutagens, such as X-ray irradiation, can increase the occurrence of chromosomal rearrangements (6). These induced rearrangements, although random, have been instrumental in developing animal models that exhibit specific chromosomal abnormalities, including segmental trisomy 16 mouse model for human trisomy 21 (7, 8). Such models are invaluable for studying the effects of chromosomal anomalies and advancing our understanding of genetic diseases.

The advent of homologous recombination technology has facilitated the targeted insertion of specific genes or chromosome fragments by using an exogenous donor template with homologous arms (**Figure 1*a***), thus circumventing the stochastic nature of spontaneous mutations (9). When combined with mouse embryonic stem cell (ESC) technology, this approach enabled the generation of diverse model animals harboring specific chromosomal rearrangements (10).

The development of site-specific recombinase systems has further enhanced the precision of chromosomal rearrangement engineering by enabling targeted genetic manipulation at specific DNA sequences. These enzymatic systems recognize and act upon specific gene sequences, facilitating the rearrangement of genetic material between defined sites. Notably among these is the Cre/LoxP system developed in 1981 (11). The Cre recombinase exhibits specific recognition and binding of the LoxP sequence, thereby facilitating precise deletion or recombination of DNA sequences flanked by two LoxP sites (12) (**Figure 1*a***). Hence, Cre/LoxP technology was used extensively in engineering chromosomal rearrangements. In 1995, a seminal study employed the Cre/LoxP system to achieve inversions, duplications, and deletions on mouse chromosome 11,

Table 1 Advancements in engineering of chromosomal rearrangements, generation of AC vectors, and de novo genome synthesis

Engineering chromosomal rearrangements		Generating artificial chromosome vectors		De novo genome synthesis	
1993	Segmental trisomy 16 mouse model for Down syndrome via induced mutagenesis (7)	1983	YAC generation (30)	1970	In vitro synthesis of 77-bp yeast alanine transfer RNA gene (80)
1995	Mouse chromosomal inversions, duplications, and deletions via Cre/LoxP (13)	1987	AC vector gene integration (31)	2002	Synthesis of Poliovirus complementary DNA (81)
1999	A chromosomal inversion spanning a length of 24 cM in mouse (14)	1991	MAC construction (37)	2005	Synthesis of T7.1 artificial bacteriophage (82)
2000	Mouse embryo–nested chromosomal deletions (15)	1992	BAC construction (35)	2008	Artificial synthesis of *Mycoplasma genitalium* genome (113)
2014	Trisomy 21 in human ESCs via ZFN and MMCT (22)	1994	P1-derived AC establishment (114)	2010	Synthesis of a fully synthetic genome-driven bacterium (JCVI-syn1.0) (83)
2017	Complete deletion of trisomy 21 in mouse ESCs via CRISPR/Cas (24)	1996	Generation of Binary BAC (115)	2011	Synthetic Yeast Genome Project (Sc2.0) (86)
2018	Functional single-chromosome yeast via CRISPR/Cas9-mediated chromosome fusions (25)	1997	Creation of HAC containing satellite DNA sequence of chromosome 17 (38)	2014	First synthetic yeast chromosome (SynIII) (87)
2022	16.8-Mb chromosomal deletion and translocation in 293T cells via primer editing (29)	1999	Construction of transformation-competent AC (116)	2016	The smallest bacterial organism capable of self-replication (JCVIsyn3.0) (84)
2022	Complete fusion of two mouse chromosomes (5)	2006	Plant AC generation in maize (117)	2016	Human Genome Project-Write (GP-Write) (118)
				2017	Synthesis of five yeast chromosomes (SynII, SynV, SynVI, SynX, SynXII) (96–100)
				2023	Synthesis of all the remaining yeast chromosomes (88–95)

Abbreviations: AC, artificial chromosome; BAC, bacterial AC; CRISPR, clustered regularly interspaced short palindromic repeats; ESC, embryonic stem cell; HAC, human AC; MAC, mammalian AC; MMCT, microcell-mediated chromosome transfer; YAC, yeast AC; ZFN, zinc-finger nuclease.

marking an important shift in the ability to manipulate genes at a subchromosomal level with a range extending beyond the 20-kb limit (13). Further advancing this technique, in 1999, researchers successfully accomplished a chromosomal inversion on mouse chromosome 11 spanning a length of 24 cM, leading to the establishment of a corresponding mouse model (14). Subsequently, a series of nested chromosomal deletions were generated in mouse embryos, with sizes ranging from thousands to millions of base pairs (15). Cre/LoxP technology's versatility has been demonstrated further through its application in creating mouse models that mimic human genetic disorders, such as DiGeorge syndrome, Duchenne muscular dystrophy, and cancer-related reciprocal translocation t(8;21), by integrating it with ESC techniques.

Site-specific nuclease technologies, such as zinc finger nucleases (ZFNs) (16–17), transcription activator-like effector nucleases (TALENs) (18, 19), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems (20, 21), have revolutionized the ability to make targeted genetic modifications by inducing DSBs at specific DNA sites. These technologies harness the cell's natural repair mechanisms to introduce genetic changes. ZFNs and TALENs are engineered endonucleases, each with a customized DNA recognition domain paired with an endonuclease domain derived from the FokI restriction endonuclease (16, 17). ZFN consists of a tandem array of Cys2-His2 zinc fingers for DNA binding, whereas TALEN is composed of tandem repeats of a 33- to 35-amino-acid sequence that allows for specific DNA recognition (18, 19). The CRISPR/Cas system, with its simplicity and high specificity, has become a critical instrument in both gene and chromosomal engineering. It uses single-guide RNA (sgRNA) to recognize target sequences and direct Cas nucleases for precise cleavage (20, 21). For instance, in 2014, ZFNs and microcell-mediated chromosome transfer (MMCT) technologies were used to construct human ESCs carrying trisomy 21 (22). Subsequently, CRISPR/Cas was employed to delete the sex chromosome, generating mouse models with Turner syndrome, and to remove the extra human chromosome 21 from cells, including aneuploid mouse ESC lines derived from Down syndrome (Tc1) mice created by chromosome transfer (23) and human induced pluripotent stem cells (iPSCs) with trisomy 21 (24). Later, CRISPR/Cas technology facilitated the rearrangement of yeast chromosomes and successfully merged all 16 chromosomes into a single entity within haploid yeast cells (25). In 2022, based on CRISPR/Cas, our group accomplished the complete and programmable fusion of two mouse chromosomes and obtained a novel karyotype of mice (5) (**Figure 1*a***). Subsequently, another team achieved chromosome fusion in mice via CRISPR/Cas system as well (26). Additionally, a Robertsonian translocation mouse model based on centromere fusion via CRISPR/Cas was developed (27).

Scientists have also harnessed the CRISPR/Cas system to develop base editing and prime editing systems, with the latter enabling targeted genomic insertion through the modification of sgRNA and Cas enzymes, complemented by reverse transcriptase (28). Recently, the wild-type Cas9 nuclease was used to delete a 16.8-Mb chromosomal segment and induce chromosomal translocation in the 293T cell line through prime editing (29). These accomplishments signify a significant breakthrough in mammalian chromosome manipulation.

Generation of Artificial Chromosome Vectors

ACs are synthetic constructs designed to mimic the functional elements of natural chromosomes, providing a versatile platform for carrying exogenous DNA fragments. These ACs are particularly advantageous because they do not integrate into the host genome, thus circumventing issues related to insertional mutagenesis and position effects that can compromise gene expression. The spectrum of ACs includes yeast ACs (YACs), bacterial ACs (BACs), mammalian ACs (MACs), and human ACs (HACs).

YACs were developed by integrating yeast telomere and autonomously replicating sequences into the pBR322 plasmid and introducing them into yeast cells (30). Their application in genome sequencing projects across various species, including humans, mice, fruit flies, *Arabidopsis thaliana*, and rice, underscored their utility (31–33). The limitations of YACs, such as chimeric clones, instability during culture, and difficulty in distinguishing them from yeast chromosomes, prompted the development of BACs (34).

BACs, derived from the *Escherichia coli* F plasmid, are engineered to include a chloramphenicol resistance marker, an origin of replication, a helicase gene for efficient gene replication, and three genetic loci that facilitate the precise transmission of low-copy plasmids to progeny (35). BAC

vectors allow for the insertion of exogenous genomic DNA, which can then be introduced into bacterial strains through electroporation. Their capacity to handle large DNA fragments, combined with their low chimera-formation rate, high transformation efficiency, and straightforward manipulation, makes BACs valuable for a range of applications. These include not only genome sequencing libraries but also fundamental research and the creation of animal models. Examples of their use include gene discovery, animal model development, and genome sequencing in genome projects (36).

Although both BACs and YACs serve as effective vectors for large DNA fragments, BACs face compatibility issues within the mammalian system due to their prokaryotic origins, and YACs' limited capacity often requires genomic integration for stable gene expression. Mammalian gene regulation is complex, demanding not only the gene itself but also the appropriate regulatory sequences. MACs, modeled after the structure of mammalian chromosomes, have been engineered to address these challenges (37). MACs can accommodate foreign DNA larger than 1,000 kb and can carry exogenous genes with introns and their regulatory sequences without genomic integration. HACs, a subset of MACs, have garnered particular interest for their potential in higher organisms. HACs represent a culmination of advancements in YAC technology, adapted specifically for the needs of mammalian biology. A landmark achievement in 1997 involved the in vitro construction of a centromere structure nearly 1 Mb in length using satellite DNA sequences from human chromosome 17 (38). This HAC has been successfully integrated into human cancer cells and stably passed on to subsequent generations. HAC construction is guided by two principal strategies: the top-down approach, which involves the modification of existing chromosomes, and the bottom-up approach, which entails the de novo assembly of new chromosomes (39–41) (**Figure 1*b***).

The top-down approach involves the transfer of genome DNA, telomere DNA, and screening markers onto human chromosomes, followed by continuous random and/or selective truncation to create mini-chromosomes carrying the target genes (37, 41–50) (**Figure 1*b***). This method can also use natural mini-chromosomes, where genes are inserted and then reduced in size through irradiation to create smaller, functional chromosomes (51). Additionally, the satellite DNA–based AC technique involves integrating a selection marker and additional ribosomal DNA into the pericentromeric region of an acrocentric chromosome, leading to the targeted amplification of this area and the creation of a "sausage chromosome" (52–55). Although sequence insertion, especially into centromeric regions, poses challenges, the advent of site-specific nucleases has provided a means to overcome these difficulties, thus refining the precision of top-down HAC assembly.

Bottom-up HAC construction leverages cell-mediated processes to assemble new chromosomes from scratch (**Figure 1*b***). It primarily incorporates typical human centromeres, mammalian selectable markers, and genomic DNA, with or without telomeres. It involves cloning synthetic or natural α-satellite sequences into a YAC, BAC, or P1-derived AC to synthesize HACs de novo (56–68). The resulting structures, whether linear or circular, consist of input DNA that is amplified and/or concatenated, along with α-satellite-based centromeres, and typically range from 1 to 10 Mb in size (69). These de novo HACs can be easily engineered to incorporate specific genomic loci and marker genes, by either co-transfecting the target gene with α-satellite DNA or combining both into a single HAC vector (59–61, 67, 68, 70). Advanced techniques, such as site-specific nucleases and transposases, facilitate the efficient recombination of BACs and P1-derived ACs with α-satellite DNA, yielding a unified HAC vector (68, 69, 71). Notably, HACs have successfully expressed genes such as *HPRT1* (*hypoxanthine-guanine phosphoribosyltransferase 1*) and *GCH1* (*guanosine triphosphate cyclohydrolase 1*) in human embryonic cells, with the transgenic cells effectively complementing defective gene copies in vitro (59, 61, 68, 71).

De Novo Synthesis of Mammalian Chromosomes

The ability to design, synthesize, and assemble large mammalian genomes has been a long-standing goal in the field of chromosome engineering (**Figure 1*c***). De novo synthesis offers a novel approach to constructing large mammalian genomes from scratch. Unlike ACs that involve specific gene assembly, de novo synthesis focuses on creating entire chromosomes, allowing for the incorporation of regulatory elements like enhancers to study gene function or compensate for gene loss. The process of de novo synthesis involves designing genomes with desired functionalities based on sequencing a genome and synthesizing DNA fragments from scratch using chemical methods. These small fragments are then assembled stepwise into larger constructs until a complete chromosome is formed (**Figure 1*c***).

Three fundamental principles guide synthetic chromosome design: simplification, expansion, and reconstruction. Simplification involves identifying the minimal set of genes and their respective functions that sustain vital biological processes across diverse conditions (72). This includes streamlining both at the gene level and within non-gene intervals (73). Expansion entails the incorporation of novel genes into existing natural genomes, thereby conferring organisms with novel functionalities and phenotypes. Reconstruction involves augmenting natural genomes in various dimensions, encompassing codon substitution, modularizing gene expression elements, rearranging gene clusters, and remodeling chromosome structures (74, 75). Furthermore, epigenetic modifications must be considered as a contributing factor when dealing with reconstructed genomes.

The chemical synthesis of DNA is categorized into three methodologies: solid-phase column-based, solid-phase chip-based, and biocatalysis processes. The first generation includes the phosphoramidite synthesis method (76) and the solid-phase phosphoramidite triester synthesis method. This approach entails immobilizing DNA onto a solid support to facilitate coupling reactions. Although this method achieves low error rates, it is constrained by its limited throughput and high costs. The second-generation method (solid-phase chip-based) also necessitates a solid-phase carrier, but it is substituted with a chip and combined with electrochemical or photochemical methods, offering the advantages of high throughput and low cost. Nonetheless, its efficiency is inconsistent, and both first- and second-generation synthesis methods require substantial amounts of toxic chemical reagents. The advent of third-generation synthetic technology, using biological enzymes like terminal deoxynucleotidyl transferase, marked a significant advancement, enhancing the efficiency and length of synthesizable DNA sequences while mitigating chemical toxicity issues.

Both in vitro and in vivo methodologies have revolutionized the assembly of large-scale DNA, encompassing hundreds of kilobases to megabases. In vitro methods, such as enzyme cutting, ligation (77, 78), and Gibson assembly (79), have been instrumental in constructing smaller DNA fragments. Conversely, high–molecular weight DNA molecules necessitate in vivo assembly, often employing microbial hosts like *E. coli*, *Bacillus subtilis*, and *Saccharomyces cerevisiae*, with the latter being favored particularly for its enhanced capacity for homologous recombination, enabling the simultaneous assembly of multiple DNA fragments.

In recent years, synthetic genomics has achieved a series of groundbreaking milestones in the innovative synthesis of genomes of lower organisms, including mycoplasma, *E. coli*, and *S. cerevisiae* (**Table 1**). The synthesis of yeast alanine transfer RNA, which consists of 77 base pairs, is a pioneering study in synthetic genomics (80). In 2002, a 7.5-kb poliovirus complementary DNA was chemically constructed (81). Furthermore, in 2005, a meticulous redesign and synthesis process led to obtainment of T7.1 synthetic bacteriophage by synthesizing an approximately 12-kb fragment from the extensive 40-kb T7 bacteriophage genome (82). Subsequently, the 1.1-Mb *Mycoplasma mycoides* JCVIsyn1.0 genome was synthesized and demonstrated to be

functional (83). To date, synthetic genomes have mostly mimicked natural template DNA. In 2016, scientists minimized the 1.1-Mb JCVIsyn1.0 genome to a functional 531-kb JCVIsyn3.0 genome using four design–build–test cycles (84). In the same year, researchers conducted experiments involving various recoding schemes using only 57 codons (74). Additionally, in 2019, scientists developed a modified *E. coli* strain called Syn61 by eliminating the TAG stop codon as well as the TCG and TCA serine codons from its genetic code, resulting in a streamlined coding system consisting of only 61 codon sequences for all coding regions (85). The Synthetic Yeast Genome Project (Sc2.0) proposed the synthesis of all 16 yeast chromosomes (86), with SynIII produced in 2014 (87), followed by SynII, SynV, SynVI, SynX, and SynXII in 2017; the remaining 10 chromosomes were synthesized successfully by 2023 (88–100). These achievements mark significant milestones in chromosome synthesis research.

Although de novo synthesis has been used successfully to construct bacterial and yeast chromosomes, technical challenges such as low efficiency and high cost have limited its application in mammals. Current research on synthetic chromosomes in mammals revolves primarily around their fundamental architecture, ensuring their stable existence and efficient functionality within cellular systems.

In mammalian systems, the centromeric regions are particularly complex, exhibiting significant length and sequence intricacies, characterized by significant variations in the lengths of arrays of higher-order repeat units among individuals (101). This complexity presents substantial challenges for sequencing and synthesis, with only a few centromeres, such as those on chromosomes 8, X, and Y, being fully sequenced and assembled within the human genome (102). The design and construction of these regions in synthetic mammalian chromosomes are crucial, with recent innovations offering new methodologies that bypass traditional constraints, such as reliance on natural alpha satellite and centromere protein B (CENP-B) box sequences (103). This innovative method circumvents the requirement for conventional bottom-up vectors that rely on natural alpha satellite and CENP-B box sequences. Instead, it harnesses a nonrepetitive centromere derived from chromosome 4q21 to substitute the repetitive centromere sequence. The vector is supplemented with LacO repeat sequences, enabling localized expression of LacI-HJURP through LacI-LacO interaction. Subsequently, HJURP recruits CENP-A, which then envelops adjacent DNA to establish a functional centromere. This research obviates the need to synthesize and assemble extensive fragments of repetitive DNA found in native centromeres, thus rendering it highly significant in the design and construction of animal AC vectors. In addition to the centromere region, efforts are also underway to design and synthesize other functional gene segments, including human antibody genes. Lessons from lower organisms' genomic design principles provide valuable insights for designing mammalian genomes, guiding future advancements in genome annotation and synthesis for biomedical and biotechnological applications.

Chromosome Transfer

In the rapidly evolving field of chromosome engineering, the ability to engineer and transfer ACs into mammalian cells is a critical milestone. Although chromosome engineering has reached a level of sophistication that allows for the creation of megabase-sized DNA fragments, the effective transfer of these ACs into mammalian cells presents a complex challenge. To date, various methods have been developed and employed to facilitate this transfer, including in vitro DNA transfer techniques and intercellular transfer techniques. The former include electroporation, lipofection, viral transfection, and microinjection, and the latter include cell fusion and MMCT.

Electroporation remains a widely used method for gene delivery (several to tens of kilobases), achieved by applying an electric pulse to temporarily disrupt cell membranes and create pores, allowing charged DNA molecules to enter cells in a process reminiscent of electrophoresis

(**Figure 1*d***). Despite the robust electric field facilitating DNA transfer, the cell's phospholipid bilayer poses a barrier that limits electric current penetration, which in turn reduces the potential for cytotoxicity. Although traditional electroporation methods face challenges in effectively delivering DNA to the cell nucleus, which hampers their efficiency, nuclear transfection technology enhances the process. This advanced technique eliminates the dependency on cell division and allows for the efficient direct delivery of exogenous genes to the nucleus (104). In a notable advancement in 2021, researchers successfully used nuclear transfection technology to electroporate a 101-kb genome into A17iCre mouse ESCs, demonstrating this approach's potential for the transfer of 100-kb-level chromosomal fragments (105).

Microinjection is a precise technique that uses a fine needle to inject exogenous genes into target cells under microscopic control (106) (**Figure 1*d***). Although more costly, more labor intensive, and limited in throughput, this method has been instrumental in transferring large DNA fragments, such as 200–500-kb YACs, into mammalian cells since 1993 (107).

Liposome-mediated transfection is currently a well-established and efficient method for the delivery of small DNA fragments, typically ranging from several to tens of kilobases. It harnesses the positive charge on liposomes' surface to bind with negatively charged DNA, resulting in the formation of liposome–DNA complexes (**Figure 1*d***). These complexes can then be effectively delivered into cells through either membrane fusion or endocytosis mechanisms. However, there have been notable exceptions. In 1999, researchers successfully employed liposome transfection to deliver a compressed 2.3-Mb YAC composed of poly-L-lysine and polyethyleneimine into HT1080 cells (108). Additionally, in 2001, scientists used liposomes as carriers to transfer a 404-kb HAC into HT1080 cells (71). However, it should be noted that liposomal materials exhibit cytotoxicity when compared to physical transfer methods.

Viral transfection is typically used to deliver small DNA fragments into cells. Currently, commonly used viral vectors include retroviruses, adenoviruses, and lentiviruses. Notably, in 2015, researchers successfully transferred a 152-kb segment into human ESCs using herpes simplex virus type 1 (HSV-1) (109). However, these vectors possess limited capacity and can be challenging to manipulate, thereby raising potential biosafety concerns.

The instability of large DNA fragments in the extracellular environment during purification and amplification poses significant hurdles for the above in vitro DNA transfer techniques. These challenges underscore the need for innovative methods that ensure DNA fragment stability and integrity throughout the transfer process. Thus, this field is focusing increasingly on intercellular transfer methods, including cell fusion and MMCT. These approaches offer a promising alternative by facilitating the direct exchange of genetic material between cells, thereby bypassing the need for extensive in vitro manipulation of large DNA segments.

Cell fusion, exemplified by yeast protoplast fusion, is a critical technique in chromosome delivery, enabling the transfer of large DNA fragments or entire chromosomes into mammalian cells (110). Yeast, often used to assemble large genetic constructs, can transfer these constructs directly into mammalian cells, enhancing the efficiency of chromosome delivery.

MMCT is based on the fusion of recipient cells and microcells derived from chromosome donor cells (111). The process initiates with the prolonged treatment of donor cells with colchicine, a compound that inhibits spindle formation, leading to the formation of micronuclei containing one or more chromosomes. Subsequently, micronuclei are enriched through cytochalasin B treatment and centrifugation (**Figure 1*d***). The resulting small microcells carrying one or several chromosomes are filtered and then fuse with recipient cells, facilitating chromosome transfer. In a seminal study from 1999, researchers employed MMCT to transfer human chromosomes into mouse A9 cell lines, resulting in a cell line library in which each cell harbors one human chromosome, enabling detailed research on epigenetic modifications of human chromosomes (112). In

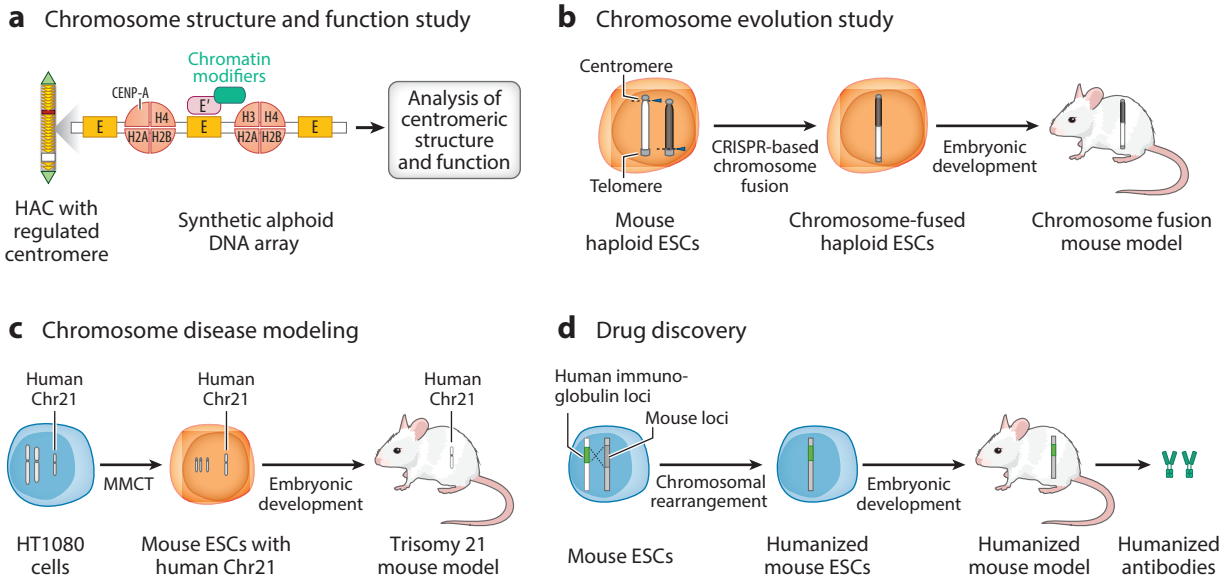


Figure 2

Chromosome engineering applications. (*a*) A HAC system with a regulatable centromere. The E′ protein is fused with another chromatin modifier to form an E′-fused protein, which can target and bind the E sequence and modify the epigenetic state of the sequence. (*b*) In the mouse sperm-like haploid ESCs (with three imprinted regions deleted), single-guide RNAs target the centromere of one chromosome and the telomere of another. The CRISPR/Cas system leads to chromosome fusion and results in the engineered haploid ESCs. Then they are injected into a metaphase II oocyte to undergo embryonic development, and a chromosome fusion mouse is established. (*c*) Human Chr21 is transferred from HT1080 cells to mouse ESCs via MMCT. Based on the embryonic developmental potential of the mouse ESCs with human Chr21, the trisomy 21 mouse model is created. (*d*) In the mouse ESCs, the mouse immunoglobulin loci are replaced by human ones through chromosomal rearrangement methods. The resulting humanized mouse ESCs can give rise to a mouse model yielding humanized antibodies. Abbreviations: CENP, centromere protein; CRISPR, clustered regularly interspaced short palindromic repeat; E, genetic regulatory element used to replace CENP-B box; E′, protein that specifically targets and binds A sequence; ESC, embryonic stem cell; HAC, human artificial chromosome; MMCT, microcell-mediated chromosome transfer.

2005, it was used to introduce human chromosome 21 into mouse ESCs and generate the mouse model of Down syndrome (23).

APPLICATIONS OF MAMMALIAN CHROMOSOME ENGINEERING

Chromosome Structure and Function Studies

Chromosome engineering in mammals has become a valuable tool for unraveling the complexities of chromosome structure and function (**Figure 2*a***). This field has extensively studied chromosome segregation, a vital cellular process during division. Notably, research involving HACs and MACs has provided significant insights. These studies have shown that HACs maintain mitotic and cytogenetic stability over time, with specific CENPs playing a crucial role in ensuring accurate chromosome segregation (38, 119).

Further investigations have deepened our understanding of centromere composition and functionality. For instance, studies have illuminated the essential role of CENP-B in centromere formation, demonstrating its necessity for de novo centromere assembly and its complex influence on chromosomal stability (120). Additionally, research has explored how modifications in chromatin states at the kinetochore affect chromosome segregation, revealing that alterations can lead

to chromosomal instability and mis-segregation (121, 122). In 2019, researchers developed HACs that function independently of the traditional constraints of centromeric DNA, challenging the established notion that repetitive α-satellite sequences and CENP-B are required for centromere specification (103). This novel HAC design, which leverages the epigenetic marking of CENP-A nucleosomes for centromere establishment, has profound implications for our understanding of chromosome structure. It suggests that the chromatin environment and the presence of specific histone variants, rather than DNA sequence itself, may be the primary determinants of centromere identity and function.

Moreover, CRISPR genome editing technology has propelled advancements in understanding the 3D organization of chromosomes. Manipulating structural DNA motifs and proteins and altering DNA looping mechanisms have provided novel methods to investigate the 3D genomic landscape. For detailed information, readers are referred to another review (123).

These comprehensive studies underscore the nuanced interplay between chromatin structure and centromere activity, crucial for maintaining chromosome stability and preventing aneuploidy in mammalian cells. Through detailed examination, chromosome engineering continues to shed light on the intricate mechanisms underpinning mammalian chromosome structure and function, significantly advancing our knowledge in the field.

Identification of Gene Regulatory Elements and Gene Function

Chromosome engineering has significantly advanced our comprehension of gene function(s) and regulatory mechanisms. This field has been pivotal in decoding the intricate relationships between genes, regulatory elements, and their environmental context, which are essential for gene expression and regulation.

MMCT has been particularly impactful in identifying tumor suppressor genes. For example, researchers created a library of mouse A9 cells, each harboring a single human chromosome transferred via MMCT, facilitating the analysis of human chromosomes in cancer cell environments (124). This approach has led to the discovery of tumor suppressor genes across various chromosomes, including the identification of *PITX1* (*paired-like homeodomain 1*) on chromosome 5, a gene that negatively regulates telomerase activity, contributing to cellular aging and cancer suppression (124).

In another study, chromosome engineering was used to investigate transcriptional regulation across species. Analysis of hepatocytes in mice carrying human chromosome 21 revealed the predominance of genetic sequence over the nuclear environment in determining gene expression and transcription factor binding (125). This finding challenges the traditional emphasis on epigenetic and cellular factors in transcriptional regulation, suggesting a fundamental role for the genetic sequence.

These studies illustrate the potential of chromosome engineering in uncovering gene functions and regulatory elements, with implications for understanding cancer biology and transcriptional regulation across species. As the field evolves, expanding research to include diverse species and genetic configurations will be crucial for dissecting the complex dynamics of gene regulation.

Chromosome Evolution Studies

Exploring the impact of chromosome rearrangements on phenotypic evolution, adaptation, and speciation has been a crucial aspect of chromosome evolution research (1). Using chromosome engineering and haploid ESC technologies, our group developed chromosome-fused mouse models (Chr4+5 and Chr1+2), providing insights into chromosomal influences on evolution (5) (**Figure 2*b***). Varied phenotypic outcomes were observed in these models, such as postnatal

overgrowth, behavioral changes, and fertility alterations. Specifically, Chr1+2 mice exhibited growth acceleration and heightened anxiety-related behaviors, whereas Chr4+5 mice showed normal growth and behavior patterns. The dysregulation of *Capn11* (*calpain 11*), associated with rearranged chromosomes, was linked to these phenotypic deviations.

In terms of chromatin 3D structure evolution, increased interactions were noted in fused chromosomes within Chr4+5 haploid ESCs, neural stem cells, and brain tissues, hinting at chromosomal interaction changes through evolution. This was accompanied by altered topologically associated domain (TAD) compactness and distributions, illustrating structural adaptations over time. Comparatively, these changes paralleled those in naturally occurring Robertsonian mice, enriching our understanding of chromosomal fusion processes across evolutionary spans (126). Studies addressing speciation have shown that chromosomal rearrangements can contribute to reproductive isolation and speciation by affecting fertility in hybrid organisms (127, 128). Mice with Chr1+2 experienced infertility, and Chr4+5 mice had reduced fertility, indicating the role of chromosome segregation errors in reproductive limitations. Moreover, other groups have also undertaken efforts to replicate chromosome fusion in mice to study karyotype evolution, with CRISPR/Cas technology facilitating the creation of haploid ESCs and mouse models with metacentric chromosomes, simulating natural karyotype evolution and providing a model to study chromosomal fusion events over evolutionary timescales (27).

Engineered mouse models have deepened our understanding of chromosome evolution, revealing the effects of rearrangements on phenotype and speciation. These studies offer valuable insights into the complex dynamics of evolutionary biology. In the future, chromosome fusion technology can be used to merge chromosomes 2A and 2B in chimpanzee iPSCs to simulate the human karyotype. Following this, the iPSCs can be differentiated into brain organoids to investigate the mechanisms underlying human brain evolution and other human-specific traits.

Human Chromosome Disease Modeling

Chromosome engineering has been pivotal in modeling human diseases, particularly through replicating chromosome rearrangements and genetic anomalies (**Figure 2*c***). The creation of disease-specific chromosome alterations in model organisms like mice has provided valuable insights into the genetic basis and phenotypic manifestation of various conditions (129, 130).

For instance, the modeling of DiGeorge syndrome involved engineering chromosome deletions in mice, mirroring the human 22q11.2 deletion associated with the syndrome. This led to the identification of critical genes, including *TBX1* (*T-box transcription factor 1*), underlying the heart defects characteristic of DiGeorge syndrome (131–136). Moreover, the development of aneuploid mouse models, like the Tc1 line carrying human chromosome 21, has advanced our understanding of Down syndrome, offering a comprehensive model to study the disorder's wide-ranging phenotypes (23) (**Figure 2*c***).

Similarly, research on mouse chromosome 11B3 deletion has shed light on the complexities of cancer development, suggesting the synergistic effects of gene losses in tumorigenesis beyond the well-known *Trp53* (*transformation related protein 53*) gene (137). Additionally, site-specific nuclease genome editing technologies have facilitated the creation of precise chromosomal translocations and inversions in cell models, directly impacting oncogenesis research. These engineered alterations replicate the genetic conditions found in cancers like Ewing sarcoma and acute myeloid leukemia [linked to translocations t (11;22) and t (8;21)] and non-small cell lung cancer [linked to inversions inv (2) (p21p23) and inv (10) (p11q11)], enhancing our ability to explore cancer biology and therapeutic interventions (138, 139). CRISPR/Cas-based chromosome engineering has established diverse chromosomal rearrangements related to cancers such as hematological malignancies

and brain, liver, lung, and intestinal cancers, as reviewed elsewhere (129). These advancements underscore the role of chromosome engineering in disease modeling, providing a robust framework for dissecting the genetic intricacies of human diseases and exploring new therapeutic avenues.

Drug Discovery

Chromosome engineering has emerged as a cornerstone in drug discovery, offering unprecedented precision in the humanization of animal models for antibody discovery (**Figure 2*d***) and drug screening. Transgenic methods were used to construct mice producing humanized antibodies by introducing an entire human immunoglobulin heavy chain gene, including the constant region, into mice (140). In 2014, another study demonstrated the creation of transgenic mice with humanized immunoglobulin loci, enabling the production of human antibodies upon immunization (141) (**Figure 2*d***). This study improved upon earlier models by integrating the comprehensive human immunoglobulin variable-gene repertoire into the mouse genome, preserving mouse constant regions, and ensuring normal immune functionality and robust antibody responses.

Further advancements in the genetic humanization of mice have been achieved by replacing extensive segments of mouse immune genes with their human counterparts, thereby greatly enhancing the production of human monoclonal antibodies (142). Another notable development involved *VelocImmune* mice, engineered to produce human antibodies efficiently, maintaining a fully functional immune system for rapid therapeutic antibody testing (143).

These studies collectively highlight the transformative role of chromosome engineering in therapeutic antibody discovery, paving the way for more effective vaccine design and antibody development. However, challenges such as ethical concerns, genetic modification complexities, and technology scalability remain.

In drug screening, HACs have been used to create cell lines expressing wild-type or mutant *EGFR* (*epidermal growth factor receptor*) genes, facilitating the assessment of tyrosine kinase inhibitors in non-small cell lung cancer. This approach provided stable gene expression and consistent genomic backgrounds, aligning cell line responses with clinical outcomes and surpassing traditional methods (144, 145). This evolution in chromosome engineering represents a significant leap forward in drug discovery, offering sophisticated tools to develop and test new therapeutics.

Chromosome engineering has revolutionized drug discovery by facilitating the precise humanization of animal models and creating advanced platforms for antibody development and drug screening. These innovations offer a promising pathway for personalized medicine, enhancing the efficacy and specificity of therapeutic interventions.

CHALLENGES AND PERSPECTIVES

Challenges

Although chromosome engineering is replete with potential, it faces formidable challenges, due primarily to the inherent complexity of mammalian genomes, the nascent state of genome editing technologies, and the daunting task of synthesizing and assembling large DNA fragments. Together, these challenges highlight the urgent need for innovative solutions to fully exploit the potential of chromosome engineering.

Chromosomal rearrangement engineering. Despite the revolutionary impact of CRISPR/Cas9 in genome editing, limitations in efficiency and specificity hamper its application to chromosomal rearrangements. Manipulating large DNA fragments within mammalian cells can result in unexpected outcomes, including off-target effects, accidental deletions, or rearrangements. Rapid identification and correction of these errors are crucial for advancing chromosome engineering.

Advanced technologies such as single-cell sequencing and artificial intelligence–powered predictive models could play important roles in overcoming these challenges, enhancing the precision, efficiency, and safety of chromosome engineering efforts. The quest for high-fidelity chromosome editing demands advancements in tools and methodologies that can navigate the intricacy of mammalian genomes with unparalleled precision and efficiency.

MAC vector generation and de novo mammalian chromosome synthesis. Generating MAC vectors and synthesizing mammalian chromosomes are complex tasks hindered by mammalian genome complexity and limited technological resources. Incomplete understanding of chromosomal architecture, especially of core structures such as centromeres, complicates the accurate design and prediction of genetic modification outcomes. This knowledge gap is acute in synthetic genome creation, often resulting in unpredictable phenotypes. Further, synthesizing and assembling large DNA fragments into functional mammalian chromosomes is technically demanding and expensive, exacerbated by the absence of appropriate systems for managing these large constructs.

Mammalian chromosome transfer. Delivering large DNA fragments and entire mammalian chromosomes into mammalian cells remains one of the most formidable challenges in mammalian synthetic genomics. The instability of large DNA fragments during in vitro processes such as purification, amplification, and delivery exacerbates this challenge, alongside the inherent limitations of current delivery techniques.

Techniques such as electroporation and microinjection for exogenous DNA transfer not only encounter issues with transfer efficiency and cell toxicity but also must address the critical problem of maintaining the stability of large DNA fragments in vitro. Moreover, techniques like MMCT and yeast protoplast fusion, crucial for the intercellular transfer of large DNA fragments and entire chromosomes, face their own sets of limitations as well. MMCT, for example, is limited by its ability to transfer ACs only from cells capable of efficiently forming micronuclei, such as DT40, A9, and HT1080 cells, into recipient cells. The low efficiency of this technique significantly hampers its practical application. Solutions to improve efficiency include using advanced chromosome engineering techniques for precise chromosome monitoring, facilitating efficient sorting and transfer, and potentially applying CRISPR/Cas systems to enhance its effectiveness. A chromosome tagging system combining dCas9 with fluorescent proteins could aid in purifying microcells containing the desired chromosome (146). Furthermore, optimizing cell fusion efficiency through mechanisms like receptor-ligand recognition and exploring new carrier tools for DNA transfer could offer avenues to circumvent current limitations. Even if the transfer is successful, ensuring the stability and reproducibility of the function of engineered chromosomes within the host organism presents another layer of complexity. Minor modifications in chromosomal architecture can unpredictably affect genomic stability and function, highlighting the need for meticulous design and evaluation of engineered chromosomes.

Ethical and biosafety considerations in mammalian chromosome engineering. As the field progresses, ethical and biosafety considerations gain importance. The potential creation of novel life forms through advanced chromosome engineering raises ethical questions and biosafety concerns. An important ethical issue is the "playing God" critique, which reflects deep societal unease with the human-driven creation of life, suggesting that such actions may overstep the natural boundaries that govern life itself. Concurrently, biosafety concerns emerge from the possibility that these engineered life forms may inadvertently be released into the environment, potentially leading to unforeseen consequences, such as horizontal gene transfer and significant disruption to existing ecosystems. Scientists must address these challenges responsibly, adhering to regulations and ethical guidelines to minimize risks and ensure the safe advancement of the field.

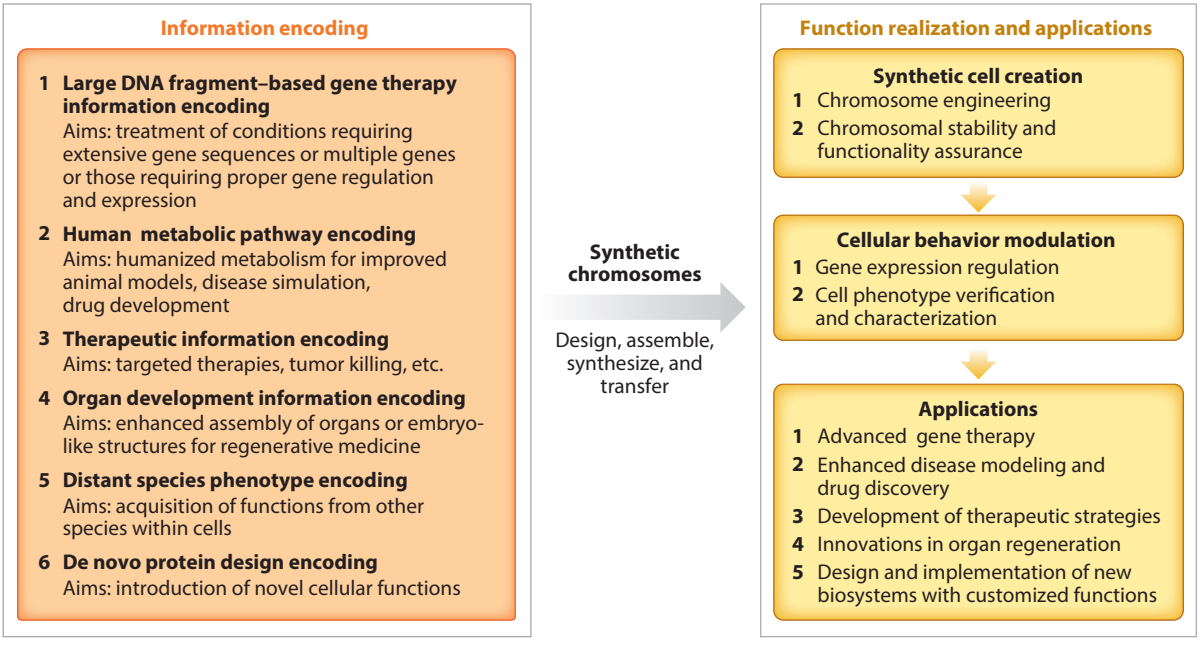


Figure 3

Perspectives on synthetic chromosomes and synthetic cells.

Perspectives

Despite these challenges, the prospects of chromosome engineering are vast, promising transformative breakthroughs in basic research and clinical applications.

Chromosome engineering for enhanced genetic disease modeling. Chromosome engineering, including substantial DNA segments, goes beyond traditional disease modeling (**Figure 3**). For instance, introducing complete human metabolic pathways can realize the simulation of the human environment in humanized animal models for related research. The engineering of animal models with complete human metabolic pathways offers an unprecedented opportunity to simulate human physiological conditions accurately. This innovation is exemplified by the introduction of both the human *CYP3A* (*cytochrome P450 family 3 subfamily*) gene region and *UGT2* (*UDP glucuronosyltransferase family 2*) gene clusters into rats (147), heralding a new era of humanized models for drug metabolism and pharmacokinetics studies. Designing and manipulating large DNA segments in mammals offers unique advantages in the study of multigene and chromosomal diseases. Future developments may include animal models embedded with comprehensive human genetic networks, enhancing our understanding of disease mechanisms and treatment responses.

Chromosome engineering for agricultural breeding. Chromosome engineering is setting the stage for an agricultural revolution, specifically by enabling the editing of large DNA segments and entire chromosomes rather than focusing on small-scale gene editing. This advanced approach allows for the introduction of comprehensive traits that significantly enhance livestock performance and resilience. By precisely manipulating chromosomal DNA, scientists can integrate traits for superior disease resistance across entire herds, reducing antibiotic dependency and lowering the risk of widespread diseases. This method also facilitates the modification of chromosomal regions controlling growth and metabolism, leading to livestock with optimized growth rates,

thus ensuring enhanced productivity and welfare without adverse effects. Furthermore, chromosome engineering's ability to adjust whole nutritional pathways may elevate the quality of animal products, contributing to global food security by offering nutritionally enriched meat. This shift from gene- to chromosome-level modifications represents a significant advancement in agricultural practices, indicating a future in which sustainable and efficient food systems may be achieved through responsible chromosome engineering.

Chromosome engineering for regenerative medicine. At the forefront of regenerative medicine, the role of chromosome engineering marks a key transformation, set to redefine therapeutic strategies for a spectrum of diseases (**Figure 3**). Unlike traditional gene editing that focuses on modifying individual genes or small genome regions, chromosome engineering excels in manipulating large DNA fragments and even entire chromosomes. This capability allows for the aggregation of multiple functionally interconnected genes and the construction of extensive DNA regions endowed with specific functionalities. When combined with stem cell therapy, chromosome engineering may provide novel insights into the treatment of complex genetic disorders. Chromosome engineering's ability to precisely edit and manage vast gene loci and clusters of genes offers a solution to the inherent complexity of numerous genetic conditions. This innovation harbors the potential for correcting or substituting entire gene clusters, heralding new paradigms in treating ailments like muscular dystrophy and cystic fibrosis, which are beyond the reach of conventional gene therapy.

The customization of PSCs through chromosome engineering introduces a new dimension to regenerative medicine. By crafting PSCs with customized phenotypes and functions, we can significantly diminish the risk of immune rejection and pave the way for personalized medical treatments. This advancement promises the regeneration of damaged tissues and organs, directly addressing the acute shortage of donor organs available for transplantation. Furthermore, chromosome engineering holds promise for advancing the field of xenotransplantation and for developing humanized animals. By integrating human-specific immunoglobulin loci and immune rejection suppressor gene clusters into the genomes of animals such as pigs, chromosome engineering may enable the production of organs highly compatible with the human immune system. Such a breakthrough could solve the persistent organ shortage in transplantation, possibly increasing the availability of life-saving organs for patients on transplant waiting lists and significantly improving their chances of survival.

Synthesis of mammalian chromosomes. Synthesizing mammalian chromosomes represents a crucial step in advancing chromosome engineering. This process enables the correction of genetic anomalies and the introduction of innovative metabolic pathways, thereby enhancing our comprehension of chromosomal structures, such as centromeres and telomeres, and the dynamic organization of chromatin within the 3D space. Moreover, it sheds light on the mechanisms of chromosome rearrangement and their significance in the evolution of species (**Figure 3**). The progress achieved in the Synthetic Yeast Genome Project (Sc2.0) has sparked important discussions about the creation of synthetic cells. Despite being the fundamental units of life, cells possess intricate compositions. Defining the core attributes necessary for a cell to function independently is essential. These attributes may vary in importance depending on the perspectives of different stakeholders, such as medical professionals seeking treatments for diseases, biotechnologists aiming to produce specific products, or biophysicists in search of the most elementary forms of life. A consensus is emerging that synthetic cells should, at minimum, exhibit capabilities for autonomous growth, metabolism, and replication. Although the complete realization of synthetic genomics and chromosome engineering presents formidable challenges, the ongoing advancements in this field hold promise for the development of synthetic cells. These could revolutionize the fields of

biology, medicine, and biotechnology by introducing transformative solutions and opening new horizons in synthetic biology (**Figure 3**).

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LITERATURE CITED

1. Damas J, Corbo M, Lewin HA. 2021. Vertebrate chromosome evolution. *Annu. Rev. Anim. Biosci.* 9:1–27
2. Mallo M. 2018. Reassessing the role of *Hox* genes during vertebrate development and evolution. *Trends Genet.* 34:209–17
3. Schatz DG, Swanson PC. 2011. V(D)J recombination: mechanisms of initiation. *Annu. Rev. Genet.* 45:167–202
4. Nowell PC. 2007. Discovery of the Philadelphia chromosome: a personal perspective. *J. Clin. Investig.* 117:2033–35
5. Wang LB, Li ZK, Wang LY, Xu K, Ji TT, et al. 2022. A sustainable mouse karyotype created by programmed chromosome fusion. *Science* 377:967–75
6. Muller HJ. 1927. Artificial transmutation of the gene. *Science* 66:84–87
7. Davisson MT, Schmidt C, Reeves RH, Irving NG, Akeson EC, et al. 1993. Segmental trisomy as a mouse model for Down syndrome. *Prog. Clin. Biol. Res.* 384:117–33
8. Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, et al. 1995. A mouse model for Down syndrome exhibits learning and behaviour deficits. *Nat. Genet.* 11:117–84
9. Smithies O, Gregg RG, Boggs SS, Koralewski MA, Kucherlapati RS. 1985. Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. *Nature* 317:230–34
10. Thomas KR, Folger KR, Capecchi MR. 1986. High frequency targeting of genes to specific sites in the mammalian genome. *Cell* 44:419–28
11. Stricklett PK, Nelson RD, Kohan DE. 1998. Site-specific recombination using an epitope tagged bacteriophage P1 Cre recombinase. *Gene* 215:415–23
12. Collins EC, Pannell R, Simpson EM, Forster A, Rabbitts TH. 2000. Inter-chromosomal recombination of *Mll* and *Af9* genes mediated by cre-*loxP* in mouse development. *EMBO Rep.* 1:127–32
13. Ramírez-Solis R, Liu P, Bradley A. 1995. Chromosome engineering in mice. *Nature* 378:720–24
14. Zheng B, Sage M, Cai WW, Thompson DM, Tavsanli BC, et al. 1999. Engineering a mouse balancer chromosome. *Nat. Genet.* 22:375–78
15. Su H, Wang X, Bradley A. 2000. Nested chromosomal deletions induced with retroviral vectors in mice. *Nat. Genet.* 24:92–95
16. Kim YG, Cha J, Chandrasegaran S. 1996. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. *PNAS* 93:1156–60
17. Bibikova M, Golic M, Golic KG, Carroll D. 2002. Targeted chromosomal cleavage and mutagenesis in *Drosophila* using zinc-finger nucleases. *Genetics* 161:1169–75
18. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, et al. 2011. A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol.* 29:143–48
19. Mak AN-S, Bradley P, Cernadas RA, Bogdanove AJ, Stoddard BL. 2012. The crystal structure of TAL effector PthXo1 bound to its DNA target. *Science* 335:716–19
20. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, et al. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 315:1709–12
21. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816–21
22. Kazuki Y, Yakura Y, Abe S, Osaki M, Kajitani N, et al. 2014. Down syndrome-associated haematopoiesis abnormalities created by chromosome transfer and genome editing technologies. *Sci. Rep.* 4:6136
23. O'Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, et al. 2005. An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. *Science* 309:2033–37

24. Zuo E, Huo X, Yao X, Hu X, Sun Y, et al. 2017. CRISPR/Cas9-mediated targeted chromosome elimination. *Genome Biol.* 18:224
25. Shao Y, Lu N, Wu Z, Cai C, Wang S, et al. 2018. Creating a functional single-chromosome yeast. *Nature* 560:331–35
26. Wang Y, Qu Z, Fang Y, Chen Y, Peng J, et al. 2023. Chromosome territory reorganization through artificial chromosome fusion is compatible with cell fate determination and mouse development. *Cell Discov.* 9:11
27. Zhang XM, Yan M, Yang Z, Xiang H, Tang W, et al. 2022. Creation of artificial karyotypes in mice reveals robustness of genome organization. *Cell Res.* 32:1026–29
28. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533:420–24
29. Tao R, Wang Y, Hu Y, Jiao Y, Zhou L, et al. 2022. WT-PE: Prime editing with nuclease wild-type Cas9 enables versatile large-scale genome editing. *Signal. Transduct. Target Ther.* 7:108
30. Murray AW, Szostak JW. 1983. Construction of artificial chromosomes in yeast. *Nature* 305:189–93
31. Burke DT, Carle GF, Olson MV. 1987. Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 236:806–12
32. Roberts L. 1992. Two chromosomes down, 22 to go: Researchers have produced detailed physical maps of human chromosomes Y and 21, providing a boost both to the Human Genome Project and to efforts to locate disease genes. *Science* 258:28
33. Sakata K, Antonio BA, Mukai Y, Nagasaki H, Sakai Y, et al. 2000. INE: a rice genome database with an integrated map view. *Nucleic Acids Res.* 28:97–101
34. Bellis M, Gérard A, Charlieu JP, Marçais B, Brun ME, et al. 1991. Construction and characterization of a partial library of yeast artificial chromosomes from human chromosome 21. *DNA Cell Biol.* 10:301–10
35. Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, et al. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *PNAS* 89:8794–97
36. Shizuya H, Kouros-Mehr H. 2001. The development and applications of the bacterial artificial chromosome cloning system. *Keio J. Med.* 50:26–30
37. Farr C, Fantes J, Goodfellow P, Cooke H. 1991. Functional reintroduction of human telomeres into mammalian cells. *PNAS* 88:7006–10
38. Harrington JJ, Van Bokkelen G, Mays RW, Gustashaw K, Willard HF. 1997. Formation of *de novo* centromeres and construction of first-generation human artificial microchromosomes. *Nat. Genet.* 15:345–55
39. Irvine DV, Shaw ML, Choo KHA, Saffery R. 2005. Engineering chromosomes for delivery of therapeutic genes. *Trends Biotechnol.* 23:575–83
40. Saffery R, Choo KHA. 2002. Strategies for engineering human chromosomes with therapeutic potential. *J. Gene Med.* 4:5–13
41. Choo KHA. 2001. Engineering human chromosomes for gene therapy studies. *Trends Mol. Med.* 7:235–37
42. Heller R, Brown KE, Burgtorf C, Brown WR. 1996. Mini-chromosomes derived from the human Y chromosome by telomere directed chromosome breakage. *PNAS* 93:7125–30
43. Saffery R, Wong LH, Irvine DV, Bateman MA, Griffiths B, et al. 2001. Construction of neocentromere-based human minichromosomes by telomere-associated chromosomal truncation. *PNAS* 98:5705–10
44. Barnett MA, Buckle VJ, Evans EP, Porter AC, Rout D, et al. 1993. Telomere directed fragmentation of mammalian chromosomes. *Nucleic Acids Res.* 21:27–36
45. Farr CJ, Bayne R, Kipling D, Mills W, Critcher R, Cooke HJ. 1995. Generation of a human X-derived minichromosome using telomere-associated chromosome fragmentation. *EMBO J.* 14:5444–54
46. Farr CJ, Stevanovic M, Thomson EJ, Goodfellow PN, Cooke HJ. 1992. Telomere–associated chromosome fragmentation: applications in genome manipulation and analysis. *Nat. Genet.* 2:275–82
47. Itzhaki JE, Barnett MA, MacCarthy AB, Buckle VJ, Brown WR, Porter AC. 1992. Targeted breakage of a human chromosome mediated by cloned human telomeric DNA. *Nat. Genet.* 2:283–87
48. Katoh M, Ayabe F, Norikane S, Okada T, Masumoto H, et al. 2004. Construction of a novel human artificial chromosome vector for gene delivery. *Biochem. Biophys. Res. Commun.* 321:280–90

49. Mills W, Critcher R, Lee C, Farr CJ. 1999. Generation of an ~2.4 Mb human X centromere-based minichromosome by targeted telomere-associated chromosome fragmentation in DT40. *Hum. Mol. Genet.* 8:751–61
50. Wong LH, Saffery R, Choo KHA. 2002. Construction of neocentromere-based human minichromosomes for gene delivery and centromere studies. *Gene Ther.* 9:724–26
51. Auriche C, Donini P, Ascenzioni F. 2001. Molecular and cytological analysis of a 5.5 Mb minichromosome. *EMBO Rep.* 2:102–7
52. Moralli D, Vagnarelli P, Bensi M, De Carli L, Raimondi E. 2001. Insertion of a *lox* P site in a size-reduced human accessory chromosome. *Cytogenet. Cell Genet.* 94:113–20
53. Carine K, Jacquemin-Sablon A, Waltzer E, Mascarello J, Scheffler IE. 1989. Molecular characterization of human minichromosomes with centromere from chromosome 1 in human-hamster hybrid cells. *Somat. Cell Mol. Genet.* 15:445–60
54. Hadlaczky G. 2001. Satellite DNA-based artificial chromosomes for use in gene therapy. *Curr. Opin. Mol. Ther.* 3:125–32
55. Csonka E, Cserpán I, Fodor K, Holló G, Katona R, et al. 2000. Novel generation of human satellite DNA-based artificial chromosomes in mammalian cells. *J. Cell Sci.* 113:3207–16
56. Masumoto H, Ikeno M, Nakano M, Okazaki T, Grimes B, et al. 1998. Assay of centromere function using a human artificial chromosome. *Chromosoma* 107:406–16
57. Ebersole TA, Ross A, Clark E, McGill N, Schindelhauer D, et al. 2000. Mammalian artificial chromosome formation from circular alphoid input DNA does not require telomere repeats. *Hum. Mol. Genet.* 9:1623–31
58. Henning KA, Novotny EA, Compton ST, Guan X-Y, Liu PP, Ashlock MA. 1999. Human artificial chromosomes generated by modification of a yeast artificial chromosome containing both human alpha satellite and single-copy DNA sequences. *PNAS* 96:592–97
59. Grimes BR, Schindelhauer D, McGill NI, Ross A, Ebersole TA, Cooke HJ. 2001. Stable gene expression from a mammalian artificial chromosome. *EMBO Rep.* 2:910–14
60. Mejía JE, Alazami A, Willmott A, Marschall P, Levy E, et al. 2002. Efficiency of *de novo* centromere formation in human artificial chromosomes. *Genomics* 79:297–304
61. Ikeno M, Inagaki H, Nagata K, Morita M, Ichinose H, Okazaki T. 2002. Generation of human artificial chromosomes expressing naturally controlled guanosine triphosphate cyclohydrolase I gene. *Genes Cells* 7:1021–32
62. Grimes BR, Rhoades AA, Willard HF. 2002. α-Satellite DNA and vector composition influence rates of human artificial chromosome formation. *Mol. Ther.* 5:798–805
63. Ohzeki J-i, Nakano M, Okada T, Masumoto H. 2002. CENP-B box is required for de novo centromere chromatin assembly on human alphoid DNA. *J. Cell Biol.* 159:765–75
64. Kouprina N, Ebersole T, Koriabine M, Pak E, Rogozin IB, et al. 2003. Cloning of human centromeres by transformation-associated recombination in yeast and generation of functional human artificial chromosomes. *Nucleic Acids Res.* 31:922–34
65. Rudd MK, Mays RW, Schwartz S, Willard HF. 2003. Human artificial chromosomes with alpha satellite-based de novo centromeres show increased frequency of nondisjunction and anaphase lag. *Mol. Cell Biol.* 23(21):7689–97
66. Alazami AM, Mejía JE, Monaco ZL. 2004. Human artificial chromosomes containing chromosome 17 alphoid DNA maintain an active centromere in murine cells but are not stable. *Genomics* 83:844–51
67. Basu J, Stromberg G, Compitello G, Willard HF, Van Bokkelen G. 2005. Rapid creation of BAC-based human artificial chromosome vectors by transposition with synthetic alpha-satellite arrays. *Nucleic Acids Res.* 33:587–96
68. Kotzamanis G, Cheung W, Abdulrazzak H, Perez-Luz S, Howe S, et al. 2005. Construction of human artificial chromosome vectors by recombineering. *Gene* 351:29–38
69. Lim HN, Farr CJ. 2004. Chromosome-based vectors for mammalian cells: an overview. *Methods Mol. Biol.* 240:167–86
70. Mejía JE, Larin Z. 2000. The assembly of large BACs by *in vivo* recombination. *Genomics* 70:165–70
71. Mejía JE, Willmott A, Levy E, Earnshaw WC, Larin Z. 2001. Functional complementation of a genetic deficiency with human artificial chromosomes. *Am. J. Hum. Genet.* 69:315–26

72. Hutchison CA III, Chuang R-Y, Noskov VN, Assad-Garcia N, Deerinck TJ, et al. 2016. Design and synthesis of a minimal bacterial genome. *Science* 351:aad6253
73. Hoshika S, Leal NA, Kim M-J, Kim M-S, Karalkar NB, et al. 2019. Hachimoji DNA and RNA: A genetic system with eight building blocks. *Science* 363:884–87
74. Ostrov N, Landon M, Guell M, Kuznetsov G, Teramoto J, et al. 2016. Design, synthesis, and testing toward a 57-codon genome. *Science* 353:819–22
75. Hochrein L, Mitchell LA, Schulz K, Messerschmidt K, Mueller-Roeber B. 2018. L-SCRaMbLE as a tool for light-controlled Cre-mediated recombination in yeast. *Nat. Commun.* 9:1931
76. Beaucage S, Caruthers MJC. 1981. Deoxynucleoside phosphoramidites—a new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Lett.* 22:1859–62
77. El-Shemy HA, Engler C, Kandzia R, Marillonnet S. 2008. A one pot, one step, precision cloning method with high throughput capability. *PLOS ONE* 3:e3647
78. Shetty RP, Endy D, Knight TF Jr. 2008. Engineering BioBrick vectors from BioBrick parts. *J. Biol. Eng.* 2:5
79. Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA 3rd, Smith HO. 2009. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* 6:343–45
80. Agarwal KL, Büchi H, Caruthers MH, Gupta N, Khorana HG, et al. 1970. Total synthesis of the gene for an alanine transfer ribonucleic acid from yeast. *Nature* 227:27–34
81. Cello J, Paul AV, Wimmer E. 2002. Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science* 297:1016–18
82. Chan LY, Kosuri S, Endy D. 2005. Refactoring bacteriophage T7. *Mol. Syst. Biol.* 1:2005.0018
83. Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang R-Y, et al. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329:52–56
84. Hutchison CA III, Chuang R-Y, Noskov VN, Assad-Garcia N, Deerinck TJ, et al. 2016. Design and synthesis of a minimal bacterial genome. *Science* 351:aad6253
85. Fredens J, Wang K, de la Torre D, Funke LFH, Robertson WE, et al. 2019. Total synthesis of *Escherichia coli* with a recoded genome. *Nature* 569:514–18
86. Dymond JS, Richardson SM, Coombes CE, Babatz T, Muller H, et al. 2011. Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. *Nature* 477:471–76
87. Annaluru N, Muller H, Mitchell LA, Ramalingam S, Stracquadanio G, et al. 2014. Total synthesis of a functional designer eukaryotic chromosome. *Science* 344:55–58
88. Zhao Y, Coelho C, Hughes AL, Lazar-Stefanita L, Yang S, et al. 2023. Debugging and consolidating multiple synthetic chromosomes reveals combinatorial genetic interactions. *Cell* 186:5220–36.e16
89. Schindler D, Walker RSK, Jiang S, Brooks AN, Wang Y, et al. 2023. Design, construction, and functional characterization of a tRNA neochromosome in yeast. *Cell* 186:5237–53.e22
90. Zhang W, Lazar-Stefanita L, Yamashita H, Shen MJ, Mitchell LA, et al. 2023. Manipulating the 3D organization of the largest synthetic yeast chromosome. *Mol. Cell* 83:4424–37.e5
91. Shen Y, Gao F, Wang Y, Wang Y, Zheng J, et al. 2023. Dissecting aneuploidy phenotypes by constructing Sc2.0 chromosome VII and SCRaMbLEing synthetic disomic yeast. *Cell Genom.* 3:100364
92. Luo J, Vale-Silva LA, Raghavan AR, Mercy G, Heldrich J, et al. 2023. Synthetic chromosome fusion: effects on mitotic and meiotic genome structure and function. *Cell Genom.* 3:100439
93. Lauer S, Luo J, Lazar-Stefanita L, Zhang W, McCulloch LH, et al. 2023. Context-dependent neocentromere activity in synthetic yeast chromosome VIII. *Cell Genom.* 3:100437
94. Foo JL, Kitano S, Susanto AV, Jin Z, Lin Y, et al. 2023. Establishing chromosomal design-build-test-learn through a synthetic chromosome and its combinatorial reconfiguration. *Cell Genom.* 3:100435
95. Williams TC, Kroukamp H, Xu X, Wightman ELI, Llorente B, et al. 2023. Parallel laboratory evolution and rational debugging reveal genomic plasticity to *S. cerevisiae* synthetic chromosome XIV defects. *Cell Genom.* 3:100379
96. Shen Y, Wang Y, Chen T, Gao F, Gong J, et al. 2017. Deep functional analysis of synII, a 770-kilobase synthetic yeast chromosome. *Science* 355:eaaf4791
97. Xie ZX, Li BZ, Mitchell LA, Wu Y, Qi X, et al. 2017. "Perfect" designer chromosome V and behavior of a ring derivative. *Science* 355:eaaf4704

98. Mitchell LA, Wang A, Stracquadanio G, Kuang Z, Wang X, et al. 2017. Synthesis, debugging, and effects of synthetic chromosome consolidation: synVI and beyond. *Science* 355:eaaf4831
99. Wu Y, Li BZ, Zhao M, Mitchell LA, Xie ZX, et al. 2017. Bug mapping and fitness testing of chemically synthesized chromosome X. *Science* 355:eaaf4706
100. Zhang W, Zhao G, Luo Z, Lin Y, Wang L, et al. 2017. Engineering the ribosomal DNA in a megabase synthetic chromosome. *Science* 355:eaaf3981
101. Hayden KE. 2012. Human centromere genomics: Now it's personal. *Chromosome Res.* 20:621–33
102. Fukagawa T, Earnshaw WC. 2014. The centromere: chromatin foundation for the kinetochore machinery. *Dev. Cell* 30:496–508
103. Logsdon GA, Gambogi CW, Liskovykh MA, Barrey EJ, Larionov V, et al. 2019. Human artificial chromosomes that bypass centromeric DNA. *Cell* 178:624–39.e19
104. Kim JY, Choi JH, Kim SH, Park H, Lee D, Kim GJ. 2021. Efficacy of gene modification in placenta-derived mesenchymal stem cells based on nonviral electroporation. *Int. J. Stem Cells* 14:112–18
105. Mitchell LA, McCulloch LH, Pinglay S, Berger H, Bosco N, et al. 2021. *De novo* assembly and delivery to mouse cells of a 101 kb functional human gene. *Genetics* 218:iyab038
106. Stewart MP, Langer R, Jensen KF. 2018. Intracellular delivery by membrane disruption: mechanisms, strategies, and concepts. *Chem. Rev.* 118:7409–531
107. Gnirke A, Huxley C, Peterson K, Olson MVJG. 1993. Microinjection of intact 200- to 500-kb fragments of YAC DNA into mammalian cells. *Genomics* 15:659–67
108. Marschall P, Malik N, Larin Z. 1999. Transfer of YACs up to 2.3 Mb intact into human cells with polyethylenimine. *Gene Ther.* 6:1634–37
109. Moralli D, Monaco ZL. 2015. Developing de novo human artificial chromosomes in embryonic stem cells using HSV-1 amplicon technology. *Chromosome Res.* 23:105–10
110. Li LP, Blankenstein T. 2013. Generation of transgenic mice with megabase-sized human yeast artificial chromosomes by yeast spheroplast-embryonic stem cell fusion. *Nat. Protoc.* 8:1567–82
111. Suzuki T, Kazuki Y, Hara T, Oshimura M. 2020. Current advances in microcell-mediated chromosome transfer technology and its applications. *Exp. Cell Res.* 390:111915
112. Kugoh H, Mitsuya K, Meguro M, Shigenami K, Schulz TC, Oshimura M. 1999. Mouse A9 cells containing single human chromosomes for analysis of genomic imprinting. *DNA Res.* 6:165–72
113. Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, et al. 2008. Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319:1215–20
114. Ioannou PA, Amemiya CT, Garnes J, Kroisel PM, Shizuya H, et al. 1994. A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. *Nat. Genet.* 6:84–89
115. Hamilton CM, Frary A, Lewis C, Tanksley SD. 1996. Stable transfer of intact high molecular weight DNA into plant chromosomes. *PNAS* 93:9975–79
116. Liu YG, Shirano Y, Fukaki H, Yanai Y, Tasaka M, et al. 1999. Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. *PNAS* 96:6535–40
117. Yu W, Lamb JC, Han F, Birchler JA. 2006. Telomere-mediated chromosomal truncation in maize. *PNAS* 103:17331–36
118. Boeke JD, Church G, Hessel A, Kelley NJ, Arkin A, et al. 2016. The Genome Project-Write. *Science* 353:126–27
119. Ikeno M, Grimes B, Okazaki T, Nakano M, Saitoh K, et al. 1998. Construction of YAC-based mammalian artificial chromosomes. *Nat. Biotechnol.* 16:431–39
120. Okada T, Ohzeki J-i, Nakano M, Yoda K, Brinkley WR, et al. 2007. CENP-B controls centromere formation depending on the chromatin context. *Cell* 131:1287–300
121. Kouprina N, Petrov N, Molina O, Liskovykh M, Pesenti E, et al. 2018. Human artificial chromosome with regulated centromere: a tool for genome and cancer studies. *ACS Synth. Biol.* 7:1974–89
122. Nakano M, Cardinale S, Noskov VN, Gassmann R, Vagnarelli P, et al. 2008. Inactivation of a human kinetochore by specific targeting of chromatin modifiers. *Dev. Cell* 14:507–22
123. Wang H, Han M, Qi LS. 2021. Engineering 3D genome organization. *Nat. Rev. Genet.* 22:343–60
124. Kugoh H, Ohira T, Oshimura M. 2016. Studies of tumor suppressor genes via chromosome engineering. *Cancers* 8:4

125. Wilson MD, Barbosa-Morais NL, Schmidt D, Conboy CM, Vanes L, et al. 2008. Species-specific transcription in mice carrying human chromosome 21. *Science* 322:434–38
126. Vara C, Paytuví-Gallart A, Cuartero Y, Álvarez-González L, Marín-Gual L, et al. 2021. The impact of chromosomal fusions on 3D genome folding and recombination in the germ line. *Nat. Commun.* 12:2981
127. Faria R, Navarro A. 2010. Chromosomal speciation revisited: rearranging theory with pieces of evidence. *Trends Ecol. Evol.* 25:660–69
128. White MJD. 1978. *Modes of Speciation*. New York: W.H. Freeman & Co.
129. Alonso S, Dow LE. 2021. Engineering chromosome rearrangements in cancer. *Dis. Models Mech.* 14:dmm049078
130. van der Weyden L, Bradley A. 2006. Mouse chromosome engineering for modeling human disease. *Annu. Rev. Genom. Hum. Genet.* 7:247–76
131. Scambler PJ, Carey AH, Wyse RK, Roach S, Dumanski JP, et al. 1991. Microdeletions within 22q11 associated with sporadic and familial DiGeorge syndrome. *Genomics* 10:201–6
132. Driscoll DA, Spinner NB, Budarf ML, McDonald-McGinn DM, Zackai EH, et al. 1992. Deletions and microdeletions of 22q11.2 in velo-cardio-facial syndrome. *Am. J. Med. Genet.* 44:261–68
133. Lindsay EA, Botta A, Jurecic V, Carattini-Rivera S, Cheah Y-C, et al. 1999. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* 401:379–83
134. Lindsay EA. 2001. Chromosomal microdeletions: dissecting del22q11 syndrome. *Nat. Rev. Genet.* 2:858–68
135. Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, et al. 2001. *Tbx1* haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 410:97–101
136. Merscher S, Funke B, Epstein JA, Heyer J, Puech A, et al. 2001. *TBX1* is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* 104:619–29
137. Liu Y, Chen C, Xu Z, Scuoppo C, Rillahan CD, et al. 2016. Deletions linked to *TP53* loss drive cancer through p53-independent mechanisms. *Nature* 531:471–75
138. Choi PS, Meyerson M. 2014. Targeted genomic rearrangements using CRISPR/Cas technology. *Nat. Commun.* 5:3728
139. Torres R, Martin MC, Garcia A, Cigudosa JC, Ramirez JC, Rodriguez-Perales S. 2014. Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR–Cas9 system. *Nat. Commun.* 5:3964
140. Taylor LD, Carmack CE, Schramm SR, Mashayekh R, Higgins KM, et al. 1992. A transgenic mouse that expresses a diversity of human sequence heavy and light chain immunoglobulins. *Nucleic Acids Res.* 20:6287–95
141. Lee EC, Liang Q, Ali H, Bayliss L, Beasley A, et al. 2014. Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery. *Nat. Biotechnol.* 32:356–63
142. Macdonald LE, Karow M, Stevens S, Auerbach W, Poueymirou WT, et al. 2014. Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes. *PNAS* 111:5147–52
143. Murphy AJ, Macdonald LE, Stevens S, Karow M, Dore AT, et al. 2014. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. *PNAS* 111:5153–58
144. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, et al. 2004. *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304:1497–500
145. Ikeno M, Hasegawa Y. 2020. Applications of bottom-up human artificial chromosomes in cell research and cell engineering. *Exp. Cell Res.* 390:111793
146. Chen B, Gilbert LA, Cimini BA, Schnitzbauer J, Zhang W, et al. 2013. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* 155:1479–91
147. Kazuki Y, Kobayashi K, Hirabayashi M, Abe S, Kajitani N, et al. 2019. Humanized UGT2 and CYP3A transchromosomic rats for improved prediction of human drug metabolism. *PNAS* 116:3072–81