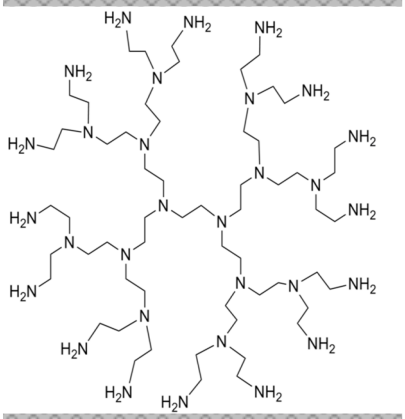

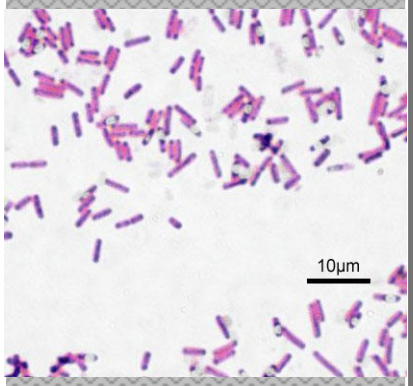

EMGEN Newsletter

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IN THIS ISSUE:

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CRISPR

1. Evolution of genome editing technology

Developments in genome regulation technologies permit scientists to make precise, directed changes to the genome to more suitably model the illnesses. The ability to modify DNA can afford useful insight into the identification of new objects for medical intervention as well as better comprehend the relationship between the genome and its functions. In 1989, the idea of gene regulating began when homologous recombination (HR) was applied to objective genes in mouse embryonic stem cells to make knock-in and knock-out cells. HR occurs infrequently in mammalian cells that affects the low frequency of the recombination. Hence, HR was not considered as an efficient genome regulating tool. In the early 1990s, Cre-lox gene regulating technology was introduced. The system permitted researchers to control gene expression both operationally and temporally. Cre is a DNA endonuclease, which occupies 34 bp loci entitled loxP. Recombination at these locations leads to knock-out of objective genes. The Cre-lox method was not impressive as the genetic distance increased between loxP locations. Just 5 years later, in order to increase gene integration in mammalian cells, double strand breaks (DSB) were introduced into the mammalian genome by either homology directed repair (HDR) or error-prone nonhomologous end joining (NHEJ). The strategies represented new and more capable methods for modifying the eukaryotic genome.

In 2013, the practice of CRISPR (Clustered Regular Interspaced Short Palindromic Repeats)/Cas began as a gene modification tool. CRISPR /Cas is a method adapted from the bacterial immune structure that is effective, rapid, and easy in usage. The type II CRISPR classes from *Streptococcus thermophilus* and *S. pyogenes* were engineered to modify mammalian genomes. To better fit the method for mammalian cells, a two-vector method was developed. The two principal components contain (1) the crRNA-tracrRNA complex and (2) a Cas9 endonuclease. When co- expressed, they create a complex that is recruited to the objective gene sequence. Besides that, the crRNA and tracrRNA may be combined to form a chimeric guide RNA (gRNA) with the same function to guide Cas9 to objective DNA sequence. The constituents can then be entered in mammalian cells through transfection or viral transduction. In addition to CRISPR, two effective genome regulating technologies have been Zinc-Finger (ZF) domains and Transcription Activator-Like Effectors (TALEs) that employ DSB-mediated repair. Both of these methods use DNA binding proteins with nuclease activity that bind to DNA and make site-specific DSBs. While effective, both of these techniques require a good knowledge in protein designing, which has been an obstacle to use of this methods by many lab researchers.

2. Discovery of CRISPR in bacterial immune systems

To evade infection by viruses and phages, prokaryotes have developed different approaches such as blocking virus attachment and avoiding DNA insertion. The CRISPR/Cas method is a bacterial immune system and lets bacteria to both inhibit foreign DNA from being inserted into the genome, and likewise, target the invasive DNA for destruction.

2.1. CRISPR

In 1987, Nakata and coworkers discovered mysterious repeat and non-repeat sequences downstream of the *iap* gene. These repeat arrays would become mentioned to as CRISPR in 2002. Just 3 years later, it was disclosed that these sequences, or "spacers", really comprised DNA from bacteriophage viruses.

2.2. Cas

Soon after this finding, the attendance of *cas* genes was similarly publicized. Cas genes express a DNA endonuclease that has a close relation to CRISPR structures. In 2005, Bolotin and et al. suggested that foreign DNA degradation may be a primary function of CRISPR/Cas.

2.3. CRISPR/Cas system

In 2008, the specialty of the CRISPR/Cas system for foreign DNA was further clarified with the discovery of protospacer adjacent motifs (PAMs). PAMs are preserved motifs inside the genome and locate just upstream of the "protospacers" or objective genomic sequences in the foreign DNA. These motifs are preferential objects for the Cas endonucleases, and permit the CRISPR/Cas system to detect between self- and non-self DNA. Three CRISPR systems, including Type I, II and III have been described in bacteria. Because of the relative simplicity, Type II CRISPR interference would become the system adjusted for genome editing in mammalian cells. CRISPR-based immunity is consisted of immunization and immunity stages. In the immunization stage, Cas proteins (Cas1/Cas2) make a complex that cuts the foreign viral DNA.

The foreign cleaved DNA is then inserted into the bacterial CRISPR loci as repeat-spacer units. The immunity stage induces following re-infection. In this step, the repeat-spacer units are transcribed to produce pre-CRISPR RNA (pre-crRNA). Consequently, a tracrRNA guides protein Cas9 to crRNA. The Cas9 endonuclease and trans-activating crRNA then attach to the pre-crRNA. Following cleavage by RNA polymerase, a mature crRNA-Cas9-tracrRNA complex is assembled. This crRNA-Cas9-tracrRNA complex is crucial to target and damage the foreign DNA.

3. Advantages of CRISPR genome editing

Regarding the molecular biology expertise, CRISPR is accurate and can be used easily in any lab. CRISPR/Cas does not need protein engineering for every objective gene and several genes can be edited concurrently. The CRISPR system only needs a small, simple DNA construct to encode the Cas9 and gRNA. However, if knock-in is being carried out, the system also requires the donor template for HR. These adaptations of CRISPR for mammalian cells have revolutionized genome editing technology.

4. Improving the specificity of CRISPR genome editing

Various organized studies attempted to experimentally determine the procedures concerning gRNA specificity and usefulness. In 2014, the gRNA design tool was introduced to improve sgRNA design for gene editing and genetic screens. The tool returns a score forecasting the activity of any sgRNA based on empirical procedures achieved by experimental studies. In 2015, the parameters regarding gRNA effectiveness were uncovered based not only on the gene sequences but also on epigenetic status. Based on the results, an interactive web tool was developed that was capable to determine CRISPR/Cas9 and assigned an anticipated activity. Despite it is rare for a 20 base pairs gRNA sequence to have 100% homology at multiple sites at the full length of the genome, sgRNA-Cas9 complexes are tolerant to various mismatches in their targets. Hence, there have been considerable efforts to advance modified CRISPR/Cas9 systems with improved specialty. Cas9 attaches to abundant locations throughout the genome that show a number of mismatches to the guide, but it only generates double strand breaks at a small subset of those locations. So far, double strand breaks have been seen at sites including five or more mismatched nucleotides respective to the gRNA sequence. Several strategies for improving gRNA-Cas9 directing specialty have been developed. Generally, Cas9n will effectively cleave DNA if it aims two loci within near vicinity, but occurring on opposite strands of the genomic DNA. Recently, two methods have been established for enhancing gRNA-Cas9 directing specificity in various approaches. The first afore mentioned system is based on the observation that an aspartate-to-alanine (D10A) mutation in the RuvC catalytic domain of Cas9 leads to it to generate single strand breaks (nicks) instead of double strand breaks. Single strand breaks are specially repaired via HDR rather than NHEJ that can potentially reduce the frequency of undesired insertion-deletion mutations from off-directed double strand breaks. The second system to improve specialty has focused on the gRNA itself. While 20 bp regions were initially utilized, it was seen that mismatches were tolerated most often in the 3′ end of the gRNA. It is shown that gRNAs with 17 or

18 supplementary nucleotides work as effectively as 20 base-pair sequences to introduce introduce mutations by using of NHEJ or HDR at on-target locations. Besides this, they represented reduced mutagenic properties at closely matched off-target locations. These truncated gRNAs (without final nucleotides of the 3′ end) can be employed with WT SpCas9 or in combination with the RNA-Fok1 nuclease. The incidence of off-target cleavage events is reduced by delivering a short-lived Cas9 protein rather than a long-lived enzyme, which indicates that the Off-target binding of Cas9 throughout the genome is concentration-dependent. A filtered Cas9 protein can be mixed to its leader RNA *in vitro* to form a Ribonucleoprotein. After transfer, Ribonucleoprotein slices chromosomal DNA quickly and then will be parsed instantly in cell, decreasing off-target properties. Another advantage is that Ribonucleoprotein can be efficiently transported to hard-to-transfect cells, such as human fibroblasts and pluripotent stem cells. Moreover, Ribonucleoprotein delivery may be less stressful for cells than plasmid transfection.

5. Improving gRNA and Cas9 delivery efficiency

Primary target models for CRISPR/Cas9 system components are mammalian cell cultures or hard-to-transfect cell lines in which transfection efficacy through lipofection or electroporation is very low. Lentiviral vectors are favorable for this kind of cells. By using modified viral vectors or any other non-viral drug delivery systems *in vivo*, CRISPR/Cas9 system components can be transported. A preferred vehicle for *in vivo* gene delivery is recombinant adeno-associated virus (rAAV) particles, but the size of the SpCas9 gene (> 4 kb) exceeds the usual cargo limit of AAV vectors. Utilizing smaller Cas9 orthologues from other species, such as *Staphylococcus aureus* (SaCas9), which are small enough to be packaged along with a single guide RNA expression cassette into a single AAV vector is one of the solutions. Other established ways are included: (1) generating transgenic animal lines that express Cas9 and transport only the guide RNAs and any crucial inducer at the trial phase, and (2) improving a split-Cas9 system performing split-inteins.

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NEXT-GENERATION SEQUENCING (NGS)

1. The evolution of genomic science

DNA sequencing has changed from the time of 2D chromatography in the 1970s. With the introduction of the Sanger chain termination strategy in 1977, scientists were able to sequence DNA in a reproducible and reliable way. Beyond the increasing of data output, the beginning of next generation sequencing (NGS) technology has transformed the manner scientists consider genetic information.

Since then, personalized genomic medicine established and supports large size sequencing for typical therapeutic aspects. Scientists can now enter a period where they are able to get the complete catalog of ailment genes and analyze numerous samples rapidly. This will permit them to monitor thousands of individuals and detect the varieties among them, to identify crucial genes that cause heart disease, cancer, autism or schizophrenia.

2. The basics of NGS

The idea of NGS knowhow is mostly related to capillary electrophoresis (CE) sequencing. Throughout successive rounds of DNA synthesis, DNA polymerase accelerates the addition of fluorescently labeled dNTPs to a DNA pattern. Through each round, at the point of insertion, nucleotides are recognized by fluorophore excitation. The important dissimilarity is that, in addition of sequencing a distinct DNA piece, NGS broadens this procedure through lots of pieces in an equal attitude. Illumina sequencing by synthesis (SBS) technique proposes a great correctness without too many faults. The Illumina NGS procedure contains 4 main steps:

2.1. Library preparation

The sequencing library is organized by random destruction of the cDNA or DNA sample, followed by 5′ and 3′ adapter ligation. Alternatively, “tagmentation” integrates the destruction and ligation reactions into a distinct stage that considerably enhances the efficacy of the library provision route. Subsequently, adapter-ligated pieces are PCR amplified and gel purified.

2.2. Cluster generation

The cluster is created by loading the library into a flow cell where pieces are seized on a lawn of surface-bound oligos complementary to the library adapters. Subsequently, each piece is amplified into distinct, clonal clusters via bridge intensification. When cluster creation is finished, the templates are ready for sequencing.

2.3. Sequencing

In SBS knowhow, an adjustable process is practiced that senses certain bases as they are added into DNA pattern. Since all 4 adjustable dNTPs are existing through every sequencing round, regular opposition inhibits adding repetitive bases and considerably minimizes raw error rates. The result is greatly precise base-by-base sequencing that essentially removes sequence-context-specific errors, even within repetitive sequence areas and homopolymers.

2.4. Data analysis

While data analysis, the newly detected sequence reads are arranged to an explicit genome. Succeeding arrangement, large number of analysis are probable as well as insertion-deletion recognition, single nucleotide polymorphism (SNP), metagenomic or phylogenetic analysis, and more.

3. NGS procedures

NGS procedures provide numerous dissimilar procedures, authorizing scholars to ask nearly any question, concerning to transcriptome, epigenome, or genome of any creature. Sequencing procedures vary mainly by how the DNA or RNA models are retrieved and by the data examination routes applied. After the provision of sequencing libraries, the real sequencing step remains essential to the same, regardless the procedure. There are several standard library provision kits that present protocols for different procedures of sequencing. Even though the number of NGS procedures is continually growing, an overview of the most usual procedures is presented here.

3.1. Genomics

3.1.1. Whole-genome sequencing

Microarray-grounded, Genome-Wide Association Studies (GWAS) is the most routine attitude for recognizing disorders in the entire of the genome.

3.1.2. Exome sequencing

The usual practiced sequencing approach is exome sequencing. The exome forms less than 2% of the human genome, but comprises many recognized ailment-causing variants, creating whole-exome sequencing as a suitable alternative to whole-genome sequencing. With exome sequencing, the protein-coding part of the genome is especially captured and sequenced. It can effectively detect variants across a broad range of utilizations, including genetic disorders, tumor studies and population genetics.

3.1.3. *De novo* sequencing

De novo sequencing mentions to sequencing a fresh genome where there is no original sequence accessible in alignment. Sequence reads are produced as contigs and the covering modality of *de novo* sequence data relies on the size and continuity of the contigs including the number of gaps in the data. Another critical issue in creating high-modality *de novo* sequences is the variety of insert sizes included in the library. Combining short-insert paired-end and long-insert mate pair sequences is the most effective technique for maximal covering across the genome.

3.1.4. Targeted Sequencing (TS)

With TS, a series of genes or fragments of the genome are isolated and sequenced. TS permits scientists to focus expenses, time and data analysis on special regions of the interest and permits sequencing at much greater coverage levels.

For example, an usual WGS study obtains coverage levels of 30×-50× per genome, while a directed re-sequencing project can efficiently cover the objective area at 500×-1000× or greater. This greater coverage allows scientists to determine rare variants that would be too expensive and too rare to discover with CE-based or WGS sequencing.

3.2. Transcriptomics

Library provision techniques for RNA sequencing (RNA-Seq) essentially start with the total RNA specimen provision followed by a ribosome exclusion phase. The total RNA specimen is then altered to cDNA (via a reverse-transcription step) before usual NGS library provision. RNA-Seq focused on mRNA, noncoding RNA, microRNAs or small RNA can be achieved by containing extra isolation or enrichment steps before cDNA synthesis.

3.2.1. Total RNA and mRNA sequencing

Transcriptome sequencing is an important advance in the investigation of gene expression since it lets a snapshot of the whole transcriptome rather than a predetermined series of genes. Whole-transcriptome sequencing gives an inclusive view of a cellular transcriptional profile at a given biological moment and considerably increases the strength of RNA discovery strategies. As with any sequencing method, an extensive dynamic range lets detection and quantification of both usual and rare transcripts. Additional capabilities contain detection of isoforms, gene fusions, and novel transcripts as well as aligning sequencing reads across splice junctions.

3.2.2. Targeted RNA sequencing

Targeted RNA sequencing is a technique for evaluating transcripts of interest for distinct expression, allele-specific expression, as well as finding isoforms, gene-mixtures and splice junctions, and cSNPs. Targeted RNA sequencing is an effective method for the study of different pathways of interest or for the validation of whole transcriptome sequencing results or gene expression microarray.

3.2.3. Noncoding RNA and small RNA sequencing

Small, noncoding RNA, or microRNAs are short nucleotide sequences (about 18-22 base pairs) that have a role in the gene regulation often as gene silencers or repressors. As the role of microRNAs in transcriptional and translational regulation has become more evident, the study of such sequences has grown.

3.3. Epigenomics

While genomics include the investigation of inheritable or acquired alterations in the DNA sequence, epigenetics is the investigation of heritable alterations in gene activity caused by mechanisms other than DNA sequence alterations. Mechanisms of epigenetic activity contain DNA methylation, DNA-protein interactions, small RNA-mediated regulation, histone modification and etc.

3.3.1. Methylation sequencing

An essential focus in epigenetics is the investigation of cytosine methylation (5-mC) states across particular regions of regulation, such as promotors or heterochromatin. Cytosine methylation can importantly modify permanent and temporal gene expression and chromatin remodeling. While there are several approaches for the investigation of genetic methylation, methylation sequencing leverages the benefits of NGS strategy and genome-wide analysis while evaluating methylation states at the single-nucleotide level. Two methylation sequencing approaches are widely utilized: reduced representation bisulfite sequencing (RRBS) and whole genome bisulfite sequencing (WGBS). During RRBS, a restriction enzyme unaffected by methylation status, digests DNA sequence to produce fragments in the 100-150 base pair size range. The fragments are isolated to enrich CpGs and promoter containing DNA areas. Subsequently, sequencing libraries are constructed applying the ordinary NGS techniques. During WGBS, sodium bisulfite changes non-methylated cytosine to uracil. Uracil is then changed to thymine in the sequence reads.

3.3.2. ChIP sequencing

Protein-DNA or protein-RNA interactions have a vital effect in many biological processes and ailment states.

These interactions can be investigated with NGS by integrating chromatin immunoprecipitation (ChIP) practices and NGS methods. ChIP practices will start with the chromatin immunoprecipitation phase. ChIP practices differ widely since they must be specific to the tissue type, species, and experimental conditions.

3.3.3. Ribosome profiling

Ribosome profiling is a method relied on deep sequencing of ribosome-protected mRNA fragments. Purification and sequencing of these fragments supply a "snapshot" of all the ribosomes that are active in a cell at a particular time. This information can reveal what proteins are being actively translated to in a cell, and can be helpful for studying translational control, determining the rate of protein synthesis, measuring gene expression, or predicting protein abundance. Ribosome profiling allows systematic studying of cellular translation processes and prediction of protein amount.

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SUGAR-BINDING PROTEIN GALECTIN-9 FOUND TO BE A NEW WEAPON TO FIGHT HIV

Galectin-9 is a humanoid sugar-binding protein. Scholars found that this protein effectively revitalizes cryptic HIV stores and displays infected cells recognizable to the immune system for annihilation in patients undergoing anti-HIV drug treatment. The last barrier to a healing for HIV infection is the appearance of cryptic HIV-infected cells, which can revitalize cryptic HIV and generate new viruses when anti-HIV treatment is stopped. During antiviral medication cure, the cryptic HIV-infected cells are hidden and unseen by the immune system. Scholars at the University of California and their workmates have found that the galectin-9 effectively forces cryptic HIV-infected cells to be revealed. Based on their previous analysis that determined vital humanoid genes engaged in controlling the cryptic state of HIV-infected cells, the lead scholar, M. Abdel-Mohsen and colleagues utilized cutting-edge plans to show that galectin-9, a beta-galactoside-binding lectin, reawakes cryptic viruses and renders infected cells detectable to the immune system. This therapeutic plan forcing cryptic HIV out of camouflage as a therapeutic concept which described as the "shock and kill" HIV elimination method.

Reference: https://www.sciencedaily.com/releases/2016/07/160713101503.htm

ALZHEIMER'S GENE MAY SHOW EFFECTS ON BRAIN STARTING IN CHILDHOOD

Scientists realized that a gene engaged in Alzheimer's disease and recovery after brain injury may display its influences on the brain and thinking skills as early as childhood. Prior studies represented that humans with the epsilon (ε)-4 variant of the apolipoprotein-E gene is more probable to develop Alzheimer's disease than humans with the ε2 and ε3, other two variants of the gene. "Investigating these genes in young children may provide us early evidences of who may be at risk for mental disorder in the future and probably even assist us find out approaches to inhibit disease from appearing or to delay the start of the disease", said study author of

the University of Hawaii. This research showed that children with the ε4 variant of the apolipoprotein-E gene had differences in their brain development compared to children with the ε2 and ε3 variants of the gene. The differences were observed in regions of the brain that are often damaged by Alzheimer's disease. "These observations mirror the steeper decline and smaller volumes of the hippocampus in the elderly who have the ε4 gene", Chang said.

Reference: https://www.sciencedaily.com/releases/2016/07/160713172713.htm

MECHANISM OF AUTOPHAGY INITIATION HAS JUST BEEN DETECTED

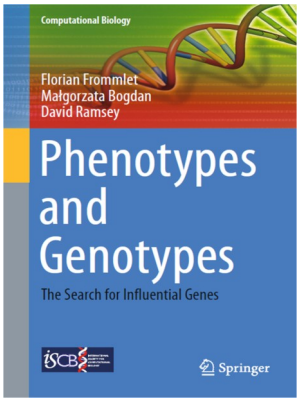
Scientists showed that Autophagy-Related Protein 13 (ATG13) joins autophagy beginning elements with help of other elements and utilizing a chain-similar construction, by that helping the association of different elements of the autophagy beginning machinery, beginning autophagosome creation via the employment of ATG9 vesicles and phosphorylation of different ATG elements. Since autophagy dysfunction is associated with serious diseases such as cancer and neurodegeneration, the artificial control of autophagy may ease the development of preventive therapy for severe diseases. Scholars at Tokyo Institute of Technology and their coworkers centralized on ATG13 and investigated its operation and construction *in vitro*. The data represented that ATG13 has a naturally chain-similar ailment in solution and that ATG13 has two specific binding sites for ATG17.

Comprehensive analyses of the interaction between ATG13 and ATG17 revealed that ATG13 joins two ATG17 molecules to each other employing two binding sites. Analysis of the size of the ATG1 composite uncovered that ATG1 composites are linked to each other by ATG13, which lead to the creation of a large autophagy initiation composite. These data show that the supramolecular composite resulting from the connection of ATG1 composites to each other by ATG13 works as the autophagy initiation machinery *in vivo*.

Reference: https://www.sciencedaily.com/releases/2016/07/160711150945.htm

Book Alert

PHENOTYPES AND GENOTYPES



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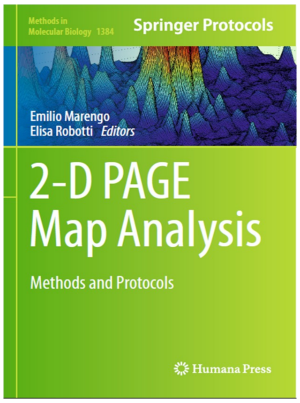

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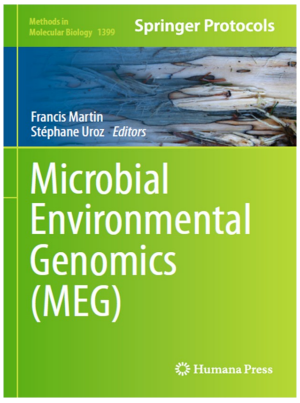
MICROBIAL ENVIRONMENTAL GENOMICS (MEG)



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Announcements

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http://globalbiotechcongress.com/

http://www.eccb2016.org/

Announcements

http://www.ieee-ichi.org/

http://cancer.madridge.com/index.php

21st World Congress on Advances in Oncology
and
19th International Symposium on Molecular Medicine

October 6-8, 2016

Metropolitan Hotel, Athens, Greece

https://www.spandidos-publications.com/pages/conference

POLYETHYLENIMINE

Polyethylenimine (PEI) is a cationic polymer that has been broadly applied for non-viral transfection *in vitro* and *in vivo*. The polymer consists of repeating units. Each unit contains the amine group and two carbon aliphatic CH_2CH_2 spacer. In the field of gene therapy, PEI has a benefit over other polycations, because of that it integrates powerful DNA compaction capacity with an intrinsic endosomolytic activity. Recently, to insert genes to a particular tumor tissue, e.g. liver or lung, PEI-centered, non-viral conveyer were locally or systemically employed. In general, gene therapy was considered as a hopeful practice for curing diverse incurable genetic or non-genetic diseases. Compared to viral conveyer, non-viral conveyer such as PEI-centered conveyer have many advantages, as they are simple to carry, considerably stable, safer and easier to modify. Unfortunately, they have some disadvantages as well as lower transfection efficacy, hence, additional tasks are required for their optimization. Moreover, in the case of PEIs, the gene transport effectiveness increases with molecular mass up to 25 kDa and then slumps, while the cytotoxicity rises linearly. To overcome such problems, different methods developed for PEI-based conveyer that includes the improvement of techniques for polyplex preparation and the fusion of nuclear localization signals or endosomolytic factors.

Reference: https://en.wikipedia.org/wiki/Polyethylenimine

CERTAIN *BACILLUS SUBTILIS* STRAINS UTILIZED TO PRODUCE POLYHYDROXYALKANOATES

Polyhydroxyalkanoates (PHAs) are recyclable linear polyesters that are made by bacteria. To store carbon and energy, bacteria convert sugar and lipids and produce PHAs. Biosynthesis of PHA is commonly induced by specific deficiency conditions such as lack of nitrogen, particular elements deficiency, absence of oxygen and surplus store of carbon sources. PHAs can be applied in the bioplastic manufacture since they are either thermoplastic or elastomeric substances, with melting points ranging from 40 to 180°C. PHAs can be utilized in a broader range of applications and it's possible to change their possessions and biocompatibility by blending, adjusting or combining them with other inorganic materials, polymers and enzymes.

To produce PHAs, a culture of a microorganism such as *Bacillus subtilis*, can be placed in a suitable medium and fed with appropriate nutrients. Recombinant *Bacillus subtilis* species were employed in PHA production with use of malt leftovers as carbon supply. Compared to other bioplastics from polymers such as polylactic acid, PHAs have many advantages. They are UV stable, show a low permeation of water and are soluble in halogenated solvents such as dichloroethane, dichloromethane or chloroform. There are many promising utilizations of PHA produced by bacteria in pharmaceutical and medical aspects, mainly due to their recyclability. For example, fixation and orthopaedic uses, as well as bone plates, sutures, meniscus repair and renewal devices, bone marrow frameworks, tendon and ligament implants, wound dressings, and etc.

Reference: https://en.wikipedia.org/wiki/Polyhydroxyalkanoates

KEFIR GRAINS

Kefir "grains" are utilized as bacterial/yeast zymosis starters to prepare kefir, an acetified milk drink. Kefir has its origins in the Caucasus Mountains. Kefir grains initiating the zymosis are a combination of the highly variable community of lactic acid bacteria and yeasts in a matrix of sugars, lipids and proteins. This symbiotic matrix (or SCOBY) builds "grains" that are similar to cauliflower. Some of lactic acid bacteria such as *Lactobacillus* species permanently exist in these grains. Kefiran is a water-solvable polysaccharide that is present in the kefir grains and forms a creamy texture and feeling in the mouth. The architecture of kefir depends highly on the composition of the milk that is acetified, in addition of the concentration of vitamin B12. Throughout zymosis, lactose, the sugar of milk, converts mainly to lactic acid by the lactic acid bacteria, which brings acidification of the product. Then *Propionibacteria* disrupt a small amount of the lactic acid into propionic acid. Some ingredients that lead to the flavor of kefir are acetic acid, pyruvic acid, diacetyl, acetoin, acetaldehyde, citric acid and amino acids resulting from protein disruption.

Reference: https://en.wikipedia.org/wiki/Kefir