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2022-2023 SSGSA STEM Sustainability Case Competition: Genetic Engineering

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Abstract
The SSGSA STEM Sustainability Case Competition is an annual research case competition hosted by undergraduate students from the STEM Students Guelph Support Association (SSGSA). The mission of this competition is to provide University of Guelph undergraduate students with an opportunity to develop their own research proposal while gaining valuable experience in innovative thinking and critical research analysis. Each year students, in teams of up to three are paired with an experienced mentor to develop and present a novel research proposal aligning with the competition's theme. During the competition, students are taught fundamental principles outlining three lab techniques which they could write about in their proposal. The theme of the competition this year was Genetic Engineering, and competitors learned about CRISPR-Cas9, CAR T-cell therapy, and PCR. In the 2022-2023 SSGSA STEM Sustainability Case Competition over 100 participants submitted abstracts for judgment, and we present the Top 20 winning submissions to be read by you in our competition abstract booklet. We hope you enjoy reading this year's best abstract submissions and encourage you to participate in the growing SSGSA community as we strive to encourage interest in novel scientific research fields surrounding STEM.

Keywords: STEM Sustainability Case Competition; SSGSA; abstract submissions; genetic engineering; undergraduate; STEM; research; University of Guelph

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Conference Abstracts

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SSGSA STEM Sustainability Case Competition Abstracts

Increasing iPSC reprogramming efficiency via CRISPR-Cas9-Mediated overexpression of *SMC1*

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The generation of induced pluripotent stem cells (iPSCs) using exogenous factors is a highly inefficient process due to epigenetic barriers impeding endogenous pluripotency gene expression. Intrachromosomal looping, orchestrated by the cohesion-mediator complex, mediates transcriptional activation of endogenous pluripotency genes by facilitating crucial promoter-enhancer interactions. Knockdown of *SMC1*, a gene which encodes for certain cohesion-associated proteins, has been shown to limit iPSC induction by abolishing DNA looping. CRISPR-Cas9-mediated overexpression of *SMC1* will be investigated for its ability to upregulate DNA looping and enhance iPSC reprogramming efficiency. Human skin fibroblasts will be transfected with a polycistronic retroviral vector containing OSKM reprogramming factors along with a green fluorescent protein *(OCT4-SOX2-KLF4-c-MYC-EGFP*). Transfected fibroblasts will be confirmed via fluorescence and grown for 4 weeks. Afterwards, the cells are characterized and sorted as either unreprogrammed cells (URCs) or iPSCs as determined by alkaline phosphatase staining, immunochemical staining, reverse transcriptase polymerase chain reaction, and karyotyping. A CRISPR-Cas9 gene editing system will then be used to overexpress *SMC1* for two, four, and six weeks in URCs and fibroblasts. Increased expression of *SMC1* in CRISPR-Cas9-edited cells will be confirmed by western blotting. Chromatin immunoprecipitation (CHiP) will be conducted to confirm binding of OSKM factors to their respective promoters. Chromosome conformation capture (3C) and quantitative polymerase chain reaction will be performed to detect and quantify the frequency of intrachromosomal interactions between the promoter and enhancer regions of the *OCT4* locus. Increased *OCT4* promoter-enhancer interactions should occur in *SMC1*-overexpressed URCs as compared to unedited URCs. As a result, *SMC1*-overexpressed URCs should express higher levels of endogenous OSKM factors, leading to a greater rate of iPSC induction than those without *SMC1*-overexpression. Increasing somatic cell reprogramming efficiency will facilitate the production of iPSCs for their various applications in regenerative medicine, disease modelling, and drug discovery.

Treatment of STAC3 disorder through the utilization of CRISPR-Cas9 technologies

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STAC3 disorder is a debilitating genetic disorder caused by a missense mutation in the *STAC3* gene in the human genome. The *STAC3* gene codes for a protein that promotes Ca^{2+} uptake by the muscles, which is crucial for healthy muscle contraction. STAC3 disorder is characterized by muscle weakness, with 36% of afflicted individuals dying before the age of 18. A missense mutation in the human genome substituting cytosine for a guanine at base pair (bp) 1046 in exon 10 in chromosome 12q13-1 is the direct cause of this disorder. Zebrafish have a similar causation of myopathy and are a good initial model for human STAC3 disorder since they share ~70% genetic information. In zebrafish, however, it is a nonsense mutation that causes STAC3 disorder. Due to the recessive nature of myopathy, mating two phenotypically affected parents will produce an affected offspring. CRISPR-Cas9 targets specific areas of a DNA strand using two elements: a guide RNA, which binds to the mutated strand, and the Cas9 enzyme, which cleaves off the targeted DNA. Immediately after fertilization, a guide RNA can be introduced to the zygote with the complementary bp to that of the mutated strand. Following the success of the guide RNA, Cas9 binds to the mutated strand highlighted by the guide RNA and cleaves off 10 to 15 bp. After cleavage, a template strand can be inserted into the zygote which will activate homology-directed repair within the cell, a safer alternative to non-homologous end joining. Using CRISPR-Cas9, the correct bp can be added to expectedly reverse the mutation causing STAC3 disorder. In the future, this research can be extended to other animal models and eventually human in vitro fertilization.

Transformation of METase and PETase via SPRINP for plastic fermentation to produce ethanol

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Plastic pollution is an ever-growing problem that has minimal and expensive solutions. In recent years the use of microorganisms has shown success in processing and degrading plastics in a natural and cost-efficient manner. The transformation of ethanol-producing microorganisms with the ability to degrade plastics presents an effective use for discarded plastic as a substrate for bioethanol production. *Pleurotus ostreatus*'s (*P. ostreatus*) ability to degrade plastic has been used as a pre-treatment to form biochar using lignocellulosic biomass. This spent fungal substrate can be exploited for use in fermentation processes by *Saccharomyces cerevisiae* (*S. cerevisiae*) to produce ethanol. Triplicates of a fungal pre-treatment (FPT) control, an FTP with untreated *S. cerevisiae* and an FTP with treated *S. cerevisiae* will be tested for their production of ethanol. *S. cerevisiae* will undergo a SPRINP treatment to transform it with METase and PETase enzymes from *Ideonella sakaiensis* (*I. sakaiensis*). All treatments and controls will undergo pre-treatment, hydrolysis and fermentation as outlined by Grover R. with the exception of the controls not being inoculated with yeast. Incubation will take place at 35°C at 150 RPM and ethanol levels will be tested every 24 hours for 96 hours using Headspace Gas Chromatography. Once ethanol production levels have surpassed 29 g/L, the fermentation of plastic will be deemed a source of ethanol production. *S. cerevisiae* that receive the SPRINP treatment are expected to surpass this ethanol level. These findings have applications worldwide to deal with the ever-growing plastic pollution in a way that is also producing an invaluable green fuel source.

The utilization of collagen bio-ink with bone marrow mesenchymal stem cell additives for the regeneration of articular cartilage in the knee

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Pre-mature connective tissue degradation can occur in articular cartilage and often progresses to osteoarthritis. The degradation rate increases due to repetitive joint impact and is complicated because self-renewability is restricted by a lack of innervation and vascularization. Advances in *in vitro* 3D bio-printing have demonstrated the ability to restore articular cartilage. In this process, the involvement of hydrogel polymeric scaffolding, a material that encases compatible cells, will ensure cell connectivity and maintain structural integrity. Bone marrow mesenchymal stem cells (BMSCs) can be added to release nutrients and signal factors to support proliferation and prevent chondrocyte dedifferentiation. The efficacy of BMSCs for cartilage growth can be tested using rabbit models that lack articular cartilage in their knees (n=20). The control group containing ten randomly selected rabbits will be treated with a collagen (COL) based hydrogel containing native chondrocytes and methacrylate anhydride for improved mechanical strength. The experimental group will be treated with

COL hydrogel, native cells, and methacrylate additives in conjunction with BMSCs. A 0.2mm thick fraction of articular cartilage from the right hind knee will be removed, with the compatible cells for containment within the collagen scaffolding. Subjects in the experimental group will have stem cells extracted in addition to the hydrogel. The 3D-printed cartilage will be implanted in the corresponding groups, and the rabbits will follow the same diet and exercise regimen for 12 weeks before removal to analyze cellular composition and growth. The tissue will be histochemically stained to compare neocartilage tissue formation, biocompatibility, and mechanical properties between the experimental and control groups. BMSCs are expected to promote chondrocyte proliferation and postpone dedifferentiation, maintaining structural integrity and growth. Therefore, current 3D-printing techniques for cartilage regeneration could be improved via BMSC additives in COL hydrogel, showing potential for treating cartilage deterioration in osteoarthritic patients.

Additive manufacturing application of 3D printing to fill atrophic scars resulting from acne, surgical procedures and trauma

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Atrophic scars are sunken pits that form when a wound heals below the regular height of the skin, causing a visible depression. They can result from a surgical procedure, diseases (such as chickenpox) and standard conditions (such as acne). Atrophic scars can be formed by inadequate production of dermal collagen and connective tissues or by missing underlying fat and muscle. These conditions prevent the body from completely filling the damaged area and producing a sunken scar. Current treatments to reduce atrophic scars include chemical peels, fillers, needling, punch excision and subcision. Most of the treatments mentioned above require several operations for significant results or include the risk of new scars or hyperpigmentation. This study will test the hypothesis that 3D printing techniques can be utilized to fill sunken areas caused by atrophic scars. The expected outcome is that this method would require little revision and have a lower chance of producing novel scars or hyperpigmentation. The atrophic scar would be sanitized and numbed. A laser would create lacerations in the scar, and a scan would be performed to determine accurate depth, height and width dimensions. The quantity of materials needed for the 3D printer would be measured and inserted. These materials would include a bio-ink mixture of hydrogels and intact cells, such as fibrinogen and stem cells. The theory behind this method involves the embedded cells interacting with cells exposed through the lacerations. These interactions would stimulate angiogenesis and melanocyte and collagen production. This method's efficacy can be assessed through later scans to evaluate whether the scar is filled and the degradation of the filling. Although 3D printing skin is still a novel practice, there is the potential for development and expansion into various treatment areas.

Application of CRISPR-Cas9 to increase secretion of gut-derived hormone peptide tyrosine-tyrosine (PYY) as a considerable therapeutic option for obesity

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Diet-related diseases pose the greatest burden on today's healthcare system. Most of these diseases result from excessive food consumption and other unhealthy eating habits. Scientists are interested in gut-derived hormones as potential targets for treating obesity due to their profound effects on satiety and appetite regulation. Scientists have recently discovered that the Peptide YY hormone plays a significant role in enhancing satiety and decreasing food intake. Attenuated levels of circulating PYY are associated with high mortality rates and hypertriglyceridemia, exacerbating the ongoing obesity pandemic. Increasing PYY secretion levels using CRISPR Cas-9 gene editing may be propitious when investigating the effects on appetite reduction and the development of obesity in mice. Mice (n=30) with similar body weight measurements and triglyceride levels will be divided into equal groups of two. One half will be defined as the control group, while the other half will undergo CRISPR treatment. The methodology of this experiment consists of the dCas9 protein complex with its sequence-specific sgRNA, targeting the region encoding for the production of PYY to increase secretions to a predefined amount. After treatment, both groups are maintained on a high-fat diet for four months while body weight, triglyceride levels, and overall food intake are monitored. The PYY transgenic mice are expected to exhibit decreased weight gain, lower triglyceride levels, and overall reduced effects of overeating compared to the control group. The findings of this study imply that a decrease in PYY levels may play a role in the etiology of obesity and may provide a novel therapeutic option as a treatment for decades to follow.

Quantifying HIV reservoirs: A comparative efficiency study of detection methods

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Human Immunodeficiency virus (HIV) is a retrovirus that integrates itself into the host's genome and infects CD4+ immune cells. In 2018, there were over 60,000 people in Canada with HIV, and 20,000 of those people died. Currently, there are no cures for HIV due to viral reservoirs, i.e., non-replicating viruses in human cells that may reactivate and infect other cells. These reservoirs cannot be detected by the immune system or be treated with antiretroviral therapy (ART). Detection methods that can precisely quantify viral reservoirs are needed. Currently, the quantitative viral outgrowth assay (qVOA) is the "golden standard" method but is costly and takes up to two weeks to perform. Thus, diagnostic techniques that are more accurate, efficient, and affordable are needed. Immunohistochemistry, a method recently applied to HIV research, is a cheaper and faster alternative to qVOA as it takes up to two hours to obtain results, excluding cell biopsy and cell preparation times. By accurately detecting these reservoirs, they can be eliminated by new treatments. The objective of this study is to evaluate the use of immunohistochemistry in the detection of viral reservoirs in HIV patients. HIV patients (n = 50) on ART for five years at the Toronto Immunodeficiency Clinic will have biopsies taken from their gastrointestinal mucosa lymph nodes via colonoscopy. Tissues will be processed for analysis using the golden standard and immunohistochemistry. Accuracy will be determined by the analytical sensitivity and specificity of the two tests, and test agreement will be analyzed using kappa statistics. Immunohistochemistry is expected to be more efficient at detecting viral reservoirs than qVOAs. Determining which detection method provides a more accurate quantification of viral reservoirs will enable further research in creating specific treatment plans to target these reservoirs.

Investigating the effects of whole intron removal on transcription levels: Improving treatment of Friedreich's ataxia with gene editing

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Friedreich's ataxia (FRDA) is a recessive, genetic neurodegenerative disease characterized by the onset of motor deficits as well as frequent comorbid arrhythmia and diabetes around 10 to 15 years of age. It has a global incidence of approximately 1 in 50,000 people, making it the most common inherited ataxia. Although the mechanisms and genetic basis of FRDA are still being investigated, it has been established that most cases are caused by long or short expanded GAA trinucleotide repeats in the first intron of the frataxin (*FXN*) gene. This mutation results in activation of maladaptive epigenetic mechanisms and DNA methylation, both of which impair the transcription of *FXN*. This has especially detrimental effects on the electron transport chain in dorsal root ganglia and cardiac muscle, causing progressive sensorimotor neuron death that leads to FRDA symptoms such as impaired posture and involuntary movement. A recent in vitro study using FRDA dorsal root ganglia models provided evidence that removal of various lengths of GAA repeats using CRISPR-Cas9 correlated with increased *FXN* transcription levels proportionate to the number of repeats removed. The present study aims to investigate whether removing the entire first intron of *FXN* has positive effects on transcription levels in FRDA models. *In vitro* modelling will be done to determine transcription and protein levels after removal of the entire first *FXN* intron by CRISPR-Cas9. Since epigenetic modulation in this region mainly results in gene silencing, its removal likely will not cause any maladaptive changes in expression. The findings from this study could improve the efficacy and accessibility of FRDA treatment by providing a homogenized intervention for patients with both long and short GAA repeats. Furthermore, this would help to reduce the burden of FRDA on the global healthcare system, given its high prevalence worldwide.

Bulbar palsy alleviation by means of CRISPR-Cas9 in mice experiencing oxidative stress of the oropharyngeal muscles

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Speech and swallowing dysfunction known as bulbar palsy, is a characteristic of Amyotrophic lateral Sclerosis (ALS), which is a serious and fatal neurodegenerative disease resulting in paralysis of both skeletal muscles and motor neurons (MN). ALS has no known cure, however treatments regarding alleviation of symptoms have begun to progress in efficacy. Genetic engineering, specifically CRISPR-Cas9, presents an encouraging new way of potentially alleviating bulbar palsy symptoms

through genetic manipulation of the superoxide dismutase 1 (*SOD1*) gene. *SOD1* encodes for SOD1 proteins which act as antioxidants in the body. However, in ALS patients it is found to be mutated, causing oxidative stress of MN of the oropharyngeal muscles. Using a mice model of C57BL/6 mice (n=100), wildtype mice (n=50) will possess the normal functioning *SOD1* gene, while mutant mice (n=50) will possess a mutant *SOD1* gene, leading to the expression of ALS-like symptoms. The mice were randomly selected using both allocation concealment and blinding and placed into their respective groups (mutant and wildtype) verified through western blotting and immunofluorescence in myofibers and satellite cells which are precursors to skeletal muscle cells. The CRISPR-Cas9 treatment was used in the mutant mice, where the mutated *SOD1* gene was removed and replaced with the newly engineered *SOD1* DNA fragment. The mice will be analyzed over a 6-month period and the onset of ALS-like symptoms of bulbar palsy will be monitored. The mice that receive the CRISPR-Cas9 *SOD1* DNA treatment are expected to display a decrease in their ALS-like symptoms, beginning to present a similar phenotype as the wildtype mice. If these findings conclude promising results, the C57BL/6 mice exhibit similar behavioural characteristics to humans and therefore may be useful for human ALS patients.

Improvement of Sub-Saharan African soil quality through the transgenic application of indigenous olive *OesDHN* gene by CRISPR-Cas9 within nitrogen fixing soybean plants

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Food security in Sub-Saharan Africa has become a major concern with the rapid population growth. The effects of climate change, poor soil, land degradation, lack of fertilizers, and poor agricultural infrastructures threaten sustainable agriculture and food production. CRISPR-Cas9 may be a promising way of generating a drought-tolerant soybean cultivar, simultaneously improving Sub-Saharan African soil quality in the process. The soil improvement is a result of soybeans, like other legume species, hosting nitrogen fixing bacteria within their rhizosphere. Drought tolerance will be driven by *OesDHN* isolated from olive (Olea europaea L. subsp. europaea, var. *Sylvestris)* grown abundantly in Northern Africa. *OesDHN* is expressed during drought stress. The *OesDHN* transgene will be extracted from olive tree leaf samples by the cetyl trimethyl ammonium bromide method. The target site will be selected for *OesDHN* immediately before the protospacer adjacent motif. Polymerase chain reaction primers will be designed to amplify the region containing the sgRNA target. Forward and reverse primers with 4bp overhangs will be obtained. Amplified DNA will be cloned into a binary vector with a Cas9 expression cassette. The vector will then be mixed with plant cells for *Agrobacterium*-mediated transformation. Control and transgenic plants will be grown in control and drought conditions. During the procedure, tissue samples will be collected from nascent leaves, macerated, and measured by gas chromatography–mass spectrometry for abscisic acid (ABA) levels. Samples will be rated on visual dryness. After reaching maturity, nitrogen levels in the sample group's soil will be measured by the Kjeldahl method. As seen with other drought tolerance genes, such as *DREB1*, *OesDHN* inclusion should increase transgenic soybean survival under drought conditions, represented by elevated ABA levels. Furthermore, an increased drought tolerance should lead to more resources dedicated to nitrogen fixing activity. With promising results, the process of soil improvement can be complemented with further research into other nutrition fixing transgenic crops modified for drought tolerance.

Biodegradation of polyurethane with *S. cerevisiae* through CRISPR-Cas9 delivered *pueA*: A step towards large-scale fungal biodegradation of global plastic pollutants

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In 2021 alone, 24.7 billion tonnes of polyurethane (PU), a plastic that takes hundreds of years to naturally degrade, were produced globally. Polyurethane esterases (Pue), such as PueA, are enzymes produced by bacteria that break down PU, which is otherwise destroyed through processes that release toxic emissions. Compared to bacteria, fungal species can grow under a plethora of conditions. Thus, using CRISPR-Cas9 to transfect fungi with the *pueA* gene will begin the development of organisms that can enzymatically degrade PU in real-world environments. We will insert the *pueA* gene into *Saccharomyces cerevisiae*, a fungal strain for which several genetic engineering techniques have been successfully developed. Fluorescent tags will be used to confirm proper gene insertion and to localize PueA expression. We are going to culture the *pueA*-modified *S. cerevisiae* and the wild type in the presence of Impranil, a PU product commonly used to assess the PU-degrading ability of microbial strains. A solid and liquid medium for growth will be prepared. After 4 weeks, the translucency of the cultures, which indicates the amount of PU degraded, will be measured using spectrophotometry. The improved ability of the *pueA*-modified *S. cerevisiae* to break down PU would support the utility of CRISPR-Cas9-delivered

pueA as a technique to engineer organisms that can combat the global PU crisis. Furthermore, this technique can be applied to native fungal strains that can degrade plastic waste in geographically diverse landfills and oceans.

Upregulation of the myelin-related growth factor NRG1 by means of CRISPR-Cas9 to remyelinate neurons in mice with multiple sclerosis

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Multiple sclerosis (MS) is a chronic, neurodegenerative condition characterized by the progressive demyelination of neurons of the central nervous system (CNS). Generally, deficiencies in neuregulin 1 (NRG1), a myelin-related growth factor, impairs oligodendrocyte differentiation, reducing myelination in the CNS. Gene-editing may allow for the restoration of NRG1 levels through manipulation of the *NRG1* gene. Adult SJL/J male mice (n=180) with Theiler's Murine Encephalitis Virus-Induced Demyelinating Disease will model the pathology of MS in humans. Mice will be randomly assigned to three groups (n=60). One group will receive an injection of the CRISPR-Cas9 complex into the corpus callosum (CC), another group will receive a placebo saline injection, and the final group will not receive any injection. The CRISPR-Cas9 complex will include a guide ribonucleic acid directed to the locus containing the constitutively expressed β-actin gene, inserting the *NRG1* gene via endonuclease activity and homology-dependent repair, enhancing *NRG1* expression. After 16 weeks, two samples of white matter from the CC will be obtained from the mice. The average axon diameter of each section will be measured using electron microscopy. The conduction velocity of axons in the CC will also be measured using recordings of compound action potentials (CAPs). Upon proper tissue preparation, a stimulating and recording electrode will be inserted 200 µm into the CC to record CAPs. The recording electrode will be moved 0.5 mm to 2.5 mm from the stimulating electrode to estimate conduction velocity, which is dependent on fiber diameter and can indicate the level of axonal myelination. Mice receiving the CRISPR-Cas9 injection are expected to show an increase in myelin thickness and conduction velocity compared to the other two groups. These findings will be fundamental in developing treatments to slow the progression of MS in humans.

Establishing HIV resistance: The application of CRISPR-Cas9 to replicate the *FREM1* gene on chromosome 9 in HIV-Resistant individuals

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Members of the Pumwani sex worker cohort in Nairobi Kenya, are resistant to Human Immunodeficiency Virus (HIV) infection despite multiple exposures. The *FREM1* gene, located on chromosome 9, is the gene implicated in this resistance. *FREM1*, specifically its Single Nucleotide Polymorphism (SNP) minor allele rs1552896, is highly expressed in cervical mucosa tissue, indicating that this gene confers HIV resistance by disrupting the process in which HIV enters the body vaginally. Therefore, we propose CRISPR-Cas9 to alter the *FREM1* gene in the germline of female *Pan troglodytes* into the SNP minor allele rs1552896 to confer HIV resistance in females that would otherwise be susceptible to contracting the disease vaginally. *FREM1* is highly conserved across all simians, and thus *P. troglodytes* are a fit animal model for this investigation. Subsequently, both the experimental and control group were vaginally infected with the Simian Immunodeficiency Virus (SIV). SIV infection in *P. troglodytes* is the most comparable analogue to HIV infection in *Homo sapiens*, as the two viruses diverged only a century ago. Now, monoclonal antibody-based antigen assay can be used to detect SIV infection. Antigen assay can be used to test for both HIV in humans and SIV in other simians. In essence, consensus sequences among the SIV and HIV genomes are used to synthesize a primer. Serum samples from the test subjects are then collected, and reverse transcriptase-polymerase chain reaction is then used to test for SIV infection. This methodological design is only applicable for vaginal HIV transmission in females. Furthermore, an ethical limitation of this methodology stems from the ability of CRISPR-Cas9 to genetically alter germline cells as altered genes may additionally give rise to undesirable changes in the genome. However, this ultimately provides a basis for the *FREM1* gene as a novel prospect of study to further understand and investigate HIV resistance in the population.

Investigating the therapeutic potential of antisense oligonucleotide downregulation of FTO demethylase in Huntington's disease model Hdh$^{+/Q111}$

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG expansion in the huntingtin (HTT) protein and characterized by early degeneration in the striatum and later degeneration in the hippocampus, amygdala and cortex. Aberrant splicing of HTT mRNA to form HTT exon 1 protein and tau hyperphosphorylation have been implicated in HD pathophysiology. Recent evidence has shown that knocking down demethylase proteins leads to a decrease in tau phosphorylation, a reduction in HTT exon 1 mRNA levels, and improves cognitive functions in HD animal models. The proposed study investigates the therapeutic potential of selectively knocking down mRNA demethylase fat mass and obesity associated (FTO) protein. Small RNA fragments known as antisense oligonucleotides (ASO) will target FTO mRNA for degradation, thereby decreasing protein expression. 4-month-old Hdh$^{+/Q111}$ heterozygous knock-in mice will receive an intraventricular injection of either FTO ASO or sterile PBS vehicle. At 8 months, motor and cognitive function will be tested before half the mice are decapitated following anesthesia. At 12 months, the remaining mice will be tested again before being decapitated following anesthesia. Following brain dissection, samples from the striatum, hippocampus, amygdala, and cortex will be collected. All samples will be incubated with labelled primary antibodies for FTO, HTT exon 1 protein, and hyperphosphorylated tau, followed by incubation with labelled secondary antibodies. Fluorescent emissions will be collected and quantified using a confocal fluorescent microscope. Behavioural testing is predicted to show significant improvements in cognitive function and motor coordination at 8 months and slight improvements at 12 months. Immunohistochemistry results are predicted to show FTO ASO significantly decreases FTO, HTT exon 1, and hyperphosphorylated tau in all brain regions compared to control at 8 and 12 months. Thus, FTO ASO injections at 4 months may have long-lasting therapeutic effects in Hdh$^{+/Q111}$ mice.

Utilizing CRISPR-Cas9 and high cell density fermentation on the AXE gene in *Bacillus subtilis* to enzymatically produce peracetic acid for lignin depolymerization

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A major goal in eco-friendly biotechnology is to replace the usage of finite fossil fuel reserves with renewable materials. Lignin, a complex aromatic heteropolymer found in lignocellulose, has proven to be a promising renewable feedstock with numerous industrial applications. The resistance of lignin to break down into usable aromatic chemicals is the main limiting factor to its potential use in biorefineries. Consequently, less than 2% of the approximately 100 million tonnes of lignin produced annually is used for the synthesis of industrial chemicals. The base monomers of lignin: sinapyl, coniferyl, and P-courmaryl alcohol have significant applications in the synthesis of various industrial and pharmaceutical materials. Considering this, there is an environmentally safe and efficient method to break down lignin into its three monomers using enzymatically derived peracetic acid (PAA). PAA depolymerizes lignin by breaking the β-aryl bonds connecting the monomers. Using *Bacillus subtilis* for the synthesis of PAA from ethyl acetate via acetyl xylan esterase (AXE), we plan to use CRISPR-Cas9 and high cell density fermentation (HCDF) to increase the efficacy of AXE-derived PAA. Treatment group [1] will utilize CRISPR-Cas9 to increase expression of AXE, group [2] will utilize HCDF to increase AXE concentration, and group [3] will feature both techniques. One control *B. subtilis* culture will be used to quantitatively compare the efficacy of the treatments by gas chromatography to measure the mole fraction of PAA in the different treatments. It is predicted that treatments [1] and [2] will have greater mole fractions of PAA than the control, with further enhanced expression in group [3] through the constructive interactions of HCDF and CRISPR-Cas9. This study will explore the practicality of CRISPR-Cas9 and HCDF in the industrial application of *in situ* synthesis of AXE-derived PAA to depolymerize lignin into its commercially viable and sustainable monomers.

Improved treatment of colorectal cancer in NQO1 609C>T recessive individuals using CRISPR-Cas9 gene editing

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Colorectal cancer is the third most common cancer in the world, and the second highest cancer-related cause of death. In 2018, there were approximately 1.8 million new cases recorded. NAD(P)H quinone dehydrogenase 1 (NQO1) is a potent superoxide scavenger known to prevent oxidative stress, and its loss of function is associated with cancer development. Individuals who are homozygous recessive for a single nucleotide polymorphism on the NQO1 gene, 609C>T, are predisposed to colorectal cancer development and recurrence. Additionally, they do not receive the benefits of chemotherapeutic agents sensitized to NQO1, such as β-lapachone. CRISPR-Cas9 treatment may hold a key therapeutic solution for these individuals. Therefore, we propose to examine the effects of CRISPR-Cas9 treatment on colorectal cancer cells homozygous recessive for NQO1 609C>T. Our study will utilize human colorectal cancer cell lines, grown in-vitro (n=96 wells). The CRISPR-Cas9 treatment will result in 609C homozygous cells, and protein expression will be confirmed with western blotting. Four equal groups will subsequently be created. These four groups will consist of: NQO1 609C>T negative control, NQO1 609C>T + β-lapachone, CRISPR-Cas9 treatment alone, and CRISPR-Cas9 treatment + β-lapachone. Wells will be assessed by tumour growth percentage over a period of 24 hours, after which treatment will cease. Cells that receive the CRISPR-Cas9 treatment and β-lapachone are expected to display improved tumour shrinkage in comparison to all other groups. This CRISPR-Cas9 treatment can lead to an improvement in colorectal cancer treatment for NQO1 609C>T individuals, as well as decrease tumour recurrence. Combined CRISPR-Cas9 NQO1 609C>T and β-lapachone treatment is an optimal target for future studies using tumour-specific precision targeting.

Effects of genetic-engineered *Bacillus subtilis* on perinatal depression

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Perinatal depression (PD) is a type of depressive disorder commonly developed during a woman's gestational period and after delivery. In this condition, there are pathophysiological changes in the brain such as reduced neuronal size, altered molecular mechanisms, and altered neurotransmitter levels of monoamines. Unresolved perinatal depression in mothers can often result in preterm birth, gestational hypertension, obstetric morbidity, and suicide. Gut dysbiosis is characteristic of perinatal depression. It is found that women with perinatal depression are associated with reduced levels of butyrate-producing gut bacteria in their gut microbiota. Butyrate is a short-chain fatty acid that has been linked to preventing brain disorders like depression due to its neuroprotective abilities. Butyrate impacts the levels of serotonin, GABA, and Brain Derived Neurotrophic Factor (BDNF) in the brain due to its ability of crossing the blood-brain barrier by passive diffusion. A genome editing tool, CRISPR Cas-9, will be used to genetically engineer *Bacillus subtilis* to enhance its ability to generate butyrate so that it can reach the blood-brain barrier. The study will use mice models of PD. The effects of butyrate on perinatal depression will be assessed on three mice groups from the beginning of pregnancy to 15 days post-labour. One group (n=20) will contain healthy pregnant mice. The second and third groups (n=20 each) will consist of mice with perinatal depression. The second group will be given the non-genetically engineered *Bacillus subtilis*, whereas the third group will be given the genetically engineered *Bacillus subtilis* intragastrically. The effects will be tested using a forced swim test (FST), sucrose preference test, immunohistochemistry for 5-hydroxytryptamine (5-HT) and tryptophan hydroxylase (TPH), and Sholl analysis. It is expected that group 3 will have higher scores in the FST and sucrose preference test, greater expression of 5-HT and TPH, and greater dendritic radius compared to group 2. These results are expected to show that the higher levels of butyrate in group 3 will reduce PD symptoms in mice. Recent studies suggest that elevated butyrate levels are beneficial in both mice and humans with PD. Therefore, the results of the study conducted on mice could be useful to humans as well.

Using adeno-associated viruses as vectors to deliver the CRISPR-Cas9 system for the treatment of prion diseases

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Prion diseases, or transmissible spongiform encephalopathies, are neurodegenerative disorders characterized by rapid neuron death upon onset of infection. They are caused by the accumulation of a misfolded version of the prion protein, PrP^{C}. Mutations in the *PRNP* gene cause PrP^{C} to misfold into the pathogenic form, PrP^{Sc}. Previously, administering antibodies in the brain was tried as a potential treatment to combat the infection. However, due to the large size of the antibodies, only a

small number could cross the blood-brain barrier, thus minimizing the effectiveness of the treatment. A study involving mice discovered that *PRNP* must be active and PrP^{c} should be present in order for the prions to accumulate. Utilizing the results of this study, a method of treatment for prion diseases could involve gene silencing of *PRNP*. An effective method for gene silencing would be to utilize the CRISPR-Cas9 system as it is able to completely remove the mutated portion of the gene. Adeno-associated viruses (AAV) are a promising method to deliver the CRISPR-Cas9 system for gene therapy. We, therefore, propose the use of these viruses as vectors for gene therapy due to their high titers and efficiency as well as having a favourable safety profile due to their lack of pathogenesis. AAV can also be re-targeted to infect specific cells using the Bispecific Antibody-Based Platform. Additionally, AAV can cross the blood-brain barrier and has been used effectively in mice to administer antibodies for PrP^{Sc}. Both the guide RNA needed to match the *PRNP* mutation and the Cas9 enzyme can be carried in one capsid. With no known effective treatments for prion diseases, this approach could extend the lives of patients or even be a cure for a previously terminal illness.

Diagnostic biomarkers for pancreatic cancer using immunohistochemistry in mouse models

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In developed countries, pancreatic cancer is 4th in causing cancer-related mortality and has a five-year survival rate of under 9%. This is due largely to the fact that symptoms start to appear in the late stages and the position of the pancreas makes it difficult to test. There are little to no early detection screening methods available to routinely check for pancreatic cancer to help solve this issue. The location of the pancreas, the late onset of symptoms and the fact that it is often misdiagnosed leads to this high mortality. The late diagnosis could be reduced by using immunohistochemistry, a method involving the use of antibodies to search for specific antigens. Some of these antigens can be used as markers to detect pancreatic cancer; however, they are not entirely reliable. These markers can often lead to a misdiagnosis because they are implicated in other pancreas related conditions, such as chronic pancreatitis. This study aims to use immunohistochemistry in genetically mutated mouse models, which have been used to study pancreatic cancer. Increased levels of CA 19-9 has been identified as a biomarker for pancreatic cancer in humans and mice models. However, this biomarker lacks sensitivity and specificity since it can also indicate other conditions. If testing in mouse models yields more definitive markers further studies could be done using pancreatic cancer patients. Finding these markers could allow clinicians to routinely screen for pancreatic cancer, leading to earlier diagnosis and better patient outcomes. It could be more inexpensive than current diagnosis methods that are not the most accurate. Moreover, further research in this area can significantly impact the treatment of pancreatic cancer by allowing physicians to better differentiate between types of pancreatic cancer leading to a more personalized treatment plan.

Determination of the efficacy of immunohistochemical enzymatic detection of monkeypox vs. CRISPR-Cas12a detection as an alternative to PCR testing

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Monkeypox virus (MPXV) is a viral illness native to Central and West Africa which, in May 2022, was declared a global outbreak by the World Health Organization. The high transmissibility and mortality associated with MPX make this iteration of the virus especially dangerous. The only currently approved diagnostic tool in Canada is a polymerase chain reaction (PCR) test, which is often performed only in the case of symptomatic disease. However, evidence of asymptomatic transmission in the United Kingdom and elsewhere highlights the need for a test for asymptomatic carriers. This study will determine which of two techniques, enzyme-based immunohistochemistry and CRISPR-Cas-12a, presents the earliest detection of MPX. Fifteen BALB/c mice will be intranasally infected with clinically obtained MPXV. Two tissue samples of 4µm will be obtained from each mouse at three-day intervals for two weeks post-infection. Immunohistochemical (IHC) detection will involve ELISA assay targeting the 3A1 capture antibody and the 2D1 detection antibody to produce stains. The G-quadruplex oligonucleotide associated with MPXV will be tagged with 6-FAM at the 3'-end and black hole-1 quencher at the 5'-end, which when degraded with CRISPR-Cas-12a cleavage, results in a visible fluorescence. Using MATLAB and colour vector analysis of slides stained using IHC and CRISPR fluorescence, a stain intensity curve as a function of the concentration of the *F3L* gene can be used to quantitatively determine which test yields a higher intensity at a lower concentration. It is expected that the test demonstrating greater intensity at a lower *F3L* concentration will more effectively detect asymptomatic MPXV. Early detection of MPX infection is crucial in controlling outbreaks and minimizing transmission to others. The expected results of this study hope to provide methods for quicker detection at the earliest stages of MPXV infection.

Conflicts of Interest
The authors declare that they have no conflict of interest. These authors include: Michael Hamilton, Meryam Tawfik, Massimo Maiuri, Amelia Rilling, Grace Basso, Sukhjot Pooni, and Mackenzie Paton-Stevens.

Authors' Contributions
MH: Co-President and founder of the SSGSA, drafted the SSGSA Genetic Engineering competition case package, peer-reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
MT: Co-President of the SSGSA, drafted the SSGSA Genetic Engineering competition case package, peer-reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
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