NYGC Institutional Biosafety Committee
Minutes
Date/Time: Friday, July 31, 2026 3:00 PM EST
Location: Zoom

Attendees:

KM: Kevin McGhee (Chair, BSO)
BA: Brian Anderson, MPH (Community representative)
MG: Matthew Geddis, PhD (Community representative)
SM: Silas Maniatis, PhD
CC: Christina Caragine, PhD
VD: Vanessa Durante
CZ: Chenxu Zhu, PhD
HL: Heather Lewis

I. Kevin McGhee (KM) opened the meeting at 3:02 PM with a quorum present.

II. KM reminded the committee of the confidentiality and conflict of interest policies. KM noted that there would be recusals needed for the Sanjana and Zhu lab protocols.

III. KM shared the minutes from the previous meeting on November 20, 2025 and asked for any comments or corrections. Having none, KM moved for approval, seconded by SM. The minutes were approved unanimously.

IV. KM shared the IBC Charter draft, noting the severance of the Institutional Stem Cell Research Oversight Committee (ISCRO) from the IBC. KM CC 2nd - all approved.

V. KM commented that the committee is voting today on all active protocols to align them on the same schedule. NYGC IBC policy requires triennial renewal to be reviewed by the IBC, allowing for approval of annual renewal by the IBC Chair as designated reviewer when there are no substantive changes to the protocol.

VI. New recombinant DNA protocol

Protocol Number	IBC020
Protocol Version	Y0-M0
Principal Investigator	John Blair
Project title	Functional Perturbation of iPSC derived neural cells
NIH Guidelines section	III-D-3-a
Project lead	John Blair
Scientific summary	The protocol uses hPSCs as an in vitro model for human central nervous system cells. CRISPR-mediated perturbation will be used to knock down selected genes, with effects assessed through transcriptomic, proteomic, morphological, and cell-identity analyses.

Vector details	Lentiviral CRISPRi system using lenti-CRISPRi-ZIM3, lentiGuide-Puro, psPAX2 packaging vector, and pMD2.G envelope plasmid. Viral promoters listed are CAG and SV40. Vectors were purchased from Addgene.
Inserts	dCas9-KRAB-ZIM3 dead nuclease, puromycin resistance, blasticidin resistance, and guide RNAs complementary to genes involved in neural differentiation and other essential genes.
Hosts / target systems	E. coli Stbl3 and Endura electrocompetent cells will be used for plasmid amplification. HEK293T cells will be used for viral production. The ultimate target is hiPSCs.
Risk attenuation	The lentiviral system is replication-defective and split across transfer, packaging, and envelope plasmids. The protocol uses a second-generation lentiviral system, with packaging functions supplied separately from the transfer vector and VSV-G supplied by a separate envelope plasmid. The transfer vectors do not contain oncogenes, and guide RNAs do not have effect in mammalian systems without co-expression of Cas9 protein.
Replication-competent virus testing	Recombinant products will not be tested for replication deficiency. Testing would require a replication-competent positive control, which may increase exposure risk.
Integration / scale / tropism	Lentiviral integration is not site-specific and tends to occur at actively transcribed genes. VSV-G confers broad human and mouse tropism.
Generation and use	Standard restriction enzyme-based and ligase-free cloning will be used, with propagation in E. coli Stbl3 or Endura competent cells. HEK293T cells will be used for viral production, and the resulting lentiviral system will be used for in vitro CRISPRi perturbation of hiPSCs.
Training	John Blair has completed all required training, including the BSL-2+ documented competency observation with EHS before conducting lentiviral work.
Biosafety level	BSL-2+ for lentiviral work.
Enhanced precautions	Lentivirus work will be restricted to a single BSC and incubator in room 447, or room 319 after relocation. Viral vector work and storage will be marked with the biohazard symbol and agent name. Secondary containment will be used when transporting virus between the tissue culture room and freezer room. During lentivirus work, the tissue culture room door will be closed and signage posted. Double gloves will be used, or single gloves will be decontaminated with 70% ethanol when hands are removed from the BSC. Disposable sleeve covers and fluid-resistant lab coats will be worn, and fluid-resistant coats will remain in the TC room.
Discussion	The Committee reviewed the proposed use of a second-generation VSV-G-pseudotyped lentiviral CRISPRi system in hiPSCs for in vitro neural-cell perturbation studies. KM discussed the integrating nature and broad tropism of the lentiviral system, and the BSL-2+ practices required for lentiviral handling.
Vote	KM moved to approve IBC020, Y0-M0, under NIH Guidelines Section III-D-3-a, with BSL-2+ containment for lentiviral work and with all personnel conducting lentiviral work required to complete BSL-2+ documented competency observation with EHS. CC seconded the motion. The vote to approve was unanimous.

VII. Recombinant DNA protocol renewals

CC was recused from the meeting for discussion and votes on protocols IBC004, IBC005, IBC008, and IBC010.

Protocol Number	IBC004
Protocol Version	Y10-M0

Principal Investigator	Neville Sanjana
Project title	Genome engineering for non-coding genomics
NIH Guidelines section	III-D-3-a
Project lead	Neville Sanjana, PhD
Scientific summary	The protocol uses lentiviral pooled-library CRISPR/Cas/Cpf systems to perform knockout, activation, repression, and chromatin modification screens in human cell lines in vitro. Virus will be produced in HEK293FT cells, used to transduce human cell lines, and followed by phenotypic selection, PCR, and deep sequencing of integrated lentiviral cassettes to identify functional genomic elements.
Vector details	Lentiviral constructs will express CRISPR/Cas/Cpf proteins and small guide RNAs. Example vectors include lentiCRISPRv2, lentiCas9-Blast, and lentiGuide-Puro. The protocol also includes SARS-CoV-2 spike-pseudotyped lentiviral particles, SARS-CoV-2 reporter fragments for Cas13 guide RNA optimization, and VLP/gag-pol fusion approaches, including possible Vpx fusion and integrase-deficient lentiviral vector approaches.
Inserts	CRISPR/Cas/Cpf proteins, guide RNAs, pooled library components, and related genome editing or perturbation elements. SARS-CoV-2 reporter work uses reporter genes tethered to short SARS-CoV-2 genome fragments, always less than 50 percent of the viral genome.
Hosts / target systems	E. coli will be used for vector and insert construction and amplification. Ultimate targets are human cell lines in vitro. NYGC IBC review is limited to in vitro work under this protocol.
Risk attenuation	Lentiviral native genes deleted include env, vif, vpr, vpu, and nef. The protocol uses SIN lentiviral vectors, split vector components, helper plasmids rather than helper viruses, second-generation packaging plasmids, and transfer plasmids that do not code for oncogenes.
Replication-competent virus testing	Absence of helper virus will be confirmed by traditional marker mobility assay, assessing transfer of transgene sequences from transduced cells to naïve cells in the absence of packaging constructs.
Integration / scale / tropism	DNA will integrate into the host genome. Cultures over 10 L will not be produced. The final product has mammalian tropism. The product may be pseudotyped, including with VSV-G.
Generation and use	HEK293FT packaging cells will be used to produce viruses by standard transfection methods, including lipofection, calcium phosphate, or PEI. Lentiviruses may be concentrated by ultracentrifugation or Amicon centrifugal filters. If high titer is not required, viral supernatants will be filtered through 0.45 μm filters and not concentrated further. Replication-incompetent virus will be used to transduce cells in vitro.
Training	Personnel conducting BSL-2+ work must complete documented competency in biosafety and microbiological practice in accordance with section 2.4 of the NYGC Biosafety Manual, in consultation with the Biosafety Officer
Biosafety level	BSL-2 and BSL-2+ are both approved. Lentiviral work is BSL-2 unless the expressed genes are known or suspected oncogenes, or the RNAi/CRISPR knockdown targets known or suspected tumor suppressors, in which case work is BSL-2+.
Enhanced precautions	For BSL-2+ work, enhanced precautions include dedicated fluid-resistant lab coats that do not leave the room, collection of used pipette tips in bleach inside the BSC with at least 20 minutes contact time, no notebooks or paper removed from the room, closed door and posted sign, lab coat and gloves for entry during work, documented competency, ethanol decontamination of nitrile gloves when hands are removed from the BSC, disposal of contaminated gloves or gloves worn when leaving the room, and disposable sleeve covers discarded in red bag at the end of each procedure.

Discussion	The Committee reviewed and discussed renewal IBC004, Y10-M0, involving lentiviral CRISPR pooled-library work in human cell lines in vitro. KM noted that there were no changes from the most recent approval.
Vote	KM moved to approve renewal IBC004, Y10-M0, under NIH Guidelines Section III-D-3-a, with BSL-2 containment for standard replication-defective lentiviral work and BSL-2+ containment for work involving known or suspected oncogene expression or RNAi/CRISPR perturbation of known or suspected tumor suppressors. Personnel conducting BSL-2+ work must complete the required documented competency observation. SM seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC005
Protocol Version	Y10-M0
Principal Investigator	Neville Sanjana
Project title	Genome engineering for non-coding genomics and neurodevelopmental and psychiatric disorders
NIH Guidelines section	III-D-3-e
Project lead	Neville Sanjana, PhD
Scientific summary	The protocol uses human ES and iPS cells, and mouse ES cells, to model genetic diseases including autism, schizophrenia, bipolar disorder, and major depression. Genome engineering tools will be used to introduce specific disease-associated mutations, including CNVs and SNPs, into isogenic cell backgrounds. Engineered cells will be differentiated into disease-relevant cell types for analysis of physiology, morphology, molecular properties, disease mechanisms, and potential therapeutic screening.
Vector details	AAV vectors based on serotypes 1, 2, 5, and 8 will be used. Genes to be expressed include CRISPR/Cas/Cpf proteins with guide RNAs, fluorescence reporters, light-sensitive ion channels, DNA-binding proteins, recombinases and transposases, and transsynaptic proteins. CRISPR plasmids were designed and cloned by Dr. Sanjana or collaborators, and other vectors are from Addgene.
Inserts	Inserts include CRISPR/Cas/Cpf proteins and guide RNAs targeting genes involved in neurodevelopmental and neuropsychiatric diseases; EGFP, EYFP, mCherry, tdTomato, GCaMP6, fluorescence voltage sensors; Channelrhodopsin-2, halorhodopsin, bacteriorhodopsin, proteorhodopsin; TALE, zinc finger, and CRISPR proteins; Cre, Dre, Flp, FokI, Gin, Sin, Tn3, Sleeping Beauty, PiggyBac; and wheat germ agglutinin. The protocol states that none of the listed genes constitute oncogenes, toxins, or viral proteins.
Hosts / target systems	E. coli will be used for vector and insert construction and amplification. The NYGC-reviewed work involves in vitro use of engineered human cell systems, including ES/iPS-derived disease-relevant cell models. Animal work referenced in the protocol occurs off-site and is outside NYGC IBC purview.
Risk attenuation	AAV structural genes are removed, rendering the vector replication-defective. The protocol describes AAV serotypes 1, 2, 5, and 8 as having low immunogenicity, no integration into the host genome, and no known human disease association. Virus will be produced using helper plasmids, not helper viruses.
Replication-competent virus testing	The protocol states that the absence of helper virus will be confirmed by traditional marker mobility assay, assessing transfer of transgene sequences from transduced cells to naïve cells in the absence of packaging constructs.
Integration / scale / tropism	The protocol states that AAV does not integrate into the host genome. Cultures over 10 L will not be produced. The final product has mammalian tropism.

Generation and use	HEK293FT packaging cells will be used to produce viruses using standard transfection techniques, including lipofection, calcium phosphate, or PEI. AAV will be concentrated by Amicon centrifugal filter units. If high titer is not required, viral supernatants will be filtered through 0.45 µm filters and not concentrated further. Replication-incompetent virus will be used to transduce cells in vitro.
Training	Personnel conducting work under this protocol must complete applicable EHS training before beginning viral vector work.
Biosafety level	BSL-2
Enhanced precautions	Work will be conducted in tissue culture rooms 434 and 435, with materials stored in room 430 wet lab and room 452 freezer room. BSCs are listed for rooms 434, 435, and 444. Disinfection will use 10 percent bleach, not ethanol. Consumables contacting biological material or liquids will be disposed of in biohazard bins.
Discussion	The Committee reviewed and discussed renewal IBC005, Y10-M0, involving in vitro AAV-based genome engineering of human ES/iPS and related cell models for neurodevelopmental and psychiatric disease research. KM noted that there were no changes to the protocol since the last approval.
Vote	KM moved to approve renewal IBC005, Y10-M0, under NIH Guidelines Section III-D-3-e, with BSL-2 containment for in vitro AAV vector production and use. SM seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC008
Protocol Version	Y9-M0
Principal Investigator	Neville Sanjana
Project title	Functional screens to understand the cancer microenvironment in immune therapies
NIH Guidelines section	III-D-3-a
Project lead	Neville Sanjana, PhD
Scientific summary	The protocol uses CRISPR/Cas9 functional screening to identify macrophage genes that maintain tumor-induced immunosuppressive traits in the cancer microenvironment. The work involves genome-wide sgRNA lentiviral libraries in macrophages, tumor cells, or other primary immune cells, with the goal of identifying pathways that may alter macrophage education toward a tumoricidal phenotype.
Vector details	Lentiviral constructs will express CRISPR/Cas/Cpf proteins and small guide RNAs. Each lentivirus will carry a guide RNA targeting a different coding sequence, and the pooled library may include constructs to knock out or otherwise modify genes across the genome. Example vectors include lentiCRISPRv2, lentiCas9-Blast, lentiGuide-Puro, GeCKOv2mouse, and lentiGuide-EGFP-Puro.
Inserts	Inserts include CRISPR/Cas/Cpf proteins, guide RNAs, and pooled lentiviral sgRNA library components. Inserts are Addgene-derived or cloned by Dr. Sanjana.
Hosts / target systems	E. coli will be used for vector and insert construction and amplification. The NYGC-reviewed work involves in vitro transduction of mouse bone marrow cells, mammalian bone marrow cells, tumor cells, macrophages, and/or other primary immune cells with genome-wide lentiviral sgRNA libraries.
Risk attenuation	Lentiviral native genes deleted include env, vif, vpr, vpu, and nef. The protocol uses SIN lentiviral vectors, second-generation packaging plasmids, helper plasmids rather than helper viruses, and transfer plasmids that do not code for oncogenes.

Replication-competent virus testing	Absence of helper virus will be confirmed by traditional marker mobility assay, assessing transfer of transgene sequences from transduced cells to naïve cells in the absence of packaging constructs.
Integration / scale / tropism	The lentiviral system is integrating and has mammalian tropism. Cultures over 10 L will not be produced.
Generation and use	Cas9-expressing macrophages, tumor cells, or other primary immune cells will be transduced with genome-wide sgRNA lentiviral libraries. HEK293FT packaging cells will be used to produce virus using standard transfection methods, including lipofection, calcium phosphate, or PEI. Lentiviruses may be concentrated by ultracentrifugation or Amicon centrifugal filters, or filtered through 0.45 µm filters if high titer is not required. Replication-incompetent virus will be used to transduce cells in vitro.
Training	Personnel conducting BSL-2+ work must complete documented competency in biosafety and microbiological practice in accordance with section 2.4 of the NYGC Biosafety Manual, in consultation with the Biosafety Officer.
Biosafety level	BSL-2 and BSL-2+ are approved. Lentiviral work is BSL-2 unless the expressed genes are known or suspected oncogenes, or the RNAi/CRISPR knockdown targets known or suspected tumor suppressors, in which case work is BSL-2+.
Enhanced precautions	For BSL-2+ work, enhanced precautions include dedicated fluid-resistant lab coats that do not leave the room, collection of used pipette tips in bleach inside the BSC with at least 20 minutes contact time, no notebooks or paper removed from the room, closed door and posted sign, lab coat and gloves for entry during work, documented competency, ethanol decontamination of nitrile gloves when hands are removed from the BSC, disposal of contaminated gloves or gloves worn when leaving the room, and disposable sleeve covers discarded in red bag at the end of each procedure. Lentivirus transported off site must be triple packaged as Biological Substance, Category B, and moved by taxi, not public transportation.
Discussion	The Committee reviewed and discussed renewal IBC008, Y9-M0, involving lentiviral CRISPR screening of macrophages, tumor cells, and other primary immune cells to study mechanisms of immune suppression in the cancer microenvironment. KM noted that there were no changes since the last approval.
Vote	KM moved to approve renewal IBC008, Y9-M0, under NIH Guidelines Section III-D-3-a, with BSL-2 containment for standard replication-defective lentiviral work and BSL-2+ containment for work involving known or suspected oncogene expression or RNAi/CRISPR perturbation of known or suspected tumor suppressors. Personnel conducting BSL-2+ work must complete the required documented competency observation. SM seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC010
Protocol Version	Y8-M0
Principal Investigator	Neville Sanjana
Project title	Interrogating Functional Elements That Regulate Expression of Immune Checkpoint Genes
NIH Guidelines section	III-D-3-a
Project lead	Neville Sanjana, PhD
Scientific summary	The protocol uses retroviral vectors to transduce human immune cells in vitro to study functional elements regulating immune checkpoint genes and other immune effectors. Modifications may include introduction of immune receptors such as T-cell receptors, followed by phenotypic selection

	and co-culture with tumor cell lines. The protocol also includes transduction of primary mouse cell types, including immune and neuronal cells, for RNA perturbation studies and molecular analyses.
Vector details	Packaging plasmids include pUMVC gag/pol plus an envelope plasmid such as RD-114 or MD2.G/VSV-G. Transfer plasmids are MoMLV- and MSCV-derived and may encode immune receptors such as CAR-T or T-cell receptor constructs, RNAi or CRISPR constructs such as Cas9 or Cas13 nucleases or guide RNAs, fluorophores, or other transgenes to modulate immune cell function. VPX-containing VLPs may also be generated using pUMVC with VPX and an envelope plasmid.
Inserts	Retroviral transfer plasmids will contain selection markers and genes to modify immune cell receptor functionality, including specific T-cell receptors. Transfer plasmids may also contain selection markers and elements to modify mouse cell gene expression, including guide RNAs for Cas9 or Cas13-mediated perturbation. Guide RNAs may target individual genes or be pooled libraries targeting many genes.
Hosts / target systems	E. coli will be used for vector and insert construction and amplification. NYGC-reviewed target systems include human and mouse primary cells and cell lines in vitro. Animal work referenced in the protocol occurs off-site and is outside NYGC IBC purview. Sanjana lab is advised by EHS to coordinate on cross-institutional approvals and to obtain documentation of NYGC-side approval from KM if needed.
Risk attenuation	Genes required for gamma-retroviral packaging and transduction are not encoded by the transfer plasmid, but are provided in trans by other plasmids or by a combination of an envelope plasmid and packaging cell line. SIN gamma-retrovirus plasmids lack most of the U3 region of the 3’ LTR to reduce promoter interference and/or drive transgene expression from an internal promoter.
Replication-competent virus testing	The protocol states that removal of viral structural genes renders the vectors replication-defective and dependent on additional proteins for packaging. Virus will be produced with helper plasmids, not helper viruses, and absence of helper virus will be confirmed by traditional marker mobility assay.
Integration / scale / tropism	The retroviral system is integrating and has mammalian tropism. Cultures over 10 L will not be produced. The product may be pseudotyped, including with VSV-G or RD-114.
Generation and use	HEK293FT or 293-GP packaging cells will be used to produce viruses using standard transfection methods, including lipofection, calcium phosphate, or PEI. Retroviruses may be concentrated by ultracentrifugation or Amicon centrifugal filters, or filtered through 0.45 µm filters if high titer is not required. Replication-incompetent virus will be used to transduce cells in vitro. For myeloid cells, VPX-containing VLPs may be made independently and mixed with replication-incompetent virus for transduction.
Training	Personnel conducting BSL-2+ work must complete documented competency in biosafety and microbiological practice in accordance with section 2.4 of the NYGC Biosafety Manual, in consultation with the Biosafety Officer.
Biosafety level	BSL-2 and BSL-2+ are approved. Retroviral work is BSL-2 unless the expressed genes are known or suspected oncogenes, or the RNAi/CRISPR knockdown targets known or suspected tumor suppressors, in which case work is BSL-2+.
Enhanced precautions	For BSL-2+ work, enhanced precautions include dedicated fluid-resistant lab coats that do not leave the room, collection of used pipette tips in bleach inside the BSC with at least 20 minutes contact time, no notebooks or paper removed from the room, closed door and posted sign, lab coat and gloves for entry during work, documented competency, ethanol decontamination of nitrile gloves when hands are removed from the BSC, disposal of contaminated gloves or gloves worn when leaving the tissue culture room, and disposable sleeve covers discarded in red bag at the end of each procedure.

Discussion	The Committee reviewed and discussed renewal IBC010, Y8-M0, involving retroviral vector transduction of human immune cells, primary mouse cells, and related in vitro cell systems to study immune checkpoint regulation and immune-effector function. KM noted that there were no changes since the previous protocol approval.
Vote	KM moved to approve renewal IBC010, Y8-M0, under NIH Guidelines Section III-D-3-a, with BSL-2 containment for standard replication-defective retroviral work and BSL-2+ containment for work involving known or suspected oncogene expression or RNAi/CRISPR perturbation of known or suspected tumor suppressors. Personnel conducting BSL-2+ work must complete the required documented competency observation. SM seconded the motion. The vote to approve was unanimous.

CC returned to the meeting following approval of IBC004, IBC005, IBC008, and IBC010.

Protocol Number	IBC018
Protocol Version	Y9-M0
Principal Investigator	Rahul Satija
Project title	Multiplexed Single-Cell Perturbations of Senescence Regulators
NIH Guidelines section	III-D-3-a
Project lead	Personnel listed are Junsuk Lee, Renqi Wang, Chao Dong, and Jessica Nouws (new lab manager).
Scientific summary	The protocol uses a multimodal single-cell CRISPR screening platform to identify regulators of cellular senescence. The study integrates genome-wide CRISPR perturbation with phenotypic enrichment and parallel single-cell measurements of RNA, protein, and guide RNA to generate hypotheses about genes that promote distinct senescence states.
Vector details	Lentiviral CRISPR system using lenti-CRISPRv2, lentiCas9-Blast, lentiGuide-Puro, psPAX2 packaging vector, and pVSVG.CMV-EGFP envelope plasmid as positive control. Viral promoters listed are CMV and SV40. Plasmids will be obtained from Neville Sanjana or commercially purchased from Addgene.
Inserts	Inserts include spCas9 nuclease, dCas9-KRAB-MeCP2 dead nuclease, puromycin resistance, blasticidin resistance, and guide RNAs from the whole-genome GeCKO-v2 library.
Hosts / target systems	E. coli Stbl3 and Endura electrocompetent cells will be used for plasmid amplification. HEK293T cells will be used for viral production. The lentiviral CRISPR system will be used for in vitro perturbation studies of cellular senescence regulators.
Risk attenuation	The protocol uses a second-generation lentiviral system split across transfer, packaging, and envelope plasmids. The transfer plasmid contains LTRs and psi packaging signal, while Gag, Pol, Rev, Tat, and VSV-G are supplied separately. The protocol states that transfer vectors do not contain oncogenes and that guide RNAs do not have an effect in mammalian systems without co-expression of Cas9 protein.
Replication-competent virus testing	Recombinant products will not be tested for replication deficiency. Testing would require a replication-competent positive control, which may increase exposure risk.
Integration / scale / tropism	DNA will integrate into the host genome. Cultures over 10 L will not be produced. VSV-G confers broad human and mouse tropism. The product is pseudotyped with a VSV-G envelope.
Generation and use	Standard restriction enzyme-based and ligase-free cloning methods will be used, with propagation in E. coli Stbl3 or Endura competent cells. HEK293T cells will be used for viral production, and the

	resulting lentiviral CRISPR system will be used for in vitro perturbation studies of cellular senescence regulators.
Training	All personnel working on this protocol must complete the BSL-2+ documented competency observation with EHS before conducting lentiviral work.
Biosafety level	BSL-2+
Enhanced precautions	Lentivirus work will be restricted to a single BSC and incubator. Viral vector work and storage will be marked with the biohazard symbol and agent name. Secondary containment will be used when transporting virus between the tissue culture room and freezer room. During lentivirus work, the TC room door will be closed and signage posted. Double gloves will be used, or single gloves will be decontaminated with 70 percent ethanol when hands are removed from the BSC. Disposable sleeve covers and fluid-resistant lab coats will be worn, and fluid-resistant coats will remain in the TC room.
Discussion	The Committee reviewed and discussed IBC018, Y9-M0, involving lentiviral genome-wide CRISPR screening to identify regulators of cellular senescence. KM noted that there were no changes since the previous approval. MG noted that files names and protocol numbers were swapped between this protocol and IBC006. KM clarified that the file titled IBC006 was protocol IBC018, while file titles IBC018 was IBC006, and that he would correct the mismatch before the next meeting.
Vote	KM moved to approve IBC018, Y9-M0, under NIH Guidelines Section III-D-3-a, with BSL-2+ containment for lentiviral work. All personnel conducting lentiviral work must complete the required BSL-2+ documented competency observation with EHS. VD seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC006
Protocol Version	Y1-M0
Principal Investigator	Rahul Satija
Project title	Multiplexed Single-Cell Perturbations of Cleavage and Polyadenylation Regulators
NIH Guidelines section	III-D-3-a
Project lead	Not separately identified. Personnel listed are Isabella Mascio, Cecilia McCormick, Junsuk Lee, Chao Dong, and Jessica Nouws.
Scientific summary	The protocol studies regulation of alternative cleavage and polyadenylation using CRISPR interference in K562 cells. A pool of guide RNAs and dCas9-KRAB-MeCP2 will be introduced into K562 cells by transfection or lentiviral delivery, with virus produced in HEK293FT cells. Gene knockdown will be measured using single-cell RNA-seq with guide capture.
Vector details	Lentiviral CRISPR system using lenti-CRISPRv2, lentiGuide-Puro, psPAX2 packaging vector, and pMD2.G envelope plasmid. Viral promoters listed are CAG and SV40. Plasmids will be obtained from Neville Sanjana or commercially purchased from Addgene.
Inserts	Inserts include spCas9 nuclease, dCas9-KRAB-MeCP2 dead nuclease, puromycin resistance, blasticidin resistance, and guide RNAs complementary to genes encoding RNA-binding proteins and CPA regulators.
Hosts / target systems	E. coli Stbl3 and Endura electrocompetent cells will be used for plasmid amplification. HEK293T cells will be used for viral production. K562 cells will be used as the target cell system for in vitro CRISPRi perturbation studies.
Risk attenuation	The protocol uses a second-generation lentiviral system split across transfer, packaging, and envelope plasmids. The transfer plasmid contains LTRs and psi packaging signal, while Gag, Pol, Rev, Tat, and VSV-G are supplied separately. The protocol states that transfer vectors do not contain

	oncogenes and that guide RNAs do not have an effect in mammalian systems without co-expression of Cas9 protein.
Replication-competent virus testing	Recombinant products will not be tested for replication deficiency. The protocol states that testing would require a replication-competent positive control, which may increase exposure risk.
Integration / scale / tropism	DNA will integrate into the host genome. Cultures over 10 L will not be produced. VSV-G confers broad human and mouse tropism. The product is pseudotyped with VSV-G.
Generation and use	Standard restriction enzyme-based and ligase-free cloning methods will be used, with propagation in E. coli Stbl3 or Endura competent cells. Guide RNAs and dCas9-KRAB-MeCP2 will be introduced into K562 cells by transfection or lentiviral delivery, with virus produced in HEK293FT cells.
Training	All personnel working on this protocol must complete the BSL-2+ documented competency observation with EHS before conducting lentiviral work.
Biosafety level	BSL-2+
Enhanced precautions	Lentivirus work will be restricted to a single BSC and incubator. Viral vector work and storage will be marked with the biohazard symbol and agent name. Secondary containment will be used when transporting virus between the tissue culture room and freezer room. During lentivirus work, the TC room door will be closed and signage posted. Double gloves will be used, or single gloves will be decontaminated with 70 percent ethanol when hands are removed from the BSC. Disposable sleeve covers and fluid-resistant lab coats will be worn, and fluid-resistant coats will remain within the TC room.
Discussion	The Committee reviewed and discussed IBC006, Y1-M0, involving lentiviral CRISPRi screening in K562 cells to identify regulators of cleavage and polyadenylation. KM noted that there were no changes to the protocol since the last approval.
Vote	KM moved to approve IBC006, Y1-M0, under NIH Guidelines Section III-D-3-a, with BSL-2+ containment for lentiviral work. All personnel conducting lentiviral work must complete the required BSL-2+ documented competency observation with EHS. VD seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC015
Protocol Version	Y3-M0
Principal Investigator	Sanja Vickovic
Project title	Systematic identification of intrinsic and extrinsic determinants of Colorectal cancer state transitions
NIH Guidelines section	III-D-3-a
Project lead	Sanja Vickovic, PhD
Scientific summary	The protocol uses lentiviral pooled-library CRISPR/Cas systems to identify genes controlling colorectal cancer cell state transitions in in vitro models, including human or mouse cell lines, stem cells, and organoids. Lentivirus will be produced in HEK293FT cells and used for genetic and epigenetic editing, lineage and cell-state tracing, phenotypic selection, and single-cell RNA profiling.
Vector details	Lentiviral constructs will express CRISPR/Cas proteins and guide RNAs. Example vectors include pLX_311-Cas9, dCAS-VP64_Blast, pXPR_118, pXPR_121, lentiGuide-Puro, lentiCRISPRv2, pXPR_050, pXPR_502, Brunello, Calabrese, and Dolcetto libraries. HEK293FT cells will be co-transfected with the transfer vector and packaging plasmids pCMV_VSVG and psPAX2. Viral promoters listed are SV40 and CMV.

Inserts	Inserts include CRISPR/Cas components, guide RNAs, selectable markers, activation or repression domains, chromatin modification tools, lineage or cell-state tracing elements, RNA sensors, and pooled CRISPR library components. Inserts are from Addgene or developed in house.
Hosts / target systems	E. coli will be used for vector and insert construction and amplification. NYGC-reviewed target systems include mouse or human cell lines, stem cells, and organoids in vitro. Animal work referenced in the protocol occurs off-site and is outside NYGC IBC purview.
Risk attenuation	Lentiviral transfer and packaging vectors have native genes deleted, including env, vif, vpr, vpu, and nef. The vectors are derived from SIN constructs with defective LTRs. The SIN design, split packaging system, helper-plasmid production, and sequence validation substantially reduce the risk of replication-competent lentivirus.
Replication-competent virus testing	The protocol states that the removal of viral structural genes renders the vectors replication-defective and dependent on additional proteins for packaging. Lentivirus will be produced with helper plasmids, not helper viruses. Source vectors are sequence-validated by Addgene, and any vectors modified in house will be validated by DNA sequencing.
Integration / scale / tropism	DNA will integrate into the host genome. Cultures over 10 L will not be produced. The final product has human and mouse tropism through a VSV-G envelope. The product is pseudotyped.
Generation and use	HEK293FT packaging cells will be used to produce lentivirus using standard transfection techniques, including lipofection, calcium phosphate, or PEI. Lentivirus may be concentrated by ultracentrifugation or Amicon centrifugal filters, or filtered through 0.45 µm filters if high titer is not required. Replication-incompetent virus will be used to transduce cells in vitro.
Training	Personnel conducting BSL-2+ work must complete documented competency in biosafety and microbiological practice in accordance with section 2.4 of the NYGC Biosafety Manual, in consultation with the Biosafety Officer.
Biosafety level	BSL-2 and BSL-2+ are approved. Lentiviral work is BSL-2 unless the expressed genes are known or suspected oncogenes, or the RNAi/CRISPR knockdown targets known or suspected tumor suppressors, in which case work is BSL-2+.
Enhanced precautions	For BSL-2+ work, enhanced precautions include dedicated fluid-resistant lab coats that do not leave the room, used pipette tips collected in bleach inside the BSC for at least 20 minutes, no notebooks or paper removed from the room, closed door and posted sign, lab coat and gloves for entry during work, documented competency, ethanol decontamination of nitrile gloves when hands are removed from the BSC, disposal of contaminated gloves or gloves worn when leaving the tissue culture room, and disposable sleeve covers discarded in red bag at the end of each procedure.
Discussion	The Committee reviewed and discussed renewal IBC015, Y3-M0, involving lentiviral pooled-library CRISPR/Cas screening and genetic or epigenetic perturbation of colorectal cancer cell state transitions in human or mouse cell lines, stem cells, and organoids in vitro. KM noted that there were no changes to the protocol since the previous approval.
Vote	KM moved to approve renewal IBC015, Y3-M0, under NIH Guidelines Section III-D-3-a, with BSL-2 containment for standard replication-defective lentiviral work and BSL-2+ containment for work involving known or suspected oncogene expression or RNAi/CRISPR perturbation of known or suspected tumor suppressors. Personnel conducting BSL-2+ work must complete the required documented competency observation. VD seconded the motion. The vote to approve was unanimous.

Protocol Number	IBC019
Protocol Version	Y1-M0

Principal Investigator	Chenxu Zhu
Project title	Unraveling Epigenetic Mechanisms and Therapeutic Strategies for FXN Silencing in Friedreich's Ataxia
NIH Guidelines section	III-D-3-a
Project lead	Xiaonan Guan, PhD, postdoc
Scientific summary	Friedreich's ataxia is caused by abnormal GAA repeat expansion in the FXN gene, leading to FXN silencing and reduced frataxin. The protocol uses single-cell multi-omics and CRISPR-based epigenetic editing tools to study whether epigenetic changes caused by GAA repeat expansion directly cause FXN silencing.
Vector details	Packaging plasmids are pCMVR8.74 and pCMV-VSV-G. Transfer plasmids are TRE3G-dCas9, TRE3G-dCas9-P300, TRE3G-dCas9-dP300, pgRNA, and rtTA. CMV is listed as a viral promoter. TRE3G-dCas9, TRE3G-dCas9-P300, and TRE3G-dCas9-dP300 were developed in house based on Addgene vectors, and pgRNA, rtTA, pCMVR8.74, and pCMV-VSV-G were ordered from Addgene.
Inserts	Transfer plasmids contain BFP for selection, P300 to modify H3K27ac, sgRNA, and dCas9 for targeting epigenetic marks around FXN. Sources include Addgene plasmids, dCas9 from Streptococcus pyogenes Cas9, and P300 core domain from human EP300.
Hosts / target systems	One Shot Stbl3 chemically competent E. coli will be used for vector construction and amplification. Ultimate targets are Friedreich's ataxia patient-derived iPSC cell lines and iPSC-derived sensory neurons.
Risk attenuation	The protocol states that tat, vif, vpr, vpu, and nef are removed from the transfer plasmid. Genes required for lentiviral packaging and transduction are not encoded by the transfer plasmid and are instead supplied in trans by pCMVR8.74 and pCMV-VSV-G.
Replication-competent virus testing	The protocol states that removal of viral structural genes renders the vectors replication-defective and dependent on additional proteins for packaging. Virus will be produced with helper plasmids, not helper viruses, and absence of helper virus will be confirmed by traditional marker mobility assay.
Integration / scale / tropism	The lentiviral system is integrating and has mammalian tropism. Cultures over 10 L will not be produced. The product is VSV-G pseudotyped.
Generation and use	HEK293FT packaging cells will be used to produce virus using standard transfection techniques, including X-tremeGENE 9 DNA Transfection Reagent. Virus may be concentrated by ultracentrifugation or Amicon centrifugal filters, or filtered through 0.45 µm filters if high titer is not required. Replication-incompetent virus will be used to transduce iPSC lines in vitro.
Training	Personnel performing work must complete documented competency observation with EHS before being cleared for lentivirus work.
Biosafety level	BSL-2+
Enhanced precautions	The system is doxycycline inducible, so dCas9, dCas9-P300, and dCas9-dP300 expression occurs only after doxycycline treatment. The lab will wait until passage 3 before conducting cell sorting after creating the cell lines and will evaluate off-target effects after cell line creation. Personnel must complete documented competency observation before lentivirus work. During lentivirus handling, the tissue culture room door will be closed and warning signage posted.
Discussion	The Committee reviewed and discussed renewal IBC019, Y1-M0, involving a replication-defective VSV-G-pseudotyped lentiviral system to deliver doxycycline-inducible CRISPR/dCas9 epigenetic editing constructs to Friedreich's ataxia patient-derived iPSCs and iPSC-derived sensory neurons in vitro. KM noted that there were no changes to the protocol since the last approval.

Vote	KM moved to approve renewal IBC019, Y1-M0, under NIH Guidelines Section III-D-3-a, with BSL-2+ containment for lentiviral work. Personnel conducting lentiviral work must complete the required documented competency observation with EHS. CC seconded the motion. The vote to approve was unanimous.

VIII. With no further business, KM concluded the meeting at 3:27

Prepared by Kevin McGhee on 10/13/2026