Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.
This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Poojan Pandya and Leo Takemaru

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 2018-19
1. Title	CCDC11 Acts as a Scaffold to Assemble the ESCRT Membrane Scission Machinery at Viral Budding Sites for HIV-1 Release: Identifying a Novel Therapeutic Strategy for Antiviral Therapy	Investigating the Role of the Novel ESCRT-III Recruiter CCDC11 in HIV-1 Viral Budding: Identifying a Potential Target for Antiviral Therapy
2. Change in goal/ purpose/objective	Establish and Validate a HeLa CCDC11 KO. Determine the effect of a CCDC11 KO in HeLa on the release of virus-like particles. Determine and quantify a growth defect in HEK293T and HeLa CCDC11 KO. Determine if CCDC11 and Gag interact at the cell surface. Determine if CCDC11 interacts with Alix/Tsg101/CHMP2A/CHMP4B/CHMP6/CHMP8.	Establish and validate a HEK293T CCDC11 KO. Determine the effect of a CCDC11 KO in HEK293T on the release of virus-like particles.
3. Changes in methodology	CRISPR-Cas9 used to generate a HeLa CCDC11 KO. Validation of KO using Western blot, DNA sequencing, and IF staining. p24 ELISA assay to quantify viral-like particle release. Phase contrast microscopy used to assess cell count and cell morphology of WT vs. KO IF staining, structured illumination microscopy, and co-IP experiments performed to assess interactions of CCDC11 with Gag/Alix/Tsg101/CHMP2A/CHMP4B/CHMP6/CHMP8.	CRISPR-Cas9 used to generate a HEK293T CCDC11 KO. Validation of KO using Western blot, DNA sequencing, and IF staining. p24 ELISA assay to quantify viral-like particle release.
4. Variable studied	The effect of CCDC11 depletion in HeLa cells on HIV budding. The growth rate and morphology of wild type cells vs. both HEK293T and HeLa CCDC11 KO. The cellular localization of ectopically expressed Flag-CCDC11 with GFP-Gag/HA-Alix/Myc-Tsg101/ HA-CHMP2A/HA-CHMP4B/HA-CHMP6/HA-CHMP8. Physical interactions between Flag-CCDC11 with /HA-Alix/ Myc-Tsg101/HA-CHMP2A/HA-CHMP4B/HA-CHMP6/ HA-CHMP8	The effect of CCDC11 depletion in HEK293T cells on HIV budding.
5. Additional changes		

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 2018-19

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Poojan Pandya and Leo Takemaru — Leo Takemaru — 1/15/2020

Student's Printed Name(s) — Signature — Date of Signature (mm/dd/yy)

18-19 ABSTRACT

OFFICIAL ABSTRACT and CERTIFICATION

Investigating the Role of the Novel ESCRT-III Recruiter CCDC11 in HIV Viral Budding: Identifying a Potential Target for Antiviral Therapy

Poojan Pandya & Leo Takemaru

Half Hollow Hills High School West, Dix Hills, NY, USA; Ward Melville High School, East Setauket, NY, USA

Acquired immunodeficiency syndrome (AIDS) is a life-threatening condition caused by infection of the human immunodeficiency virus (HIV). Compromising the immune system, HIV reduces the body's ability to fight the organisms that cause disease. In an effort to discover potential targets for antiviral therapies, we explored the role of coiled-coil domain-containing 11 (CCDC11) in HIV-1 budding. CCDC11 plays an important role in ciliogenesis and cytokinesis. During cytokinesis, it recruits the endosomal sorting complex required for transport III (ESCRT-III) membrane scission complex to the midbody to mediate the physical separation of two dividing daughter cells. The ESCRT-III machinery is also integral to the viral budding process of HIV-1 and many other viruses. Therefore, we hypothesized that CCDC11 is also required for viral budding. To investigate this possibility, we established CCDC11-knockout human embryonic kidney (HEK) 293T cells using the CRISPR-Cas9 technology. Indels of the CCDC11 gene were confirmed by DNA sequencing, and protein levels were assessed via western blotting and immunofluorescence staining. To determine the effect of CCDC11-knockout on HIV-1 budding, we employed the Enzyme-linked immunosorbent assay (ELISA) p24 capture assay to indirectly assess viral release by measuring the relative concentration of HIV-1 Gag structural protein (p24) or mutant P7L-Gag in cell media. Our results demonstrate that ectopic overexpression of CCDC11 markedly enhances whereas depletion of CCDC11 in the knockout cells dramatically reduces viral particle release. The defective viral budding in CCDC11-knockout cells was restored when wild-type CCDC11 was re-expressed. Collectively, our data suggest that CCDC11 is critical for efficient HIV-1 budding.

Category
Pick one only—
mark an "X" in box
at right

Animal Sciences	☐
Behavioral & Social Sciences	☐
Biochemistry	☐
Biomedical & Health Sciences	☐
Biomedical Engineering	☐
Cellular & Molecular Biology	☐
Chemistry	☐
Computational Biology & Bioinformatics	☐
Earth & Environmental Sciences	☐
Embedded Systems	☐
Energy: Chemical	☐
Energy: Physical	☐
Engineering Mechanics	☐
Environmental Engineering	☐
Materials Science	☐
Mathematics	☐
Microbiology	■
Physics & Astronomy	☐
Plant Sciences	☐
Robotics & Intelligent Machines	☐
Systems Software	☐
Translational Medical Sciences	☐

1. As a part of this research project, the student directly handled, manipulated, or interacted with (check ALL that apply):

   ☐ human participants ■ potentially hazardous biological agents

   ☐ vertebrate animals ■ microorganisms ■ rDNA ■ tissue

2. I/we worked or used equipment in a regulated research institution or industrial setting: ■ Yes ☐ No

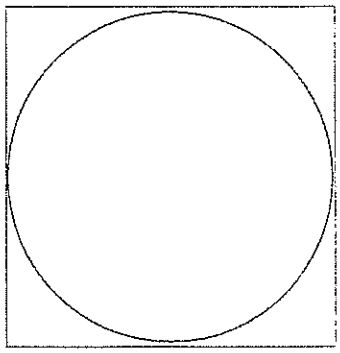
3. This project is a continuation of previous research. ☐ Yes ■ No

4. My display board includes non-published photographs/visual depictions of humans (other than myself): ☐ Yes ■ No

5. This abstract describes only procedures performed by me/us, reflects my/our own independent research, and represents one year's work only ■ Yes ☐ No

6. I/we hereby certify that the abstract and responses to the above statements are correct and properly reflect my/our own work. ■ Yes ☐ No

This stamp or embossed seal attests that this project is in compliance with all federal and state laws and regulations and that all appropriate reviews and approvals have been obtained including the final clearance by the Scientific Review Committee.



18-19 Rosters Plan + Addendum

Investigating the Role of the Novel ESCRT-III Recruiter CCDC11 in HIV Viral Budding: Identifying a Potential Target for Antiviral Therapy

Research Plan

Poojan Pandya and Leo Takemaru

a) Rationale

Viral infections affect billions of people worldwide each year and are one of the most important public health problems. However, molecular and cellular mechanisms of viral infections are highly complex and not fully understood. The cellular Endosomal Sorting Complex Required for Transport (ESCRT) plays important roles in a variety of biological processes associated with internal membrane fission, ranging from cytokinetic abscission to viral budding. Viral budding is a process that viral particles been released from the host cells to proliferate in the host organism. In the case of HIV-1, when the primary structural protein Gag is recruited to the plasma membrane of the host cells and the viral particles are assembled, ESCRT III is required for the membrane scission and the release of the viral particles. Recent studies from the mentor's laboratory suggest that the coiled-coil domain-containing protein 11 (CCDC11), which is involved in ciliogenesis, plays a critical role in cell division through recruitment of the ESCRT-III membrane scission complex to the midbody. However, whether CCDC11 is required for other ESCRT-III-mediated biological processes is currently unknown. Elucidation of the potential role of CCDC11 in the HIV-1 viral budding process will provide valuable knowledge in the antiviral drug design and infection treatment.

b) Hypothesis

Recent studies suggest that CCDC11 is required for efficient cell division through recruitment of the ESCRT-III component Charged Multivesicular Body Protein 2A (CHMP2A) to the midbody. Since ESCRT-III complex is also known to be important for HIV-1 viral budding, we hypothesize that CCDC11 is required for the viral budding from the plasma membrane of host cells.

Research Questions

1. Is it possible to generate CCDC11-knockout cells? Can cells survive without CCDC11?
2. If so, do these cells show defects in ciliogenesis and cell division?
3. Is CCDC11 required for HIV-1 budding?
4. What is the function of CCDC11 in the HIV-1 viral budding process?

Engineering Goals:

We aim first to successfully generating CCDC11 knockout cell lines the human embryonic kidney 293T (HEK293T) cells. Next, we will evaluate defects of the knockout cells in ciliogenesis and cell division. Then, we will investigate effects of depleting CCDC11 on HIV-1 Gag protein release and explore mechanism of CCDC11's role in HIV-1 viral budding. Our overall goal is to demonstrate function of CCDC11 in the viral budding process.

Expected Outcomes

We expect that CCDC11-knockout cells are most likely viable and show defective ciliogenesis and cell division. We also expect that the frequency of HIV-1 Gag protein release are significantly lower in the CCDC11 knockout cells, provide evidence that CCDC11 is important for viral budding by recruiting the ESCRT-III membrane scission complex to the site of viral budding at plasma membranes.

c) Procedure, Risk and Safety, Data Analysis
I. Procedure:

Bacterial transformation:
CCDC11-related plasmids will be transformed into DH5alpha *E. coli* competent cells to amplify plasmid DNAs. The Qiagen Plasmid Plus Midi Kit will be used to purify the plasmid DNAs.

Cell Culture:
HEK293T cells will be purchased from American Type Culture Collection (ATCC, CRL-3216) and grown in Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) with 100 U/ml penicillin-streptomycin at 37 °C in a 5% CO_2 incubator.

Transient transfection:

The day before transfection, cells will be trypsinized and counted. $2X10^5$ cells will be seeded into 6-well tissue culture plates with 2ml DMEM and 10% FBS each well. After incubating for 18-24 hrs, the cells should be at 70-90% confluent at the time of transfection. 2.5 μg total DNA will be transfected using Lipofectamine 3000 (*Invitrogen*) following the manufacturer's protocol.

Generation of CCDC11-knockout HEK293T cells using the CRISPR/CAS9 system:
HEK293T cells will be transfected with pSpCas9(BB)-2A-Puro (PX459) V2.0 containing non-targeting or targeting human CCDC11 gRNA using Lipofectamine 3000. Transfected cells will be selected in the presence of puromycin (2.5 ug/ml). Two different gRNAs targeting CCDC11 will be used: gRNA-1, GCGGTTTGGCACCGTACAGC, gRNA-2, AGCGGTTTGGCACCGTACAG. Following 1-2 weeks of continued culture, when transfected cells have formed multiple single colonies, the colonies will be isolated and further grow for experiments. To determine CCDC11-knockout mutations, genomic DNA will be isolated from the cell colonies, and PCR fragments encompassing the gRNA sequence will be generated and used for sequencing.

Western blotting:
Wild-type (WT) and CCDC11-knockout HEK293T cells will be harvested and lysed in the Radioimmunoprecipitation assay (RIPA) buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Nonidet P-40 [NP-40], 0.5% Sodium deoxycholate, 0.1% SDS) with protease cocktail inhibitors (Roche) added before use, and the cell lysates will be sonicated to disrupt cellular membranes and release the cell contents. The lysates will be centrifuged at 13000-rpm and supernatant will be collected and added 5xSDS sample buffer followed by denaturing the proteins at 95 °C for 3 mins. The samples will be loaded into a 10% SDS-Polyacrylamide gel electrophoresis (PAGE) and the gel will be run for 2 hrs at 90 V. Proteins will be transferred from the gel onto the Nitrocellulose membrane (0.45um) with the

power supply running at 16V for 14 hrs. The membrane will then be washed with Tris buffer saline with 0.1% Tween (TBST) and blocked for 1 hr at room temperature in 5% skim milk. Primary antibody CCDC11 will be added and allowed to incubate for 1 hr. The membrane will be washed 3 times for 15 mins each with TBST. Secondary antibodies conjugated with horseradish peroxidase will be added and allowed to incubate for 1 hr at room temperature. Antibodies will then be removed and the membrane will be washed with TBST 3 times for 15 mins each. Chemiluminescent substrates (SuperSignal™ West Pico Stable Peroxide Solution and SuperSignal™ West Pico Luminol Enhancer Solution, ThermoFisher Scientific) will be used to detect the protein followed by film exposure to visualize the specific protein transferred on the membrane.

Immunofluorescence staining:

HEK293T cells will be grown on glass coverslips in a 12-well plate 24-48 hrs. The cells will then be washed and fixed with cold 50% methanol/50% acetone. The fixed cells will be incubated for one hr with 300 µl of blocking buffer containing 5% goat serum in diluent solution (2% bovine serum albumin [BSA] and 0.2% Triton X-100 in PBS). The blocking buffer will be discarded, and the cells will be incubated at 4 °C overnight with 300 µl of the diluent solution containing the following primary antibodies: rabbit anti-CCDC11 (Sigma-Aldrich, HPA040595, 1:300) and mouse anti-acetylated alpha-tubulin (Sigma-Aldrich, T7451, 1:300). Subsequently, the cells will be washed and incubated for one hr with appropriate anti-rabbit or anti-mouse IgG secondary antibodies conjugated with DyLight 488 and DyLight 549 (Vector Laboratories). The cells will then be washed, counterstained with 4′,6-diamidino-2-phenylindole (DAPI), used as a nuclear counterstain, for two mins at room temperature, washed again, and mounted onto a glass slide using Fluoromount-G (SouthernBiotech) for microscopic analyses.

Method to exam HIV-1 viral budding:

The HIV-1 Gag protein p24 (MW 24kD) is the structural protein of the HIV-1 capsid. It is initially synthesized as a polyprotein precursor, which is necessary and sufficient for the produce and assembly of non-infectious, virus-like particles (VLP) in the absence of other viral proteins or packageable viral RNA. Therefore, detectable p24 in the cell culture supernatants is strongly correlated to released VLP. The antigen capture enzyme-linked immunosorbent assay (ELISA) is currently the most common method used to demonstrate virus replication both *in vivo* and *in vitro* for quantitation of the HIV-1 Gag p24 protein. We plan to utilize the HIV-1 p24 Capture ELISA kit (Immuno DX LLC) to measure the accumulation of Gag p24 in the culture supernatants by reading the optical densities (O. D.) at wavelength of 450nm using a microplate reader to monitor effects of CCDC11 on HIV-1 viral budding.

Quantification of Gag p24 release using ELISA:

CCDC11 knock-out HEK293T cells and control cells will be transfected in triplicate with plasmid bearing HIV-1 WT-Gag p24 or P7L-Gag. P7L-Gag is a p24 mutant containing a single amino acid substitution that impairs Tsg101 binding during viral budding. Tsg101 is a component of ESCRT-I, which functions in a sequential manner with ESCRT-0,-II and-III in a series of membrane fusion and budding events. An expression plasmid for Renilla luciferase (pRL-TK) will be cotransfected with WT- or P7L-Gag to normalize transfection frequency. 24-48 hrs following DNA transfection, tissue culture media and cells will be harvested. The

tissue culture media will be clarified by centrifiguation to remove cellular debris and used for ELISA assay following the manufacturer's protocol. The cells will be lysed using RIPA buffer with sonication and used for measuring Renilla luciferase activities by the Dual-Luciferase Reporter Assay System (Promega) and a Berthold luminometer (Berthold Technologies). The value of Gag p24 production will be normalized by the value of Renilla luciferase activity from each sample. P7L-Gag release will be compared with the WT-Gag release, and the effects of CCDC11 depletion on WT-Gag and P7L-Gag release will be examined. As an alternative way of monitoring Gag p24 expression and release, we will perform Western blotting using the cell culture supernatants and lysates, followed by densitometric measurements of bands on the immunoblot corresponding to Gag using the NIH ImageJ software.

II. Risks and Safety:

1) Human subjects: N/A

2) Vertebrate animals: N/A

3) Potentially hazardous biological agents (PHBA):

The human embryonic kidney (HEK) 293Tcell cell line will be purchased from American Type Culture Collection (ATCC, CRL-3216) and handled in a biological safety cabinet under proper supervision. Appropriate safety equipment will be used including gloves, goggles, and lab coats. This requires biosafety level 2 practices and containment facilities. DH5alpha *E. coli* cells are classified as biosafety level 1. Personal safety equipment should be worn at all times when handling.

4) Hazardous chemicals/activities/devices:

Safety precautions must be taken when handling chemicals, and appropriate protective attire will be used at all times. These include gloves, goggles, lab coats, close-toed shoes, and long pants. Infectious/hazardous wastes will be properly disposed of in designated containers. Reagents will be handled with care as described below.

Phosphate-buffered saline (PBS):

It may cause irritation to the eyes and skin through contact. If ingested, PBS may cause irritation to the digestive tract. If inhaled, it may cause irritation to the respiratory tract.
If one's eye comes in contact with PBS, one can use water to flush out the chemical. In the case of skin irritation, the chemical can be washed off with water. In the case of ingestion, one may rinse the mouth with water.

Dulbecco's modified eagle medium (DMEM):

It may cause irritation to the eyes, skin, mucous membranes and respiratory tract if inhaled. If eye irritation occurs, one must wash their eye thoroughly with water and seek medical assistance. In the case of skin irritation, the affected area must be washed thoroughly with a large amount of soap and water. If swallowed, one must wash their mouth with water.

Penicillin-streptomycin antibiotics:

It may cause allergic skin reactions, eye irritations, and breathing difficulties. If skin contact occurs, immediately wash off with water for at least 15 mins. If eye contact occurs, rinse immediately with water, particularly under the eyelids, for 15 mins. If ingested, call a poison control or a physician immediately. If inhaled, immediately move to fresh air. Give artificial respiration if necessary and call poison control or a physician immediately.

Ampicillin antibiotic:
It is a potential skin and eye irritant. Prolonged exposure may cause damage to organs. Proper protective equipment should be used when handling.

10% Fetal bovine serum (FBS):
It may cause irritation if inhaled, swallowed, or comes in contact with skin and eyes. Eye protection and appropriate clothing should be used when handling.

Tris-buffered saline (TBS):
It is a serious eye irritant. It may also be dangerous if inhaled or swallowed. Eye protection and proper gear should be worn.

Acrylamide:
It can permeate skin and irritate eyes. If eye contact occurs, flush eyes immediately with water for 15 mins. If skin contact occurs, flush affected area with water for 15 mins. If inhaled, get fresh air and if needed, give artificial respiration. If ingested, seek medical attention.

4′,6-diamidino-2-phenylindole (DAPI):
It may cause skin and eye irritation. It may also be harmful if swallowed or inhaled. DAPI is a carcinogen or potential carcinogen as it binds to regions in DNA. In the case of eye contact, flush eyes out with water. In the case of skin contact, thoroughly wash and cover the contaminated area with an emollient. It is important to avoid prolonged exposure. If ingested, wash out the mouth with water, and if inhaled, move to an area with fresh air.

Qiagen Plasmid Plus Midi Kit buffers:
These buffers are proprietary components and therefore have no associated concentrations. They can cause serious skin and eye irritation upon exposure. Protective gloves, goggles, and lab coats should be worn. Hands should be thoroughly washed after handling

III. Data Analysis:
1) Immunofluorescence microscopy: Immunostained HEK293T cells will be viewed under a Leica DM6000B epifluorescence microscope with a Leica DFC7000 T camera and LAS X Software. The cellular localization of CCDC11 and cilia formation will be carefully evaluated.
2) Western blotting: Protein bands will be visualized using the enhanced chemiluminescent (ECL) method, and analyzed based on their molecular weights and intensities. Densitometric measurements of bands on the immunoblot corresponding to the protein of interests will be analyzed using the NIH ImageJ software.

3) ELISA assay: Effects of CCDC11 on budding of HIV-1 virus-like particles (VLP) will be measured based on a standard curve prepared from several different concentrations of recombinant HIV-1 p24 protein.

4) Quantification analysis: The standard error of the mean will be calculated using the Excel software and error bars will be shown in results graph.

Bibliography

1. Martin-Serrano J, Zang T, Bieniasz PD. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. Nat Med. 2001;7(12):1313-9. doi: 10.1038/nm1201-1313. PubMed PMID: 11726971.

2. Pincetic A, Medina G, Carter C, Leis J. Avian sarcoma virus and human immunodeficiency virus, type 1 use different subsets of ESCRT proteins to facilitate the budding process. J Biol Chem. 2008;283(44):29822-30. doi: 10.1074/jbc.M804157200. PubMed PMID: 18723511; PMCID: PMC2573067.

3. Morita E, Sandrin V, McCullough J, Katsuyama A, Baci Hamilton I, Sundquist WI. ESCRT-III protein requirements for HIV-1 budding. Cell Host Microbe. 2011;9(3):235-42. doi: 10.1016/j.chom.2011.02.004. PubMed PMID: 21396898; PMCID: PMC3070458.

4. Guizetti J, Gerlich DW. ESCRT-III polymers in membrane neck constriction. Trends Cell Biol. 2012;22(3):133-40. doi: 10.1016/j.tcb.2011.11.007. PubMed PMID: 22240455.

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6. Christ L, Raiborg C, Wenzel EM, Campsteijn C, Stenmark H. Cellular Functions and Molecular Mechanisms of the ESCRT Membrane-Scission Machinery. Trends Biochem Sci. 2017;42(1):42-56. doi: 10.1016/j.tibs.2016.08.016. PubMed PMID: 27669649.

Investigating role of the Novel ESCRT-III recruiter CCDC11 in HIV Viral Budding: Identifying a Potential Target for Antiviral Therapy

Updated Research Plan Addendum

Poojan Pandya and Leo Takemaru

II. Risks and Safety:

3) Potentially hazardous biological agents (PHBA):

The human embryonic kidney (HEK) 293Tcell cell line will be purchased from American Type Culture Collection (ATCC, CRL-3216) and handled in a biological safety cabinet under proper supervision. Appropriate safety equipment will be used including gloves, goggles, and lab coats. This requires biosafety level 2 practices and containment facilities. DH5alpha *E. coli* cells are classified as biosafety level 1. Personal safety equipment should be worn at all times when handling. **Tissue culture and bacterial wastes including culture media will be treated with bleach (a final concentration of >10%) for 30 mins and discarded into the laboratory sink. The sink will be thoroughly rinsed after waste discharge.**

4) Hazardous chemicals/activities/devices:

Safety precautions must be taken when handling chemicals, and appropriate protective attire will be used at all times. These include gloves, goggles, lab coats, close-toed shoes, and long pants. Infectious/hazardous wastes will be properly disposed of in designated containers. Reagents will be handled with care as described below.

Phosphate-buffered saline (PBS):

It may cause irritation to the eyes and skin through contact. If ingested, PBS may cause irritation to the digestive tract. If inhaled, it may cause irritation to the respiratory tract. If one's eye comes in contact with PBS, one can use water to flush out the chemical. In the case of skin irritation, the chemical can be washed off with water. In the case of ingestion, one may rinse the mouth with water.

Dulbecco's modified eagle medium (DMEM) and Opti-MEM I:

These are common media for tissue culture cells. It may cause irritation to the eyes, skin, mucous membranes and respiratory tract if inhaled. If eye irritation occurs, one must wash their eye thoroughly with water and seek medical assistance. In the case of skin irritation, the affected area must be washed thoroughly with a large amount of soap and water. If swallowed, one must wash their mouth with water.

Penicillin-streptomycin antibiotics:

It may cause allergic skin reactions, eye irritations, and breathing difficulties. If skin contact occurs, immediately wash off with water for at least 15 mins. If eye contact occurs, rinse immediately with water, particularly under the eyelids, for 15 mins. If ingested, call a poison control or a physician immediately. If inhaled, immediately move to fresh air. Give artificial respiration if necessary and call poison control or a physician immediately.

Ampicillin antibiotic:

It is a potential skin and eye irritant. Prolonged exposure may cause damage to organs. Proper protective equipment should be used when handling.

10% Fetal bovine serum (FBS):
It may cause irritation if inhaled, swallowed, or comes in contact with skin and eyes. Eye protection and appropriate clothing should be used when handling.

Tris-buffered saline (TBS):
It is a serious eye irritant. It may also be dangerous if inhaled or swallowed. Eye protection and proper gear should be worn.

Acrylamide:
It can permeate skin and irritate eyes. If eye contact occurs, flush eyes immediately with water for 15 mins. If skin contact occurs, flush affected area with water for 15 mins. If inhaled, get fresh air and if needed, give artificial respiration. If ingested, seek medical attention.

4′,6-diamidino-2-phenylindole (DAPI):
It may cause skin and eye irritation. It may also be harmful if swallowed or inhaled. DAPI is a carcinogen or potential carcinogen as it binds to regions in DNA. In the case of eye contact, flush eyes out with water. In the case of skin contact, thoroughly wash and cover the contaminated area with an emollient. It is important to avoid prolonged exposure. If ingested, wash out the mouth with water, and if inhaled, move to an area with fresh air.

Qiagen Plasmid Plus Midi Kit buffers:
These buffers (**lysis buffer L7 containing RNaseA, Precipitation buffer N3, and Elution buffer E4**) are proprietary components and therefore have no associated concentrations. They can cause serious skin and eye irritation upon exposure. Protective gloves, goggles, and lab coats should be worn. Hands should be thoroughly washed after handling

Polyethyleneimine (PEI):
PEI is a cationic polymer used for transient transfection of HEK293T cells. 1 mg/ml may cause skin and eye irritation. It is important to wear protective gears when handling PEI.

Methanol:
Methanol is toxic when ingested. It may also cause serious skin and eye irritation when exposed. Protective gears should be worn at all times. It is flammable and should be stored in a flammable storage cabinet.

Acetone:
Acetone may cause skin and eye irritation upon exposure. Protective gloves, goggles, and lab coats should be worn at all times. It is flammable and should be stored in a flammable storage cabinet.

TE buffer:
This is a Tris-based buffer and can cause skin and eye irritations. If eye contact occurs, flush eyes immediately with water for 15 mins. If skin contact occurs, flush affected area

with water for 15 mins. If inhaled, get fresh air. Seek medical attention. If ingested, do not induce vomiting.

Lysogeny Broth (LB), RNAiMAX, and lipofectamine:
LB is a neutritional medium for bacteria. RNAiMAX and lipofectamine are cationic lipids used for transient transfection of tissue culture cells. These are not hazardous but potentially irritate skin and eye upon exposure.