HUMBOLDT STATE UNIVERSITY

Risk Management and Safety Services

Biosafety Committee Notes
December 14, 2016
Meeting called to order 1:07 P.M.

Present: Amy Sprowles, Patricia Siering, David Baston, Jefferey Schineller, Michelle Dostal, John Steele, Sabrina Zink, Shannon Townsend, Emily Benvie, Jose Hernandez

Guest Presenters: Amy Sprowles, Jianmin Zhong

Absent: Richard Brown, Steven Karp

- 1:07 P.M.
    - Meeting Began
    - Agenda passed out
    - Current Draft of Biosafety Pre-award Checklist
    - Sprowles is not available until presentation to be done at 1:30 P.M.
- 1:08
    - Review for Amy Sprowles' Biological Use Authorization Application (BUA)
    - Sabrina's findings
        - Working with Lentiviral Vectors
        - Requires Biosafety Level 2 (BSL-2) even if safe
        - Why should it be reduced to BSL-1?
        - Looking at the regulatory information and agencies (e.g.- NIH, CDC)
        - Final recommendation – BSL-2
        - Floor open to committee feedback
- 1:14
    - Steele suggested changing the nomenclature to be more specific and offers definitions
        - BSL-2 requirements trigger when talking about viral particles, not plasmids
        - The plasmid doesn't affect the cells unless injected into the nucleus
        - No viral transmission, just expression vector
    - Zink asks about risk of oncogenes
        - Steele states risk level is low to human
        - Needs to be injected into cells to potentially be a problem or lipid transfected
        - Even if inhaled or ingested, will not cause a problem
        - If packaged into a live virus, then maybe it could be a problem

- Sprowles is using this protocol for viral transfection because it is better than other methods for her research.
- Steele brings up adding a checkbox for the pre-award checklist that will trigger the discussion for BSL-2 requirements, which include
    - Toxins, pathogens, co-transfections with live viruses
    - Any plasmid from a list of potentially problematic genes such as:
        - GAG- (Group Specific Antigen)- encodes viral matrix, capsid and nucleoproteins
        - Pro- encodes protease that cleaves the premature gag protein
        - Pol- Polymerase that encodes the reverse transcriptase and integrase
        - Env- encodes the surface of glycoproteins to target cells receptors.

- 1:25
    - Zink asks why does packaging for kit recommend BSL-2
    - Steele- recommended by the company under the assumption of work with viruses.
    - Unless it has the sequence, will not replicate
    - ~72 hours, plasmid won't be expressed by cells anymore
- 1:26
    - Zink inquiries about other considerations
    - Bastion brings up dangers of working with open wounds
        - Issue not specific to BSL-2
        - BSL-1 deals with those concerns
    - Siering brings up that the kit will allow a person to make viruses
    - Steele states that you need all 4 of the genes to make a virus
    - Sprowles is only working with one of those and is not making viruses
- 1:29
    - Steele asks that the wording to be stronger and more defined
    - May want to add check boxes
        - Does application involve something that can be made into a virus?
        - Can a pathogen or toxin result from experiments?
    - Zink will take feedback regarding the Pre-award Checklist
    - Will also send a digital copy for everyone to review and propose changes
- 1:31
    - Sprowles enters meeting

- 1:37
    - Amy Sprowles begins her presentation of her research
        - Review of cell biology processes
        - Review of plasmids worked with
        - DNA can be specified to work with certain species, or eukaryotes and prokaryotes.
        - Review of transfection
        - What the plasmid is encoded with tells it what to do
        - What the protein made does determines what biosafety level is needed
        - Won't spread unless it is told to
        - From her research
            - Plasmids stay in the cell and shown by the green glow of Green Fluorescent Protein.
            - Cas 9 /CRISPR review
            - Steele
                - Placing U6 to GFP into plasmid wouldn't change function
                - Plasmid DNA cannot pass through cell membrane for mammalian cells
                - Only cells that get the plasmid will get the mutation, which are all in the dish
            - Transfection Review
                - BSL-1 for transfections
                - Viral components targeting humans take precautions to BSL-2
                - In eukaryotic cells, need certain protein interactions to be in place to be interacted with
            - Can do electroporation
                - But sterility issues and not as reliable
            - Viral transduction
                - Just a way to get plasmid into cell
                - To make into a virus, requires knowledge in how to make virus
            - For class labs, BSL-2 lab practices are used mostly to protect cells from students
            - Students come with all sorts of contaminants and are learning hygienic lab skills
            - Hepa-Filters are used in these rooms

- Presentation Ends
  - Siering Votes for BSL-1 for Sprowles research
  - BSL-2 options discussed
  - Sprowles only has the one of the components
  - To do other virus work, will need to order other kits
  - The Stem Cell class doesn't use lentiviruses
  - Not using to make viral particles, but using as a DNA delivery system
  - Motion called for approval for BSL-1 practices to be used for Sprowles research
    - All in favor
  - Sprowles will send everyone a copy of the powerpoint
- Zink has started the website
  - Will add more resources to it

- 2:12
  - Housekeeping
    - Dostal
    - Approval of the minutes from the previous meeting
      - All in favor
      - No further discussion required
    - Steele
      - Karp looked into CITI options
      - Looked at CItI for training options
        - Collaborative Institutional Training Initiative
      - They cover the training costs with subscription
      - This includes training for PI's, students, and committee members
      - Have to register
    - Zink
      - Should it be a part of BUA?
        - Yes
      - Documentation built into system
      - Anyone listed on BUA must be trained
      - On website will be requirements for students to look at
    - Steele
      - If submitted BUAs for lab
      - Should be mechanism for unifying to addendum

- 2:17
  - Sprowles

        - BUAs should have a numbers on it to find the people involved with the project and check if they meet requirements
    - Steele
        - Set term limitations on BUAs
        - Such as 3 years
- 2:18
    - Pre-award Checklist –Draft
    - Siering
        - What are Microbiological Agents
        - Wording vague
    - Dostal
        - Contact if you are interested in making any changes to checklist before next meeting
    - Steele
        - Talked about paperwork he has to do for other agencies for human cell work
    - Siering
        - Likely pathogenic agents instead of just microbiological agents on draft
- 2:23
    - Dostal
        - Any other issues?
        - Next meeting will be sometime in January
        - Concern raised
            - Meeting requirements and attendance
            - Quorum requires five members
                - Includes two community members
            - If we need to have a vote, we need people
            - Zhong issue resolved, no real meeting required
            - Only voting on issues BSL-2 or higher
            - If agree on issues/ no issues and no voters, may not need to meet
- 2:26
    - Zink
        - Talk about Zhong's research
        - *Rickettsia parkeri*
            - Responds to doxycycline
            - Not all risk for BSL-3
            - *parkeri* not listed
            - Considered BSL-2 or 3 depending on if it responds to treatment

        - Meeting is for endosymbiont work
            - Zhong wants both of them reviewed
            - *R. parkeri* and endosymbiont work
            - Zhong needs to submit BUA for endosymbiont work
    - Dostal
        - Zhong coming in to explain his research and answer questions
        - Will be late do to hosting a final until 2:35 PM
    - Siering
        - Has Zhong defined endosymbiont by 16S rRNA sequencing?
            - Is defined as *Rickettsia*
            - Not transmitted horizontally
            - Doesn't grow above 32^{o} C
                - Can't live in host
            - Only can grow in a tick cell.
        - No immediate danger in vitamin producing organism, but still BSL-2 due to being *Rickettsia*
        - Who are his collaborators?
            - Rocky Mountain Lab
                - Gave Zhong plasmid
            - What are their protocols?
- 2:35
    - Zhong enters meeting
    - Opening remarks and thanks
    - *Rickettsia* endosymbiont
    - Nonpathogenic according to him and his lab results
    - Has been working on *Rickettsia* for 18 years
    - Collected ticks from 7 different counties in California
    - Evidence
        - Prevalence to be 100% based on qPCR
        - Rabbit grown ticks
            - 4 stages of ticks
            - Passed from mother to offspring
            - Also passed from life stage to life stage
        - Invitro tick cell lines (ISE)
            - Incubated tick cells at different temperatures.
            - Between 32-34^{o} C, *Rickettsia* will die
            - Humans at 37^{o} C
        - University of Minnesota

        - Found that *Rickettsia* can't infect at temperatures greater than 32 degree C
    - It is under BSL-2 lab practices
- Siering
    - Zhong looked at bacteria in dogs
    - Couldn't get deer samples
    - Doesn't have proof that *Rickettsia* in other animals in our ecosystem
- Bastion
    - Is it possible due to survive past 32°C and then transmit when conditions are favorable?
        - Zhong did not know if infections for sure, we wouldn't have evidence that supports that

- 2:44
    - Steele
        - What do collaborators do?
            - They work with both pathogenic and nonpathogenic samples
        - What level do they use?
            - BSL-2, even for nonpathogenic
    - Bastion
        - Concern
            - Cells can't infect at 32° C, can they still live at 37° C but then reinfect when temperature goes to 32° C
        - Zhong
            - At 37° C, can't get bacteria to work at that temperature. Didn't do a controlled study of it.
    - Sprowles
        - How do you quantify infection?
        - Zhong
            - Whether we observe viral particles in cytoplasm
        - mRNA can't get bacteria
            - Host cell replicates, but no trace of bacteria
        - Steele
            - If we grow *Rickettsia* to 25° C, take temperature to 37°C, and then take temperature down to 25° C again, then check for it
            - We should potentially try that
        - Zhong
            - Does it matter because I am using BSL-2 practices even if not pathogenic
    - Zink

        - Do we feel comfortable with this being BSL-2 for BUA
        - Playing catchup due to him already working on it
    - Baston
        - Uncharacterized endosymbiont
        - Zhong
            - Submitted data to be characterized to
                - International Journal of Systematic and Evolutionary Microbiology (IJSEM)
                - ATCC safe deposit bacteria collection
                - Naming the species
    - Siering
        - Full genome?
        - Lab sequencing date available online
    - Zhong thanks everyone for time and support
- 2:52
    - Zink
        - Still dumping waste down sink
        - 10% bleach
        - 100-600 mL
        - Due to small amount based on literature it will be allowed
    - Dostal
        - Chemistry department doesn't drop anything down sink
        - Zink will keep an eye out for issues
- 2:55
    - Zink
    - Next steps to take
        - BSL-2 for submitting
    - Dostal
        - Do we want to meet in January for BUA and *Rickettsia*
        - Holding off vote until more information is available
    - Siering
        - Can't be a part of next meeting until it is after January 23, 2017.
        - Zink
            - Zhong already doing it, not with compliance
            - Now operating with higher degree of scrutiny
- 2:58
    - Sprowles brings up liability issues
    - Zink
        - Liability

        - Zhong has 10 years experience
        - Even if BSL-2, needs to go to committee
        - What should we do in between?
    - Sprowles
        - Collaborate with others
        - Are their *R. parkeri* research at BSL-2?
        - Zink
            - BSL-2 or with BSL-3 practices
        - College wide meeting first week of school
        - CRNS welcome
        - Tell everyone we have a biosafety committee, draft, and website
        - Need representative
    - Dostal
        - Spring meeting is only faculty?
        - For staff also?
        - Will ask dean
    - Zink
        - BUA for Kinesiology and Psychology
            - Blood work
            - Lactic Acid
- 3:02
    - Zink
        - Voting for BSL-2 practices for endosymbiont now
        - All agree
        - Not for approval, but to be continued to be used as BSL-2.
        - Still needs to submit BUA
- 3:03
    - Meeting adjourned