TRANS FUTURES

AN OPEN SOURCE CURRICULUM

V1 organized in collaboration with MediaLab Mexico City

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workshop ✺ blurb

This workshop is an introduction to Synthetic Biology and plant transgenesis from a queer perspective. We will go over the basic concepts of cell biology and learn techniques for in-silico (on a computer) DNA design. We will also do an in-vivo (in a living plant) genetic modification procedure called Agroinfiltration. Together we will look at the violent colonial patriarchal structures that have produced these technologies and ask if and how it's possible to, "repurpose technologies for progressive gender political ends..." as challenged by the 2016 Xenofeminist Manifesto.

materials ✺ equipment ✺ & ✺ collaborators

1. Equipment:
    1. Incubator
    2. Shaker or Rocker
    3. Colorimeter or Spectrophotometer and Cuvettes
    4. 1000uL, 200uL, and 10uL micropipettes
    5. Microcentrifuge
    6. Vortexer
    7. UV light wavelength output between 300 and 400nm for GFP excitation
    8. Growth chamber to sequester transformed plants
    9. Access to a pressure cooker or autoclave for sterilization of materials
    10. Bunsen burner, ethanol lamps, or a sterile hood for creating a clean workspace
    11. Larger autoclavable bottles for sterile H2O and to be used in preparing MMA Buffer
    12. Grow lights for transformed plants
2. General Supplies:

    1. Plastic sheeting ~ plastic painters dropcloth works well
    2. Duct tape
    3. Permanent Marker
    4. Lab tape or masking tape
    5. Autoclave tape helpful but not necessary
3. Materials:
    1. MMA Buffer (see section on “Solutions and Media to Prep Beforehand” below)
    2. LB powder and Agar
    3. 15mL conical tubes
    4. Microcentrifuge tubes
    5. 1mL sterile syringes
    6. Sterile needles
    7. Sterile pipette tips for 1000uL, 200uL, and 10uL micropipettes
    8. Sterile culture flasks
    9. Autoclavable biohazard bag
    10. Sterile H2O
    11. Spray bottle and 70% isopropyl or ethanol solution for sterilizing surfaces
    12. Inoculation loops (can be reusable metal loop, sterile toothpicks, disposable sterile loops, or sterile pipette tips)
4. Collaborators:
    1. Preferable to have a partner with access to a university, industrial, or DIY biology lab who can facilitate access to an autoclave, specific materials difficult to acquire without an affiliation such as an appropriate plasmid containing agrobacterium strain, and assist with proper disposal of biohazard waste.
    2. People to perform this technoscientific choreography with
    3. An agrobacterium strain transformed with an appropriate transfection plasmid (we used strain AGL1 with a plasmid containing a GFP and CYP11A1 expression cassette)
    4. Tobacco plants for transformation ~ and possibly other plants for experimentation

workshop ✺ outline

Day 1: INTRO TO SYNTHETIC BIOLOGY

1. Introduce yourself briefly -- how/where you learned biology (BUGSS) -- personal reference point for hormones and hormone hacking
    1. Introductions of participants, ask what contact with biology//cell biology//molecular biology people have had to gauge prior knowledge and experience, why interested in this workshop
2. Introduce core concepts of agrobacterium induced transgenesis
    1. Molecular Biology 101
        1. Genes, transcription, translation, proteins, and enzymes
            1. Metabolic pathways
        2. Synthetic biology: DNA from a design standpoint

        1. Coding regions, promoters, terminators, transport tags, etc.
    3. Plasmids! Trans border crossings
        1. Horizontal gene transfer ~ horizontal gene transfer as queer production ~ the queering of biofamily, heterosex, and species boundaries
        2. Bacterial and fungal transgenesis: heat shock transformation, electroporation
        3. Plant transformation: gene gun, agrobacterium
    4. Agrobacterium induced transformation ~ Queering the notion of the engineered as synthetic or unnatural
        1. Agro as a wild genetic engineer, genetic architect and genetic farmer
            1. Mechanism of action and wild type virulent behavior
        2. History of the relationship between humans, agrobacterium, and plants
    5. Patents on transgenic plants, patenting as neocolonial practice ~ Ikechi Mgbeogi
        1. Copyleft biotech and pharma practices
            1. Scientists who discovered insulin
            2. Open Source Insulin project
            3. Paul Preciado's call for a copyleft politics for hormones and Xenofeminism's call for a transfeminist intervention in technoscience
            4. Open Source Gendercodes
3. Inoculate liquid cultures with agrobacterium strain containing plasmid. This is what will be prepared for plant transformation on Day 2 (refer to steps 1-3 of the *transfection choreography* listed below). Each person gets their own tube so that everyone has a chance to innoculate.

Day 2: PLANT TRANSGENESIS

1. Discussion of tobacco as model organism: why they are used
    1. Tobacco as a sacred plant for Indigenous Peoples all over Turtle Island important for medicinal and ceremonial uses, colonial monetization of tobacco, early western medicinal uses, monetization of tobacco as the primary driver of the early slave trade in north america (shift from indentured servant laborers to people stolen from their homes and forced into slavery)
    2. The shift from a leisure product to a biotechnical cash crop
    3. Tobacco as, "the cinderella of plant biotechnology"
2. Together perform the remaining movements of the agrobacterium mediated plant transgenesis process, refer to steps 4-10 of the *transfection choreography* listed below.
3. Plants and hormones
    1. Indigenous plants used as abortifacients, birth control, menopause therapeutics, to induce labor, and sexual stimulants
    2. Brief history of pharmaceutical hormones

            1. Gonad transplantation, organotherapy, self experimentation, and humans/animals as biotechnical products
            2. The shift to molecular extracts and chemical synthesis from animal products
            3. Petrolocene! The creation of synthetic hormones produced from petroleum
            4. 1950s and the search for plant sources
            5. African poison arrow vine, Mexican yam (barbasco), Agave, and the creation of the pill
            6. Present day ~ microbial fermentation/transformation of soy, yam, pine, and other plant sterols
            7. Could a hormonal soy plant create a libre trans politic?
                1. Side note -- pomegranate and date palm accumulate estrogens in the seeds
4. Brief Introduction to my methods for OSG
    1. Open source databases, software, and analysis tools for molecular biology
        1. Benchling
        2. KEGG databases and metabolic pathways
        3. Pubchem
        4. Etc.
5. Assignment is to think of a modification you would create in a plant.Think simple, what proteins could you express that would induce production of a medicine, new material properties, a new appearance, some ability or trait borrowed from another organism…

[3-5 day waiting period between workshop day 2 and day 3 in order to allow gene transfer, integration and protein production to take place]

Day 3: IN-SILICO, GENE DESIGN, ORGANISM DESIGN, AND DIRECTED EVOLUTION

1. Transformants can be checked for successful transformation and gene expression by observing under a black light to reveal fluorescence in infiltrated areas of the leaves
2. In Silico workflow case study
    1. OSG from concept to materialization
        1. Conception
        2. Initial research and pathway analysis
        3. Patent and Scientific literature analysis (looking at enzyme isoforms and what has already been done)
        4. Finding the necessary genes
        5. Determining a suitable vector (destination plasmid)
        6. Adding any other components
            1. Promoters
            2. Terminators
            3. His tags
            4. Protein targeting
3. and re-coding for your target organism

    1. Removing cryptic intron splice sites (if working with a plant)
    2. Codon optimization
4. Sourcing the gene
    1. Synthesis: tickets for free gene synthesis through _____ initiative
    2. Addgene
    3. iGEM databank
    4. Searching through papers and approaching researchers as a fellow “researcher”
    5. PCR amplification directly from DNA extracted from organism

agroinfiltration ✺ protocol

SOLUTIONS AND MEDIA TO PREP BEFOREHAND

{NOTE: these antibiotics are particular selective agents for the plasmid and agrobacterium strain we were working with, but might be different for other strains/plasmids}

- Kanamycin Antibiotic Stock at 50ug/uL concentration
    1. Weigh .5g Kanamycin, add to 10mL sterile H2O and dissolve completely
    2. Prewet a .22um syringe filter by passing 5-10mL sterile H2O through and discard water
    3. Sterilize Kanamycin stock through the syringe filter, stock can be stored at -20C for up to a year.
- Rifampicin Antibiotic stock at 50ug/uL concentration
    1. Weigh .5g Rifampicin, add to 10mL DMSO and dissolve completely
    2. Stock can be stored at -20C for up to a year. Rifampicin might precipitate out of solution while in storage, vortex to resuspend before use.
- 200mL MMA Buffer (for agrobacterium resuspension before injection)
    1. 2mL of 1M MgCl2
        1. Weigh 2.03g MgCl2 and dissolve in 10mL sterile H2O to create 10mL of 1Molar stock
        2. Autoclave stock to sterilize
    2. 4mL of a .5M MES Buffer
        1. Weigh .9762g MES free acid and dissolve in 10mL sterile H2O
        2. Filter sterilization is recommended, or autoclave if filter sterilization not feasible.
    3. 200uL of a 100mM Acetosyringone stock
        1. Weigh 196mg Acetosyringone and dissolve in 6mL Ethanol
        2. Bring to volume (10mL) with sterile H20
        3. Filter sterilize
    4. Bring MMA Buffer to volume with 193.8mL sterile H2O

transfection ✺ choreography

1. Prepare solutions and media as indicated above prior to workshop

2. On day 1 of the workshop go over sterile technique and biosafety with participants and have them prepare 1 or more flasks containing 100mL of LB and add the appropriate antibiotics for selection of your particular agrobacterium strain and plasmid.
3. After preparing the media, you can use an inoculation loop to select a single colony of agrobacterium from a petri dish and swirl the colony into the prepared flask to innoculate. Do this step about 24 hours before you will be running the workshop the next day to ensure you will have a high density culture ready.
4. When Agrobacterium liquid culture reaches high density, each person should pipette 2mL culture into a microcentrifuge tube.
5. Centrifuge samples at 4,000G (10,000rpm) for 10 minutes to pellet the cells and discard the supernatant (liquid fraction).
6. Add sterile water to your tube and resuspend the pelleted cells into the water using the vortexer. This step is meant to wash off the antibiotics which could harm the plant cells when the solution is injected.
7. Repeat steps 2 and 3 for a second wash
8. Repeat step 2 and this time resuspend pelleted bacteria in 1mL MMA buffer.
9. Take a 15mL conical tube and add 6mL MMA buffer to it. Then pipette the contents of your microcentrifuge tube, along with one other person's and combine them into the 15mL conical tube. You should have a final volume of 8mL agrobacterium suspension that can be used for syring agroinfiltration of plant leaves.
10. For the final step we will form 2 groups that will use different techiques
    1. One group should add 8uL of Acetosyringone solution to their Agro-suspension and let tube incubate for 30min-1hr. before injection.
    2. The other group should incubate for 30min-1hr. without Acetosyringone before injecting the solution into plant leaves