Plasmid retention & Biocontainment Notebook

WEDNESDAY, 7/3/2019

- Miniprepped Chris Barnes' pUC with Axe/Txe (AT), Hok/Sok (HS) and Microcin V (MCC) that was transformed into TOP10 competent cells respectively.
- Chris Barnes' pUC --> Kanamycin resistant
- Primer used: Retention seq primer
- Sent for DNA sequencing
    - pUC-GFP-Axe/Txe (6475bp): **Successful**
    - pUC-GFP-Hok/Sok (5350bp): **Unsuccessful**
    - pUC-GFP-MicrocinV (9521bp): **Unsuccessful**

THURSDAY, 7/11/2019

- Ran 1.5% agarose gel to determine plasmid separation of SGRS (**Failed**)

FRIDAY, 7/12/2019

- Defined and designed gBlock with David and Salva
    - Comprising of pCon(strong)-antitoxin-pCon(weak)-toxin-pCon(normal)-GFP/RFP
    - Using 2 plasmids - David's pAC and Chris Barnes' pUC
- Repeated on 1.5% agarose gel to test plasmid separation capability

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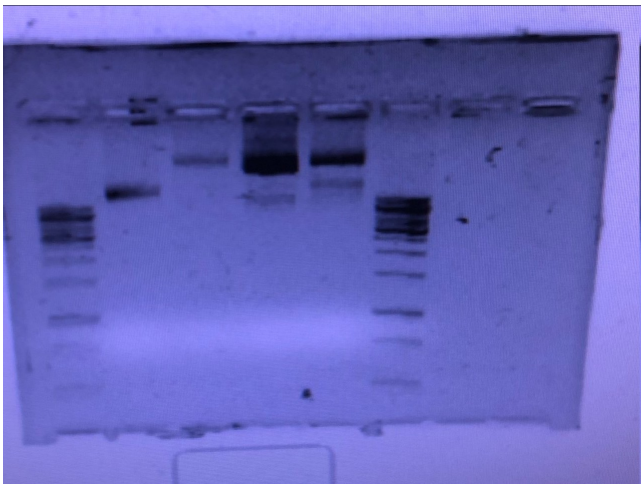

1. 10Beta-E2K-SGRS-GFP
2. HS
3. MCC
4. Mixed (10Beta-E2K-SGRS-GFP + HS + MCC)
    - Cannot really differentiate
    - Will be running again next Tuesday
        - Fragments are too big to migrate down the 1.5% gel, repeating experiment in a 1% gel
    - Made both MG1655 Wild type (WT) and MG1655 PCon GFP competent cells
        - MG1655 PCon GFP --> Kanamycin resistant
    - Designed primers for cloning out antitoxin from pUC and cloning into David's pAC

TUESDAY, 7/16/2019

- Transformed David's pAC into MG1655 WT competent cells
    - David's pAC --> Chloramphenicol resistant
- Repeated on 1% gel to test plasmid separation capability

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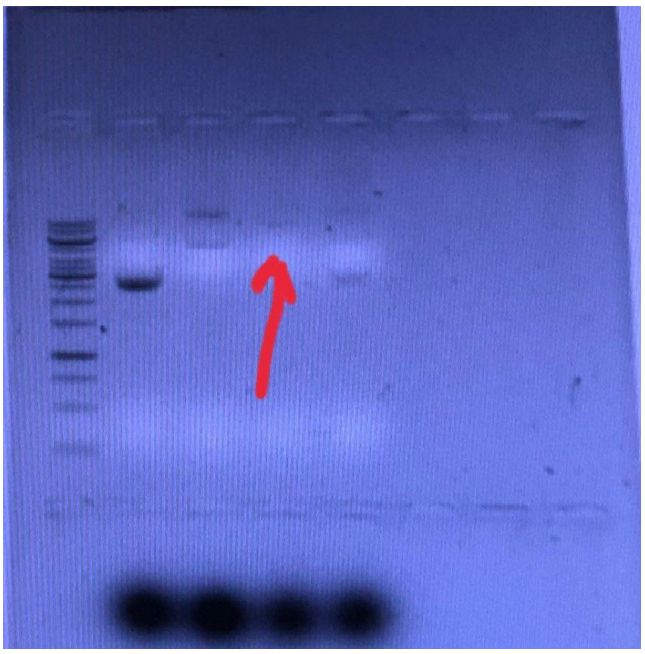

1. 10Beta-E2K-SGRS-GFP
2. HS
3. MCC
4. Mixed (10Beta-E2K-SGRS-GFP + HS + MCC)
    - Bands are too faint due to plasmid yield.
    - Seems like MCC and be seperated from MCC if plasmid yield is low. Too much plasmid loaded into gel will cause the bands to merge (refer to gel obtained on **7/12**).

WEDNESDAY, 7/17/2019

- Picked successful colonies and grew them in Chloramphenicol LB broth for miniprep .
- Miniprepped David's pAC
- Primers for cloning out antitoxin from pUC recieved

FRIDAY, 7/19/2019

- Conducted PCR for Chris Barnes' pUC to construct backbone template for Gibson
    - pUC-GFP-Axe/Txe (6475bp)
        - (insert primer)
    - pUC-GFP-Hok/Sok (5350bp)
        - (insert primer)

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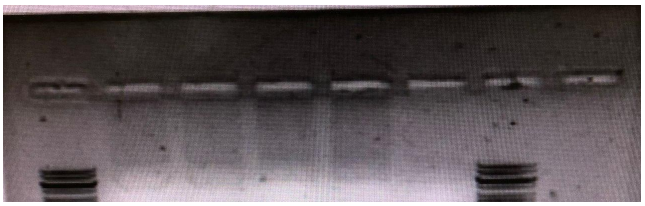

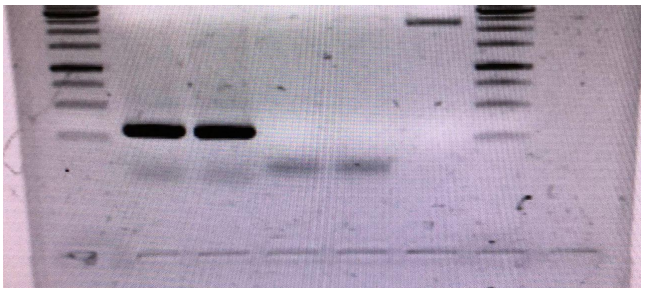

1. and 2. Axe
3. and 4. Hok
5. David's pAC
   - AXE cloning: **Successful**
   - HOK cloning: **Unsuccessful**
     - HOK might be problematic therefore subsequent experiments was done using AXE only.

FRIDAY, 7/26/2019

- Conducted gibson with AXE/TXE with David's pAC on 7/27/19, transformed into MG1655 and plated on chloramphenicol plates
- No colonies (Gibson failed)
  - Troubleshooted --> gel extraction method was not optimized

MONDAY, 7/29/2019

- Ran Gibson again with AXE/TXE and David's pAC; no colonies (Gibson failed)
  - Experiment put on hold
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TUESDAY, 7/30/2019

- Prepared 1. AXE/TXE in LB + Kanamycin and 2. MG1655 WT for passaging experiment (Chris Barnes' paper)
  - Respective colonies are passaged for 6 days and GFP was measured at a single timepoint

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THURSDAY, 8/1/2019

- Conducted PCR for pAC-J23100 and 3Frag_Ram to construct backbone template for Gibson
  - pAC-J23100 = 1721 bp (J)
    - pAC-J23100 Rev* and Fwd*
  - 3Frag_Ram = 1883 bp (G)
    - 3Frag_Ram_GFP Rev* and Fwd*
- Ran gel to check PCR product
  -

J on left G on right

- Did gel extraction
  - J = 32.1 ng/ul
  - G = 68.7 ng/ul
- Conducted Gibson with PCR product and gBlocks
  - pCon-Axe-pCon-Hok-pCon-RFP + pAC-J23100
  - pCon-Sok-pCon-Txe-pCon-GFP + 3Frag_Ram

FRIDAY, 8/9/2019

- Conducted PCR on Gibson products using overlapping PCR protocol
  - Ampli-PCR Fwd and 3Frag_Ram Rev* and 3Frag_Ram Fwd*
- Ran gel on product
  -

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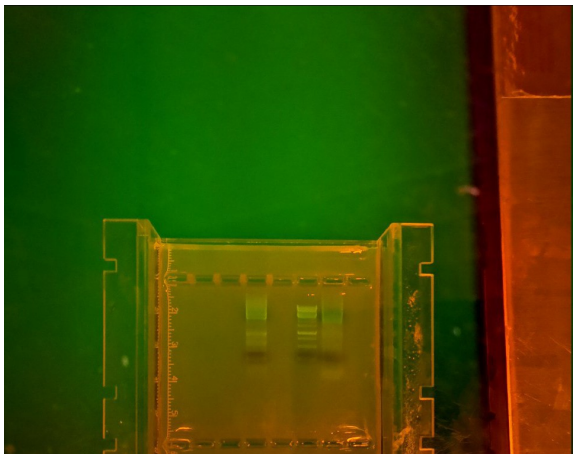
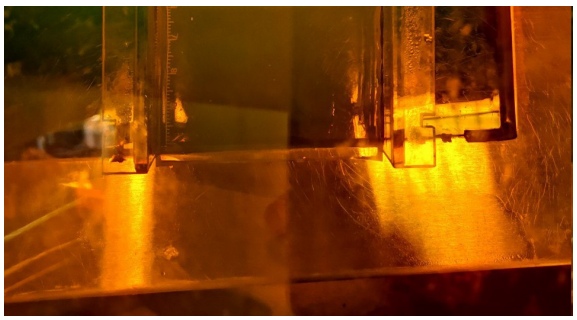


Left G, middle ladder, Right J



- Transformed everything onto chloramphenicol plates

SATURDAY, 8/10/2019

- Picked 2 colonies from each plate. Only gibson products (G* and J*) had colonies which were green. Inoculated 1 colony in C and 1 colony in CK media

SUNDAY, 8/11/2019

- Only G* and J* inoculated tubes contained bacteria with green fluorescence for both C and CK tubes. MIniprepped.
  - C tube - 275.7 ng/ul
  - CK tube - 230.5 ng/ul
  - Will send for sequencing tomorrow

MONDAY, 8/19/2019

- Conducted Gibson with PCR product and gBlocks
  - pCon-Axe-pCon-Hok-pCon-RFP + 3Frag_Ram

TUESDAY, 8/20/2019

- Got multiple colonies, some red and some green and some orange. Picked and inoculated for sequencing orange colonies.

WEDNESDAY, 8/21/2019

- Sent for sequencing

THURSDAY, 8/22/2019

- Orange colonies were mostly unsuccessful except for a few

MONDAY, 9/2/2019

- One colony was transformed into MG1655 to try obtaining clones. Plated on CK plates

TUESDAY, 9/3/2019

- Colony grew significant numbers of orange colonies. 3 picked and sent for sequencing

FRIDAY, 9/6/2019

- Sequencing result came back. Colonies were successful for Txe, but mutation occurred in Hok. Decided to retransform to check viability of Hok and Txe in K plates and C plates.

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MONDAY, 9/9/2019

- K plates had both plasmids (orange), indicating toxin effect of Txe is present. However, C plate had only red, indicating lack of retention of K plasmid (green) and thus poor effect of Hok.

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