FINAL SCRIPT: APPROVED FOR FILMING

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Title: Standardized Modular Assembly of Polycistronic Operons with Modular Cloning (MoClo) *In-Cloning*

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 14
Number of Shots: 33

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Jelmar de Vries:** I develop methods to allow modular DNA assembly from individual molecular parts all the way up to chromosome-sized constructs and reprogram cells with these artificial chromosomes.
   1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.4*

What technologies are currently used to advance research in your field?

1.2. **Jelmar de Vries**: One important innovation in the field is the development of Modular cloning toolkits, that allow modular assembly of multi-gene constructs from a library of standardized molecular parts.
   1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research gap are you addressing with your protocol?

1.3. **Timon Lindeboom:** Modular Cloning toolkits are typically designed to clone monocistronic transcription units. This protocol demonstrates a generalizable way for the assembly of polycistronic transcription units with the *In-Cloning* toolkit.
   1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.14*

What advantage does your protocol offer compared to other techniques?

1.4. **Timon Lindeboom:** This strategy of cloning a polycistronic transcription unit allows coding sequences to flexibly be used either in individual transcription units or in polycistronic arrangements.
   1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Modular Cloning of a Transcription Unit Using Golden Gate Assembly

Demonstrator: Jelmar de Vries

2.1. To begin, select the level 0 parts to be assembled into a transcription unit **[1]**. These must include at least an RBS, a coding sequence, and a terminator **[2]**.

2.1.1. WIDE: Talent reviewing sequences on a computer.

2.1.2. SCREEN: 68103Shot-2.1.2.mp4 00:00-00:13

2.2. Choose the position in which the transcription unit will be assembled in the operon **[1]**. Based on the chosen position, select the corresponding acceptor plasmid **[2]** and simulate the Golden Gate Assembly in Snapgene **[3]**.

2.2.1. SCREEN: 68103Shot2.2.1.mp4 00:00-0:09

2.2.2. SCREEN: 68103Shot2.2.2.mp4 00:00-00:08

2.2.3. SCREEN: 68103Shot2.2.3.mp4 00:00-00:07

2.3. In the golden gate reaction, use the enzyme SapI (*Sap-One*) to release the ribosome binding site, coding sequence, and terminator from their respective plasmids and cut open the acceptor plasmid **[1]**. Assemble the ribosome binding site, coding sequence, and terminator into the cut acceptor plasmid **[2]**.

2.3.1. SCREEN: 68103Shot2.2.3.mp4 00:07-00:32

2.3.2. SCREEN: 68103Shot2.2.4.mp4 00:00-00:06

2.4. Next, for the Golden Gate reaction, use a pipette to add 50 femtomoles of each desired insert, and 50 femtomoles of the acceptor plasmid **[1-TXT]**, 1 microliter each of 10x T4 DNA ligase buffer, T4 ligase, and SapI to a reaction tube **[2]**. Then add nuclease-free water to bring the final volume to 10 microliters **[3]**.

2.4.1. Talent pipetting 50 fmol of DNA inserts and plasmid. **TXT: GG: Golden Gate**

2.4.2. Shot of 1 µL each of enzymes, and buffer being pipetted into a PCR tube.

2.4.3. Talent adding nuclease-free water to adjust the reaction volume to 10 µL.

2.5. Place the tube in a thermocycler **[1].** Run 25 cycles of 37 degrees Celsius for 3 minutes, 16 degrees Celsius for 4 minutes, and 50 degrees Celsius for 20 minutes, then heat-

inactivate the enzymes at 80 degrees Celsius for 20 minutes **[2]**.

2.5.1. Talent placing the tube in a thermocycler.

2.5.2. Shot of Thermocycler interface showing the temperature program being input with cycling and final digestion steps.
Videographer: Please film the instrument screen for this shot

2.6. Optionally, pipette 0.5 microliters of SapI to the GG reaction **[1]** and incubate at 37 degrees Celsius for 1 hour **[2]**.

2.6.1. Talent pipetting 0.5 microliters of SapI into the reaction tube.

2.6.2. Talent placing the tube at 37 degrees Celsius.

2.7. Pipette 5 microliters of the reaction mix into 50 microliters of chemically competent *E. coli* cells **[1]**. Perform a heat shock transformation by plunging the tube into a water bath at 42 degrees Celsius **[2]**, then transfer the tube to ice **[3].** Incubate the cells in 1 milliliter of media for 1 hour to help recovery **[4]**.

2.7.1. Talent adding 5 µL of the reaction mix into a labeled tube with 50 µL competent *E. coli* in a microcentrifuge tube in ice.

2.7.2. Talent adding the labeled tube with competent cells and reaction mix into a waterbath at 42 °C.

2.7.3. Talent removing tube from heat and plunging into ice.

2.7.4. Talent adding 1 mL of LB media to the cells

2.8. Plate 100 microliters of the transformation outgrowth onto an LB agar plate containing 100 micrograms per milliliter ampicillin **[1]**. Incubate the plate overnight at 37 degrees Celsius **[2]**.

2.8.1. Talent spreading transformation mix onto a labeled LB ampicillin plate.

2.8.2. Talent placing the plate into an incubator set at 37 degrees Celsius.

2.9. The next day, pick two white colonies using a pipette tip **[1].** Inoculate each into 5 milliliters of LB medium containing 100 micrograms per milliliter of ampicillin **[2]**. Incubate overnight at 37 degrees Celsius in a shaking incubator at 250 revolutions per minute **[3]**.

2.9.1. Talent picking white colonies with a pipette tip.

2.9.2. Talent dipping the pipette tip into tubes with 5 mL selective LB medium.

2.9.3. Talent placing the culture tubes in a shaking incubator.

2.10. Perform a plasmid extraction according to the manufacturer's instructions **[1].**

2.10.1. Talent pours the mix into a column for extraction.

2.11. Digest 1 microgram of extracted plasmid DNA by adding 1 microliter of 10x digestion

buffer and 1 microliter of BbsI *(B-B-S-One)* **[1]**. Add nuclease-free water to make the total volume 10 microliters and incubate **[2-TXT].**

2.11.1. Talent adding buffer and enzyme to the DNA sample in a microtube.

2.11.2. Shot of nuclease-free water being added to the tube. **TXT: Incubation: 37 °C, 15 min**

2.12. Now, load the digested plasmid on a 1 percent agarose gel **[1]** and run electrophoresis at 100 volts for 35 minutes **[2]**.

2.12.1. Talent carefully pipetting DNA samples into gel wells.

2.12.2. Talent starting the electrophoresis system.

2.13. Then, image the gel and confirm the correct assembly of the plasmid **[1]**.

2.13.1. Talent considers gel on the screen of gel imager.

2.14. To assemble a Level M construct, choose the Level 1 transcription units to be assembled **[1]**. Simulate the Golden Gate assembly by releasing the first and second transcription units and the endlinker using the enzyme BbsI **[2]**. Simultaneously, cut the acceptor plasmid open **[3].** Then assemble all 3 parts into the acceptor plasmid to form the polycistronic transcription unit **[4].**

2.14.1. SCREEN: 68103Shot3.1.1.mp4 00:00-00:09

2.14.2. SCREEN: 68103Shot3.1.3.mp4 00:00-00:18

2.14.3. SCREEN: 68103Shot3.1.3.mp4 00:19-00:24

2.14.4. SCREEN: 68103Shot3.1.4.mp4 00:00-00:06

Results

3. Results

3.1. The negative control for Level 1 plasmid assembly, which lacked a terminator part, yielded no colonies **[1]**, whereas the complete reaction produced 292 white colonies and no red colonies **[2]**.

3.1.1. LAB MEDIA: Figure 5. *Video editor: Please highlight 5A*

3.1.2. LAB MEDIA: Figure 5. *Video editor: Please highlight 5B*

3.2. Assembly of the multi-gene construct without the second cassette at 37 degrees Celsius yielded only 2 white colonies **[1]**, while the complete assembly yielded 9 large colonies and 435 small colonies **[2]**.

3.2.1. LAB MEDIA: Figure 6 *Video editor: Please highlight 6A*

3.2.2. LAB MEDIA: Figure 6 *Video editor: Please highlight 6B*

3.3. At 30 degrees Celsius, the negative control for multi-gene assembly yielded 4 white colonies **[1]**, while the complete reaction yielded more than 1,500 colonies **[2]**.

3.3.1. LAB MEDIA: Figure 6 *Video editor: Please highlight 6C*

3.3.2. LAB MEDIA: Figure 6 *Video editor: Please highlight 6D*

3.4. BsaI digest of the acceptor plasmid pMA60 gives the expected single band at 5,500 base pairs **[1].** BsaI digestion of plasmids from 24 colonies revealed that 23 out of 24 showed the correct restriction pattern of two bands at approximately 4,300 base pairs and 1,700 base pairs **[2]**.

3.4.1. LAB MEDIA: Figure 7. *Video editor: Please highlight the pMA60 lane*

3.4.2. LAB MEDIA: Figure 7. *Video editor: Highlight the gel lanes C1-C12, C14 to C24*

3.5. Fluorescence imaging showed strong green and cyan fluorescence in colonies from plates containing the OC6 inducer **[1]**, whereas plates without an inducer showed minimal or no fluorescence **[2]**.

3.5.1. LAB MEDIA: Figure 8 *Video editor: Please highlight 8B and D*

3.5.2. LAB MEDIA: Figure 8 *Video editor: Please highlight A and C*

1. **transcription**
   Pronunciation link: https://www.merriam-webster.com/dictionary/transcription
   IPA: /trænˈskrɪpʃən/
   Phonetic Spelling: tran-SKRIP-shun
2. **terminator**
   Pronunciation link: https://www.merriam-webster.com/dictionary/terminator
   IPA: /tɜrˈmɪneɪtər/
   Phonetic Spelling: ter-mi-NAY-tur
3. **operon**
   Pronunciation link: https://www.merriam-webster.com/dictionary/operon
   IPA: /ˈɑpərɑn/
   Phonetic Spelling: OP-uh-ron
4. **acceptor (plasmid)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/acceptor
   IPA: /ækˈsɛptər/
   Phonetic Spelling: ak-SEP-tur
5. **Golden Gate Assembly**
   - **Golden**: /ˈgoʊldən/ (go-ld-uhn)
   - **Gate**: /geɪt/ (gayt)
   - **Assembly**: /əˈsɛmblɪ/ (uh-SEM-bluh)
     Pronunciation link (Golden Gate): https://www.howtopronounce.com/golden-gate
     (For "Assembly": https://www.merriam-webster.com/dictionary/assembly)
6. **SapI**
   Pronunciation link: (no standard dictionary, but see enzyme resources)
   IPA: /ˈsæp aɪ/
   Phonetic Spelling: SAP-eye
7. **femtomole**
   Pronunciation link: https://www.merriam-webster.com/dictionary/femtomole
   IPA: /ˈfɛmtoʊˌmoʊl/
   Phonetic Spelling: FEM-toh-mohl
8. **microliter (µL)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
   IPA: /ˈmaɪkroʊˌliːtər/
   Phonetic Spelling: MY-kroh-LEE-tur
9. **thermocycler**
   Pronunciation link: https://www.howtopronounce.com/thermocycler
   IPA: /ˌθɜrmoʊˈsaɪlər/
   Phonetic Spelling: THUR-moh-SYler
10. **ligase**
    Pronunciation link: https://www.merriam-webster.com/dictionary/ligase

IPA: /ˈlaɪˌgeɪs/
Phonetic Spelling: LY-gays

11. **nuclease**
    Pronunciation link: https://www.merriam-webster.com/dictionary/nuclease
    IPA: /ˈnjuːkliːeɪs/
    Phonetic Spelling: NOO-kle-ays
12. **competent** (as in "competent cells")
    Pronunciation link: https://www.merriam-webster.com/dictionary/competent
    IPA: /ˈkɒmpətənt/ or /ˈkɑmpətənt/
    Phonetic Spelling: KOM-puhtent
13. **electrophoresis**
    Pronunciation link: https://www.merriam-webster.com/dictionary/electrophoresis
    IPA: /ɪˌlɛktroʊfəˈriːsɪs/
    Phonetic Spelling: ih-LEK-troh-foh-REEsis
14. **polycistronic**
    Pronunciation link: https://www.howtopronounce.com/polycistronic
    IPA: /ˌpoʊliːsɪˈstrɒnɪk/
    Phonetic Spelling: POL-ee-sis-TROH-nik
15. **BbsI**
    Pronunciation link: (enzyme databases)
    IPA: /bi-bi-ess-eye/
    Phonetic Spelling: B-B-S-eye