**STANDARD OPERATING PROCEDURE**

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| **Principle Investigator:** | **Date Created: 10-05-2015** |
| **Lab Location:** St. John 151 | **Approved by:** |
| **Prepared By: Leah Tooman** | **Signature:** |

**SOP 11: Rehydrating and Diluting Primers**

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| **Section 1: Risk Assessment**  *Potentially hazardous processes that will be performed* |

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| **Section 6: Engineering Controls**  *Description of the engineering controls that will be used to prevent or reduce the likelihood of exposure to the hazards.* |

Please do this work IN THE HOOD to avoid contaminating your stocks. Also, ALWAYS use clean PCR water and FILTER tips.

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| **Section 7: Biological Spill Response and Lab Accident Response Procedures**  *Procedures for cleanup of spills and accidental releases* |

**Location of:**

**Eye Wash:** left down the hall out the lab door

**First Aid Kit:** Shelf directly left of the main exit from the lab

**Fire Extinguisher:** Mounted on the wall directly right of the main exit from the lab

**Spill Kit:** Shelf directly left of the main exit from the lab

**Emergency Shower:** hallway outside the lab to the left

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| **Section 8: Decontamination Procedures**  *Procedures that will be used to decontaminate lab equipment, glassware and clothing.* |

· Part of the procedure

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| **Section 10: Protocol** |

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| **Protocol Title:**  Rehydrating and diluting primers | **Page 1 of 1** |
| **SOP No. 11** | **Date:**  10-05-15 |
| **Object: same as the title.** | |
| **Required Supplies:**  Filter tips, pipettes  PCR water  Your primers | |
| **Procedure:**   1. Ordering primers: ( Skip to step 3 if you do not need to do this) and sign in   <https://www.idtdna.com/site/account>. Username tooman.l, password: leah Under custom Synthesis pick DNA Oligos and go from there, its very intuitive. Leave the defaults ticked so there is Standard Desalting, 25nmole Oligos and no normalization. Then click Add to Order and proceed through the checkout. The account was created under Anthony Amend so the bill will come to him and you just remember to bring it to Nicole for a signature and a billing account and give it to Cindy   1. Once they arrive you will be sent an email ( if you have attached your email to the IDT account) and you can go and pick up your tube of lyophilized primers from 315 Snyder hall**.** 2. **To make stocks:** I like to keep my stocks at 100um. If you have a particularly large volume you may want to make it 1000, or you may want to go straight to 50um as this is the concentration we use for fungal primers and it doesn’t usually yield a tonne so you may as well. Always write the concentration on the lid of the bottle. 3. On the side you will see written some number like, for example 24.4nm (or 24.4nmole) = 0.17mg or something like that. Multiply the number in front of the nm by 10 and add that amount of water to make 100um stock. Remember to change this amount of water if you want a final concentration other than 100um. 4. **To make Dilutions:** Then dilute this stock into aliquots at whatever concentration you are working at. For Hynson lab Fungal PCRs we use primers at 50um so I would dilute 1:2 and make all your stocks now and freeze them in single use volumes 5. For example if you are doing lots of PCRs in quick succession (as in a couple DAYS) you can make your aliquots larger, but the safest way to do it is to decide how much primer you would need for one plate (96 reactions) if that is what you are likely to be doingand aliquot out that much in to individual tubes 6. Label your aliquots with the name of the primer (Full name, not an abbreviation- if it cant all fit on the lid put some of it and make sure you put the full name on the side of the tube where you have more room to write), the date, and the concentration.   EG: ITS1F-Tul, 50um, 5.10.15   1. Freeze the aliquots in the aliquot box in the freezer and take one out as needed for your PCR. | |

**STAFF ACKNOWLEDGEMENT**

I have read and understand the SOP and agree to adhere to the requirements listed

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