This Google document is a template for your graded electronic notebook. Go to File > Make a Copy to get an editable copy of this template in your own Google Drive. You are not permitted to work offline. The Revision History will be used to time-stamp your entries. Timely entry in the notebook is a factor in your ELN grade. Each week, you will complete a pre-lab quiz on Canvas, read over the protocol in the ELN before lab, make sure you complete all observations in red by the end of the lab period, and complete the analysis questions in lab or shortly after.

Note: This ELN template was created using Labii's template for formatting and headers.

(https://www.labii.com/)

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Genomes Week 1: DNA Extraction

Note: This experiment was created using Labii's template.

Overview

The first step of the genomics research project is to extract DNA from a sample. We will follow the protocol from Thermo Fisher for isolating DNA from soil samples:

In addition to the protocols below, use our shared results spreadsheet to prepare your lab report.
https://docs.google.com/spreadsheets/d/16lGJ7_t3Yt_15s85gfxjxhy0CdHDvltCdIhbi8pOPA/edit?usp=sharing
Materials and equipment

[List all reagents, equipment, and instruments used]

Procedure 1: Lyse DNA

1. On weigh paper, measure out approximately 0.2±0.05 g of chewable tablets. [ENTER EXACT WEIGHT HERE]
2. Add 600 µL of S1—Lysis Buffer to the Bead Tube.
3. Add cells (powder or resuspended pellet) to Bead Tube, cap securely, then vortex.
4. Add 100 µL of S2—Lysis Enhancer, cap securely, and vortex briefly.
5. Incubate at 65°C for 10 minutes.
6. Homogenize by bead beating for 10 minutes at maximum speed on the vortex mixer. Use the DIY hands-free adapter.
7. Centrifuge at 14,000 × g for 5 minutes.
8. Transfer up to 400 µL of the supernatant to a clean microcentrifuge tube. IMPORTANT! A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.
9. Add 250 µL of S3—Cleanup Buffer, and vortex immediately. Vortex immediately to ensure even dispersion of S3—Cleanup Buffer and uniform precipitation of inhibitors.
10. Incubate on ice for 10 minutes.
11. Centrifuge at 14,000 × g for 1 minute.
12. Transfer up to 500 µL of the supernatant to a clean microcentrifuge tube, avoiding the pellet.
13. Add 900 µL of S4—Binding Buffer, and vortex briefly.
1. Load 700 µL of the sample mixture onto a spin column-tube assembly, and centrifuge at 14,000 × g for 1 minute.
2. Discard the flow-through and repeat step 2b with the remaining sample mixture. Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample remains in the column, centrifuge again at 14,000 × g for 1 minute.
3. Place the spin column in a clean collection tube, add 500 µL of S5—Wash Buffer, then centrifuge the spin column-tube assembly at 14,000 × g for 1 minute.
4. Discard the flow-through then centrifuge the spin column-tube assembly at 14,000 × g for 30 seconds. The second centrifugation optimizes removal of S5—Wash Buffer, which could interfere with downstream applications.
5. Place the spin column in a clean tube, add 100 μL of S6—Elution Buffer, then incubate at room temperature for 1 minute.
6. Centrifuge the spin column-tube assembly at 14,000 × g for 1 minute, then discard the column. The purified DNA is in the tube.
7. As a class, prepare new working solution for the Qubit and two standards for the broad sensitivity assay.

[RECORD AMOUNT OF DYE AND BUFFER USED TO MAKE WS]

8. Test 2 μL of your DNA sample on the Qubit by mixing with 198μL of WS in a Qubit tubes.
9. Move the rest of your DNA to a capped tube for next week. Label your sample and place in the -20°C freezer.

Results

[RECORD CALCULATED CONCENTRATION OF ORIGINAL SAMPLE FROM QUBIT]

[ADD TABLE WITH CAPTION SHOWING CONCENTRATIONS OF ALL TUBES IN YOUR LAB SECTIONS]

Conclusion / Discussion

Thermo-Fisher reports 0.5 to 15 micrograms of DNA isolated per 0.2 g of sample.

1. [Estimate the final volume in your DNA tube]
2. [Calculate amount of DNA isolated using the Qubit concentration]
3. [Compare yield to expected result]
4. [What was the QC step in today’s lab?]
Genomes Week 2: Covaris Fragmentation and Fragment Analyzer

Overview

In between our last lab and today, your samples were brought to North Shore Innovenure at the Cummings Center for mechanical shearing on the Covaris ultrasonicator. In today’s lab, we will compare electrophoregrams of the DNA before and after fragmentation to ensure that the DNA meets size standards for starting library prep. We expect that the concentration of DNA will remain the same before and after fragmentation, but that the size distribution of DNA will have shifted after mechanical shearing. A broader size distribution will ensure that our run has enough library diversity for successful cluster identification.

Materials and Equipment

[List all reagents, equipment, and instruments used]

Procedure

1. For the remainder of the genomes lab, each student has been assigned a sample ID corresponding to a 96 well-plate location.
[Make a table to show which samples each student loaded onto 96-well plate]
2. Open fragment analyzer and ensure fresh mixtures of Gel 1 and Conditioning Solution:
   Gel 1: dissolve 4 µL Intercalating Dye in 40 mL NGS Gel
   5X Conditioning Solution: Dilute with DI water to 1X
3. Load Buffer Tray: 1st row only of inlet buffer plate with 1mL 1X inlet buffer.
2. Load Marker Tray: Rinse buffer in rinse buffer tray. 100 \( \mu \text{L} \) in 1st row only.
3. Go to Utilities> Prime (do for any new solutions).
4. Go to Utilities > Check Solution Levels: Manually enter volumes in 50mL tubes
5. Load Sample Tray:
   a. Load every well with 22 \( \mu \text{L} \) SS diluent marker or 24 \( \mu \text{L} \) blank or 24 \( \mu \text{L} \) ladder
      i. To make ladder, mix 550\( \mu \text{L} \) of HS with 50 \( \mu \text{L} \) of NGS Ladder. Mix well. Pipette 24 \( \mu \text{L} \) of each into ladder wells.
   b. Add 2 \( \mu \text{L} \) of sample to every sample well. Pipette up and down to mix sample and diluent.

[Record plate layout in this notebook and in software]

Results

[Paste table of fragment sizes from shared results Google spreadsheet here]

Conclusion/Discussion

1. [Next week, the NEBNext Ultra II kit requires 500 pg - 1 \( \mu \text{g} \) of Fragmented DNA. How much larger is 1 \( \mu \text{g} \) than 500 pg.
2. [Use your tube’s Qubit concentration to estimate the amount of input DNA in 50 \( \mu \text{L} \) of undiluted sample.]

Genomics Week 3: End Repair and Adaptor Ligation

Overview
During the third and fourth week in the multi-week genomes lab, you will modify your fragmented DNA to prepare it for sequencing. This sequence of chemical modifications is known as library prep. NEB’s protocol will divided into three parts to fit within the time constraints of lab. At an internship or job, you would want to complete the protocol in one time period.

Part I: End Prep (completed by instructor immediately before lab period). You will find your tubes in the thermal cycler.

NEBNext Ultra II protocol is a pdf of Canvas

---

**Materials and Equipment**

[List all reagents, equipment, and instruments used]

---

**Procedure**

[MODIFY ACCORDING TO STEPS DONE IN YOUR LAB SECTION. FOR EXAMPLE, DID YOUR SECTION PERFORM SIZE SELECTION OR JUST CLEANUP BASED ON LAST WEEK’S CONCENTRATION MEASUREMENT]

1. Professor Kaufman performed End Prep reaction on all tubes immediately prior to lab. Each tube had 2 uL of 1XTE, 3 uL of End Prep Enzyme Mix, and 7uL of End Prep Reaction Buffer added to it. The tubes were then held for 30 minutes @ 20°C, for 30 minutes @ 65°C, and held until the beginning of class at 4°C.
2. Remove your group’s tube from the thermal cycler. Each PCR tube has 60 uL of End Prep Reaction mix. Start at Step 2.2 in NEB protocol and 30 µl of NEBNext Ligation master mix (red) and 1 uL NEB Ligation Enhancer(red) and 2.5 uL NEBNext Adaptor (red).

1. Before completing ligation steps, take AMPURE beads out of freezer to thaw.
2. Mix your tube contents by pipetting up and down with fresh tip. Spin tubes.
3. Incubate at room temp (20°C) for 20 minutes.
4. Add 3 μl of (red) USER™ Enzyme to the ligation mixture.

5. Incubate at 37°C for 15 minutes with lid set to >47°C.

6. Vortex beads to resuspend.

7. Pipette 87uL of beads into the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used.

8. Incubate at room temp for 5 minutes.

9. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. When the solution is clear, carefully remove and discard the supernatant.

10. Add 200 μl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

11. Repeat Step 12 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

12. Air dry the beads for 5 minutes while the tube/plate is on the magnetic stand with the lid open. Caution: Do not overdry the beads. This may result in lower recovery of DNA target.

13. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry. Elute by removing the tube/plate from the magnetic stand. Add 17 μl of 10 mM Tris-HCl or 0.1XTE.

14. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand. 3.2.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 μl to a new PCR tube. The DNA is no longer on the beads, but is in the solution you have moved to your new tube.
Results

[Record observation of beads at the end of air drying. Do they look glossy and brown?]

Conclusion/Discussion

[Discuss any pitfalls in the completion of this protocol. If you don't have DNA in the final trace, what are possible causes of DNA loss in the adapter ligation?]

[Calculate amount of DNA in your tube at each step]

Genomes Week 4: Indexing PCR and Clean-up

Overview

During this fourth week in the multi-week genomics lab, you will modify your library to add indices, also known as bar codes. After this step, all of the classes samples can be mixed in a single tube and identified on the MiSeq by their barcode. During the PCR run, we will enter sample information including barcodes onto the MiSeq in preparation for next week’s run.

NEBNext Ultra II protocol and NEBNext Indexing Kit (we’re using Set 2) posted on Canvas.

Good guide of actual base changes with NEBNext kit:

http://bioinformatics.cvr.ac.uk/blog/illumina-adapter-and-primer-sequences/
Materials and Equipment

[List all reagents, equipment, and instruments used]

Procedure

1. I used the Illumina MiSeq software to check that the following combination of indices would be allowed for a run. **ENTER Index names and sequences**

<table>
<thead>
<tr>
<th>Sample</th>
<th>NEBNext Index</th>
<th>Equivalent TruSeq LT</th>
<th>Expected Index Read</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

2. Each PCR tube has 15uL of Adaptor-ligated DNA. Start at Step 4.1.1A in NEB protocol and add:
   
   - 25 μl of Ultra Q5 master mix (blue)
   - 5 μL Universal Primer(blue)
   - 5 μL Index X Primer (blue) --- Check table to make sure you have the correct tube [EDIT PROTOCOL FOR YOUR LAB SECTION]

1. Before completing PCR reaction, take AMPURE beads out of freezer to thaw.
2. Mix your tube contents by pipetting up and down with fresh tip. Spin tubes.
3. Place in thermal cycler for 17 minute PCR run.

[Record thermal profile as an image or table]

1. Vortex AMPURE beads to resuspend.
2. Pipette 45uL of beads into the reaction tube. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used.
3. Incubate at room temp for 5 minutes.
4. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. When the solution is clear, carefully remove and discard the supernatant.
5. Add 200 μl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
6. Repeat Step 5 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.
7. Air dry the beads for 5 minutes while the tube/plate is on the magnetic stand with the lid open. Caution: Do not overdry the beads. This may result in lower recovery of DNA target.
8. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry. Elute by removing the tube/plate from the magnetic stand. Add 33 μl of 0.1XTE.
9. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.
10. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 30 μl to a new PCR tube. The DNA is no longer on the beads, but is in the solution you have moved to your new tube.

Results

[Draw a picture of how the beads look on the side of the tube before the final step of moving your DNA to a new tube]
Conclusion/Discussion

1. [Describe the library molecules in your tube. How have they chemically changed since we first isolated gDNA? You can answer this question as an ordered list.]

2. [Read https://www.neb.com/-/media/catalog/Datacards%20or%20Manuals/manualE7500.pdf and list the best index combinations for 2, 3 and 4 samples. What do you notice about the bases at each cycle of the indexing read?]

Genomes Week 5: Fragment Analyzer/Library Normalization

Overview
During this fifth week in the multi-week genomics lab, we will test our samples to see their concentration and size distribution. The best sample from both lab sections will be chosen for the run.

Materials and Equipment
[List all reagents, equipment, and instruments used]

Procedure

1. Before lab, fragment analyzer software was set up and fresh mixtures of Gel 1 and Conditioning Solution were made
   Gel 1: dissolve 4 µL Intercalating Dye in 40 mL NGS Gel
   5X Conditioning Solution: Dilute with DI water to 1x

2. Load Sample Tray:
a. Load every well with 22 μL HS diluent marker or 24 μL blank or 24 μL ladder
   i. To make ladder, mix 550 μL of HS with 50 μL of NGS Ladder. Mix well. Pipette 24 μL of each into ladder wells.
   
   b. Add 2 μL of sample to every sample well. Pipette up and down to mix sample and diluent.

3. Load Buffer Tray: 1st row only of inlet buffer plate with 1mL 1X inlet buffer.
4. Load Marker Tray: Rinse buffer in rinse buffer tray. 200 μL in 1st row only.
5. Go to Utilities> Prime (do for any new solutions).
6. Go to Utilities >Check Solution Levels : Manually enter volumes in 50mL tubes

[Record Plate Layout for your lab section]

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
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<td>Ladder</td>
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<tr>
<td>Student(s)</td>
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</tr>
</tbody>
</table>

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Results

Results will be shared on Canvas after class. Record the concentration and average size for the largest peaks in the electrophoregram from the sample that will be run.

1. [Add image of the electrophoregram from the sample tube that your section will sequence]
2. [Add table listing size and concentration of largest peaks for that sample]

---

Conclusion/Discussion

1. Draw the ideal electrophoregram for a genomic library.
2. [How much water would you add to 5μL of your section’s DNA to make a 4nM solution? (HINT: Use MiSeq calculator from Announcements) ]
Genomics Week 6: Library Pooling and Run

Overview
During the sixth week of the multi-week metagenomics lab, we will start the sequencing run. All of the samples will be pooled during the 10AM lab session and the run will be set up on the MiSeq. The samples will be denatured, diluted and loaded onto the MiSeq cartridge during the 2PM lab session.

Illumina MiSeq Dilute and Denature Libraries Guide

Guide to Making Sample Sheet

One sample will be loaded onto the run for BIO311L. L1 will run a cleaning cycle, load flow cell and buffer, and dilute samples. L2 will denature the library and PhiX and load.

Materials and Equipment
[List all reagents, equipment, and instruments used]

Procedure
L1 SECTION
1) Run post-run wash cycle.
2) Move HT1 buffer and PhiX moved to 2-8ºC to thaw for L2.
3) Check sample sheet for the run.
[RECORD genome used and index for every sample in the pool.]

<table>
<thead>
<tr>
<th>Sample</th>
<th>NEBNext Index</th>
<th>Equivalent TruSeq LT</th>
<th>Expected Index Read</th>
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<tr>
<th>Sample</th>
<th>NEBNext Index</th>
<th>Equivalent TruSeq LT</th>
<th>Expected Index Read</th>
</tr>
</thead>
</table>
4) Move sample 17 to 2-8C for 2PM lab section. The sample does not need to be diluted prior to denaturing and dilution at 2PM.
5) Label a clean microcentrifuge tube DAL (Diluted Amplicon Library) for L2 to denature and dilute the library.
6) Prepare fresh 0.2 N NaOH (note: 1 N = 1 molar for a molecule like NaOH with only one hydroxide) by mixing 10 uL 2N NaOH and 90 uL DI water. This will be used by L2 to denature the phiX control DNA and the pooled library.

---

**L2 SECTION**

1) Clean flow cell with alcohol wipe. Dry completely and check gaskets. Load flow cell.
2) Load buffer.
3) Obtain 10nM phiX, PAL(pooled amplicon library), TrisHCl/Tween, HT1 hybridization buffer, and 0.2 N NaOH from 2-8C refrigerator.
4) Dilute and denature phiX control.
   a) Dilute 10nM phiX to 4 nM by combining 2uL of 10nM PhiX with 3uL of TrisHCl/Tween
   b) Add 5 uL of freshly prepared 0.2 NaOH to centrifuge tube with 5uL of 4nM PhiX
   c) Vortex briefly. Centrifuge for 1 min at 280 x g
   d) Incubate at room temperature for 5 minutes
   e) Add 990 uL of HT1 buffer to tube with denatured PhiX. Invert 10X to mix.
5) Denature PAL and dilute to form DAL
   a) Add 5 uL of freshly prepared 0.2 N NaOH to centrifuge tube with 5uL of 4nM pooled library
   b) Vortex briefly. Centrifuge 1 min at 280 x g
   c) Incubate at room temperature for 5 minutes
   d) Add 990 uL of HT1 buffer to tube with denatured library. Invert 10X to mix.
6) Keep the denatured and diluted phiX and library tubes on ice.

**Note:** It is required to perform this denaturation step immediately before loading into the MiSeq reagent cartridge to ensure efficient template loading on the MiSeq flow cell.

7) In a fresh 1.5 mL tube, pipette 30uL of 20pM denatured PhiX and 570uL of denatured 20pM DAL. Invert 10X to mix. Keep to rerun if error.
8) In a fresh 1.5 mL tube, combine 360 uL of 20pM library with 240 uL of HT1 buffer to dilute to 12pM. Load 600 uL of 12pM sample into the Load Samples reservoir of the MiSeq reagent cartridge.
9) Load reagent cartridge with sample well full.
10) Sequence your library as indicated in the MiSeq System User Guide (part # 15027617). 2 x 76bp run.
Results/Conclusion/Discussion

Results of this run will be analyzed in the next lab session.

The fragment analyzer measured 1.39 ng/uL of DNA with an average size of 303 bp. qPCR measured 4.2 nM of DNA with adaptors. We expect that the fragment analyzer will overestimate the amount of adaptor-ligated DNA. Calculate the concentration from the fragment analyzer using the MiSeq calculator. Was the fragment analyzer an overestimate?

What was the final loading concentration for the libraries for this MiSeq run? Start with concentration and size from qPCR and show your work to show the concentration at every denature and dilution step.

Denature

Dilution 1

Dilution 2