Download virtualbox and winscp. Then download the ligbuilder folder from github.

Follow instructions for virtualbox below. In winscp, transfer to the virtualbox desktop the ligbuilder folder. And you should be able to run the tasks. The tasks will be outputted in the results folder. Compare the new files/files with more info with the old ligbuilder folder on your desktop/downloads (THAT IS NOT MODIFIED) to see what to upload using the diff command shown below.

**Step 1: Create a New Ubuntu Virtual Machine in VirtualBox**

1. **Open VirtualBox**.
2. Click on **"New"**.
3. Name your virtual machine (e.g., UbuntuVM), select **Type** as "Linux", and **Version** as "Ubuntu (64-bit)".
4. Click **"Next"**.
5. Allocate RAM to your virtual machine (at least 2048 MB recommended).
6. Click **"Next"**.
7. Select **“Create a virtual hard disk now”** and click **"Create"**.
8. Choose **VDI (VirtualBox Disk Image)** and click **"Next"**.
9. Choose **“Dynamically allocated”** or **“Fixed size”** (Dynamically allocated is usually fine).
10. Set the size of the virtual hard disk (at least 25 GB recommended) and click **"Create"**.

I used 7000 memory and 5 cpu to run 5 tasks.

**Step 2: Install Ubuntu on the Virtual Machine**

1. With the new VM highlighted, click on **“Settings”**.
2. Go to **“System”** and uncheck **“Floppy”** in the Boot Order section.
3. Go to **“Storage”**, click on the empty disk icon next to “Controller: IDE”, then choose **“Choose a disk file…”**.
4. Select the **ubuntu-22.04.3-desktop-amd64.iso** file and click **“Open”**.
5. Click **“OK”** to close the Settings window.
6. Start the VM and follow the on-screen instructions to install Ubuntu. During installation, create a user and set a password.
7. Change the network to bridged adapter.
8. hostname -I to get ip 10.66.177.80 to login winscp

**Step 3: Configure SSH in Ubuntu**

Since you don't have permission to install Guest Additions, we can use SSH and SFTP to transfer files. To do this, you need to install and configure an SSH server in Ubuntu:

1. Once Ubuntu is installed, open a terminal.
2. Run sudo apt update and sudo apt install openssh-server.
3. Run sudo systemctl start ssh to start the SSH service.
4. Run sudo systemctl enable ssh to enable the SSH service to start on boot.
5. Find your VM’s IP address with ip a or ifconfig.

**Step 4: Transfer Files using WinSCP**

1. **Download and Install WinSCP** on your Windows machine.
2. **Open WinSCP** and enter the following details:
   * **File Protocol**: SFTP
   * **Hostname**: Your VM's IP address
   * **Port Number**: 22
   * **User Name**: The username you created during the Ubuntu installation
   * **Password**: The password you set for your user during the Ubuntu installation
3. Click **“Login”**.
4. Once connected, you can drag and drop files between your Windows machine and your Ubuntu VM.
5. To prevent Ubuntu running in VirtualBox from locking the screen and requiring a password after being idle, you can disable the screen lock feature.

To check differences, get the unmodified folder, and the modified folder and run

1. diff -r /home/ubuntu/Desktop/testcompare/ /home/ubuntu/Desktop/create/LigBuilderV3 > /home/ubuntu/Desktop/coolstuff.txt

**Step 5: Perform Calculations in Ubuntu**

Use the terminal or any other tool you prefer to perform your calculations on the data you transferred.

Make two+ windows in ubuntu, the first is for the build server, the second is for the calculations for every virtual cpu, I put 5 tasks

wget https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86\_64.sh

chmod +x Miniconda3-latest-Linux-x86\_64.sh

./Miniconda3-latest-Linux-x86\_64.sh

source ~/miniconda3/bin/activate

conda init

conda create -n openbabel\_env python=3.8 openbabel -c conda-forge

sudo apt install openbabel

sudo ln -s /usr/bin/obabel /usr/bin/babel

chmod +x ./build

./build -Automatic build.input

chmod +x run\_goldpocket

**Step 6: Retrieve the Data**

Upload the results from your Windows desktop to your Google Drive as you normally would.

1. After completing the calculations, use WinSCP again to connect to your Ubuntu VM.
2. Navigate to the folder where the results are stored.
3. Drag the results from the Ubuntu VM back to your Windows desktop.
4. **output\_goldpocket**: This directory will contain the initial output files from the LigBuilder run.
5. **process\_goldpocket**: This directory will contain files that result from processing the initial output, such as filtered and scored ligands.
6. **cluster\_goldpocket**: This directory will contain the results of any clustering analysis performed on the processed ligands.
7. **report\_goldpocket**: This directory will contain the final reports, likely in HTML format, which summarize the results of the LigBuilder run.
8. **synthesize\_goldpocket**: This directory will contain files related to the synthesis analysis of the ligands.

**Step 7: Finding the Optimal Molecule**

1. **Target Analysis**:
   * Understand the structure and function of the repressor protein binding to DNA and inhibiting TERT expression.
   * Use crystallography data, if available, to study the DNA-binding pocket of the repressor protein.
2. **In Silico Modeling**:
   * Use LigBuilder or similar software to generate a library of potential molecules that could fit into the DNA-binding pocket of the repressor protein.
   * Optimize the interactions within the pocket, focusing on hydrogen bonds, hydrophobic interactions, and Van der Waals forces.
   * Then continue to the next steps and for an iterative process, we need to select the parts of the molecule that are successful, perhaps from the patent and we need to see where the parts of the molecule in the patent bind to. But assume at the first run that we would have generated a molecule that works.
3. **Virtual Screening**:
   * Employ software like AutoDock to perform docking simulations to predict how the generated molecules will bind to the target.
   * Analyze the binding poses to identify molecules with optimal interactions and binding affinity.
4. **Molecular Dynamics Simulations (MDS)**:
   * Utilize YASARA or similar molecular dynamics software to simulate how the molecules behave in a dynamic environment.
   * Confirm the stability of the ligand-receptor complex over time and assess the binding free energy.
5. **Structure-Activity Relationship (SAR) Studies(AND BELOW best to look at 1.docx for info)**:
   * Synthesize the most promising molecules from the virtual screening.
   * Test these molecules in vitro and in vivo for activity against the repressor protein and effect on TERT expression.
   * Use the data to identify crucial structural features contributing to efficacy and potency.
   * For simpler testing can we just buy a human cell petri dish that is senescent and divide those human cells into other petri dishes and treat each petri dish with the chemical and see if the amount of cell divisions it can make with the chemical can make it immortal? And for animal testing, we can test it on mice, for toxicity, then dogs, because they age by telomere shortening like we do, but they may not have the same protein like us, they may have similar.
6. **Lead Optimization**:
   * Based on SAR and MDS data, modify the molecules to enhance properties like specificity, stability, and cell permeability.
   * Consider the drug-likeness of the molecules using Lipinski's rule of five as a guide.
   * Test these new variants again using the same virtual and actual testing protocols.
7. **Toxicity and Off-target Effects and Effectiveness**:
   * Conduct preliminary toxicity screens to identify any cytotoxicity.
   * Use cheminformatics tools to predict off-target binding and potential side effects.
   * To directly assess the potential of your compound to extend cellular lifespan and reverse senescence, you could focus on a specific set of in vitro assays:
8. **Cell Proliferation Assay**:
   * Treat normal human cells with a finite replication capacity with your compound and monitor their ability to divide beyond the typical Hayflick limit of around 50 divisions.
   * Count cell divisions over time using an automated cell counter or by manual counting with microscopy.
9. **Iterative Refinement**:
   * Based on the feedback from the biological testing, refine the molecule further.
   * Focus on improving the pharmacokinetic and pharmacodynamic profiles.
10. **Preclinical Testing**:
    * Once a lead compound is identified, proceed with preclinical testing to evaluate its safety and efficacy in more complex biological systems.
    * Assess the compound's impact on cell division, telomere length, and cellular senescence.
11. **Clinical Considerations**:
    * If preclinical testing is successful, plan for clinical trials.
    * Develop a comprehensive understanding of the potential impact on human health, considering the ethics and long-term effects of telomere extension.