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A do-it-yourself low-cost agarose gel documentation and DNA quantification system

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ABSTRACT

This protocol describes how to build your own agarose gel documentation system to create digital images for quantification of DNA stained with dyes that fluoresce under blue LED light. Free software and inexpensive materials are used that can save tens of thousands of dollars when compared to similar commercial products.

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KEYWORDS

DNA quantification, Gel documentation, ImageJ, Blue light transilluminator, DIY molecular biology

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GUIDELINES

This protocol includes instructions for a self-made blue light box. Of course, commercially available blue light transilluminators may also be used, if available.

The materials listed for building the light box are meant to serve as examples only. We encourage the user to be creative in sourcing materials. Many of these items are available at local hardware or electronics stores. You may also upcycle or repurpose free materials (such as a cardboard box) if available.

MATERIALS

NAME	CATALOG #	VENDOR
Agarose low EEO (Agarose Standard)	A21140100	AppliChem
Lambda DNA	SD0011	Thermo Fisher
Tiffen 55 mM 21 filter (orange)		Amazon

NAME	CATALOG #	VENDOR
Entangle: tethered camera control and capture		
Blue light blocking orange safety glasses		Amazon
Image J image processing and analysis software		
3W High Power SMD LED COB Chip Lights royal blue 440-450 nm with PCB		ELE Doctor, via Ebay
3.9 Ohm 5 Watt 5% Wire Wound Cermet Sandblock Resistor		LF Components Inc
10K Ohm 5 Watt 5% Ceramic Resistor Resistor 5W		LAP-Electronic-GbR
12V 2 A power source		Ranpo lighting mall
Polycarbonate sheet	PC1218	Amazon
Aluminum strip	er MZZ016	Muzata
Electrical wire		Amazon
Thermal paste Hutixi HT-WT160		Amazon
Nikon D3400 Digital SLR camera		
SYBR™ Safe DNA Gel Stain	S33102	Thermo Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Tiffen 55 mM 21 filter (orange)		Amazon

MATERIALS TEXT

Prepare lambda DNA dilutions of 2, 4, 8, 16, and 32 µg/ml.

This protocol includes instructions for a self-made blue light box. Of course, commercially available blue light transilluminators may also be used, if available.

The materials listed for building the light box are meant to serve as examples only. We encourage the user to be creative in sourcing materials. Many of these items are available at local hardware or electronics stores. In addition to the items listed above, you may need some plastic cable ties, small screws, a drill, and soldering supplies (soldering iron of at least 30 Watts and soldering wire). If using aluminum strips to mount the LEDs, a small hacksaw is needed to cut them to the desired lengths. A recycled cardboard box can be used as the light box housing.

We use a Nikon D3400 DSLR camera for image acquisition. Most digital SLR cameras are capable of taking images in raw format and can be controlled by the Entangle software. A camera producing Raw format images is recommended for digital quantification of gel images. Check the information on the Entangle website to find a list of suitable cameras

<https://entangle-photo.org/>. If using an alternative camera, check the diameter of the lens and be sure to purchase the correct diameter of orange lens filter (Tiffen 21).

We recommend a laptop computer with a minimum of 4 GB RAM, running the free Linux Ubuntu operating system. We encourage reuse of old or otherwise unused computers. Outdated Windows computers can easily be wiped and have Ubuntu installed.

Price list for Gel Doc components as of 6/4/2020

Tiffen filter	\$46.26
Orange safety glasses, 1 pair	\$1.58
Nikon D3400 digital camera, renewed	\$372.00
Thermal grease paste, 30g	\$7.99
Electrical wire	\$10.99
Polycarbonate sheet	\$9.99
Aluminum lighting strip	\$23.99
3W High Power SMD LED COB Chip Lights royal blue 440-450 nm with PCB, 10 pieces	\$6.75
3.9 Ohm 5 Watt 5% Wire Wound Cermet Sandblock Resistor, 2 pieces	\$3.30
10K Ohm 5 Watt 5% Ceramic Resistor 5W, 2 pieces	\$3.30
12V 2A power source, 1 unit	\$4.17
Cardboard box, recycled	\$0
Laptop with minimum 4GB RAM,	~\$300

Total	\$790.33
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SAFETY WARNINGS

Always follow laboratory safety procedures. Wear appropriate PPE, including lab coats, gloves, and safety glasses. While blue LED light is less dangerous than UV, the high intensity blue light can still be harmful to eyes, and it is recommended to wear blue-blocking safety goggles (see materials list).

BEFORE STARTING

Download and install all software according to instructions.

Prepare lambda DNA dilutions of 2, 4, 8, 16, and 32 µg/ml.

Pour a 1% agarose gel with enough lanes to run your samples + 5 lanes for lamda standards.

Build an LED transilluminator

1 Build the LED transilluminator.



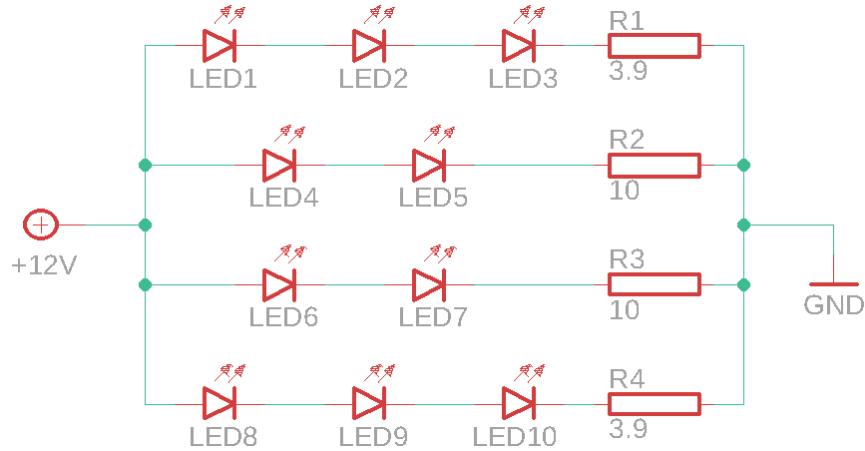
The instructions provided here give directions to mount LED lights on aluminum strips, which will dissipate any heat generated by the lights. Although this provides maximum safety for the unit, it is probably not necessary if the lights are not left running for extended periods of time. The simplest system would be to glue or tape the lights directly to the cardboard box. However, if this method is used, the unit should be monitored to ensure excessive heat does not build up to produce a fire hazard. We encourage users to be creative and resourceful in sourcing materials to create a simple, functional, low cost light box.

- 1.1 Cut a rectangular hole in a cardboard box to the desired size of your illuminated window. Make sure this hole is smaller than the clear plastic sheet that you will cover it with. In our prototype, we cut the hole to 15 x 30 cm.

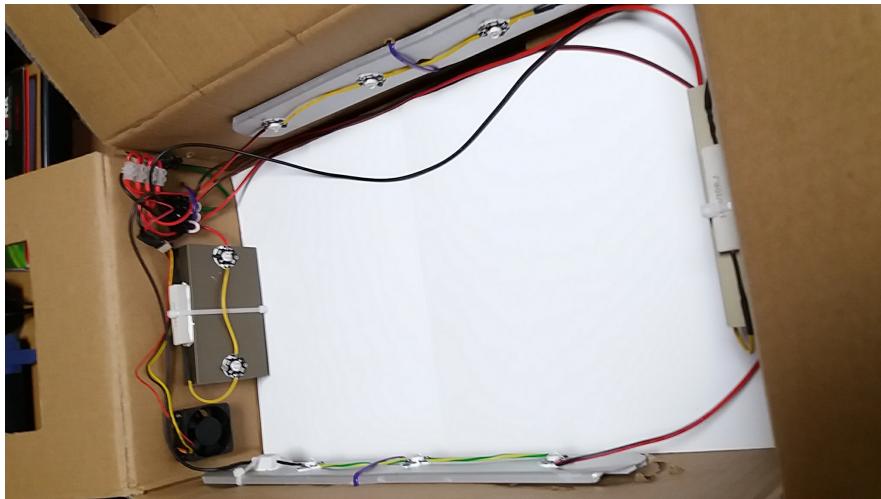


- 1.2 Cut aluminum strips to serve as a base for the LED lights. These serve to dissipate heat from the LEDs. Drill holes in the strips to attach the LEDs. Make two strips with 3 LEDs for each long side of the box, and two strips with 2 LEDs for each short side.

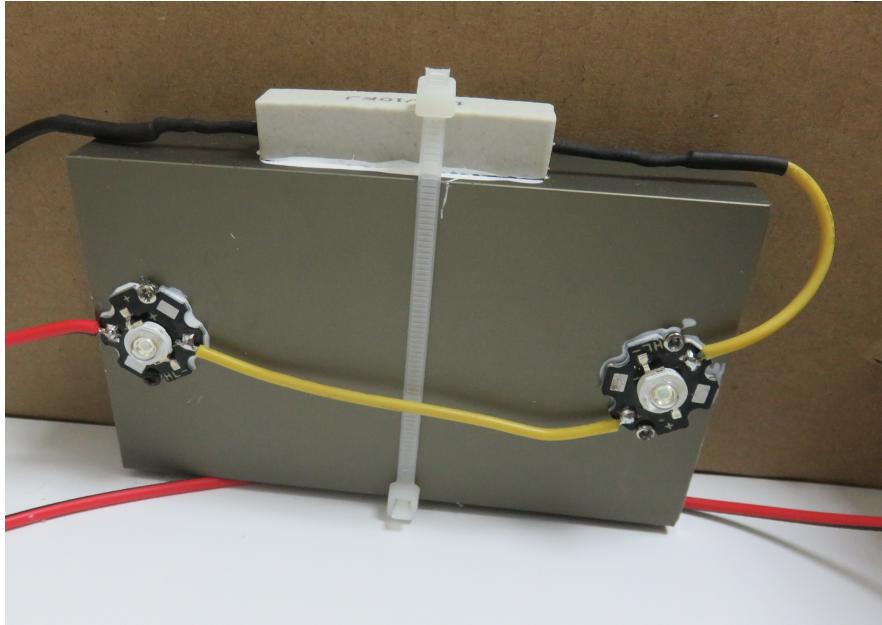
- 1.3 Connect the LEDs, resistors, and power source according to the circuit diagram shown below. Solder the wires to the LEDs and resistors as shown in the photos below, then apply thermal grease to the back of the LEDs and fix them into place on the aluminum strips with small screws. Finally, poke holes through the cardboard box and use plastic cable ties to secure the aluminum strips holding the LEDs.



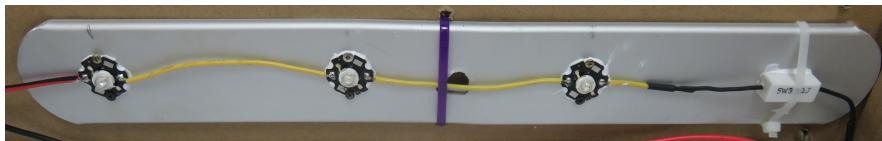
Circuit diagram for connecting the LED lights, resistors, and power source.



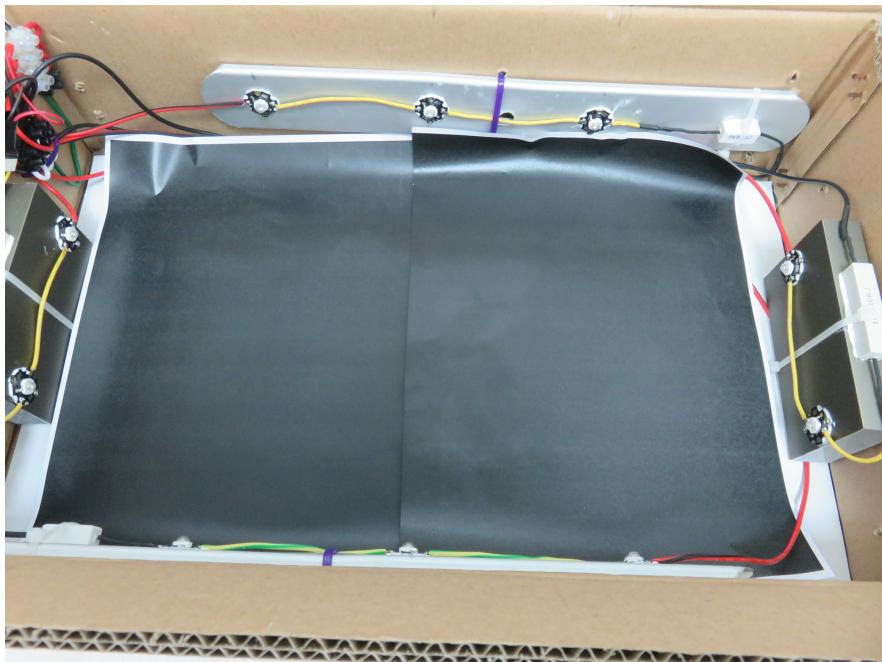
Our prototype transilluminator. LEDs are affixed to aluminum strips, which are attached to the box sides using plastic cable ties. In this prototype, a small fan is used for circulating air to reduce the temperature inside the box. This is optional as the box is generally not run for long periods of time. Note that this picture shows a white background inside the box to provide better contrast to show the wiring. Black paper is later added to reduce background reflection on gel images.



A close up of LED and resistor wiring. The resistor is the light gray block on top of the aluminum box.



A close up of three LED lights and a resistor attached to an aluminum strip. This is one long side of the light box.



Line the bottom of the box with black paper. This reduces reflection of the light and keeps the background noise of the digital image lower.

1.4 Close the box and place the clear plastic (polycarbonate or equivalent) sheet over the top of the box to

cover the hole.

Install software.

2 Prepare a computer for image aquisition and image analysis.



A computer and operating system are needed to control the digital camera, collect digital images and run ImageJ. ImageJ is platform independent. We favor the Linux operating system because it is free and commonly used in bioinformatics analysis pipelines (for example, see <https://dx.doi.org/10.17504/protocols.io.bdji6kn>). The Linux Ubuntu operating system will work on most desktops and laptops (<https://ubuntu.com/download/desktop>). This enables an up-to-date operating system to be installed on old hardware, thus equipment can be reused that is otherwise destined for a landfill. The prototype described in this protocol used an 8 year old laptop with 8Gb of RAM with Ubuntu 18.04 LTS installed. Software installation is described for this operating system.

2.1 Install software.

A. ImageJ downloads and instructions are found at <https://imagej.nih.gov/ij/download.html>. If you are using ImageJ for other applications, you may consider Fiji, which is ImageJ bundled with many useful plugins (<https://imagej.net/Fiji/Downloads>).



Fiji (Image J)

by NIH

B. Install software to control the digital camera. This protocol uses Entangle for Linux that is available from the GNOME/Ubuntu Software repository.



Entangle 2.0

source by Daniel P. Berrangé

C. Install spreadsheet software. If you have installed Ubuntu, the free LibreOffice Suite comes preinstalled. LibreOffice is available for most operating systems. If you are using a commercial software for text, presentations and spreadsheets, why not try a free alternative?



LibreOffice 6.4.2

Linux, macOS, Windows

by The Document Foundation

D. Install software to convert RAW images to TIFF format. Ufraw-batch is used in Linux. To install, open a terminal window (found in applications in Ubuntu) and type the command below. You will be prompted to enter your password.



ufraw-batch

sudo apt install ufraw-batch

Used to convert image formats. This includes the conversion of RAW (NEF) images to the standard TIFF format.
Ubuntu 18.04 LTS

DNA electrophoresis

1h

- 3 Load 5 µl of your genomic DNA sample on a 1% agarose gel. Also run 5 µl aliquots of lambda DNA standards of 2, 4, 8, 16, and 32 µg/ml (ng/µl). This will allow you to create a standard curve to compare your sample to. Run gel approximately 20 minutes at 100 V. The DNA should be migrated into the gel 0.5-1.0 centimeter. Longer running times can spread the DNA band more, and reduce accuracy of quantification.

- 3.1 Stain gel 30 minutes in 1X SYBR dye (Dilute concentrate 1 in 10,000 in gel running buffer, i.e. TAE or 0.5X TBE). Stain can be included in the gel matrix when pouring the gel, but it generally increases the background fluorescence. Staining solution is stable for at least two weeks and can be reused for several gels.

Photographing gel

5m

- 4 Photograph the gel using a suitable camera that can produce uncompressed raw format or TIFF (.tif) images. Directions are given below for using a self made system and free software. Ideally, the gel documentation system should be set up in a dark room, or other small room or closet that can be made relatively dark. Alternatively, the camera and transilluminator can be covered with a thick cloth that blocks out light.

- 4.1 Make sure the computer and camera are switched on. The orange filter



Tiffen 55 mM 21 filter (orange)

by Amazon

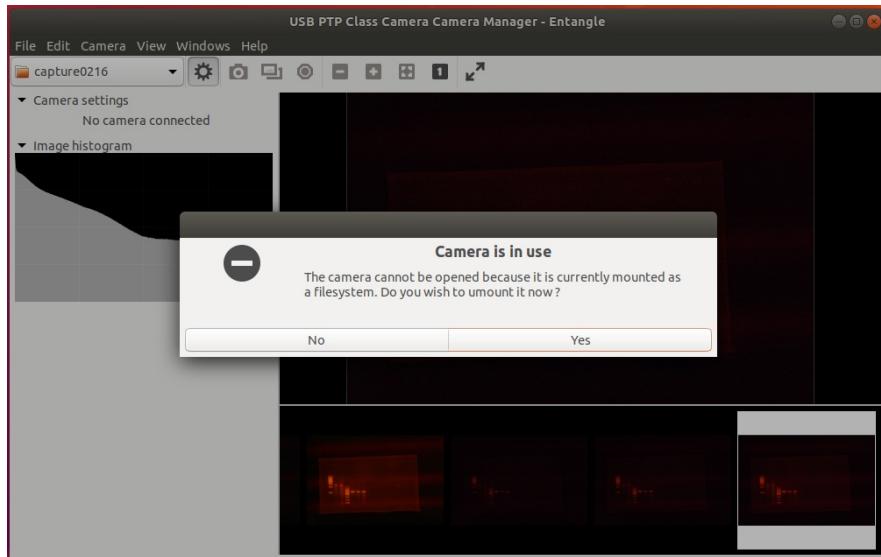
[View](#)

should be screwed to the camera lens. Make sure the camera is set to save raw images. Connect the camera to the computer with a mini-USB cable or whatever is appropriate for your camera. A camera icon should appear on the desktop to show that the computer recognizes the camera (when using Ubuntu). The camera can be held over the light box with a ring stand or tripod to allow hands free imaging.



"Do-it-yourself" gel documentation system.

- 4.2 Open the Entangle program. A window will pop up that says "Camera is in use". Click Yes to unmount the camera.



4.3 Activate continuous capture mode by clicking on the small button in the toolbar that looks like two rectangles stacked together. Zoom and focus the gel by manually adjusting the camera with the light box turned off. To view the gel: make sure you are wearing orange safety glasses, turn off any overhead lighting, then turn on the light box.

4.4 Under the camera settings, adjust the exposure time. Usually 1/16 to 1/8 sec exposure is sufficient. Capture image by clicking the camera button. Image will display in the window. Take additional exposures as necessary. Images often look darker after converting from raw format to .tif, so it is better to take a slightly brighter exposure than you think is necessary.

Convert images to TIFF format

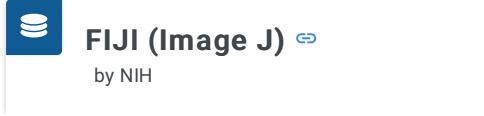
5 Convert image from RAW (e.g. NEF) format to TIFF, if necessary.

5.1 Move images to be converted into new folder. Open a Terminal window, cd into the folder containing the image files, and enter the following command line:

```
ufraw  
find . -type f -name "*.nef" -exec ufraw-batch --out-type=tif --out-depth=16  
'{}' \;
```

Measure band intensities

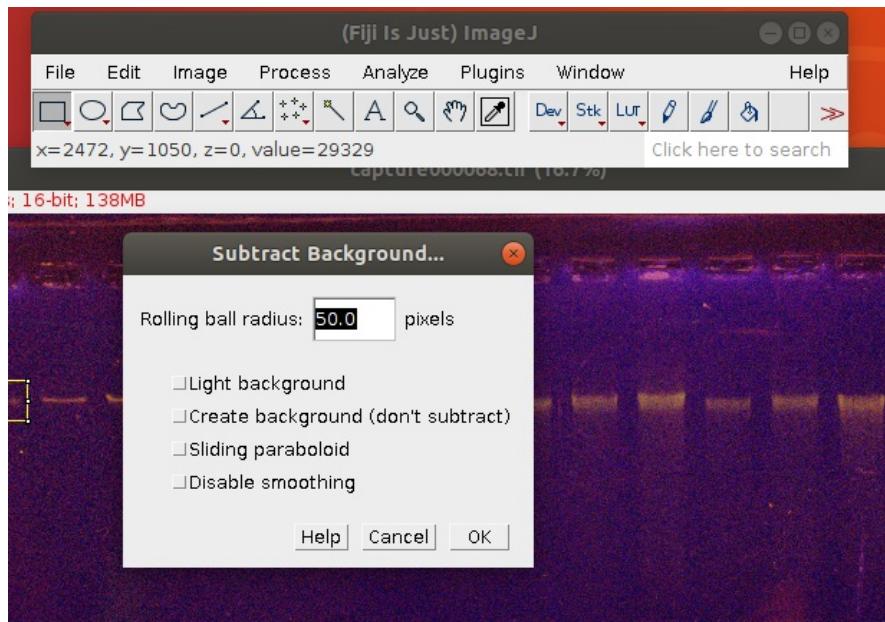
6 Measure band intensities.



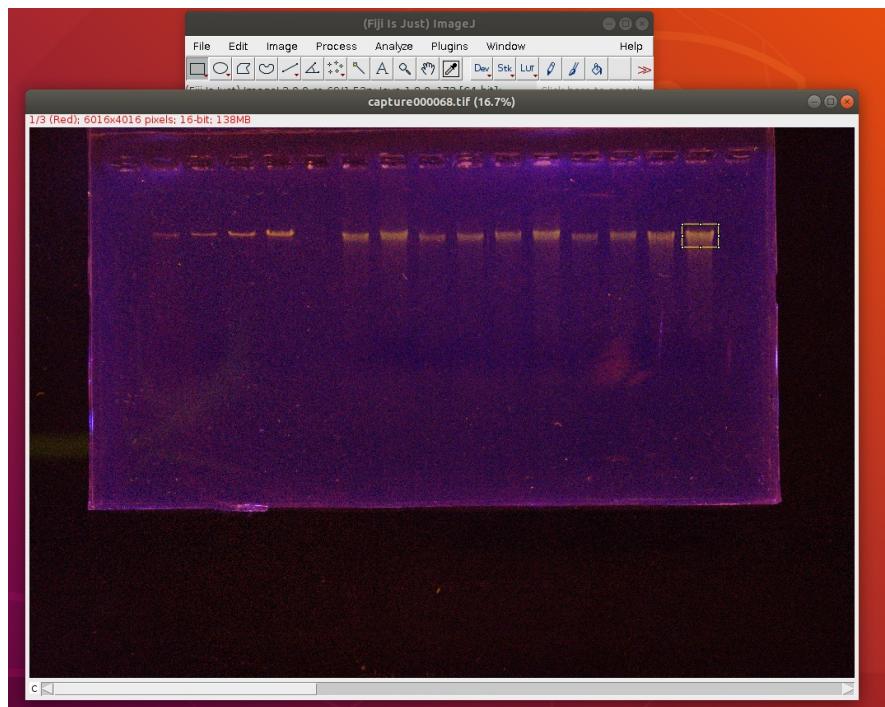
<https://imagej.nih.gov/ij/docs/menus/analyze.html#measure>

6.1 Make sure Image J or FIJI is installed and running. Open the image of your gel using the File > Open drop down, Control + O, or drag and drop the image onto the toolbar.

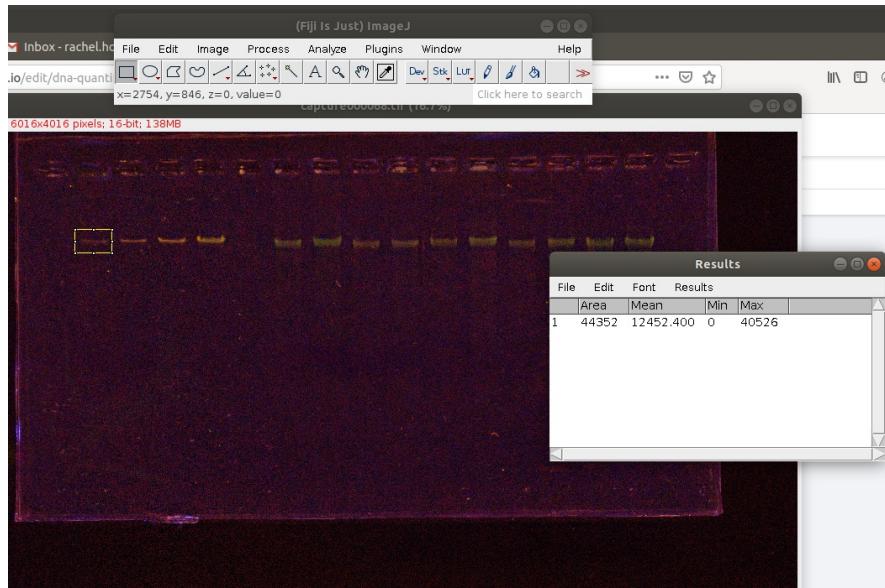
6.2 From the process menu, select subtract background. In the popup window, rolling ball radius should be set at 50 pixels. Click OK. You should see the background signal reduce from the image. Background subtraction can vary quite a bit depending on the image. See the Image J manual for more information <https://imagej.nih.gov/ij/docs/guide/146-29.html#sub:Subtract-Background...>. Additional information for use in quantifying DNA in agarose gels can be found at https://link.springer.com/chapter/10.1007/978-3-319-45021-6_14.



- 6.3 Using the rectangle tool (top left button), draw a small box around the brightest band in your gel. Then move the box to the first band in your gel (it is easiest to work left to right). You move the box by placing the cursor over the box, and it should turn to an arrow. Click and hold, then drag to the desired position. You can also use the arrow keys to move the box.



- 6.4 Measure the number of pixels in the band by selecting Measure from the Analyze menu, or **Ctrl + M**. A Results window should open displaying Area, Mean, Min, and Max values. Repeat the measurement process for each band on the gel. The size of the measurement box (Area) should be the same for all bands. If you accidentally resize the box while moving it, you need to start again.



- 6.5** After you have measured all the bands, save the values by copying and pasting into a spreadsheet, or select Save as from the File menu (Ctrl + S), and the values will be saved as comma separated values that can be imported into spreadsheet software.

Analyze values in Spreadsheet.

- 7** Create a standard curve in a spreadsheet software using the "mean" values (pixel densities) measured from the bands of the Lambda DNA standards in your gel. Chart these values against the known concentrations of the Lambda samples.

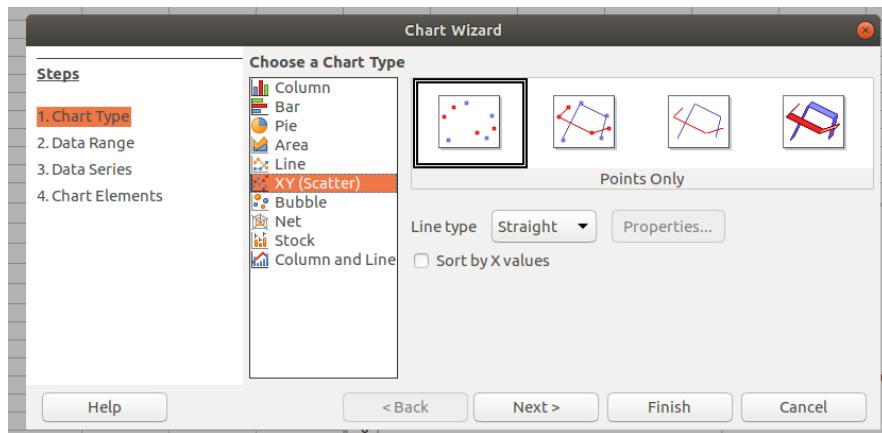


LibreOffice comes pre-installed with Ubuntu 18.04 and the examples shown in this section use LibreOffice Calc.

- 7.1** Highlight the data to be charted.

Density	Concentration (ng/μl when load 5 μl)
6657.738	16
5168.9	8
4364.791	4
3939.259	2

Select Insert > Chart. Use the chart wizard to create an XY Scatter plot.



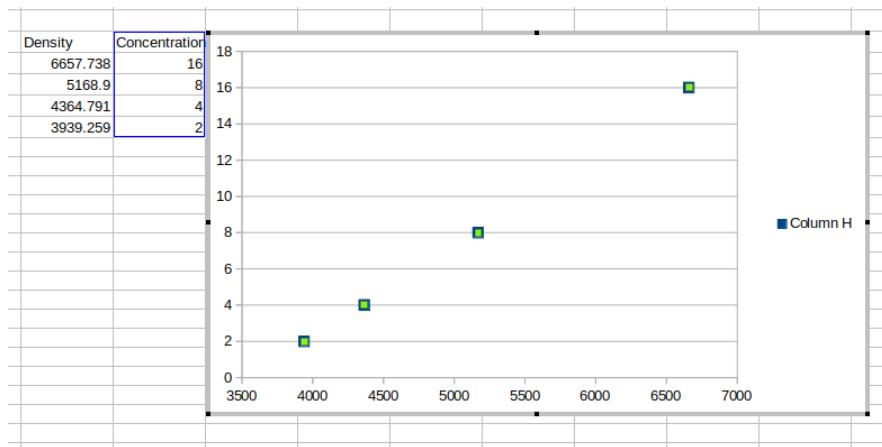
Select XY Scatter plot, points only.



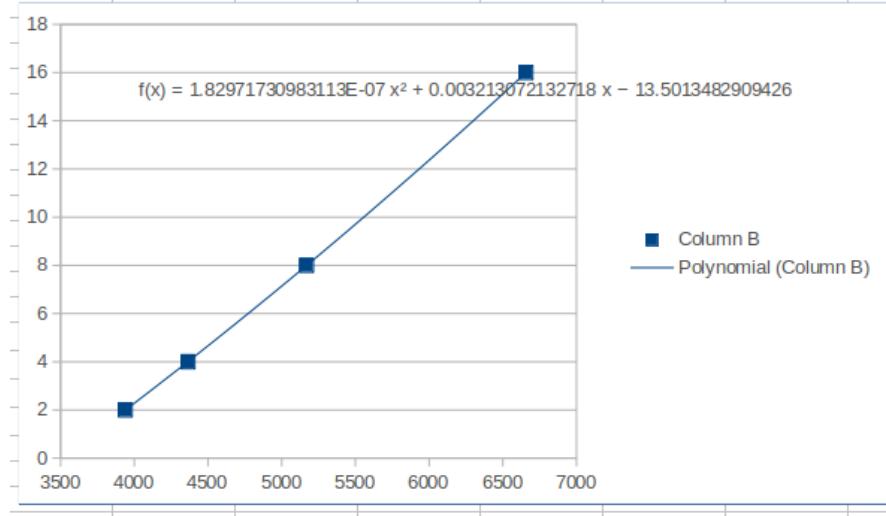
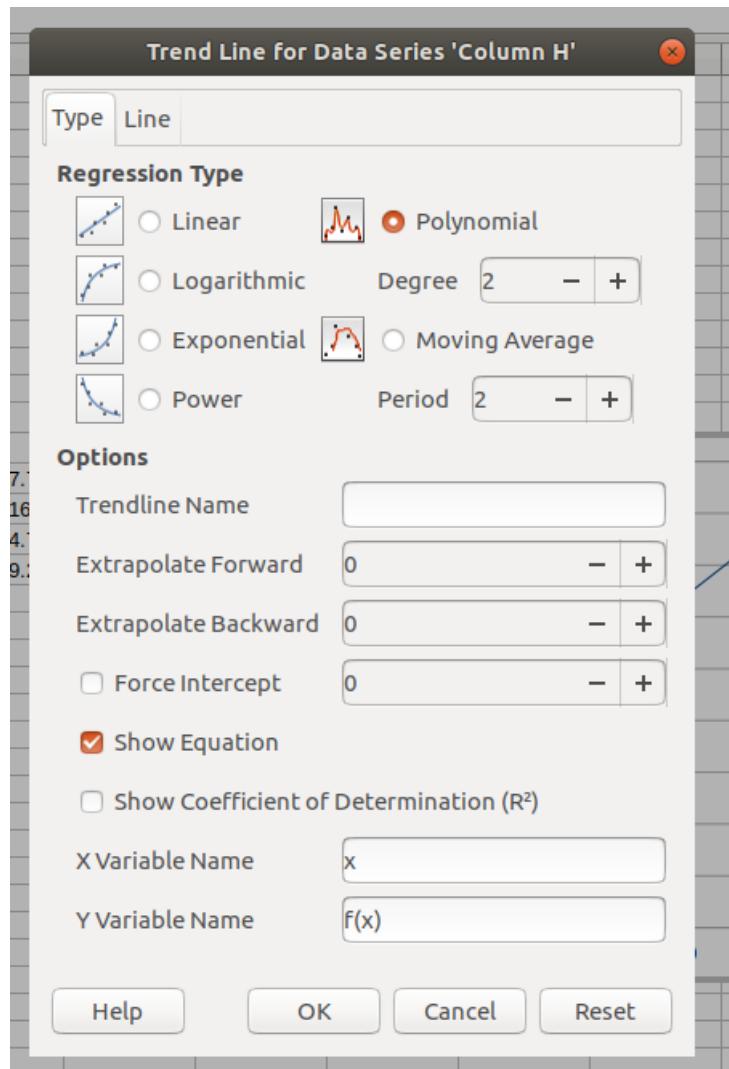
Choose data series in columns, then click Finish.

7.2 Fit a trend line to the charted data.

Click on the chart, then click on a point in the chart to select. The point should turn green to indicate it is selected.



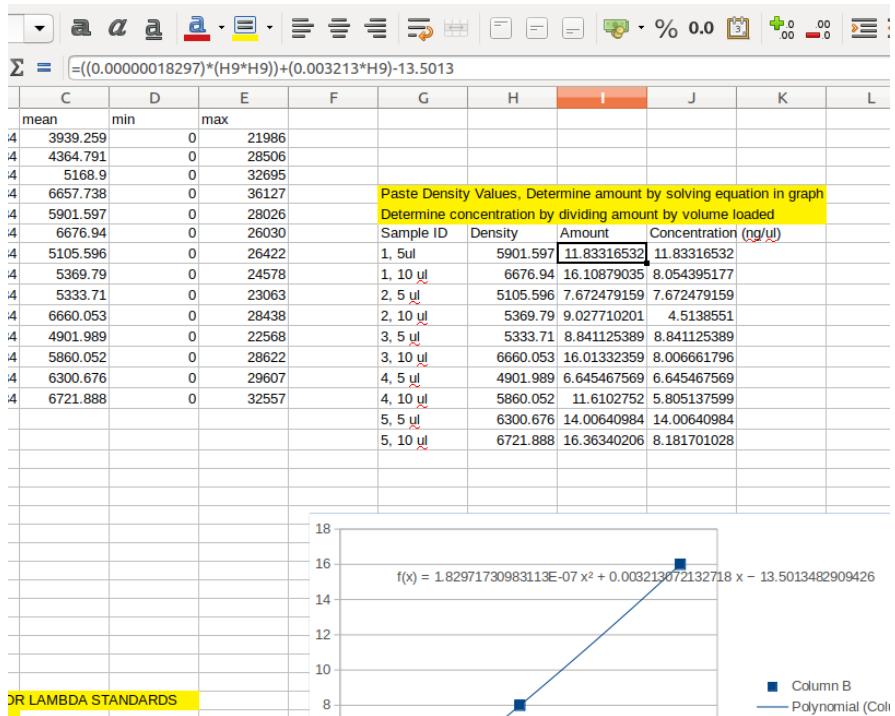
Select Insert > Trend Line from the menu. A window will appear. Select 2nd degree polynomial function. Click Show equation to display the equation of the line on the chart.



A line will appear through the points.

- 7.3 Use the polynomial equation to calculate the concentrations of your gDNA samples by plugging the measured densities (mean values) into the equation. Unfortunately you can't copy/paste the equation

from the chart, so you have to enter it manually into the formula bar. If the loading volume of your lambda samples and your gDNA samples are the same, you don't need to adjust the values to determine the concentration. If you loaded more or less, multiply or divide by the appropriate factor to calculate the concentrations of your samples.



Genomic DNA should be stored in TE to prevent degradation. It is also best to avoid multiple freeze thaw cycles. Therefore, DNA can be aliquoted so that a portion is stored at - 20° C, and a working stock is stored at 4° C. Please see our protocol for the low cost extraction of genomic DNA from plant tissues (<https://dx.doi.org/10.17504/protocols.io.bdg9i3z6>).