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DENG LAB HANDBOOK

VERSION 1

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Organisational Matters

To do list

Intern/Bachelors Student/Masters Student

What	When
<input type="checkbox"/> Collect your keys and lab-coat. You should have 2 forms – one for the keys and one for the lab-coats – from Judith	The first Tuesday or Thursday after you start, at 10 am – Building 21, room 901
<input type="checkbox"/> Check your university requirements (do you have to write a report? Do you have to present your results? Do you have to do a project proposal?) and communicate them with you supervisor	ASAP
<input type="checkbox"/> Reading. You may even want to put together a literature review, especially if you need to write a report as this can form the basis of your introduction	When you have time in the office
<input type="checkbox"/> Clear out your fridge and bench etc. Check with your supervisor if there are any stocks etc they would like to keep. Make sure anything left is well labelled.	When you leave
<input type="checkbox"/> Send all raw data, data analysis, scans of your labbook etc to you supervisor	When you leave
<input type="checkbox"/> Give your lab book to your supervisor	When you leave
<input type="checkbox"/> Autoclave your lab coat and put it into the laundry (by the locker)	When you leave – make sure you this early enough that you have time for the autoclave to finish so you can take it to the laundry.
<input type="checkbox"/> Return your keys	When you leave

PhD

What	When
<input type="checkbox"/> Collect your keys and lab-coat. You should have 2 forms – one for	The first Tuesday or Thursday after you start, at 10 am – Building 21, room 901

the keys and one for the lab-coats – from Judith		
<input type="checkbox"/> Register at the university (DocGS)		ASAP, within the first 6 months
<input type="checkbox"/> Organise your TAC committee and register it with HELENA		Within the first 6 months
<input type="checkbox"/> Organise your first TAC meeting, and prepare a report and slides for it		After approx. 9 months

Lab Meetings

Lab meetings are at 10 am every Wednesday in the seminar room 221 (see map below). You can find the schedule on the N: drive.

Progress report:

- Every Doctoral Researcher will ideally present their progress every ~2 month
- Senior PostDocs will present every ~6 month
- Junior PostDocs will present every ~3 month
- Students will present their results at the end of their internship and/or thesis
- Doctoral Researchers as well as students should discuss their progress report in advance with their direct supervisor and adjust the structure if necessary (e.g. present little progress on many projects when there is not much to present for one project alone)
- Structure:
 - Gantt chart
 - Brief update on projects you didn't lay focus on (max. 2-3 slides)
 - Present the project you worked the most on in detail, meaning
 - Introduction
 - Aim/Hypothesis
 - 1 slide where you remind everyone what your progress was at your last presentation
 - Results
 - Discussion & Outlook
 - 1 slide where you point out things you would like to discuss or need help with to get discussions started

Journal club:

- Who should do those? → Doctoral Researchers & Master students
- The group presenting will choose an interesting, quite new paper relevant to our lab and prepare a presentation for it
- The paper must be sent to the entire group a week in advance
- Structure:
 - Which paper, journal & impact factor
 - One slide introducing the lab which published the paper
 - Main part: Introduction – Aims/Hypothesis – Results – Discussion – Summary
 - One slide where you explain why you think this paper is interesting & what our lab could learn/use from that paper

Chair:

- Who is going to be a chair of the lab meeting? → Every Doctoral Researcher & PostDoc the week before their own progress report

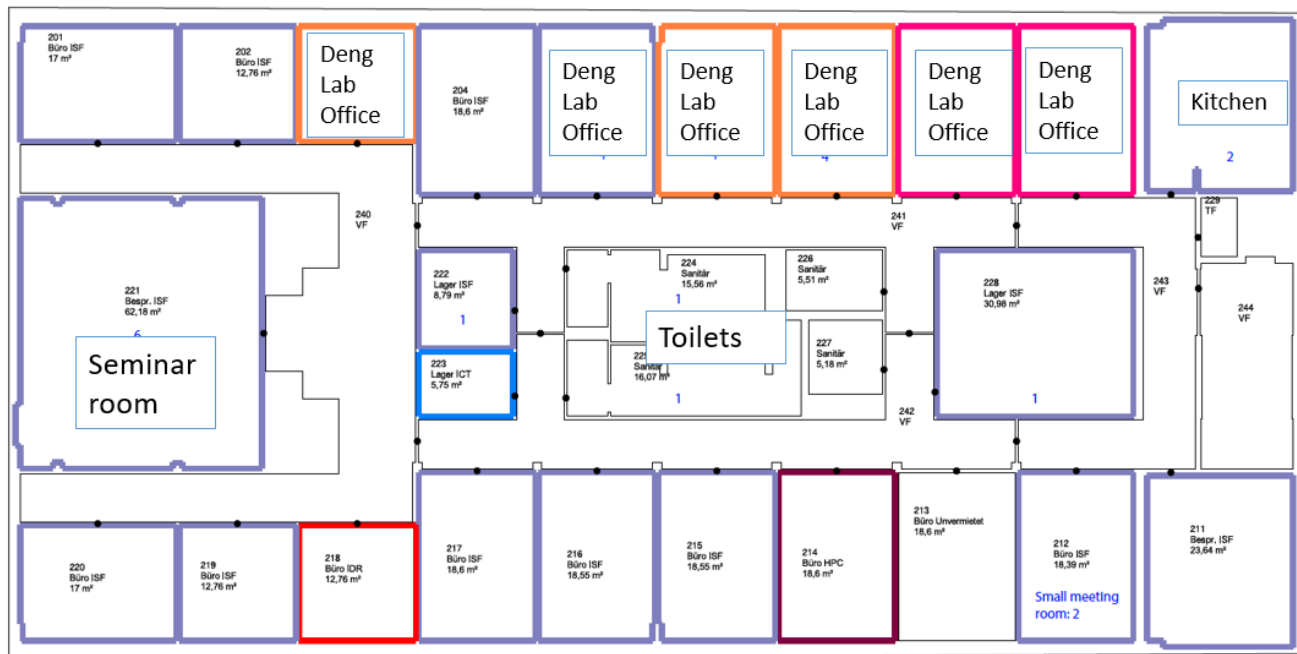
- What does the Chair do? → As it says, chair the lab meeting, meaning: Start discussion by asking questions if no one else has a question in the beginning, pick people to ask questions/comment
- Everybody is encouraged to ask questions and the chair must ask questions
- When students present their work, their direct supervisor is also chair as he/she knows the work of his/her student best

Abstract presentation:

- Who should do those? → Doctoral Researchers
- Present an abstract of a paper you think might be interesting for the lab
- Prepare 1-2 slides, where you show the abstract & briefly summarise it in max 5 min
- If you miss the lab meeting (due to sickness or courses), abstract presentation won't be rescheduled

General things:

- Master thesis students will take part in the Journal Club to get more experience in presenting
- **Everyone** is encouraged to asked questions, no matter how stupid you think they are – We are all here to learn and probably others were wondering the same things
- The structure of the meeting is always:
 - 1 Discuss general things important for the lab
 - 2 Progress report
 - If students also present their thesis/internship they will present first
 - 3 Journal club / abstract presentation
- If you can't present your progress report due to sickness or cancelled lab meetings, you will present in the next lab meeting where we don't have a journal club
- If you know well in advance that you will not be able to present at your supposed date due to already booked holiday or courses which are happening on that date you are responsible to find somebody to switch with
 - **However, this should be an exception and should be discussed with your direct supervisor in before!**
 - If you successfully swapped with someone, please tell the responsible person so that the lab meeting schedule can be updated
- The lab meeting schedule can be found on the N: drive

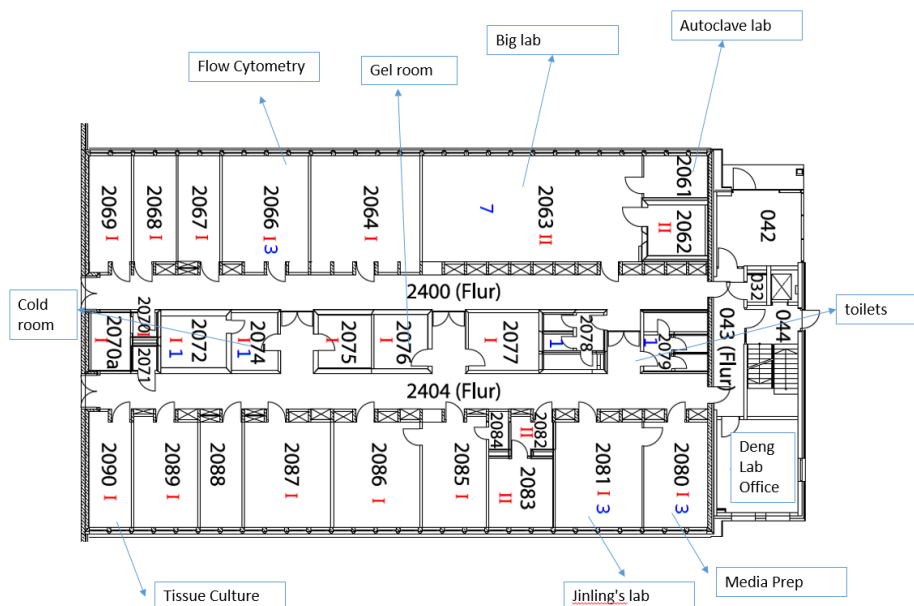


Cleanbench Booking

Since we are a big, busy lab group and we want to make sure that everyone can work smoothly, we have a booking system for the cleanbenches and some of the more commonly used instruments in place. Please ask your supervisor for access to the form.

The following rules apply.

1. You can book the hood at most one week in advance
2. Only book when you are planning to use it (do not book it whilst you have your lunch etc)
3. Mention on the booking form if you need the hood for less than the full hour slot
4. Do not double book more than one hood at the same time
5. Do not book longer than 4 hours (between 9am – 4pm)
 - if you have longer experiments communicate it and either start early or late
6. If you do not show up within the first 15 minutes of your booking, the hood can be used by someone else until the full hour
7. Stick to your bookings and do not overrun (communicate if it should happen and rearrange)
8. Delete booking immediately if not needed (ask others to do so for you when you are sick)
9. Also include your booking, even if space/lab is not commonly used
10. Stay in your assigned lab – see map of labs below



Lab Duty

Information about who is in charge of what tasks and how often those tasks must be performed can be found on the whiteboard outside the TAs office. There are some tasks that we all should be doing all the time:

- When the autoclave bins are half full, you should empty them and put the bag in the metal case just outside the autoclave room. The bins should be refilled with 2 clear autoclave bags, which can be found under the sink.
- When the plate bin is half full, you should empty it. This bin should be refilled with 3 clear autoclave bags.

Collecting parcels

Parcels should be collected from the end of the corridor – anything with the name of someone in our lab (usually Pfeiffer-Nigisch or Bernhoeft). You need to open the parcel and compare the contents with the delivery note – make sure that everything that is supposed to be inside is inside – and count each item to make sure, ticking it off on the delivery note. Put the item away (check storage instructions and make sure it is stored at the correct temperature etc) and then write on the delivery note where you have stored it. You should then place the note in the tray in the TAs office.

Some things will be delivered on dry ice. If this is the case, please place the dry ice in the storage container in the cold room.

This task should be done twice a day – parcels are delivered at 10am and 2pm.



Dry ice storage

Putting glassware away

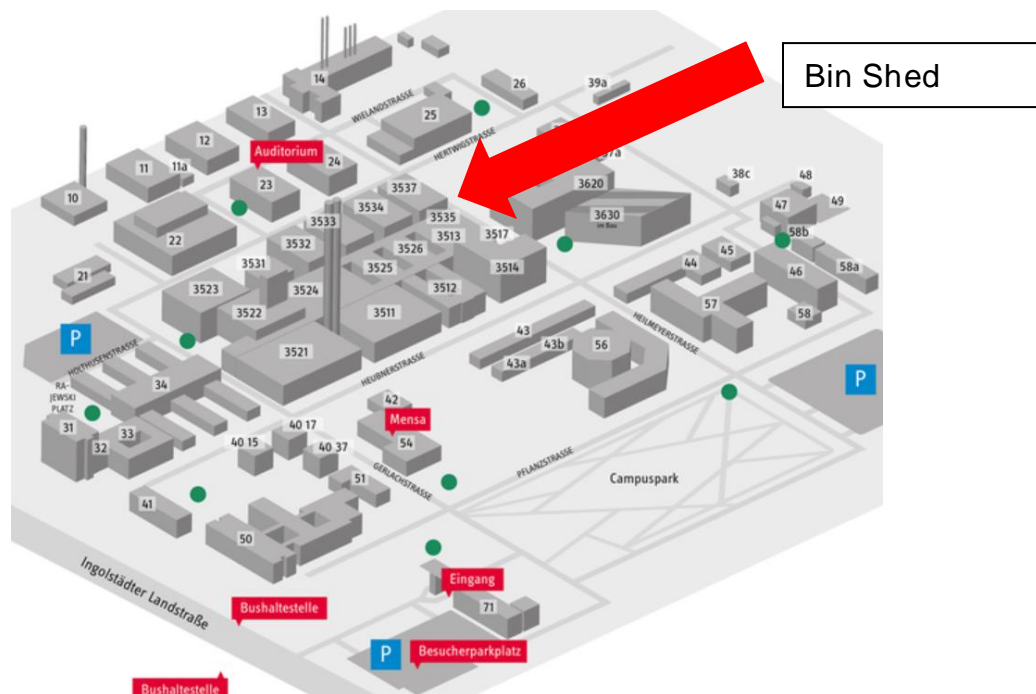
When clean glassware is brought into the lab, it will be placed on the bench nearest the autoclave room. You should put it away in the cupboards in the corridor. You should also check the oven in 2080 for any autoclaved glass tubes and return them to their

place on the shelf in the autoclave room. Make sure you return the tray you have used to carry them to its place in the autoclave room.

This task should be done daily.

Taking cardboard out

Boxes and empty cardboard will be placed in the corridor outside the lab. This should be taken out to the outside bins. If there is a lot of it, you can use a trolley (stored under the stairs) to help. This should be done every day.



Refilling Milli-Q & Ethanol

Mili-Q

There are two tanks of Milli-Q in 2080 which need to be refilled in the Virology Department. You should use the trolley to transport them. You should arrange with Jutta to have training on how to use the Mili-Q machine – it may help to bring someone who speaks German for this.

Ethanol

Refilling surface wipes etc

- Serological pipettes
- Filters
- Tips

Cleaning autoclave
Cleaning centrifuges
Clean the fume hood
Gel room
Water bath
Empty broken glass bins

Key Safety Rules











You must have a safety induction when you first start in the lab, and attend yearly ...

Here is a reminder of some of the key rules that you will use on a daily basis.

Labelling

All of your bottles/tubes etc should be clearly labelled with your name, the date, and what they contain (not just on the autoclave tape). If they contained hazardous material, they should also be labelled with the appropriate pictograms.



	Exploding bomb (for explosion or reactivity hazards)		Flame (for fire hazards)		Flame over circle (for oxidizing hazards)
	Gas cylinder (for gases under pressure)		Corrosion (for corrosive damage to metals, as well as skin, eyes)		Skull and Crossbones (can cause death or toxicity with short exposure to small amounts)
	Health hazard (may cause or suspected of causing serious health effects)		Exclamation mark (may cause less serious health effects or damage the ozone layer*)		Environment* (may cause damage to the aquatic environment)
 Biohazardous Infectious Materials (for organisms or toxins that can cause diseases in people or animals)					

* The GHS system also defines an Environmental hazards group. This group (and its classes) was not adopted in WHMIS 2015. However, you may see the environmental classes listed on labels and Safety Data Sheets (SDSs). Including information about environmental hazards is allowed by WHMIS 2015.

Waste

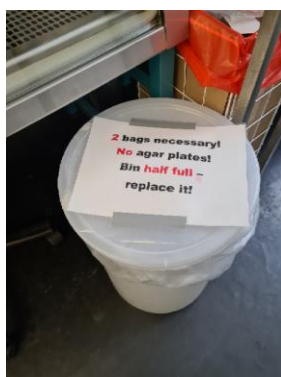
Autoclavable waste

Anything that has come into contact with bacteria should be autoclaved. There are different bins for liquid waste, solid (non agar) waste, and agar/agar plates.

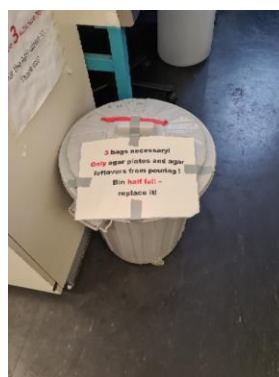
liquids



solids



plates



Non-autoclavable waste

Sterile waste can be put into the non autoclavable waste, which is emptied by the cleaners.



Special Waste

Chemical waste is disposed of separately. Please discuss with Sarah before you put anything into these bins.

liquids



solids



Glassware and sharps are also disposed of separately. Small glass such as plaque assay tubes can be put in the sharps bin even if it is contaminated, however larger broken glassware should be disinfected before placing in the bin.

Sharps, needles,
small broken
glassware



Big broken
glassware



Data Backup

Data and scans of your lab book should be backed up once a month.

- One the first day of the month, you should upload your raw data and the scan of your lab book on H: Drive or in a password protected folder on N: Drive.
- Each month, you should create a new version without overwriting previous versions
- When this is done, you should send confirmation to Judith:
judith.brehme@helmholtz-munich.de
- Supervisors should check the plausibility of the data every six months

Useful links

- [Cleanbench booking form](#)
- [Google calendar with lab meetings and seminars](#)
- [Notion Template with TUM and HELENA Requirements \(Doctoral Researchers\)](#)
- [The Helmholtz Intranet, HINT](#)
- [Freedcamp](#)
- [the Helmholtz Munich Grad School, HELENA](#)
- [Courses and training offered by HELENA and the Careers Centre](#)
- [the Campus Management System](#)

Training on Instruments

Before using any of the instruments, you MUST be trained on them. You only need to organise training for the instruments you need to use. After you have been trained, you will be given the SOP, and both you and the trainer will sign in a training book. Please contact the following responsible person for training:

Instrument	Responsible person	Date of training	Do you have the SOP?	Have you signed the training book?
Cleanbenches	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
Fume Hood	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
Autoclave	TAs		<input type="checkbox"/>	<input type="checkbox"/>
Benchtop centrifuges	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
High-speed Centrifuge	Sophie/Wanqi		<input type="checkbox"/>	<input type="checkbox"/>
Ultra-speed Centrifuge	Sophie/Wanqi/Ali		<input type="checkbox"/>	<input type="checkbox"/>
PCR machine	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
qPCR machine	Wanqi		<input type="checkbox"/>	<input type="checkbox"/>
Gel electrophoresis	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
Qubit	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
Nanodrop	TAs/your supervisor		<input type="checkbox"/>	<input type="checkbox"/>
Nanosight	Jinling		<input type="checkbox"/>	<input type="checkbox"/>
Plate Reader	Wanqi		<input type="checkbox"/>	<input type="checkbox"/>
Flow-Cytometer	Kawtar		<input type="checkbox"/>	<input type="checkbox"/>
Anaerobic chamber	Ali		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>

Common Protocols

Below you will find some protocols for commonly used experiments and procedures in the Deng Lab. For more protocols not included here, check the shared folder on the N: drive, the shared folder on HMGU box, or freedcamp.

Enrichment of Phages

In order to grow a new stock of a phage, the phage must be enriched. This can be done in liquid (easier, especially for higher volumes) or on a plate (more likely to reach a higher titre, especially for some phages).

Liquid Enrichment

Required:

- Overnight culture of the host bacteria

Procedure:

- Add 1 mL of overnight culture to 50 mL of media in an Erlenmeyer flask.
 - These volumes can be scaled up or down as required – but you should always use a flask much bigger than the volume of LB you are using.
- Place the flask on a shaker in an incubator at 37 °C (or the correct temperature for your bacteria) and incubate until the OD₆₀₀ reaches 0.3.
 - The specific OD is not super important here, but the bacteria should be in exponential phase
 - You can estimate the OD by eye – the culture should be slightly turbid, but not so much as an overnight culture
- Add 50-100 µL of phage sample to the flask.
 - If your phage sample is particularly low titre you may need to add more.
- Return the flask to the incubator and incubate overnight.
- Transfer the culture to a 50 mL falcon tube and pellet the bacteria by centrifuging at 6000 x g for at least 10-15 minutes.
 - Some centrifuges will go at a maximum speed of 4000 x g – in this case, centrifuge at 4000 x g for at least 15 minutes
- Use a syringe to pass the supernatant through a 0.2 µm filter membrane filter into a fresh falcon tube
 - If your phage is a particularly large one, then you may wish to use a 0.45 µm filter instead.

Plate Enrichment

Required:

- Plates with at least 100 plaques each

Procedure:

- Add media directly on top of the plate.
 - At least 1 mL, probably 3-5 mL. The more liquid you add, the higher the volume of your final phage stock, but the lower the concentration.
- Incubate at room temperature for approx. 1 hour.
- Collect the liquid from the top of the plate into a fresh falcon tube.
- Using a cell spreader, scrape the top layer of overlay agar from the plate into the same falcon tube.
- Vortex for 10 – 30 mins.
- Centrifuge and filter as described for liquid enrichment.

Isolation of Phages

Phages can be isolated from anywhere where bacteria grows (so, lots of possible sources). The basic principle of classical phage isolation is that you take a possible source, mix it with the target bacteria, and if there is a phage present that can infect that bacteria, then it will replicate to a detectable level.

Required:

- Clinical or environmental samples
- 2 x LB (or correct media for the target bacteria)

Preparation of samples

Solid samples, eg faeces

- Weigh your sample
- Add 10 mL PBS for each 1g of sample
 - Less liquid might be better, depending on the sample you could use as low as 1 mL per 1g of sample
 - The standard for faecal samples is 1:10 weight:volume, whereas for soil it is 1:1
- Vortex for 1 hour
 - This is to ensure that the sample is well suspended in the liquid
- Centrifuge for 1 minute at 700 x g and transfer the supernatant into a fresh falcon tube and briefly vortex
 - This is to ensure that all organic faecal matter is separated from bacteria and virus like particles (VLPs)
- Centrifuge for 45 minutes at 6000 x g
 - This is to pellet the bacteria, leaving the VLPs in the supernatant
- Using a syringe and a syringe filter, filter supernatant through a 0.45µm filter

Liquid samples, eg wastewater

- Add to 50 mL falcons and centrifuge at 6000 x g for at least 10-15 minutes to pellet the bacteria and biological material
 - Some centrifuges will go at a maximum speed of 4000 x g – in this case, centrifuge at 4000 x g for at least 15 minutes
- Using a syringe and a syringe filter, filter supernatant through a 0.45µm filter

It may be beneficial to concentrate a large volume of your sample at this stage. Please refer to the protocol for concentrating phages with a high speed centrifuge.

Screening

- Add 50 mL of prepared sample to 50 mL of 2 x LB in a 1 L Erlenmeyer flask.
- Add 15 mL of overnight culture

- These volumes can be scaled up or down as required – but you should always use a flask much bigger than the volume of LB you are using.
- Incubate shaking at 37 °C (or the correct temperature for your target bacteria) overnight
- Transfer the culture to 50 mL falcon tubes and centrifuge at 6000 x g for 10-15 minutes to pellet the bacteria
 - Some centrifuges will go at a maximum speed of 4000 x g – in this case, centrifuge at 4000 x g for at least 15 minutes
- Using a syringe and syringe filter, pass the supernatant through a 0.45 µm filter.
 - Some people prefer to use a 0.22 µm filter for this (and previous filtration steps). Whilst this will ensure the sample is completely free from bacteria, it may also exclude some very large phages.
- Use a plaque assay, spot plate or streak plate to test for the presence of phages.

Plaque Assay

A plaque assay is used to measure the concentration of phages. You can also do spot plates or streak plates, which will not tell you the concentration of the phage but will tell you whether or not a phage is present, which can be useful for experiments like isolating novel phages or host range assays.

Preparation:

- Turn the water bath on at a temperature of approx. 50°C
- Plug the heat block in inside the hood and set it to a temperature of 45 - 48°C
 - High temperatures will make the process easier, but some bacteria require the lower temperature to grow
- Microwave the soft agar until it is completely melted and put it into the water bath
 - Make sure the lid is loose in the microwave
 - The agar should sit in the water bath for long enough that the agar has cooled down a little – if it is used straight from the microwave it will be too hot and will kill the bacteria
- Label plates and epis
 - 6 plates per plaque assay – 3 dilutions in duplicate
- Dilute your phages
 - Do a 1:10 serial dilution.
 - We are going to plate out 3 different dilutions. You want to plate the dilution which are going to give you between 30 and 300 plaques on the plate. You can work this out from the expected approximate titre, or if you're not sure, the 10^6 , 10^7 , and 10^8 dilutions are a good place to start

Procedure:

- Add 3 mL of molten soft agar to a glass tube and place it in the heat block
 - 1 for each plate
- Add 100 μ L of phage and approx. 100-200 μ L of bacterial overnight culture to each tube and vortex
 - You can alter the amount of bacteria you use (it should be enough to make a lawn) but the amount of phage added must stay consistent
 - Vortexing at this step is very important!
- Pour the contents of the appropriate tube onto the labelled plate and swirl it around until the plate is fully covered.

- You have to move quickly at this stage so that the plate is fully covered before the agar sets
- Incubate overnight at appropriate temperature for the bacteria
- Count the plaques and calculate the phage titre using the following formula:

$$\text{PFU/mL} = \text{no. of plaques} \times \text{dilution factor} \times 10$$

- The extra $\times 10$ is to account for the 'extra dilution' you do when you add 100 μL into the soft agar

Spot Plates

Preparation:

- Turn the water bath on at a temperature of approx. 50°C
- Plug the heat block in inside the hood and set it to a temperature of 45 - 48°C
 - High temperatures will make the process easier, but some bacteria require the lower temperature to grow
- Microwave the soft agar until it is completely melted and put it into the water bath
 - Make sure the lid is loose in the microwave
 - The agar should sit in the water bath for long enough that the agar has cooled down a little – if it is used straight from the microwave it will be too hot and will kill the bacteria
- Label plates and epis
 - 1 plate per phage
 - Your plate should be labelled for each of your dilutions (eg 0-7). Here is an example of how you could set up your plate:



- Dilute your phages
 - Do a 1:10 serial dilution.
 - We want to dilute until we see single plaques – generally a dilution down to 10^{-8} is sufficient and all fits on a single plate

Procedure:

- Add 3 mL of molten soft agar to a glass tube and place it in the heat block
 - 1 for each plate
- Add approx. 100-200 μ L of bacterial overnight culture to each tube and vortex
 - You can alter the amount of bacteria you use but it should be enough to make a lawn
 - Vortexing at this step is very important!
- Pour the contents of the appropriate tube onto the labelled plate and swirl it around until the plate is fully covered.
 - You have to move quickly at this stage so that the plate is fully covered before the agar sets
- Once the agar is set, spot 10 μ L of each of your phage dilutions directly on top of the agar
 - Make sure where you are spotting each dilution matches with the way you have labelled your plate
- Incubate overnight at appropriate temperature for the bacteria
- If there are phages present, single plaques should be visible at at least one of the dilutions.
 - Sometimes, a phage which is not specific for a host can still kill the host at a high enough concentration. If you cannot see individual plaques, you cannot conclude definitively that the phage infects the bacteria.

Phage streak plates

Preparation:

- Turn the water bath on at a temperature of approx. 50°C
- Plug the heat block in inside the hood and set it to a temperature of 45 - 48°C

- High temperatures will make the process easier, but some bacteria require the lower temperature to grow
- Microwave the soft agar until it is completely melted and put it into the water bath
 - Make sure the lid is loose in the microwave
 - The agar should sit in the water bath for long enough that the agar has cooled down a little – if it is used straight from the microwave it will be too hot and will kill the bacteria
- Label plates and epis
 - 1 plate per phage

Procedure:

- Add 3 mL of molten soft agar to a glass tube and place it in the heat block
 - 1 for each plate
- Add approx. 100-200 μ L of bacterial overnight culture to each tube and vortex
 - You can alter the amount of bacteria you use but it should be enough to make a lawn
 - Vortexing at this step is very important!
- Pour the contents of the appropriate tube onto the labelled plate and swirl it around until the plate is fully covered.
 - You have to move quickly at this stage so that the plate is fully covered before the agar sets
- Once the agar is set, spot 50 μ L of each of your phage dilutions directly on top of the agar
- Using an inoculation loop, spread the phage lysate around the plate exactly as if you were streaking out a plate of bacteria
- Incubate overnight at appropriate temperature for the bacteria

→ If there are phages present, single plaques should be visible on the plate, eg:



Plugging Plaques and Plaque Purification

Plaque purification is used when isolating a novel phage or when phage contamination is suspected in order to ensure a pure stock of a single phage.

- Add 500-800µl LB to an Eppendorf tube
- Place the tip of a P1000 pipette tip into the centre of a plaque and aspirate – a small disc of agar should stay in the pipette tip when you take it away from the plate
 - The chosen plaque should be as isolated as possible from other plaques on the plate
- Eject the agar into the Eppendorf tube and vortex
- Incubate the tube for 1h at room temperature or at 4°C; this step will release phages into LB
- Filter the phage supernatant through 0.45µm or 0.22 µm syringe filter into a new tube
- Perform a plaque assay or spot plate using this filtrate and the target host
- Repeat this process until there is only one plaque morphology present and you are confident that there is only one phage present
 - Note that one phage can produce similar plaques with different sizes
- Store the phage stock at 4°C and with 25% glycerol at -80°C

Concentrating of phage samples

Sometimes a high concentration of phages are required and it can be useful to concentrate a large volume into a more highly concentrated smaller volume. There are two main ways to do this: by centrifugation or by ultrafiltration.

High-speed

These instructions are for the Beckman Avanti JE high-speed centrifuge, others may vary. You **MUST** be trained on the high-speed centrifuge before you are allowed to use it.

- Add 30-35 mL of sample to each high speed tube and balance
 - High-speed centrifuges should be balanced by weight and not just by volume
 - Add or remove 100 μ L to change the weight by 0.1g
 - You should not run the high-speed with tubes that contain less than 30 mL
 - If the high-speed you are using is in a different lab, take note of the safety rules – if the centrifuge is in a BSL1 lab, phages infecting BSL2 bacteria **MUST** be filtered through a 0.22 μ m filter beforehand.
- Turn on the centrifuge using the button on the right hand side.
- If the rotor is not already in the centrifuge, put it in.
- Add the tubes into the rotor. Check the lids are tightened.
- Put the lid of the rotor on and screw it tight. If the lid and rotor is installed correctly, you should not be able to lift the rotor now.
- Press rotor, enter the rotor number (25.5) and press enter.
- Enter the speed, time and temperature the same way.
 - Rotor: 25.5
 - Speed: 35,000 x g
 - Temperature: 4C
 - Time: 2 h 30 m
- Press enter
- Press start, and wait for the centrifuge to get up to speed before leaving.

After the spin:

- Remove the tubes, marking where the pellet should be with a pen before pouring away the supernatant.
- Return to the lab before re-suspending pellet in 3 mL SM buffer and transferring to a 15 mL falcon for storage at 4 °C.

Amicon

- Add phage sample into the amicon column
- Centrifuge the column until one tenth of the initial volume remains

- Different samples go through at different speeds, so start 3000 x g for 3 minutes and if the sample is not passing through, slowly increase the speed and time.
- Wash the filter membrane by adding PBS up to the original volume and resuspending the phages, before centrifuging as above until one tenth of the original volume remains
- Resuspend the sample in the desired buffer and volume, and transfer into a 15 mL falcon for storage at 4°C.

Caesium Chloride Gradient Purification

CsCl gradients can be used to remove impurities from phage preparations.

Making up CsCl Concentrations

- Make up 4 different CsCl concentrations – add the CsCl to a falcon and then fill the falcon up to 50 mL using 1 x TE buffer.
 - P1.2 : 11.19g
 - P1.4 : 26.94g
 - P1.5 : 33.74g
 - P1.65 : 43.78g
- When the CsCl has dissolved, double check by placing the tube on the scales and zeroing it. Then, remove 1 mL – P1.2 should then weigh -1.2g, P1.4 should weigh -1.4g etc etc
- Filter sterilize into fresh falcons using 0.22 µm filters and store at room temperature.

Making the gradient

- Starting with P1.65, add the different concentrations to the ultracentrifuge tube. Pipette very slowly down the side of the tube to prevent the concentrations from mixing. Use the following amounts:
 - P1.2 : 1 mL
 - P1.4 : 3 mL
 - P1.5 : 3 mL
 - P1.65 : 2 mL
- Once each density has been added, mark with a line on the outside of the tube where each density comes up to.
- Add the 3 mL of phage sample at the top of the tube.
- Balance the tubes – weigh them, then add additional SM buffer to each one which is lighter than the heaviest, until they all weigh the same.
- Add the tubes to the rotor, sliding them into the sleeves using tweezers and hooking them onto the body of the rotor, making sure the numbers line up.

Using the ultracentrifuge

NOTE: these instructions are for the ultracentrifuge in the INET building, others may vary. You MUST be trained on the ultracentrifuge before you can use it.

- Turn on using button on right hand side

- Put rotor in, carefully checking to make sure it lines up. Twist it and listen for the click as it slots into place.
- The control screen is a touch screen – touch each parameter to change it.
- Within the speed setting, click select rotor and select the correct rotor and tubes. The rotor should be SW40 Ti. Check the package for the serial number of the tubes.
- Close the lid and press start – the vacuum will take a moment to get up to pressure before it starts spinning
- Wait for it to get up to speed before leaving.
- For phage purification, the parameters are as follows:
 - Speed: 24,000 rpm
 - Time: 4 hours
 - Temperature: 4°C
 - Rotor: SW40 Ti

After the spin

- Carefully remove the tubes from the rotor using the tweezers
- Examine the contents of the tube – you are looking for a thin greyish blue line – this is your phage. Mark it on the outside of the tube with a pen, you want to have half a centimeter either side of it.
- Carefully remove all the liquid above the top line and add it to the waste bottle
- Carefully remove the liquid between the two drawn lines and add it to a labelled 15 mL falcon.
- Pour the remaining liquid into the waste.
- If no line is visible, save all of the liquid between the earlier marked lines showing p1.4 and p1.5.

DNA Extraction

Before DNA extraction, phages should be at a sufficiently high concentration and should be filtered and free from bacteria.

From Phages

- Add 2 μL DNase I and 20 μL of 10 x DNase buffer to up to 600 μL of prepared phage sample and incubate at 37 °C for 1h 30 mins
- Inactivate DNase I at 65 °C for 30 mins
- Add 10 μL of 20% SDS and 40 μL of proteinase K (20mg/mL) and incubate for 1h at 37 °C
 - Double check the concentrations and incubation temperatures for the enzymes and as they may differ from different suppliers
 - You may want to use a different heatblock here, or at least consider that it will take time for the heat block to cool down from the previous step
 - Briefly spin down any condensed liquid after each incubation
- Mix the sample with an equal amount of phenol:chloroform:isoamyl alcohol in a phase lock gel (PLG) light tube.
 - Green tubes on Ali's bench
 - Centrifuge before use.
 - This should be 672 μL if 600 μL were used to start.
- Mix and centrifuge at 12000xg for 5 mins
- Add 500-600 more phenol:chloroform:isoamyl to the tube and centrifuge at 12000xg for 5 mins
- Repeat the previous step a second time
 - You may need to use a second PLG tube, depending on the starting volume
- Transfer the aqueous phase to a new Eppendorf tube and add up to 1200uL of cold 100% ethanol to the tube and incubate overnight at -80 °C
 - 100% ethanol should be kept at -20 °C before use
 - Sample can also be incubated at -20 °C
- Centrifuge the tubes at 16000-1800xg at 4 °C for 1h
- Remove the supernatant and resuspend the pellet in 100 μL of TE buffer
- Purify the extracted DNA using ZYMO kit
 - Genomic DNA Clean & Concentrator
 - Use kit protocol as described

- Measure DNA concentration
 - If using nanodrop, record the ratios as well as the concentration
 - Qubit is more accurate

Wizard Kit

The Wizard kit can be used to extract DNA in a way that is less time and labour intensive than the method listed above. However, the yield is lower and it is only appropriate when the DNA is required for PCR or other experiments that do not require high quality DNA.

- Treat the sample with DNase and Proteinase K as described in the first 3 steps above.
- Mix 1 mL DNA Purification Resin with up to 1 mL phage sample
 - The ratio of resin should be between 2:1 and 1:1.
 - Shake the resin to mix before use.
 - Using more than 1 mL of phage sample will significantly decrease yield of DNA
 - if you have more than this, you can use more Wizard columns, or you can concentrate prior to DNA extraction using Amicon Ultra Concentrators
- Attach mini-column to bottom of 3 mL or 5 mL sterile syringe that has had the plunger removed.
- Add resin with sample to the syringe and push the solution through the column
 - You can save the flow-thru just in case you think you overloaded the resin.
- Remove mini-column from the syringe and pull out plunger. Reattach mini-column to the syringe and add 2 mL of 80% isopropanol to the syringe. Use the plunger push through the isopropanol to wash the resin.
 - you can repeat the isopropanol washing step to reduce impurities
- Remove mini-column from syringe and place in a sterile 1.5 mL centrifuge tube. Centrifuge at 10,000 x g for 2 minutes to remove any residual liquid.
- Place mini-column in new sterile 1.5 mL centrifuge tube.
- Add 100 µL 80 °C TE buffer to top of mini-column.
- Place tube lid over top of column and vortex gently for 10 seconds.
- Wait one minute and then centrifuge at 10,000 x g for 30 seconds to elute DNA.
 - You can repeat this a second time using 50 µL warm TE in order to recover an additional 10-20 % of DNA, but do not pool the 2 elutions until you have quantified the amount of DNA so as not to dilute the sample.
 - I normally use 50 + 25 µL TE buffer for elution
- Quantify DNA with nanodrop or qubit

PCR and gels

Protocols for PCR vary a lot and this is only an example. Check the specific volumes and timings with your supervisor, and you can also check the official protocols of the company who produce the Taq master mix (in this case Promega GoTaq Green Master Mix, but this can vary, so check!)

Reconstitute Primers

- Briefly centrifuge to put powder at the bottom
- Dilute to 100 μM stock – For every 1 mole, add 10 μL of PCR-grade water
 - The number of moles and the volume should add will be written on the side of the tube as well as in the paperwork that came with the primers
- Dilute 1:10 to create a working solution – 10 μM
- Store both at $-20\text{ }^{\circ}\text{C}$

Make A Master-Mix

Option 1: you are testing multiple DNA samples with the same primers

- Create a master-mix by adding the following amounts for each sample +1 to an autoclaved Eppendorf
 - GoTaq MasterMix 12.5 μL
 - 10 μM Forward Primer 2 μL
 - 10 μM Reverse Primer 2 μL
 - dH₂O 3.5 μL
- add 20 μL of master-mix to each autoclaved PCR tube
- add 5 μL of the appropriate DNA sample to tubes

Option 2: you are testing the same DNA sample with multiple primer sets

- Create a master-mix by adding the following amounts for each sample +1 to an Eppendorf
 - GoTaq MasterMix 12.5 μL
 - DNA 5 μL
 - dH₂O 3.5 μL
- Add 21 μL of master-mix to each PCR tube
- Add 2 μL of 10 μM Forward Primer and 2 μL of 10 μM Reverse Primer to each PCR tube

Run Thermocycler

- Run the following program on the thermocycler:

○ Initial denaturation	95 $^{\circ}\text{C}$	10 minutes	} 40 x
○ Denaturation	95 $^{\circ}\text{C}$	30 seconds	
○ Annealing	T_a	50 seconds	
○ Extension	72 $^{\circ}\text{C}$	E+	
○ Final Extension	72 $^{\circ}\text{C}$	2 minutes	

- Storage 4 °C ∞

- T_a = annealing temperature. This is calculated based on the primers you are using and can be calculated using an online calculator
- $E+$ = the extension time. This is calculated based on the size of the PCR product and should be approx. 1 minute per kb.

Gel

- Weigh out the correct amount of agarose powder and mix it with TAE buffer. Microwave to combine.
 - 0.7g of agarose in 70 mL will make enough for a small 1% gel
 - Do not use water to make the gel, however you can use TBE instead of TAE for smaller fragments (<1kb) as it resolves them better
 - Different percentage gels are used depending on the length of the fragment. Larger fragments move more slowly through the gel, and so lower percentages are used to help them. The opposite is true for smaller fragments.

Gel percentage	Range of efficient separation (bp)
0.5	2,000–50,000
0.6	1,000–20,000
0.7	800–12,000
0.8	800–10,000
0.9	600–10,000
1.0	400–8,000
1.2	300–7,000
1.5	200–3,000
2.0	100–2,000
3.0	25–1,000
4.0	10–500
5.0	10–300

- - Add DNA stain to the dissolved gel solution
 - SYBR Safe should be added in the ratio 1:10000, eg 1 μ L per 10 mL
 - Pour into gel tray with comb and leave to set
 - Leave the agarose to cool for a few minutes before pouring the gel in order to avoid damaging the gel tray

- The gel will take 15 – 30 mins to set
- Make sure the gel is not leaking before you leave it
- Remove comb and place into gel tank. Fill the tank above the level of the gel with TAE buffer
- Carefully pipette each sample into a well and run the gel
 - The gel will run from black to red
 - The sockets should be on the left side
 - Check if you can see rising bubbles in the buffer to be sure it is working
 - Higher voltages can melt the gel so reduce the voltage if it starts to get too hot
 - You can stop the gel in the middle of the run to check how far down the gel the bands have run – this is to prevent the smallest bands from running off of the end of the gel
 - Choose electrophoresis conditions according to the recommendations below:

Size of the DNA	Voltage	Buffer
<1 kb	5-10 V/cm	TBE
1-5 kb	4-10 V/cm	TAE or TBE
> 5 kb	1-3 V/cm	TAE
Up to 10 kb, fast electrophoresis with Express DNA ladders	up to 23 V/cm	TAE

- Image the gel.
 - To log into the computer use the username sarah.schulz and the password Moorepark2.
 - It may help to invert the colours or alter the contrast to better see the bands
 - Save the image to a USB to transfer it to your own computer.
 - Agarose gel and anything contaminated with SYBR safe should be disposed of in the special chemical waste bin

Bacterial Growth Curve

In order to use specific quantities of bacteria in later experiments we must correlate the amount of bacteria in a sample with the OD measurement using a growth curve.

- This experiment should be done in triplicate with three overnight cultures from 3 separate bacterial colonies
- Add 15 mL of LB (or appropriate media for your bacteria) in each of four 50 mL Falcon-tubes,
- These will be 3 replicates and one as negative control
- Vortex the overnight cultures before adding 1.5 – 3 mL to the LB. Measure the OD₆₀₀ of this culture before incubating it, shaking, at the appropriate temperature for the bacteria.
- At the following time points transfer an aliquot of the sample into an Eppendorf tube and return it to the incubator. For each aliquot, follow the following procedure:
 - Store the aliquot on ice
 - Measure the OD₆₀₀
 - Do a serial dilution and plate out 100 uL of each dilution onto an LB plate, spreading it using a cell spreader.
- The dilutions used will vary depending on the speed of bacterial growth
- Time points can be extended for slow growing bacteria

Time Point	Time after initial inoculation
T0	30 mins
T1	1.5h
T2	2.5h
T3	3.5h
T4	4.5h
T5	5.5h
T6	6.5h
T7	7.5h

Tips and Tricks

- If you need to incubate a culture overnight but it's the weekend, most of the time its fine to incubate it at room temperature over the weekend instead
- You can find shared documents (including a welcome package) on the N: drive, in the folder AGDeng -> Group Documents
- If you need to pipette something viscous (eg glycerol), cut the tip off of the pipette tip
- Go through your protocol before you start and make a list of all the things you're going to need. Then, make sure you collect all of those things and have them ready before you start working.
- RPM \neq RCF – RCF is consistent across different centrifuges with different size rotors, RPM is not