Slide 1:
In this lecture, I will discuss where and how to isolate phages and how to obtain pure isolates and stocks.

Slide 2:
Phages can be found everywhere in the environment. Water is a common isolation site. High concentrations of phages are present in sea water, freshwater and waste treatment plants. Additionally phages are found in soil samples attached to the soil particles. Furthermore, infected animals are an important isolation source. Sewage from farms, intestine samples, faecal samples and stable bedding are used in the isolation process. A common source of phages for human treatment is infected patients. As these patients have the target bacterium, there should also be some phages present that infect these bacteria. These phages are isolated from a stool sample, a skin swab, saliva and even dental plaques. Similarly, healthy humans can be a rich source of phages for commensal pathogens. These phages can be readily isolated from nasal secretions. Phages can also be isolated from places more hostile to life such as hot springs and glaciers.

Slide 3:
When the sample is available, the phages are isolated. First, the sample is pre-treated both physically, for example by extraction in a buffer solution, by centrifugation or by filtration, as chemically by chloroform killing the bacteria present in the sample. The pre-treatment is followed by an enrichment step. This involves the incubation of the sample along with the desired host(s) in liquid culture, often overnight depending on the host, to amplify any phages in the sample. This will result in a higher concentration of desired phages in the sample that would allow for easier isolation. Subsequently, a double agar overlay plaque assay is performed in which a suspension of bacteria and sample are mixed in a semi-solid agar solution followed by cultivation. If phages infect the target bacteria with sufficient efficiency, plaques will form.

Slide 4:
After the isolation of the phages from the sample, pure isolates are generated via plaque purification. Plaques are formed via the previously described double agar overlay plaque assay. Next, the obtained plaques are extracted from the solid media, eluted, then diluted before being incubated with fresh target bacteria to form new individual plaques. This plaque purification process is repeated a minimum of three times to ensure that the final plaque does not contain phage contaminants.

Slide 5:
When a pure isolate is available, a phage stock containing pure phage particles in a high concentration is generated. A purified plaque is isolated and incubated along with the desired host(s) in liquid culture. After overnight incubation chloroform is added to kill the bacteria present in the culture. Cellular debris is removed by centrifugation. The supernatant containing the phages is separated and chloroform is added again. A plaque assay of a dilution series of the generated phage stock is performed to determine the titer (or the number of plaques per mL) of the stock.

Slide 6:
Phages can also be isolated from lysogens. Lysogens are bacteria in which the phage genome is integrated into the bacterial chromosome. The phage is then present as a prophage. This prophage can be induced and thus liberated from the host chromosome via DNA damage as this will result in an SOS response activating the RecA protein. This protein will repress the CI repressor that normally blocks the lytic promoters. By interrupting this repressor, the lytic promoters are expressed and the

prophage can leave the bacterial chromosome. Common methods to induce DNA damage are for example UV treatment and the addition of mitomycin C.