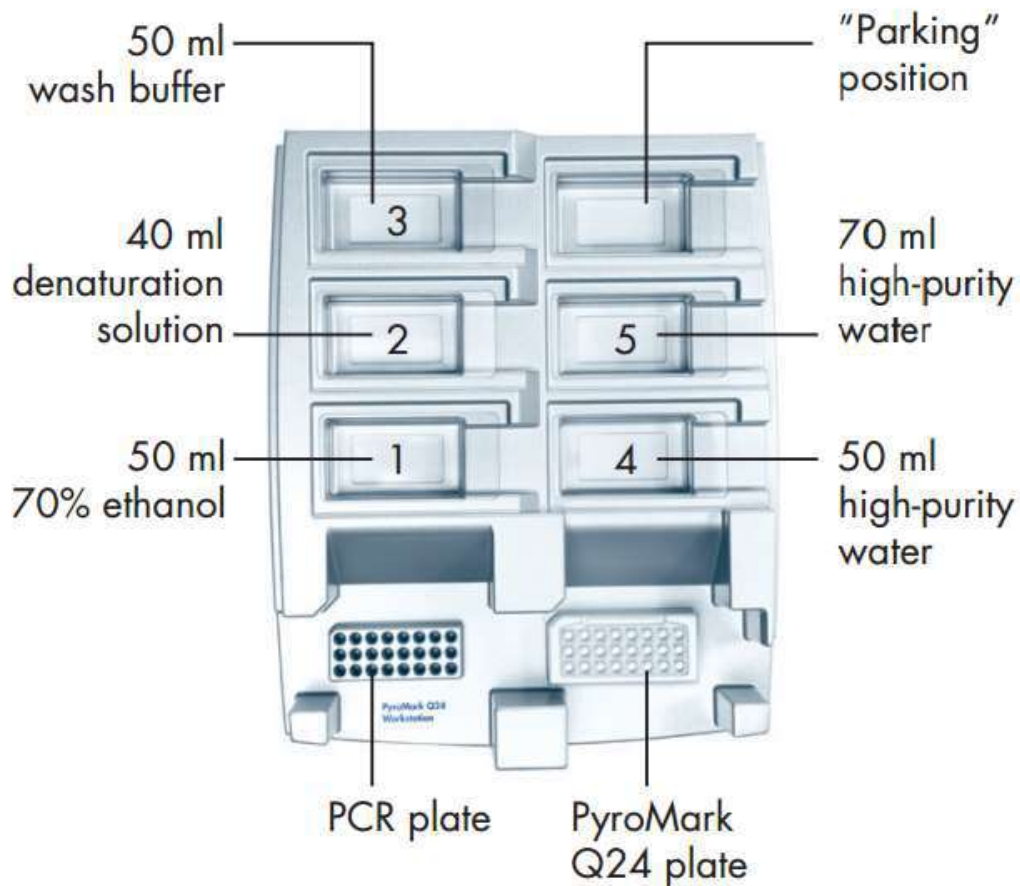


Preparation

- Switch on the Q24 sequencer.
- Set the heater to 85°C (the actual temperature should read 80°C on the thermometer) and put one of the Q24 plate holders on this to heat up.
- Prime the cartridge with ddH₂O by filling up the wells and pressing down with your thumb. Make sure there is a straight stream of water coming out.
- Place the required solutions into the workstation:



- Switch on the pump for the vacuum. Use the vacuum to flush all of the water in tray 5 (just to clean the vacuum). Switch off the vacuum then refill the water in tray 4.
- Get the following reagents out to reach room temperature:

Fridge

Binding buffer
Annealing buffer
Sephacrose beads

-20°C Freezer

Substrate mixture
Enzyme mixture
Sequencing primer (stock)
PCR samples

- Log on to the computer and create a new run file. Open the **PyroMark Q24 Advanced software**. Go to **File > New Run**. Enter the Instrument Method (this number is found on the cartridge). Load the assay onto the plate by **right-clicking > Load Assay**. Enter the sample ID's if you want.

Sample preparation

- Invert the Sepharose beads until they go into solution. **DO NOT** vortex.
- In a 2mL Eppendorf, make a master mix containing the beads, binding buffer and ddH₂O:

	<u>1 reaction</u>	<u>24 reactions (+ 10%)</u>
Sepharose beads	1 μ L	26.4 μ L
Binding buffer	40 μ L	1056 μ L
PCR-grade water	29 μ L	765.6 μ L

- Get a 24-well PCR plate (cut up from a 96-well plate) and pipette **70 μ L** of this master mix into each well.
- Add **10 μ L** of PCR sample into each well. Put the strip caps onto these.
- Place the plate on the shaker with tape. Shake at **1400 RPM** for **10 mins** (make sure the heat setting is not switched on!). Whilst this is shaking proceed to the next step.

Diluting the sequencing primer

- Dilute the sequencing primer to **0.3 μ M**.
- In an Eppendorf, add **2 μ L** of sequencing primer stock to **648 μ L** of annealing buffer. Vortex to mix.
- Add **25 μ L** of diluted sequencing primer to each well of a Q24 sequencing plate.
- Place this on the workstation.

Processing the samples

- Take the samples off the shaker and onto the workstation. Remove the lids carefully. Turn on the vacuum and process the samples with the following:

<u>Tray</u>	<u>Time</u>
PCR samples	15 seconds
70 % ethanol	5 seconds
Denaturation buffer	5 seconds
Wash buffer	10 seconds

- Invert the vacuum 90° for 5 seconds to clear the tubes.
- As you place the vacuum over the Q24 plate turn **OFF** the vacuum and place the filters into the plate containing the sequencing primer.
- Shake the handle from side-to-side for approximately **10 seconds** to deposit the beads.
- Transfer the Q24 plate onto the heated Q24 plate holder and leave for **2 minutes**. Put the vacuum handle into the first water tray (numbered 4 on the image) and shake from side-to-side for 10 seconds to clean the filters. Then switch the vacuum ON and suck up all of the water in the second tray (numbered 5 on the image).
- While waiting, load the sequencing reagents into the sequencing cartridge. To find out how much you need to load go back onto the run file. Go to **Tools > Pre Run Information**. A window should appear to inform you how much reagents to put into the cartridge and where to load them. Be careful not to introduce any air bubbles.
- After the 2 minutes is up take the plate off the heated Q24 plate holder and place inside the sequencer.
- When you have finished loading the cartridge load this into the machine. Save the run on the USB stick and insert this into the machine to run the file.