Cxbladder™

How it works

The Cxbladder analysis process

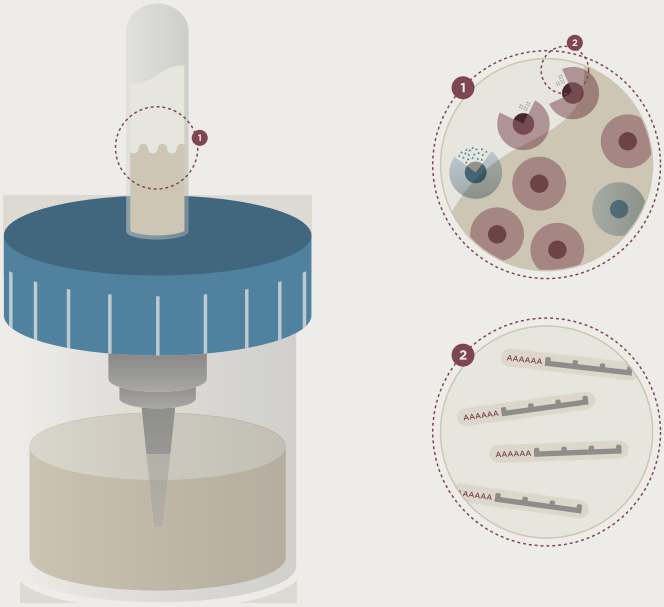
Because what happens at the molecular level is so crucial to the development of cancer, Cxbladder detects bladder cancer at that molecular level by measuring specific messenger RNA, representing a bladder cancer molecular signature.

1

Sample collection

When a patient's mid-stream urine sample is collected in the Cxbladder urine collection cup, it is aspirated into the Cxbladder tube and mixed with the proprietary Cxbladder liquid (RNA preservative reagent).

This liquid disrupts cells found in the patient's urine sample, releasing cell contents including the mRNA which is preserved for transport to our CLIA approved laboratory.



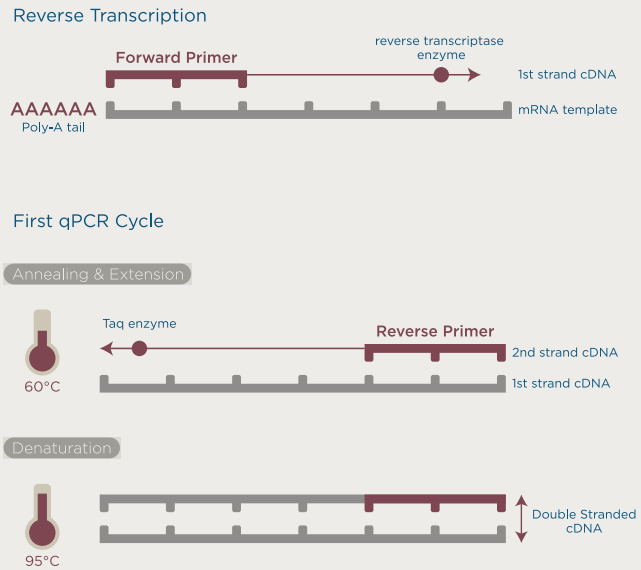
2A

Introducing RT-qPCR

At the lab, the mRNA is purified, extracted and converted to its stable DNA copy. We use a technology called Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) to amplify the biomarker genes of interest.

The RT-qPCR process works by binding specific primers to the mRNA. Primers work like a combination lock: only binding to the biomarker genes needed to identify if the correct combination is present. Once connected, primers provide a location for the enzymes to bind, allowing a first strand copy of DNA to be generated (cDNA) from the mRNA biomarker template (Reverse Transcription) and then to perform the qPCR process.

Information on RT-qPCR is continued on next page



Cxbladder™

How it works

The Cxbladder Analysis Process Continued

2B

RT-qPCR

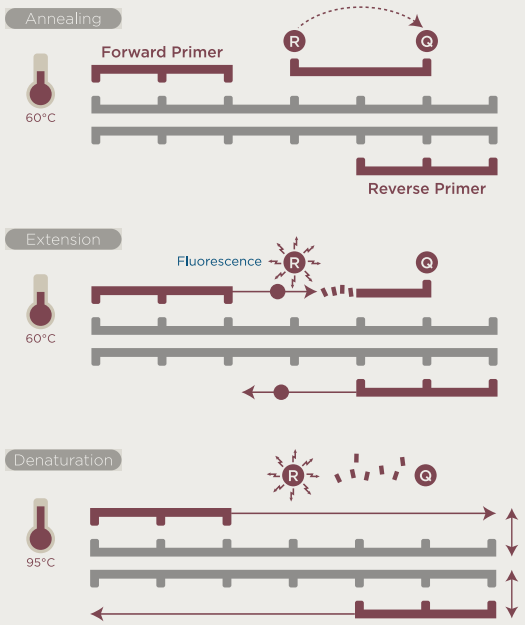
PCR amplification begins with denaturation of the double stranded cDNA into two templates at 95°C.

The amplification of biomarker cDNA is quantified in real time by detection of a probe which, like the primer, attaches itself only to the specific biomarker gene of interest; this is known as the annealing phase. The probe consists of a fluorescence emitting dye called a reporter (R), and a quencher (Q) that absorbs the reporter's fluorescence.

In the extension phase the Taq enzyme extends the primer using the cDNA as a template. The Taq enzyme degrades the probe during extension, separating the reporter from the quencher allowing the emission of a florescence flash.

The Taq enzyme then completes extension and the newly formed double stranded cDNA are denatured by reheating to 95°C for the next PCR cycle.

Second qPCR Cycle Onwards



3

Biomarker gene measurement

With each PCR cycle, the probe emits a fluorescence flash. As the replication process increases, the fluorescence accumulates until it hits a key detection point (Cp). The Cp is the PCR cycle where the fluorescence of the individual biomarker gene significantly exceeds the background fluorescence.

In the Cxbladder test, each of the five biomarker genes is measured by a different probe. It is the quantity of these biomarker genes in relation to each other that is used to calculate a Cxbladder score.

PACIFIC EDGE DIAGNOSTICS NZ LTD

Pacific Edge Diagnostics NZ Ltd
Centre for Innovation
87 St David St, PO Box 56
Dunedin, 9016 NZ
Tel: +64 3 479-5800
Fax: +64 3 479-5801
www.pacificedgedx.com