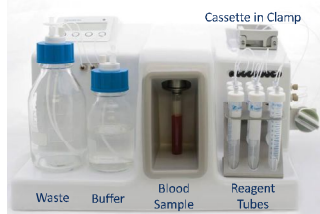


## Introduction

### Parsortix system

The Parsortix™ system (Figure 1), ANGLE plc, is designed for the capture and harvest of circulating tumour cells (CTCs) from peripheral blood. The system enriches CTCs based on the larger size and lower compressibility of CTCs compared with other blood components. The system does not use antibodies for enrichment and hence captures a spectrum of phenotypes, including mesenchymal CTCs and clusters of cells. Captured cells can be identified and enumerated inside the capture cassette and/or harvested out of the cassette for a range of downstream analyses, such as the AdnaTest ProstateCancerPanel AR-V7 qPCR assay.



**Figure 1: Parsortix system.** Blood is mounted onto the sample port, sample is separated and CTCs are captured on the steps of the Parsortix cassette.

### AdnaTest ProstateCancerPanel AR-V7

QIAGEN's AdnaTest Combination of Combinations Principle (COCP), combines immunomagnetic cell capturing using a mixture of different antibodies to overcome potential lack of EpCAM antigens with profiling of several molecular targets in parallel, resulting in highly sensitive CTC determination and characterization. This workflow provided the basis for the detection of the splice variant of the androgen receptor AR-V7 by Jun Luo and E. Antonarakis (NEJM 2014). Based on these findings QIAGEN developed a qPCR based AdnaTest ProstateCancerPanel AR-V7 that has already been successfully functionally tested in several research studies. However, the test does not enable enumeration of CTCs.

In this study we aim to combine the label-free Parsortix technology (which harvests all phenotypes of CTCs and enables their enumeration) with the AdnaTest ProstateCancerPanel AR-V7 qPCR assay (gene expression analysis) in one workflow.

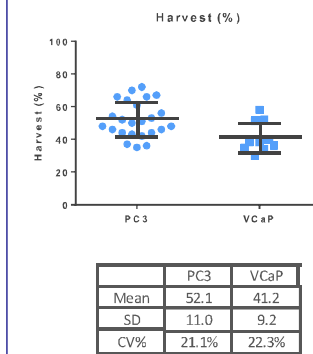
## Methodology: Parsortix system - AdnaTest ProstateCancerPanel AR-V7 Workflow

- Blood was drawn from Healthy Donors (HD) into K2-EDTA blood stability tubes and spiked with AR-V7 positive VCaP or AR-V7 negative PC3 Prostate Cancer (PC) cells
- Spiked blood samples were separated through the Parsortix system (unspiked HD samples were processed as a control) and cells were captured in the cassette; cells were either harvested immediately after separation in PBS (120 µl) or cells were labelled *in situ* with an antibody cocktail via the reagent tubes (Immunofluorescence [IF]), enumerated and subsequently harvested in PBS (120 µl)
- Harvested cells were lysed using the AdnaTest lysis/binding buffer and stored at -20°C until downstream processing
- The AdnaTest ProstateCancerPanel AR-V7 qPCR assay was performed following the manufacturer's recommendations

### Workflow:



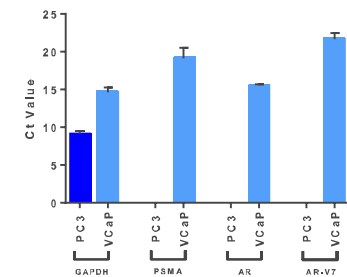
### Parsortix enrichment of PC3 and VCaP Prostate Cancer cell lines



**Figure 2 Capture and harvest of PC3(AR-V7) and VCaP (AR-V7\*) PC Cells.** The percentage harvest of CellTracker™ prelabelled PC3 (AR-V7) and VCaP (AR-V7\*) cells.

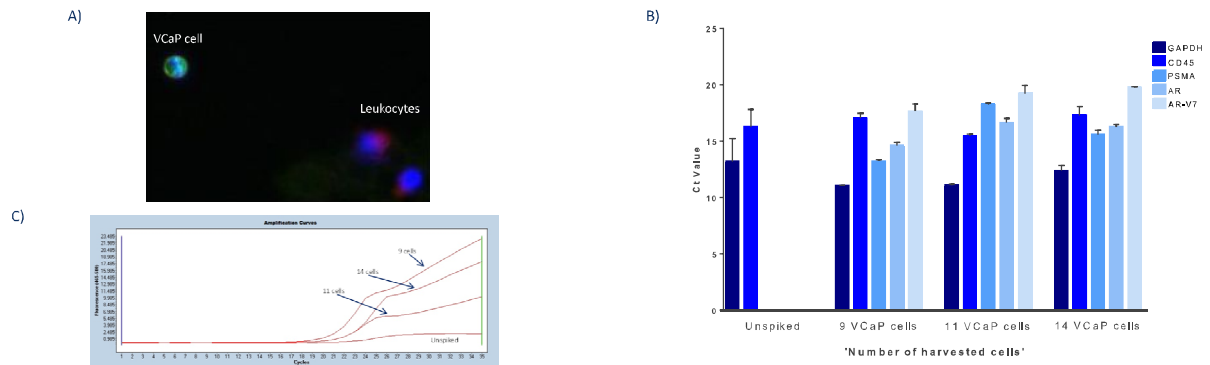
### VCaP Prostate Cancer Cell Line is Positive for AR-V7

AR-V7 qPCR Ct Values using PC3 (AR-V7) and VCaP (AR-V7\*) PC cell lines



**Figure 3 AdnaTest ProstateCancerPanel AR-V7 qPCR using PC3 and VCaP PC cell lines.** Approximately 200 cells were lysed. Ct values from ProstateCancerPanel AR-V7 qPCR assay using PC3 (AR-V7) and VCaP (AR-V7\*) PC cell lines (18 preamplification cycles plus 35 PCR cycles performed). Panel includes housekeeper gene (GAPDH) and PC specific genes (PSMA, AR and AR-V7).

## Parsortix enrichment, In-cassette staining plus AdnaTest ProstateCancerPanel AR-V7 qPCR



**Figure 4 Staining of captured VCaP cells in-cassette and subsequent analysis by the AdnaTest ProstateCancerPanel AR-V7 qPCR assay.** A) Representative image of stained VCaP cells. The cells were stained using antibodies (EpCAM, HER-2, CK8, CD45 and DAPI) visualised using a fluorescence microscope with 20x magnification. Blue = DAPI (nuclear marker), Green = EpCAM, HER-2 and CK8 (CTC markers) and Red = CD45 (leukocyte marker). B) Ct values from ProstateCancerPanel AR-V7 qPCR assay on samples containing low numbers of VCaP cells (18 preamplification cycles plus 35 PCR cycles performed), cell numbers were determined by in-cassette staining. Panel includes housekeeper gene (GAPDH), leukocyte marker (CD45) and PC specific genes (PSMA, AR and AR-V7). C) Representative AR-V7 amplification curves from the ProstateCancerPanel AR-V7 qPCR assay

## Results

- Both the AR-V7 negative PC3 and AR-V7 positive VCaP cell lines can be captured and harvested using the Parsortix system. The efficiency is lower for the VCaP cell line, 41%, in comparison to the PC3 cell line, 52%, due to differences in compressibility and size (*In a cell culture plate: PC3 cells have a mean diameter of 20 µm; VCaP cells have a mean diameter of 12 µm*).
- The VCaP cells are a representative PC cell line to use in the AdnaTest ProstateCancerPanel AR-V7 qPCR assay, as shown by the expression of PSMA, AR and AR-V7 genes.
- The PC3 cells are a suitable negative control for the AR-V7 qPCR assay, as shown by the lack of PSMA, AR and AR-V7 gene expression.
- VCaP cells enriched by the Parsortix system can be used in the AR-V7 qPCR assay; samples containing VCaP cells express the AR-V7 gene and unspiked HD samples do not express AR-V7.
- VCaP cells can be stained in the capture cassette and enumerated prior to harvesting the cells for use in the ProstateCancerPanel AR-V7 qPCR assay.
- A low number of VCaP cells (~10 cells) can be detected by the AR-V7 qPCR assay (Figure 4) after staining of the cells with cell surface marker antibodies (EpCAM, HER-2 and CK8).

## Conclusions

- The AdnaTest ProstateCancerPanel AR-V7 qPCR assay distinguishes between AR-V7 negative and positive PC cell lines (i.e. PC3 and VCaP).
- VCaP cells spiked into K2-EDTA blood can be captured in the Parsortix capture cassette and stained using cell surface marker antibodies EpCAM, HER-2 and CK8.
- Stained VCaP cells can be harvested from the Parsortix system and subsequently undergo gene expression analysis using the AdnaTest ProstateCancerPanel AR-V7 kit.
- AR-V7 gene expression can be detected in harvests containing as low as ~10 stained VCaP PC cells- further work will include investigation of the assay's limit of detection in combination with the Parsortix system.
- The label-free capture and enumeration of cancer cells from blood using the Parsortix system can be combined with gene expression analysis of those cells by the AdnaTest ProstateCancerPanel AR-V7 qPCR assay.
- This combination of technologies provides a powerful new workflow for liquid biopsy research.