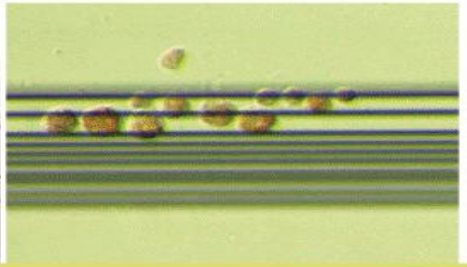
ANGLE



CELLS FOR PRECISION MEDICINE.

Application Note

Introduction

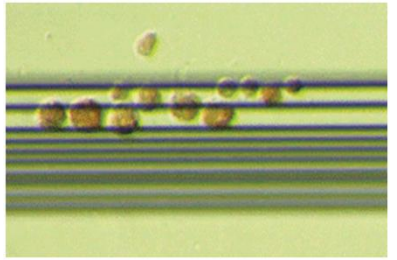
ANGLE plc: UK publicly traded company (UK AIM:AGL)

- Commercial patent protected epitope-independent circulating tumor cell (CTC) harvesting technology

ANGLE Biosciences: Toronto based subsidiary of ANGLE

- Novel downstream analysis systems for cost effective, highly multiplexed analysis of nucleic acids and proteins
- Combined focus on sample to answer, liquid biopsy testing
- Other technology applications exploited through corporate partnerships with established industry leaders.

Parsortix system



Captured rare cells in Parsortix cassette.

Ziplex benchtop analysis system for laboratory use.

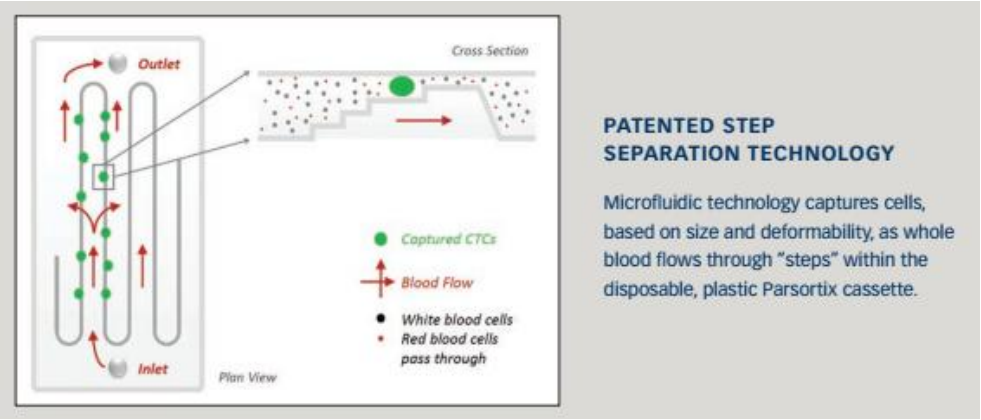
Ziplex SA analysis system for distributed testing (under development)

Isolation of RNA from ~1 -100 cells captured by the cassette

Downstream Applications

Parsortix Technology:

- Capture and harvest of CTCs and other cells of interest from 100 µL to 30 mL of blood sample
- Addresses the needs for:
  - Highly enriched cell populations
  - Epitope independence
  - Viable cells
  - Research flexibility
  - Simple and easy process
- Several health conditions investigated using captured cancer cells including breast, ovarian, lung, prostate, colorectal, pancreatic, melanoma and others



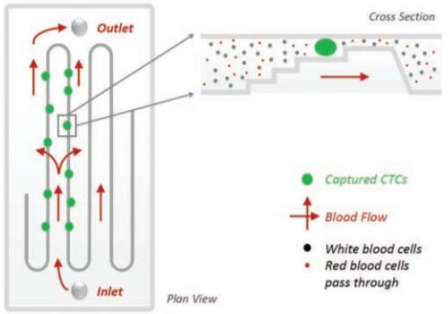
Downstream applications include in-vitro staining or harvesting of cells for molecular analyses on the Ziplex platform

- Highly multiplexed target detection from a few CTC cells
- High specificity to avoid spurious amplification and false positives
- Rapidly adaptable to new gene targets and disease applications
- Low complexity and affordable

Parsortix/HyCEAD/Ziplex Workflow

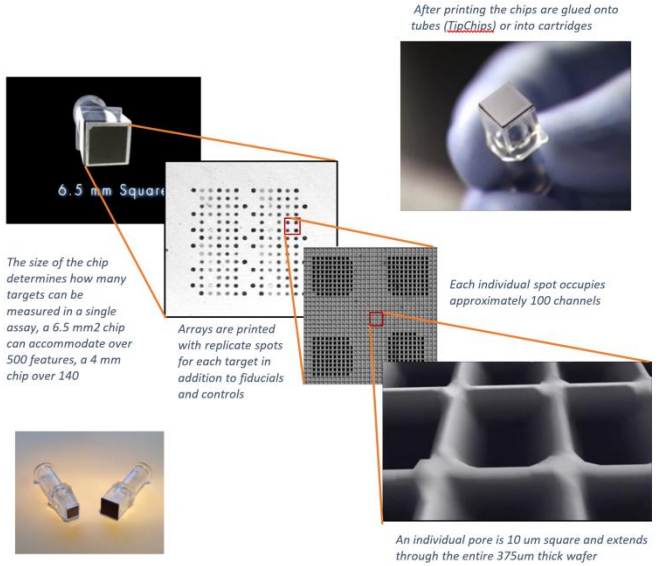
- Draw blood
- Parsortix processing
- Recover harvest enriched for CTCs
- Lyse cells
- Multiplex HyCEAD processing
- De-multiplexing on array of probes
- CTC expression profiles

CTCs become trapped in narrow gaps within the Parsortix cassette while most blood cells pass through the gap to waste



Target specific oligonucleotide probes are spotted on flow-through microarrays (TipChips). TipChips are immersed in solutions that are repeatedly passed through the microarray using pressure and vacuum. Images of chemiluminescent light emission are automated analysed to generate expression profiles.

- Whole blood processed through Parsortix to yield a cell suspension enriched in circulating tumour cells (CTCs).
- Cells are lysed and amplicons from multiple mRNA targest are amplified from the lysate (no RNA/DNA detection); RNA yield from ~ 1 -100 cells
- Mixture of amplicons (100+ targets) are de-multiplexed on a flow-through microarray of target-specific probes.
- Amplicons from eight HyCEAD amplifications (CTC samples) are automatically hybridized on individual flow-through arrays to produce quantitative expression data for all targeted genes of interest.



VERSA 1100 Gene by Aurora Biomed

Automation of the sample preparation procedure for hybrid capture, extension and detection (HyCEAD) by a VERSA 1100 Gene instrument

Operator sample processing time (**12 samples**)
~ 4 hours

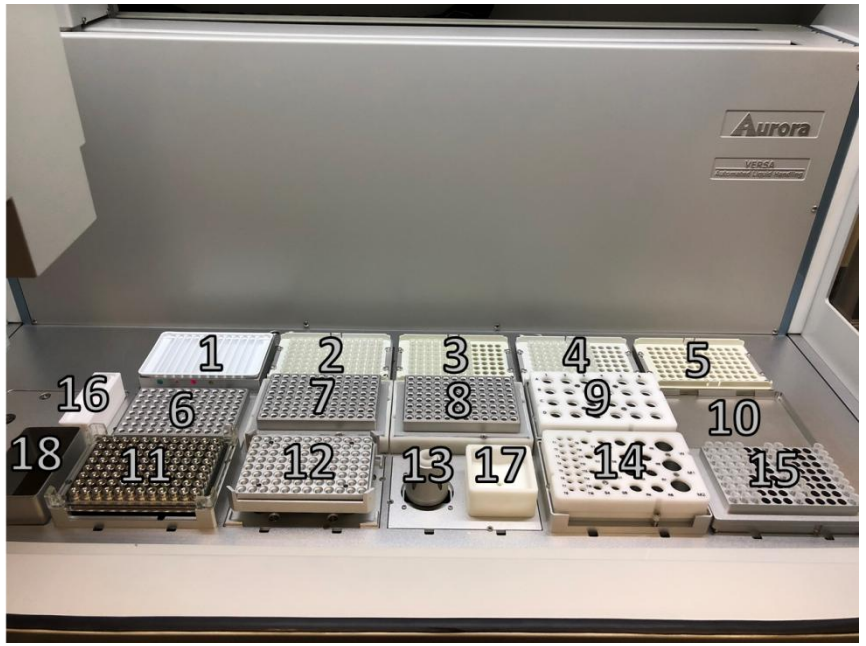
VERSA 1100A sample processing time (**24 samples**)
~3.5 hours

Double the efficiency of manual operation

Aurora
ILLUMINATING SOLUTIONS

aurorabiomed.com

Deck Layout



1. Reservoir
2. Tips (200 µL)
3. Tips (200 µL)
4. Tips (50 µL)
5. Tips (50 µL)
6. PCR Plate Adaptor (Ambient)
7. PCR Plate Adaptor (0-100°C)
8. PCR Plate Adaptor (0-100°C)
9. Reagent Cooler (6x4)
10. Tips (20 µL)
11. Magnetic Deck
12. Plate Shaker (RT-100°C)
13. Bead Shaker
14. Reagent Cooler B
15. Single Tip Box
16. Liquid Waste Station
17. Reagent Drop Priming Station
18. Tip Disposal Chute
19. Reagent Drop Lines

Deck Layout in Software (VERSAware)

Verification of Automated 8 Sample HyCEAD Processing on VERSA 1100 Gene

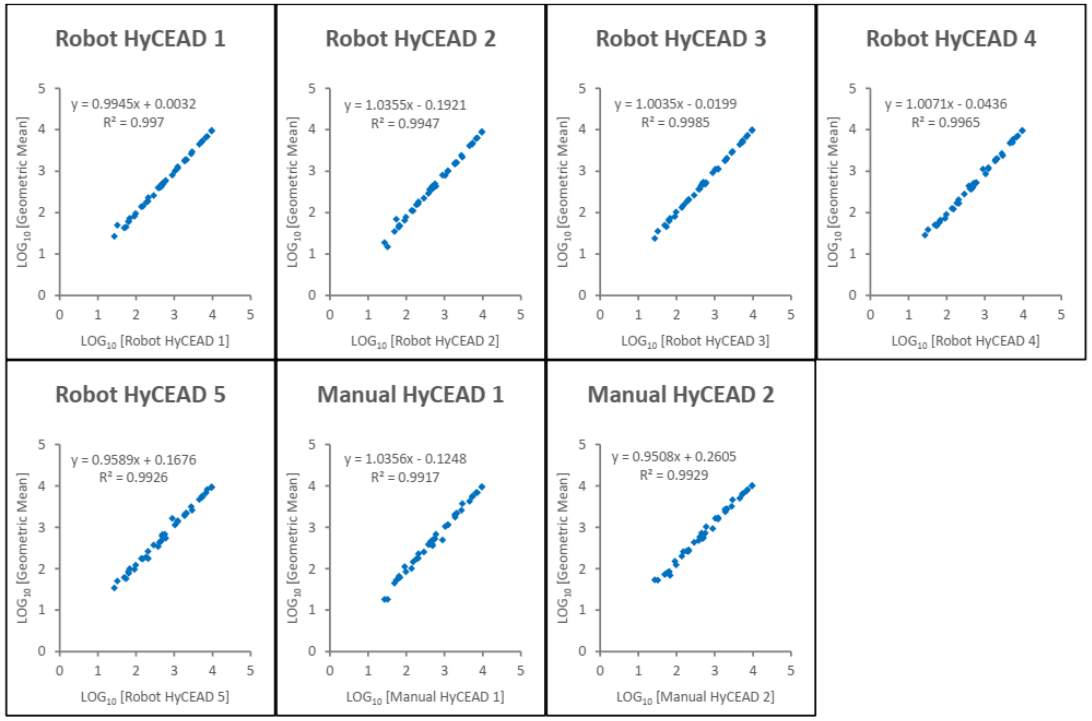


Figure 2A: Comparison of robot and manual HyCEAD runs. Geometric means were calculated from 56 individual measurements for each probe. HyCEAD data represents the mean of 8 measurements for each probe.

Method

- Single sample was repeatedly run on the VERSA using automated HyCEAD protocol and manually by operator.
- Each sample was probed for 68 genes of interest on a single microarray (TipChip); multiplexed targeting.

Results

- Results from individual HyCEAD runs were compared to the geometric means of all probes with intensities > 20 AU.
- Excellent correlation between each run and the geometric mean with R^2 values > 0.99
- Probe signal intensities in each of the 8 chips across the 5 automated HyCEAD runs showed no apparent positional bias which can occur due to temperature edge effects in heating blocks or small misalignments on the robot deck

Verification of Automated 24 Sample HyCEAD Processing on VERSA 1100 Gene

Inter-sample Variance

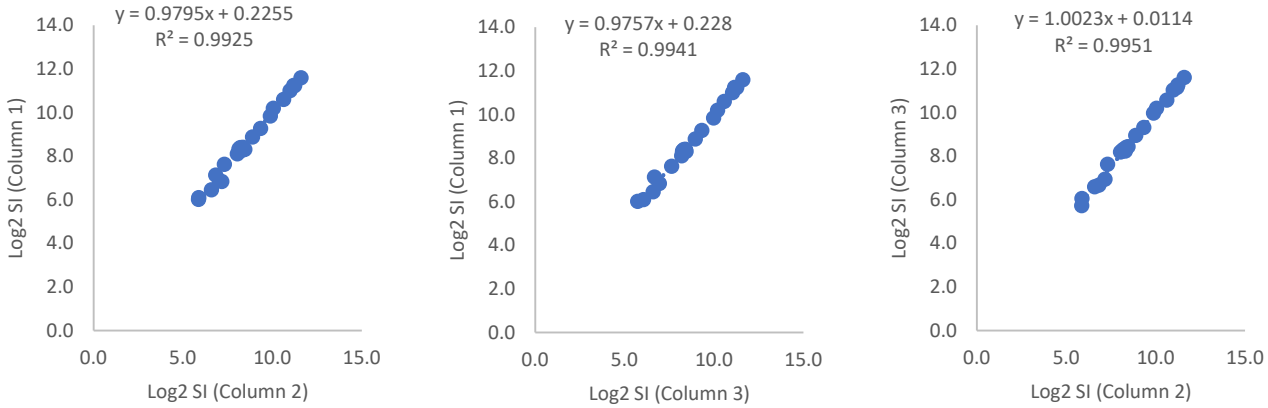


Figure: Correlation plots of probe geomean signal intensities for 24-sample automated HyCEAD. Means calculated from 8 individual measurements for each probe.

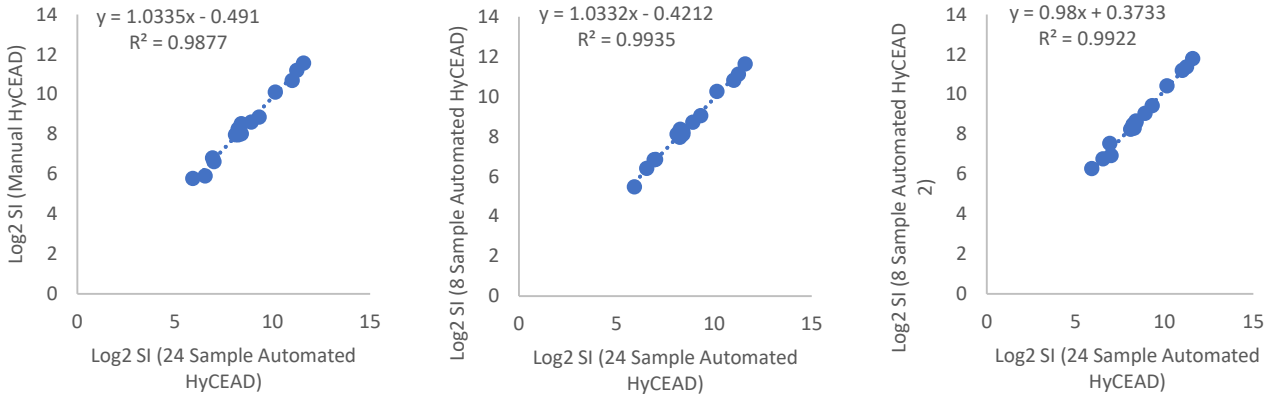


Figure: Comparison of automated and manual HyCEAD. Means calculated from 8 individual measurements for each probe.

Method

- Single sample was run on the VERSA using automated HyCEAD protocol for simultaneous processing of 24-samples.
- Each sample was probed for 68 genes of interest on a single microarray (TipChip); multiplexed targeting.

Results

- Assessing inter-sample variance that may be increased due to longer processing time for 24 samples (measure indication of positional bias)
- Excellent correlation between geomean SI of each set of 8 samples with R^2 values > 0.99; minimal inter-sample variance
- Probe signal intensities in each of the 24 chips across the single HyCEAD run showed no apparent positional bias which can occur due to temperature edge effects in heating blocks, small misalignments on the robot deck, or longer-processing time for 24-sample analysis
- Comparison of automated and manual HyCEAD runs showed good correlation of geomean SI of 24-sample automated HyCEAD with manual HyCEAD with R^2 values > 0.98.

CELLS AND ANALYSIS FOR PRECISION MEDICINE

ANGLE Europe Ltd

10 Nugent Road
The Surrey Research Park
Guildford GU2 7AF
United Kingdom

ANGLE Biosciences

50 Ronson Drive
Suite 105
Toronto, ON M9W 1B3
Canada

ANGLE North America Inc.

3711 Market Street
University Science Center 8th floor
Philadelphia PA 19104
USA

www.ANGLEplc.com

For Research Use Only: Not for Use in *In-vitro* diagnostics