HOLOGIC®

High Throughput Genotyping using the Invader® and InvaderPlus® Platform Paul Docherty¹ PhD, Robert Holt¹ PhD, Mike Kaiser² PhD, David Reed² BSc

Tepnel Pharma Services

Tepnel Pharma Services is an independent CRO that specialises in the provision of pharmaceutical testing and molecular genetic services in support of drug development.

As a Molecular Genetic Services provider, we offer a full range of services for biomarker discovery, assay development, assay validation and companion diagnostics development in support of pre-clinical, clinical and personalised medicine studies. We offer a complete biomarker discovery and validation package from initial study design through to companion diagnostics development.

Our Molecular Genetic Services include gene expression, genotyping, copy number and epigenetic analysis using a broad range of technologies including our proprietary Invader and InvaderPlus chemistries.



Invader and InvaderPlus Chemistry Platforms

The Invader chemistry platform is a homogeneous, isothermal, DNA probebased system for highly sensitive, quantitative detection of specific nucleic acid sequences. Invader is an accurate and specific method for the analysis of SNPs, InDels, CNV and Gene Expression. Invader and InvaderPlus chemistries are:

- A proprietary, patented technology (45 issued patents to date)
- Detects specific nucleic acid sequences
- Template can be DNA, RNA or microRNA
- Signal amplification reaction
- Invader is a direct isothermal genomic assay
- InvaderPlus combines sensitivity of PCR and specificity of Invader
- Structure specific recognition and cleavage by Cleavase[®] family of enzymes
- Fluorescence detection

1 Tepnel Pharma Services, Manchester, UK 2 Hologic AgBio, Madison, WI, USA

Invader Assay for SNP Detection Biplex FRET Format



Consists of two simultaneous isothermal reactions, a primary reaction specifically and accurately detects single-base changes, insertions, deletions and changes in gene and chromosome number whilst a second reaction is used for signal amplification and generic readout.

1st reaction:

- Two oligos, a probe and Invader oligo, hybridize to the target - If a specific target is present, a one base pair overlapping structure is generated
- This triplex is recognised and cut by proprietary Cleavase enzymes
- 5' flap oligo released
- Number of flaps is proportional to amount of target in sample

2nd reaction:

- Cleaved flaps from the primary reaction combine with a FRET cassette generating a fluorescent signal
- No target, no cleavage, no flap, no signal
- Different 5' flaps and corresponding FRET probe allows for multiple sequences to be detected in a single well

InvaderPlus Chemistry

Combines the sensitivity of PCR and the specificity of Invader in a single closedtube reaction. Set-up is one-step and requires no addition to or transfer from the reaction, eliminating PCR cross contamination issues. The PCR cycle allows for a shorter Invader incubation time and the combination of PCR and Invader allows the use of lower concentration and quality input samples.

Initial reaction:

- . PCR portion the target is amplified with specific primers using PCR (A)
- 2. Heat denaturation of the polymerase enzyme (B)
- 3. Amplified target is then detected using the Invader reaction (C)





Sample InvaderPlus Results

DNA Samples Assay 1





Summary and Conclusions

Benefits of using Tepnel Pharma Services' Invader Service Scalable technology (HT >30,000 samples/hr)

- Simple assay design
- Affordable price
- Improved turnaround

Invader is the ideal platform for

- SNP screening
- High-throughput genotyping
- Transgene detection
- Copy number determination

