A Comparison of the Invader® and InvaderPlus® Platforms with other Genotyping Technologies

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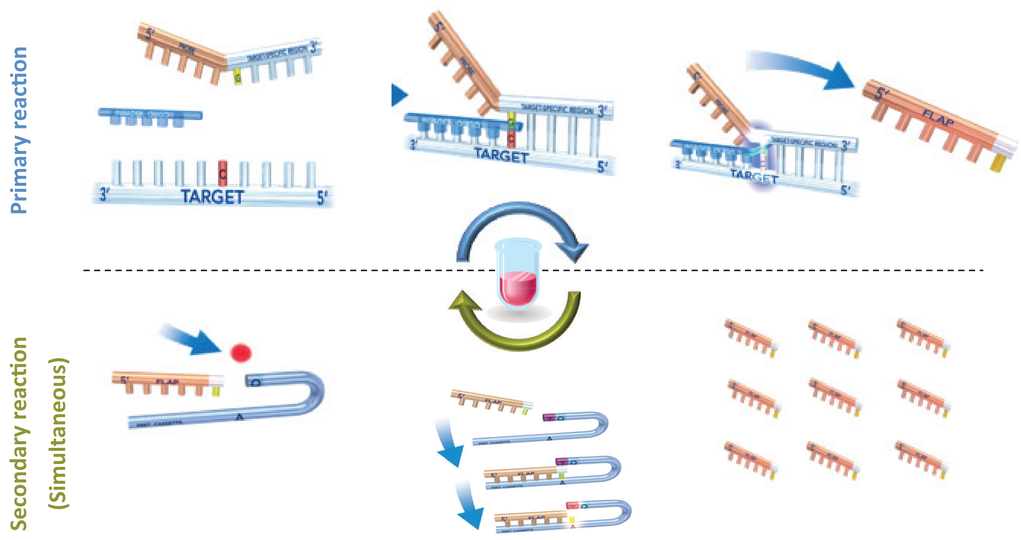
Invader and InvaderPlus Chemistry Platforms

- The Invader chemistry platform is a proprietary, cost effective and isothermal probe-based system used in a wide range of applications
- Invader and InvaderPlus assays are suitable for high-throughput genotyping, copy number variation, gene expression and detection of InDels
- Invader chemistry is currently used in multiple FDA-approved and CE-marked products and is used extensively by laboratories worldwide for their clinical, diagnostic and research needs
- Invader is a proprietary technology with 45 patents and over 200 publications to date
- Invader is a direct isothermal genomic assay whereas InvaderPlus combines sensitivity of PCR and the specificity of Invader
- Both Invader and InvaderPlus assays are available via the Tepnel Pharma Services ICP service to support your pre-clinical, clinical and personalised medicine studies
- This study compares four different genotyping technologies; Invader, InvaderPlus, TaqMan® and KASP™

Invader Chemistry



1st reaction:

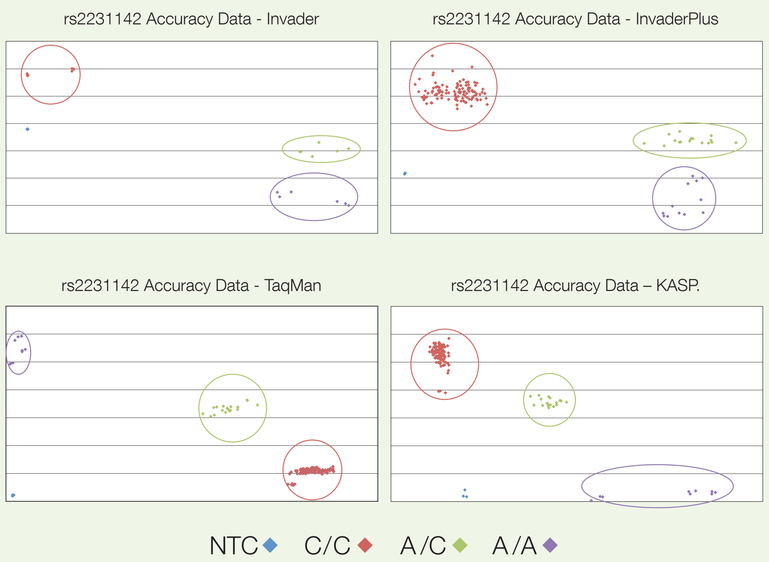
Two oligos, a probe and Invader oligo, hybridise to the target to create a one-base overlapping structure. This triplex is recognised and cut by proprietary Cleavase enzymes and a 5' flap oligo is released. Number of flaps is proportional to amount of target in sample – quantitative detection.

2nd reaction:

Flaps serve as 'Invader' oligo resulting in specific cleavage of labelled universal oligo – the FRET probe – and 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.

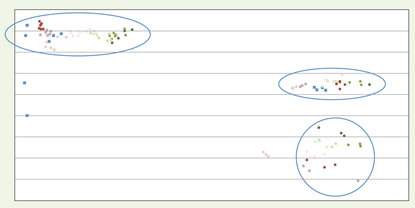
Accuracy Results

Cluster plots showing concordance of results between Invader, InvaderPlus, TaqMan and KASP:

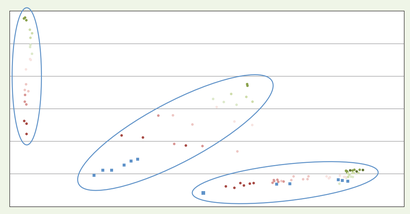


Limit of Detection Results

Cluster plots showing concordance of LOD results, examples show concordance between InvaderPlus and TaqMan:



Limit of Detection Data – InvaderPlus



Limit of Detection Data – TaqMan

Colour coding indicates DNA concentrations, Green = High, Red = Low.
Buccal samples prepared using hotshot DNA preps are shown in Blue.

Percentage of Samples Genotyped Correctly

SNP	Invader	InvaderPlus	TaqMan	KASP
rs2231142	100%	100%	100%	97.7%
rs2032582	100%	100%	100%	97.7%
rs4148323	100%	100%	100%	100%

Advantages of Invader, InvaderPlus, TaqMan and KASP

	Invader	InvaderPlus	TaqMan	KASP
Cost	Low	Low	High	Low
Throughput Capability	High	High	Medium	High
Assay Conversion Rate	High	High	High	Medium
Use Poor Quality DNA	No	Yes	Yes	Yes
Use Low DNA Input	No	Yes	Yes	Yes

Materials and Methods:

Technologies: **We compared four different technologies; Invader, InvaderPlus, TaqMan and KASP using four SNP targets:**
- rs2231142 (Designed to test manufacturing process)
- rs2032582 (Tri-allelic, requires two assays)
- rs4148323 (UGT1A1 *1/*6)

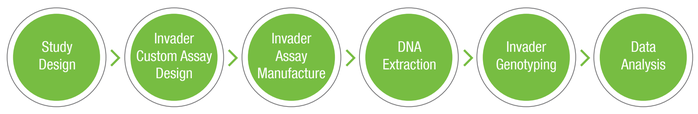
Work Package 1: Accuracy. Concordance of results between Invader, InvaderPlus, TaqMan and KASP. 44 samples from the ECACC Ethnic Diversity Panel were processed (in triplicate) for each assay. N.B. Invader assays tested on reduced panel.

Work Package 2: Limit of Detection. Testing was carried out with eight dilutions of reference DNA in triplicate to determine the lowest amount of input DNA that can be used in the InvaderPlus and Taqman assays. Reactions were carried out with the DNA concentrations ranging from 2 ng/µL (Taqman standard input) down to 0.015625 ng/µL

Work Package 3: Buccal Samples. Comparison of assay performance with poor quality DNA. DNA extractions carried out from three buccal swab samples using Isohelix DNA isolation kit. DNA samples genotyped directly from lysates.

Tepnel Pharma Services

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



Tepnel Pharma Services ICP Service

Tepnel Pharma Services offers a fully customisable service utilising Invader and InvaderPlus chemistries for biomarker discovery, assay development, assay validation and companion diagnostics development in support of pre-clinical, clinical and personalised medicine studies.

Conclusions

- This study compared four different genotyping technologies; Invader, InvaderPlus, TaqMan and KASP genotyping assays across multiple targets
- Invader and InvaderPlus assays represent a viable alternative to TaqMan and KASP chemistries
- Invader and InvaderPlus assays are:
  - **Cost effective**
  - **Scalable**
  - **Automatable**
- InvaderPlus assays are suitable for use with lower quality, lower concentration DNA, whereas Invader assays are suited to high-throughput isothermal applications

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INVADER CHEMISTRY

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