3base™
The Science Behind EasyScreen™
Molecular Diagnostic Assays

Nucleic Acid Conversion Technology

Topics

- 3base™ Technology Overview
- Technical Advantages of 3base™
- Validation of 3base™ Specificity
- Validation of *EasyScreen* Assays
  - C. difficile, screening panels
- Validation of GS1 automation
- Future developments

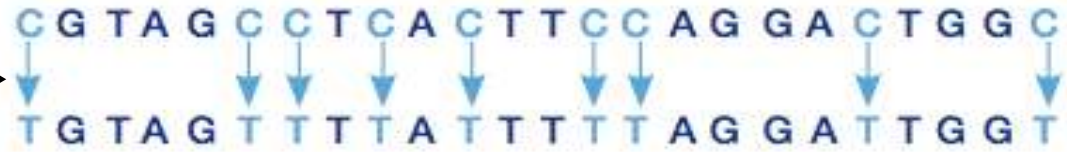
Overview of 3base™

Basic technical functionality: Moving from 4base to 3base™

Regular Cell Lysis and Preparation Steps

Native Sequence

3base™ *proprietary Sodium Bisulfite Treatment*



Original (Native) 4-base Microbial genome

Converted 3base™ Microbial Genome

3base™ Sequence

Standard Real-Time PCR Instrument

Genetic Signatures

Developed to Improve Subtype Similarity

e.g. Non-Converted Influenza Sequences

```
Influenza A virus H5N1  TGTGTGTGCA GGGATAATTG
Influenza A virus H7N3  TGTATATGTA GGGACAATTG
Influenza A virus H5N8  TGTGTTTGTA GAGACAACTG
Influenza A virus H5N3  TGTATATGTA GGGACAATTG
Influenza A virus H5N2  TGTGTTTGCA GAGATAATTG
Influenza A virus H6N6  TGCATTTGCA GGGACAATTG
Influenza A virus H2N9  TGCACTTGCA GGGATAATTG
Influenza A virus H6N5  TGCGTTTGCC GAGATAATTG

            Consensus  TGYRYDTGYM GRGAYAAYTG
```

768 Possible combinations

55% Homology

Genetic Signatures

Conversion to 3base™ Improves Subtype Similarity and Reduces Variation – Enabling Analysis via PCR

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3base™ Converted Influenza Sequences

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Influenza A virus H6N6	TGTATTTGTA GGGATAATTG
Influenza A virus H2N9	TGTATTTGTA GGGATAATTG
Influenza A virus H6N5	TGTGTTTGTT GAGATAATTG
Consensus	TGTRTDTGTW GRGATAATTG

24 Possible combinations

80% homology

Cytosines are converted to Thymines – *resulting in non-natural 3-base DNA/RNA*

Sufficient information is retained after conversion for genotyping equivalent to native (4base) genomic assays

No Loss of clinical specificity is observed by this base conversion

e.g. HPV clinical trial showed superior performance vs. Digene HC2 Assay in reducing False Positives

Genetic Signatures

3base™ Technology

The cornerstone of EasyScreen™ *assays*

- Universally applicable to all specimen types - converts and detects both DNA and RNA
  - Works with multiple specimen types simultaneously
  - Stool is first commercially available
- Allows for simple multiplexed assays
  - More targets detected per specimen - e.g. 22 gastroenteritis causing targets are simultaneously detected from stool
- Real-Time PCR format
  - No post-PCR handling; virtually eliminates potential for contamination
- Open platform
  - Suits all laboratories – no need to purchase new equipment

Technical Advantages of 3base™ Technology

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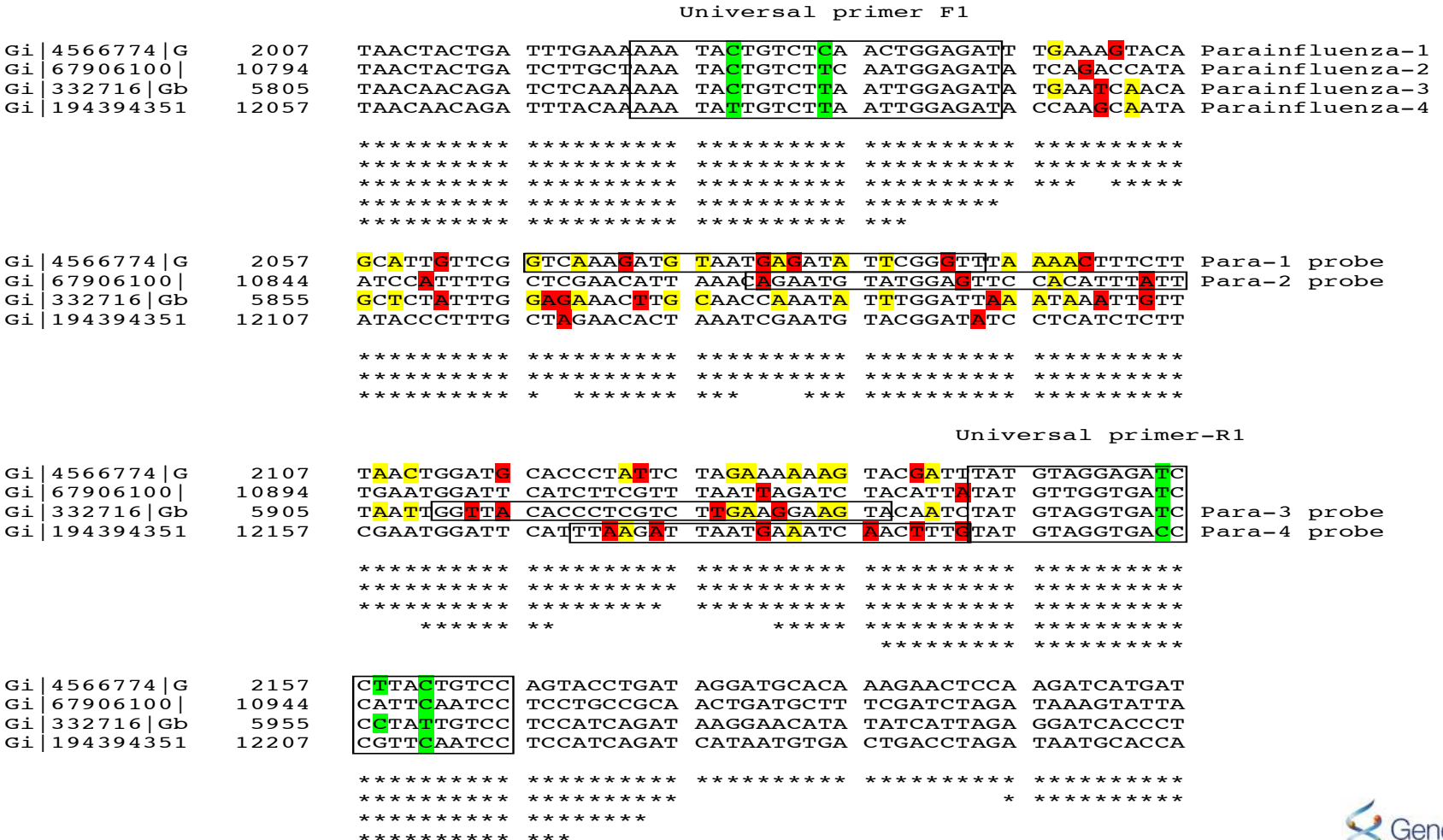
Technical Advantages of 3base™ Assays

One primer set detects multiple species



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Validation of 3base™ Specificity

Validation of Specificity

- Single copy genes may be amplified from a 3base™ converted human genome
- High-Risk HPV identified as hardest challenge

ELSEVIER

Journal of Clinical Virology 42 (2008) 22–26

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Comparison of a novel HPV test with the Hybrid Capture II (hcII) and a reference PCR method shows high specificity and positive predictive value for 13 high-risk human papillomavirus infections

Cristina Baleriola[a], Douglas Millar[b], John Melki[b], Neralie Coulston[b], Phillip Altman[c], Nikolas Rismanto[a], William Rawlinson[a,*]

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[c] *Altman Biomedical Consulting Pty. Ltd., 152 Cammeray Road, Cammeray, NSW 2062, Australia*

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HPV Validation Study Summary

- The HGS tests had a statistically significant higher PPV than the HC2 (Digene) test ($P < 0.001$)
- The HGS test has a lower rate of false positives at the same level of sensitivity as the HC2 (Digene) test ($P< 0.001$)
- The HGS test and the HC2 test showed no statistical difference in NPV for presence of virus ($p=0.677$) or false negatives (sensitivity; $P=0.398$)

Validation of *EasyScreen*™ Clinical Performance

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EasyScreen™ *C. difficile* Detection and Reflex Kits

- Rapid real-time PCR kit for detection of *C.difficile*
- Reflex kit to identify hypervirulent subtypes

Product #	Description	Microorganisms Detected
CDD001	*EasyScreen*™ *C. difficile* Detection Kit	Toxigenic *C. difficile* (targets both *tcdA* and *tcdB*)
CDD002	*EasyScreen*™ *C. difficile* Reflex Kit	Hypervirulent *C. difficile* incl. 027 & 078 (targets (i) *tcdC* gene deletion at position 117 and (ii) binary toxin gene (iii) *gyrA* gene mutation (fluroquinolone resistance))

Tom Riley/Kerry Carson to present at ECCMID 2014:
sensitivity 93.7% PPVs 96.8%

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Hypervirulent Strains of *C. difficile*

Identified in Australia 2010

New bacteria found in NSW

From: *AAP* December 17, 2010 2:53PM **5 comments**

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A NEW strain of a bacteria, which causes diarrhoea, has been found for the first time in NSW.

NSW Health said samples from 21 patients tested positive for the new strain of Clostridium difficile (C difficile) bacteria, also known as 027.

"The 027 strain of C difficile, while common in North America and Europe since 2003, was identified in Australia earlier this year," NSW Health said in a statement.

"C difficile is a bacteria present naturally in the gut of many healthy children under the age of two years and some adults without suffering any ill effects."

EasyScreen™ Gastrointestinal Screening Panels

Detection Kit	Targets
EasyScreen™ Enteric Bacterial Detection Kit (REF: EB003)	*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia entercolitica, Listeria monocytogenes, C. difficile*, *Aeromonas hydrophila*, *Vibrio cholera*/*parahaemolyticus*, Shiga toxigenic *E. coli* (*stx*1/*stx*2), Extraction control and Internal Positive Control
EasyScreen™ Enteric Protozoan Detection Kit (REF: EP001)	*Giardia intestinalis, Cryptosporidium spp, Entamoeba histolytica, Dientamoeba fragilis, Blastocystis hominis*, Extraction control and Internal Process Control
EasyScreen™ Enteric Viral Detection Kit (REF: EV001)	Norovirus GI, Norovirus GII, Adenovirus hexon, Adenovirus 40/41, Rotavirus A and B, Astrovirus (group 1-7), Sapovirus, and an Extraction control

EasyScreen™ Protozoan Panel

High performance vs microscopy and RT-PCR

Method	No. Samples	No of Positive samples and sensitivity					Overall Sensitivity[a]	Overall Specificity[b]
		Blastocystis	*Cryptosporidium*	*D. fragilis*	*E. complex*	*Giardia*		
EasyScreen	358	96% (51/53)	100% (9/9)	95% (41/43)	92% (22/24)	92% (24/26)	92-100%	100%
RT-PCR	358	96% (51/53)	89% (8/9)	95% (41/43)	100% (6/6)*	96% (25/26)	89-100%	100%
Microscopy	358	66% (35/53)	55% (5/9)	74% (32/43)	75% (18/24)	73% (19/26)	55-77%	95-100%

[a] Calculated as follows: (number of true positives/[number of true positives + number of false negative]) x 100

[b] Calculated as follows: (number of true negatives/[number of true negatives + number of false positives]) x 100

- The RT-PCR method used for comparison only targeted *E. histolytica*. A conventional and nested PCR was performed for further confirmation of *E. dispar* and *E. moshkovskii*

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Clinical Sensitivity and Specificity

As presented by Lee Thomas, Westmead Hospital, Sydney, at ASM 2013

Pathogen detected	*EasyScreen*™	Sensitivity %	Specificity %	Additional pathogens
Viruses (Noro, Rota, Adeno, Astro)	69	100	97.1%	25
C. difficile	58	84.8	99.4	9
Campylobacter spp.	48	100	100	0
Salmonella spp.	42	97.7	100	1
Shigella spp.	11	100	99.5	0
L. monocytogenes	1	NA	NA	1
Y. enterocolitica	3	100	100	2
D. fragilis	10	100	100	10
B. hominis	17	100	100	16
G. intestinalis	12	92.3	100	7
Cryptosporidium spp.	3	100	100	3
Entamoeba complex	5	NA	NA	5
Totals	279			79

- *EasyScreen*™ assays **identified 79 infections that were missed using traditional methods** and following SOP (28% extra)
- *EasyScreen*™ results were **generated in ~4 hours, compared to up to 4 days for traditional methods**
- **High sensitivity and specificity** across all targets

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Validation of GS1 automation

The New GS1

Optimised and easy to use for improved workflow

- Single platform to perform all sample processing steps (nucleic acid extraction) & set-up of PCR plates (96 or 384 well format)
- Optimised for all *EasyScreen*™ Assays
- Improves sample throughput
- Eliminates set up errors
- Open system for general lab use
- Developed in partnership with Hamilton Robotics - based on the Nimbus platform

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Clinical Validation of GS1

Ongoing evaluation by Damien Stark, SydPath, Sydney, 221 Specimens

Pathogen	Conventional Methods*	*EasyScreen*™
Campylobacter	7	9
Salmonella	8	9
Shigella	5	6
C. difficile	3	7
Yersinia	-	1
Cryptosporidium	-	1
Giardia	9	12
Dientamoeba fragalis	4	20
Blastocystis hominis	16	21
Entamoeba histolytica	1	1
Norovirus group II	-	7
Adenovirus	-	1
Adenovirus 40/41	-	1
Sapovirus	-	1
Total	53	97

*Viruses were not tested routinely using conventional methods

- *EasyScreen*™ assays **identified 44 infections that were missed using traditional methods** and following SOP (20% extra)
- Extra infections detected are still being validated
- *EasyScreen*™ results were **generated with <2 mins hands-on time per specimen**

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Future Developments

- Exciting new screening assays in development:
  - MRSA
  - *Mycobacterium tuberculosis/avium*
  - Meningitis
  - STIs
  - Pneumonia
  - Influenza Detection and Typing
  - Respiratory Tract Viral Infections

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- Thomas Karagiannis and Peter Huntington PaLMS
- Damien Stark and Tamalee Roberts SydPath

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  - Ken McPhee, Viral Laboratory, CIDMLS, Westmead Hospital, Westmead
  - Susie Roczo-Farkas, Enteric Virus Group, The Royal Children's Hospital, Victoria

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