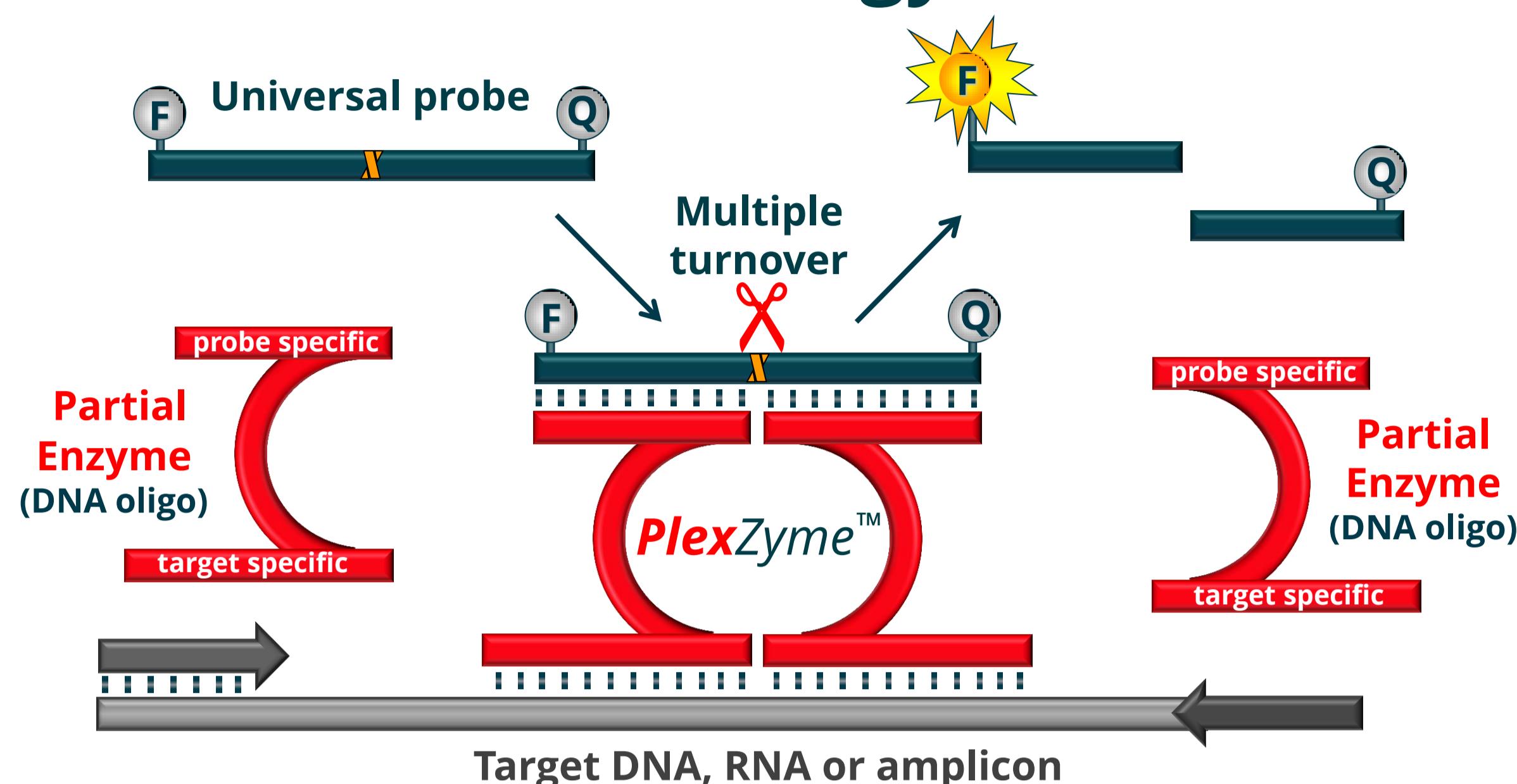


Multiplexing STI causing pathogens using **PlexPCR™**: superior multiplexing capability

Mokany E, Tan LY, Erskine SM, Njuguna P, Walker SM, Denver C & Todd AV.

SpeeDx Pty Ltd, National Innovation Centre, Sydney, Australia.

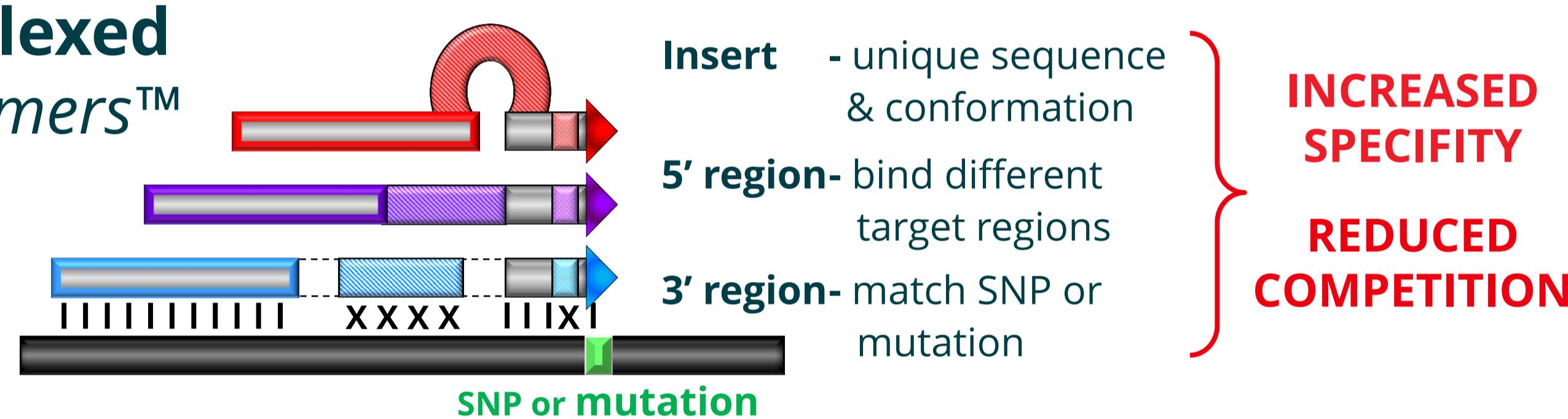
PlexPCR™ technology for NAATs



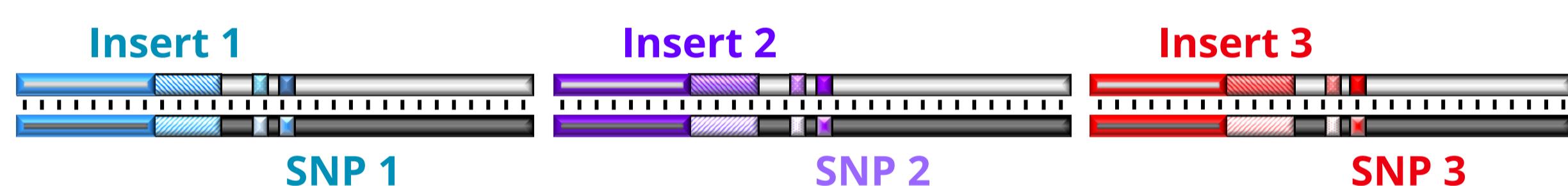
Highly specific, sensitive, universal probes and superior multiplexing capacity

Flexible PlexPrime™ design for multiplexing

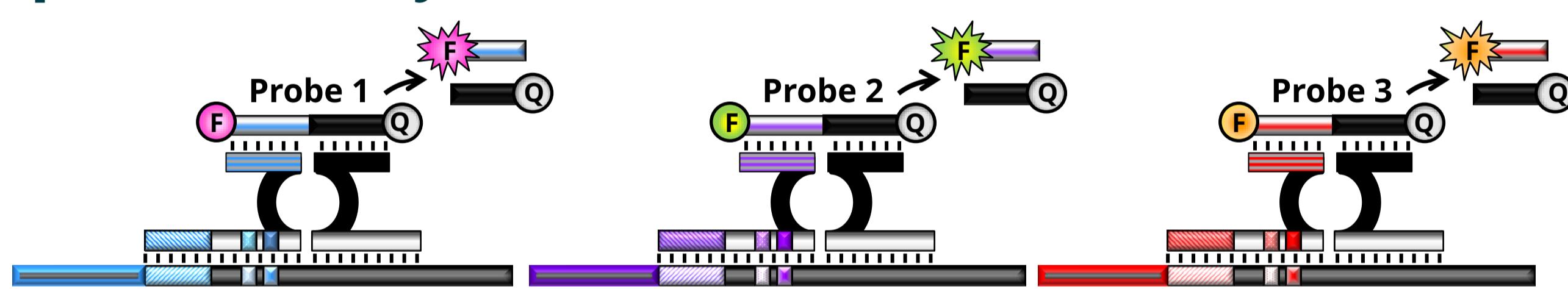
Multiplexed PlexPrimers™



PlexPrime™ amplicons are distinctly different



Allele-specific PlexZyme™ detection

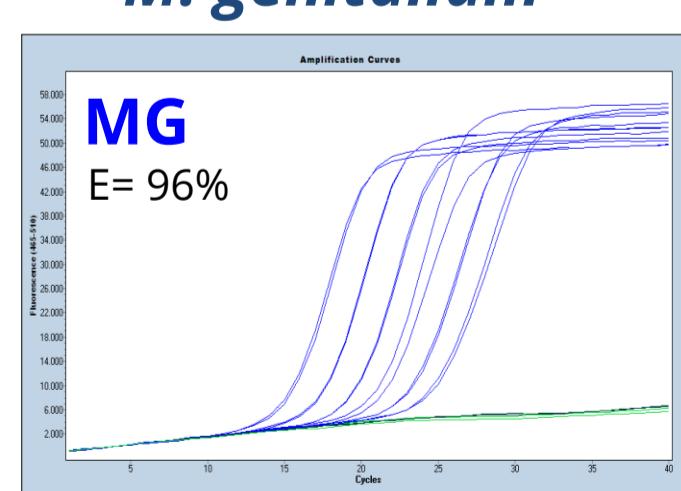


Superior multiplex capacity for clustered mutations

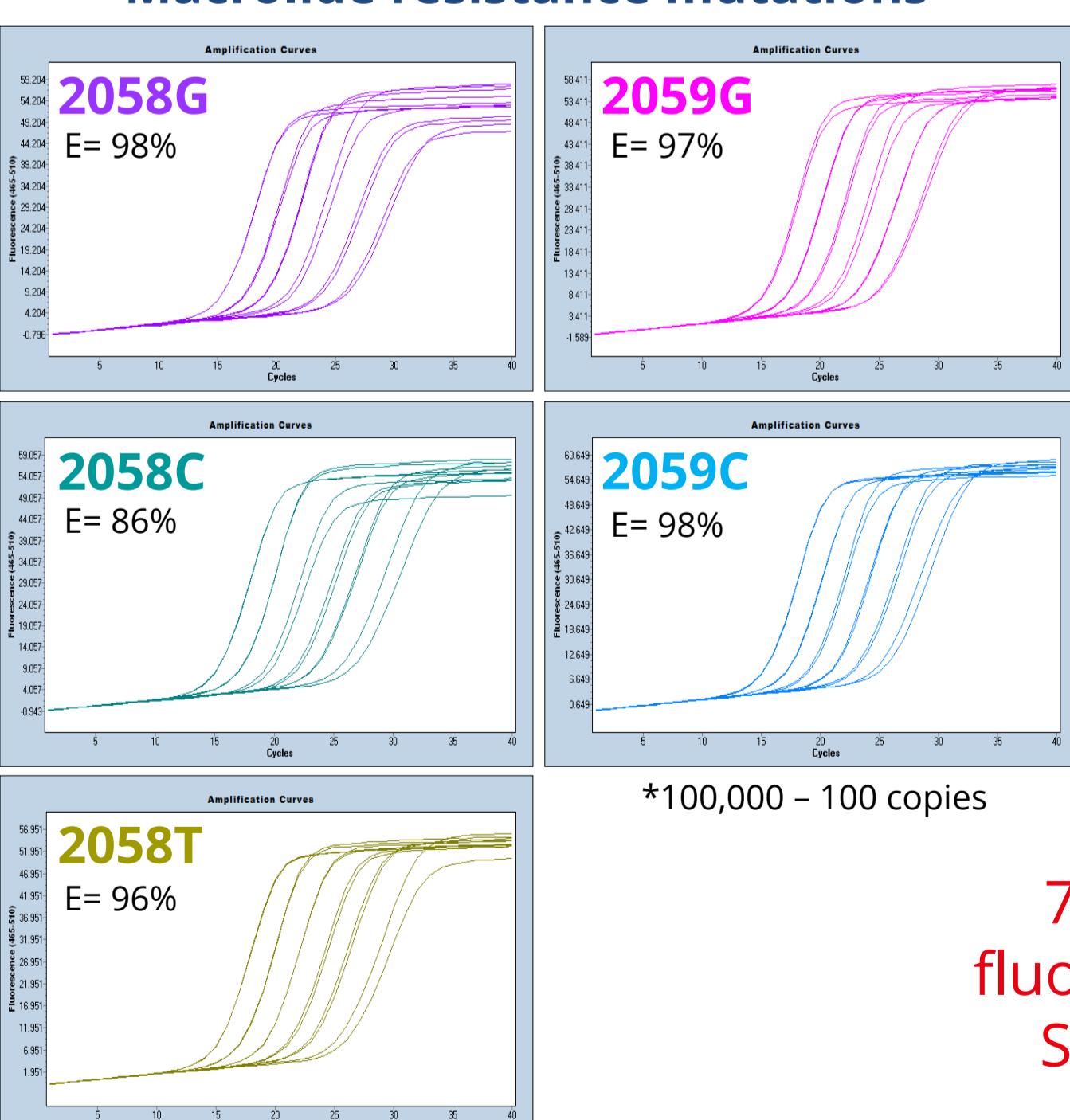
Multiplexed Mgen ResistancePlus™ assay

Simultaneous detection of *M. genitalium* and 5 mutations associated with macrolide resistance

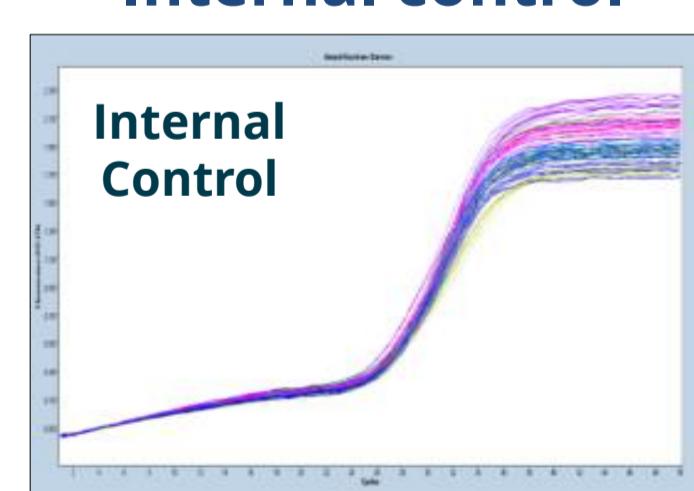
Readout 1 *M. genitalium**



Readout 2 Macrolide resistance mutations*



Readout 3 Internal control



7-plex

7 targets read in 3 fluorescent channels (5 SNPs in 1 channel)

**Efficient and sensitive detection of 5 mutations
Compatible with conventional qPCR machines**

STI assays

CT, NG(x2), LgV

Chlamydia trachomatis (CT)
Neisseria gonorrhoeae (NG) porA
Neisseria gonorrhoeae (NG) omp
Lymphogranuloma Venereum (LgV)

Mgen ResistancePlus™

Mycoplasma genitalium (MG)
23S macrolide resistance

STI-1

Mycoplasma genitalium (MG)
Mycoplasma hominis (MH)
Ureaplasma parvum (UP)
Ureaplasma urealyticum (UU)
Trichomonas vaginalis (TV)

Coming Soon

- Gonorrhoeae ResistancePlus™
- Syphilis

All assays include an internal extraction/amplification control

Herpes Simplex Proficiency Panel

HSV-1&2, VZV assay tested on RCPA Quality Assurance Program HSV panel

Sample^	Expected results	Approx. cps/ml	SpeeDx PlexPCR™		Focus Diagnostics Simplexa^	
			HSV 1*	HSV 2*	HSV 1*	HSV 2*
9A	HSV-1	10 ⁵	15.8		28.9	
9B	HSV-2	10 ³		19.9		31.2
9C	negative	-	-	-	-	-
9D	HSV-1	10 ⁵	16.2		29.0	
9E	HSV-1 & HSV-2	10 ⁴ & 10 ²	18.3	22.6	32.1	36.0
9F	HSV 2	10 ³		20.1		32.5

*Samples and results provided by RCPAQAP (molecular.infectious@rcpaqap.com.au)

* Cq value (SpeeDx protocol has 10 cycle touchdown upfront)

**Correct identification of 5 positive and 1 negative sample
Strong signals compared to other commercial kit**

HSV-1&2, VZV assay Independent Evaluation

HSV-1&2, VZV assay tested on 120 clinical samples

HSV-1	Independent		HSV-2	Independent		VZV	Independent				
	+	-		+	-		+	-			
SpeeDx	+	18	0	SpeeDx	+	10	0	SpeeDx	+	19	1
	-	0	102		-	0	110		-	0	100

Very high clinical sensitivity and specificity

HSV-1: 100% and 100%, HSV-2: 100% and 100%, VZV: 100% and 99%, respectively

99.2% concordance with independent assay

Only discrepancy was a mixed infection not detected by the independent assay

STI assay Independent Evaluation

M. genitalium	Independent		M. hominis	Independent				
	+	-		+	-			
SpeeDx	+	7	0	5	4	SpeeDx	+	-
	-	0	44	0	42		-	0
U. urealyticum	Independent		U. parvum	Independent				
	+	-		+	-	SpeeDx	+	-
SpeeDx	+	14	0	19	2		-	0
	-	1*	36	0	29		-	0

M. genitalium correlated 100% between the assays tested.

U. urealyticum, *U. parvum* & *M. hominis* correlated 98%, 96% & 92% respectively between the assays.

Highly sensitive SpeeDx assay detected 4 extra *M. hominis* and 2 extra *U. parvum*.

* Upon repeat of independent assay the result was not detected

Sensitive assay; good concordance with Independent assay

PlexPCR™ is a flexible, rapid & cost-effective technology for multiplexed detection of targets and genetic variants

If you are interested in multiplexing your assay and/or wanting to achieve specific single base discrimination contact info@speedx.com.au
or for more information about **PlexPrime™** & **PlexPCR™** technology visit www.speedx.com.au