



Fonterra's experience with the GENE-UP



Why Change?

- Historical
 - ISO or FDA-BAM for final product pathogen testing
 - TECRA for environmental pathogen testing
- Phasing out of the TECRA method an opportunity to re-evaluate all pathogen testing being carried out by Fonterra.
- Decisions, Decisions!
 - Environmental and/or Final Product
 - Test in-house or by contract laboratories

Selection Process



- ~10 different methods evaluated as a paper based exercise
- Ranking of methods based on:
 - Approvals
 - Time to result
 - Ease of use
 - Support
 - Cost

Work Flow

- Easy work flow for the GENE-UP for both *Listeria* and *Salmonella*

- Pre-enrich the sample 37°C for 21 ± 3 h.

- 20 µL to lysis tube



- Vortex 5 min

- 5 µL of lysed sample to 5 µL of PCR reagent in a 96-well plate

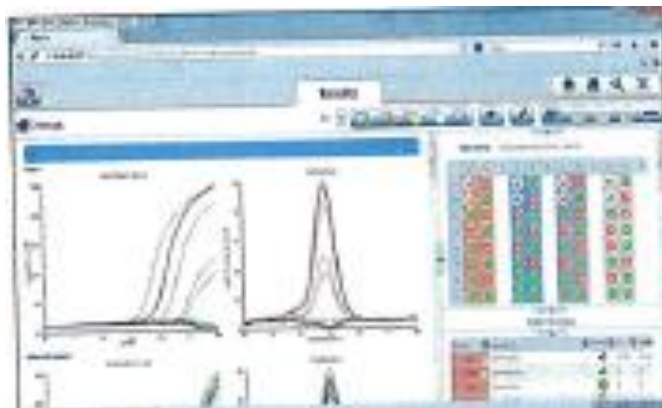
- Add strip caps



- Spin 5 seconds

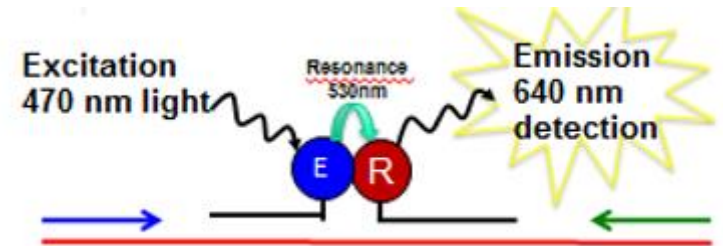
- Run the plate (< 1 h)

- View the results



Technology

- Mechanical lysis method
- GENE-UP detection is a real time PCR technique based on FRET technology.
- FRET Components
- FRET signal & Melting point both used to determine a result.



Target call or external control call	Internal Control call	Final result status (target+IC)	Test Result interpretations
+	+	+	Positive test Result
+	-	-	Negative test Result
-	+	!	Invalid test Result
-	-	✓	Successful negative or positive external control
+	+	✓	Successful negative or positive external control
-	-	X	Failed negative or positive external control
-	-	X	Failed negative or positive external control

Handwritten annotations in red:

- A red oval encircles the 'Sample results' column (the 'Final result status' column), encompassing the +, -, and ! icons.
- A red oval encircles the 'External control results' column, encompassing the ✓ and X icons.
- A red box labeled 'Sample results' is placed next to the '-' icon in the second row.
- A red box labeled 'External control results' is placed next to the 'X' icon in the sixth row.

GENE-UP Verification *Salmonella*



- Inclusivity & sensitivity

Organism ID	0 cfu/swab		5 cfu/swab		15 cfu/swab		50 cfu/swab	
	GeneUp	TSA Count cfu	GeneUp	TSA Count cfu	GeneUp	TSA Count cfu	GeneUp	TSA Count
<i>S. enterica</i> Menston NZRM 383	-ve	0	+ve	10	+ve	8	+ve	46
<i>S. Adelaide</i> FMX1	-ve	0	+ve	5	+ve	23	+ve	68
<i>S. typhimurium</i> FMX38	-ve	0	+ve	3	+ve	18	+ve	65
<i>S. Mbandaka</i> FMX2	-ve	0	+ve	8	+ve	13	+ve	59
<i>S. typhimurium</i> FMX3	-ve	0	+ve	4	+ve	25	+ve	59
<i>S. Uganda</i> FMX54	-ve	0	+ve	2	+ve	8	+ve	8
<i>S. typhimurium</i> FMX60	-ve	0	+ve	7	+ve	23	+ve	67
<i>S. spp.</i> FMX53	-ve	0	+ve	5	+ve	9	+ve	35
<i>S. typhimurium</i> FMX4	-ve	0	+ve	8	+ve	16	+ve	58
<i>Salmonella</i> Infantis FMX5	-ve	0	+ve	6	+ve	21	+ve	68

GENE-UP Verification *Salmonella*



- Exclusivity & competition with non-target organisms

Organism ID	1000 cfu/swab		1000 cfu/swab 10 cfu/swab <i>Salmonella</i> Menston		
	GeneUp	TSA Count	GeneUp	TSA Count	TSA Cnt Menston
<i>S. aureus</i>	-ve	737	+ve	737	7
<i>B. cereus</i>	-ve	20	+ve	20	7
<i>E. coli</i>	-ve	730	+ve	730	7
<i>E. cloacae</i>	-ve	674	+ve	674	7
<i>E. aerogenes</i>	-ve	810	+ve	810	7
<i>E. faecalis</i>	-ve	1187	+ve	1187	7
<i>L. seeligeri</i> A77	-ve	628	+ve	628	7
<i>P. aeruginosa</i>	-ve	1002	+ve	1002	7
<i>S. epidermidis</i>	-ve	50	+ve	50	7
<i>C. sakazakii</i>	-ve	943	+ve	943	7

GENE-UP Verification *Listeria*



- Inclusivity & sensitivity

Organism	0 cfu/swab		5 cfu/swab		15 cfu/swab		50 cfu/swab	
	GeneUp	TSA Count	GeneUp	TSA Count	GeneUp	TSA Count	GeneUp	TSA Count
<i>L. seeligeri</i> A77	-ve	0	+ve	4	+ve	12	+ve	30
<i>L. innocua</i> 4968010	-ve	0	+ve	5	+ve	11	+ve	31
<i>L. seeligeri</i> A76	-ve	0	+ve	3	+ve	12	+ve	34
<i>L. innocua</i> #88	-ve	0	+ve	2	+ve	9	+ve	33
<i>L. innocua</i> #87	-ve	0	+ve	7	+ve	23	+ve	68
<i>L. innocua</i> #86	-ve	0	+ve	5	+ve	10	+ve	37
<i>L. ivanovi</i> NZRM 797	-ve	0	+ve	1	+ve	14	+ve	48
<i>L. innocua</i> 4970468	-ve	0	+ve	5	+ve	16	+ve	34
<i>L. innocua</i> FMX6	-ve	0	+ve	2	+ve	17	+ve	58
<i>L. innocua</i> FMX7	-ve	0	+ve	6	+ve	11	+ve	50
<i>L. innocua</i> FMX8	-ve	0	+ve	4	+ve	13	+ve	48

GENE-UP Verification *Listeria*



- Exclusivity & competition with non-target organisms

Organism ID	1000 cfu/swab		1000 cfu/swab 10 cfu/swab <i>L. seeligery</i> A77		
	GeneUp	TSA Count	GeneUp	TSA Count	TSA Cnt <i>L.seeligery</i>
<i>S. aureus</i>	-ve	737	+ve	737	8
<i>B. cereus</i>	-ve	20	+ve	20	8
<i>E. coli</i>	-ve	730	+ve	730	8
<i>E. cloacae</i>	-ve	674	+ve	674	8
<i>E. aerogenes</i>	-ve	810	+ve	810	8
<i>E. faecalis</i>	-ve	1187	+ve	1187	8
<i>Salmonella</i> Menston	-ve	843	+ve	843	8
<i>P. aeruginosa</i>	-ve	1002	+ve	1002	8
<i>S. epidermidis</i>	-ve	50	+ve	50	8
<i>C. sakazakii</i>	-ve	943	+ve	943	8

Lab Setup and Accreditation

- Great support from Biomerieux in training and setting up the system.
- Use a dedicated room for GENE-UP testing
- IANZ Accreditation 1st June.
- Routine testing of environmental swab samples from 28th June.



FRET components

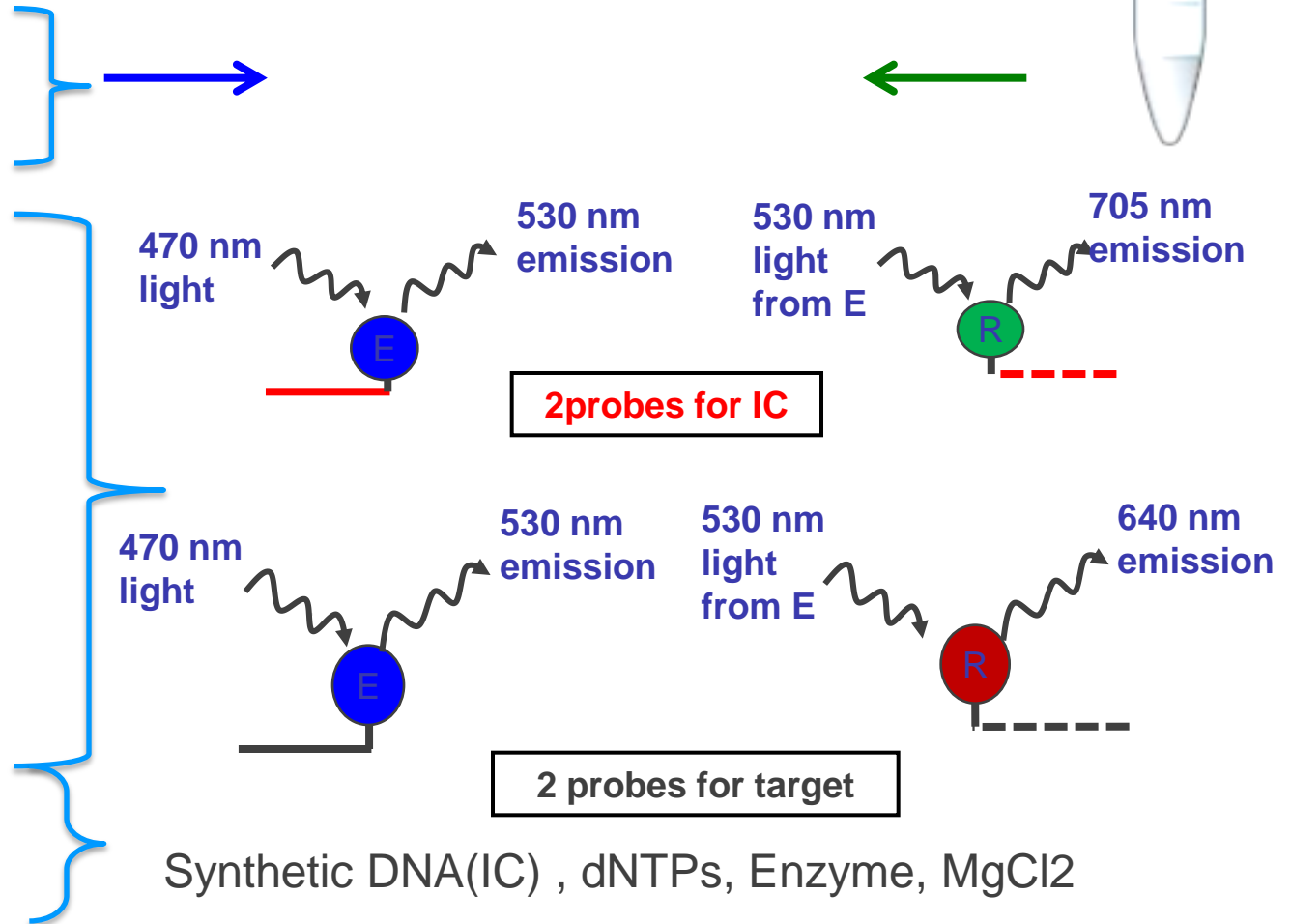
- Several components In PCR reagents :PCR MIX (pellet)



2 primers same for IC and target

4 probes with 3 different fluorophores

And other components



GENE-UP results per sample

