

A Complete Solution for Bacterial Genotyping:

- Thermo Scientific KingFisher System for DNA Purification Followed by High Resolution Melting Analysis with Thermo Scientific PikoReal qPCR Instrument

Chuck Maw

Sample Preparation & Analysis

Microbial Genotyping Workflow

Thermo Scientific™ products for:

DNA purification



KingFisher Duo
instrument

DNA
concentration
and quality

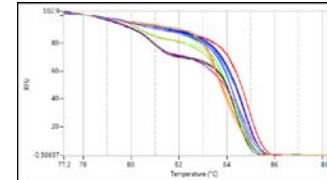


Multiskan GO
instrument

Melting curve
genotyping

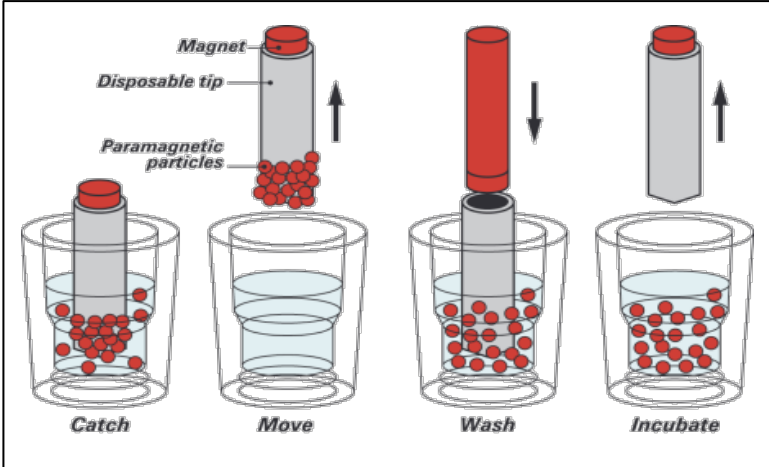


PikoReal qPCR
instrument



Microbial DNA Purification

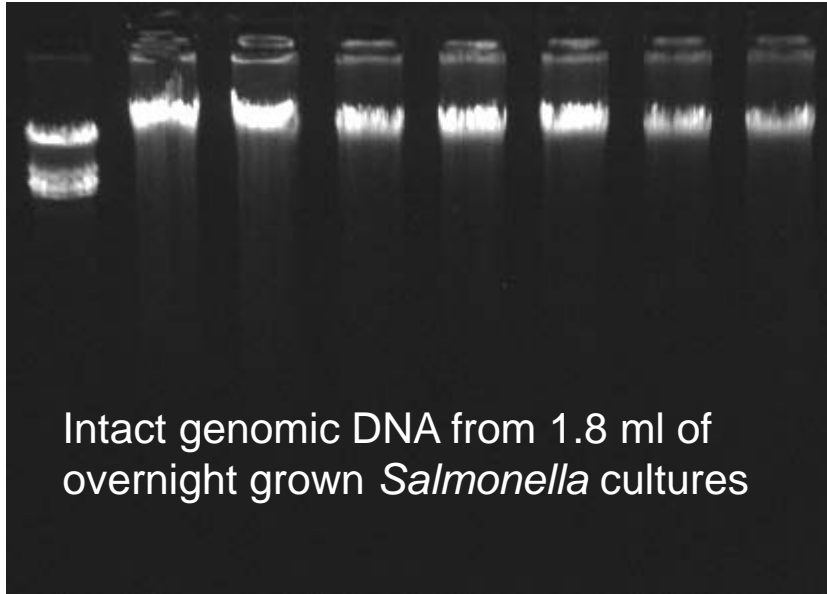
Thermo Scientific™ KingFisher™ instruments are magnetic particle processors for DNA, RNA, protein and cell isolation from variable starting materials. We offer eight Thermo Scientific KingFisher Kits optimized for DNA or RNA purification from different sample types.



The idea of KingFisher is to move the magnetic particles instead of liquids.

Microbial DNA Purification

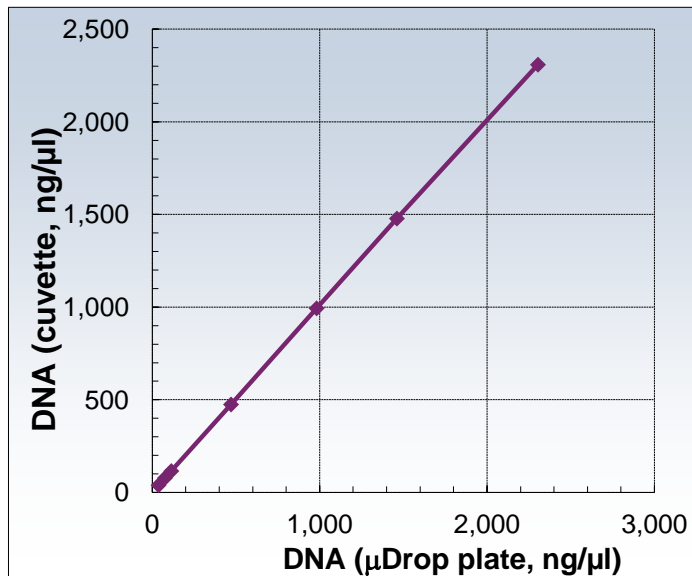
- Thermo Scientific KingFisher Cell and Tissue kit is suitable for purification of high quality genomic DNA from bacteria.



DNA Concentration and Purity Assessment



- Thermo Scientific™ Multiskan™ GO
 - Spectrophotometer
 - Scalable throughput
 - Cuvettes, plates and μ Drop Plate
- Thermo Scientific™ μ Drop™ Plate
 - Quick and easy
 - Volume range 2-10 μ l
 - No sample dilution necessary
 - 16 samples measured simultaneously



Cuvette

μ Drop Plate

96-well UV plate

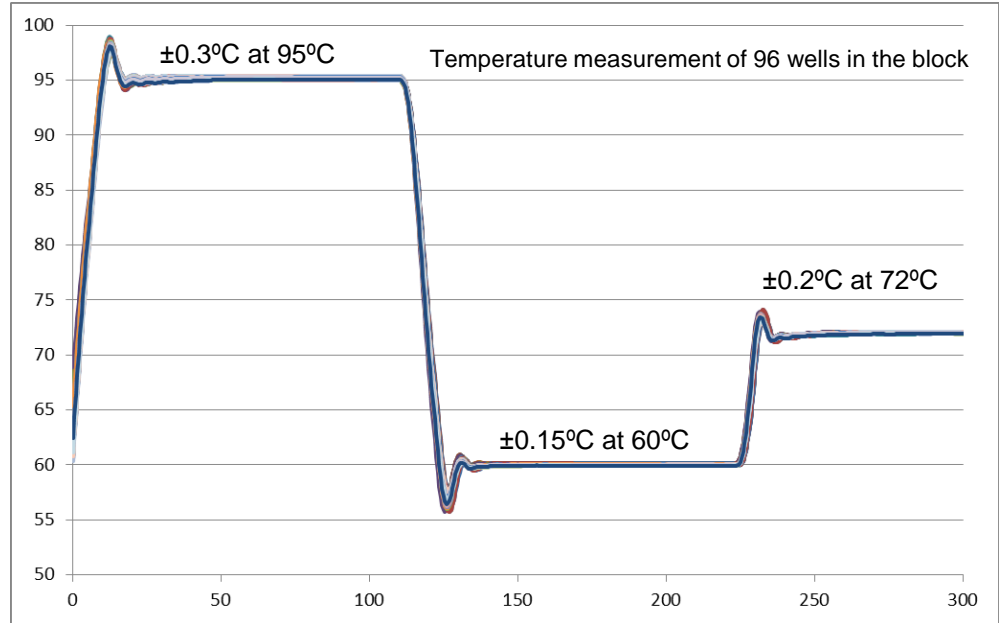
384-well UV plate

Scalable Throughput

Precise Block Temperature Uniformity



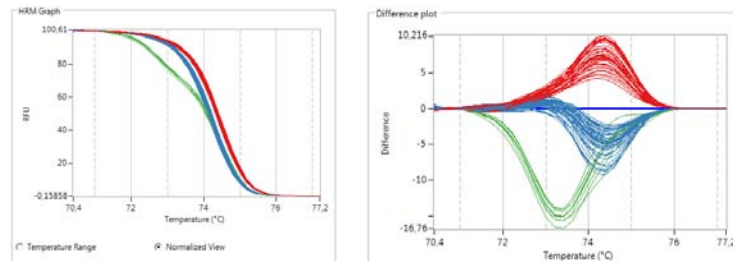
Thermo Scientific™
PikoReal™
qPCR



High Resolution Melt (HRM) - Easy, Fast and Affordable

- Similar analysis to traditional melt curve analysis with SYBR Green intercalating dye.
- HRM master mixes with saturating fluorophores, such as EvaGreen are used to gain additional resolution.
- Instrument and software capable of HRM analysis are required to perform HRM experiments.

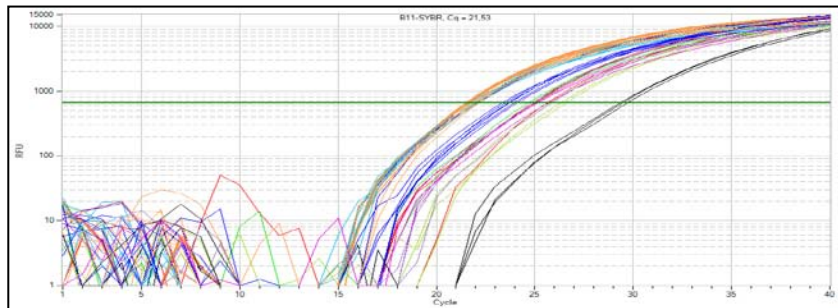
Thermo Scientific PikoReal
Software includes free HRM
analysis module!



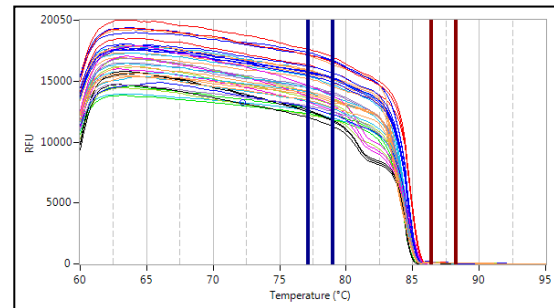
Class4 (A/T) SNP detection

Simple Pre-steps for HRM Analysis

1. Amplify targets

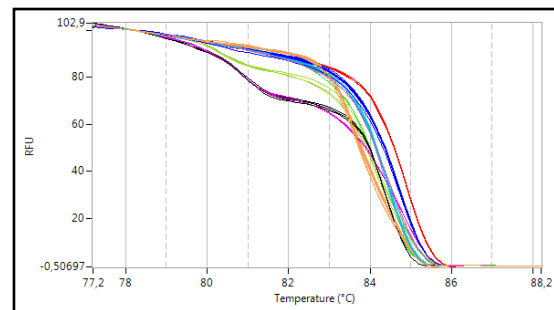


2. Melt amplified targets



Thermo Scientific
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3. Normalize melt curves

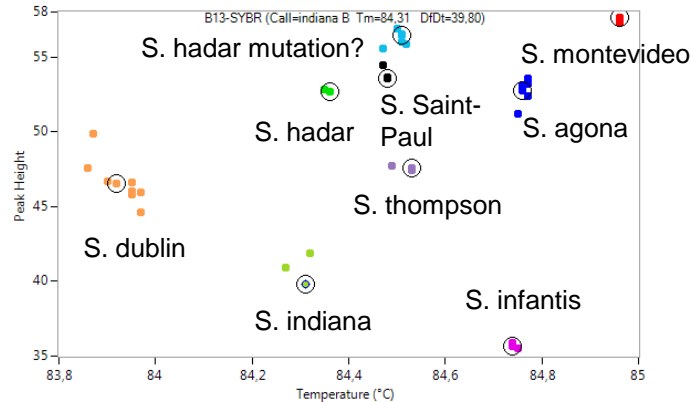
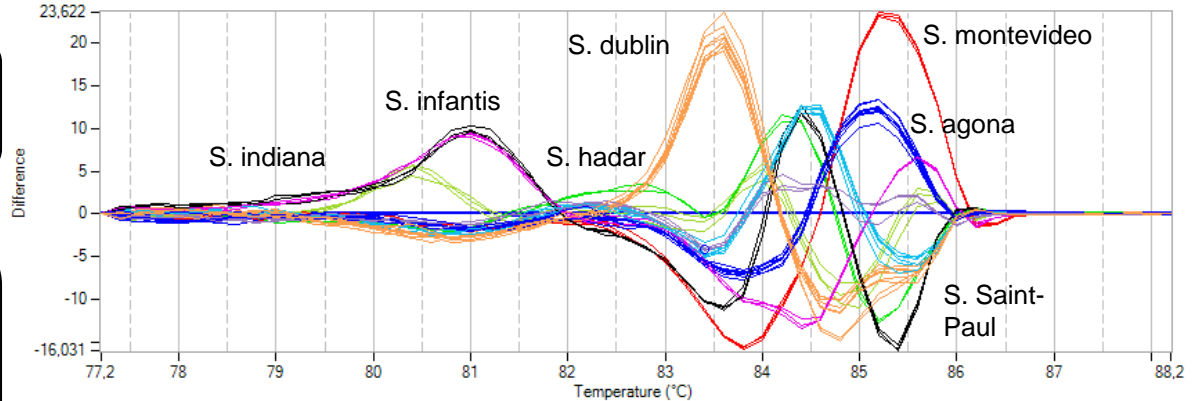


Detection of *Salmonella* Genotypes with HRM Analysis



Difference Plot Analysis

Scatter Plot Analysis - quick and intuitive clustering - **only available in Thermo Scientific PikoReal Software!**

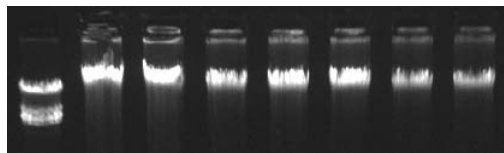


Sample Type	Call	Call Efficiency
AD / HRM Standard	S. indiana B	100,0
Unknown	S. indiana B	87,0
Unknown	S. indiana B	84,4
AD / HRM Standard	S. infantis C1	100,0
Unknown	S. infantis C1	96,1
Unknown	S. infantis C1	96,4
AD / HRM Standard	S. Montevideo C1	100,0
Unknown	S. Montevideo C1	93,5
Unknown	S. Montevideo C1	96,3
AD / HRM Standard	S. Saint-Paul	100,0
Unknown	S. Saint-Paul	95,2
Unknown	S. Saint-Paul	96,0
AD / HRM Standard	S. Thompson	100,0

Summary



Thermo Scientific KingFisher Duo



Purify

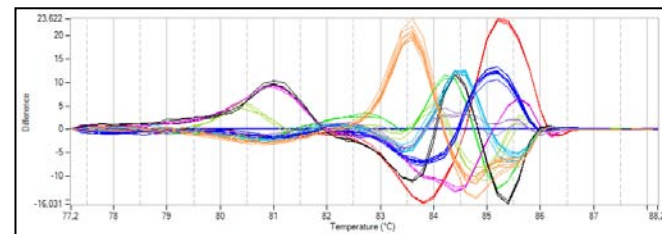
Thermo Scientific Multiskan GO



Sample	μDrop plate	
	(ng/μl)	CV%
1	4.8	10
2	23.5	5
3	118.5	1
4	588.1	1

Analyze

Thermo Scientific PikoReal



Identify