Stochastic Profiling in Mycobacteria

Will Matern

Summary

- Extracted Mtb total RNA using Trizol/RNeasy
  - gDNA should me minimal but there is no DNase treatment.
- Primed RT with:
  - TTTTTTTTTTTTTTTTTTTTTTTTNNNNNNNNNNNNNNN
  - Adds a poly-T “cap” to the cDNA.
  - Rest of protocol is the same.

Remarks

- Necessary as many mRNAs in Mtb are believed to lack polyadenylation.
  - However, many ARE poly-adenylated.
- As the RT primer binds randomly to a transcript there are many potential cDNA products.

Results

No RT and No Template controls

Plate 1

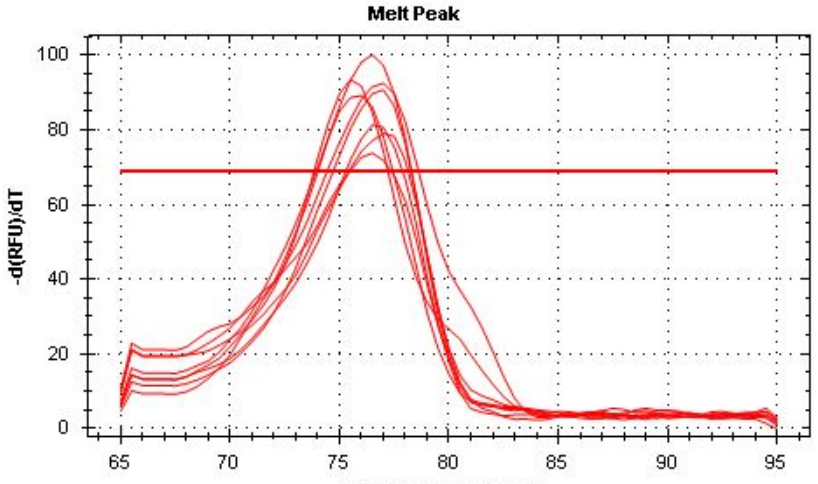
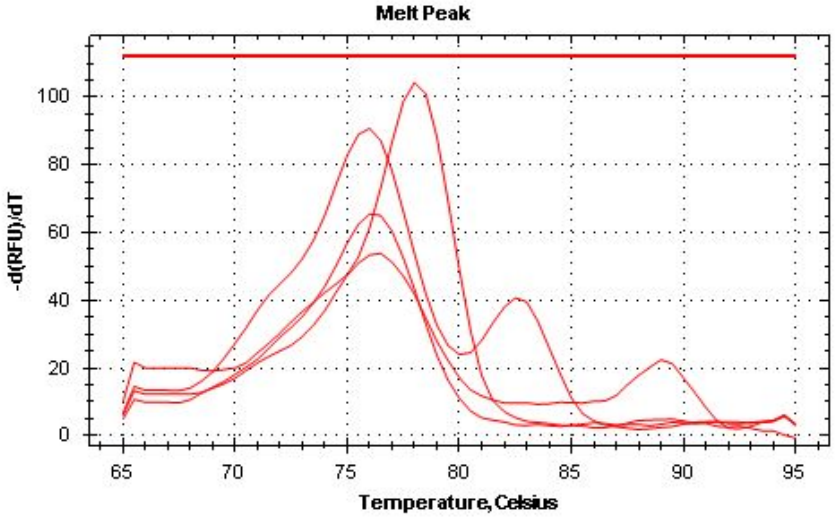
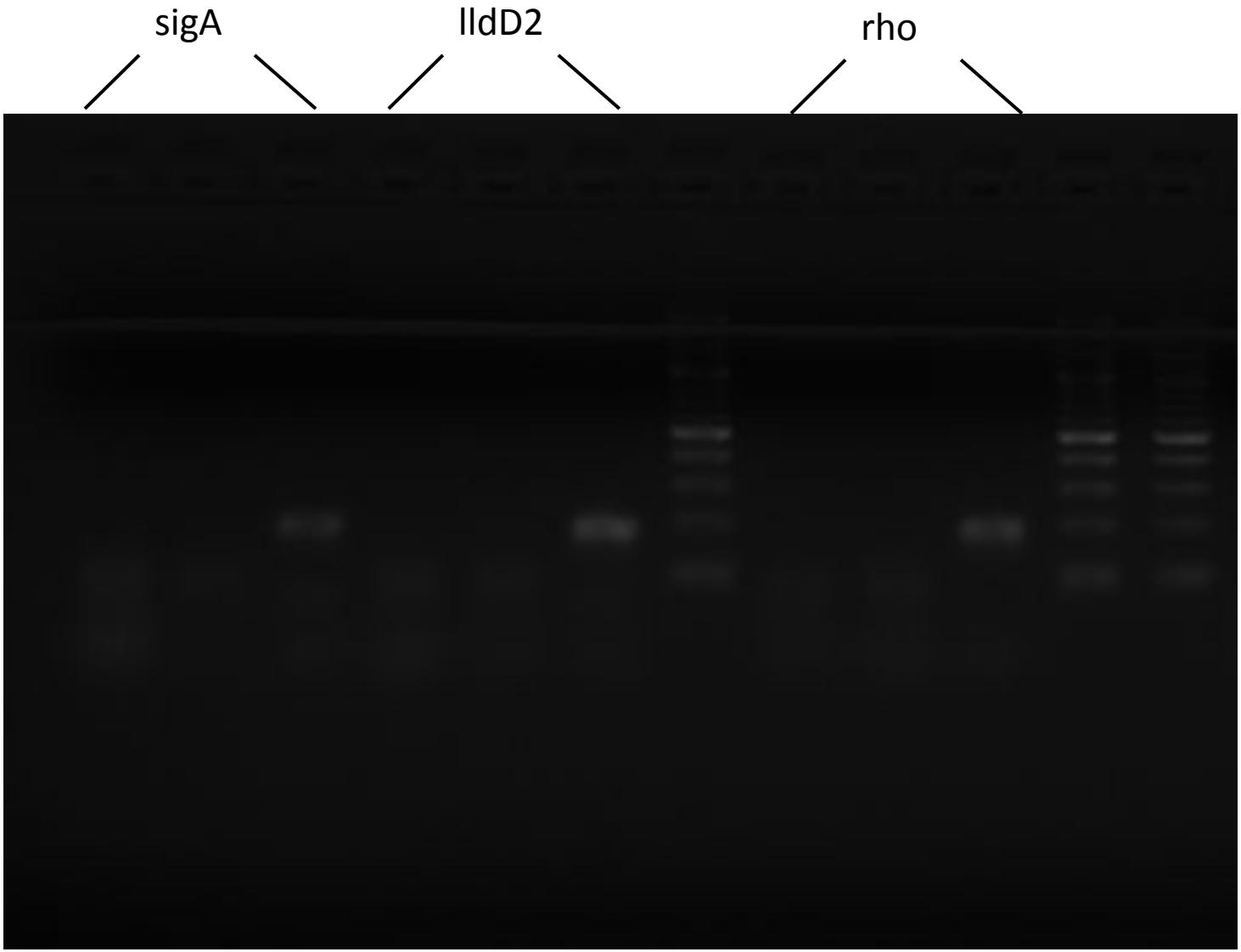


Plate 2



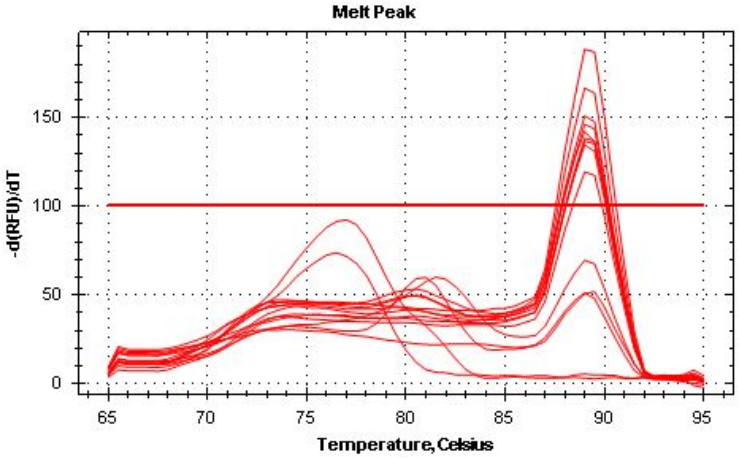
No RT and No Template seem to have the same product. Means that gDNA does not show a large effect.

Results

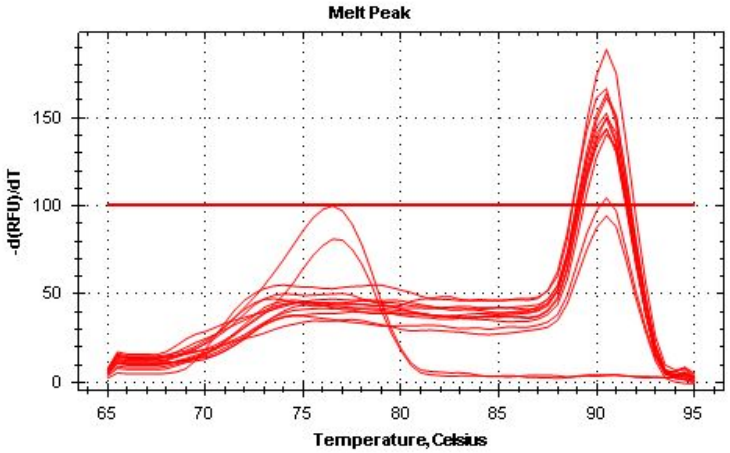


Results

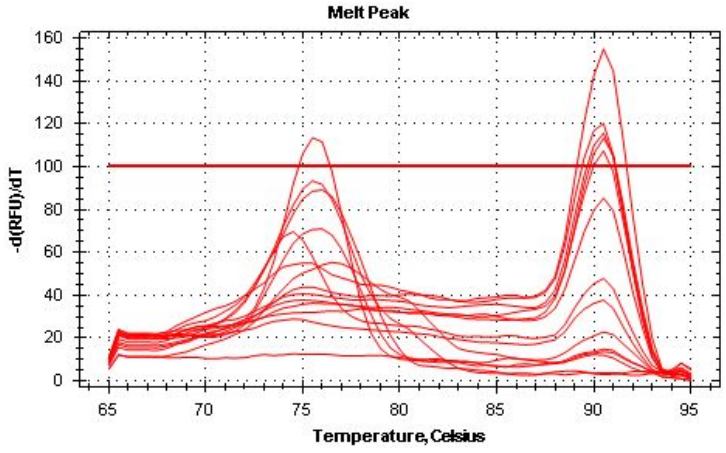
sigA_v1



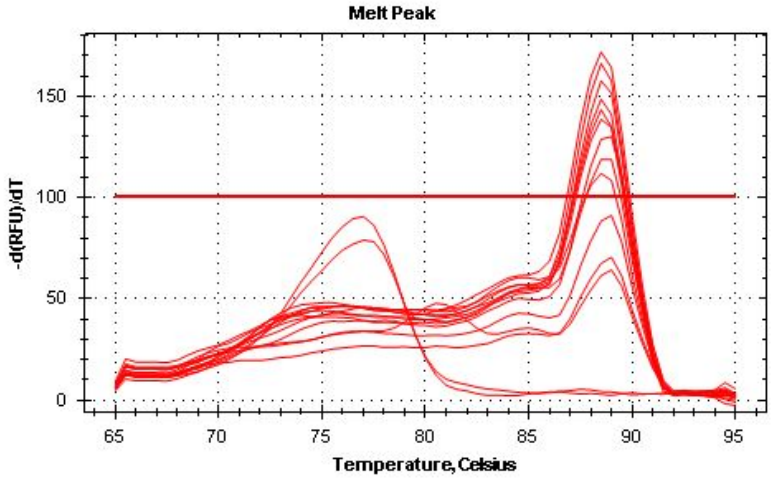
lldD2



sigA_v2



rho



Conclusions

- sigA_v1 seem to be better at amplifying than sigA_v2 primers.
- Optimal settings: ~15ug of AL-1 primer @ 30 cycles.
- Can do stochastic profiling in Mtb with random priming of RT (pending some new ways to extract RNA).
- gDNA does not appear to be an issue with our current extraction technique.