Train Malta

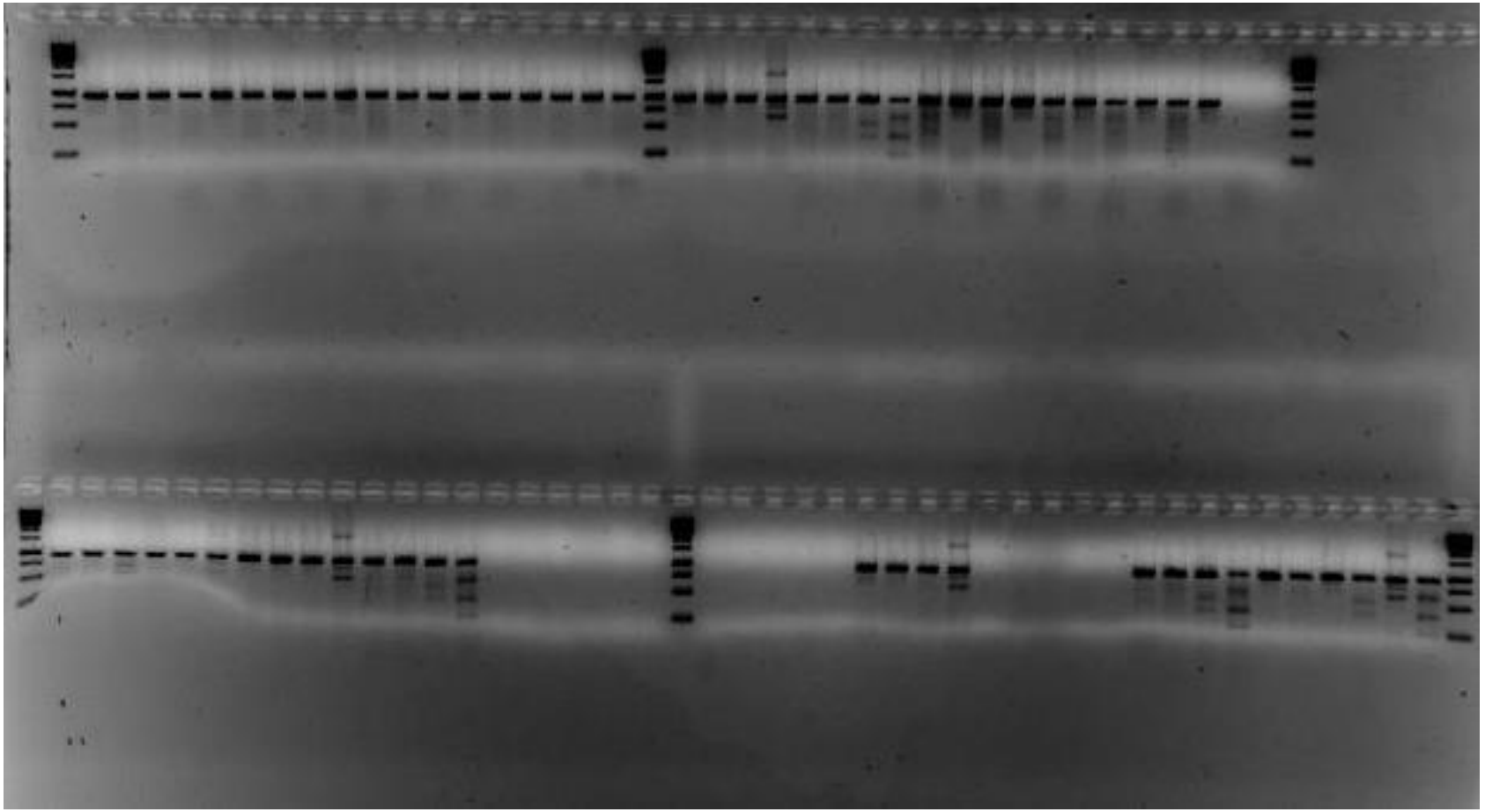
Final Results

September 2017

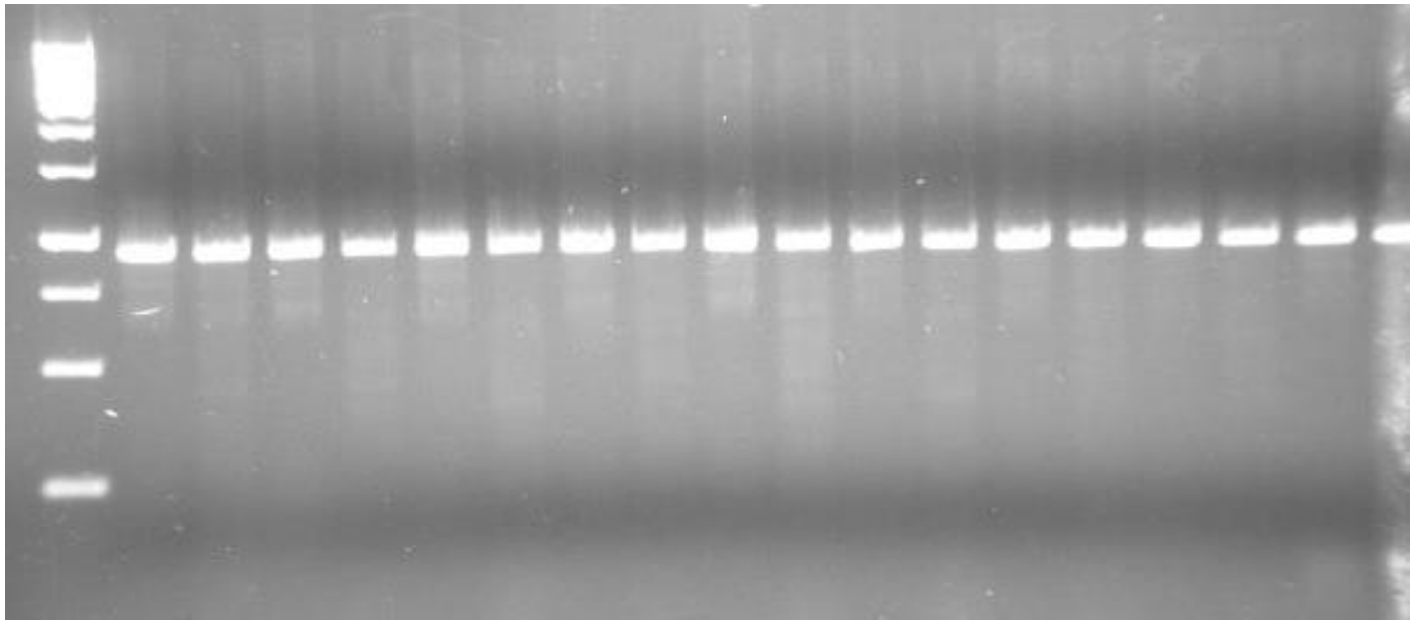
Big well done!

It was a tough week and everyone really got into the experiments. This was not an easy set of experiments and we asked a lot. Overall, it was fun and successful; we all learnt something from the process and this is the reason we run it. Granted, not all experiments worked perfectly but then again this is a normal part of the process (this is why we include controls! So when we repeat we can work out why this step did not work correctly).

Final Gel Electrophoresis Image



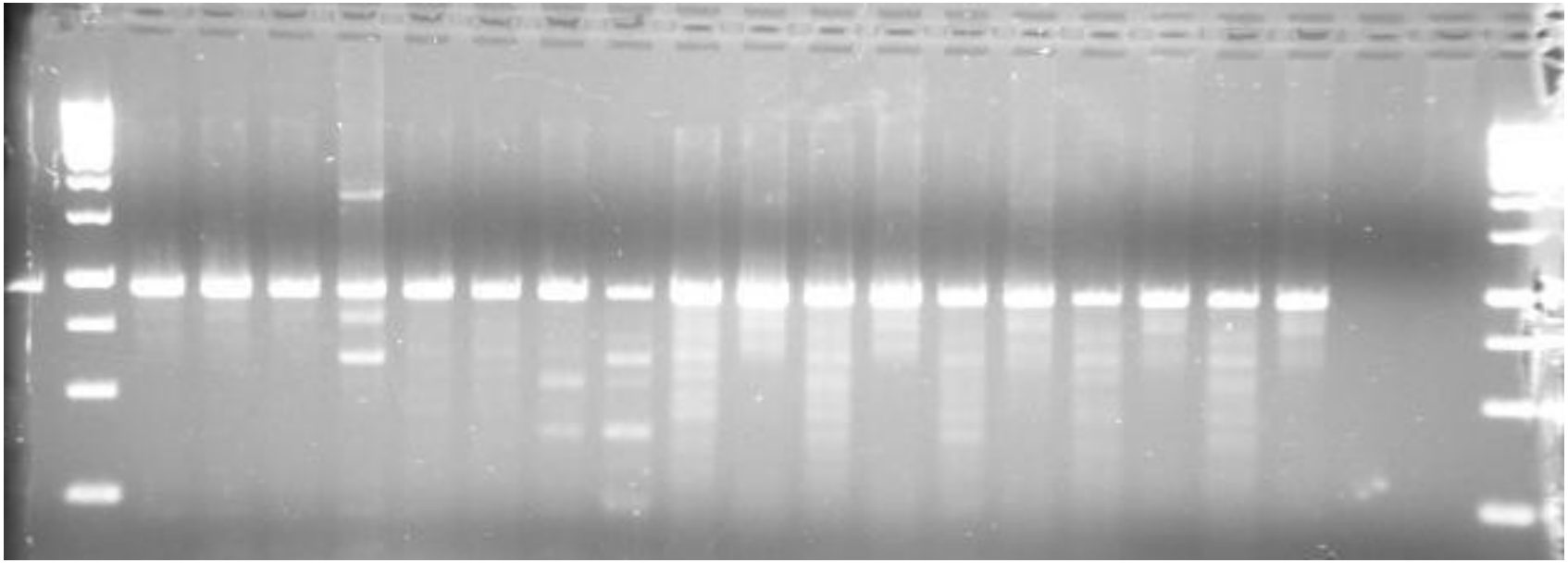
Final: Top Left Corner



ALEX

A0 A0(T7) A2 A2(T7) A3 A3(T7) B0 B0(T7) B2 B2(T7) B3 B3(T7) C0 C0(T7) C2 C2(T7) C3 C3(T7)

Final: Top Right Corner

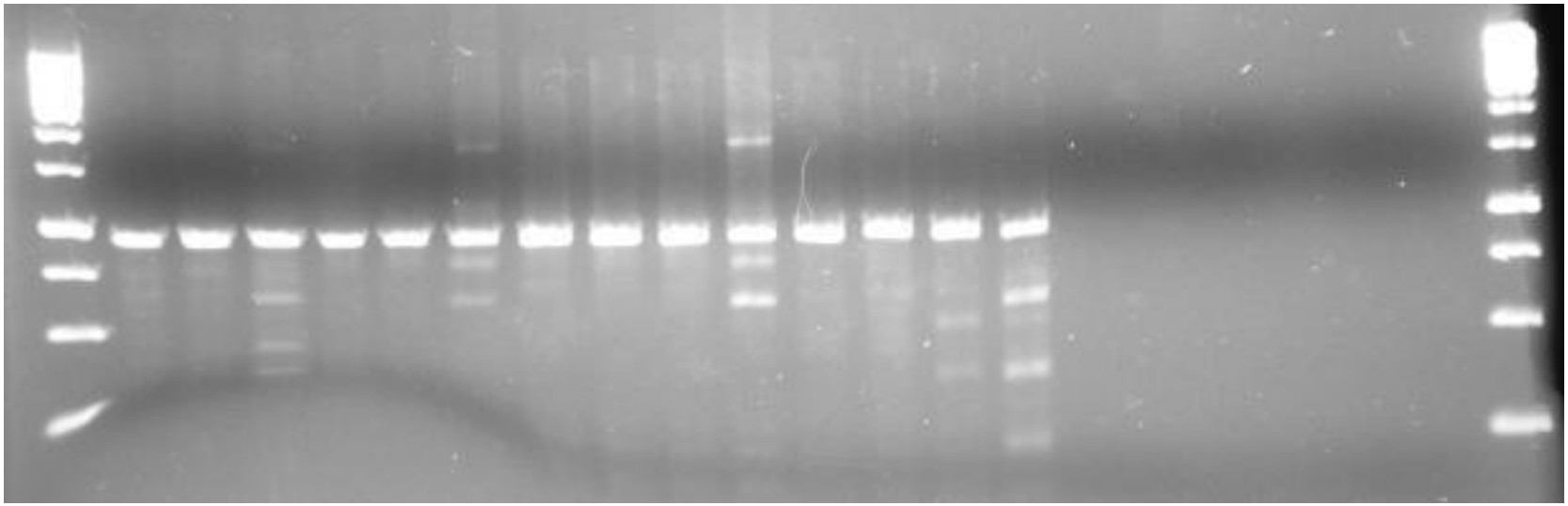


RS
1(NE) 2(NE) 3(NE) 4(NE) 1(T7) 2(T7) 3(T7) 4(T7)

BV
1E !N 2E 2N 3E 3N

MP
1 1’ 2 2’ 3 3’

Final: Bottom Left Corner

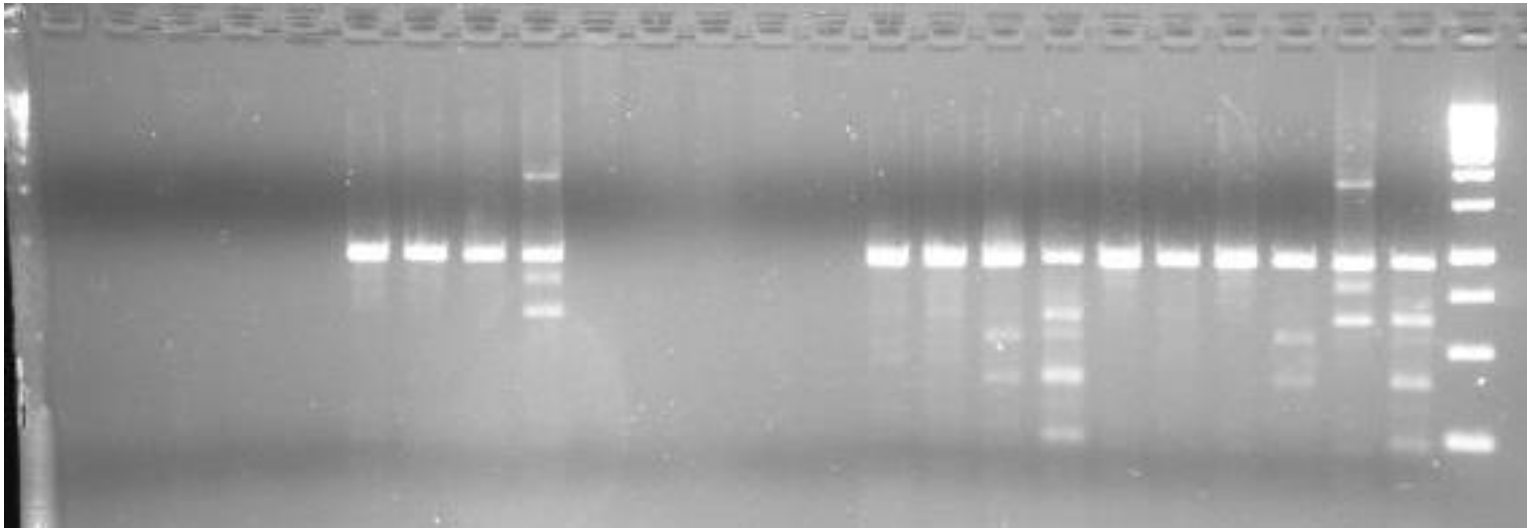


SI
1+ 2+ 3+ 1- 2- 3-

JAZ
0(NE) 1(NE) 2(NE) 3(NE) 0(T7) 1(T7) 2(T7) 3(T7)

IR
1 2 3 4 5 6

Final: Bottom Right Corner



Av (T7)
0 1 2 3 NT

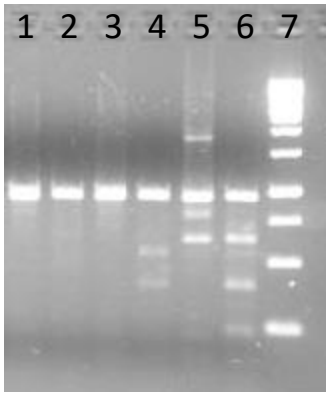
O (T7)
-ve 1 2 3

IN or AN (No Enzyme)
0 1 2 3 NT

O (T7)
-ve 1 2 3

M2=KR
1a 1b 2a 2b 3a 3b

What do we expect?



Something like this!

1	2	3	4	5	6	7
Cas9-only Undigested	Cas9-only T7 Digested	Guide1+Cas9 Undigested	Guide1+Cas9 T7 Digested	Guide1+2+Cas9 Undigested	Guide1+2+Cas9 T7 Digested	1kb DNA Ladder

In Summary

LANES 1-2) As there is no guide to lead the enzyme to cut then we expect there to be no difference between the Cas9-only digested and undigested (which we observe in Lanes 1 and 2).

LANES 3-4) You will see that when we have added a single guide with the Cas9, then we see that the guide is directed to the region of interest and there is an obvious difference between the digested and undigested (Lanes 3 and 4); what we observe is a single consistent cut/repair site, which results in two smaller bands that equal the same molecular weight as the original PCR product.

LANES 3-5) When we add two guides (that are close enough together – like in this example) we expect to see cleavage, and removal, of the region and therefore expect to see multiple PCR products with differing lengths (depending on how consistently the cells repair this region), so in this example we should be comparing the Undigested Guide1+Cas9 (Lane 3) against the Undigested Guide1+2+Cas9 (Lane 5) – which we observe multiple bands that indicate double cleavage of this region; concluding that both guides are working with reasonable efficiency. As you will observe in the Undigested Guide1+2+cas9 (Lane 5) we have multiple unexpected bands and at this point in time we can only assume this is due to the denaturing and re-annealing of the fragments causing aberrant fragments/loops (we did not do the post-PCR gel electrophoresis so with our results we can only speculate about this).

There is another comparison between the Guide1+2+Cas9 undigested and digested (lanes 5 and 6), but due to having two cutting sites there are many permutations of PCR products and it is difficult to explain every band that is present – you can however compare the Digested Guide1+Cas9 (Lane 4) to Guide1+2+Cas9(Lane 6), this clearly shows that the introduction of the second guide has an effect and is functional. For a completely robust check of the second guide, then we would have also tested this guide without the presence of the other guide, but to reduce the workload we omitted it this time.

CONCLUSION) Ultimately we are trying to ascertain if both guides efficiently target the region of interest and from this test we can conclude that they both are directed to our region of interested. Note this does not tell us anything about off-targets, if you wanted to check predicted off-targets regions (as ascertained whilst originally designing the sgRNAs), then it would involve designing primers to PCR these regions specifically and then checking them for cut/repair sites by the same methodology.

Grazzi!

Big thank you from Isabel and myself, it was an intense week and we enjoyed running these practicals. We hope you got something from them and if you have any questions then please send them our way:

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