Cloning the *norV* promoter and *nrfA* gene into the iGEM Foundation Biobrick Standard to optimise *E. coli* for the conversion of nitric oxide (NO) to ammonia.

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Results

Colony PCR

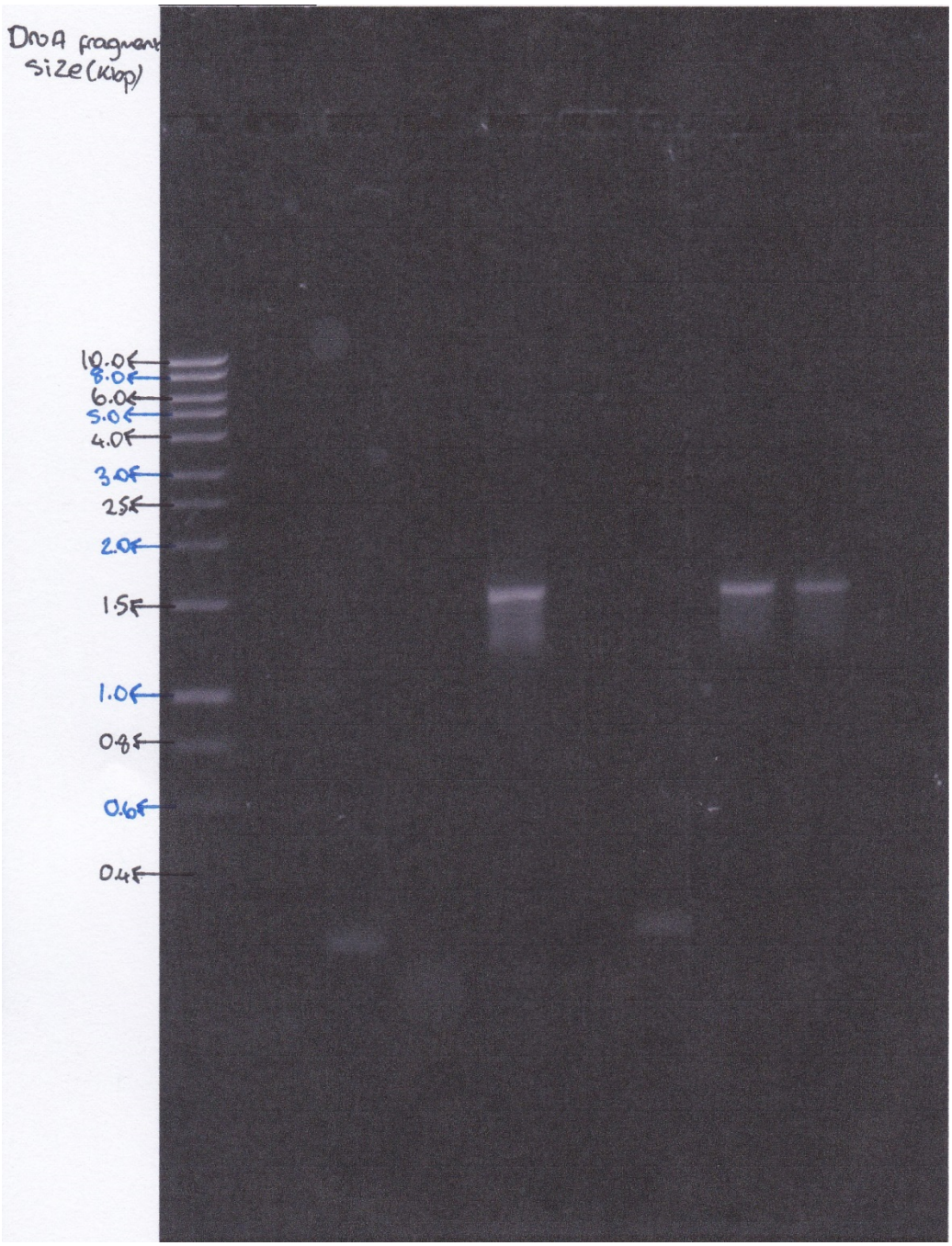

Figure 6: **Agarose gel of PCR product, from process outlined in experiment 3**
lane 1: empty, lane 2: ladder, lane 3: norV 0.1 (1), lane 4: nor V 1 (1), lane 5: NrfA 0.1 (1), lane 6: NrfA 1 (1), lane 7: norV 0.1 (2), lane 8: nor V 1 (2), lane 9: NrfA 0.1 (2), lane 10: NrfA 1 (2), lane 11 empty

Figure 6 shows successful PCR products of nor V in lanes 4 and 8. Whilst successful products for NrfA are shown in lanes 6, 9 and 10. PCR purification was carried out on successful products, in order to use within further experiments.

Ligations

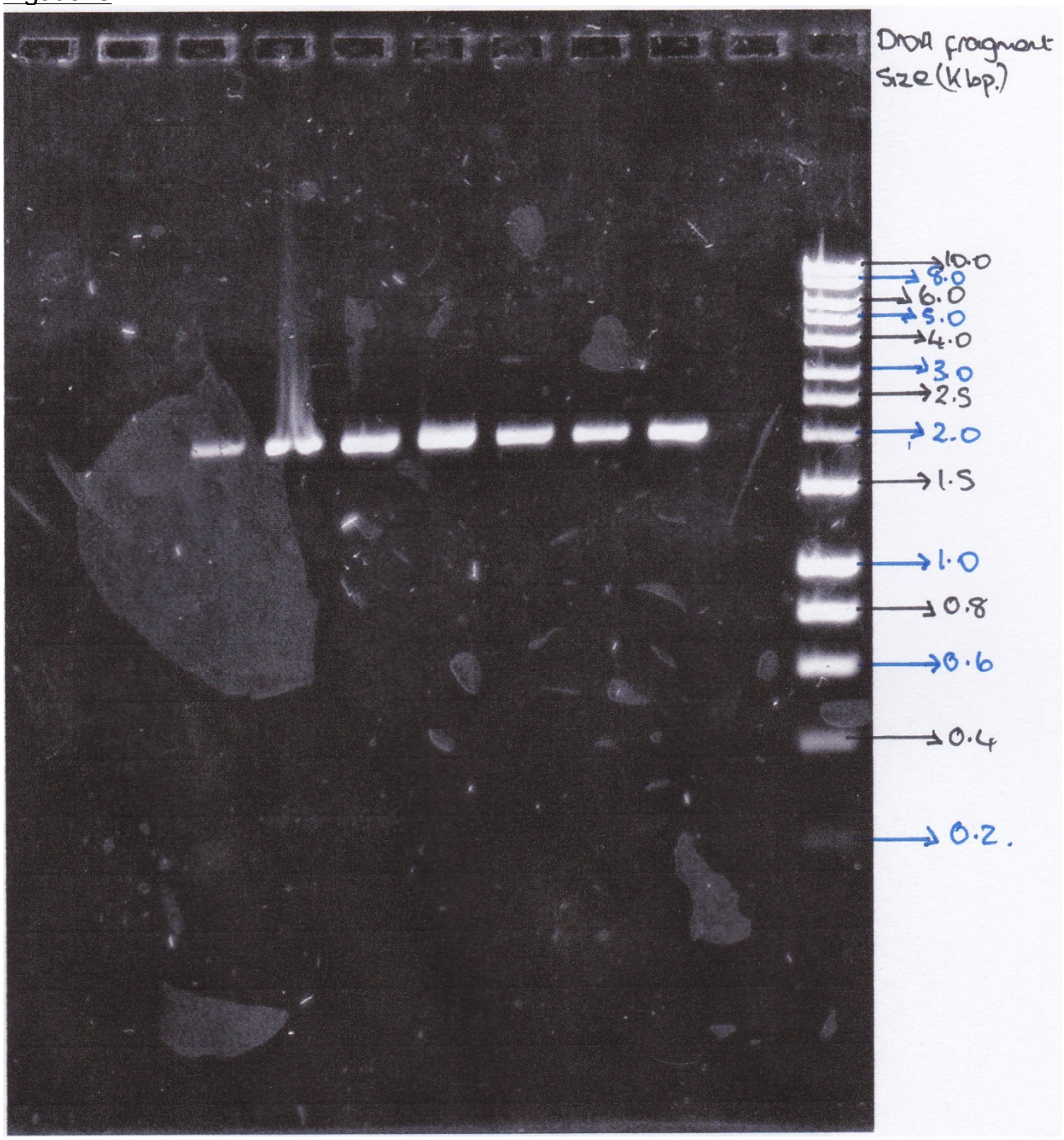

Figure 7**: Agarose gel showing diagnostic digests of ligations with plasmid (pSB1C3), norV and NrfA.** Lane 1 and 2: empty, lane 3: linearised plasmid, lane 4: norV ligation (1:1), lane 5: norV ligation (1:3), lane 6: norV ligation (1:5), lane 7: NrfA ligation 1:1, lane 8: NrfA ligation 1:3 (1), lane 9: NrfA ligation 1:5 (2), lane 10: empty, lane 11: ladder.

Figure 7 shows two successful ligations of norV, they can be observed in lanes 4 and 5 on the gel. In both of these lanes, there are bands at approximately 2000bp, which corresponds to our plasmid (pSB1C3) and 200bp which corresponds to our insert (norV).

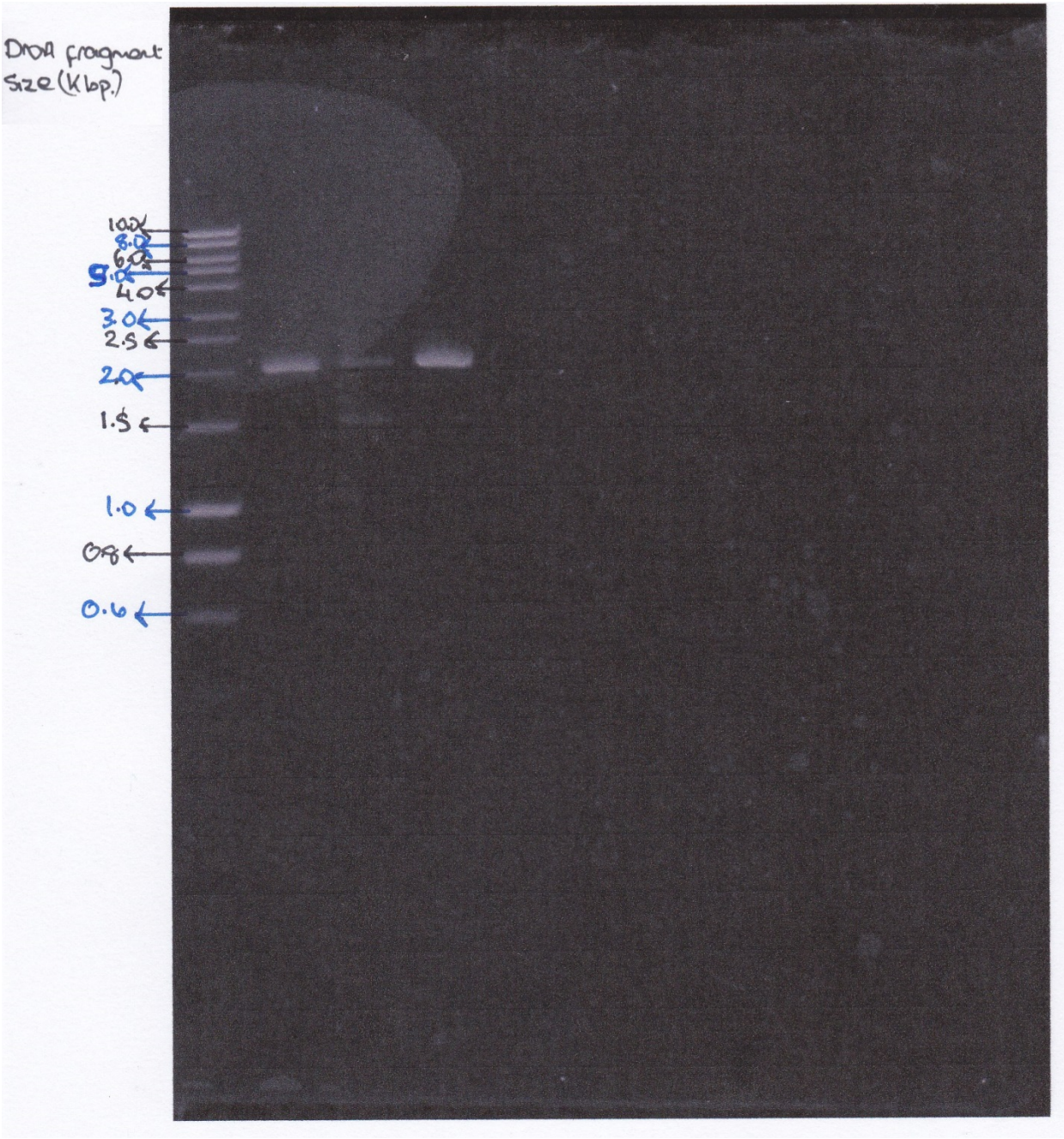

Figure 8: **Agarose gel showing diagnostic digests of ligations with plasmid (pSB1C3), norV and NrfA**
lane 1: ladder, lane 2 : NrfA ligation 1:1, lane 3: NrfA ligation 1:3 (1), lane 4: NrfA ligation 1:3 (2), lanes 5-11: empty

Figure 8 shows a successful ligation of NrfA and plasmid, it can be observed in lane 1 on the gel. In this lane , there are bands at approximately 2000bp, which corresponds to our plasmid (pSB1C3) and 1500bp which corresponds to our insert (NrfA)