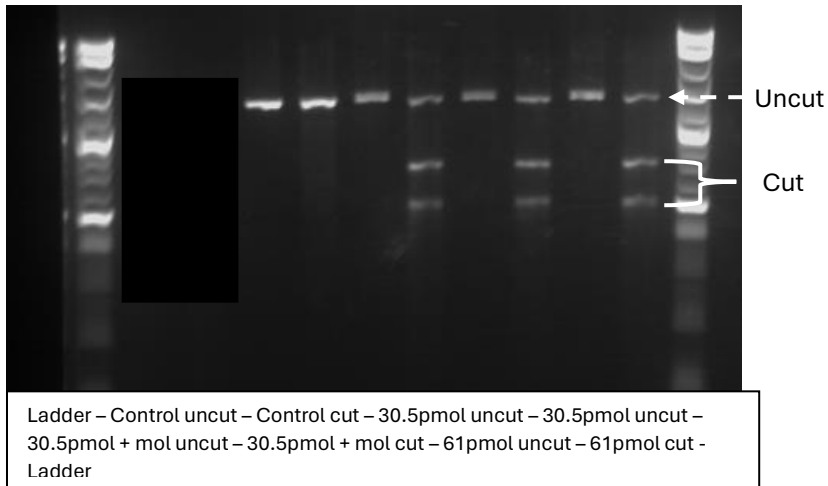


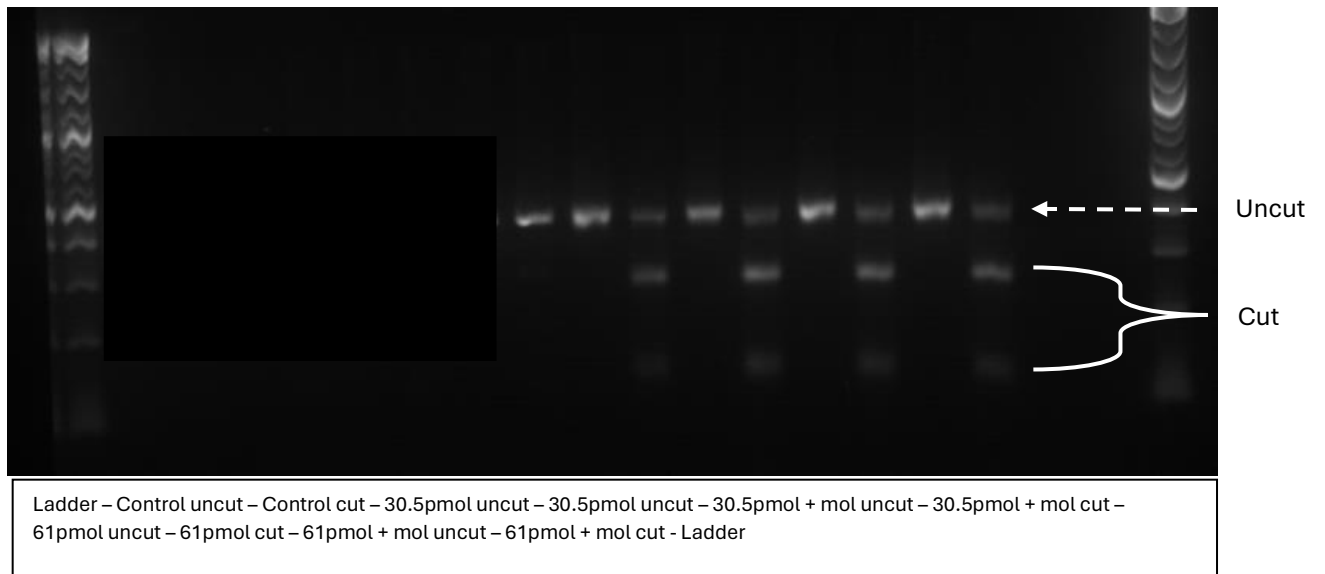
Source data gels for efficiency assessment:

**Figure S1A: SRSF2 RNP efficiency assays:**

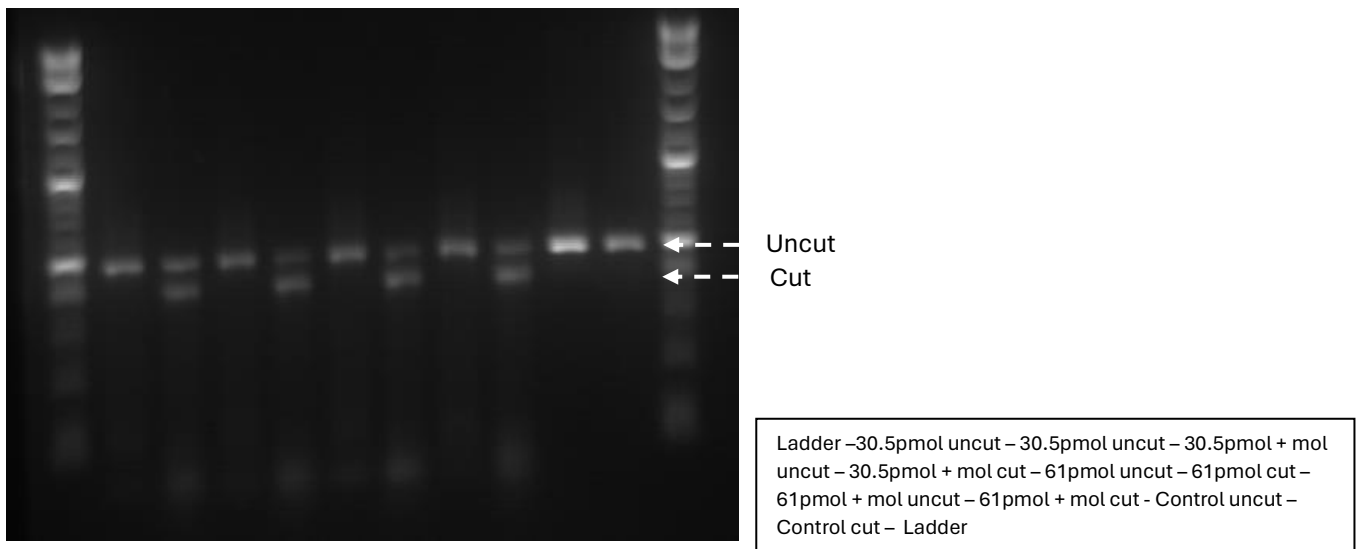
SRSF2 replicate 1:



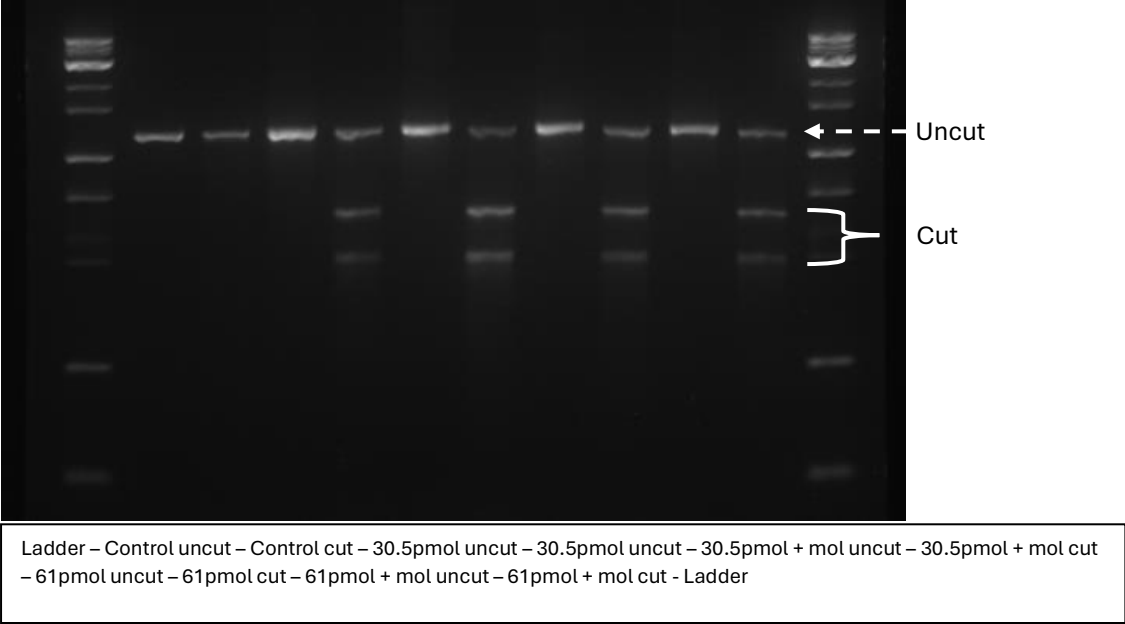
SRSF2 replicate 2:



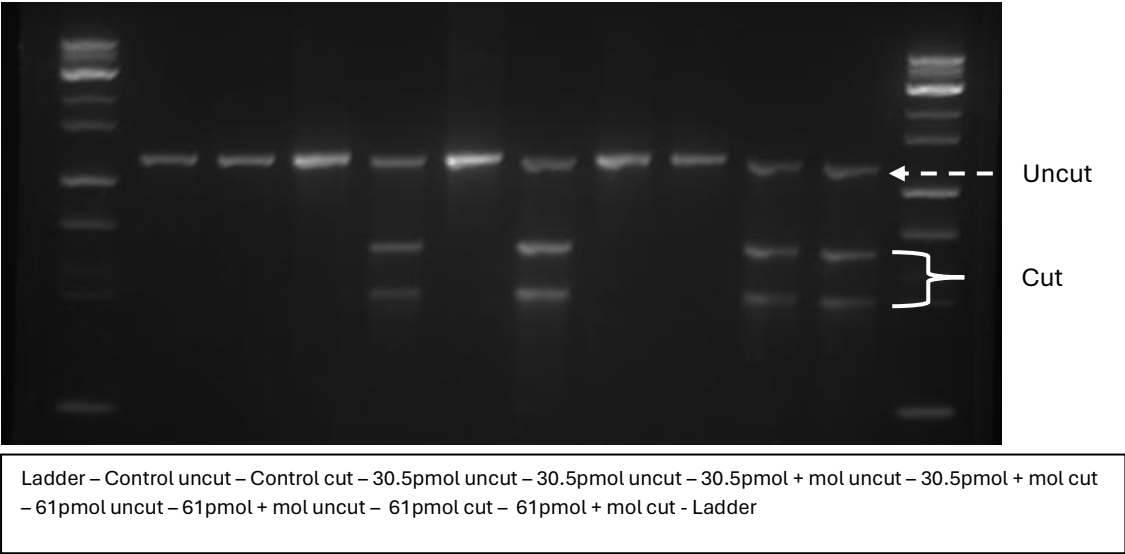
SRSF2 replicate 3:



SRSF2 replicate 4:

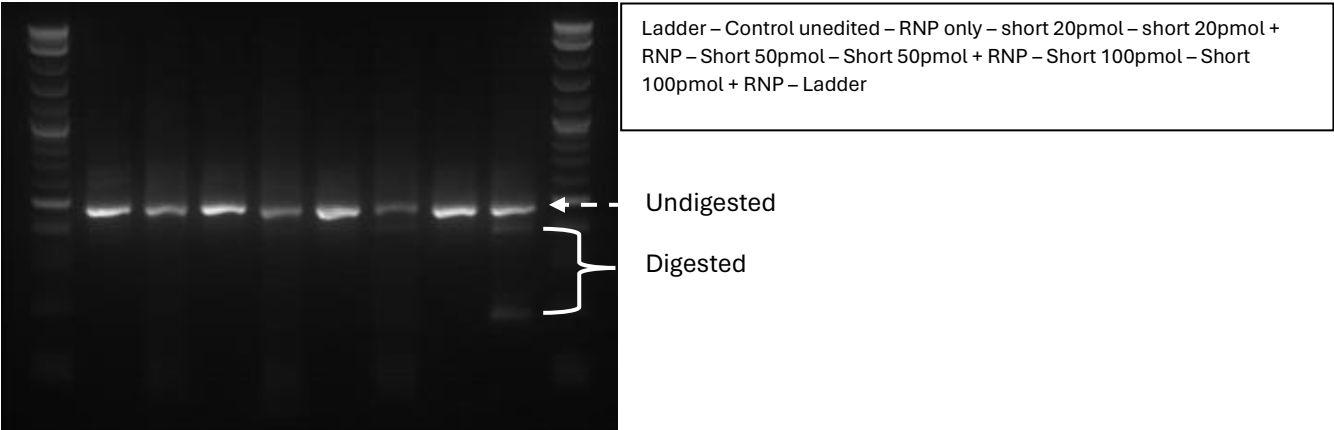


SRSF2 replicate 5:

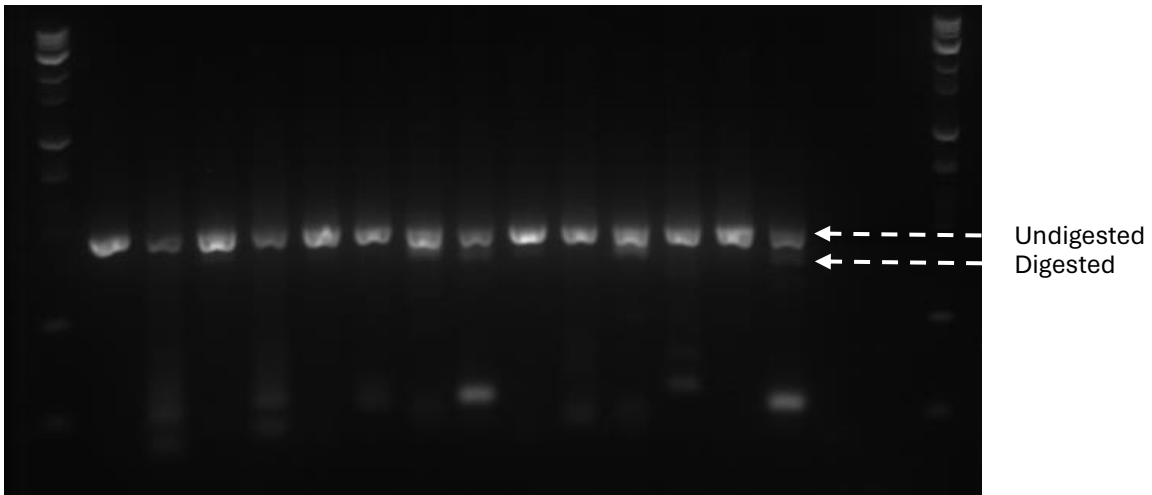


**Figure 1E: SRSF2 short and long donor assay:**

Replicate 1 for short donor:

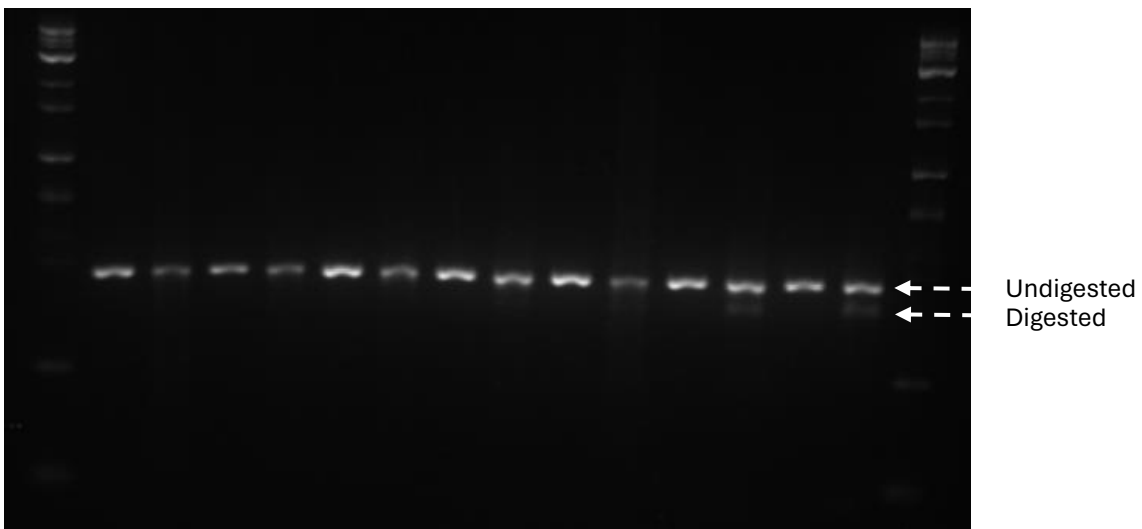


Replicate 2 for short donor and 1 for long donor:



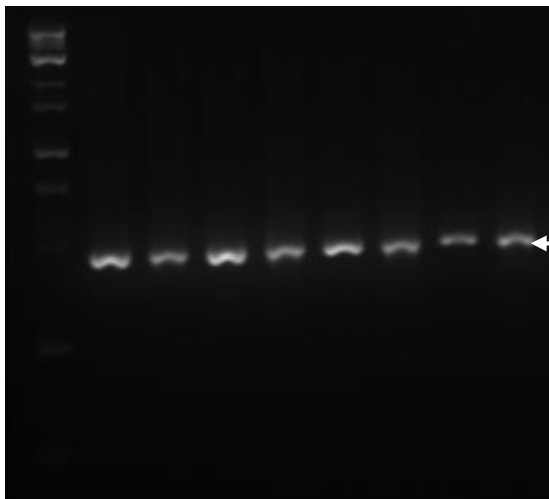
Ladder – Control unedited – RNP only – Long 5pmol – Long 5pmol + RNP – Long 10pmol – Long 10pmol + RNP – Long 20pmol – Long 20pmol + RNP – short 20pmol + RNP - Short 20pmol – Short 50pmol + RNP - Short 50pmol – Short 100pmol – Short 100pmol + RNP – Ladder

Replicate 3 for short donor and 2 for long donor:



Ladder – Control unedited – RNP only – Long 5pmol – Long 5pmol + RNP – Long 10pmol – Long 10pmol + RNP – Long 20pmol – Long 20pmol + RNP – short 20pmol – short 20pmol + RNP – Short 50pmol – Short 50pmol + RNP – Short 100pmol – Short 100pmol + RNP – Ladder

Replicate 3 for long donor:

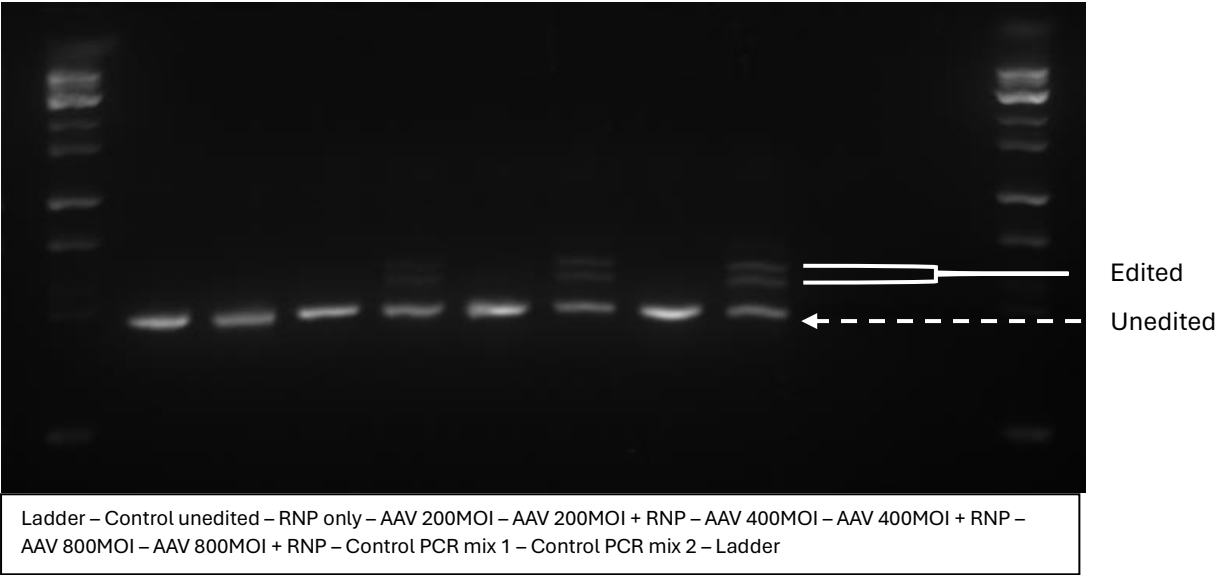


Ladder – Control unedited – RNP only – Long 5pmol – Long 5pmol + RNP – Long 10pmol – Long 10pmol + RNP – Long 20pmol – Long 20pmol + RNP – Ladder

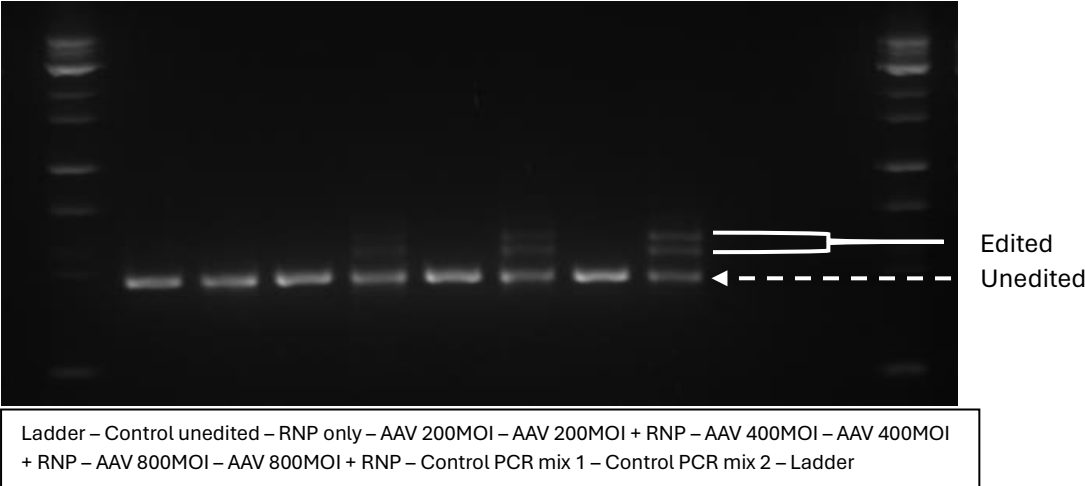
Undigested

**Figure 1C: SRSF2 AAV MOI assay:**

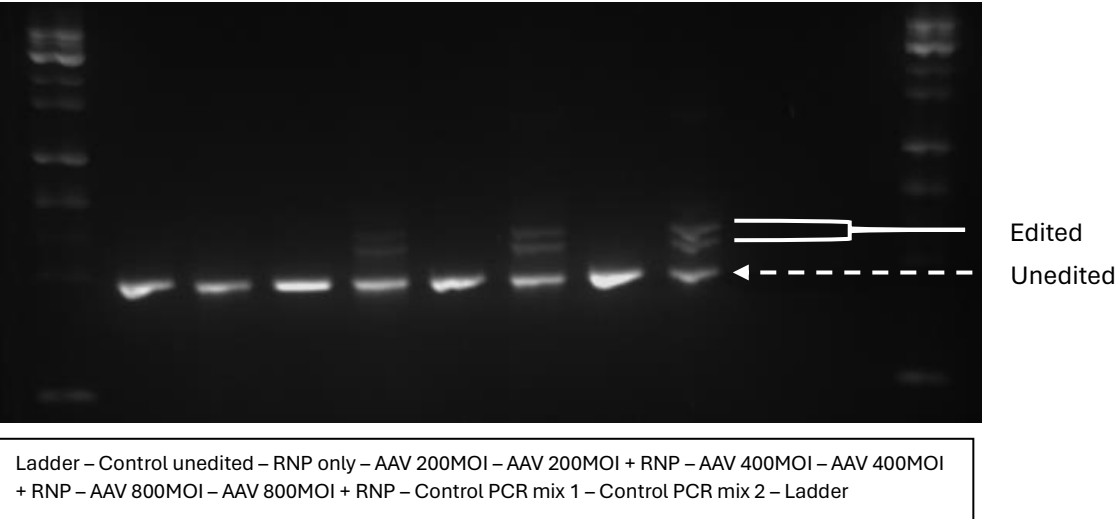
AAV MOI replicate 1:



AAV MOI replicate 2:

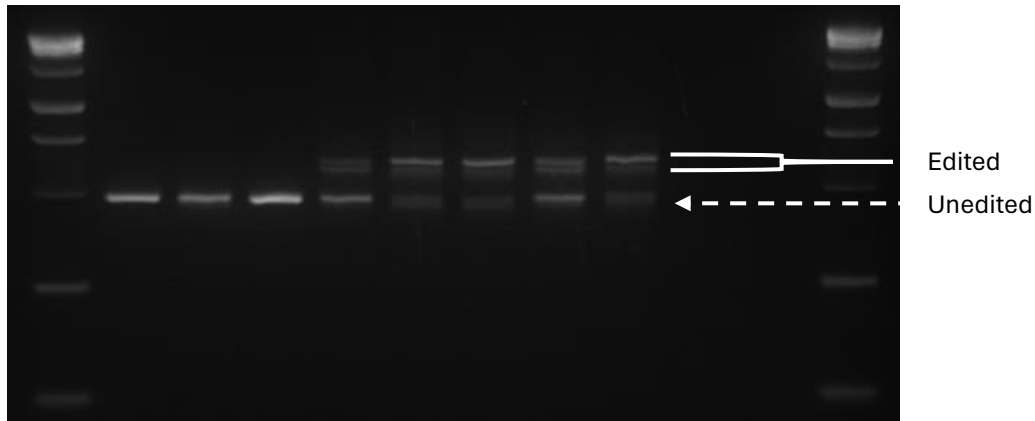


AAV MOI replicate 3:



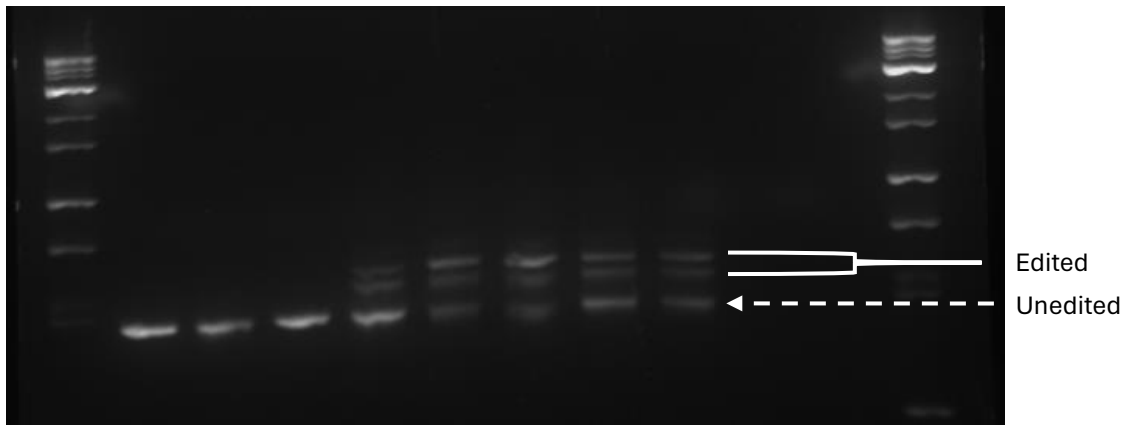
**Figure 2A : AZD7648 vs M3814 comparison assay:**

AZD7648 vs M3814 replicate 1:



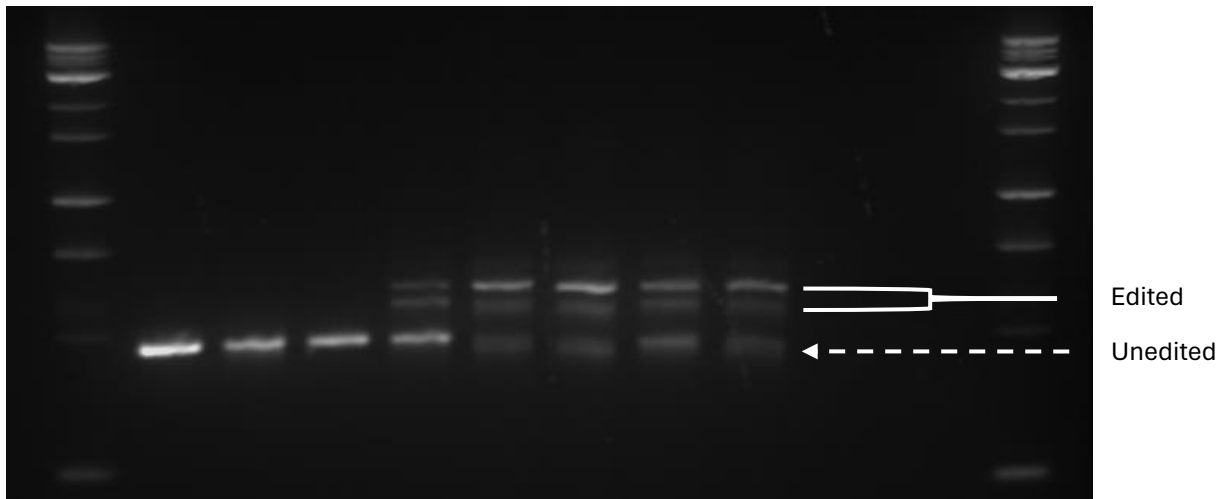
Ladder – Control unedited – RNP only – Donor only – Donor + RNP – Donor + RNP + M3814 0.5µM – Donor + RNP + M3814 5µM – Donor + RNP + AZD7648 0.5µM – Donor + RNP + AZD7648 5µM - Control PCR mix 1 – Control PCR mix 2 – Ladder

AZD7648 vs M3814 replicate 2:



Ladder – Control unedited – RNP only – Donor only – Donor + RNP – Donor + RNP + M3814 0.5µM – Donor + RNP + M3814 5µM – Donor + RNP + AZD7648 0.5µM – Donor + RNP + AZD7648 5µM - Control PCR mix 1 – Control PCR mix 2 – Ladder

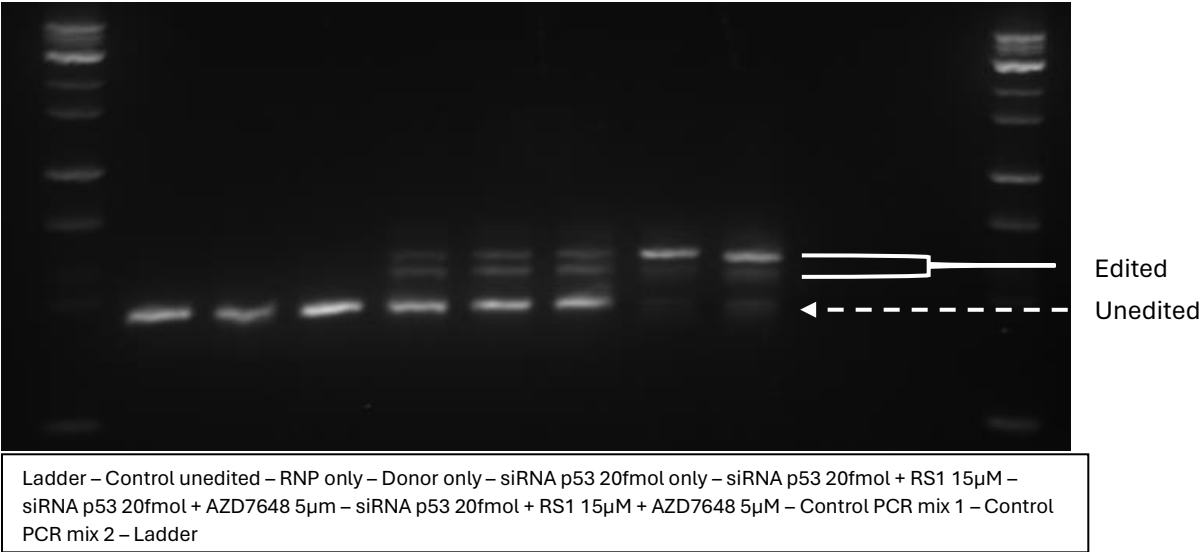
AZD7648 vs M3814 replicate 3:



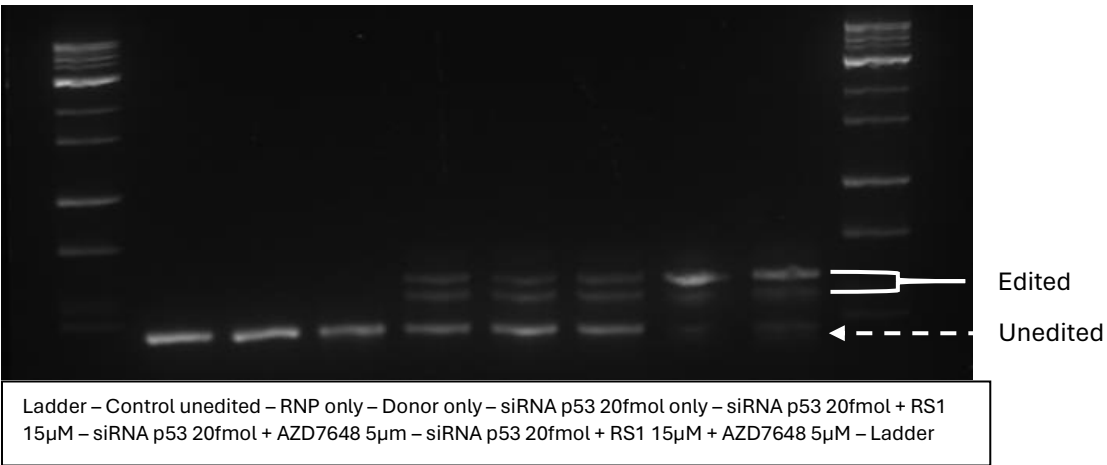
Ladder – Control unedited – RNP only – Donor only – Donor + RNP – Donor + RNP + M3814 0.5µM – Donor + RNP + M3814 5µM – Donor + RNP + AZD7648 0.5µM – Donor + RNP + AZD7648 5µM - Control PCR mix 1 – Control PCR mix 2 – Ladder

**Figure 2C: AZD7648 and RS1 additive effect assay:**

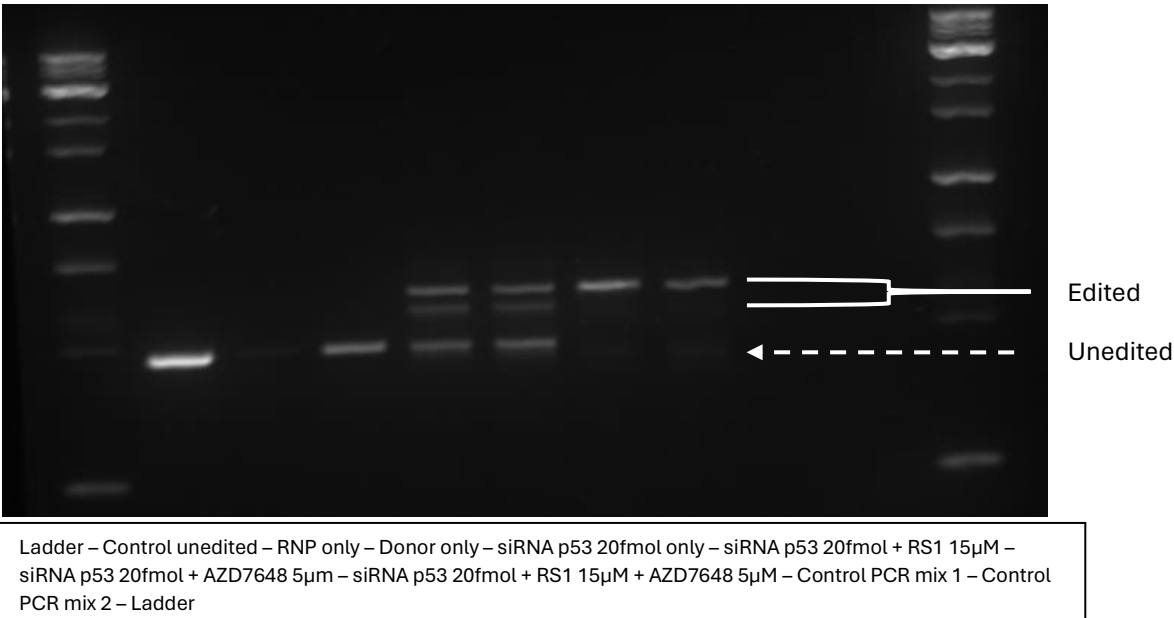
AZD7648 and RS1 additive effect assay replicate 1:



AZD7648 and RS1 additive effect assay replicate 2:

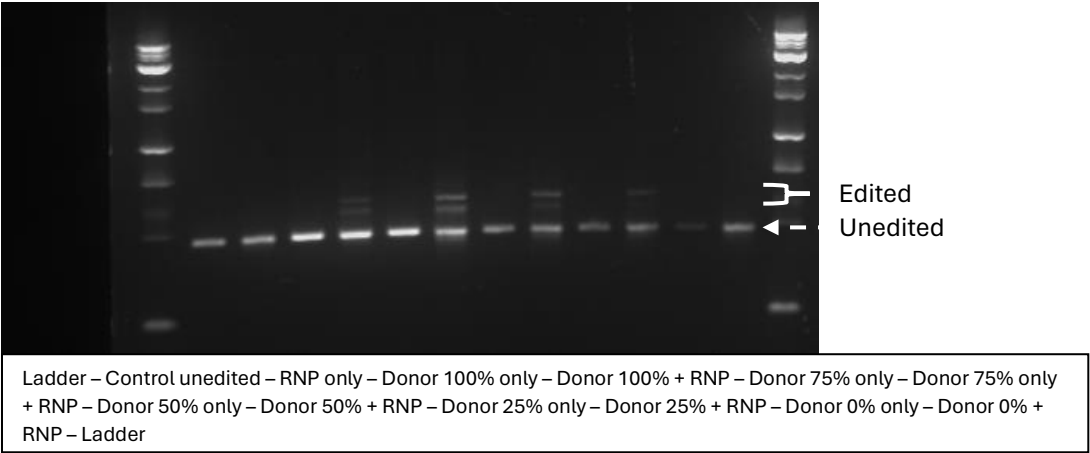


AZD7648 and RS1 additive effect assay replicate 3:

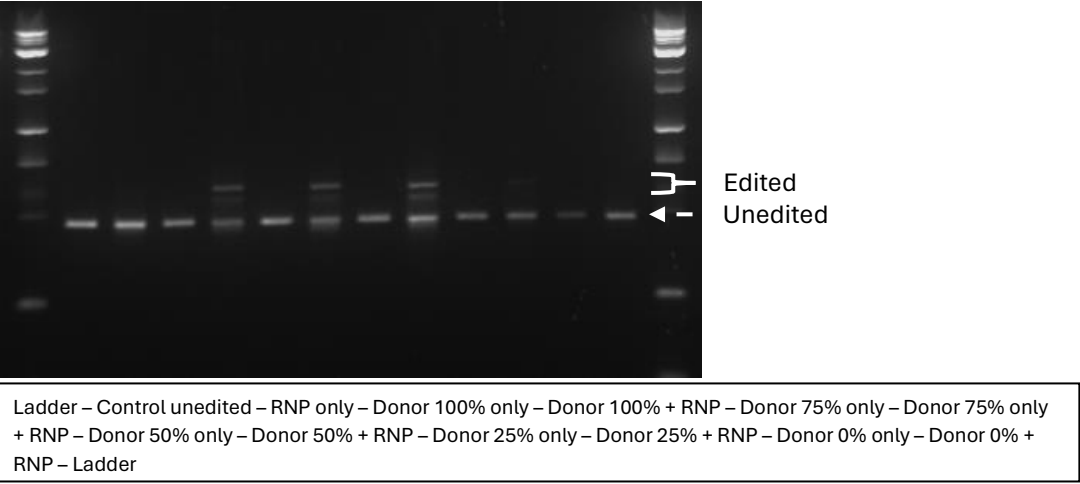


**Figure 4B: Bulk assessment for tuning experiment:**

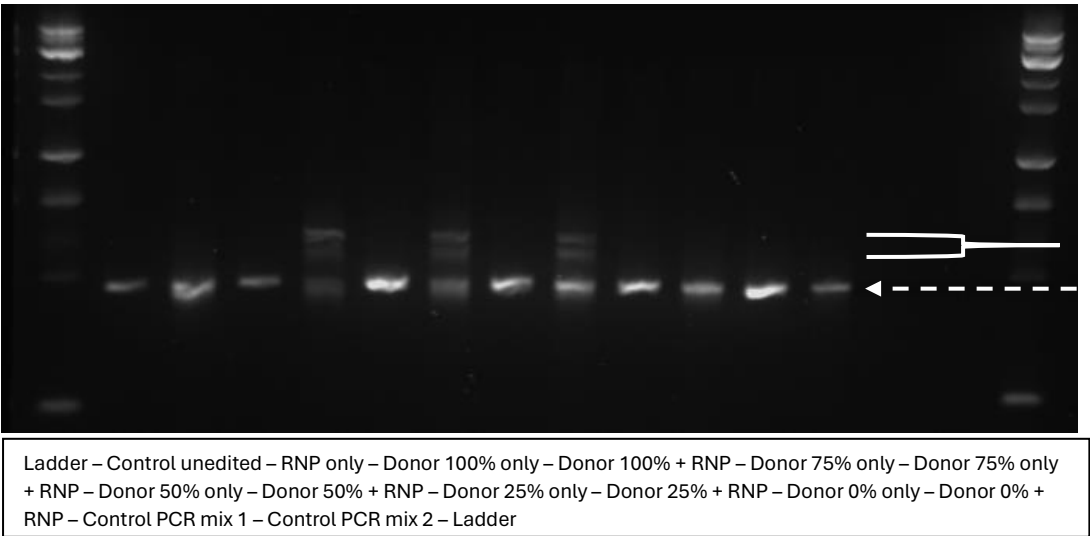
Integration rate in the bulk assessment replicate 1:



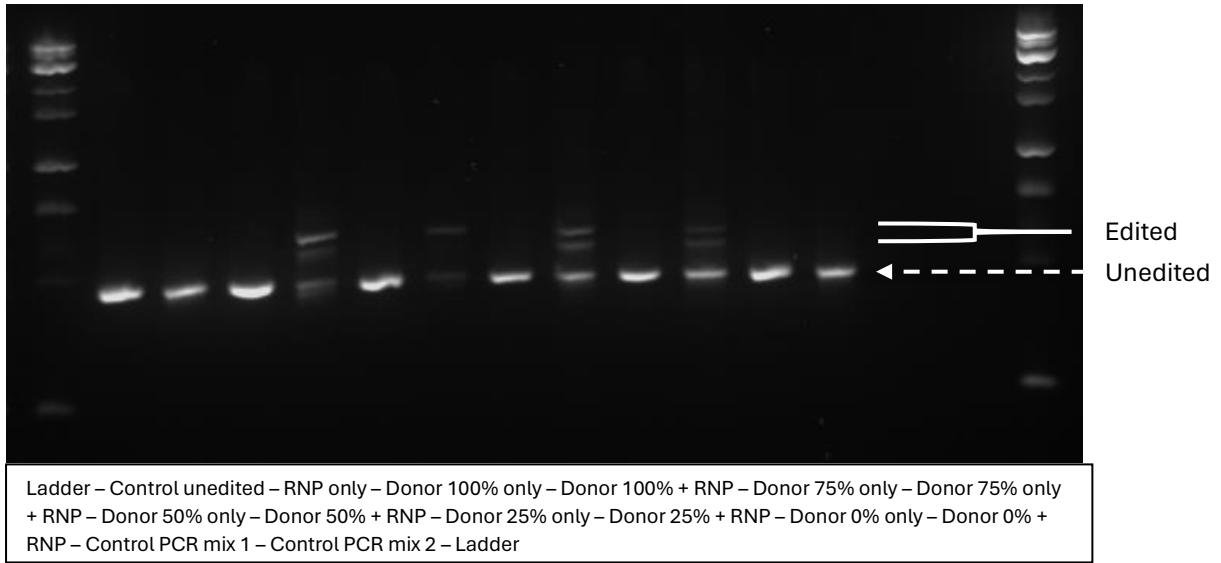
Integration rate in the bulk assessment replicate 2:



Integration rate in the bulk assessment replicate 3:

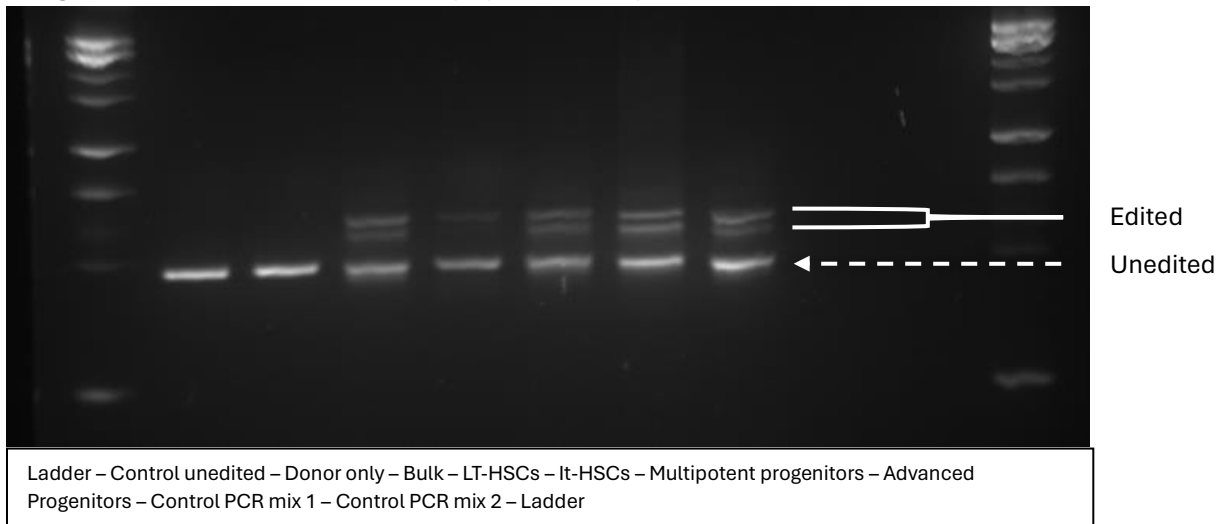


Integration rate in the bulk assessment replicate 4:

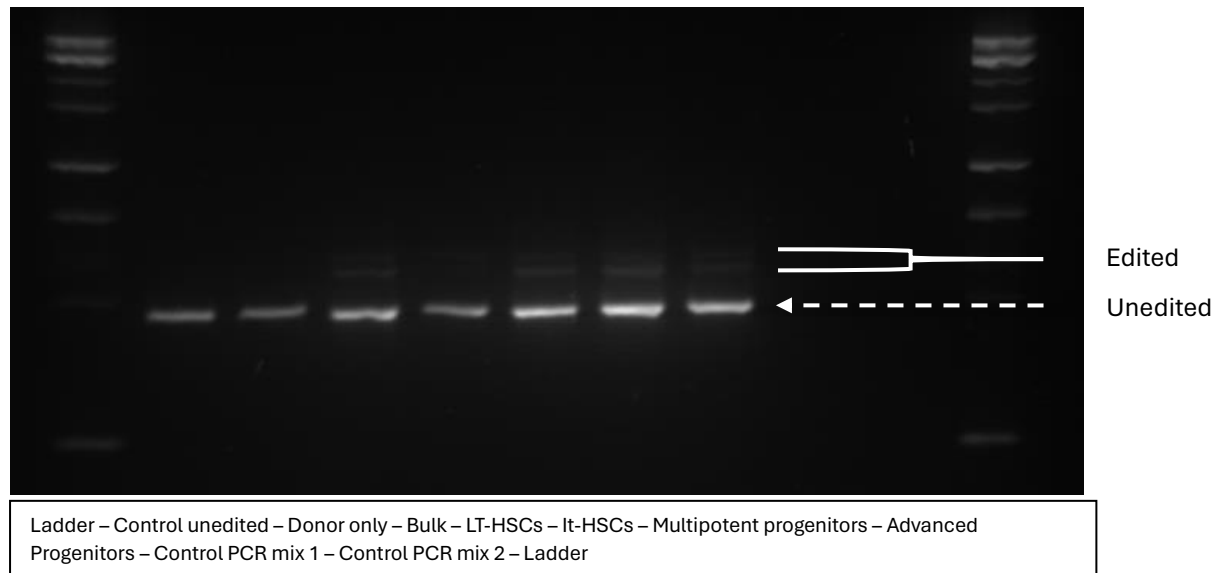


**Figure 3A: Assessment of integration in all 4 sub-populations in CD34+ cells**

Integration assessment in all 4 sub-populations replicate 1:

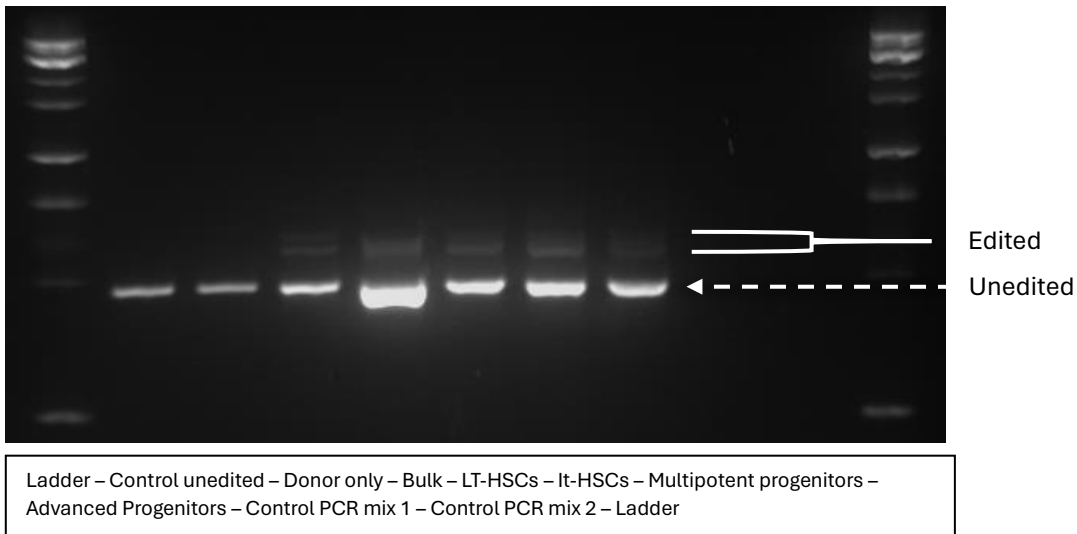


Integration assessment in all 4 sub-populations replicate 2:



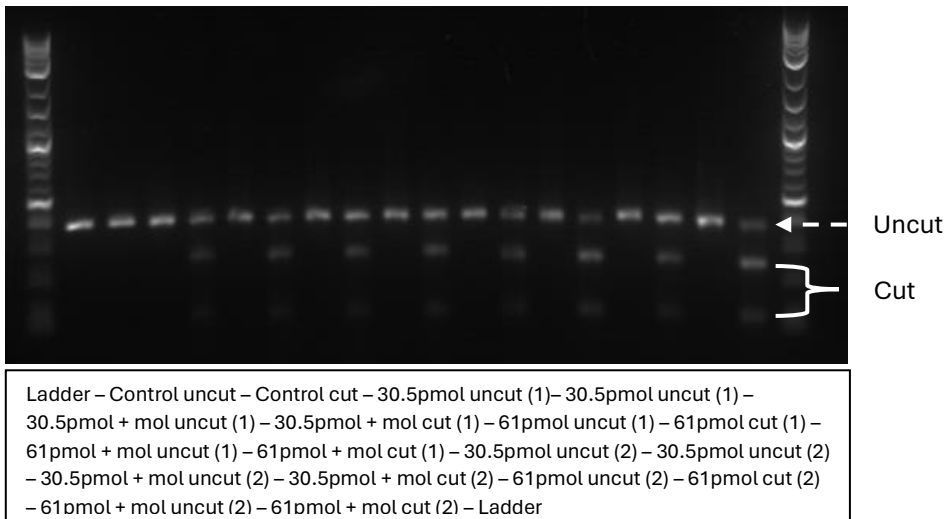


Integration assessment in all 4 sub-populations replicate 3:

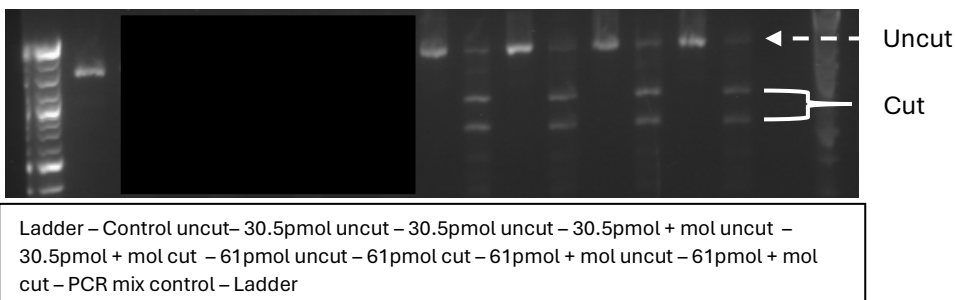


**Figure S1C: RNP cutting efficiency for SF3B1 gene:**

RNP cutting efficiency for SF3B1 gene replicate 1 and 2:

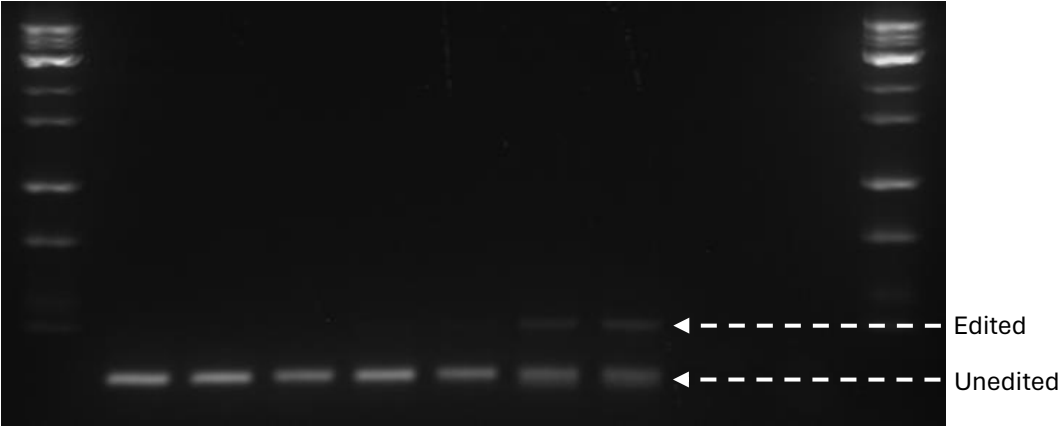


RNP cutting efficiency for SF3B1 gene replicate 3:



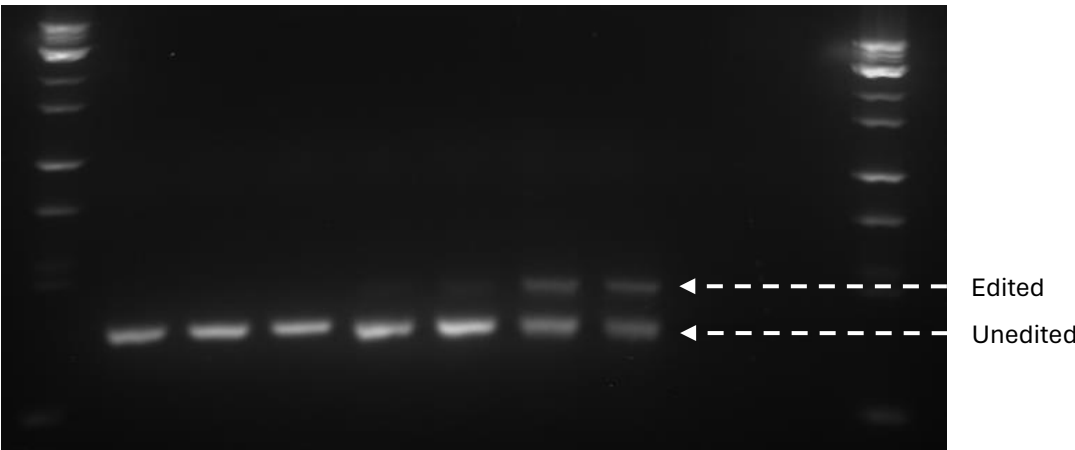
**Figure S3A: Integration rate for SF3B1 gene using AZD7648 and RS1 molecules:**

Integration rate for SF3B1 gene replicate 1:



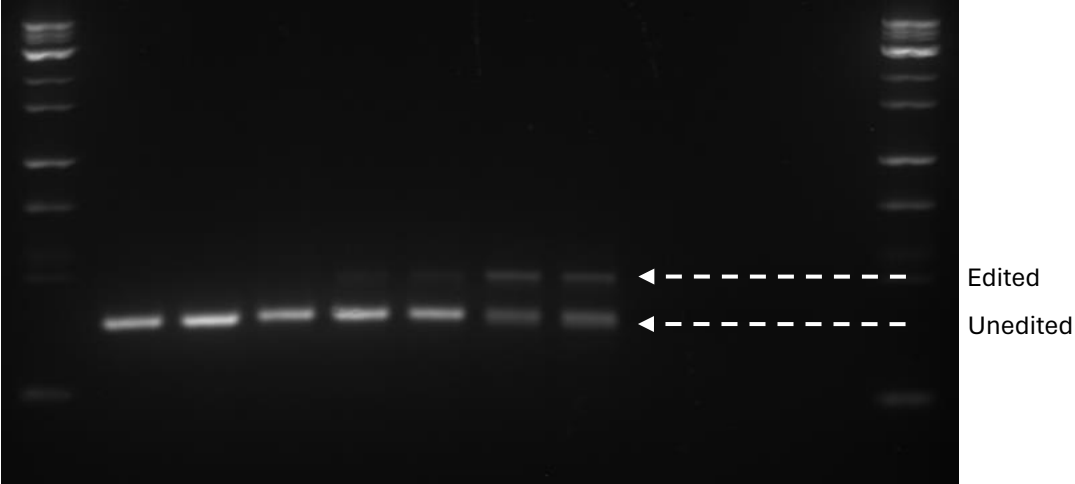
Ladder – Control unedited – RNP only – Donor only – siRNA p53 20fmol only – siRNA p53 20fmol + RS1 15µM – siRNA p53 20fmol + AZD7648 5µm – siRNA p53 20fmol + RS1 15µM + AZD7648 5µM – Control PCR mix 1 – Control PCR mix 2 – Ladder

Integration rate for SF3B1 gene replicate 2:



Ladder – Control unedited – RNP only – Donor only – siRNA p53 20fmol only – siRNA p53 20fmol + RS1 15µM – siRNA p53 20fmol + AZD7648 5µm – siRNA p53 20fmol + RS1 15µM + AZD7648 5µM – Control PCR mix 1 – Control PCR mix 2 – Ladder

Integration rate for SF3B1 gene replicate 3:



Ladder – Control unedited – RNP only – Donor only – siRNA p53 20fmol only – siRNA p53 20fmol + RS1 15µM – siRNA p53 20fmol + AZD7648 5µm – siRNA p53 20fmol + RS1 15µM + AZD7648 5µM – Control PCR mix 1 – Control PCR mix 2 – Ladder