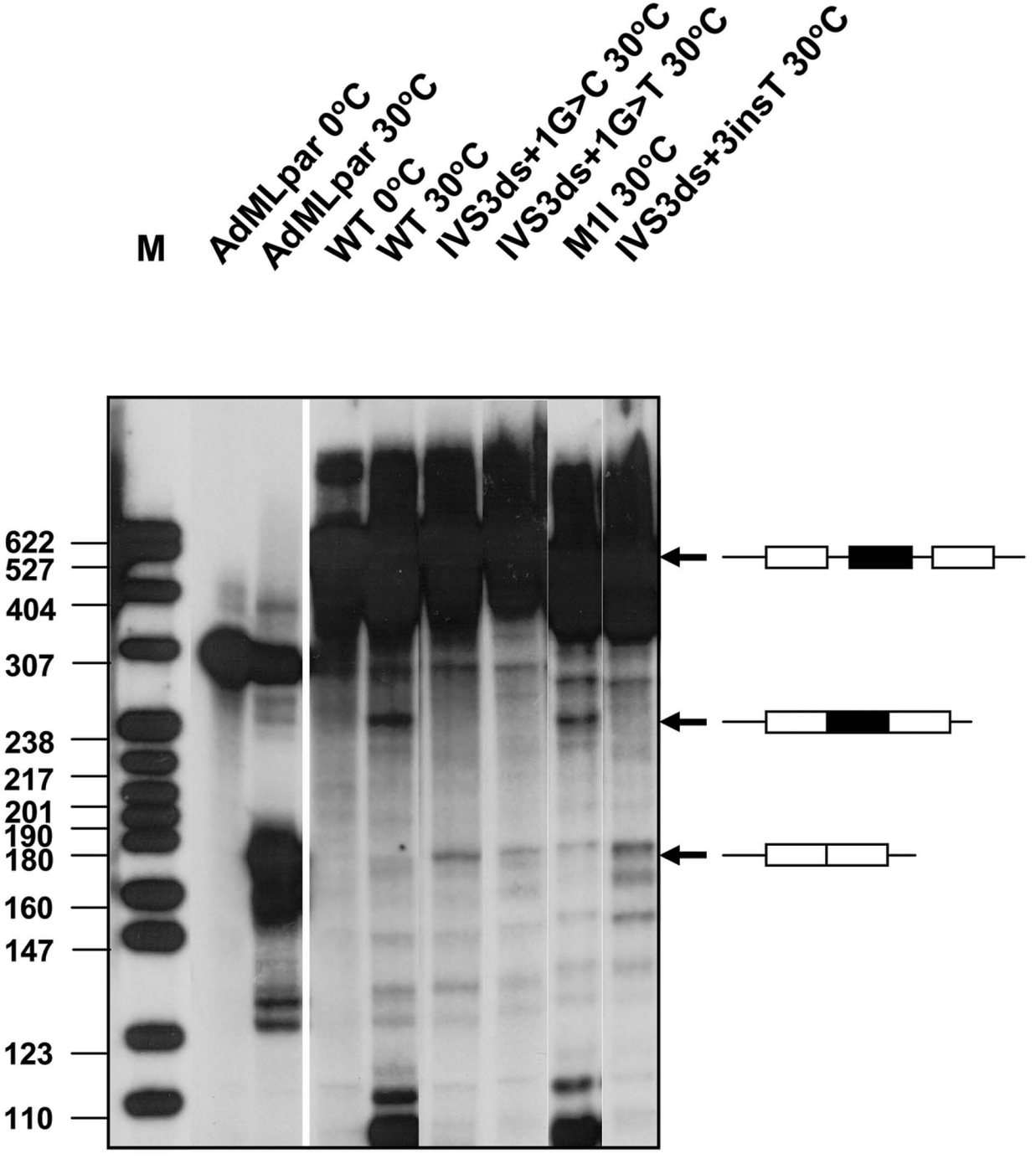
M
AdMLpar 0°C
AdMLpar 30°C
WT 0°C
WT 30°C
IVS3ds+1G>C 30°C
IVS3ds+1G>T 30°C
M1I 30°C
IVS3ds+3insT 30°C
622
527
404
307
238
217
201
190
180
160
147
123
110

Figure 2. *In vitro* splicing assay demonstrating that mutation of the IVS3 donor splice site impair splicing. The wild type or mutant exon 3 with 5' and 3' flanking intronic regions (filled rectangle) was introduced into the intron of the adenovirus major late first and second leader exon (unfilled rectangles) as described[11,12]. Constructions were confirmed by DNA sequencing and were then used to transcribe capped pre-mRNA *in vitro* in the presence of ^{32}P-labelled GTP using T7 RNA polymerase. After gel purification, 20 fmol RNA was incubated for 60 minutes on ice (0°C) (control) or at 30°C with HeLa nuclear extract, 0.5 M ATP and 1.6 - 3.2 mM $MgCl_2$ before phenol separation and ethanol precipitation and separation on a denaturing 5% polyacrylamide gel. AdMLpar alone was used as a positive control. AdMLpar splices efficiently to a 177 nt RNA species (two left lanes), whereas the wild type exon 3 (WT) and the M1I mutant exon 3 are spliced into this RNA to give a 283 nt product as indicated by the three box diagram on the right. The IVS3ds+1G C, IVS3ds+1G T and the IVS3ds+3insT mutant exons however are excluded from the spliced product and produce an identical 177 nt product to that from AdMLpar alone. M = DNA size markers.