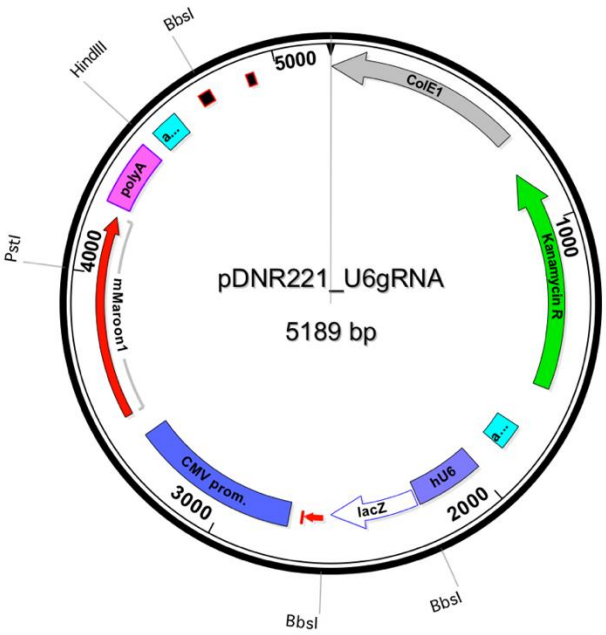
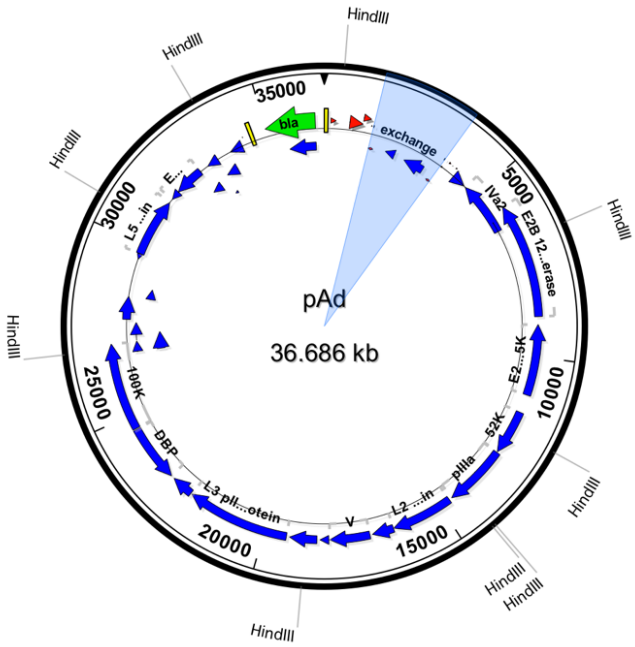
Supplementary Figure S1

A

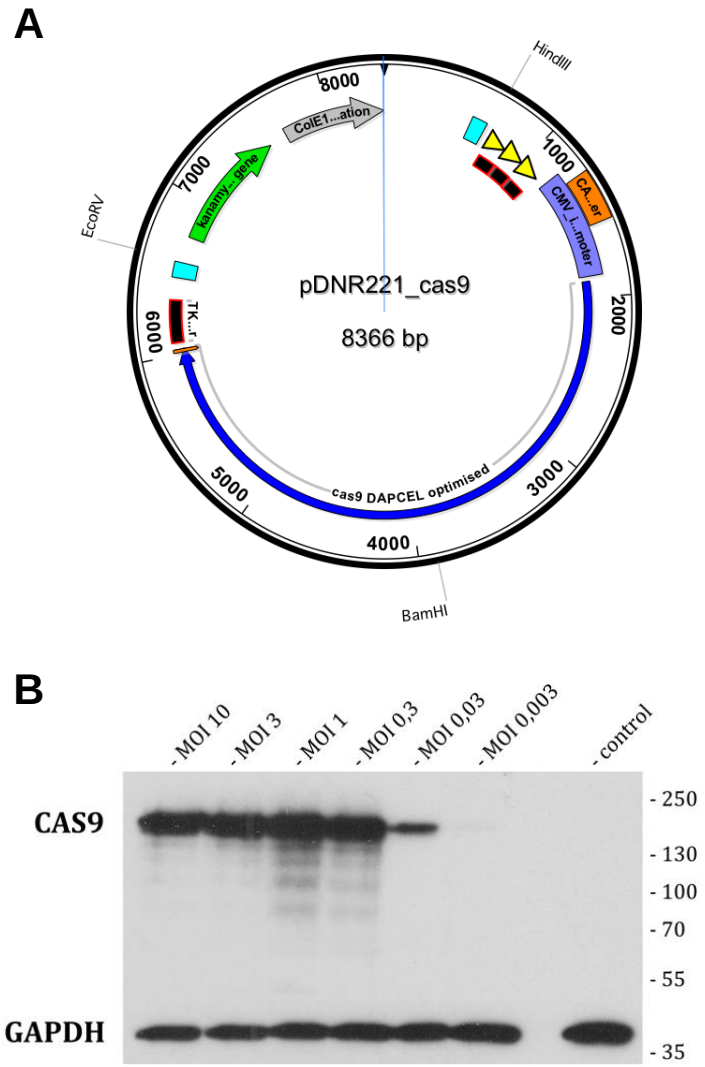


B



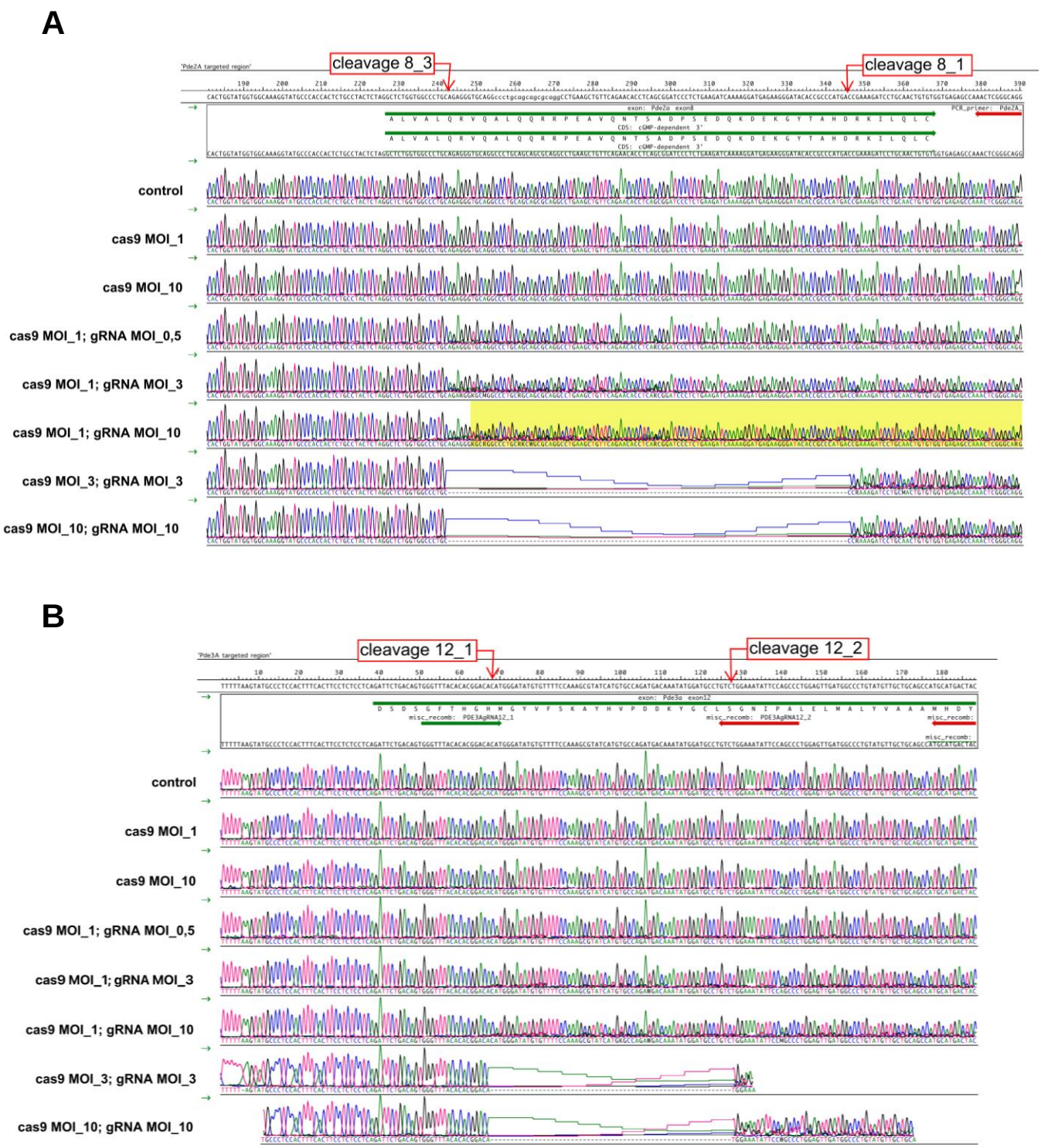
Supplementary Figure S1. Schematic structure of the shuttle (pDNR221) and adenoviral (pAd) vectors used for gRNA adenovirus generation. (**A**) Genetic cassette encoding LacZ under the control of the U6 promoter followed by cytomegalovirus (CMV) promoter and mMaroon1 sequences was cloned into pDNR221 vector between the attL sites (turquoise). Next, LacZ was exchanged by individual gRNA sequences via BbsI restriction site and corrected sequence/orientation was verified by sequencing. Full sequence of the construct can be found in supplementary files. (**B**) gRNA containing cassette from the pDNR221 vector was recombined into adenoviral vector using the LR reaction of the gateway cloning protocol as per manufacturer instructions.

Supplementary Figure S2



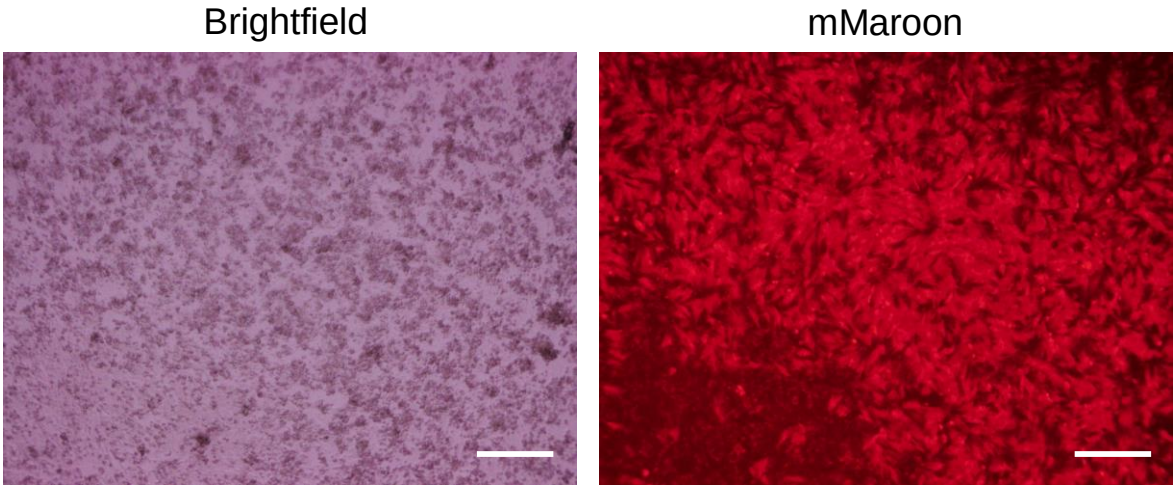
Supplementary Figure S2. Cas9 expression in neonatal cardiomyocytes transduced with different amounts of Cas9 adenovirus. (A) Schematic structure of the shuttle vector encoding Cas9. An optimized Cas9 sequence under the control of CMV promoter was cloned after three transcription stop signals (yellow arrows). Full sequence of the construct can be found in supplementary files. **(B)** Rat neonatal cardiomyocytes were transduced for 5 days with Cas9 adenovirus at different multiplicity of infection (MOI) and collected for immunoblot analysis. Representative blots, n=4.

Supplementary Figure S3



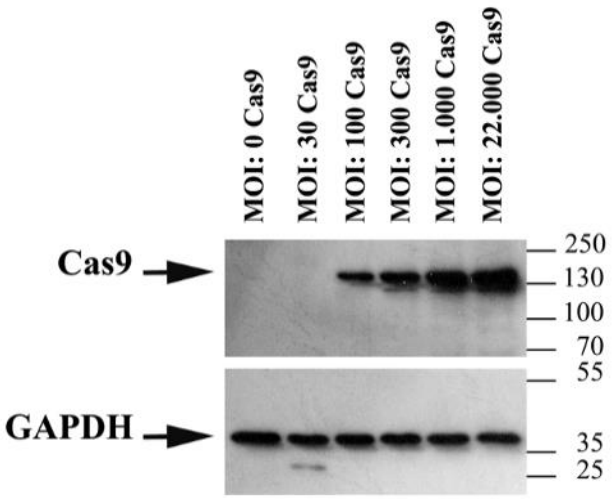
Supplementary Figure S3. Genomic rearrangements in neonatal cardiomyocytes with PDE2A and PDE3A-KO. Rat neonatal cardiomyocytes were transduced for 14 days with Cas9 plus gRNA adenoviruses for PDE2A-KO (**A**) or PDE3A-KO (**B**) as described in Figure 2. Targeted genomic loci of both PDEs were amplified by PCR and sequenced. The cleavage sites for CRISPR/Cas9 complexes with gRNA's for PDE2A and PDE3A are highlighted by arrows above. The MOI of adenoviruses for Cas9 and gRNAs are presented on the left. The 100% deletion of the targeted regions in PDE2A and PDE3A cardiomyocytes can be achieved at MOI 3 or higher.

Supplementary Figure S4



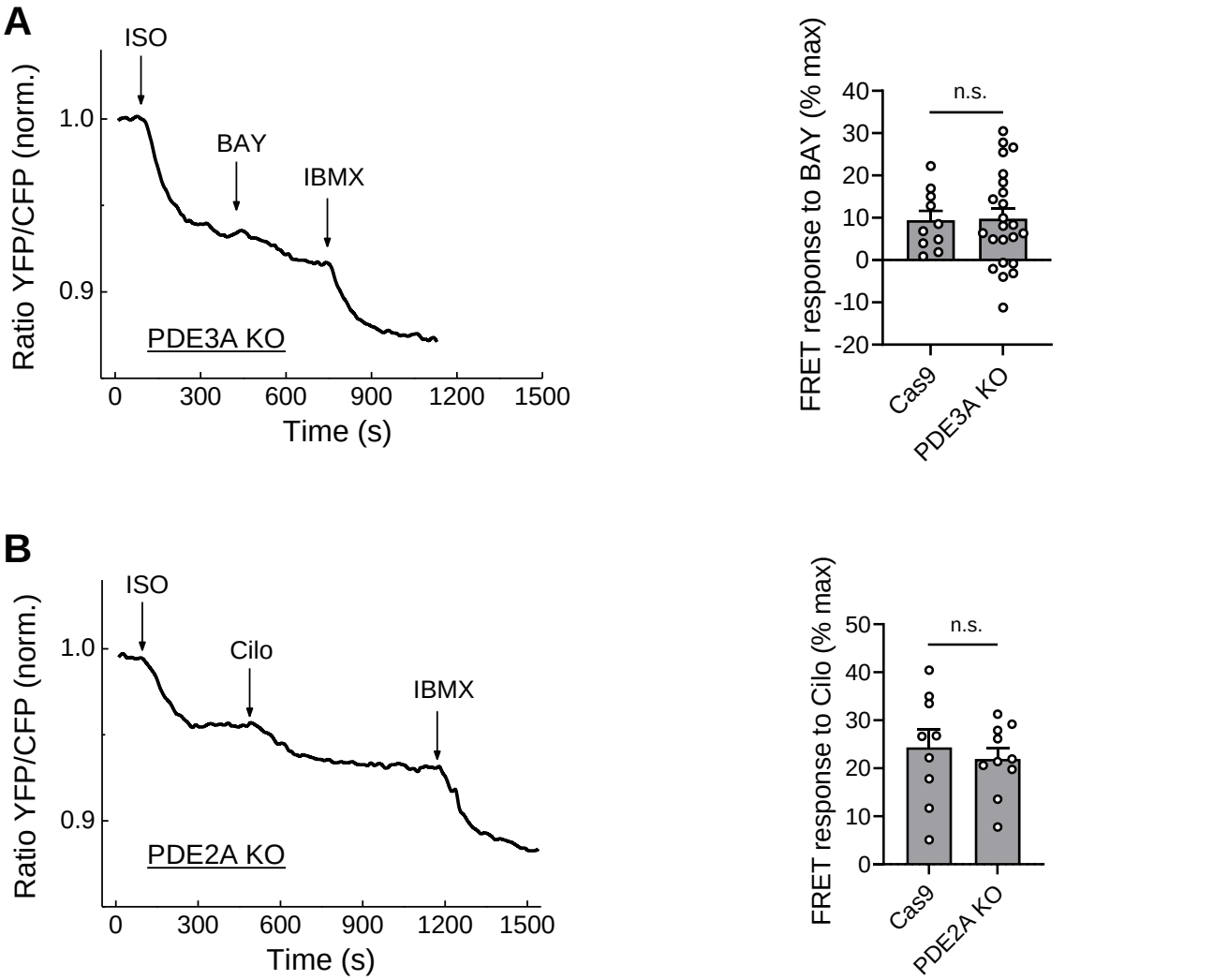
Supplementary Figure S4. Control of expression for gRNA constructs in neonatal cells. Neonatal cardiomyocytes were transduced for 3 days with Cas9 and PDE2A gRNA adenoviruses as described in Figure 3. mMaroon fluorescence was detected in red fluorescent channel of the Keyence BZ microscope. Representative images, n=10. Scale bar, 100 µm.

Supplementary Figure S5



Supplementary Figure S5. Cas9 expression in adult cardiomyocytes transduced with different amounts of Cas9 adenovirus. Adult rat ventricular cardiomyocytes were transduced for 3 days with Cas9 adenovirus at different multiplicity of infection (MOI) and collected for immunoblot analysis. Representative blots, n=4.

Supplementary Figure S6



Supplementary Figure S6. Control experiments performed to assess the effects of PDE2A knockout on PDE3A and *vice versa*. Adult rat ventricular cardiomyocytes were transduced for 6 days with Cas9 or Cas9 plus gRNA adenoviruses as described in Figure 5. The effect of the PDE2 inhibitor BAY 60-7550 (100 nM) in PDE3A-KO cells (A) and of the PDE3 inhibitor Cilostamide (Cilo) in PDE2A-KO cells (B) were measured as described in Figure 5. Representative FRET traces and data analysis, n.s. – not significant by mixed ANOVA followed by Chi-squared test.

Table S1

Primer sequences used for RT-PCR of individual PDE2A and PDE3A isoforms.

Primer name	Primer sequence
PDE2A_totD1	CCTGCAGGTGGTGGAGAA
PDE2A_totR2	AGAGGGATCCGCTGAGGT
PDE2A_A1_D1	GCCAGCAGGTCTTCCTCA
PDE2A_A1_D2	GACCCCCTTGCACAGAAG
PDE2A_A1_R1	AGGCTCAGCAAAGCATCC
PDE2A_A2_D1	AGCATGGTCCTGGTGTTGC
PDE2A_A2_D2	TACGAGCATGGTCCTGGTGT
PDE2A_A2_R1	TTGGACAACAGCGATGAGG
PDE2A_A2_R2	GAATTGGACAACAGCGATGA
PDE2A_A3_D1	GGCAGATGATGAGGAGTGATG
PDE2A_A3_D2	CAGGAGCCAGCAGTACCC
PDE2A_A3_R1	GGCTTGAGGAAGACCTGCT
PDE2A_A3_R2	AGGCTCAGCAAAGCATCC
PDE3A_totD2	GGAGTTGATGGCCCTGTATG
PDE3A_totR2	GGAACGGTCATTGTACAGCA
PDE3A_ A1_D1	GATTCCGGGGTGGAAGAG
PDE3A_ A1_D2	AGACCTTACCTGGCATACTTGG
PDE3A_ A1_R1	CTCTCTTGTGGTCCCATTCTG
PDE3A_ A1_R2	CGCCTCCTCCTCTTCCAC
PDE3A_ A23_D1	GAAAGGAGAGCAAGATACCAATTT
PDE3A_ A23_D2	GAAGGCAAGAAGCAAAGGAA
PDE3A_ A23_R1	TCTCTTGTGGTCCCATTCTGA

Table S2

Primer combinations used for RT-PCR of individual PDE2A and PDE3A isoforms.

Isoforms	Primer combinations	PCR product size
PDE2A all isoforms	PDE2A_totD1 + PDE2A_totR2	103 bp
PDE2A1	PDE2A_A1_D1 + PDE2A_A1_R1	141 bp
PDE2A1	PDE2A_A1_D2 + PDE2A_A1_R1	71 bp
PDE2A2	PDE2A_A2_D1 + PDE2A_A2_R1	45 bp
PDE2A2	PDE2A_A2_D1 + PDE2A_A2_R2	48 bp
PDE2A2	PDE2A_A2_D2 + PDE2A_A2_R1	49 bp
PDE2A2	PDE2A_A2_D2 + PDE2A_A2_R2	52 bp
PDE2A3	PDE2A_A3_D1 + PDE2A_A3_R1	116 bp
PDE2A3	PDE2A_A3_D1 + PDE2A_A3_R2	173 bp
PDE2A3	PDE2A_A3_D2 + PDE2A_A3_R1	66 bp
PDE2A3	PDE2A_A3_D2 + PDE2A_A3_R2	123 bp
PDE3A all isoforms	PDE3A_totD2 + PDE3A_totR2	118 bp
PDE3A1	PDE3A_ A1_D1 + PDE3A_ A1_R1	149 bp
PDE3A1	PDE3A_ A1_D1 + PDE3A_ A1_R2	27 bp
PDE3A1	PDE3A_ A1_D2 + PDE3A_ A1_R1	289 bp
PDE3A1	PDE3A_ A1_D2 + PDE3A_ A1_R2	167 bp
PDE3A2 + PDE3A3	PDE3A_ A23_D1 + PDE3A_ A23_R1	78 bp
PDE3A2 + PDE3A3	PDE3A_ A23_D2 + PDE3A_ A23_R1	95 bp