

Supplementary Material

Article

EpiCRISPR targeted methylation of Arx gene initiates mouse pancreatic alpha cells reprogramming into insulin-producing cells

Marija Đorđević¹, Peter Stepper², Clarissa Feuerstein³, Clarissa Gerhauser³, Verica Paunović⁴, Anja Tolić¹, Jovana Rajić¹, Svetlana Dinić¹, Aleksandra Uskoković¹, Nevena Grdović¹, Mirjana Mihailović¹, Renata Jurkowska⁵, Tomasz Jurkowski⁵, Jelena Arambašić Jovanović¹, Melita Vidaković¹

¹Department of Molecular biology, Institute for Biological Research “Siniša Stanković” - National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, Belgrade, Serbia; marija.sinadinovic@ibiss.bg.ac.rs, anja.tolic@ibiss.bg.ac.rs, jovana.rajic@ibiss.bg.ac.rs, sdinic@ibiss.bg.ac.rs, auskokovic@ibiss.bg.ac.rs, nevenag@ibiss.bg.ac.rs, mista@ibiss.bg.ac.rs, jelena.arambasic@ibiss.bg.ac.rs, melita@ibiss.bg.ac.rs

²Institute of Biochemistry and Technical Biochemistry, University of Stuttgart, Stuttgart, Germany; current address: Research Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna, Austria; pstepper@cemm.oeaw.ac.at

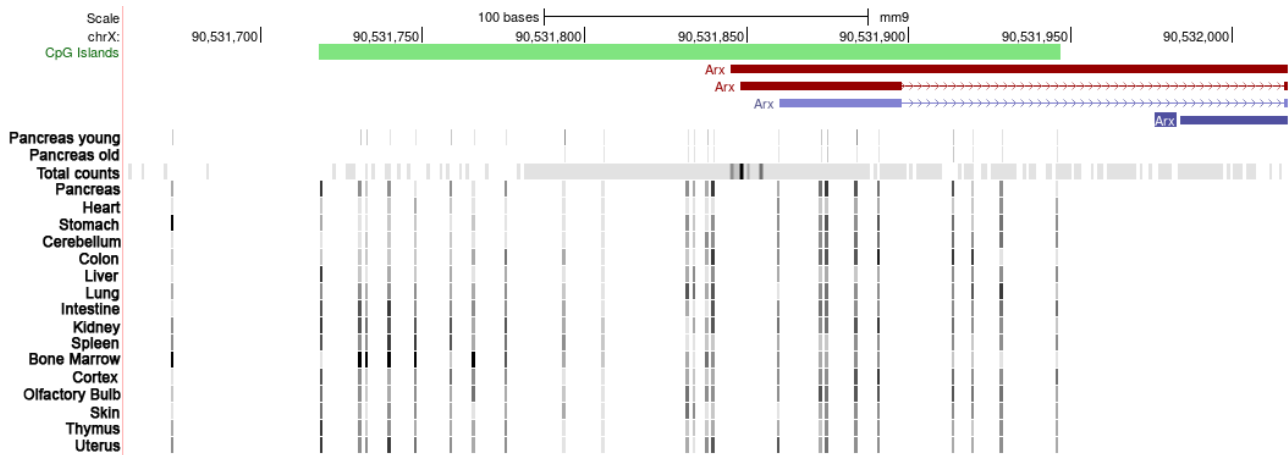
³Division of Epigenomics and Cancer Risk Factors, DKFZ, Im Neuenheimer Feld 280, Heidelberg, Germany

⁴Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Dr. Subotica 1, Belgrade, Serbia; vericapaunovic@gmail.com

⁵Cardiff University, School of Biosciences, Museum Avenue, CF10 3AX Cardiff, Wales, UK jurkowskit@cardiff.ac.uk

Correspondence: Tomasz P. Jurkowski jurkowskit@cardiff.ac.uk, Jelena Arambašić Jovanović jelena.arambasic@ibiss.bg.ac.rs, Melita Vidaković melita@ibiss.bg.ac.rs.

Supplementary Figure



Supplementary Figure 1. UCSC genome browser views of murine *Arx* gene's DNA methylation sites in the pancreas and other tissues. If compared with other tissues, *Arx* gene in pancreatic tissue (young and old) exhibits low DNA methylation. CpG island was shown as a green box.

Supplementary Tables

Table 1. Targeted sequences for sgRNAs

Targeting sequences of sgRNAs	
Arx sgRNA	1 GCAAAGCGCAAAGCGCGAAC
	2 TAACAAGTGTAGTGAGCCGC
	3 AGGGTGGGAGCCCGCAACCG
	4 GATGCTGTCGATGCAGTAGG

Table 2. Primers used for RT-qPCR

Gene	Primer sequence (5'-3')	
REEP 5	fw	TCATCGGACTGGTGGCTTTG
	rev	GTTGGGACTCTCGATGGCTT

Cas 9	fw	TCAGGCGGCAAGAGGATTC
	rev	AGTCATCCACGCGAATCTGG
Arx	fw	CGGAGAAGCCCATGCAAAAT
	rev	TGCAGCTCAGCCTCGAAC
Pax 4	fw	GCTCGAATTGCCAGCTAAAG
	rev	TTACTGTGGGGACTGGGAAGA
Ins 2	fw	GGAGCGTGGCTTCTTCTACA
	rev	TTCATTGCAGAGGGGTAGGC
Slc2a2	fw	TGCACCATCTTCATGTCGGT
	rev	ATTGCAGACCCAGTTGCTGA
Maf A	fw	TTCTGGAGAGCGAGAAGTGC
	rev	ACAGAAAGAAGTCGGGTGCG
Pax6	fw	TGTCTACCAGCCAATCCCAC
	rev	CATTCACTGACGGGCTGGT
Pdx1	fw	CCTTTCCCGAATGGAACCGA
	rev	GGGCCGGGAGATGTATTTGT
Nkx2-2	fw	TTCCGGACACCAACGATGAA
	rev	CCGTGCAGGGAGTATTGGA
Nkx6-1	fw	GAGAGCACGCTTGGCCTATT
	rev	TCGTCGTCATCCTCCTCATTC

Table 3. Primers used for HRM

Primers			Sequence (5'-3')	Primer length	Amplicon length
R1	M	Fw	AATTTTAAGGATAAGAAGGAGCGATA	26	198
		Rev	CGAACTACGTAAACCTACCG	20	
	U	Fw	TTTTAAGGATAAGAAGGAGTGATA	24	200
		Rev	AAACCAAACCTACATAAACCTACCAA	25	
R2	Fw	GTATTAGGAAGAGGGTTGTTTTGAG	25	287	
	Rev	ATATCTCCAACCTAACAAATCCCAA	25		

Table 4. Touchdown PCR program for amplification of bisulfite converted DNA, starting at 55 °C.

bisulfite PCR program		
95	15 min	
94 °C	30 s	6 x
x °C	30 s	10 x
72 °C	40 s	
94 °C	30 s	
x-5 °C	30 s	35 x
72 °C	40 s	
94 °C	30 s	
x-10 °C	30 s	
72 °C	40 s	
72 °C	4 min	
4 °C	hold	

Table 5. Primers for NGS library preparation

Primers	Sequence (5'-3')
mArx_bis_1_f	TTT TTT GTT TTT ATA TTT ATA GGT TTT TTT T
mArx_bis_1_r	TTA CTC ATA ACT AAT ACT TTT TCC TTA AAC
mArx_bis_2_f	GTT TAA GGA AAA AGT ATT AGT TAT GAG TAA
mArx_bis_2_r	TAT CTC CAA CTT AAC AAA TCC CA
mArx_biscg2_3_f	AGG TTG AGT TGT ATT TGT TAT TTA AGT
mArx_biscg2_3_r	CTA ACT AAT CTT AAA CRT ATC CCA AAC
mArx_biscg2_4_f	GTT TGG GAT AYG TTT AAG ATT AGT TAG
mArx_biscg2_4_r	AAT ACA ACA ACA ACT CTT CCT TAA A

Table 6. Antibodies used for Immunoblot analysis (IBA) and Immunocytochemistry (ICC)

	Antibody	Host species	Dilution IBA/ICC	Supplier
Primary antibody	anti-glucagon	Goat	1:500 / 1:50	C-18 / Santa Cruz Biotechnology
	anti-insulin	Rabbit	1:500 / 1:200	H-86 / Santa Cruz Biotechnology
	anti-Arx	Rabbit	1:1000 / 1:100	ab48856 / Abcam
	anti-beta Actin	Rabbit	1:2000 / 1: -100	ab8227 / Abcam
Secondary antibody	anti-rabbit IgG-HRP	Cow	1:2000 / -	sc-2379 / Santa Cruz Biotechnology
	anti-goat IgG-HRP	Donkey	1:2000 / -	sc-2020 / Santa Cruz Biotechnology
	anti-goat IgG, F(ab') ₂ -TRITC	Donkey	1:100	sc-3855 / Santa Cruz Biotechnology
	anti-rabbit IgG (H+L), Alexa Fluor 555	Donkey	1:4000	A-31572 / Invitrogen

Table 7. Primers for PCR reaction after ChIP

		Sequence (5'-3')	Amplicon length
ChIP primers	fw	TTAGGGTTTGCCACCCCATCTA	208
	rev	CAATCAGCCTCCCCAAATGAAG	