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Supplementary information

Direct cell reprogramming: approaches, mechanisms and progress

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Direct cell reprogramming: approaches and mechanisms

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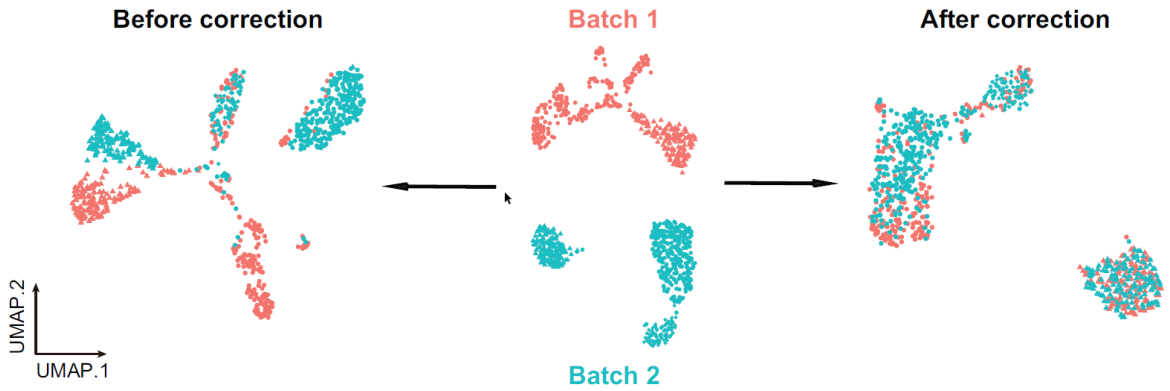
Supplementary information

Supplementary Box 1 | **Batch effect correction for scRNA-seq data**

Systematic differences in gene expression profiles across batches, which is also known as 'batch effect', pose big challenges to integration analysis across multiple scRNA-seq datasets, especially those produced using different experimental protocols, and/or by different laboratories. Without proper correction, batch effects may result in misleading findings and/or failure in the identification of novel cell type(s) and differentially expressed genes[1,2] (left panel of the figure, two batches are shown in different colors, and the two different cell types are represented by solid circles and triangles, respectively. The data showed in the figure is pseudo data generated by computer simulation.). To correct the batch effect, many methods have been specifically developed for scRNA-seq data. One popular strategy, as employed by MNNcorrect[3] and Seurat v3[4], is to correct the batch effect via adopting the information of mutual nearest neighbors (MNNs) between different batches. Here, MNNs refers to pairs of cells having mutually similar gene expression profiles across batches, which are assumed to be from the same biological state. This strategy is ideally suited for the datasets where the variation from the batch effect is less than or comparable to the true biological differences. LIGER is another widely used batch effect correction method that is designed for jointly inferring cell types across multiple scRNA-seq datasets[5]. Not only characterizing shared features among batches, but LIGER also takes batch-specific features into consideration, which can maximally recover the latent differentiation among different batches. These methods have been shown to achieve more accurate and robust batch effect corrections than traditional methods used in analyzing bulk RNA-seq data (right panel of the figure).



Supplementary table 1 | **Examples of successful direct reprogramming**

Delivery method	Reprogramming cocktail	Efficiency	In vitro, in vivo	Advantage	Disadvan-tage	Starting cell	Target cell type	Ref
Sendai virus	GMT	1.50%	In vivo	Non-integration and low cytotoxicity		Resident cardiac fibroblast	Cardiomyocytes	6
Retrovirus	GHMT	~2.40%	In vivo	Easy to package, Large packaging capacity(~9kb)	Integrated into the genome, only infect proliferating cells	Resident cardiac fibroblast	Cardiomyocytes	7
	GMT	10-15%	In vivo			Resident cardiac fibroblast	Cardiomyocytes	8
	Ascl1/Sox2/NeuroD1	-	In vivo			Glial cells	Neurons	9
	Neurog2/FG2+EGF	-	in vivo			non-neuron cells in neocortex and striatum	Neurons	10
	NeuroD1	-	In vivo			Reactive glial cells	Neurons	11
	FOXA3, HNF1A, HNF6		In vitro			Vein- and blood-derived endothelial	Hepatic progenitor cells	12
Retrovirus/intraperitoneally injection	GMT/SB431542+XAV939	-	In vivo	Easy to package	Integrated into the genome	Resident cardiac fibroblast	Cardiomyocytes	13
Lentivirus	miRNAs 1,133,208,499	1.5-7.7%	In vivo	Easy to package, Large packaging capacity(~9kb)	Integrated into the genome	Resident cardiac fibroblast	Cardiomyocytes	14
	Sox2	-	In vivo			Astrocytes	Neuroblasts	15
AAV	FOXA3, GATA4, HNF1A and HNF4a	4%	In vivo	Specific targeting, could target both dividing and non-dividing cells	small packaging capacity (~4.7kb)	Hepatic myofibroblast	Hepatocytes	16
	Ascl1,Lmx1a, Nurr1	66.81%	In vivo			Resident glial cells	Neurons	17
	Ngn3, Pdx1, Mafa	20%	In vivo			Pancreatic exocrine	α cell, β cell, δ cells	18,19
	Mafa and Pdx1	70%	In vivo			α cell	β cell	20
	shPTB	80%	In vivo			Astrocytes	Neurons	21
Nanoparticle	GMT	-	In vivo	Low cytotoxicity, Non-integrating		Resident cardiac fibroblast	Cardiomyocytes	22
Hydrodynamic tail vein injection	Pdx1, Neurog3 and MafA	-	In vivo	Non-integration	Could only target liver	Liver cells	Insulin producing cell	23
Small molecules	CHIR99021, RepSox, Forskolin, VPA and TTNPB	-	in vitro	Non-integration, easy to	Easy to diffuse, short half-life in vivo	Tail-tip fibroblast	Cardiomyocytes	24

	VPA, CHIR99032, RepSox, Forskolin, i-Bet151 and ISX-9	8%	in vitro	produce and administrate		Astrocytes	Neurons	25
	Forskolin,dorsomorphin	99%	in vitro			Fatal lung fibroblast	Cholinergic Neurons	26
	CHIR99021, A83-01, BIX01294, AS8351, SC1, Y27632, and OAC2	6.60%	in vivo			Fibroblast	Cardiomyocytes	27
	LDN193189, SB431542, TTNPB, thiazovivin, CHIR99021, VPA, DAPT, SAG, and purmorphamine	54%	in vitro			Astrocytes	Neurons	28
mRNA	GMT	0.50%	in vitro	Non-integration, No immune response	Multiple transfection required	Cardiac fibroblast	Cardiomyocytes	29
sgRNA	Myod1	-	in vitro	Activate endogenous gene expression directly	Required co-expression of CRISPRa protein	Fibroblast	Myoblasts	30
	Ngn1, Brn2, Ezh2 and Foxo1	83%	in vitro			Fibroblast	Neurons	31
Protein	Gata4, Hand2, MEf2c and Tbx5	80.92%	in vitro	Non-integration	Low efficiency	Fibroblast	Cardiac progenitor cells	32

Supplementary table 2 | **Non-coding RNAs in direct reprogramming**

miRNA	Cocktail	Target	Conversion cell type	Target cell type	In vitro, in vivo	Efficiency	Ref
miR-124	miR-124, MYT1L, BRN2	/	Human postnatal fibroblasts	iNs	In vitro	4-8%	33
			Human primary dermal fibroblasts			1.5%-2.9% (abdominal skin) 9.5%-11.2% (breast skin)	
miR-9/9*, miR-124	miR-9/9*, miR-124, NEUROD2, ASCL1, MYT1L	USP14, EZH2, and REST, BAF53b	human neonatal foreskin fibroblasts	iNs	In vitro	~10%	34,35
miR-124	miR124 and RA	PTBP1	P19 cells	iNs	In vitro	MAP2+ 72.7%	36
miR-9/9*, miR-124	miR-9/9*, miR-124, BCL11B, DLX1, DLX2, MYT1L, BCL-XL	/	Human postnatal fibroblasts	iNs	In vitro	MAP2+ 90%	37
			Human adult dermal fibroblasts			MAP2+ 82%	
miR-302/367	miR-302/367, VPA	/	mice astrocytes	Neuroblasts	In vivo	/	38
	miR-302/367		Human astrocytes with miR injected to mice		In vivo	/	

	miR-302/367		Human astrocytes		In vitro	80%(TUJ1)	
miR-302/367 cluster, miR-9/9*, miR-124	miR-302/367 cluster, miR-9/9*, miR-124	/	Human fibroblasts	iNs	In vitro	/	[39]
miR-9/9*, miR-124	miR-9/9*, miR-124, Ascl1	Ctdsp1 and Ptbp1	murine Müller glia	iNs	In vitro	MAP2+ 63%	[40]
miR-1, miR-133	/	miR-133 represses SRF protein, so repress prolifiration; miR-1 represses HDAC4, so promote differentiation	C2C12 myoblast cells	skeletal muscle	In vitro	/	[41]
miR-133	miR-133, Gata4, Mef2c, Tbx5	Snai1	Mouse MEFs	iCM	In vitro	0.35	[42]
	miR-133, Gata4, Mef2c, Tbx5		Mouse adult cardiac fibroblasts			0.12	
	miR133, Gata4, Mef2c, Tbx5, Mesp1, Myocd,		Human cardiac fibroblasts			23%-27%	
miR-1 miR-133, miR-208, miR-499	Combination of miR-1 miR-133, miR-208, miR-499, with/without JAK inhibitor I	affects H3K27 methylation	Mouse neonatal cardiac fibroblasts	iCM	In vitro	1.5%-7.7% without JAK inhibitor I ~28% with JAK inhibitor I	[14]
			Mouse adult heart with myocardial infarction		In vivo	1%	[43]
miR-1, miR-133	Gata4, Hand2, Tbx5, myocardin, miR-1, miR-133	may play a role in development of sarcomere structure and suppression of smooth muscle gene expression	Neonatal human foreskin fibroblasts	iCM	In vitro	~20%	[44]
			Human adult cardiac fibroblasts			~13%	
			Human adult dermal fibroblasts			~9.5%	

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