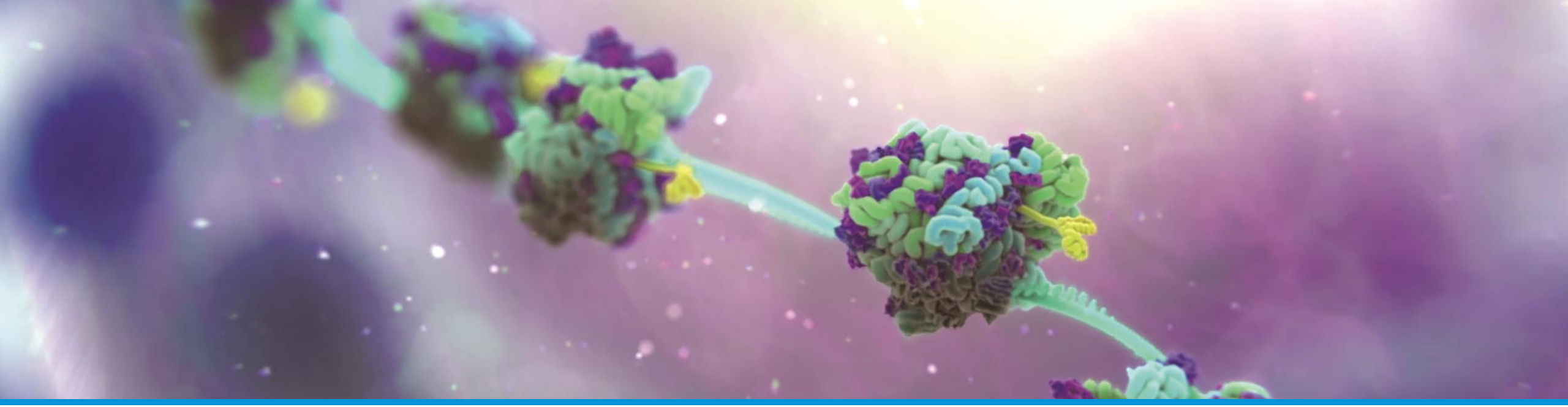

Lipid-stripped albumin enables high-efficiency mRNA reprogramming of adult human fibroblasts

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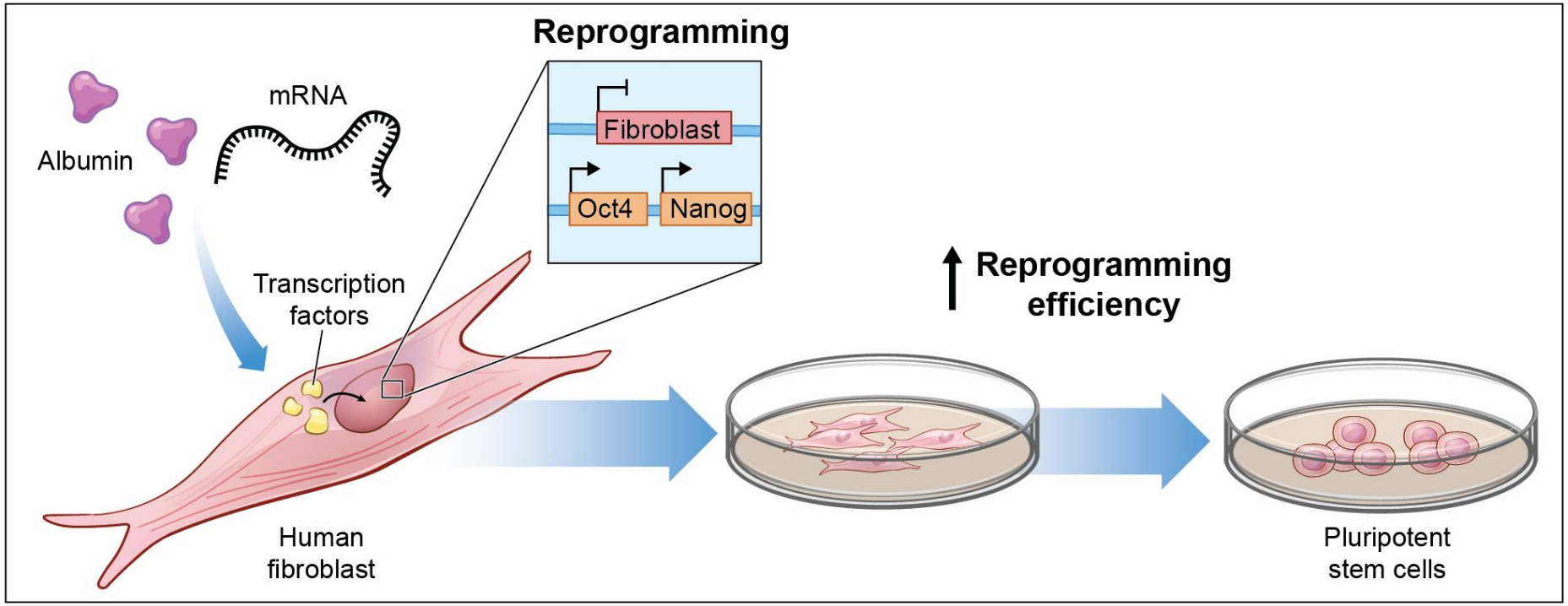
5/12/2021

Standardizing mRNA reprogramming medium



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mRNA reprogramming medium formulation

Reprogramming medium
DMEM/F12
L-ascorbic acid-2-phosphate
ITSE
bFGF
recombinant human serum albumin (HSA)
pyridoxal hydrochloride
Y-27632
choleserol
hydrocortisone
polyoxyethylenesorbitan monooleate
D-alpha-tocopherol acetate
HEPES

Essential 8
DMEM/F12
L-ascorbic acid-2-phosphate
ITSE
bFGF
TGFβ1

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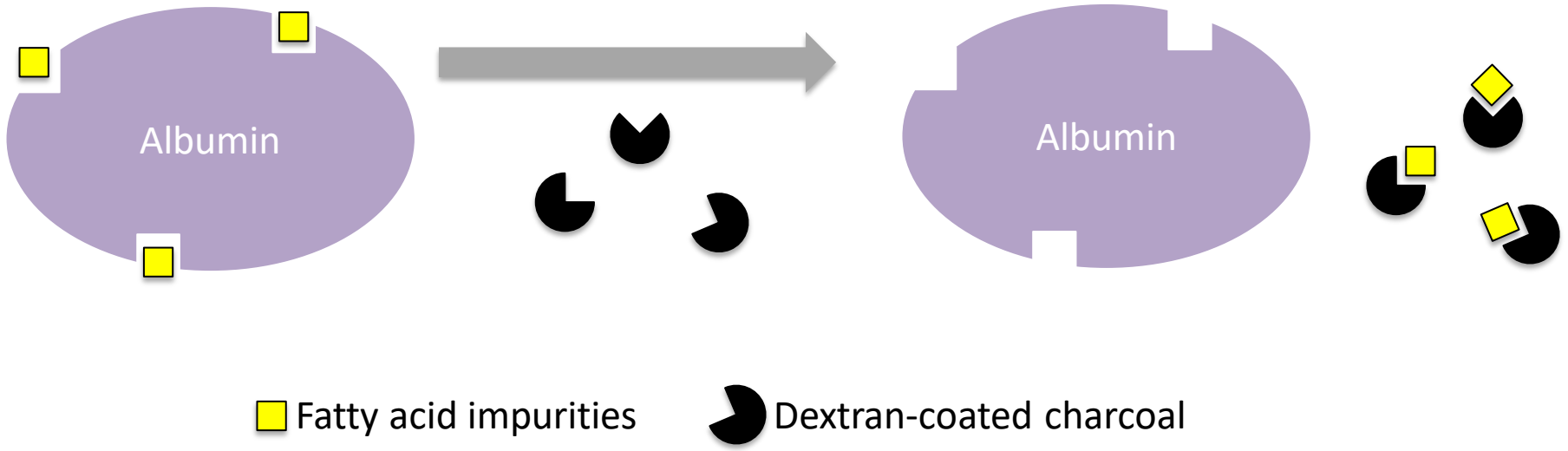
Recombinant human serum albumin treatment

1. Sodium octanoate used to stabilize HSA during heat treatment
2. Mixed-bed resin used to deionize HSA
3. Dextran-coated activated charcoal used to strip fatty acids from HSA



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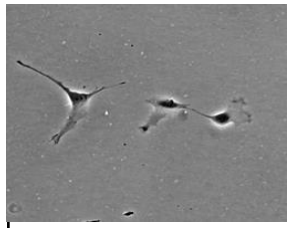
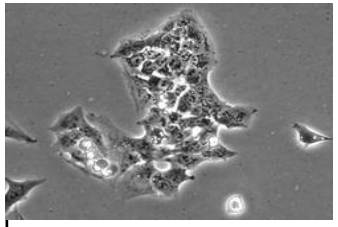
Reprogramming timeline

DMEM + 10% FBS
HSA reprogramming medium
Essential 8 medium

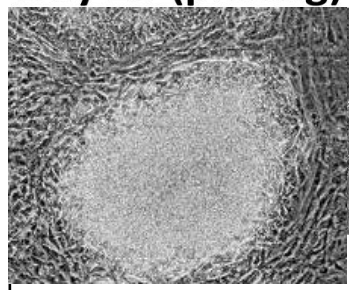


Day 1
0.25ug transfection



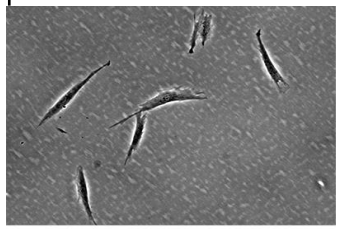
Day 6, 7, & 8
2ug transfections

Day 15 (picking)

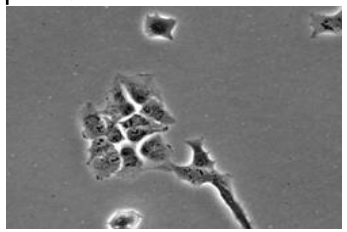


.25ug | 1ug | 1ug | 2ug | 2ug | 2ug

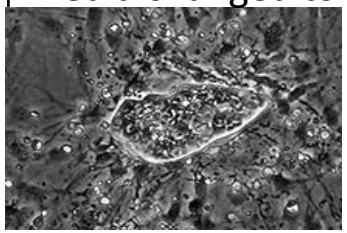
Day 0
Plate at low density



Day 4 & 5
1ug transfections



Day 13
Media changed to E8



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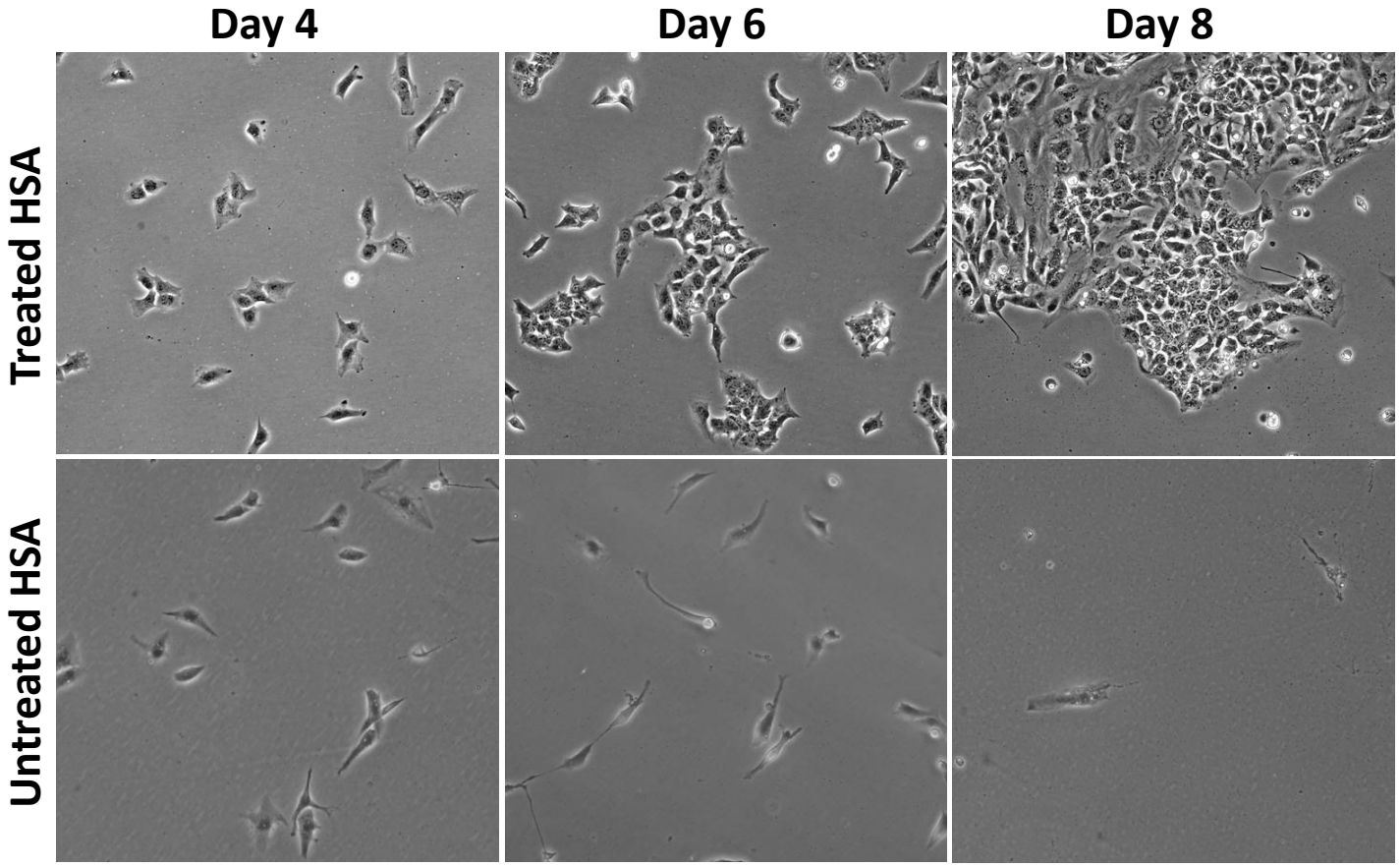
Reprogramming comparison



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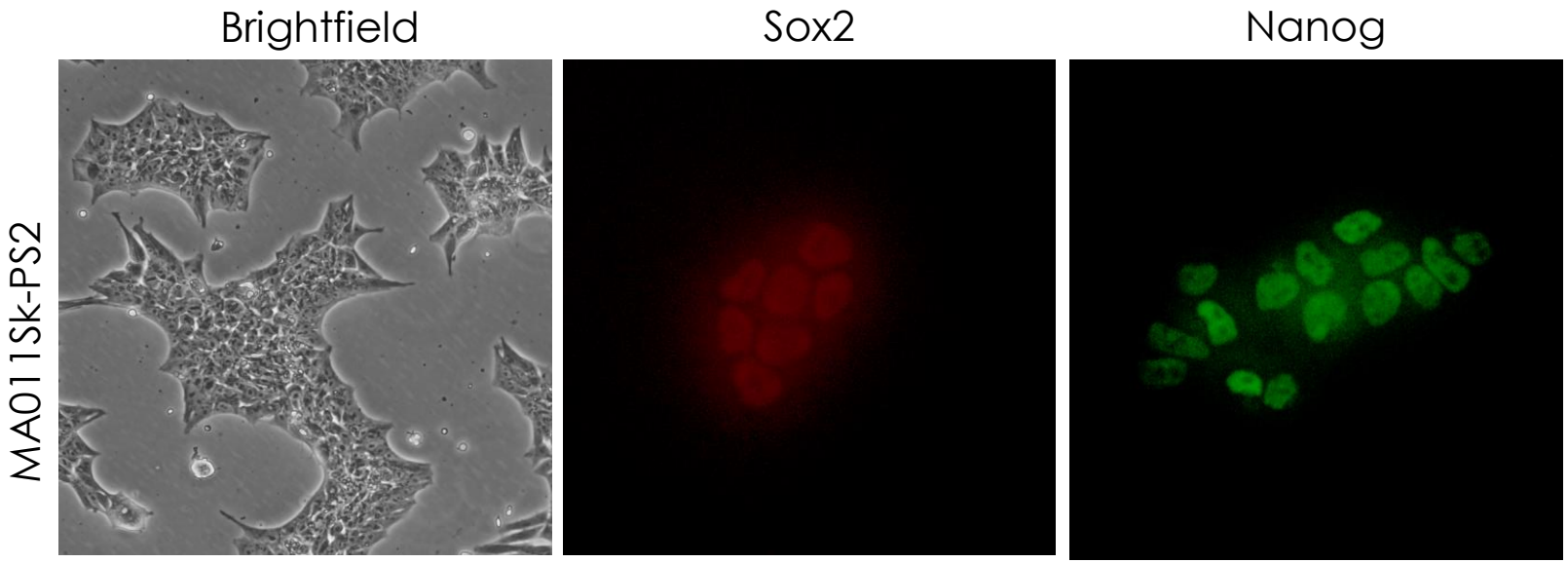
Characterization of resulting pluripotent stem cells

>6% efficiency was achieved using 6 transfections. Colonies were stained for SSEA4 on day 20.



mRNA-reprogrammed cells (MA011sk PS2) were picked and cultured before being fixed with 4% Paraformaldehyde, permeabilized with triton, and then blocked with casein. Cells were stained for Sox2 and Nanog.

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Whole genome sequencing

- mRNA-reprogrammed cells were sequenced using Illumina HiSeq to obtain an average of 24x Coverage
- Human Reference genome 38 (Hg38) used for comparison
- No reprogramming factors were found duplicated in the genome

Variants by Sequence Context

	SNVs	Insertions	Deletions
In Genes	1,535,883	173,382	169,437
In Exons	52,494	3,905	3,809
In Coding Regions	22,909	226	291
In UTR Regions	29,585	3,679	3,518
In Splice Site Regions	2,635	261	261
In Mature microRNA	0	0	0

Genes include exons, introns and UTR regions. Exons include coding and UTR regions. UTR regions include 5' and 3' UTR regions. Splice site regions include regions annotated as splice acceptor, splice donor, splice site or splice region.

Variants by Consequence

	SNVs	Insertions	Deletions
Frameshifts	-	59	86
Non-synonymous	10,864	162	199
Synonymous	11,931	-	-
Stop Gained	94	5	0
Stop Lost	20	0	3

Variation consequences are calculated following the guidelines at http://uswest.ensemble.org/info/genome/variation/predicted_data.html #consequences

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Discussion

- Non-immunogenic synthetic mRNA can be used for high-efficiency reprogramming with a reduced transfection schedule
- Dextran-coated charcoal-treated HSA is vital to successful mRNA reprogramming
- Future studies should examine the roles of individual HSA-associated molecules to determine the reprogramming-limiting factor

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Acknowledgements

I'd like to thank the team at Factor Bioscience Inc for their support and assistance. I greatly appreciate the feedback and guidance I've received on this project and others I've worked on over the years.

Conflict of interests

JH, FK, CR, and MA are inventors on U.S. Pat. Appl. No. 63,016,626. CR and MA are inventors on U.S. Pat. Nos. 8,497,124; 9,127,248; 9,399,761; 9,562,218; 9,695,401; 9,879,228; 9,969,983; 10,131,882; 10,201,599; 10,443,045; and others.

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