A xeno-free media formulation that supports efficient mRNA reprogramming of human fibroblasts into induced pluripotent stem cells under feeder-free conditions

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Introduction

Human induced pluripotent stem cells (iPSC) play an important role in disease modeling as well as drug discovery and hold great promise for future clinical applications. Cellular reprogramming with mRNA is considered to be the most straightforward method for generation of high-quality iPSC, since the procedure eliminates the risk of genomic integration. Importantly, the potential clinical use calls for protocols and cultivation media that are compliant with GMP cell production.

To meet this demand, we have developed a xeno- and serum-free media formulation for mRNA-based reprogramming. Our media supports rapid and efficient reprogramming of human fibroblasts into iPSC within 12–16 days using a 6-factor mRNA cocktail. Reprogramming is done under non-hypoxic, feeder-free conditions without the need of conditioned medium, and is compatible with various cultivation matrices, thereby enabling a safe and convenient way for generating iPSC.

Results

1 StemMACS™ Repro-Brew XF supports rapid and efficient mRNA-based reprogramming of human fibroblasts into iPSC

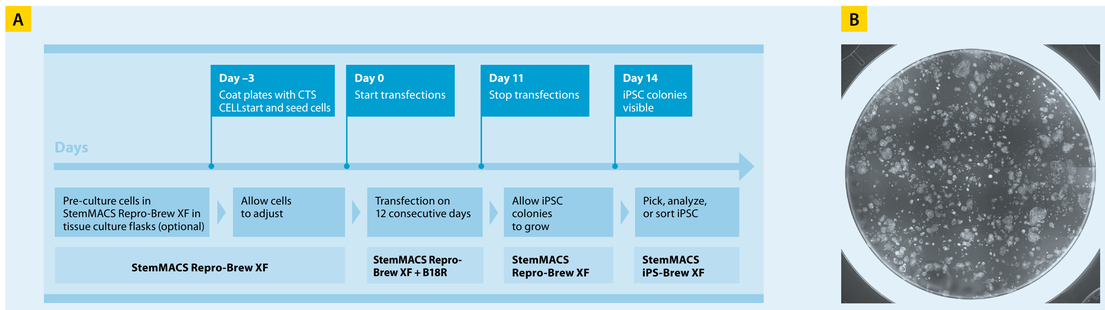
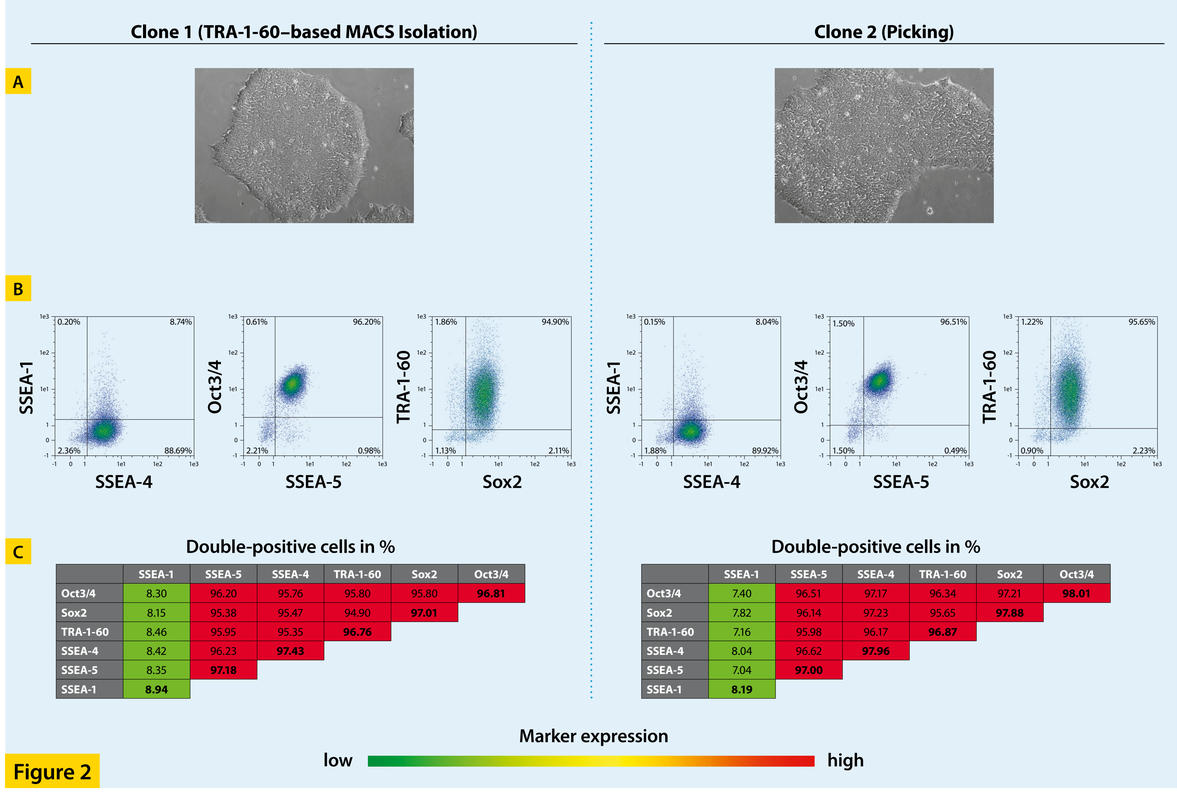


Figure 1

The protocol for mRNA-based reprogramming of human fibroblasts **(A)** starts with the seeding of fibroblasts on a matrix (e.g. CTS™ CELLstart™, Matrigel, or Vitronectin) 2–3 days prior to the first mRNA transfection. After adaption to the StemMACS™ Repro-Brew XF reprogramming medium, cells are transfected on 12 consecutive days with a 6-factor mRNA cocktail (StemMACS mRNA Reprogramming Kit: Oct4, Sox2, Klf4, c-Myc, Nanog, Lin28). At the end of the reprogramming procedure fully reprogrammed iPSC colonies can either be visualized by immunofluorescent staining of, e.g., Oct3/4 **(B)** or isolated by magnetic cell sorting (MACS® Technology) based on the marker TRA-1-60 or picking. Isolated cells can then be expanded to establish a new iPS cell line.

2 After reprogramming in StemMACS™ Repro-Brew XF, cells show typical iPSC morphology and marker expression



	SSEA-1	SSEA-5	SSEA-4	TRA-1-60	Sox2	Oct3/4
Oct3/4	8.30	96.20	95.76	95.80	95.80	**96.81**
Sox2	8.15	95.38	95.47	94.90	**97.01**	
TRA-1-60	8.46	95.95	95.35	**96.76**		
SSEA-4	8.42	96.23	**97.43**			
SSEA-5	8.35	**97.18**				
SSEA-1	**8.94**					

Double-positive cells in %

	SSEA-1	SSEA-5	SSEA-4	TRA-1-60	Sox2	Oct3/4
Oct3/4	7.40	96.51	97.17	96.34	97.21	**98.01**
Sox2	7.82	96.14	97.23	95.65	**97.88**	
TRA-1-60	7.16	95.98	96.17	**96.87**		
SSEA-4	8.04	96.62	**97.96**			
SSEA-5	7.04	**97.00**				
SSEA-1	**8.19**					

Double-positive cells in %

Figure 2

Cell lines generated in StemMACS™ Repro-Brew XF showed a typical iPSC morphology **(A)**. To confirm the quality of the generated cell lines marker expression was monitored closely over several passages using flow cytometry. The two shown cell lines displayed a high and persistent expression of pluripotency markers such as TRA-1-60, SSEA-4, SSEA-5, Oct3/4, and Sox2 while maintaining a low expression level of differentiation markers such as SSEA-1 **(B)**. A heat map of an iPSC marker panel shows the percentage of double-positive cells for various iPSC markers **(C)**. In summary, iPSC generated in StemMACS Repro-Brew XF show typical stem cell characteristics.

3 iPSC generated in StemMACS™ Repro-Brew XF can be differentiated into all three germ layers and maintain a normal karyotype

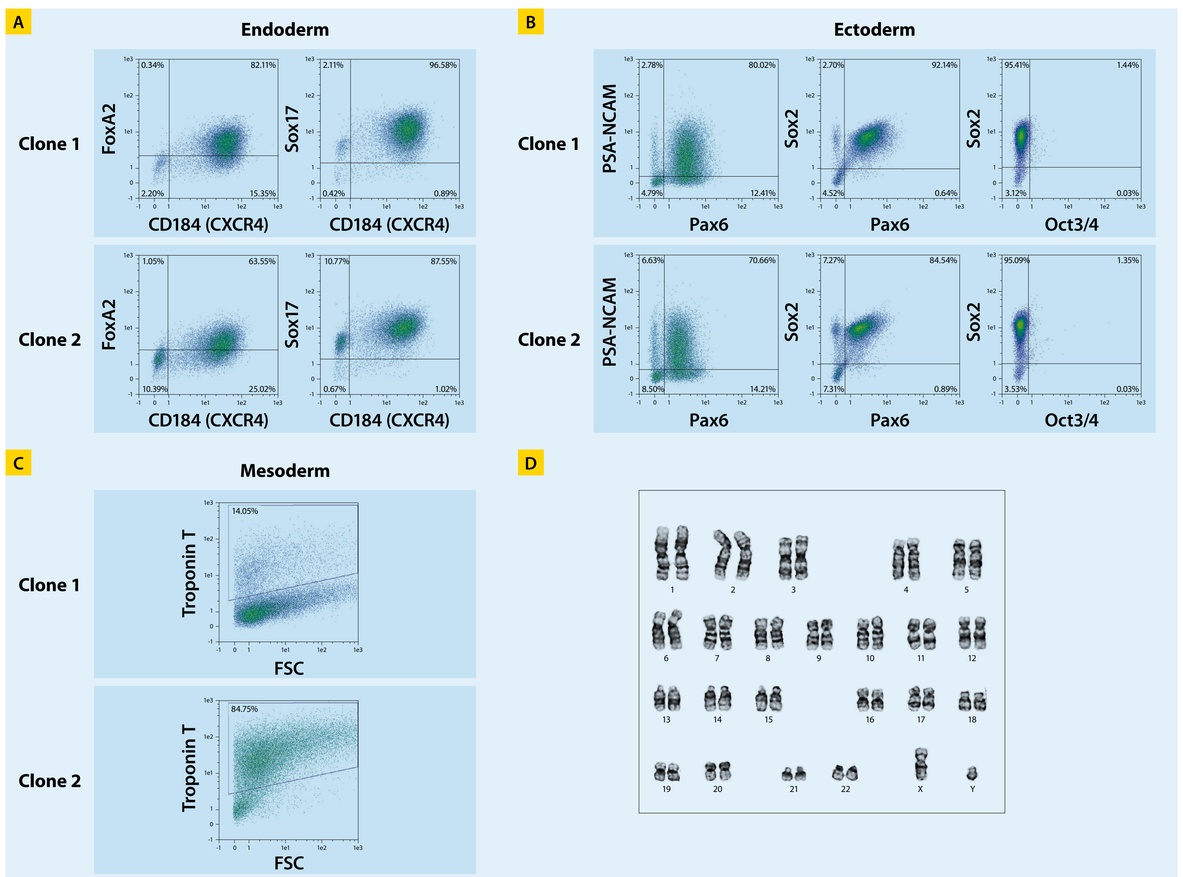
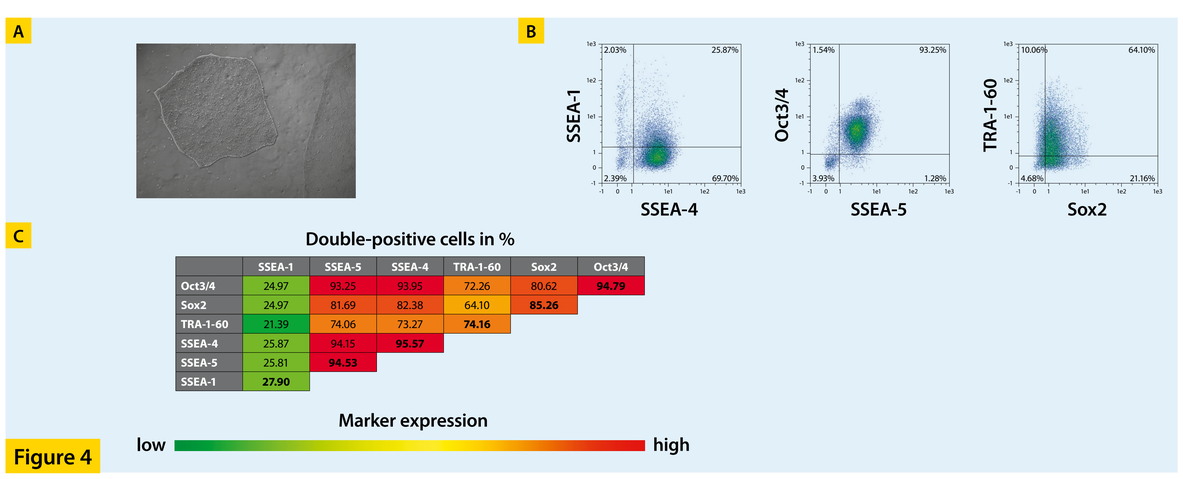


Figure 3

Next, we determined the differentiation potential of two generated cell lines. **(A)** Endodermal differentiation was done using Activin A combined with the GSK3 inhibitor CHIR99021 in StemMACS™ iPS-Brew XF to derive FoxA2$^+$CXCR4$^+$ definitive endoderm cells. **(B)** For ectodermal differentiation Sox2$^+$Pax6$^+$ neural stem cells were generated by inhibition of TGF-β and BMP signaling, using SB431542 in combination with LDN-193189. **(C)** Mesodermal differentiation was done using a monolayer cardiac differentiation protocol based on the temporally regulated activation and inhibition of Wnt signaling by the small molecules CHIR99021 and IWR-1, thereby generating Troponin T–positive, beating cardiomyocytes. **(D)** To determine the genomic stability of iPSC generated in our medium, we analyzed the karyotype of generated cell lines in regular intervals (example shown for clone 1 in p20). All in all, cells reprogrammed in StemMACS Repro-Brew XF have a high differentiation potential while maintaining a normal karyotype.

4 StemMACS™ Repro-Brew XF allows for mRNA-based reprogramming of renal epithelial cells (REC) into iPSC



	SSEA-1	SSEA-5	SSEA-4	TRA-1-60	Sox2	Oct3/4
Oct3/4	24.97	93.25	93.95	72.26	80.62	**94.79**
Sox2	24.97	81.69	82.38	64.10	**85.26**	
TRA-1-60	21.39	74.06	73.27	**74.16**		
SSEA-4	25.87	94.15	**95.57**			
SSEA-5	25.81	**94.53**				
SSEA-1	**27.90**					

Double-positive cells in %

Figure 4

With modifications of the above protocol (fig. 1A) StemMACS™ Repro-Brew XF also allowed for the reprogramming of human REC isolated from urine. The generated iPSC colonies showed a typical morphology **(A)**, expressed various pluripotency markers such as TRA-1-60, SSEA-4, SSEA-5, Oct3/4, and Sox2 while maintaining a low expression level of differentiation markers such as SSEA-1 **(B** and **C)**. Hence, StemMACS Repro-Brew XF also enables the reprogramming of other cell types besides fibroblasts.

Conclusion and outlook

We have developed a xeno- and serum-free medium that:

- supports rapid and efficient mRNA-based reprogramming of human fibroblasts into iPSC,
- enables the generation of iPSC with typical morphology and marker expression,
- allows for the resulting iPSC to be differentiated in all three germ layers while maintaining a normal karyotype,
- also supports reprogramming of REC into iPSC.