Neuro
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Standardized QC workflow for analyzing the quality and differentiation potential of human iPSCs

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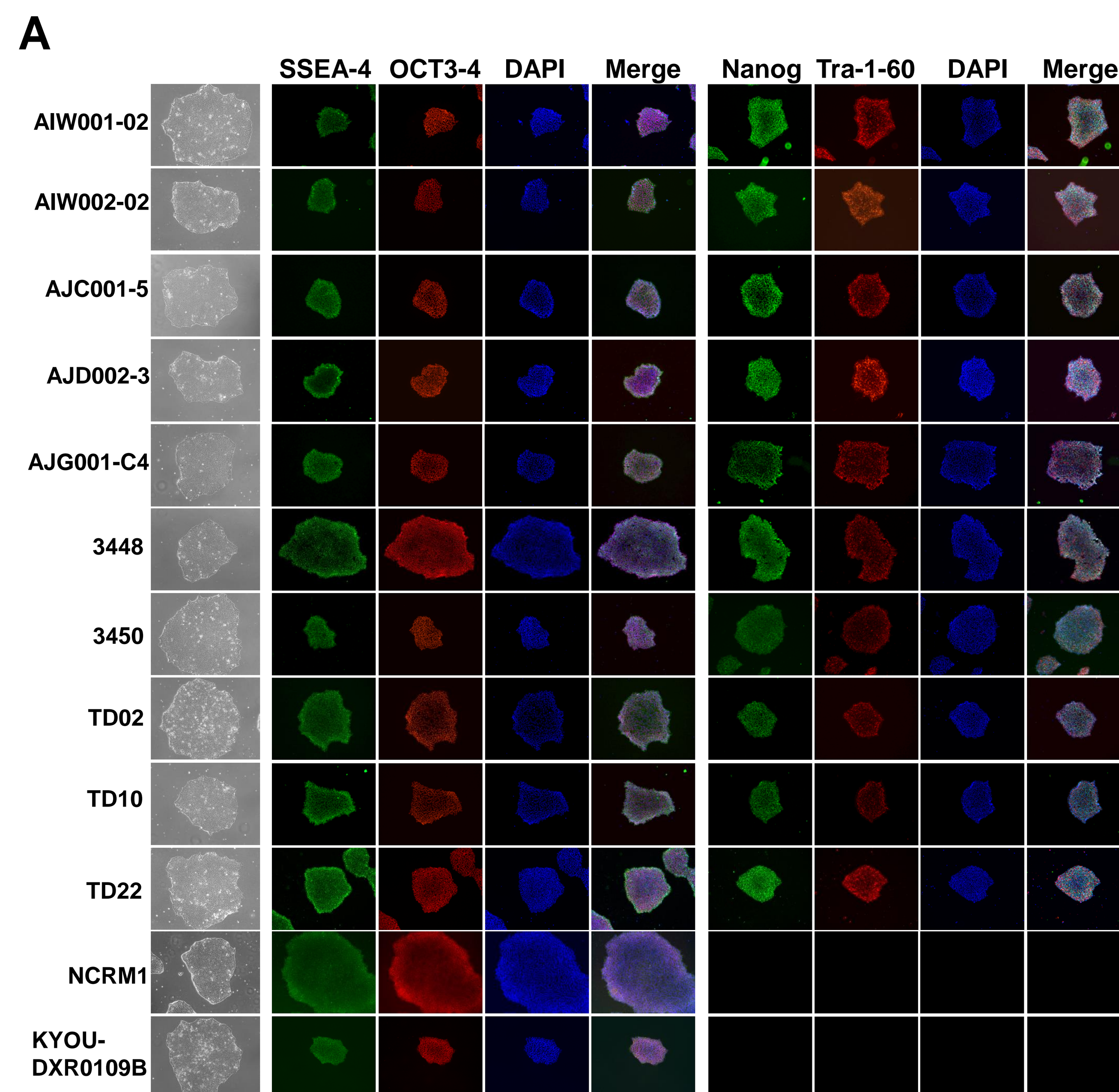
Introduction

Human induced pluripotent stem cells (hiPSCs) derived from human somatic cells have given scientists new opportunities to model and investigate human diseases, and to develop new therapeutics. The routine use of hiPSCs and functional cells derived from hiPSCs in high throughput applications for drug discovery will require a constant supply of pluripotent, well characterized and quality controlled cell stocks. However, the lack of standardized quality control of IPSC hinders laboratory efficiency and experimental reproducibility. We have established a workflow to monitor hiPSC morphology and proliferation using longitudinal time-lapse imaging, coupled with assessment of pluripotency through immunofluorescence staining and qPCR analysis of pluripotency markers. We also assess embryoid body (EB) formation and differentiation into three germ layers by qPCR and IF staining, and karyotypic abnormalities by qPCR.



B

Relative Expression

C-MYC
KLF4
NANOG
OCT3/4
SOX2
ZFP42

H9
AIW001-02
AIW002-2
AJC001-5
AJD002-3
AJG001-C4
3448
3450
TD02
TD10
TD22
NCRM1
KYOU-DXR0109B

Fig. 1: HiPSCs exhibit pluripotency. *(A) Representative phase contrast images and immunostaining for pluripotency markers SSEA-4, Oct3-4, Nanog and Tra1-60. (B) qPCR for mRNA expression of pluripotency markers. The expression of pluripotency markers in all hiPSCs is similar to H9 (embryonic stem cells).*

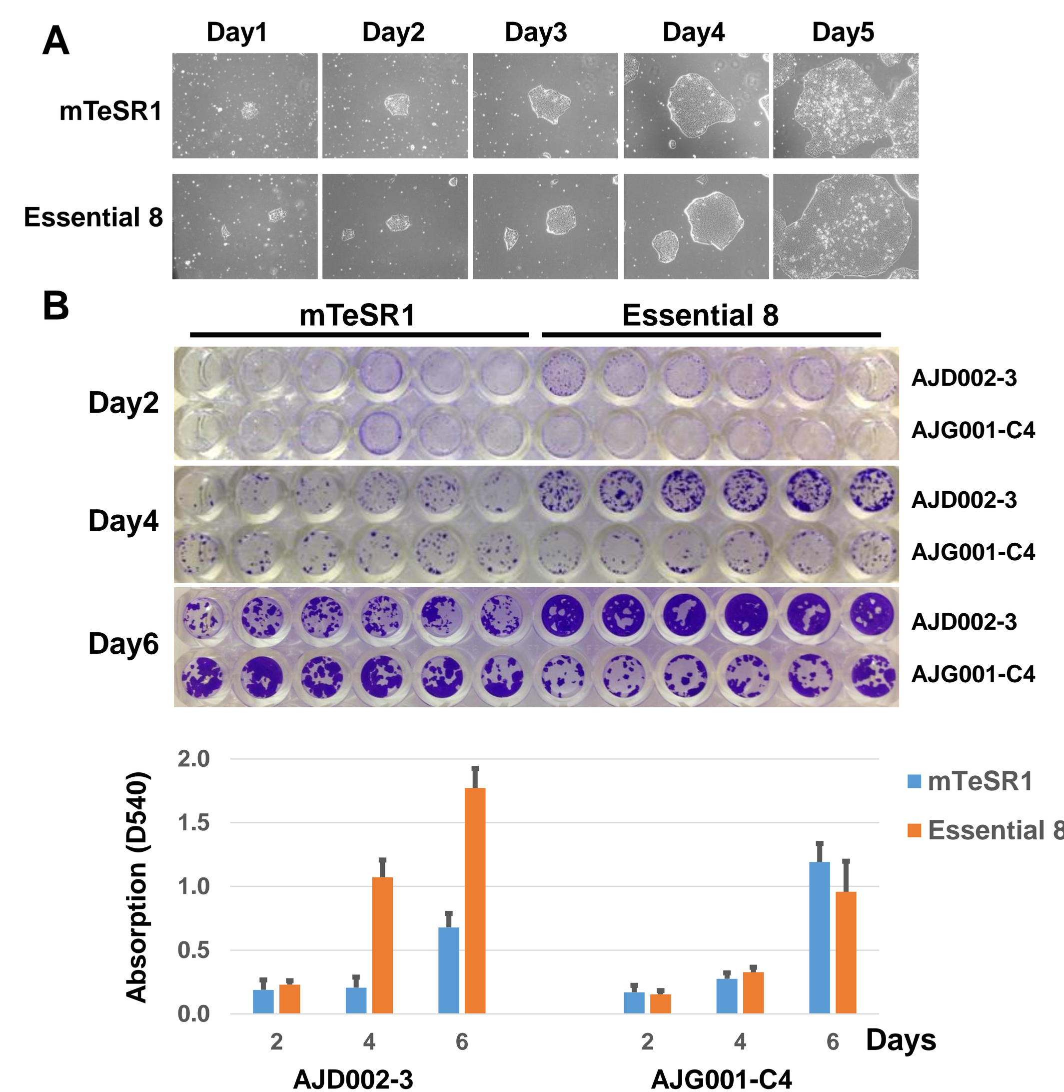


Fig. 2: HiPSC growth and proliferation under different conditions. *(A) Representative daily bright field images of hiPSCs. (B) Crystal violet assay shows that AJG001-C4 grows better in mTeSR1 media (Stemcell Tech.) while AJD002-3 cells proliferate best in Essential 8 media (ThermoFisher).*

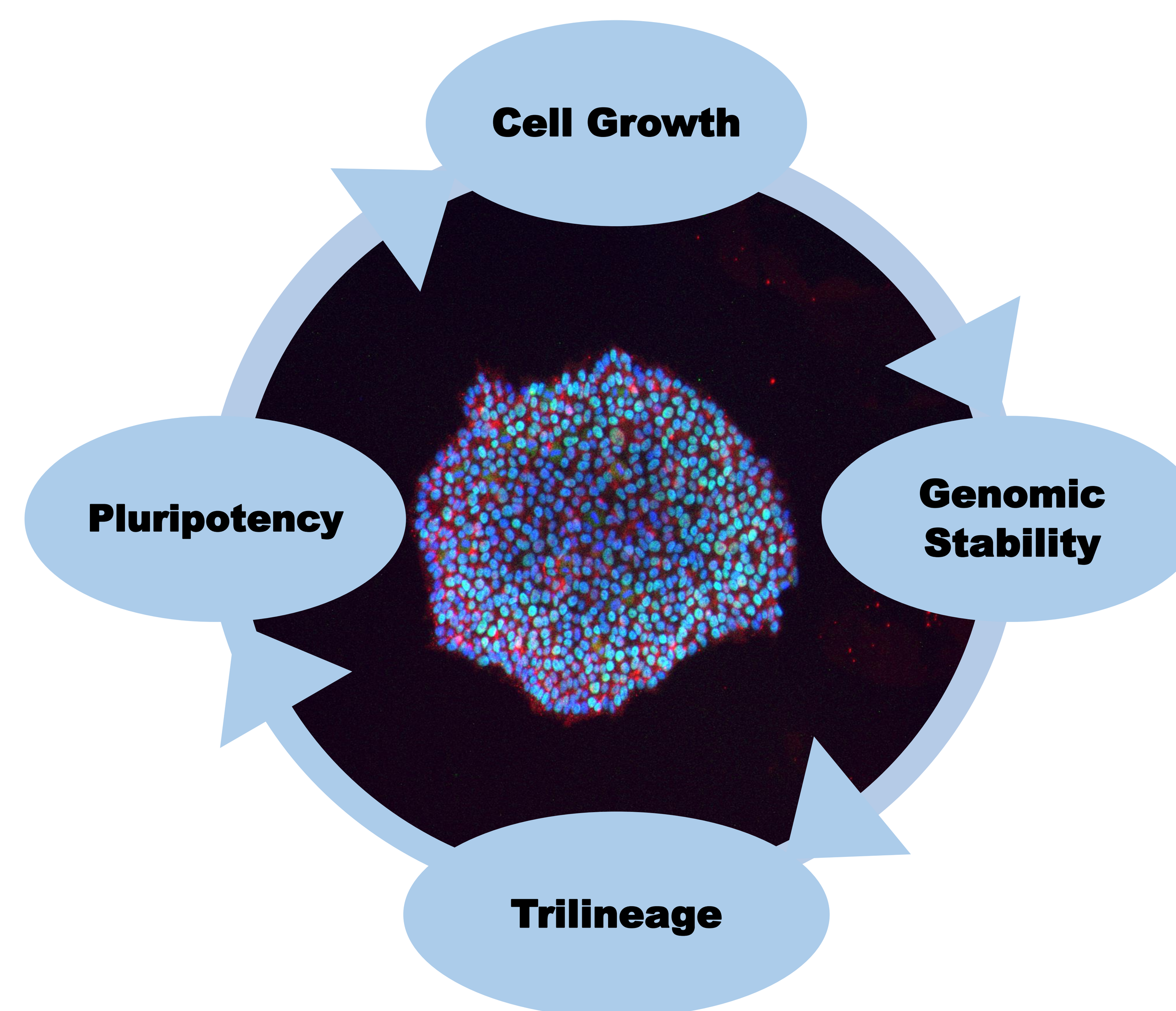


Table 1. Overview of healthy control hiPSC profiles

Hipsc lines	Source	Gender	Age of donor	Reprogramming	Karyotype	Genomic stability	Pluripotency	Trilineage	Culture media
AIW-001-02	MNI	Female	48	Retrovirus	Normal	Normal	+	+	mTeSR1
AIW-002-02	MNI	Male	37	Retrovirus	Normal	Normal	+	+	mTeSR1
AJC001-5	MNI	Male	37	Retrovirus	Normal	Normal	+	+	mTeSR1
AJD002-3	MNI	Male	44	Retrovirus	Normal	Normal	+	+	Essential 8
AJG001-C4	MNI	Male	37	Episomal	Normal	Normal	+	+	mTeSR1
3448	MNI	Male	48	Episomal	Normal	Normal	+	+	Essential 8
3450	MNI	Male	37	Episomal	Normal	Normal	+	+	Essential 8
TD02	MNI	Male	37	Episomal	Normal	Normal	+	+	mTeSR1
TD10	MNI	Female	64	Episomal	46,X,t(X;2)(q26;p13)	Normal	+	+	Essential 8
TD22	MNI	Male	59	Episomal	Normal	Normal	+	+	Essential 8
NCMR1	NIH	Male	Unknown	Episomal	In progress	20q duplication	+	+	mTeSR1
KYOU-DXR0109B	ATCC	Female	Unknown	Retrovirus	In progress	20q duplication	+	+	mTeSR1

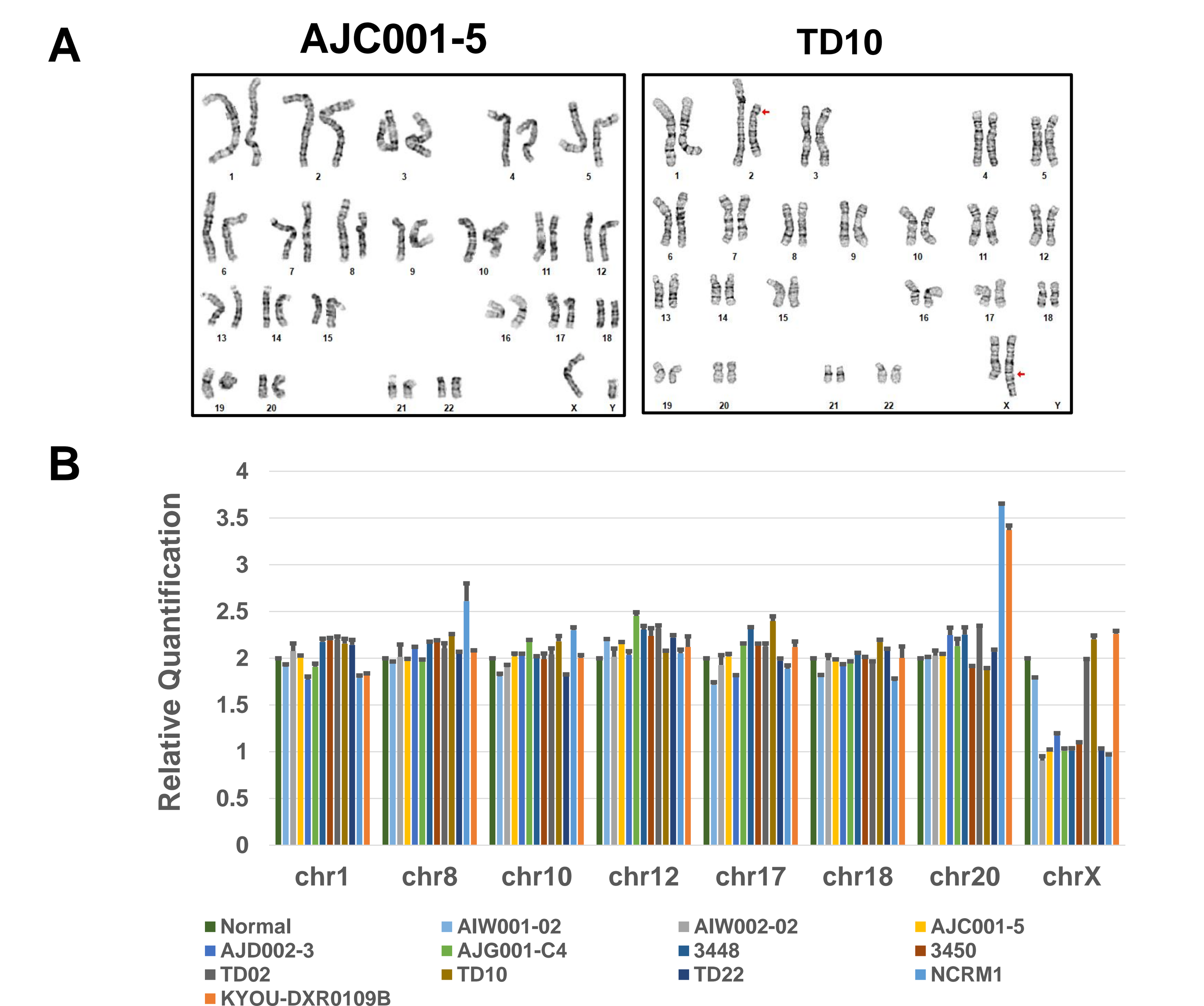


Fig. 3: Genomic stability analysis. *(A) Karyotyping and G-band analyses showing examples of one normal (left) and one abnormal hiPSC karyotype. An apparently balanced translocation between the long (q) arm of chromosome X and the short (p) arm of chromosome 2 is present in TD10 (red arrow). (B) qPCR-based genetic analysis to check for small chromosome abnormalities in all hiPSC lines.*

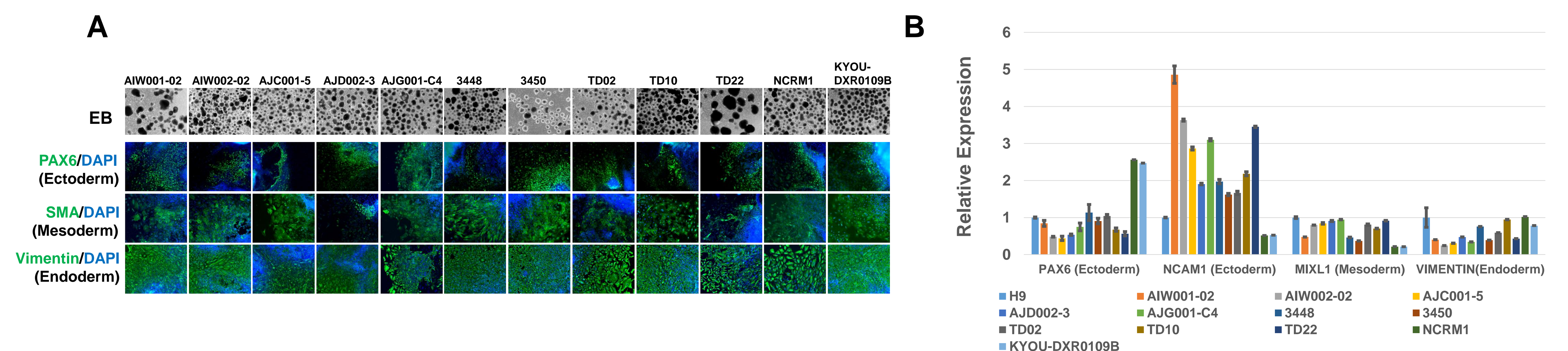


Fig. 4: Differentiation of hiPSCs into three germ layers. *(A) Representative phase contrast images of embryoid bodies (EB) of hiPSCs and immunostaining for the three germ layers markers PAX6, SMA and Vimentin. (B) qPCR for mRNA expression of three germ layer markers. The expression of the three germ markers in hiPSCs is similar to H9 cells.*

Conclusion

The workflow outlined here provides routine quality control of hiPSCs through simple, standardized characterization and can be adapted by researchers for their own labs. This approach ensures high quality hiPSCs are available for research and translational work.

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