Supplementary information

Regeneration of the entire human epidermis by transgenic stem cells

Tobias Hirsch, Tobias Rothoeft, Norbert Teig, Johann W. Bauer, Graziella Pellegrini, Laura De Rosa, Davide Scaglione, Julia Reichelt, Alfred Klausegger, Daniela Kneisz, Oriana Romano, Alessia Secone Seconetti, Roberta Contin, Elena Enzo, Irena Jurman, Sonia Carulli, Frank Jacobsen, Thomas Luecke, Marcus Lehnhardt, Meike Fischer, Maximilian Kueckelhaus, Daniela Quaglino, Michele Morgante, Silvio Bicciato, Sergio Bondanza, Michele De Luca

Supplementary Tables

Supplementary Table 1. List of I7-index primers and I5 LTR-primers used for library preparation.

Primer set	*Primer name*	*Primer sequence*
I7	Linker_primer_701_N	CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGA CGTGTGCTCTTCCGATCTGTAATACGACTCACTATAGGGC
	Linker_primer_702_N	CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGA CGTGTGCTCTTCCGATCTGTAATACGACTCACTATAGGGC
	Linker_primer_703_N	CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAG ACGTGTGCTCTTCCGATCTGTAATACGACTCACTATAGGGC
I5	MuLV_LTR-3pIN_501_N	AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCC TACACGACGCTCTTCCGATCTGACTTGTGGTCTCGCTGTTCCTTGG
	MuLV_LTR-3pOUT_502_N	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCC CTACACGACGCTCTTCCGATCTGGGTCTCCTCTGAGTGATTGACTACC

I7 primers (701/702/703) anneal on the common universal adapter introduced during ligation step and allow to multiplex up to three samples per lane. I5 primers (501/502) anneal on LTR specific region of MuLV vector and allow to multiplex two different priming sites.

Supplementary Table 2. List of primers used for LAM-PCR on holoclones.

Primer name	*Primer sequence*
MLV 3´LTRlin_biotin	GGTACCCGTGTATCCAATAA
MLV 3´LTR_biotin	GACTTGTGGTCTCGCTGTTCCTTGG
LCrv	GTAATACGACTCACTATAGGGC
MLV 3´LTR nested	GGTCTCCTCTGAGTGATTGACTACC
LCrv	AGGGCTCCGCTTAAGGGAC
LC1 TAlinkerMse(+)	GTAATACGACTCACTATAGGGCTCCGCTTAAGGGAC
LC2 TAlinkerMse(-)	TAGTCCCTTAAGCGGAG

Supplementary Table 3. List of primers used for PCR on meroclones and paraclones in PGc, 4Mc, and $8Mc_1$.

Culture	*Primer name*	*Primer sequence*
PGc	MLV 3´LTR control F	GGACCTGAAATGACCCTGTG
	Chr.5a	ACCCACAGCTCCTGTCTCAT
	Chr.2a	TTCTTTCAGTCTGGTGGGGTG
	Chr.4a	TGGTGGTGGAGTATCTGGAG
	Chr.4b	GTGGTGGTGGAGTATCTGGAG
	Chr.19a	CTCACCATCATGAGGAGCAA
	Chr.19b	CTCACCATCATGAGGAGCAA
	Chr.5b	GAGCAATTTGAGGGTCAGAGA
	Chr.17c	GAAATCAAGATTGTATCACGTTCC
	Chr.16	CTGCACACATGCCCTCTTT
	Chr.2b	TCCCAGGAACTTTGTTCAGA
	Chr.3	CCCTAAGGAGCTCCAACTGA
	Chr.Y	CTGAGGATGGTGGCAGAAAT
	Chr.6	GCCAATTAACACTCGTTCACC
	Chr.14b	GGCTCCCAGGTATGTTCTCA
4Mc	Chr.1	CCTGATGTTCTGTCCCCCTA
	Chr.9a	GCATGCACAACAGCTCAAAC
	Chr.14a	GCCTCCATTTGGAGAGAAAAT
	Chr.15a	CCTCCTCCTCTTCCCTTGAT
$8Mc_1$	Chr.8	CGGCAACCACTTTAAAGGAC
	Chr.9b	GCCTCACTTTCTTTCTCTGTAAATG
	Chr.17a	GGCTCACTGCAACCTTCATC
	Chr.X	CTGGAGCTGGGTGAGATAAAG
	Chr.5c	GGAATGGGGCATAAGAGACA
	Chr.17d	TTGAGATAGTCTTACGCTGTCACC

Supplementary Table 4. List of independent integrations identified by NGS analysis.

The libraries of integrations were obtained using two independent LTR-primers (3pIN, 3pOUT). The .xlsx file contains the list of independent integrations found in PGc, 4Mc, $8Mc_1$ and $8Mc_2$ and merged data (all_integrations) showing integrations retrieved across samples.

Supplementary Data

To investigate the presence of spontaneous mosaic revertants, NGS analysis was performed on pre-graft cultures. NGS sequencing was performed with the PGM (Life Technologies) with a coverage of 692 reads. We detected 12 reads (1.7%) with a G at position c.1977-1G>A in conjunction with 1977delG. The deletion leads to a frameshift in exon 15 resulting in a premature termination codon. These results were confirmed by cloning PCR products of exon 15 of transduced pre-graft cultures into a standard TA cloning vector (Stratagene). PCR products were digested with DdeI restriction enyzme, which recognizes only the wildtype or any revertant sequence (CTCAG) but not the mutant sequence. DdeI was able to cut a wildtype control but was unable to cut any of the amplified samples, hence confirming the absence of a reversion.