SOP: Propagation of Engineered K562 cells
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Source Information

Engineered K562 cells were received from either Sangamo BioSciences, Inc., Richmond, CA or from Sigma-Aldrich Corp., St. Louis, MO, USA. The parental K562 cell line (K562_P5) used for genomic engineering was originally obtained from ATCC (catalog #CCL-243; chronic myelogenous leukemia derived cell line with erythroid properties).

Engineered cells were derived using sequence-specific Zinc finger nucleases (ZFNs) for targeted genome editing. At user-specified locations, these enzymes create double-strand DNA breaks that are subsequently altered by endogenous DNA repair mechanisms so as to generate precisely targeted genomic edits (deletions, integrations, or sequence alterations; see http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology.html, Chen, et. al., 2011, Nature Methods 8:753–755, and Miller et al., 2007, Nature Biotechnology 25:778–785).

The targeted regions in K562 cells include the well-known regulatory sites in the beta-globin locus control region (LCR) characterized by five DNaseI hypersensitive sites (DHSs) that are active in K562 cells; other targeted sites include DHS regions with potential regulatory function that are also active in K562 cells.

Notes:
These cell lines grow in suspension.

K562 Parental Cells and ZFN-Treated Clone List

Cell Line	*ZNFs Target Region*	*Genomic Alteration*	*All Alleles Affected?*
K562_P5	None (Parental)	None (Parental)	Not applicable
K562_2C10_C5	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	Yes
K562_4C5_C4	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	Yes
K562_4G7_D3	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	Yes
K562_A4.1_C6	chr11:5295966-5314244 globin LCR	None (treated nonmutation control)	Not applicable
K562_A4.1_E2	chr11:5295966-5314244 globin LCR	None (treated nonmutation control)	Not applicable
K562_B3.4_A8	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	No
K562_E10.3_C6	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	No
K562_E6.1_A1	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	No
K562_F4.1_B2	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	No
K562_G5.4_A11	chr11:5295966-5314244 globin LCR	18.3 kb deletion (LCR HS1–5)	No
K562_ZFN_12-11	chr17:19222172-19222177 DHS site	10 bp deletion	Yes
K562_ZFN_29-17	chr2:201689777-201689783 DHS site	29 bp deletion	No

(GRCh37/hg19 coordinates)

Materials List

1. Iscove's DMEM, 1X, (Iscove's modification of DMEM) Medium, with L-glutamine and 25mM HEPES, without α-thioglycerol and β-mercaptoethanol (Cellgro, Cat# 10-016-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH-30071-03)
3. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
4. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
5. T25, T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated serological pipets (1, 5, 10, 25, 50mL)
8. Freezing Medium (growth medium containing 10% DMSO)
9. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
10. CryoVials (Nunc, Cat# 368632)
11. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)

12. Eppendorf Centrifuge 5810R
13. Revco UltimaII -80°C Freezer
14. Thermolyne Locator 4 Liquid Nitrogen Freezer
15. Hemocytometer
16. Micropipet w/ P20 tips
17. Microscope

Growth Medium for Engineered K562 Cells

Iscove's DMEM Medium, 1X, with L-glutamine and 25mM HEPES, without α-thioglycerol and β-mercaptoethanol
10% Characterized FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Cultures

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in 37°C water bath until ice crystals disappear.
3. Swab outside of the ampoule with 70% ethanol and then dispense contents of ampoule into a 15mL Corning conical centrifuge tube.
4. Add 10mL cold growth medium, drop wise, into the centrifuge tube containing cells.
5. Pellet cells gently at 200 x g 4°C 5 minutes and remove DMSO-containing supernatant.
6. Resuspend pellet at $2x10^5$cells/mL with pre-warmed growth medium and grow in a 37°C, 5% CO_2 humidified incubator. **Concentration of cells should never exceed $1x10^6$cells/mL.**

B. Sub-culture and Maintenance

1. Take cell counts with a hemocytometer every 24-48 hours to maintain the culture at a cell density between $2x10^5$cells/mL and $1x10^6$cells/mL. The cells have a fairly rapid doubling time and the concentration of cells should not exceed $1x10^6$cells/mL.
2. Add fresh warm medium when appropriate to maintain cell density and expand the culture to the desired number of cells.
3. Record each subculture event as a passage.

C. Generation of Seed Stocks

1. At an early stage of expansion and with sufficient number of cells to continue maintenance, a small portion of the cells should be set aside as a seed stock, if needed.
2. Cells for the seed stock should be placed in a conical centrifuge tube and centrifuged at 500 x g (4°C) for 5 minutes.
3. Aspirate supernatant and resuspend the cell pellet in 1X PBS to wash. Centrifuge again under same conditions.
4. Resuspend the cell pellet in freezing medium (growth medium containing 10% DMSO) at a concentration yielding 2 million cells per 1mL aliquot.
5. Dispense 1mL cell suspension per cryovial. Place cryovials in a Nalgene Cryo 1°C freezing container and store overnight at -80°C.
6. Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1. Passage cells until the desired number of cells for experimentation is reached in a logarithmic growth phase.
2. Pellet cells and rinse with 1X PBS as in "Generation of Seed Stocks" section.
3. Examine viability using Trypan blue staining (SOP TP-7).