



NeuraCell Contract Research Update – TAU Project G389R Donor

April 12th, 2017 Steve Lotz



G389R (MHF-100) Reprogramming Status Report *MHF-100-SeV-hiPSC Clones 1, 2 and 3*

- Reprogramming summary:
 - Expand Fibroblast and Tested for Myco (negative)
 - Length = ~7 weeks
 - All lines were derived using Sendai Virus 2.0 (Transgenic free system)
 - Length = 5 weeks; Split at Day 10 onto MG (Corning)
 - Format: 25K and 50K; 12w plate
- 3 lines were obtained for MHF-100. Each has been expanded and banked. Three clones from the patient's fibroblasts will be fully characterized



G389R (MHF-100) Characterization Summary

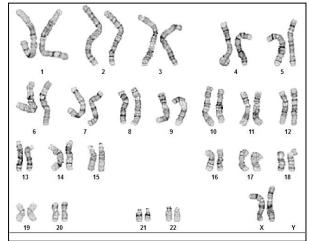
KT and FP confirms genomic integrity and identification

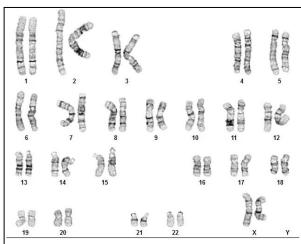
MHF-100-SeV-hiPSC

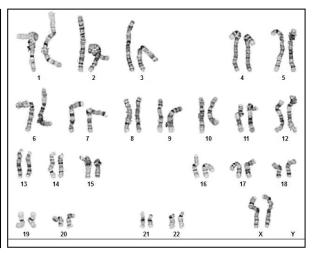
Clone 1 (p3)

Clone 2 (p3)

Clone 3 (p3)







MHF-100 -SeV-hiPSC clones 1, 2 and 3 have been assayed by WiCell to confirm a normal karyotype and no clonal abnormalities are detected at the stated band level of resolution.

G389R (MHF-100) -SeV-hiPSC Characterization Summary KT and FP confirms genomic integrity and identification

| Label on tube | MHF-100 p8 (70911) | MHF-100 C1 p3 (70912) | MHF-100 C2 p3 (70886) | MHF-100 C3 p3 (70887) |
|-----------------------|--------------------|-----------------------|-----------------------|-----------------------|
| Label on Report | MHF-100 p8 (70911) | MHF-100 C1 p3 (70912) | MHF-100 C2 p3 (70886) | MHF-100 C3 p3 (70887) |
| conc (ng/μL) | 29.8 | 104.9 | 42.2 | 21.6 |
| A260/280 | 1.44 | 1.79 | 1.59 | 1.51 |
| Assay Date | 3/21/2018 | 3/21/2018 | 3/21/2018 | 3/21/2018 |
| File Name | STR 180322 wmr | STR 180322 wmr | STR 180322 wmr | STR 180322 wmr |
| Report Date | 3/27/2018 | 3/27/2018 | 3/27/2018 | 3/27/2018 |
| FGA | 22,23 | 22,23 | 22,23 | 22,23 |
| ТРОХ | 8,12 | 8,12 | 8,12 | 8,12 |
| D8S1179 | 13,15 | 13,15 | 13,15 | 13,15 |
| vWA | 14,18 | 14,18 | 14,18 | 14,18 |
| Amelogenin | X,X | X,X | X,X | X,X |
| Penta_D | 11,12 | 11,12 | 11,12 | 11,12 |
| CSF1PO | 10,12 | 10,12 | 10,12 | 10,12 |
| D16S539 | 9,13 | 9,13 | 9,13 | 9,13 |
| D7S820 | 9,11 | 9,11 | 9,11 | 9,11 |
| D13S317 | 11,13 | 11,13 | 11,13 | 11,13 |
| D5S818 | 13,13 | 13,13 | 13,13 | 13,13 |
| Penta_E | 5,12 | 5,12 | 5,12 | 5,12 |
| D18S51 | 17,19 | 17,19 | 17, <mark>19</mark> | 17,20 |
| D21S11 | 29,29 | 29,29 | 29,29 | 29,29 |
| TH01 | 6,9.3 | 6,9.3 | 6,9.3 | 6,9.3 |
| D3S1358 | 14,16 | 14,16 | 14,16 | 14,16 |
| Allelic Polymorphisms | 28 | 28 | 28 | 28 |
| Matches** | 70886, 70912 | 70886, 70911 | 70911, 70912 | |
| | | · | >80% match criteria | >80% match criteria |

<u>Results:</u> Based on the cells submitted by WiCell Cytogenetics for Neural Stem Cell Institute dated and received on 03/19/18, these samples define the STR profiles of the human stem cell lines as indicated by name. The genotypic profiles comprising a range of 25-29 allelic polymorphisms across the 15 STR loci analyzed.

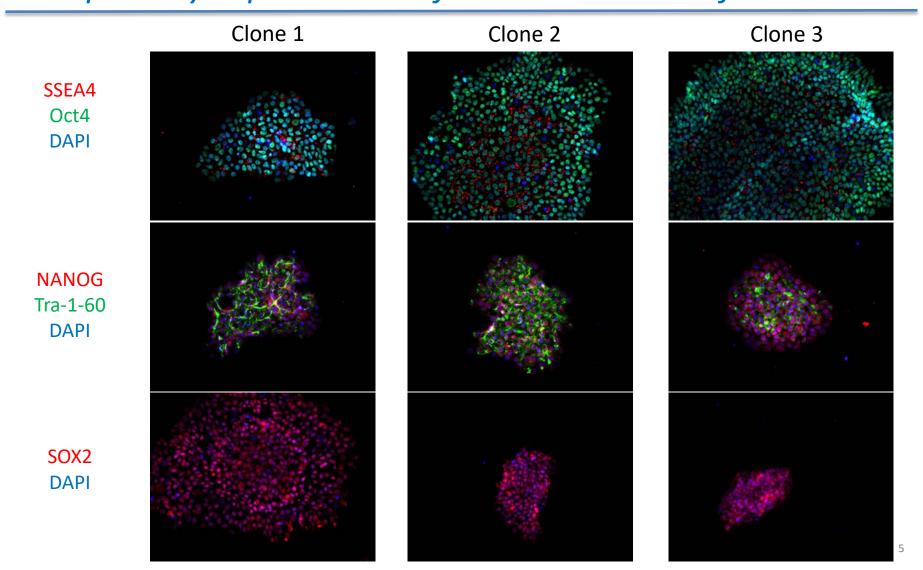
<u>Interpretation:</u> The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggests that the stem cells submitted correspond to the cell lines as named and were not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~2-5%.

** Note: The STR profile of the following sample is an exact match for the given sample/samples. Sample 70886 is a match to sample 70887 with the exception of the D18S51 loci (17,19) where in sample 70887 17,20 is observed. Standards for cell line authentication have been promoted with suggested algorithms to determine degrees of "relatedness" and have been recommended as a simple and effective way to interpret results from STR profiling of human cell lines. In general, >80% match is common between related samples, whereas < 50% match is unrelated (Int. J. Cancer: 132, 2510-2519. 2013). Using this criteria, across the 15 microsatellite STR loci, determined the 70886 sample displays a 29/30 allelic match (97% match), thus we would call this sample a match to sample 70887.



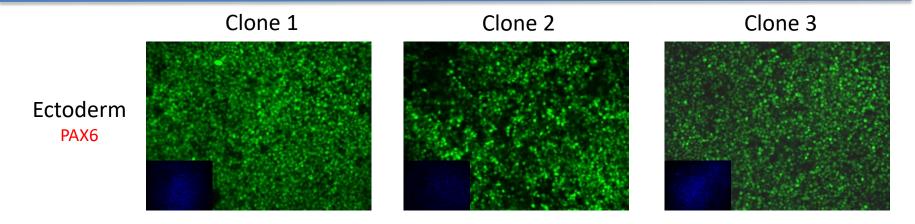
G389R (MHF-100) -SeV-hiPSC Characterization Summary Pluripotency expression confirmed via immunoflorescence



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G389R (MHF-100) -hiPSC Characterization Summary

Differentiation potential confirmed via directed tri-lineage



Mesoderm Brachyury

Endoderm SOX17