Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource
November 2019

Genotyping Shared Resources Verification Set

GACVS was performed at the Mayo Clinic Genotyping Shared Resource. Briefly, a total of 80ng of genomic DNA was processed through the following main steps: (i) DNA was amplified using two multiplex polymerase chain reactions (PCR) each consisting of a 12 µL reaction that contained 1.25 U of AmpliTaq Gold® (Thermo Fisher Scientific), 3 µL of custom primer multiplex mix (each consisting of 7 primer pairs*), and the genomic DNA. The thermocycler conditions were: 5 mins at 95°C, 25 cycles of 1 m at 95°C, 1m at 62°C, 1 m at 72 °C, followed by 25 mins at 60 °C. (ii) 2ul of PCR product is added to 11ul of a master mix containing 1ml HiDi formamide (Thermo Fisher Scientific) and 15ul Liz 500 Size Standard (Thermo Fisher Scientific). Total volume in each well 13ul. (iii) Fragment analysis is completed on the ABI3730 (Applied Biosystems). (iv) Analysis is completed using GeneMarker v2.4.0.

The Gene Analysis Core Verification Set is a custom-designed primer set based on the CODIS primer set. This set contains two primer pools used to amplify genomic DNA in two separate reactions. Each primer pool contains seven markers corresponding to seven of the markers in the CODIS set, providing 14 markers total. Markers included in the set are: Amelogenin, CSF1PO, D13S317, D16S539, D18S51, D21S11, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX and vWA. Allele locations were verified using allele calls from Corriell CEPH cell line control DNA. This cell line DNA is used as controls for runs.

Locus	**Forward (top row) & Reverse (bottom row) Oligonucleotide Sequences**
FGA Primer Pair (TMR)	GGCTGCAGGGCATAACATTA **A**TTCTATGACTTTGCGCTTCAGGA
TPOX Primer Pair (TMR)	GCACAGAACAGGCACTTAGG CGCTCAAACGTGAGGTTG
D8S1179 Primer Pair (TMR)	**ATT**GCAACTTATATGTATTTTTGTATTTCATG ACCAAATTGTGTTCATGAGTATAGTTTC
vWA Primer Pair (TMR)	GCCCTAGTGGATGATAAGAATAATCAGTATGTG GGACAGATGATAAATACATAGGATGGATGG
Amelogenin Primer Pair (TMR)	CCCTGGGCTCTGTAAAGAA ATCAGAGCTTAAACTGGGAAGCTG
D18S51 Primer Pair (Fluorescein)	TTCTTGAGCCCAGAAGGTTA ATTCTACCAGCAACAACACAAATAAAC
D21S11 Primer Pair (Fluorescein)	ATATGTGAGTCAATTCCCCAAG TGTATTAGTCAATGTTCTCCAGAGAC

TH01 Primer Pair (Fluorescein)	GTGATTCCCATTGGCCTGTTC ATTCCTGTGGGCTGAAAAGCTC
D3S1358 Primer Pair (Fluorescein)	ACTGCAGTCCAATCTGGGT ATGAAATCAACAGAGGCTTGC
CSF1PO Primer Pair (JOE)	CCGGAGGTAAAGGTGTCTTAAAGT ATTTCCTGTGTCAGACCCTGTT
D16S539 Primer Pair (JOE)	GGGGGTCTAAGAGCTTGTAAAAAG GTTTGTGTGTGCATCTGTAAGCATGTATC
D7S820 Primer Pair (JOE)	ATGTTGGTCAGGCTGACTATG GATTCCACATTTATCCTCATTGAC
D13S317 Primer Pair (JOE)	ATTACAGAAGTCTGGGATGTGGAGGA GGCAGCCCAAAAAGACAGA
D5S818 Primer Pair (JOE)	GGTGATTTTCCTCTTTGGTATCC AGCCACAGTTTACAACATTTGTATCT

After 2018, STR was analyzed using the Promega GenePrint 24 system. Information on this system can be found here: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/tm465-geneprint-24-system.pdf?la=en