# SERVICE GUIDE

# Human Cell Line Authentication Service

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SVG2306HCL

# **Service Guide:**
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## 1.0 Overview

AGRF's Human Cell Line Identification service is a PCR-based DNA fingerprinting system using short tandem repeat (STR) profiling of samples using the Promega GenePrint 10 system.

Cell lines have the potential to be misidentified or contaminated with other cells. As a result, major journals now recommend or require cell line authentication before manuscripts are accepted for publication. Cell line authentication is recommended upon receipt of a new cell line and at regular intervals during passages.

The American Tissue Culture Collection (ATCC) Standards Development Organization Workgroup issued standard ASN-0002, which recommends the use of at least eight STR loci (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317 and D5S818), plus Amelogenin for gender identification, for human cell line authentication.

The International Cell Line Authentication Committee (ICLAC) recommends including the use of at least eight core STR loci and application of match criteria (80% match threshold) to allow for a small amount of genetic drift in some cell lines.

The Promega GenePrint-10 System co-amplifies ten loci, including the ASN-0002 loci (TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317 and D5S818) as well as D21S11. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92 × 109. This assay is only suited to human samples. If you require a higher resolution assay, please contact us to discuss options.

## 2.0 Applications

Applications include:

- Authentication, identification and verification of human-derived cell lines.
  Confirming the identity of cell lines is increasingly required for publication. The GenePrint 10 system meets the ANSI/ATCC-0002 standard.
- Detection of co-culture contamination
  The presence of multiple genetic profiles in the one sample indicates contamination.
- Sample identification and tracking (bio-banking).
  Genotyping the same sample over time can detect sample mix-ups prior to experimentation.
- Forensic DNA analysis (for research purposes only)
- Human identity testing (sample ID confirmation)
- Paternity testing (for research purposes only)

## 3.0 Submission Types and DNA Requirements

AGRF offers two service options: full data service or fragment analysis only service.
For the full data service, you submit your DNA samples for amplification with the GenePrint-10 assay reagents. Fragment Analysis Service users submit a PCR sample that has been already amplified by the client using the GenePrint-10 assay reagents.

### 3.1 GenePrint 10 Service

Submit DNA template and AGRF performs the multiplex GenePrint 10 reaction, capillary separation on the 3730 and allele calling in GeneMapper ID software. Each reaction is accompanied by a positive and negative control for quality assurance.

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The service requires a total of 50ng DNA per sample (10µL of 5ng/µL or similar) in 1.5mL tubes or 96-well V bottom, plates. For samples submitted in full 96-well plates please leave well position A01 empty for the internal control sample and label your plate with your Contract ID & Name

When submitting samples please ensure quality and quantity of DNA template by at least measuring concentration and 260/280nm absorbance using a spectrophotometer (e.g. nanodrop). A 260/280 reading between 1.8–2.0 is recommended. High quality genomic DNA can also be determined using fluorescence-based quantitation (PicoGreen or similar) or agarose electrophoresis. A high-quality sample will produce a high molecular weight band using agarose electrophoresis.

### 3.2 Custom Amplification Products

Submit 10ul PCR product in 1.5mL tubes or 96-well V bottom, plates. An aliquot of the allelic ladder and Promega size standard needs to be provided. AGRF will add the HiDi formamide, perform the capillary separation on the 3730 and allele calling in GeneMapper ID software.

## 4.0 Sample Data and Storage

DNA samples are stored with AGRF for 3 months after you receive your data. If you require your samples to be returned to you post-processing, please let your Account Manager know at the time of quoting. At the completion of processing, we will return your samples by post, if requested, using an Australia Post satchel at ambient temperature. If required, we can return samples using Dry Ice - this will incur a $160 minimum (metropolitan areas) charge. Samples submitted as PCR products will be discarded 2 weeks after you receive your data.

AGRF maintains an archive of client data, however clients are advised to back up their data themselves. Data will remain available for download for one month, prior to archive. If you require past data, please contact AGRF. Please note that charges will apply for restoring files to the server for data more than six months old.

## 5.0 Shipping

- DNA samples must be shipped to AGRF in tubes or 96 well, V-bottom plates and be clearly labelled and sealed.
- Samples can be shipped at room temperature via express post or courier, or shipped on icepack or dry ice via courier
- To prevent leakage in transit please use parafilm to seal tubes, and ensure plates are heat-sealed or sealed with strip caps
- AGRF can organise dry ice shipment for your samples, at the cost to the client. Please contact us for further details.

**Physical address (courier) and postal address (mail):**
AGRF Ltd
PLANT GENOMICS CENTRE
WAITE CAMPUS
HARTLEY GROVE
URRBRAE, SA, 5064

## 6.0 Turnaround time

The turnaround time is 10 working days from receipt of samples.

## 7.0 Data Output

The service includes electrophoresis and allele calling using Genemapper ID software. Final data is supplied in tabulated format (.xls) to the client for interpretation. The excel file contains allele size (in base pairs) and predefined bin name (allele category).

To interpret your data, access the ATCC database and match the STR profile provided at www.atcc.org/STR_Database.aspx

## 8.0 Quality Assurance

All works carried out during the course of the project follow strict requirements of ISO15189: 2013. AGRF Ltd is accredited as a Medical Testing laboratory according to the ISO15189: 2013 standard by the National Association of Testing Authorities (NATA). Our staff follow Standard Operating Procedures, which define their responsibilities and provide guidance on achieving standards. Compliance is monitored at regular reviews and internal audits. The work is supervised by a person with relevant qualifications and checked while in progress and upon completion to ensure the necessary standards are met.

GenePrint-10 reaction performance is assessed by analysis of an internal quality control. Should analysis fail to meet our quality standards the samples will be re-processed. Should a sample fail to amplify and the analysis passes quality control, a re-submission may be performed at the client's expense.

## 9.0 Technical Considerations

TR profiles from some cell lines may vary slightly as cells are cultured, and this is a potential limitation of the technique. Cell lines displaying microsatellite instability appear particularly susceptible to genetic drift with continued culture.

Allelic dropout (failure to detect an allele within a DNA sample) in the form of loss of heterozygosity (LOH) is particularly observed in tumour cell lines. The LOH may be due to mutation at one of the alleles that prevents PCR amplification or loss of the chromosome or chromosomal region containing the dropout allele. Allelic dropout may also be caused by degraded DNA. False homozygous genotypes can be obtained if one of the alleles fails to amplify.

Allelic ladders represent the most common alleles at each locus within the population, however, allelic ladders in commercial kits do not represent all possible alleles. Alleles that lie outside the allelic bins/categories are often referred to as off-ladder 'OL' alleles. In some cases, off-ladder alleles are considered microvariants. It is not unusual to observe microvariants in continuous cell lines, especially tumour cell lines and some cell lines are known to have microvariant alleles.

For further information see http://www.cstl.nist.gov/biotech/strbase/var_tab.htm.

Cell lines with greater than an 80% match are considered related, derived from a common ancestry. Cell lines with between 55% to 80% match are most likely unrelated and may require further profiling for authentication of relatedness. If you require a higher resolution assay option, please contact AGRF to discuss options. Authentication using STR profiling is currently available for human identification and will not indicate presence of DNA from non-human cell contamination.