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Automated PCR Setup for Cell Line Authentication Using the Eppendorf ep*Motion*® 5075 Liquid Handling Workstation with Promega *GenePrint*® Systems and Eppendorf Mastercycler® Thermal Cyclers

Promega Corporation

Abstract

Cell line misidentification or cross-contamination between cell lines can happen frequently, resulting in a significant waste of time, money and resources due to potential misinterpretation of corresponding data (1). Inability to reproduce results based on preventable errors has led the National Institutes of Health (NIH) to require regular authentication of biological resources such as cell lines to ensure their identity and validity, and many scientific journals now require authentication prior to publication (2,3). As a result, cell line identification by short tandem repeat (STR) analysis has become the gold standard for researchers because it is a fast, easy and highly discriminatory method. This Application Note describes a cell line authentication workflow for Promega *GenePrint*® 24 and *GenePrint*® 10 Systems with automated PCR setup on the Eppendorf ep*Motion*® 5075 Liquid Handling Workstation and amplification using the Eppendorf Mastercycler® pro S and Mastercycler® nexus SX1 thermal cyclers. The steps to confirm authentication are then described.

Cross-Reference Cell Lines

Before beginning a cell line authentication workflow, it is important to perform due diligence on the cell lines of interest to determine if there is existing evidence that the cell lines may be contaminated or incorrect. Searching for the cell line name within the scientific literature is an easy step to determine if other investigators have reported authentication issues. Furthermore, the International Cell Line Authentication Committee (ICLAC) has compiled a database of misidentified or cross-contaminated cell lines (standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac_/Cross_Contaminations_v7_2_2.pdf) that can be easily searched to identify suspect cell lines to avoid using. The list can be downloaded for search. If evidence of cross-contamination or misidentification is found, discard the cell line and inform others with stocks. If the initial investigation does not indicate an already known issue, you can use a cell line authentication workflow like the one shown in Figure 1.

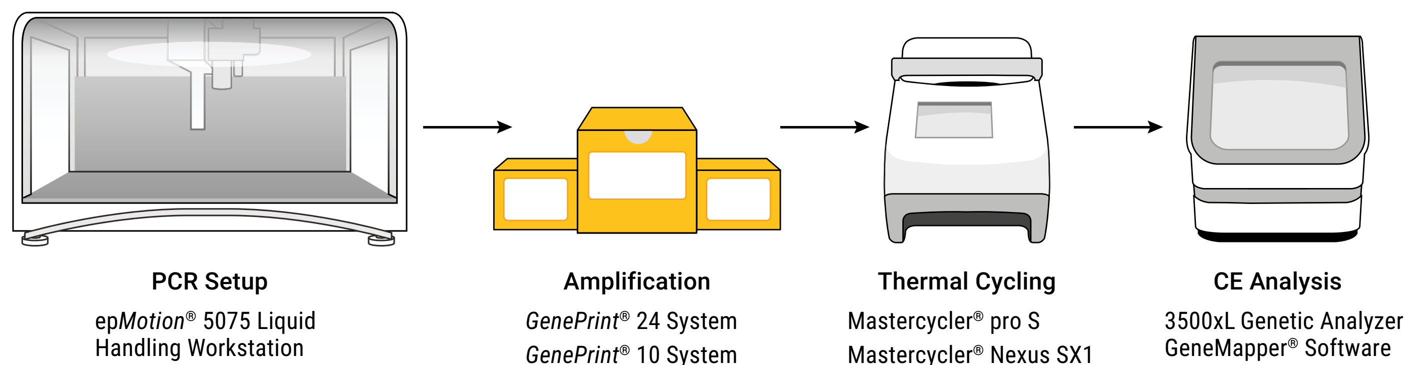

Figure 1. A cell line authentication workflow including automated PCR setup on the ep*Motion*® 5075 Instrument, DNA amplification using *GenePrint*® Systems on Mastercycler® thermal cyclers and analysis by capillary electrophoresis on the Applied Biosystems 3500xL Genetic Analyzer using GeneMapper® software.

The workflow used here is shown in Figure 1. The ep*Motion*® 5075 Instrument was programmed to dispense *GenePrint*® 24 and *GenePrint*® 10 System amplification reagents from the source tubes into a 1.5ml microtube to create the Amplification Reaction Mix (Figure 2). The reaction mixture was dispensed into the Reaction Plate followed by the transfer of 2800M Control DNA from the DNA template plate to the Reaction Plate. For *GenePrint*® 24, a 12.5µl reaction mixture consisting of 2.5µl of 5X Master Mix, 2.5µl of 5X Primer Pair Mix, 2.5µl of Amplification-Grade Water and 5µl of DNA (2.5ng) was used for amplification. For *GenePrint*® 10, a 25µl reaction mixture consisting of 5µl of 5X Master Mix, 5µl of 5X Primer Pair Mix, 5µl of Amplification-Grade Water and 10µl of DNA (10ng) was used. Water dispensed from the source tube served as a no-template control (NTC). DNA samples were amplified on the Mastercycler® pro S and Mastercycler® nexus SX1 thermal cyclers as per the thermal cycling protocol in the respective *GenePrint*® technical manuals. Ramp rates for each thermal cycler were adjusted to match those of the GeneAmp® PCR System 9700, which was used for development of *GenePrint*® Systems (Table 1).

Table 1. Ramp rate adjustments of Mastercycler® thermal cyclers for use with *GenePrint*® Systems.

***GenePrint*® Cycling Step**	**Mastercycler® pro S Ramp Rate**	**Mastercycler® nexus SX1 Ramp Rate**	**GeneAmp® PCR System 9700 Ramp Rate**
94°C to 59°C	33%	2.2°C/second	Max Mode
59°C to 72°C	20%	2.3°C/second	Max Mode
72°C to 94°C	26%	2.3°C/second	Max Mode

ep*Motion*® 5075 Instrument and *GenePrint*® PCR Setup

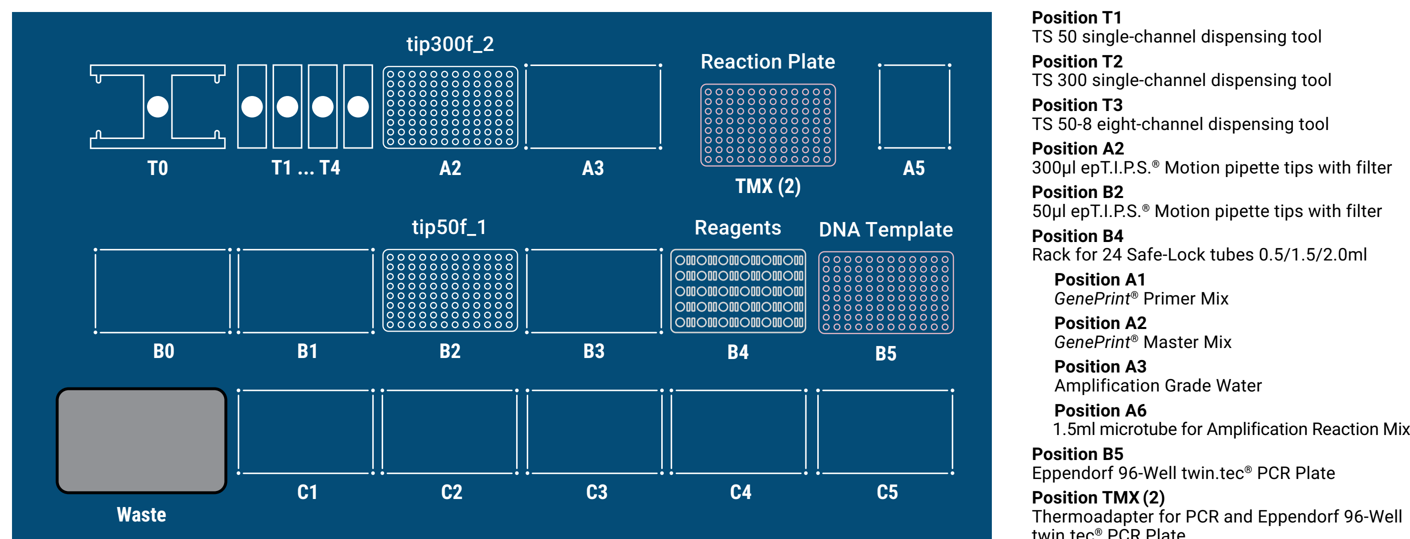

Figure 2. Deck layout of the ep*Motion*® 5075 Instrument and protocol for dispensing *GenePrint*® reagents for automated PCR setup. T0 represents the gripper; T1–T4 are the locations for the dispensing tools; A2–C5 are the locations for labware. The steps required to set up *GenePrint*® amplification reactions follow.

1. Transfer Amplification-Grade Water to a 1.5ml microtube for the Amplification Reaction Mix (Deck B4, position A3).
2. Transfer *GenePrint*® Primer Mix to the 1.5ml microtube and mix by pipetting (Deck B4, position A1).
3. Transfer *GenePrint*® Master Mix to the 1.5ml microtube and mix by pipetting (Deck B4, position A2).
4. Transfer the Amplification Reaction Mix (Deck B4, position A6) to wells in the Reaction Plate (Deck TMX (2)).
5. Transfer samples from the DNA Template Plate (Deck B5) to the wells containing Amplification Reaction Mix in the Reaction Plate.
6. Transfer Amplification-Grade Water to the Reaction Plate for No-Template Controls (NTC).
7. Shake on Thermomixer at 1200rpm for 1 minute.

Following amplification, 1µl of each reaction was injected on an Applied Biosystems 3500xL Genetic Analyzer to detect amplified fragments. For *GenePrint*® 24 System, injection conditions included a 15-second sample injection with voltage set at 1.6kV using POP-7™ polymer and a 50cm capillary array. Data analysis was performed using GeneMapper® 5.0 Software. For *GenePrint*® 10 System, injection conditions included a 24-second sample injection with voltage set at 1.2kV using POP-4™ polymer and a 36cm capillary array. Data analysis was performed using GeneMapper® *ID*-X Software.

STR Results

The ep*Motion*® 5075 Liquid Handling Workstation offers accurate automated pipetting, which helps to eliminate manual pipetting errors. This instrument was used to prepare *GenePrint*® 24 and *GenePrint*® 10 System PCR for cell line authentication followed by amplification on the Mastercycler® pro S and Mastercycler® nexus SX1 thermal cyclers. This automated PCR setup resulted in highly robust and reproducible STR results. Full profiles from 2800M Control DNA were obtained for all samples analyzed (Figure 3). Amplification on the Mastercycler® instruments resulted in balanced peak height ratios, helping to make data interpretation easier (Figure 4).

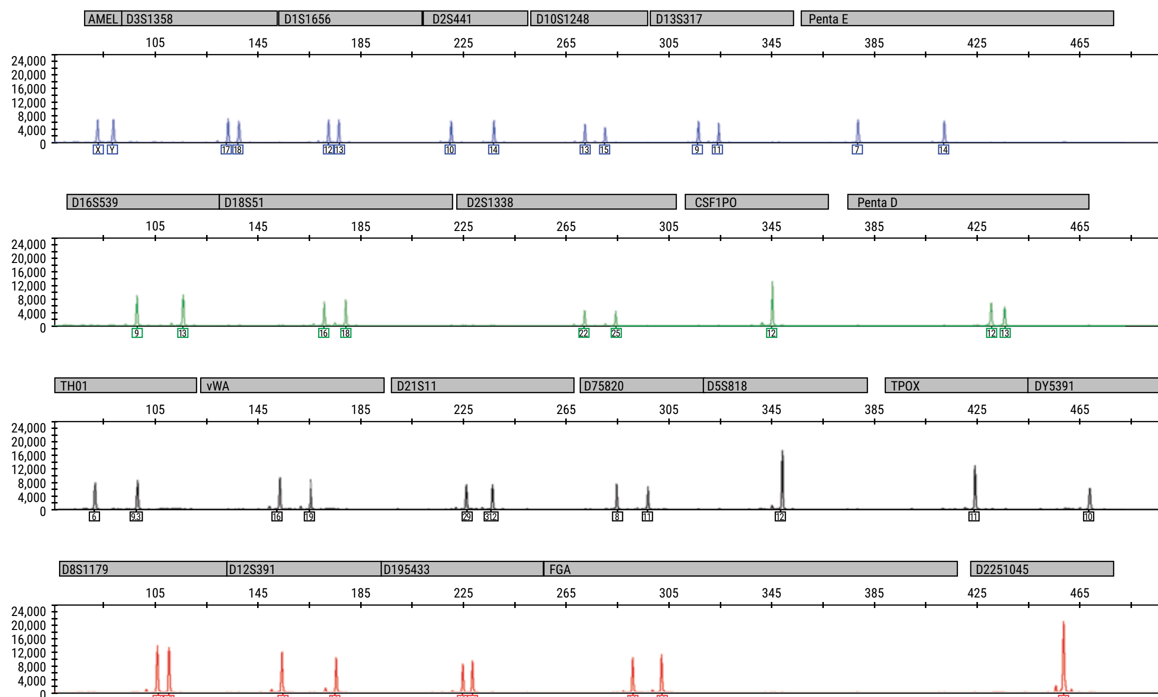

Figure 3. Example electropherogram of 2800M Control DNA using *GenePrint*® 24 System with PCR setup on the ep*Motion*® 5075 and amplified on the Mastercycler® nexus SX1.

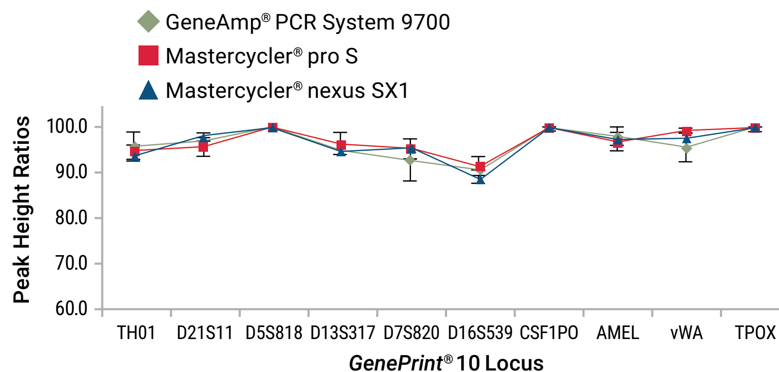

Figure 4. Peak height ratios of 2800M Control DNA using *GenePrint*® 10 System with PCR setup on the ep*Motion*® 5075 and amplified on the GeneAmp® PCR System 9700, Mastercycler® nexus SX1, and Mastercycler® pro S. N=5.

STR Profile Comparison

The STR profile results of each allele were recorded (Table 2). Multiple peaks (e.g., ≥3 alleles) for a given locus, typically consistent with a mixture, were not observed within the test sample. The experimental STR profiles from 2800M Control DNA were first compared to the known 2800M Control DNA reference STR profile in the GenePrint® *24 System Technical Manual* TM465. The alleles between the experimental STR profile and the reference matched perfectly, indicating the authenticity of the sample. The experimental STR profiles were also compared to the ATCC STR database (www.atcc.org/STR_Database.aspx) and the DSMZ STR database (www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.html) by manually entering allele results into the entry form of each database, respectively, separating each allele entry with a comma (e.g., D5S818 = 12, 12). A third database, the NCBI BioSample database (www.ncbi.nlm.nih.gov/biosample?term=human+str+profile[Filter]), was available but was not used because it did not allow for automated STR profile comparisons.

Examples of the top reported match from the ATCC STR and the DSMZ STR databases are shown in Table 3 along with the matched alleles for each locus. The ICLAC recommends match criteria from the ANSI/ATCC ASN-0002-2011 standard for human cell line authentication to interpret the comparisons and offers a match criteria worksheet (iclac.org/wp-content/uploads/ICLAC_Match-Criteria-Worksheet_v1_3.docx) to aid in the analysis. The following match algorithm used was as per the worksheet:

$$\text{Percent Match} = \frac{\text{SHARED ALLELES} \times 2}{\text{TOTAL ALLELES in Test Sample} + \text{TOTAL ALLELES in Reference Sample}} \times 100$$

The alleles underlined and in bold in Table 3 represent the shared alleles to the 2800M Control DNA test sample. The matches to the top ATCC and DSMZ cell lines were calculated with this information to be 67% (24/36) and 72% (26/36), respectively. The ANSI guidelines indicate comparisons of 0–55% match are consistent with the two samples being unrelated (i.e., different donors), those with 56–79% match being indeterminate and possibly requiring further testing, and those with 80–100% match being related (i.e., same donor). Using these guidelines the results indicated that the 2800M Control DNA test sample was not related to any of the lines in the databases tested, as one would predict if the 2800M cell line was not yet in those databases.

Table 2. *GenePrint*® Systems Reference and Experimental Loci Allele Results.

Loci	2800M Control DNA Reference	*GenePrint*® 10 Results	*GenePrint*® 24 Results
AMEL	X, Y	X, Y	X, Y
D5S818	12, 12	12, 12	12, 12
D13S317	9, 11	9, 11	9, 11
D7S820	8, 11	8, 11	8, 11
D16S539	9, 13	9, 13	9, 13
vWA	16, 19	16, 19	16, 19
TH01	6, 9.3	6, 9.3	6, 9.3
TPOX	11, 11	11, 11	11, 11
CSF1PO	12, 12	12, 12	12, 12
D21S11	29, 31.2	29, 31.2	29, 31.2
D3S1358	17, 18	—	17, 18
D1S1656	12, 13	—	12, 13
D2S441	10, 14	—	10, 14
D10S1248	13, 15	—	13, 15
Penta E	7, 14	—	7, 14
D18S51	16, 18	—	16, 18
D2S1338	22, 25	—	22, 25
Penta D	12, 13	—	12, 13
DYS391	10	—	10
D8S1179	14, 15	—	14, 15
D12S391	18, 23	—	18, 23
D19S433	13, 14	—	13, 14
FGA	20, 23	—	20, 23
D22S1045	16, 16	—	16, 16

Table 3. Examples of Top STR Database Matches to 2800M Control DNA Test Sample.

		Loci								
Database	Cell Line	AMEL	D5S818	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO
ATCC	CRL-5888	**X**, X	**12**, **12**	11, **11**	11, **11**	**9**, 11	15, **16**	**6**, 9	**11**, **11**	**12**, **12**
DSMZ	SU-DHL-4	**X**, **Y**	11, **12**	**11**, 12	**8**, **11**	11, **13**	18, **19**	**6**, **9.3**	9, **11**	**12**, **12**

Actions Upon Failure to Authenticate

The 2800M Control DNA test sample that was used for these experiments did not show evidence of a mixture (i.e., ≥3 alleles), matched the known STR profile of 2800M Control DNA and did not match any known cell lines in the reference databases. These results support the authenticity of the sample tested in this case. However, cell lines that are considered to have failed authentication are those that are tested and shown to have evidence of a mixture, do not match a known reference from which they were derived or match a database cell line other than what is expected with a ≥80% match. Take the following actions to minimize future use of contaminated or misidentified cell lines:

1. Discard stocks.
2. Inform colleagues with stocks.
3. Publish novel cross-contamination to alert scientific community.
4. Contact ICLAC (standards@atcc.org) to update database.

Summary

Cell line authentication of biological resources to enhance reproducibility of research is now an expectation for grant applications submitted to the NIH as well as a publication requirement of many scientific journals. STR analysis is a simple and definitive process used for cell line authentication that provides assurance for researchers concerned about the integrity of their research based on the data generated from cell lines. The information presented here demonstrates the use of the Eppendorf ep*Motion*® 5075 Liquid Handling Workstation, Mastercycler® pro S and Mastercycler® nexus SX1 thermal cyclers along with the *GenePrint*® 24 and *GenePrint*® 10 PCR amplification reagents in cell line authentication workflows. STR data generated using the Mastercycler® thermal cyclers were equivalent to those generated using the GeneAmp® PCR System 9700. The combination of accurate and consistent Eppendorf instrumentation along with dependable Promega STR reagents provides a reliable solution to the problem of misidentification of cell lines that continues to affect research.

References

1. Clement V. *et al.* (2013) Retraction: Marker-independent identification of glioma-initiating cells. *Nature Methods* **10**, 1035.
2. NIH Notice Number: NOT-OD-15-103 “Enhancing Reproducibility through Rigor and Transparency” (2015).
3. (2015) Announcement: Time to tackle cells’ mistaken identity. *Nature* **520**, 264.

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