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Comparison of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants

Final Report

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The following statement applies to submissions to the United States Environmental Protection Agency (US EPA).

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Manger, Regulatory Affairs

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The portion of this study conducted in the United States was conducted in accordance with the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Good Laboratory Practice Standards (40 CFR Part 160, US EPA 1989) with the following exceptions:

- Planting and cultivation of the seed test and control substances in the greenhouse was not conducted according to GLP standards.
- Plant tissue was collected before the initiation of this study.
- The purchased standards were characterized by the manufacturer prior to use in this study.
- The seed control test substance (10SG900137) was not characterized according to GLP standards.
- The anti-AvHPPD-03 immunoaffinity column was not prepared according to GLP standards.

The peptide mass mapping and N-terminal sequencing portions of this study were conducted in accordance with the Organization for Economic Co-operation and Development Good Laboratory Practice Standards.

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QUALITY ASSURANCE STATEMENT

Study Title: Comparison of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants

Study Director: [redacted]

Study Number: TK0031229

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Audit Protocol	August 13, 2010	August 13, 2010
Inspect Analytical	August 19, 2010	August 20, 2010
Inspect Analytical	September 2, 2010	September 3, 2010
Audit Final Report (1st Audit)	May 25, 29, & June 5 – 6, 2012	June 8, 2012
Audit Final Report (2nd Audit)	June 25, 2012	June 26, 2012

Please reference the analytical phase reports for additional audit/inspection dates.

Prepared by: [redacted] Date: June 29, 2012

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GENERAL INFORMATION

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The peptide mass mapping analysis was conducted by Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK, and the N-terminal amino acid sequence analysis was conducted by SGS M-Scan Ltd., 3 Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK. Additional personnel associated with these analyses are listed in Appendices A and B.

Study Dates

Study initiation date:	August 13, 2010
Experimental start date:	August 17, 2010
Experimental end date:	October 13, 2011

Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta, 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA. Facility records for SGS M-Scan Ltd. are archived SGS M-Scan Ltd., 3 Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK. The original peptide mass mapping phase report will be retained in the GLP Archives at Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK.

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LIST OF ACRONYMS AND ABBREVIATIONS

Definitions of International System of Units (SI) base units and derived units may be found in NIST (2011).

AST	active site titration
AvHPPD-03	*p*-hydroxyphenylpyruvate dioxygenase enzyme encoded by *avhppd-03* gene
BCA	bicinchoninic acid
BCIP	5-bromo-4-chloro-3-indolyl phosphate
Bis-Tris	bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane
Bq	Becquerel
BTP	Bis-Tris propane
^{14}C	carbon 14
dpm	disintegrations per minute
ECL™	enhanced chemiluminescence
ELISA	enzyme-linked immunosorbent assay
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLPS	Good Laboratory Practices Standards
HGA	homogentisic acid or homogentisate
HPLC/βRAM®	high performance liquid chromatography/ β-RAM®
HPP	*p*-hydroxyphenylpyruvate
HPPD	*p*-hydroxyphenylpyruvate dioxygenase enzyme
k_{cat}	turnover number
kDa	kiloDalton
K_i	inhibition constant
LC-MS/MS	liquid chromatography – mass spectrometry (tandem mass spectrometry)
LSC	liquid scintillation counting
min	minute
MOPS	3-(N-morpholino)propane-sulfonic acid
N	normal
NBT	p-nitro blue tetrazolium chloride
NSB	nonspecific binding
PAT	phosphinothricin acetyltransferase enzyme encoded by *pat*
PVDF	polyvinylidene difluoride
Q-TOF	quadrapole time-of-flight
RSD	relative standard deviation
RT-PCR	real-time polymerase chain reaction
SB	specific binding
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	second
SQC	Stewardship Quality Control
TB	total binding
U	unit
US EPA	United State Environmental Protection Agency
v/v	volume/volume
w/w	weight/weight
§	section

1.0 EXECUTIVE SUMMARY

In order to assess the biochemical and functional equivalence of the AvHPPD-03 proteins produced in a recombinant *Escherichia coli* expression system and in soybean derived from Event SYHT0H2, the proteins from both sources were compared with respect to identity, integrity, specific enzymatic activity, and glycosylation status.

Western blot analysis of the microbially produced and plant-produced AvHPPD-03 proteins showed mobility consistent with the predicted molecular weight of 47 kDa. Additionally, the microbially produced and plant-produced AvHPPD-03 cross-reacted with the same AvHPPD-03-specific antibody, confirming similar immunoreactivity for both proteins. A specific enzymatic activity assay revealed that the microbially produced and plant-produced AvHPPD-03 were comparable in their specific activity. The microbially produced AvHPPD-03 was assessed in the presence of nontransgenic, near-isogenic soybean seed extract to simulate the experimental extraction conditions of the plant-produced AvHPPD-03. The specific activity was 1.38 Units/mg AvHPPD-03 and 1.22 Units/mg AvHPPD-03 for the microbially produced AvHPPD-03 and the plant-produced AvHPPD-03, respectively. There was no evidence of post-translational glycosylation of the microbially produced or plant-produced AvHPPD-03. Peptide mass mapping identified 65% and 55% of the predicted amino acid sequence of the microbially produced and plant-produced AvHPPD-03 respectively, confirming the identity of the protein from both sources. Except for the cleavage of the first four amino acids from the N-terminus of the plant-produced protein, the N-terminal peptide of AvHPPD-03 from both sources was consistent with the predicted sequence.

The results of this study demonstrate that the microbially produced AvHPPD-03 is biochemically and functionally equivalent to AvHPPD-03 produced in SYHT0H2 soybean and supports the conclusion that the microbially produced AvHPPD-03 is a suitable surrogate to evaluate the safety of AvHPPD-03 produced in SYHT0H2 soybean.

2.0 INTRODUCTION

The purpose of this study was to assess the biochemical and functional equivalence of microbially produced AvHPPD-03 and AvHPPD-03 produced in transgenic soybean plants derived from Event SYHT0H2.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the genes *avhppd-03* derived from oat *(Avena sativa* L.*)* and *pat* derived from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid (HGA), the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in the transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of Event SYHT0H2 soybeans.

In this study, key biochemical and functional parameters were evaluated to assess whether the microbially produced AvHPPD-03 is a suitable surrogate for AvHPPD-03 produced in SYHT0H2 soybean. Microbially produced and plant-produced AvHPPD-03 were compared with respect to identity, integrity, specific enzymatic activity, and glycosylation status. Establishing functional and biochemical equivalence supports the use of microbially produced AvHPPD-03 in studies evaluating the safety of AvHPPD-03 in SYHT0H2 soybean.

3.0 MATERIALS AND METHODS

3.1 Microbially Produced AvHPPD-03

Microbially produced AvHPPD-03 was prepared from an *Escherichia coli* expression system. The gene *avhppd-03* was introduced into a pET24a vector and transformed into *E. coli* strain BL21 (DE3) cells. The genes expressed in the microbial system and in SYHT0H2 soybean encode proteins identical in amino acid sequence.

Prior to this study, AvHPPD-03 was prepared from *E. coli* cell paste by Syngenta Protein Science (Jealott's Hill International Research Centre, Bracknell, UK). Briefly, *E. coli* cells were ruptured and the cell debris removed by centrifugation. The supernatant was filtered and the AvHPPD-03 protein was further purified using anion exchange chromatography, hydrophobic interaction chromatography and size exclusion chromatography. The purified protein was pooled, concentrated, aliquoted, and lyophilized. The resulting dry formulation was designated test substance AVHPPD-03-0209. The test substance was shipped on dry ice to Syngenta Product Safety (Research Triangle Park, NC, USA), where it was stored at -20ºC ± 8°C.

The test substance AVHPPD-03-0209 was characterized in detail and determined to contain 72.2% AvHPPD-03 by weight (w/w); the molecular weight of AvHPPD-03 was consistent with the predicted molecular weight of 47.0 kDa (Winslow 2009). The microbially produced AvHPPD-03 was resolubilized in water and included in Western blot, glycosylation status, peptide mass mapping, and N-terminal sequencing analyses. The microbially produced AvHPPD-03 was resolubilized in 25 mM ascorbic acid containing 4 µg/ml bovine catalase for use within the specific enzymatic activity analysis.

3.2 Seed Test and Control Substances

The seed test substance for this study was soybean seed lot 10SG900903 (SYHT0H2 soybean). The control substance was nontransgenic, near-isogenic soybean seed in the same genetic background as the seed test substance. Table 1 shows the descriptions and pedigree codes for the seed test and control substances.

TABLE 1 Seed test and control substances

Seed identification	Material identification	Pedigree code
SYHT0H2 soybean (test)	10SG900903	SYHT0H2
Nontransgenic (control)	10SG900137	JACK

The seed test substance was characterized by real-time polymerase chain reaction (RT-PCR) analysis (Ingham *et al.* 2001) to confirm identity and purity (Burgin 2011). Identification of the nontransgenic, near-isogenic soybean seed was also verified by RT-PCR for Stewardship Quality Control (SQC) testing according to the current Syngenta standards.

3.3 Preparation of Plant-produced AvHPPD-03 and Nontransgenic Plant Controls

Prior to this study, the seed test and control substances (Table 1) were generated under standard greenhouse conditions. Seed from SYHT0H2 soybean and nontransgenic, near isogenic control soybean were collected, ground into a fine powder, and stored at 2-8°C.

3.3.1 Extracts of Event SYHT0H2 soybean seed and nontransgenic control soybean seed for Western blot analysis

Protein for Western blot analysis was extracted from the SYHT0H2 soybean powder by resuspending it in 100 mM borate buffer (pH 7.5) containing 0.2% (v/v) polyvinylpyrrolidone, 7.7 mM sodium azide, 0.5% (v/v) Tween 20® surfactant, 1.2% (v/v) hydrochloric acid, and supplemented with protease inhibitor cocktail (Roche, 1 tablet/50 ml). The mixture was homogenized and incubated on ice. After incubation on ice, the extract was centrifuged and filtered through a 0.22µm Millipore filter unit; the resulting extract was designated SYHT0H2 extract. The concentration of AvHPPD-03 and total protein were determined by enzyme-linked immunosorbent assay (ELISA) and the bicinchoninic acid (BCA) protein assay, respectively.

Nontransgenic, near-isogenic control soybean powder, was extracted in parallel with the preparation of the SYHT0H2 extract, employing the same method. The resulting preparation was designated as the nontransgenic extract. The nontransgenic extract was analyzed by BCA to determine the concentration of total protein in the sample. The nontransgenic extract was used as a negative control in the Western blot (Table 2).

3.3.2 Purified AvHPPD-03 preparation from SYHT0H2 extract

SYHT0H2 extract was prepared as described in **§3.3.1** and the AvHPPD-03 protein was immunopurified from the SYHT0H2 seed extract. An immunoaffinity column, prepared with anti-AvHPPD-03 monoclonal antibodies, was used to purify the AvHPPD-03 from the SYHT0H2 extract. The SYHT0H2 extract was applied to the equilibrated immunoaffinity column, the column was washed to remove unbound proteins, and AvHPPD-03 was eluted in 100 mM glycine buffer (pH 2.5) and neutralized. Fractions containing AvHPPD-03 were further purified using hydrophobic interaction chromatography (HiTrap Phenyl HP column, GE Healthcare) according to manufacturer instructions. For use within Western blot, glycosylation status, and N-terminal sequencing analyses, the purified protein was desalted using PD-10 columns and concentrated by ultrafiltration. The resulting sample was analyzed by ELISA to determine the concentration of AvHPPD-03, and stored at -20°C ± 8°C until further use. For use within the peptide mass mapping analysis, the purified protein was concentrated by ultrafiltration, analyzed by ELISA to determine the concentration of AvHPPD-03, and stored at -20°C ± 8°C until further use. The purified AvHPPD-03 preparation from SYHT0H2 extract was used in Western blot, glycosylation status, peptide mass mapping, and N-terminal sequencing analyses (Table 2).

3.3.3 Extracts of Event SYHT0H2 soybean seed and nontransgenic control soybean seed for specific enzymatic activity analysis

For use in the specific enzymatic activity assays, protein was extracted from the SYHT0H2 soybean powder by resuspending it in 50 mM Bis-Tris Propane (BTP) (pH 7.0), 4 mM dithiothreitol, 5 mM 4-(2-aminoethyl)-benzenesulfonylfluoride HCl, and 1% polyvinylpolypyrrolidone. The mixture was mixed thoroughly, incubated on ice and centrifuged. After centrifugation, the soluble portion of the extract was eluted into 50 mM BTP (pH 7.0), 50 mM potassium chloride using a PD-10 column. The concentration of total active HPPD protein (AvHPPD-03 and endogenous HPPD) in the resulting extract was immediately determined by an active site titration (AST) assay.

Nontransgenic, near-isogenic control soybean powder was extracted in parallel with the preparation of the SYHT0H2 extract, employing the same method. The resulting preparation was designated as the nontransgenic extract. The nontransgenic extract was included in the AST assay and used as a control in the enzymatic activity assay to account for the endogenous HPPD activity (Table 2).

3.3.4 Microbially produced AvHPPD-03 fortified nontransgenic extract

Nontransgenic soybean seed extract was prepared as described in **§3.3.3** and fortified with microbially produced AvHPPD-03. This sample was analyzed by an AST assay prior to enzymatic activity analysis, in order to investigate if the plant matrix affects specific enzymatic activity (Table 2). Inclusion of this sample allowed for the comparison of the microbially produced and plant-produced AvHPPD-03 in the same matrix.

TABLE 2 Protein preparations and use in subsequent analysis

Analysis	Samples included in the analysis	Purpose of the analysis
Western blot	• microbially produced AvHPPD-03 • SYHT0H2 extract • purified preparation from SYHT0H2 extract • nontransgenic extract	Examine AvHPPD-03 apparent molecular weight, intactness, and relative immunoreactivity
Specific enzymatic activity	• microbially produced AvHPPD-03 • SYHT0H2 extract • nontransgenic extract fortified with microbially produced AvHPPD-03 • nontransgenic extract	Confirm functional equivalence of both proteins. Confirm correct folding of both proteins.
Glycosylation	• microbially produced AvHPPD-03 • purified preparation from SYHT0H2 extract	Confirm the absence of glycosyl residues
Peptide mass mapping	• microbially produced AvHPPD-03 • purified preparation from SYHT0H2 extract	Confirm the identity of both proteins
N-terminal sequencing	• microbially produced AvHPPD-03 • purified preparation from SYHT0H2 extract	Confirm the N-terminal amino acid sequence of both proteins

3.4 AvHPPD-03 Quantification

3.4.1 Quantification of total AvHPPD-03 by ELISA

The concentration of AvHPPD-03 was determined by ELISA (Tijssen 1985) prior to conducting Western blot, glycosylation, peptide mass mapping, and N-terminal sequencing analyses. Two ELISA methods were employed to quantify AvHPPD-03.

One ELISA method is a double-antibody sandwich assay in which the AvHPPD-03 protein was captured in the wells of a microtiter plate using a polyclonal goat antibody that binds to AvHPPD-03. An additional polyclonal rabbit antibody was then used to bind to the AvHPPD-03 captured on the microtiter plate. Detection of AvHPPD-03 was accomplished by binding of a polyclonal donkey anti-rabbit antibody conjugated with alkaline phosphatase enzyme, which catalyzes the conversion of the colorimetric substrate, *p*-nitrophenylphosphate. The concentration of AvHPPD-03 is proportional to the measured absorbance values. Samples were then quantified relative to a standard curve of known AvHPPD-03 concentrations. Samples and standards were applied to the microtiter plate in triplicate. The absorbance values were measured with a spectrophotometer at dual wavelengths of 405 and 490 nm and the results were analyzed with Molecular Devices SoftMax Pro® 5.2 software, revision C using a four-parameter algorithm. For each sample, the mean concentration of dilutions within the quantitative range of the ELISA was calculated.

Alternatively, AvHPPD-03 quantification was performed using the Qualiplate™ ELISA Kit for HPPD in Soy. AvHPPD-03 was captured on ELISA plates pre-coated with the capture antibody. An antibody-enzyme conjugate was used to bind the AvHPPD-03 protein and detection was accomplished through conversion of a colorimetric substrate. The concentration of AvHPPD-03 is proportional to the measured absorbance values. Samples were then quantified relative to a standard curve of known AvHPPD-03 concentrations. Samples and standards were applied to the microtiter plate in triplicate. The absorbance values were

measured with a spectrophotometer at dual wavelengths of 450 and 650 nm. The results were analyzed with Molecular Devices SoftMax Pro® GxP software, version 5.4.1 using a four-parameter algorithm. For each sample, the mean concentration of dilutions within the quantitative range of the ELISA was calculated.

3.4.2 Quantification of active AvHPPD-03 by AST

To allow for a suitable comparison of the specific enzymatic activity of the microbially produced and plant-produced AvHPPD-03, the concentration of active HPPD was determined by an AST assay prior to conducting the specific enzymatic activity. The active HPPD from 1) the microbially produced AvHPPD-03, 2) SYHT0H2 soybean seed extract, 3) nontransgenic extract fortified with microbially produced AvHPPD-03, and 4) nontransgenic soybean seed extract was quantified using the AST assay. Nontransgenic soybean seed extract was included in the AST assay to control for the presence of active endogenous HPPD protein. A single AST assay was conducted for each sample.

The AST assay employs a substrate competitive radiolabeled inhibitor, ^{14}C-2-(2-chloro-3-ethoxy-4-methanesulfonyl-benzoyl)-5-methyl-cyclohexane-1,3-dione (^{14}C-R243604) (Hawkes *et al*. 2001a), with a known specific activity, to measure the amount bound to HPPD under defined conditions. The ^{14}C-inhibitor bound HPPD was then separated from unbound ^{14}C-inhibitor by rapid buffer exchange using a desalting column. The radioactivity of ^{14}C-inhibitor bound HPPD was measured by liquid scintillation counting (LSC). The concentration of active HPPD was determined using the measured radioactivity of the ^{14}C-inhibitor bound HPPD after any nonspecific binding signals detected had been subtracted.

The compound 2-(2-chloro-3-ethoxy-4-methanesulfonyl-benzoyl)-5-methyl-cyclohexane-1,3-dione (R243604) is an HPPD-specific, substrate competitive inhibitor of HPPD (Schulz *et al*. 1993) with an inhibitor binding affinity (K*i*) value of < 1 nM (Hawkes *et al*. 2001b). This inhibitor was used as a binding agent to bind the active site of HPPD. The amount of active HPPD (the amount of HPPD bound to R243604) was then measured.

The specific binding (SB) of R243604 to HPPD was determined by conducting both total binding (TB) and nonspecific binding (NSB) reactions.

The TB reactions were conducted at 25°C ± 0.5°C, and contained 0.5 µM ^{14}C-radiolabeled inhibitor prepared in 50 mM BTP buffer (pH 7) containing 25 mM sodium ascorbate and 4 µg/ml bovine catalase. The reactions were initiated by the addition of various volumes (0, 100, 200 and 300 µl) of each test solution, mixed, and stopped after 5 min by adding 200 µM of unlabeled R243604. An aliquot of each reaction was removed and the protein bound fraction was separated from the unbound fraction using a NAP™-5 rapid gel filtration column. The radioactivity in disintegrations per minute (dpm) of the protein bound fraction was then measured by LSC.

The NSB reactions were performed in parallel using the same method described above with the exception of the order in which ^{14}C -R243604 and unlabeled R243604 were added. The NSB reactions measured the nonspecific binding of R243604 to HPPD.

3.4.2.1 Calculation of specific binding

The TB and NSB of R243604 to HPPD were measured, and the SB was calculated:

Specific Binding (SB) = Total Binding (TB) – Nonspecific Binding (NSB)

3.4.2.2 Calculation of amount of active HPPD

The amount of active HPPD of each reaction was calculated:

$$\text{active HPPD (pmol)} = \frac{\text{Specific Binding(dpm)}}{0.9 \times (250\ \mu l/400\ \mu l) \times 60\ (\text{dpm/Bq}) \times 1.77\ (\text{Bq/pmol})}$$

0.9 = NAP-5 column elution efficiency (per manufacturer's instructions)
$(250\ \mu l/400\ \mu l)$ = ratio of reaction volume applied to column
$60\ (\text{dpm/Bq})$ = conversion factor for disintegrations per minute (dpm) to Becquerel (Bq)
$1.77\ \text{Bq/pmol}$ = specific radioactivity of ^{14}C-R243604

The concentration of active HPPD was determined by plotting the active HPPD (pmol) *vs*. the volume (µl) of prepared HPPD used in each assay. The slope of the trendline represents the concentration of active HPPD (µM) in the solution.

3.5 Total Protein Determination

Total protein was quantified via the BCA method (Hill and Straka 1988), using bovine serum albumin as the reference protein standard. The results were analyzed with Molecular Devices SoftMax Pro® GxP software, version 5.4.1 using a four-parameter algorithm. For each sample, the mean concentration of all dilutions within the quantitative range of the BCA assay was calculated.

3.6 Immunoreactivity and Molecular Weight Determination

Western blot analysis was used to investigate the identity and integrity of the microbially produced and plant-produced AvHPPD-03, as well as an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract. Based on ELISA analysis, aliquots containing 10 ng of AvHPPD-03 prepared in lithium dodecylsulfate sample buffer were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using a 4-12% Bis-Tris gel and 3-(N-morpholino)propane-sulfonic acid (MOPS) running buffer. Based on BCA analysis, an aliquot of the nontransgenic, near-isogenic seed extract, with total protein equivalent to the plant-produced AvHPPD-03 sample prepared for Western blot, was included in the analysis as a negative control. The molecular weight standard was SeeBlue® Plus2 pre-stained standard.

The protein was transferred to a polyvinylidene fluoride (PVDF) membrane via electroblotting. The membrane was probed with a polyclonal rabbit antibody capable of detecting AvHPPD-03. Detection of AvHPPD-03 was accomplished by binding of a polyclonal donkey anti-rabbit antibody conjugated with alkaline phosphatase enzyme, which catalyzes the conversion of the colorimetric substrate BCIP/NBT. The Western blot was visually examined for the presence of intact immunoreactive AvHPPD-03 or other immunoreactive AvHPPD-03-derived fragments.

3.7 Specific Enzymatic Activity

An aliquot of the microbially produced AvHPPD-03 was sent on dry ice to Syngenta Crop Protection, LLC (Greensboro, NC, USA), where it was stored at -20°C ± 8°C. Ground seed from SYHT0H2 soybean and nontransgenic, near isogenic soybean, were shipped to Syngenta Crop Protection, LLC (Greensboro, NC, USA), where they were stored at ambient temperature. All analyses required for determining the specific enzymatic activity were conducted at Syngenta Crop Protection, LLC (Greensboro, NC).

3.7.1 Enzymatic activity assay

The specific enzymatic activity of active HPPD from 1) the microbially produced AvHPPD-03, 2) SYHT0H2 soybean seed extract, 3) nontransgenic soybean seed extract fortified with microbially produced AvHPPD-03, and 4) nontransgenic soybean seed extract was determined using a radioactive $^{14}CO_2$ trapping HPPD enzymatic activity assay. Nontransgenic soybean seed extract was used as a control in the enzymatic activity assay to account for the endogenous HPPD activity. The HPPD enzyme catalyzes the formation of HGA and carbon dioxide from *p*-hydroxyphenylpyruvate (HPP) and molecular oxygen (Figure 1). The HPPD enzymatic activity assay determines the amount of radiolabeled $^{14}CO_2$ generated from a ^{14}C-labeled HPP substrate during the enzymatic reaction (Barta and Boger 1996).

FIGURE 1 Reaction catalyzed by HPPD

The enzymatic activity for AvHPPD-03 is reported as U/mg HPPD where one unit (U) of HPPD activity is defined as the amount of enzyme required to catalyze the conversion of one µmol of HPP to produce one µmol of HGA and one µmol of CO_2 per minute under the described reaction conditions. The enzymatic activity of HPPD, in which the production of radioactive $^{14}CO_2$ was measured during the enzymatic reaction, was validated as suitable for the purpose of quantifying HPPD activity (Emborsky 2010). Specific enzymatic activity values differing by ≤ 30% are considered comparable, and support a conclusion of functional equivalence.

The enzymatic activity assays were performed at 25°C ± 0.2°C in duplicate at four time points (0, 1, 3, and 6 minutes).

A substrate mixture consisting of 63 µM unlabeled HPP in 50 mM BTP buffer (pH 7) containing 25 mM sodium ascorbate, 4 µg/ml bovine catalase, and approximately 15 µM ^{14}C-HPP was prepared. Prior to use, the purity of ^{14}C-HPP was determined using a high pressure liquid chromatography (HPLC)/βRAM® method.

The substrate mixture was transferred into individual reaction chambers and capped with a tightly fitted rubber stopper. The specific radioactivity of the freshly prepared substrate mixture was measured prior to conducting enzymatic activity assays. Each enzymatic activity assay was initiated by adding HPPD to the substrate mixture. A suspended filter soaked with 1N sodium hydroxide was used to trap the CO_2 generated during the reaction. Reactions were then stopped after 0, 1, 3, and 6 minutes by addition of 0.6 N trichloroacetic acid.

Upon stopping each reaction, CO_2 trapping was allowed to continue for 90 minutes at 25°C ± 0.2°C. Radioactivity trapped within the filter was measured by LSC. The total disintegrations per minute (dpm) for each time point was corrected for background by subtracting the measured radioactivity (dpm) for time zero.

3.7.2 Calculation of enzymatic activity

The total pmol of CO_2 produced was calculated:

$$\text{Total } CO_2 \text{ (pmol)} = \frac{(\text{dpm measured at a given time} - \text{dpm measured at time zero})}{60 \left(\frac{\text{dpm}}{\text{Bq}}\right) \times 0.67 \times \text{specific radioactivity of the substrate mix} \left(\frac{\text{Bq}}{\text{pmol}}\right)}$$

0.67 = the predetermined extraction efficiency of measuring the trapped $^{14}CO_2$ from the nozzle filter by LSC, which is 67%

60 (dpm/Bq) = conversion factor for dpm to Bq

The CO_2 production rate (pmol/sec) was determined by plotting the amount of total CO_2 produced *vs*. time. The slope of the trendline represents the CO_2 production rate of HPPD.

The slope of the trendline was calculated:

$y = mx + b$
m = slope
b = y-intercept

Using the CO_2 production rate and the amount of active HPPD as determined by AST, the k_{cat} was calculated:

$$k_{cat}(\text{sec}^{-1}) = \frac{CO_2 \text{ production rate } (\frac{\text{pmol}}{\text{sec}})}{\text{active HPPD (pmol)}}$$

k_{cat} = rate constant

A mean k_{cat} value was calculated from duplicate activity assays for all samples. Specific enzymatic activity was then calculated as follows:

$$\text{specific enzymatic activity} = \frac{k_{cat} \left(\frac{1}{\text{sec}}\right) \left(\frac{\text{mol of } CO_2}{\text{mol of HPPD}}\right) \times 60 \left(\frac{\text{sec}}{\text{min}}\right)}{94000 \left(\frac{\text{g}}{\text{mol}}\right) \times 10^{-6} \left(\frac{\text{mol}}{\mu\text{mol}}\right) \times 10^{3} \left(\frac{\text{mg}}{\text{g}}\right)}$$

94000 g/mol[1] = molecular weight for two subunits of HPPD.

[1] The HPPD enzyme is a dimer consisting of two subunits. One R243604 inhibitor binds to one dimer of HPPD (Garcia et al. 2000). Therefore, a molecular weight of 94.0 kDa (94000 g/mol) is used to calculate the specific enzymatic activity.

3.8 Glycosylation Analysis

The microbially produced AvHPPD-03 and AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract were analyzed with the ECL™ Glycoprotein Detection Module Kit to confirm the absence of glycosyl residues. Samples were subjected to SDS-PAGE under reducing conditions using a 4-12% Bis-Tris gel and MOPS running buffer. The AvHPPD-03 protein was applied to the gel at 500 and 1000 ng. Transferrin, a glycosylated protein, was applied to the gel at 10, 25, 50, and 100 ng as a positive control. Soybean trypsin inhibitor, a nonglycosylated protein, was applied on the gel at 1000 ng as a negative control. The molecular weight standard was SeeBlue® Plus2 pre-stained standard.

The AvHPPD-03 protein was electroblotted onto a PVDF membrane. While on the membrane, glycan moieties were oxidized using sodium metaperiodate, labeled with biotin, and detected with alkaline-phosphatase-linked streptavidin.

3.9 Peptide Mass Mapping Analysis

Aliquots containing approximately 0.3 μg of the microbially produced AvHPPD-03 and an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract were subjected to SDS-PAGE under reducing conditions using a 4-12% Bis-Tris gel and MOPS running buffer. The gel was stained with Coomassie® blue, and the protein band, consistent with the predicted molecular weight of AvHPPD-03, was excised from the gel. The protein was reduced, alkylated with iodoacetamide, and enzymatically digested using trypsin and chymotrypsin. A separate digestion was conducted with each enzyme. The digested samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a quadrupole time-of-flight mass spectrometer (Q-TOF Premier, Waters/Micromass) coupled to a capillary liquid chromatography instrument (Waters CapLC). The detected peptide masses were searched using MASCOT® Software against a protein database containing the expected amino acid sequence of AvHPPD-03 (Appendix A). Peptide mass mapping analysis was conducted by Syngenta Jealott's Hill International Research Centre (Bracknell, UK).

3.10 N-Terminal Amino Acid Sequence Analysis

The N-terminal amino acid sequence of the microbially produced AvHPPD-03 and an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract was determined and compared with the predicted amino acid sequence. The AvHPPD-03 protein from both sources was subjected to SDS-PAGE under reducing conditions using a 4-12% Bis-Tris gel and MOPS running buffer, followed by electroblotting to a PVDF membrane. The blot was stained with amido black, and the protein bands corresponding to the predicted molecular weight of AvHPPD-03 were excised, and sent to SGS M-Scan Ltd. (Wokingham, UK).

The samples were applied to an automated pulsed-liquid sequencer for N-terminal amino acid sequence analysis. The methodology used was developed for proteins immobilized on a PVDF membrane and optimized for automated Edman degradation analysis (Appendix B). The N-terminal amino acid sequencing was conducted by SGS M-Scan Ltd. (Wokingham, UK).

3.11 Control of Bias

Any rejected data, and the documented reasons for the rejection of those data, will be retained in the study file.

3.12 Statistical Methods

Means and relative standard deviations were calculated using Microsoft Office Excel® 2007 software.

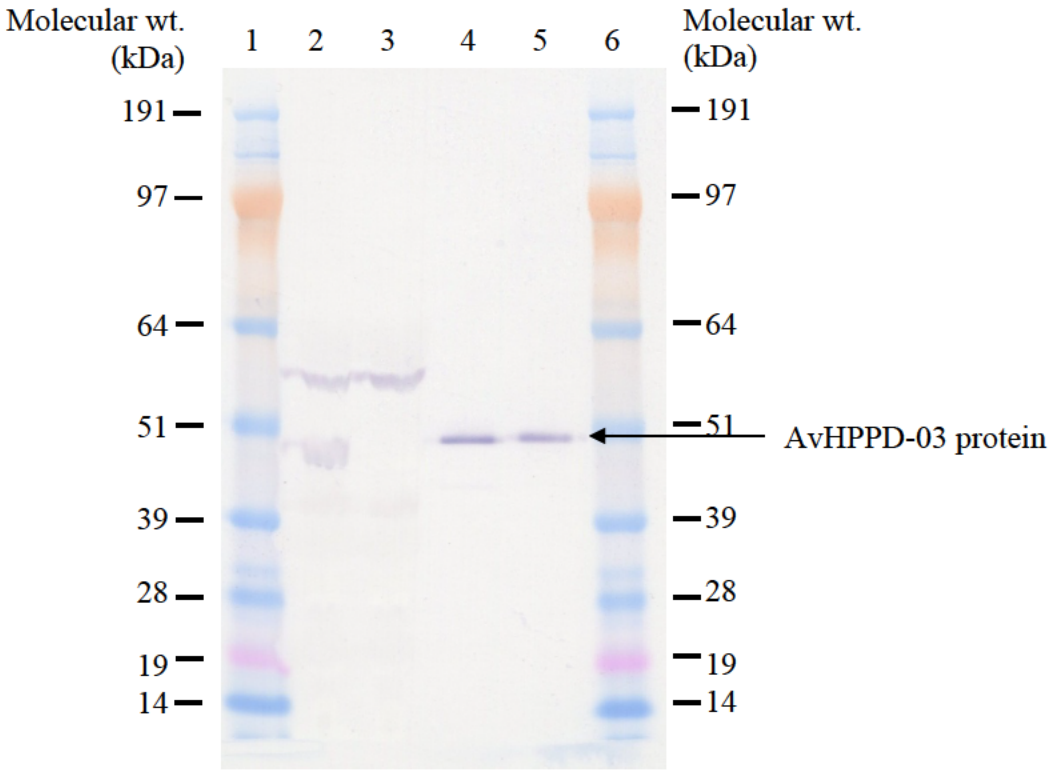
4.0 RESULTS AND DISCUSSION

4.1 Immunoreactivity and Molecular Weight

Western blot analysis of the microbially produced and AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract revealed a single and sharp immunoreactive band consistent with the predicted molecular weight of the AvHPPD-03 protein (Figure 2, Lanes 4 and 5) confirming the identity and integrity of AvHPPD-03 in both sources.

The crude SYHT0H2 extract, which contained AvHPPD-03 in the presence of the plant matrix, revealed a diffuse immunoreactive band compared to AvHPPD-03 in the microbially produced test substance and an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract (Figure 2, Lane 2). This difference is most likely a result of matrix interference, due to the high concentration of endogenous seed storage proteins in the crude soybean seed extract. Immunoreactive bands of higher molecular weight (between 51 kDa and 64 kDa) observed in the crude SYHT0H2 extract are also observed in the nontransgenic, near-isogenic seed extract, indicating that these bands are most likely due to nonspecific binding with endogenous proteins contained in the matrix (Figure 2, Lanes 2 and 3).

FIGURE 2 **Western blot analysis of plant-produced AvHPPD-03, nontransgenic soybean seed extract, an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract, and the microbially produced AvHPPD-03.**



Lane 1: Molecular weight standard
Lane 2: Crude SYHT0H2 soybean seed extract (10 ng AvHPPD-03, 83 µg total protein)
Lane 3: Nontransgenic soybean seed extract (83 µg total protein)
Lane 4: AvHPPD-03 purified preparation from SYHT0H2 extract (10 ng AvHPPD-03)
Lane 5: Microbially produced AvHPPD-03 (10 ng AvHPPD-03)
Lane 6: Molecular weight standard

4.2 Specific Enzymatic Activity

The microbially produced AvHPPD-03 showed a mean specific activity of 2.58 U/mg HPPD and the plant-produced AvHPPD-03 in crude SYHT0H2 soybean seed extract showed a mean specific activity of 1.22 U/mg HPPD (Table 3). To simulate the experimental extraction conditions of the plant-produced AvHPPD-03, the microbially produced AvHPPD-03 was added to the nontransgenic, near-isogenic soybean seed extract. The specific activity for this sample was found to be 1.38 U/mg HPPD (Table 3), indicating that the plant extract had an apparent inhibitory effect on the HPPD enzymatic activity. However, correcting for the described inhibitory effect of the plant extract places the detected specific activities of the microbially produced and plant-produced AvHPPD-03 into comparable ranges (1.38 U/mg and 1.22 U/mg HPPD, respectively) with specific activity differing by ≤ 30% between the two proteins (11.6%), thus confirming equivalent functional activity of both proteins.

TABLE 3 Specific enzymatic activity of the microbially produced and plant-produced AvHPPD-03

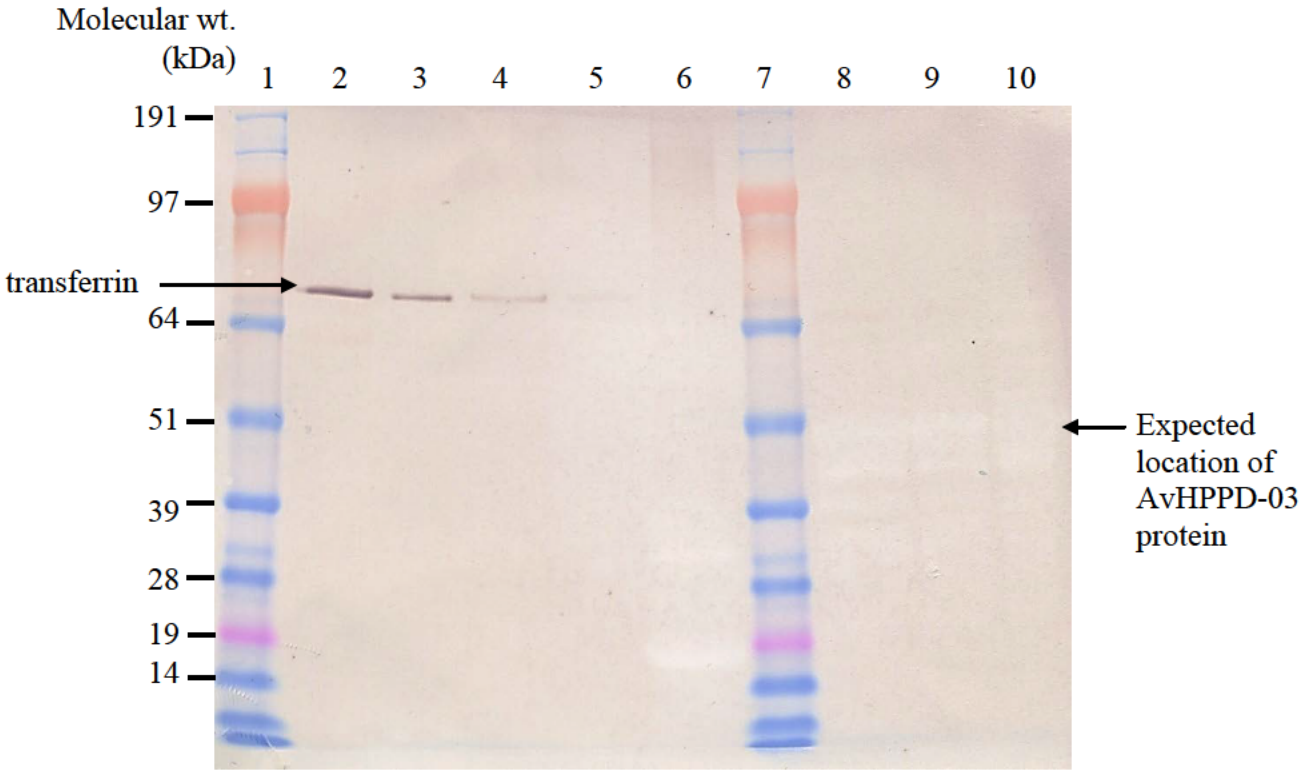
	Assay replicate	HPPD specific activity (U/mg HPPD)[a]	Mean HPPD specific activity (U/mg HPPD)	RSD (%)
Microbially produced AvHPPD-03	1	2.45	2.58	7.17
	2	2.71		
Plant-produced AvHPPD-03	1	1.26	1.22	4.36
	2	1.18		
Nontransgenic extract + microbially produced AvHPPD-03	1	1.44	1.38	6.80
	2	1.31		
Nontransgenic extract	1	0.39	0.41	6.74
	2	0.43		

[a] one unit of HPPD activity is defined as the amount of enzyme required to catalyze the conversion of one µmol of HPP to produce one µmol of HGA and one µmol of CO_2 per minute

4.3 Glycosylation Analysis

Transferrin, the positive control, generated a visible band when applied to the gel at 100, 50, 25, and 10 ng (Figure 3, Lanes 2, 3, 4 and 5). Transferrin has a molecular weight of approximately 80 kDa and contains approximately 5% glycan moieties by weight. This corresponds to approximately 25 glucose equivalents per molecule transferrin (based on a calculated molecular weight of 162 Da for the glycan moiety). The band visualized for 10 ng of transferrin (Figure 3, Lane 5) represents 0.5 ng of glycan moieties. The maximum amount of AvHPPD-03 from both the microbial and plant sources loaded on the blot was 1000 ng. If 0.5 ng of glycan were detected in AvHPPD-03, this would correspond to 0.05% by weight, or 0.145 glucose equivalents per molecule AvHPPD-03 (based on a calculated molecular weight of 47 kDa per molecule AvHPPD-03). In other words, if AvHPPD-03 bands were detected as strongly as 10 ng of transferrin in Figure 3, Lane 5, this would indicate glycosylation of about one in 6.9 AvHPPD-03 molecules. No bands corresponding to the presence of glycosylated AvHPPD-03 were visible in the microbially produced (Figure 3, Lane 10) or plant-produced AvHPPD-03 samples (Figure 3, Lanes 8 and 9); therefore, the results support the conclusion that neither the microbially produced nor the plant-produced AvHPPD-03 is glycosylated.

FIGURE 3 **Glycosylation analysis of an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract and the microbially produced AvHPPD-03**



Lane 1: Molecular weight standard
Lane 2: Transferrin (positive control), 100 ng
Lane 3: Transferrin (positive control), 50 ng
Lane 4: Transferrin (positive control), 25 ng
Lane 5: Transferrin (positive control), 10 ng[2]
Lane 6: Soybean trypsin inhibitor (negative control), 1000 ng
Lane 7: Molecular weight standard
Lane 8: AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract, 1000 ng
Lane 9: AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract, 500 ng
Lane 10: AvHPPD-03 in the microbially produced test substance, 1000 ng

[2] Due to limitations in printer resolution, the faint band visible at approximately 80 kDa may not be visible on the printed copy.

4.4 Peptide Mass Mapping

The analysis of the microbially produced AvHPPD-03 yielded coverage of 65% of the total predicted AvHPPD-03 amino acid sequence (Figure 4). The analysis of an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract yielded coverage of 55% of the total predicted AvHPPD-03 amino acid sequence (Figure 5). The identified peptides corresponded to regions throughout the sequence of AvHPPD-03. The results of the peptide mass mapping analysis confirmed the identification of the purified proteins from both sources as AvHPPD-03. Additionally, peptide mass mapping results confirmed the intact N-terminus of the microbially produced AvHPPD-03 protein.

FIGURE 4 Amino acid sequence identified by peptide mass mapping analysis of the microbially produced AvHPPD-03

```
  1    MPPTPATATG AAAAAVTPEH AARSFPRVVR VNPRSDRFPV LSFHHVELWC
 51    ADAASAAGRF SFALGAPLAA RSDLSTGNSA HASLLLRSGA LAFLFTAPYA
101    PPPQEAATAA TASIPSFSAD AARTFAAAHG LAVRSVGVRV ADAAEAFRVS
151    VAGGARPAFA PADLGHGFGL AEVELYGDVV LRFVSYPDET DLPFLPGFER
201    VSSPGAVDYG LTRFDHVVGN VPEMAPVIDY MKGFLGFHEF AEFTAEDVGT
251    TESGLNSVVL ANNSEAVLLP LNEPVHGTKR RSQIQTYLEY HGGPGVQHIA
301    LASNDVLRTL REMRARTPMG GFEFMAPPQA KYYEGVRRIA GDVLSEEQIK
351    ECQELGVLVD RDDQGVLLQI FTKPVGDRPT FFLEMIQRIG CMEKDEVGQE
401    YQKGGCGGFG KGNFSELFKS IEDYEKSLEV KQSVVAQKS
```

Identified AvHPPD-03 fragments are bold and underlined

FIGURE 5 Amino acid sequence identified by peptide mass mapping analysis of an AvHPPD-03 purified preparation from SYHT0H2 soybean seed extract

```
  1    MPPTPATATG AAAAAVTPEH AARSFPRVVR VNPRSDRFPV LSFHHVELWC
 51    ADAASAAGRF SFALGAPLAA RSDLSTGNSA HASLLLRSGA LAFLFTAPYA
101    PPPQEAATAA TASIPSFSAD AARTFAAAHG LAVRSVGVRV ADAAEAFRVS
151    VAGGARPAFA PADLGHGFGL AEVELYGDVV LRFVSYPDET DLPFLPGFER
201    VSSPGAVDYG LTRFDHVVGN VPEMAPVIDY MKGFLGFHEF AEFTAEDVGT
251    TESGLNSVVL ANNSEAVLLP LNEPVHGTKR RSQIQTYLEY HGGPGVQHIA
301    LASNDVLRTL REMRARTPMG GFEFMAPPQA KYYEGVRRIA GDVLSEEQIK
351    ECQELGVLVD RDDQGVLLQI FTKPVGDRPT FFLEMIQRIG CMEKDEVGQE
401    YQKGGCGGFG KGNFSELFKS IEDYEKSLEV KQSVVAQKS
```

Identified AvHPPD-03 fragments are bold and underlined

4.5 N-Terminal Amino Acid Sequence Analysis

The N-terminal amino acid sequence analysis of microbially produced AvHPPD-03 and plant-produced AvHPPD-03, confirmed the identity of both proteins.

Predicted sequence:	MPPTPATATGAAAAAV
Microbially produced AvHPPD-03:	MPPTPATATGAA
Plant-produced AvHPPD-03:	PATATGAAAAAV

The N-terminal sequencing analysis revealed that the plant-produced AvHPPD-03 lacked the first four amino acids at the N-terminus of the protein. The lack of the first four amino acids for the plant derived sequence is further confirmed by peptide mass mapping results (Figure 5). The presence of the additional four amino acids at the N-terminus of the microbially produced protein were confirmed to have no impact on the biochemical and functional properties of the AvHPPD-03 protein based on the comparable specific enzymatic activities observed between microbially produced and plant-produced AvHPPD-03 proteins.

4.6 Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

5.0 CONCLUSIONS

Western blot analysis of microbially produced and plant-produced AvHPPD-03 revealed immunoreactive bands that were consistent with the predicted molecular weight of the protein. Microbially produced AvHPPD-03, in the presence of nontransgenic, near-isogenic soybean seed extract, and plant-produced AvHPPD-03 had comparable specific enzymatic activity. There was no evidence of post-translational glycosylation of microbially produced or plant-produced AvHPPD-03. Peptide mass mapping analysis confirmed the identity of the proteins from both sources as AvHPPD-03. Apart from the cleavage of the first four amino acids from the N-terminus of the plant-produced protein, the N-terminal peptide of AvHPPD-03 from both sources was consistent with the predicted sequence. The lack of the first four amino acids from the N-terminus of the plant-produced AvHPPD-03 is not considered to have a significant impact on the overall integrity of the protein, based on the observation of comparable specific enzymatic activity with the fully intact microbially produced AvHPPD-03.

The results of this study support the conclusion that microbially produced AvHPPD-03 is biochemically and functionally equivalent to plant-produced AvHPPD-03, and provide the experimental evidence that the microbially produced AvHPPD-03 is a suitable surrogate for AvHPPD-03 produced in SYHT0H2 soybean.

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APPENDICES SECTION

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Peptide Mass Mapping Phase Report

Study Number:	TK0031229
Study Title:	Comparison of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
Test Site:	Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK, RG42 6EY
Principal Investigator:	[redacted] Syngenta Jealott's Hill International Research Centre [redacted]

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5	[redacted] (Master Schedule Administrator, Jealott's Hill)

This phase report contains 11 pages

Study Title: Comparison of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.

Study Number: TK0031229

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

ANALYTICAL PHASE

I, the undersigned, declare that the objectives laid down in the protocol for this analytical phase of the study were achieved and that the raw data generated are valid. This phase report fully and accurately reflects the procedures used and the raw data generated in the analytical phase of this study.

The analytical phase of this study was carried out in compliance with United Kingdom Good Laboratory Practice Regulations 1999. These regulations are in accordance with the Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice [Revised 1997].

[redacted]

21 | Mar | 2011
Date

Principal Investigator, Analytical Sciences, Jealott's Hill

Report issue authorized for Management by:

[redacted]

21st March 2011
Date

Test Site Management, Analytical Sciences, Jealott's Hill

Study Title: Comparison of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.

Study Number TK0031229

QUALITY ASSURANCE STATEMENT

In accordance with the Syngenta policy and procedures for Good Laboratory Practice, the conduct of the phase of this study has been inspected/audited by the Quality Assurance Section at Jealott's Hill International Research Centre, Bracknell, Berkshire, UK.

Date of Inspection	Phase Inspected	Date of Inspection Report
28 Aug 2010	Protocol	01 Sep 2010
28 Sep 2010	Analysis	30 Sep 2010
01 Feb 2011	Analytical Phase Report	10 Feb 2011

The laboratory facilities used to conduct this study were routinely inspected according to a programme described in Quality Assurance SOPs.

So far as can be reasonably established, the analytical phase report accurately reflects the raw data produced during the analytical phase of the study.

[redacted] [redacted] 21 March 2011

Quality Assurance Date

Contents

Summary Statement

The combination of trypsin and chymotrypsin digestion with peptide mass mapping confirmed the identity of the microbially produced AvHPPD-03 and AvHPPD-03 produced in SYHT0H2 soybean by producing evidence for the amino acid sequence of the proteins. Evidence for 64.9% of the amino acid sequence (beginning Met_1 and ending Lys_{403}) was obtained for the microbially produced AvHPPD-03. Evidence for 55.1% of the amino acid sequence (beginning Pro_5 and ending Lys_{403}) was obtained for the AvHPPD-03 produced in SYHT0H2 soybean.

1. Purpose of Phase

To confirm the identity of the microbially produced AvHPPD-03 and AvHPPD-03 produced in Event SYHT0H2 derived soybean plants using peptide mass mapping.

2. Materials and Methods

2.1. Materials

One SDS-PAGE gel (TK0031229 SYHT0H2 9/7/2010) containing microbially produced AvHPPD-03 and AvHPPD-03 produced in SYHT0H2 soybean, was received from Ellen Lentz on 10th September 2010 (Figure 2). The expected amino acid sequence of AvHPPD-03 is shown below.

MPPTPATATGAAAAAVTPEHAARSFPRVVRVNPRSDRFPVLSFHHV
ELWCADAASAAGRFSFALGAPLAARSDLSTGNSAHASLLLRSGALA
FLFTAPYAPPPQEAATAATASIPSFSADAARTFAAAHGLAVRSVGV
RVADAAEAFRVSVAGGARPAFAPADLGHGFGLAEVELYGDVVLRFV
SYPDETDLPFLPGFERVSSPGAVDYGLTRFDHVVGNVPEMAPVIDY
MKGFLGFHEFAEFTAEDVGTTESGLNSVVLANNSEAVLLPLNEPVH
GTKRRSQIQTYLEYHGGPGVQHIALASNDVLRTLREMRARTPMGGF
EFMAPPQAKYYEGVRRIAGDVLSEEQIKECQELGVLVDRDDQGVLL
QIFTKPVGDRPTFFLEMIQRIGCMEKDEVGQEYQKGGCGGFGKGNF
SELFKSIEDYEKSLEVKQSVVAQKS

Figure 1. Amino acid sequence (M_1-S_{439}) of the protein AvHPPD-03

2.2 SDS-PAGE analysis and enzymatic digestion

For peptide mass mapping analysis the main AvHPPD-03 protein bands from lanes 6, 7, 9 and 10 (as indicated in Figure 2) were excised from the SDS-PAGE gel. The protein was reduced using dithiothreitol, alkylated with iodoacetamide and digested separately with trypsin and chymotrypsin. The digested samples, which were used for all subsequent analyses described in this report, were assigned the unique reference numbers shown in Table 1.

Sample name	Microbial AvHPPD-03 (AvHPPD03-0209)		AvHPPD-03 from Event SYHT0H2	
Enzyme	Trypsin	Chymotrypsin	Trypsin	Chymotrypsin
Gel band reference	J9002/005/1	J9002/005/2	J9002/005/3	J9002/005/4
Unique digest sample reference	J9002/010/1	J9002/010/2	J9002/010/3	J9002/010/4

Table1. Shows the unique nomenclature for each of the samples submitted for mass spectrometric analysis

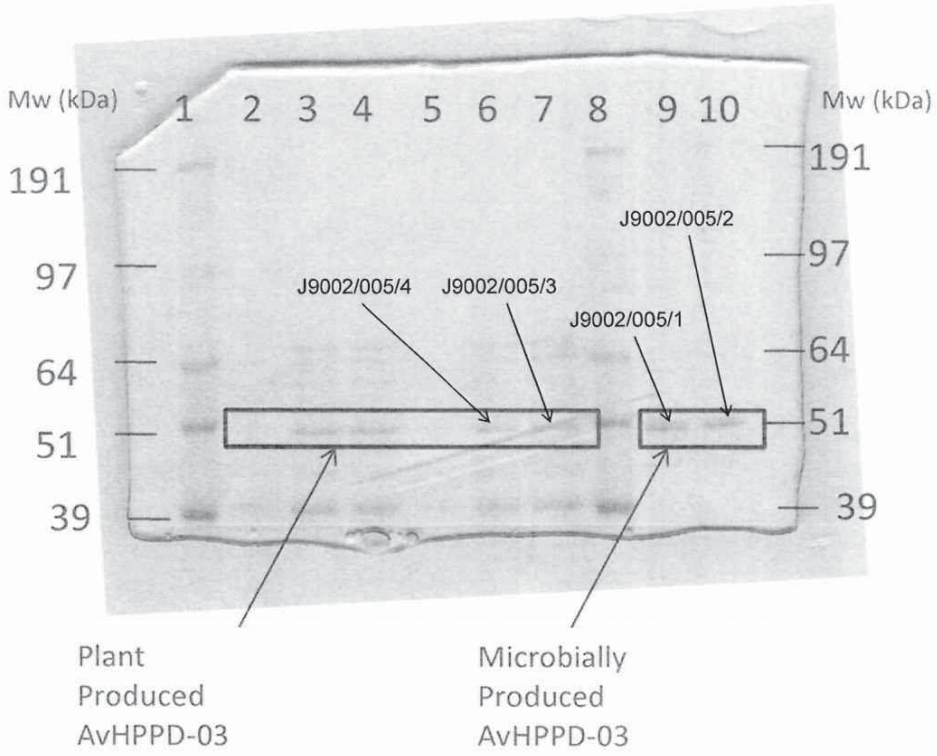


Figure 2. SDS-PAGE gel (TK0031229 SYHT0H2 9/7/2010) of microbially produced AvHPPD-03 and AvHPPD-03 produced in SYHT0H2 soybean. Lanes 1 & 8 MW markers. Lanes 2-7 AvHPPD-03 from SYHT0H2. Lanes 9-10 microbially produced AvHPPD-03. The uniquely labelled bands were excised from the gel for enzymatic digestion.

2.3 Liquid Chromatography-Mass Spectrometry / Mass Spectrometry (LC-MS/MS)

All mass measurements were made using a quadrupole time-of-flight mass spectrometer (Q-TOF Premier, Waters, UK – serial number HAB230). Samples and standards were delivered to the mass spectrometer via a nano-UPLC instrument (nano-Acquity™, Waters). The instruments were controlled and data was collected using MassLynx software version 4.1 (SCN 639). The mass spectrometer was operated in positive ion mode using a nanoflow ionisation source.

Samples were introduced into the mass spectrometer via a sample-trapping column (Waters Symmetry C18, 5 μm particle size, 180 μm x 20 mm) in conjunction with an analytical capillary column (Waters 1.7 μm BEH130 C18, 75 μm x 100 mm,). A flow rate of 300 nl min^{-1} was used for all separations.

Prior to conducting the analysis the instrument was calibrated using the product ions from the fragmentation of doubly charged glu-fibrinopeptide B (500 fmol μl^{-1}, in acetonitrile: water 1:1 v/v containing 0.1 % by volume formic acid) introduced using a nano-UPLC instrument (nano-Acquity™, Waters) at a flow rate of 0.5 μl min^{-1}. The spectra from the digest samples were lock-mass corrected using the doubly charged parent ion of the peptide glu-fibrinopeptide B (Glu-Fib). 500 fmol μl^{-1} Glu-Fib was infused at a rate of 500 nl min^{-1} and the lock mass spray channel was sampled every 30 seconds. The lock-mass correction was applied following data acquisition using Proteinlynx Global Server (version 2.4) from Waters.

36 μL of each sample was loaded onto the UPLC system. The gradient conditions for the separation are described in Table 2. The temperature of the autosampler tray was regulated at 4 °C and the column temperature was maintained at 30 °C throughout the course of these experiments.

Time (min)	Solvent B (%)
0.00	0
30.0	40
32.0	95
35.0	95
35.5	1
50.0	1

Table 2. Gradient conditions used for all sample injections. Solvent A was water containing 0.1 % by volume formic acid. Solvent B was acetonitrile containing 0.1 % by volume formic acid.

The mass spectrometer was configured to detect peptide ions in survey mode. Automatic charge state recognition was used to select up to three ions of charge states 2, 3 or 4 for subsequent fragmentation by MS/MS. Only the three most intense ions of intensity > 70 counts s^{-1} were selected. These ions were not selected again for a period of 60 s. The energy applied to fragment each ion was taken from preset tables and depended on m/z and charge state. MS/MS fragmentation data was collected from each of the three ions for 3 s before the instrument acquired another survey scan in order to select further ions for fragmentation. All MS/MS spectra were processed by Mascot™ as text (peaklist -.pkl) files, which were created using ProteinLynx Global server version 2.4 from Waters.

3. Results and Discussion

The peaklist files for the tryptic and chymotryptic digests of the microbially produced AvHPPD-03 protein were combined to produce a peaklist file containing tryptic and chymotryptic peptides. Using Mascot this combined peaklist file was searched against a database containing the amino acid sequence shown in Figure 1. The parameters used for this Mascot search are described in the Appendices (Section 10.1). The Mascot search results were manually verified using the acceptance criteria shown in the appendices (Section 10.2)[1,2]. An example of a manually verified MS/MS spectrum is shown in Section 10.3 (Figure A1). Manual verification generated the sequence coverage map shown in Figure 3 for the microbially produced AvHPPD-03 protein. Evidence for 64.9 % of the amino acid sequence was obtained.

```
  1 MPPTPATATG AAAAAVTPEH AARSFPRVVR VNPRSDRFPV LSFHHVELWC
 51 ADAASAAGRF SFALGAPLAA RSDLSTGNSA HASLLLRSGA LAFLFTAPYA
101 PPPQEAATAA TASIPSFSAD AARTFAAAHG LAVRSVGVRV ADAAEAFRVS
151 VAGGARPAFA PADLGHGFGL AEVELYGDVV LRFVSYPDET DLPFLPGFER
201 VSSPGAVDYG LTRFDHVVGN VPEMAPVIDY MKGFLGFHEF AEFTAEDVGT
251 TESGLNSVVL ANNSEAVLLP LNEPVHGTKR RSQIQTYLEY HGGPGVQHIA
301 LASNDVLRTL REMRARTPMG GFEFMAPPQA KYYEGVRRIA GDVLSEEQIK
351 ECQELGVLVD RDDQGVLLQI FTKPVGDRPT FFLEMIQRIG CMEKDEVGQE
401 YQKGGCGGFG KGNFSELFKS IEDYEKSLEV KQSVVAQKS
```

Figure 3. Amino acid sequence coverage map for the microbially produced AvHPPD-03. The sequence highlighted and underlined represents peptides identified. Evidence for 64.9 % of the amino acid sequence was obtained.

A combined peaklist file containing both tryptic and chymotryptic peptides from the plant expressed AvHPPD-03 protein, as contained in SYHT0H2 soybean, was generated as described above and searched against a database containing the amino acid sequence shown in Figure 1. A listing of the Mascot search parameters is given in the Appendices (Section 10.4). The Mascot search results were manually verified using the acceptance criteria shown in the Appendix (Section 10.2). Manual verification generated the sequence coverage map shown in Figure 4 for the protein AvHPPD-03 from SYHT0H2. Evidence for 55.1 % of the amino acid sequence was obtained.

```
  1 MPPTPATATG AAAAAVTPEH AARSFPRVVR VNPRSDRFPV LSFHHVELWC
 51 ADAASAAGRF SFALGAPLAA RSDLSTGNSA HASLLLRSGA LAFLFTAPYA
101 PPPQEAATAA TASIPSFSAD AARTFAAAHG LAVRSVGVRV ADAAEAFRVS
151 VAGGARPAFA PADLGHGFGL AEVELYGDVV LRFVSYPDET DLPFLPGFER
201 VSSPGAVDYG LTRFDHVVGN VPEMAPVIDY MKGFLGFHEF AEFTAEDVGT
251 TESGLNSVVL ANNSEAVLLP LNEPVHGTKR RSQIQTYLEY HGGPGVQHIA
301 LASNDVLRTL REMRARTPMG GFEFMAPPQA KYYEGVRRIA GDVLSEEQIK
351 ECQELGVLVD RDDQGVLLQI FTKPVGDRPT FFLEMIQRIG CMEKDEVGQE
401 YQKGGCGGFG KGNFSELFKS IEDYEKSLEV KQSVVAQKS
```

Figure 4. Amino acid sequence coverage map for the AvHPPD-03 protein purified from SYHT0H2 soybean. The sequence highlighted and underlined represents peptides identified. Evidence for 55.1 % of the amino acid sequence was obtained

4. Conclusions

The combination of trypsin and chymotrypsin digestion with peptide mass mapping confirmed the identity of the microbially produced AvHPPD-03 and AvHPPD-03 produced in SYHT0H2 soybean by producing evidence for the amino acid sequence of the proteins. Evidence for 64.9 % of the amino acid sequence (beginning Met_1 and ending Lys_{403}) was obtained for the microbially produced AvHPPD-03 (Figure 3). Evidence for 55.1% of the amino acid sequence (beginning Pro_5 and ending Lys_{403}) was obtained for the AvHPPD-03 produced in SYHT0H2 soybean (Figure 4).

5. Personnel and Test Site

[redacted]

[redacted]The study phase was performed at Syngenta, Analytical Sciences, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK.

6. Study Phase Dates

The experimental work was performed between 10th September and 29th September 2010. The interpretation was conducted between 25th October and 28th October 2010.

7. References

1) Bunkenborg J. and Matthiesen R. (2007) Interpretation of collision-induced fragmentation tandem mass spectra of post-translationally modified peptides. From Methods in Molecular Biology, vol 367: *Mass spectrometry data analysis in proteomics.* Edited by R. Matthiesen. pp.169-194
2) Chen Y., Kwon S.W., Kim S.C. and Zhao Y. (2005). Integrated Approach for Manual Evaluation of Peptides Identified by Searching Protein Sequence Databases with Tandem Mass Spectra. *Journal of Proteome Research* 4, 998-1005

8. Raw Data and Report Retention

All electronic data has been archived to the GLP electronic data archive (ADAGE) at Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. On completion of the phase report all bound and indexed phase records and a copy of the original phase report will be transferred to the GLP Archives at Syngenta Biotechnology, Inc., Regulatory Science and Trait Safety, 3054 East Cornwallis Road, PO Box 12257, Research Triangle Park, NC 27709.

A copy of all bound and indexed phase records and the original phase report will be retained at Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK.

9. Sample Disposal

Samples will be disposed upon completion of the study.

10. Appendices

10.1 Mascot search parameters for the microbially produced AvHPPD-03 protein

The following Mascot parameters were used when searching the combined peaklist file for the microbially produced AvHPPD-03.

Type of search	MS/MS Ion Search
Enzyme	None
Fixed Modifications	Carbamidomethyl (C)
Variable modifications	Oxidation (M)
Mass Values	Monoisotopic
Protein Mass	Unrestricted
Peptide Mass Tolerance	+/- 50 ppm
Fragment Mass Tolerance	+/- 0.1 Da
Missed Cleavages	2

10.2 Manual Verification of Mascot Data

In order for the peptides identified by Mascot to be accepted (successfully verified) 3 consecutive y- or b- fragment ions are required[1,2]. Successful verification is evidence for the presence of at least two consecutive amino acids from the sequence. Successfully verified spectra should not contain unassigned peaks of significant intensity. Peptides identified by Mascot that do not meet these criteria are rejected.

10.3 Example of a manually verified MS/MS spectrum of AvHPPD-03 from SYHT0H2

Figure A1 is a copy of the Mascot search results for the tryptic peptide FSFALGAPLAAR and Figure A2 is a copy of the raw data from which this Mascot search result was derived. Figure A1 is an example of a MS/MS spectrum that was manually verified and accepted using the verification criteria described in Section 10.2. The MS/MS spectrum shows a clear evidence of a y- ion series the masses of which are highlighted in the table. The consecutive y- ions y_7, y_8 and y_9 were used to accept this spectrum.

10.4 Mascot search parameters for the AvHPPD-03 protein from SYHT0H2

The following Mascot parameters were used when searching the combined peaklist file for the AvHPPD-03 protein purified from Event SYHT0H2.

Type of search	MS/MS Ion Search
Enzyme	None
Fixed Modifications	Carbamidomethyl (C)
Variable modifications	Oxidation (M)
Mass Values	Monoisotopic
Protein Mass	Unrestricted
Peptide Mass Tolerance	+/- 50 ppm
Fragment Mass Tolerance	+/- 0.1 Da
Missed Cleavages	2

Mascot Search Results: Peptide View

Page 1 of 1

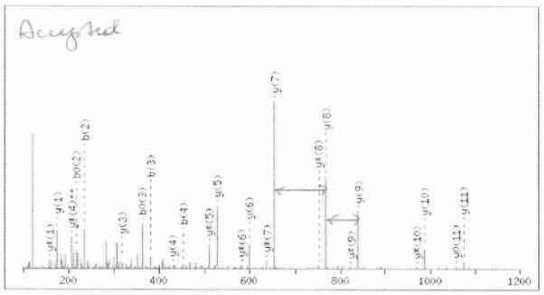
Mascot Search Results

Peptide View

MS/MS Fragmentation of FSFALGAPLAAR
Found in RQ0909, AvHPPD-03 p-hydroxyphenylpyruvate dioxygenase enzyme derived from oat (Avena sativa). Expression of avhppd-03 in plants confers a mesotrione-tolerance phenotype. The gene avhppd-03 encodes a p-hydroxyphenylpyruvate dioxygenase (AvHPPD-03)

Match to Query 140: 1219.653248 from(610.833900,2+) intensity(17380.8000)
Data file C:\MassLynx\Default.pro\pkl\files\Combined J9002-010-3 and J9002-010-4_251010.txt

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 1200 Da Full range



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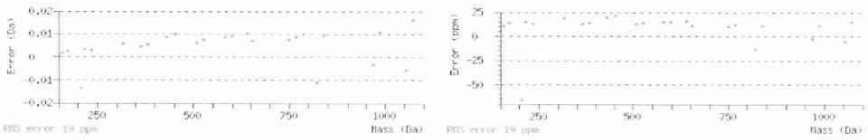
26/10/10

Monoisotopic mass of neutral peptide Mr(calc): 1219.671
Fixed modifications: Carbamidomethyl (C)
Ions Score: 56 Expect: 0.00014
Matches (Bold Red): 24/88 fragment ions using 59 most intense peaks

#	b	b^{++}	b^0	b^{0++}	Seq.	y	y^{++}	y^*	y^{*++}	y^0	y^{0++}	#
1	148.08	74.54			F							12
2	235.11	118.06	217.10	109.05	S	1073.61	537.31	1056.58	528.80	1055.60	528.30	11
3	382.18	191.59	364.17	182.59	F	986.58	493.79	969.55	485.28			10
4	453.21	227.11	435.20	218.10	A	839.51	420.26	822.48	411.75			9
5	566.30	283.65	548.29	274.65	L	768.47	384.74	751.45	376.23			8
6	623.32	312.16	605.31	303.16	G	655.39	328.20	638.36	319.68			7
7	694.36	347.68	676.35	338.68	A	598.37	299.69	581.34	291.17			6
8	791.41	396.21	773.40	387.20	P	527.33	264.17	510.30	255.66			5
9	904.49	452.75	886.48	443.74	L	430.28	215.64	413.25	207.13			4
10	975.53	488.27	957.52	479.26	A	317.19	159.10	300.17	150.59			3
11	1046.57	523.79	1028.56	514.78	A	246.16	123.58	229.13	115.07			2
12					R	175.12	88.06	158.09	79.55			1



NCBI BLAST search of FSFALGAPLAAR
(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST web gateways

All matches to this query

Score	Mr(calc):	Delta	Sequence
56.2	1219.671	-0.018	FSFALGAPLAAR
3.9	1219.671	-0.018	HHYLQLSHLP
1.0	1219.635	0.018	RVNVPVYSWT
0.7	1219.620	0.034	RVSSPGAVDYGL

Mascot: http://www.matrixscience.com/

http://mascot.rt.intra/mascot/cgi/peptide_view.pl?file=../data/20101025/F041630.dat&... 26/10/2010

Figure A1. Example of a manually verified MS/MS spectrum from AvHPPD-03 produced in SYHT0H2 for the tryptic peptide FSFALGAPLAAR.

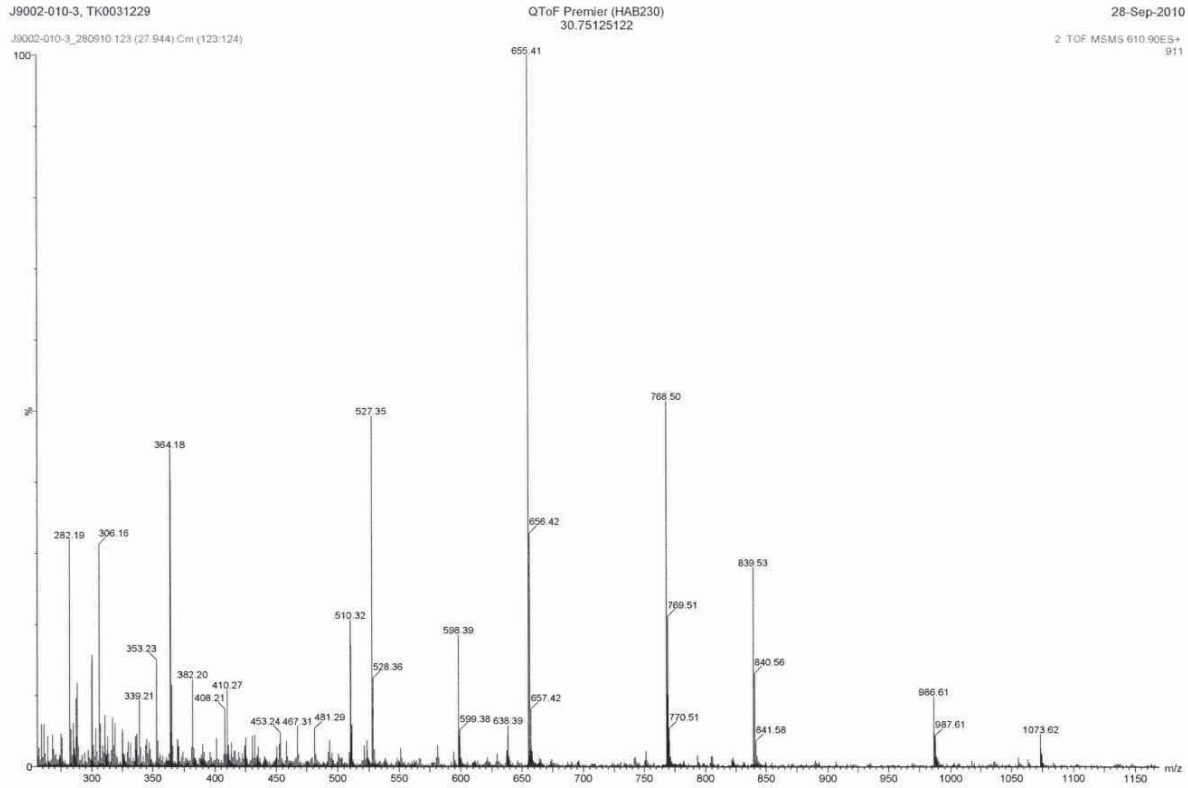


Figure A2. A copy of the raw data (without lock mass correction) from AvHPPD-03 produced in SYHT0H2 for the tryptic peptide FSFALGAPLAAR. The Mascot search result shown in Figure A1 is derived from this data.

Confidential

STUDY TITLE:	Comparison of *p*-hydroxyphenylpyruvate Dioxygenase AvHPPD-03 Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
PROTOCOL NUMBER:	TK0031229
ANALYTICAL PHASE TITLE:	N-Terminal Amino Acid Sequencing of AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
TEST SITE:	SGS M-SCAN LIMITED 2-3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK
ANALYTICAL PHASE STUDY PLAN NO:	10090101 & AMENDMENT ONE
ANALYTICAL PHASE STUDY PLAN REPORT NO:	1110/22745 Addendum one
STUDY SPONSOR:	Syngenta Crop Protection, LLC Syngenta Biotechnology, Inc 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA
STUDY DIRECTOR:	[redacted]

Report Prepared By: [redacted] Date: 28th June 2012

Team Leader, Ancillary Techniques

Report Reviewed By: [redacted] Date: 28th June 2012

Senior Biochemist

PRINCIPAL INVESTIGATOR'S STATEMENT

Report No: 1110/22745 Addendum one

Title: Comparison of *p*-hydroxyphenylpyruvate Dioxygenase AvHPPD-03 Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.

Analytical Phase Study no: 10090101 &10090101Am1

Syngenta crop Protection, LLC study no: TK0031229

The work described in this report was conducted in accordance with the principles laid down in the Good Laboratory Practice Regulations, SI 3106 (1999) and subsequent amendment SI 994 (2004) which are themselves based on the principles of good laboratory practice contained in Annex 2 to the Decision of the Council of the Organisation for Economic Co-Operation and Development (OECD), ENV/MC/CHEM (98)17. They are in conformity with, and implement the requirements of, Directives 2004/10/EC and 2004/9/EC.

No deviations from these principles were made and the report describes the procedures used and is an accurate reflection of the raw data generated during the performance of this study.

Signed: [redacted] Date: 28th June 2012

[redacted] **(Principal Investigator)**

1. Reason for Addendum One

After finalisation of the report the QA unit of the Study Sponsor requested the Sponsor address on Page 1 to be changed from:

SYNGENTA CROP PROTECTION, LLC
Syngenta Biotechnology, Inc
3054 East Cornwallis Road, P.O. Box 12257
Research Triangle Park, NC 27709, North Carolina, USA

To:

SYNGENTA CROP PROTECTION, LLC
Syngenta Biotechnology, Inc
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

This Report 1110/22745 Addendum one was produced to include the above request.

There is no impact on the results generated or the GLP compliance of the study.

QUALITY ASSURANCE STATEMENT

Report Number: 1110/22745 Addendum one

Analytical Phase Study Plan Number: 10090101 and Amendment One

Protocol Number: TK0031229

Title: Comparison of *p*-hysroxyphenylpyruvate Dioxygenase AvHPPD-03 Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.

Quality Assurance reviewed the study in accordance with the Good Laboratory Practice Regulations, 1999 SI 3106 and subsequent amendment (2004) SI No. 994, as administered by the UK Medicine and Healthcare Products Regulatory Agency.

The following phases of the study were inspected and the findings reported to study management:

Date Inspected	Phase	Date written report made to Management and and Principal Investigator	Date written report made to Study Director
28 Jun 2012	Draft Analytical Phase Study Report Add One	28 Jun 2012	28 Jun 2012

Details of the analytical phase inspected are listed above. Additional laboratory procedures are inspected on a routine basis in accordance with SGS M-Scan Limited standard operating procedures.

Facility inspections are conducted according to an approved schedule as described in SGS M-Scan Ltd standard operating procedures.

As far as can be reasonably established, the report was considered to be an accurate reflection of the raw data generated during the conduct of the study.

Signed: [redacted]
(Assistant QA Manager)

Date: 28 June 2012

1. INTRODUCTION

ICH Topic Q6B (1) provides a uniform set of internationally accepted principles for characterisation of biotechnological products to support new marketing applications. The document suggests that analyses should be performed to provide the following information for biological or biopharmaceutical products:

Amino acid sequence

The guidelines state "The amino acid sequence of the desired product should be determined to the extent possible using approaches such as amino acid composition, terminal amino acid sequence, peptide map, sulphydryl group(s) and disulphide bridge analysis and then compared with the sequence of the amino acids deduced from the gene sequence of the desired product".

If the client requests a full confirmatory sequence analysis this is normally achieved by analysis of peptides from proteolytic digests of the protein/glycoprotein using (A) Gas-phase sequencing via Edman degradation (2) and/or (B) Mass Spectrometric sequence analysis.

Terminal amino acid sequence

The guidelines state "Terminal amino acid analysis is performed to identify the nature and homogeneity of the amino- and carboxy-terminal amino acids. If the desired product is found to be heterogeneous with respect to the terminal amino acids, the relative amounts of the variant forms should be determined using an appropriate analytical procedure. The sequence of these terminal amino acids should be compared with the terminal amino acid sequence deduced from the gene sequence of the desired product".

Sequencing of the amino-terminal amino acids for a biotechnological/biological product is usually performed using manual or automated gas phase sequencing using Edman chemistry.

2. OBJECTIVE

The aim of this analytical phase study plan was to analyse the supplied AvHPPD-03 test samples using automated Edman degradation chemistry and report the N-terminal sequences obtained. The supplied AvHPPD-03 test samples were provided by the Study Sponsor blotted and stained on a PVDF membrane. Edman degradation was performed on the supplied bands using an automated pulsed-liquid sequencer. Automated pulsed-liquid sequencing for twelve residues of the N-terminal sequence was undertaken.

The study reported here was performed under analytical phase study plan no. 10090101 Amendment One, which is reproduced in Appendix I, with analytical phase study plan no. 10090101.

3. EXPERIMENTAL

3.1 Samples

Samples were received on the 12th Sept 2011 from Syngenta Crop Protection LLC and were given the unique SGS M-Scan number described:

Product	SGS M-Scan No.
TK0031229 SYHT0H2 09/06/11 LKS AvHPPD-03 Vial 1	97246
TK0031229 SYHT0H2 09/06/11 LKS AvHPPD-03 Vial 2	97247
TK0031229 MICROBIAL 09/06/11 LKS AvHPPD-03-0209	97248

The samples were stored between -10 and -30°C until required.
Analyses of the received samples were performed between the 7th and 10th October 2011.
Only SGS M-Scan sample numbers 97246 and 97248 were analysed in this report.

3.2 N-Terminal Sequencing

Pulsed-Liquid N-terminal Sequencing was carried out using an Applied Biosystems (ABI) Procise automatic protein sequencer 492.

System Suitability Test: Sequencing of β-lactoglobulin

An aliquot (10 pmol/10 µL) of prepared β-lactoglobulin solution was loaded on a precycled filter on to the Procise Sequencer and 15 residues were sequenced by Edman degradation. The released phenylthiohydantoin (PTH-) amino acid derivatives were identified by reversed phase HPLC analysis.

Sample Analysis

Precut PVDF membrane blots of each sample (two lanes per sample) were loaded on to the Procise Sequencer and twelve residues were sequenced by Edman degradation. The released phenylthiohydantoin (PTH-) amino-acid derivatives were identified by reversed-phase HPLC analysis.

3.3 Key Personnel

[redacted]

3.4 Reagents

All the reagents used during the course of the study are listed in the workfile.

3.5 Archive

The working file and original final report will be transferred to the Study Sponsor. The analytical phase study plan, a copy of the working file and final analytical phase report will be stored in an archive as described by SOP GP-013. The sample will be stored according to SOP BI-015 and M-Scan Standard Terms and Conditions.

4. RESULTS AND DISCUSSION

4.1 N-Terminal Sequencing of β-Lactoglobulin Standard

Pulsed-Liquid sequencing was performed on β-lactoglobulin standard. Fifteen residues were sequenced by Automatic Edman degradation. The data obtained from the analysis is shown in Appendix II and summarised in Table 1.

Table 1: N-Terminal Sequencing of β-Lactoglobulin Standard

Residue Number	β-Lactoglobulin Standard	
	PTH-AA Sequence Observed	pmoles Observed*
1	L	8.1
2	I	8.1
3	V	7.1
4	T	5.8
5	Q	6.7
6	T	5.1
7	M	5.8
8	K	5.6
9	G	5.1
10	L	6.5
11	D	4.7
12	I	6.2
13	Q	5.4
14	K	4.4
15	V	4.9

* Calculation based on 10 pmoles containing standard mixture. For information only, as N-terminal sequencing is not a quantitative technique.

The data obtained for a standard of β-Lactoglobulin were consistent with the following N-terminus sequence:

LIVTQ TMKGL DIQKV

The data obtained are consistent with the known sequence of β-Lactoglobulin.

The expected 19 PTH-amino acid peaks and dptu peak were present in the chromatogram obtained from analysis of the standard mixture of PTH-amino acid.
The run was completed successfully and all amino acids and dptu were identified correctly.

The average background corrected repetitive yield was calculated for L, I and V as 96.4 %. This value is within the bounds of the acceptance criteria of ≥94.0 %.

This analysis has met SGS M-Scan's SOP acceptance criteria.

4.2 N-Terminal Sequencing of SYHT0H2 AvHPPD-03 sample (SGS M-Scan No. 97246)

PVDF Pulsed-Liquid sequencing was performed on the SYHT0H2 AvHPPD-03 sample (SGS M-Scan No. 97246), with the 'expected' proline cycle method inserted for residue one, as detailed in the Analytical phase study plan. Twelve residues were sequenced by Automatic Edman degradation. The data obtained from the analysis is shown in Appendix III and summarised in Table 2.

Table 2: N-Terminal Sequencing of SYHT0H2 AvHPPD-03 sample (SGS M-Scan No. 97246)

Residue Number	SYHT0H2 AvHPPD-03 sample (SGS M-Scan No. 97246)	
	PTH-AA Observed	pmoles Observed**
1	P/A	2.6/ 3.0
2	A/T	5.2/*
3	T	3.1
4	A/G	4.7/2.7
5	T/G	2.6/2.8
6	G/A	3.8/4.0
7	A	5.7
8	A/G	5.8/2.7
9	A	6.0
10	V	*
11	A/V/G	5.0/2.0/2.9
12	V/T	2.9/*

* Please note the Protocol states that values below 2pmoles (based on 10 pmoles containing standard mixture) will not be reported. Please see the raw data for actual values.

** Calculation based on 10 pmoles containing standard mixture. For information only, as N-terminal sequencing is not a quantitative technique.

The N-terminal Edman sequencing data obtained for this sample was complex with several residues present in each cycle. However, upon comparison with the supplied expected sequence (as shown below), the data obtained can be linked to possible N-terminal trimming of the provided expected sequence.

Please note that as well as the above sequence there were high values of PTH-Glycine throughout the 12 residues sequenced, which made calling PTH-Glycine difficult throughout the analysis. Also in the first four to five residues sequenced other PTH derivitised peaks were observed not eluting with known PTH-amino acids, eluting between the PTH-Alanine and PTH- Arginine. These are likely to be contaminants from glycine/tris buffers often used during PVDF membrane blotting procedures.

The Edman sequencing data for this sample was relatively weak with some PTH-amino acid values below the ABi recommended 2pmole detection limit. However it could be confirmed that there was evidence of the 'expected' sequence (as supplied in the analytical phase study plan) with the sequence starting at residue 5: Proline. There was also weak evidence for further N-terminal trimming of the 'expected' N-terminus.

Supplied Expected sequence (for plant material)	**PATATGAAAAAVTPEH**
Observed residues in each cycle	**PATATGAAAVAV** **AT GGA G VT** **G**

4.3 N-Terminal Sequencing of Microbial AvHPPD-03-0209 (SGS M-Scan No. 97248)

PVDF Pulsed-Liquid sequencing was performed on Microbial AvHPPD-03-0209 (SGS M-Scan No. 97248). Twelve residues were sequenced by Automatic Edman degradation. The data obtained from the analysis is shown in Appendix IV and summarised in Table 3.

Table 3: N-Terminal Sequencing of Microbial AvHPPD03-0209 (SGS M-Scan No. 97248)

Residue Number	Microbial AvHPPD-03-0209 (SGS M-Scan No. 97248)	
	PTH-AA Observed	pmoles Observed*
1	P/M/A	10.4/6.6/2.6
2	P/T/A	12.7/4.0/4.4
3	T/A	7.8/4.1
4	A/P/T	7.6/8.4/8.2
5	A/P	13.0/7.4
6	A/T	15.1/7.7
7	A/T	15.6/9.7
8	A/T/G	15.0/9.3/6.2
9	G/T/A	8.5/8.7/14.3
10	G/A	8.7/17.5
11	A	22.0
12	A	24.7

* Calculation based on 10 pmoles containing standard mixture. For information only, as N-terminal sequencing is not a quantitative technique.

The N-terminal Edman sequencing data obtained for this sample is complex with several residues present in each cycle. However, upon comparison with the supplied expected sequence (as shown below), the data obtained can be linked to the expected sequence, as well as evidence for possible N-terminal trimming.

Supplied Expected sequence	**MPPTPATATGAAAAAVTPEH**
Observed residues in each cycle	**PPTAAAAAGGAA** **MTAPPTTTTA** **AA T GA**

5. CONCLUSIONS

Analysis of the N-terminal Sequence for β-Lactoglobulin

Analysis of β-Lactoglobulin standard met the acceptance criteria and therefore the system was fit for use.

Analysis of the N-terminal Sequence for SYHT0H2 AvHPPD-03 sample (SGS M-Scan No. 97246)

The data suggests the confirmation of the expected sequence from Residue 5 Proline. There was also weak evidence for further N-termini trimming of the expected sequence from Residue 6 Alanine and Residue 7 Threonine.

Analysis of the N-terminal Sequence for Microbial AvHPPD-03-0209 (SGS M-Scan No. 97248)

The data suggests the expected N-terminal sequence of the sample was present but also present were other N-terminal trimmed sequences.

The N-termini of both samples were consistent with the sequence supplied by the Study Sponsor indicating positive identity of the sample.

6. REFERENCES

1. ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.

2. Edman, P. and Begg, G. (1967) Eur. J. Biochem, **1**, 80-91.

Appendix I – Protocol No. 10090101 and Amendment One

CONFIDENTIAL

SGS M-Scan

STUDY TITLE:	Comparison of *p*-hydroxyphenylpyruvate Dioxygenase AvHPPD-03 Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
PROTOCOL NUMBER:	TK0031229
ANALYTICAL PHASE TITLE:	N-Terminal Amino Acid Sequencing of AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
TEST SITE:	SGS M-SCAN LIMITED 3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK
ANALYTICAL PHASE STUDY PLAN NO:	10090101 AMENDMENT ONE
STUDY SPONSOR:	SYNGENTA CROP PROTECTION, LLC Syngenta Biotechnology Incorporated 3054 East Cornwallis Road, P.O. Box 12257 Research Triangle Park, NC 27709 North Carolina, USA
STUDY DIRECTOR:	[redacted]

This study will be conducted in accordance with the principles laid down in the Good Laboratory Practice Regulations SI 3106 (1999) as amended by SI 994 (2004) which are themselves based on the principles of good laboratory practice contained in Annex 2 to the Decision of the Council of the Organisation for Economic Co-Operation and Development (OECD), ENV/MC/CHEM (98)17. They are in conformity with, and implement the requirements of, Directives 2004/10/EC and 2004/9/EC. The Study will also be conducted in accordance with the OECD Monograph No.13 "The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies", (ENV/JM/MONO(2002)9), OECD Monograph No.13.

Key Personnel

[redacted] (Principal Investigator)
[redacted] (Quality Assurance Unit)
(Other key personnel will be listed in the final report)

Issued on behalf of SGS M-Scan Limited (Principal Investigator)	QA Reviewed on behalf of SGS M-Scan Limited	Accepted on behalf of Syngenta (Study Sponsor)
By [redacted]	By [redacted]	By [redacted]
Date 8th Sept 2011	Date 08th Sept 2011	Date 20th Sept 2011

M-SCAN IS NOW PART OF SGS, THE WORLD'S LEADING INSPECTION, VERIFICATION, TESTING AND CERTIFICATION COMPANY.

CONFIDENTIAL

Reasons for Amendment One

Analytical Phase Study Plan 10090101 amendment one has been issued in order to account for the Study Director name change and the change in the Study Sponsor as requested by the Study Sponsor. The name of the test site has also been updated.

Study Director has changed from: Terrie Moore, to: [redacted]

Test site name has changed from: M-Scan, to: SGS M-Scan

Study Sponsor name has changed from Syngenta Biotechnology Incorporated, to: Syngenta Crop Protection, LLC

No additional changes have been made.

CONFIDENTIAL

M-Scan

STUDY TITLE:	Comparison of *p*-hydroxyphenylpyruvate Dioxygenase AvHPPD-03 Protein Produced in Recombinant *Escherichia coli* and AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
PROTOCOL NUMBER:	TK0031229
ANALYTICAL PHASE TITLE:	N-Terminal Amino Acid Sequencing of AvHPPD-03 Protein Produced in Event SYHT0H2 Derived Soybean Plants.
TEST SITE:	M-SCAN LIMITED 3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK
ANALYTICAL PHASE STUDY PLAN NO:	10090101
STUDY SPONSOR:	SYNGENTA BIOTECHNOLOGY, INCORPORATED 3054 East Cornwallis Road, P.O. Box 12257 Research Triangle Park, NC 27709 North Carolina, USA
STUDY DIRECTOR:	[redacted]

This study will be conducted in accordance with the principles laid down in the Good Laboratory Practice Regulations SI 3106 (1999) as amended by SI 994 (2004) which are themselves based on the principles of good laboratory practice contained in Annex 2 to the Decision of the Council of the Organisation for Economic Co-Operation and Development (OECD), ENV/MC/CHEM (98)17. They are in conformity with, and implement the requirements of, Directives 2004/10/EC and 2004/9/EC. The Study will also be conducted in accordance with the OECD Monograph No.13 "The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies", (ENV/JM/MONO(2002)9), OECD Monograph No.13.

Key Personnel

[redacted] (Principal Investigator)
[redacted] (Quality Assurance Unit)
(Other key personnel will be listed in the final report)

Issued on behalf of M-Scan Limited (Principal Investigator)	QA Reviewed on behalf of M-Scan Limited	Accepted on behalf of Syngenta (Study Sponsor)
By [redacted]	By [redacted]	By [redacted]
Date 14th Dec 2010	Date 14th Dec 2010	Date 3rd Jan 2011

1. Objective

The aim of this analytical phase study plan is to analyse the supplied AvHPPD-03 test samples using automated Edman degradation chemistry and report the N-terminal sequences obtained. The supplied AvHPPD-03 sample will be provided by the Study Sponsor blotted and stained on a PVDF membrane. Edman degradation will be performed on the supplied band(s) using an automated pulsed-liquid sequencer. Automated pulsed-liquid sequencing for twelve residues of the N-terminal sequence will be undertaken.

2. Samples

The test samples are described by the Study Sponsor as follows:

Chemical Name(s):	plant produced AvHPPD-03.
Batch/Lot:	As described on sample receipt documentation received with the samples, this is expected to include a transfer of custody document.
Form:	PVDF membrane blotted sample.
Storage conditions:	As described on sample receipt documentation received with the samples, this is expected to include a transfer of custody document. Otherwise the sample(s) will be stored at the same temperature as received. Storage conditions available at M-Scan Ltd are: RT, Cold (1 to 10°C), or Frozen (-10 to -30°C).

M-Scan advises that at least 10 picomoles of each sample should be supplied on no more than five pre-stained PVDF membrane pre-cut bands in order to obtain the required sample level for obtaining N-terminal sequence data for the first twelve residues of the N-terminus of the sample.

The expected sequence for the AvHPPD-03 protein is given below. Prior experiments, not conducted to GLP, suggest the plant produced AvHPPD-03 protein is likely to start with the Proline, at residue 5:

Expected sequence: MPPTPATATGAAAAAVTPEH

3. Analytical Methods

a. Equipment

Analyses will be performed using the following equipment:

Applied Biosystems Procise 492 automated N-terminal sequencer equipped with HPLC consisting of 140C pumps, a 200 Perkin Elmer series detector, a reverse phase column PTH-C18 cartridge 2.1 x 220 mm (SOPs PS001 to PS014).

b. Procedures

The latest record of M-Scan's standard performance verification system suitability test protein (β-Lactoglobulin) will be provided (analysed for fifteen cycles) and reported.

The supplied PVDF bands will then be subjected to automated Edman degradation and cycles sufficient to sequence 12 residues of the N-termini will

be undertaken. M-Scan will undertake the pulsed liquid sequencing using our standard PVDF pulsed liquid methodology, including a specific Proline cycle for Residue 1 (for the plant derived material only)(M-Scan SOPs PS001 to PS014).

4. Recording Observations

All descriptions of experimental details and methods together with all spectra, print-outs and chromatograms will be collected in a working file labelled with the appropriate job number as described in SOP GP-008.

5. Reporting

All PTH-amino acids showing a typical sequencing pattern and above the standard limit of detection will be assigned.

The residues raw pmoles based on peaks height will be reported for information only (the technique is not strictly quantitative). Please note that the standard limit of detection for the sequencer is 2pmoles and therefore residues below 2pmoles will not be reported.

The latest Performance Verification system suitability analysis performed before the analysis of the samples will also be included in the analytical phase report .

A draft analytical phase report including the raw data will be submitted to the Study Sponsor and to Syngenta, Jealott's Hill International Research Centre, Bracknell, UK, for review.

6. Time Scale

The timescale for the study is three to four weeks from receipt of samples (providing the analytical phase study plan is formally accepted by the Study Sponsor). A draft analytical phase report will then be submitted (as above).

7. Quality Assurance

This study will be conducted in compliance with the Good Laboratory Practice Regulations, SI 3106 (1999) as amended by SI 994 (2004), as enforced by the United Kingdom Good Laboratory Practice Monitoring Authority, MHRA, and in accordance with M-Scan Standard Operating Procedures. The phase protocol and final analytical phase study plan will be audited by the M-Scan Quality Assurance Unit. The QA Unit will audit selected experimental phases of the GLP studies (initial phase audit). Laboratory procedures are inspected on a routine basis in accordance with M-Scan SOPs.

On completion of the study, one copy of the draft analytical phase study plan (prior to QA review) will be supplied to the Study Sponsor. After a period of no longer than four weeks, during which the Study Sponsor may comment and have changes made if necessary, the analytical phase study plan will be fully reviewed by the QA Unit and one bound copy and a PDF version of the final analytical phase study plan will be supplied to the Study Sponsor. A PDF version of the Final analytical phase study plan will also be sent to Syngenta, Jealott's Hill International Research Centre, Bracknell, UK.

8. Archives

The working file and original final analytical phase study plan and report will be transferred to the Study Sponsor. The analytical phase study plan , a copy of the working file and final analytical phase report will be stored in an archive as described by SOP GP-013. The sample will be stored according to SOP BI-015 and M-Scan Standard Terms and Conditions.

Appendix II – Raw Data for 15 Residues of N-terminal Sequencing of β-Lactoglobulin

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RK

SAMPLE INFORMATION

Sample Name:	110928_3A_bLG	Std Amount:	10.000 pmols
ID Code:		Sample Amount:	0.000 pmols
		Detector Scale:	0.005 AUFS

Comments:

SEQUENCER INFORMATION

Name:	PROCISE	Model Number:	492
Method:	Pulsed liquid	Cartridge:	A
Operator:	RK		

CHEMICAL INFORMATION

R1	1105171	23 September, 2011	X3	0	01 September, 2001
R2	1105087	12 September, 2011	PTH Column	G110608031	27 September, 2011
R3	1012198	01 September, 2001	Solvent A	1106619	27 September, 2011
R4	1009128	23 September, 2011	Solvent B	1105351	01 September, 2001
R5	1011047	27 September, 2011	Premix	1104103	27 September, 2011
S1	0	01 September, 2001	Guard Column	0	01 January, 2002
S2	1106705	28 September, 2011	Cartridge Seals	0	01 January, 2002
S3	1106494	16 September, 2011	Glass Fiber Filter	0	01 January, 2002
S4	1105166	16 September, 2011	pth standards	1007028	01 January, 2002
X1	0	01 September, 2001		12345678	01 January, 2002
X2	0	01 September, 2001	Total Cycles Count		01 January, 2002

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SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RH

ORIGINAL METHOD TEMPLATE: D:\Program Files\AppliedBiosystems\Procise\SequencePro\Methods\Procise1.met

CALIBRATION TABLE

COMPONENT	RTIME	RESPONSE	REFERENCE	INTERNAL STD	ABS WND	REL WND
Aspartic Acid	4.19	1.000	---	---	0.20	0.00
Asparagine	4.70	1.000	---	---	0.20	0.00
Serine	5.38	1.000	---	---	0.20	0.00
Glutamine	5.64	1.000	---	---	0.20	0.00
Threonine	5.93	1.000	---	---	0.20	0.00
Glycine	6.16	1.000	---	---	0.20	0.00
Glutamic Acid	6.62	1.000	---	---	0.20	0.00
Histidine	7.70	1.000	---	---	0.20	0.00
Alanine	8.51	1.000	---	---	0.20	0.00
Arginine	9.84	1.000	---	---	0.20	0.00
Tyrosine	10.80	1.000	---	---	0.20	0.00
Proline	13.06	1.000	---	---	0.20	0.00
Methionine	13.79	1.000	---	---	0.20	0.00
Valine	14.10	1.000	---	---	0.20	0.00
dptu	15.27	1.000	---	---	0.20	0.00
Tryptophan	16.38	1.000	---	---	0.20	0.00
Phenylalanine	17.02	1.000	---	---	0.20	0.00
Isoleucine	17.49	1.000	---	---	0.20	0.00
Lysine	17.79	1.000	---	---	0.20	0.00
Leucine	18.02	1.000	---	---	0.20	0.00

GLOBAL INTEGRATION EVENTS

EVENT	TIME	VALUE
Peak Detect Off	0.00	---
Valley to Valley On	3.90	---
Peak Detect On	3.90	---

EVENT	TIME	VALUE
Valley to Valley Off	5.30	---
Peak Detect Off	19.00	---

INTEGRATION PARAMETERS

PEAK DETECTION PARAMETERS

Bunching Factor	4	Noise Threshold:	0.954 µVolts
Max Peaks	128	Area Threshold:	41.000 µVolts

PEAK SEPARATION CRITERIA

Width Ratio:	0.20	Peak Height Ratio:	5.00
Valley to Peak Ratio:	0.01	Adjusted Height Ratio:	4.00
Tangent Width:	1000.00	Valley Height Ratio:	3.00

SEQUENCE CALLING PARAMETERS

Use Pmol Heights, Allow Negative Background Off, Refine Data On

SEQUENCE SCORING VALUES

Raw Slope 1:	2.00	Raw Yield	1.00
Raw Slope 2:	1.00	Bkgd Yield	1.00
Bkgd Slope 1:	2.00	Lag Yield	1.00
Bkgd Slope 2:	1.00	Rep Yield	1.00
Max Slope:	1.50	Low Yield	1.00
Rule Book:	0.60	Bkgd Sensitivity	1.00
Dev Mult:	3.00		

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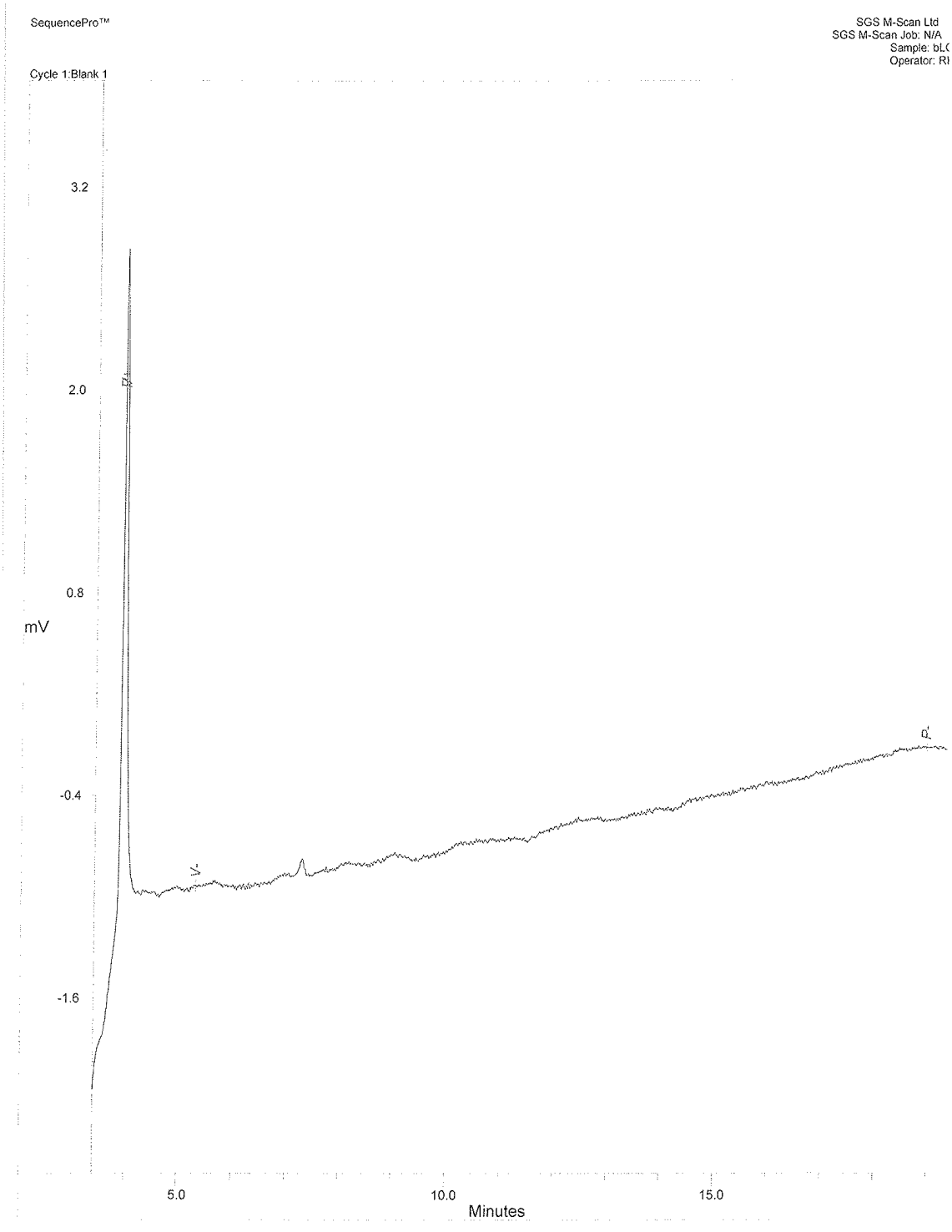
RK 29sep11


SequencePro™
SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RI
Cycle 1:Blank 1
3.2
2.0
0.8
mV
-0.4
-1.6
5.0
10.0
15.0
Minutes
Thursday, September 29, 2011 11:26:21
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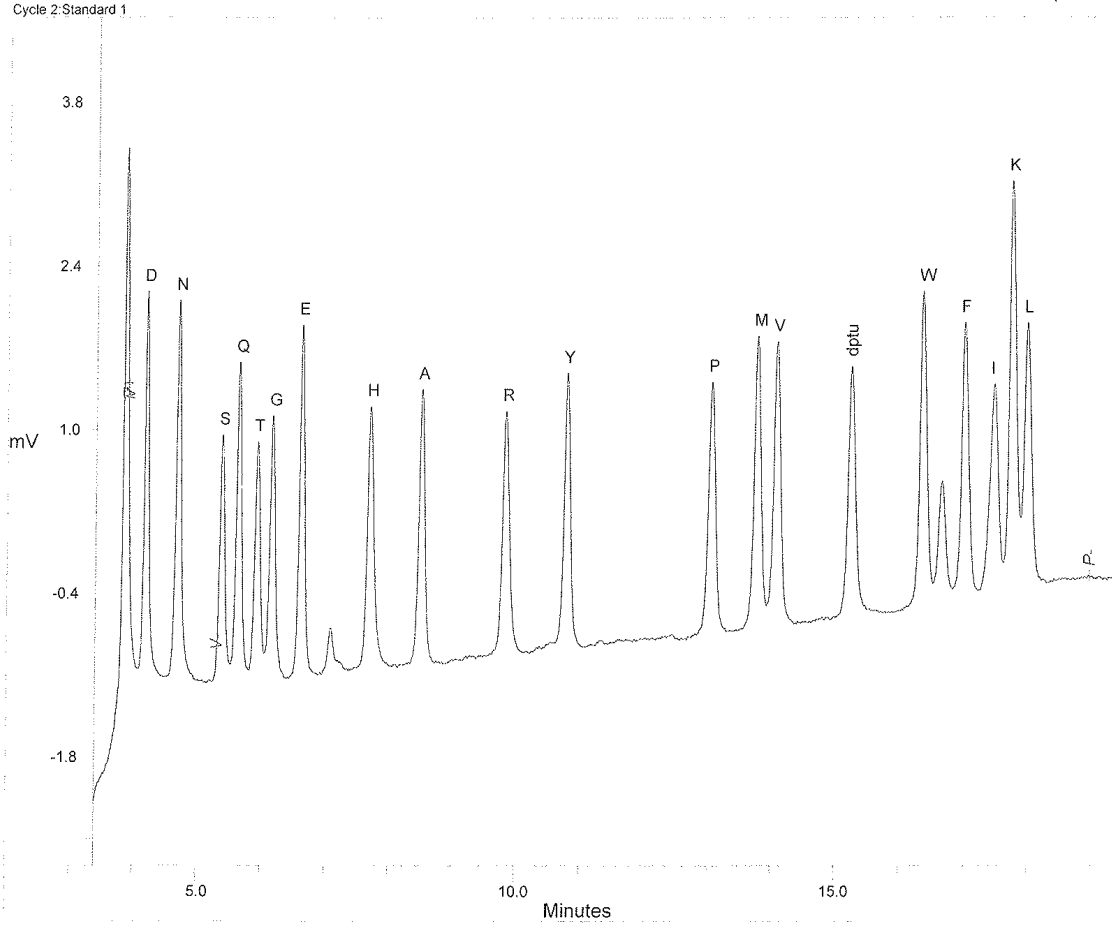
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bL
Operator: R

Cycle 2:Standard 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.19	4.19	3.254	10.000
N	4.70	4.70	3.238	10.000
	5.20		0.024	
S	5.38	5.38	2.121	10.000
Q	5.64	5.64	2.738	10.000
T	5.93	5.93	2.046	10.000
G	6.16	6.16	2.264	10.000
E	6.62	6.62	3.019	10.000
	7.08		0.393	
H	7.70	7.70	2.233	10.000
	8.21		0.025	
A	8.51	8.51	2.358	10.000
	9.00		0.019	
	9.23		0.034	
	9.33		0.033	
	9.46		0.031	
	9.53		0.023	
R	9.84	9.84	2.095	10.000
	10.38		0.057	
	10.55		0.077	
Y	10.80	10.80	2.367	10.000
	11.17		0.024	
	11.35		0.038	
	11.62		0.028	
	11.95		0.022	
	12.02		0.026	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	12.13		0.022	
	12.24		0.021	
	12.45		0.042	
	12.58		0.014	
	12.68		0.028	
P	13.06	13.06	2.153	10.000
M	13.79	13.79	2.508	10.000
V	14.10	14.10	2.438	10.000
	14.43		0.026	
	14.81		0.033	
	14.87		0.028	
	15.00		0.019	
dptu	15.27	15.27	2.123	10.000
	15.72		0.014	
W	16.38	16.38	2.659	10.000
	16.67		0.969	
F	17.02	17.02	2.291	10.000
I	17.49	17.49	1.748	10.000
K	17.79	17.79	3.461	10.000
L	18.02	18.02	2.225	10.000
	18.38		0.015	
	18.57		0.030	
	18.75		0.019	
	18.82		0.017	
	18.92		0.021	

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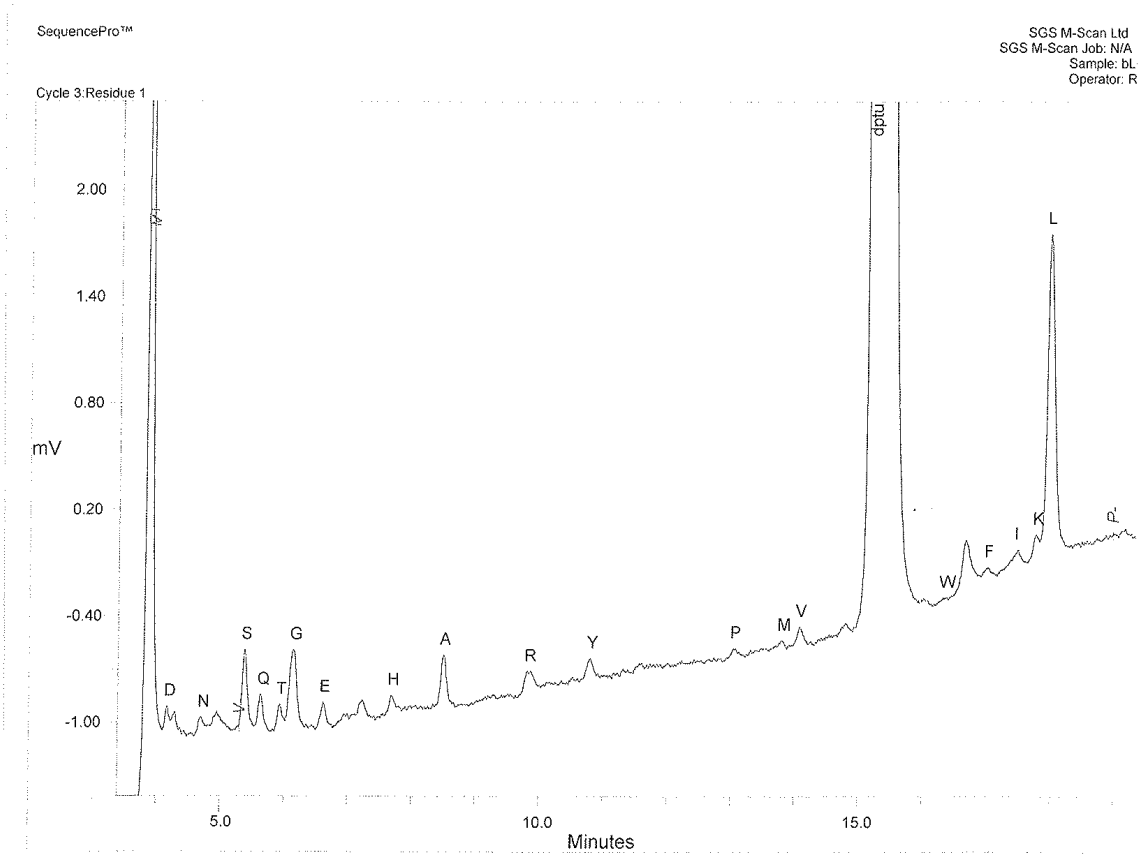
RK 29Sep11



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.108	0.331
	4.29		0.078	
	4.43		0.028	
	4.55		0.021	
N	4.70	4.70	0.081	0.250
	4.95		0.097	
S	5.38	5.38	0.463	2.182
Q	5.63	5.64	0.210	0.768
T	5.94	5.93	0.151	0.736
G	6.14	6.16	0.449	1.983
	6.43		0.027	
E	6.61	6.62	0.154	0.512
	6.94		0.023	
	7.10		0.027	
	7.22		0.104	
H	7.68	7.70	0.121	0.543
	7.90		0.040	
	7.99		0.041	
	8.07		0.035	
	8.15		0.026	
	8.26		0.019	
	8.34		0.025	
A	8.50	8.51	0.289	1.225
	8.79		0.011	
	8.95		0.018	
	9.03		0.026	
	9.16		0.037	
	9.28		0.045	
	9.43		0.037	
	9.53		0.031	
R	9.83	9.84	0.027	0.130
	10.13		0.010	
	10.35		0.027	
	10.53		0.035	
	10.60		0.023	
Y	10.81	10.80	0.112	0.472

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	10.98		0.018	
	11.18		0.021	
	11.32		0.034	
	11.61		0.047	
	11.69		0.032	
	11.74		0.031	
	12.00		0.023	
	12.23		0.026	
	12.42		0.023	
	12.65		0.024	
	12.74		0.028	
	12.84		0.023	
P	13.04	13.06	0.042	0.195
	13.31		0.030	
	13.37		0.019	
	13.48		0.012	
M	13.80	13.79	0.052	0.208
	13.92		0.023	
V	14.08	14.10	0.116	0.476
	14.25		0.026	
	14.43		0.028	
	14.55		0.032	
	14.80		0.062	
dptu	15.27	15.27	16.869	79.471
	15.46		36.228	
	16.02		0.028	
W	16.33	16.38	0.027	0.101
	16.69		0.254	
F	17.02	17.02	0.053	0.230
I	17.50	17.49	0.098	0.560
K	17.79	17.79	0.139	0.401
L	18.02	18.02	1.792	8.054
	18.43		0.026	
	18.58		0.020	
	18.74		0.026	
	18.88		0.029	

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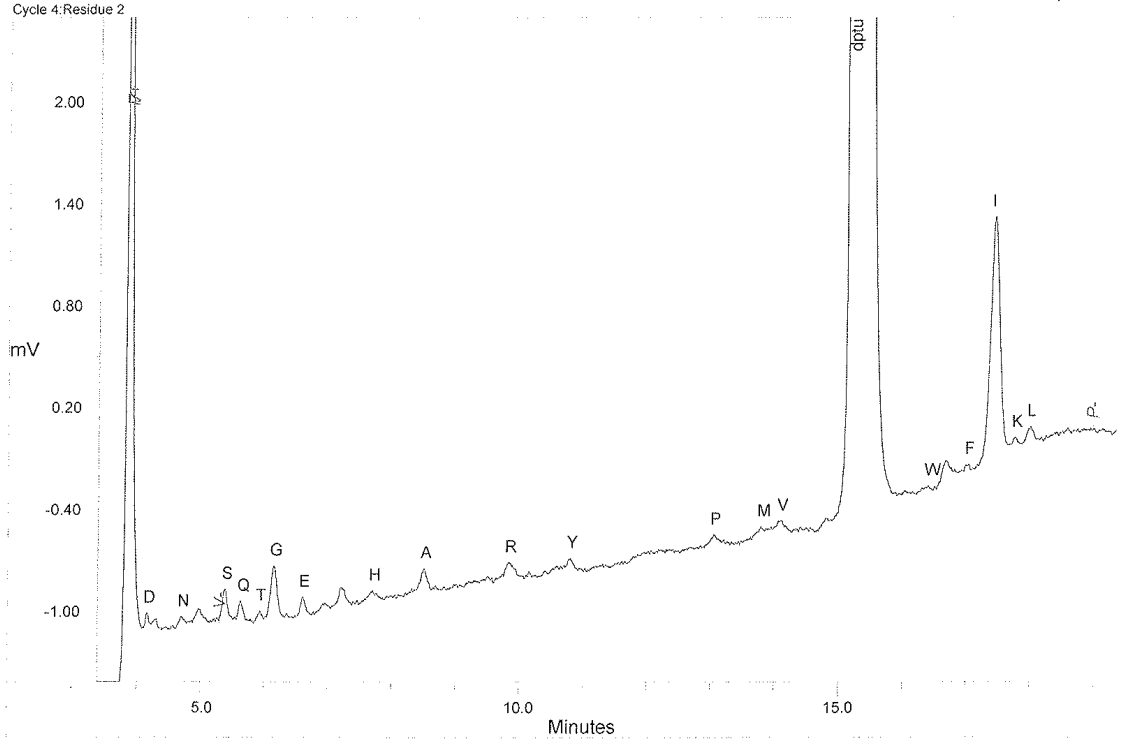
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bL
Operator: R

Cycle 4:Residue 2



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.082	0.253
	4.30		0.052	
	4.45		0.017	
	4.57		0.024	
N	4.70	4.70	0.058	0.179
	4.96		0.087	
	5.26		0.028	
S	5.39	5.38	0.194	0.914
Q	5.64	5.64	0.117	0.427
T	5.93	5.93	0.069	0.335
G	6.15	6.16	0.324	1.430
	6.36		0.035	
E	6.61	6.62	0.116	0.386
	6.78		0.020	
	6.95		0.057	
	7.22		0.126	
	7.46		0.030	
H	7.70	7.70	0.039	0.174
	8.00		0.022	
	8.06		0.023	
	8.22		0.026	
	8.32		0.041	
A	8.51	8.51	0.147	0.625
	8.69		0.034	
	8.81		0.021	
	9.02		0.024	
	9.22		0.020	
	9.50		0.028	
	9.65		0.030	
R	9.83	9.84	0.110	0.523
	10.15		0.036	
	10.39		0.031	
	10.59		0.051	
	10.65		0.041	
Y	10.81	10.80	0.079	0.335
	11.03		0.021	
	11.20		0.023	
	11.31		0.023	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.56		0.023	
	11.62		0.022	
	11.87		0.033	
	11.96		0.033	
	12.08		0.027	
	12.37		0.014	
	12.69		0.025	
	12.85		0.016	
P	13.06	13.06	0.041	0.189
	13.22		0.022	
M	13.80	13.79	0.047	0.188
	13.90		0.013	
V	14.11	14.10	0.043	0.176
	14.34		0.026	
	14.42		0.032	
	14.50		0.026	
	14.57		0.026	
	14.81		0.061	
dptu	15.28	15.27	18.551	87.393
	15.47		21.039	
	15.90		0.040	
	16.00		0.029	
	16.06		0.028	
	16.16		0.016	
W	16.42	16.38	0.035	0.132
	16.70		0.105	
	16.94		0.013	
F	17.05	17.02	0.039	0.170
I	17.49	17.49	1.424	8.149
K	17.79	17.79	0.065	0.189
L	18.03	18.02	0.087	0.392
	18.20		0.021	
	18.26		0.029	
	18.42		0.035	
	18.52		0.034	
	18.60		0.043	
	18.70		0.020	
	18.86		0.015	

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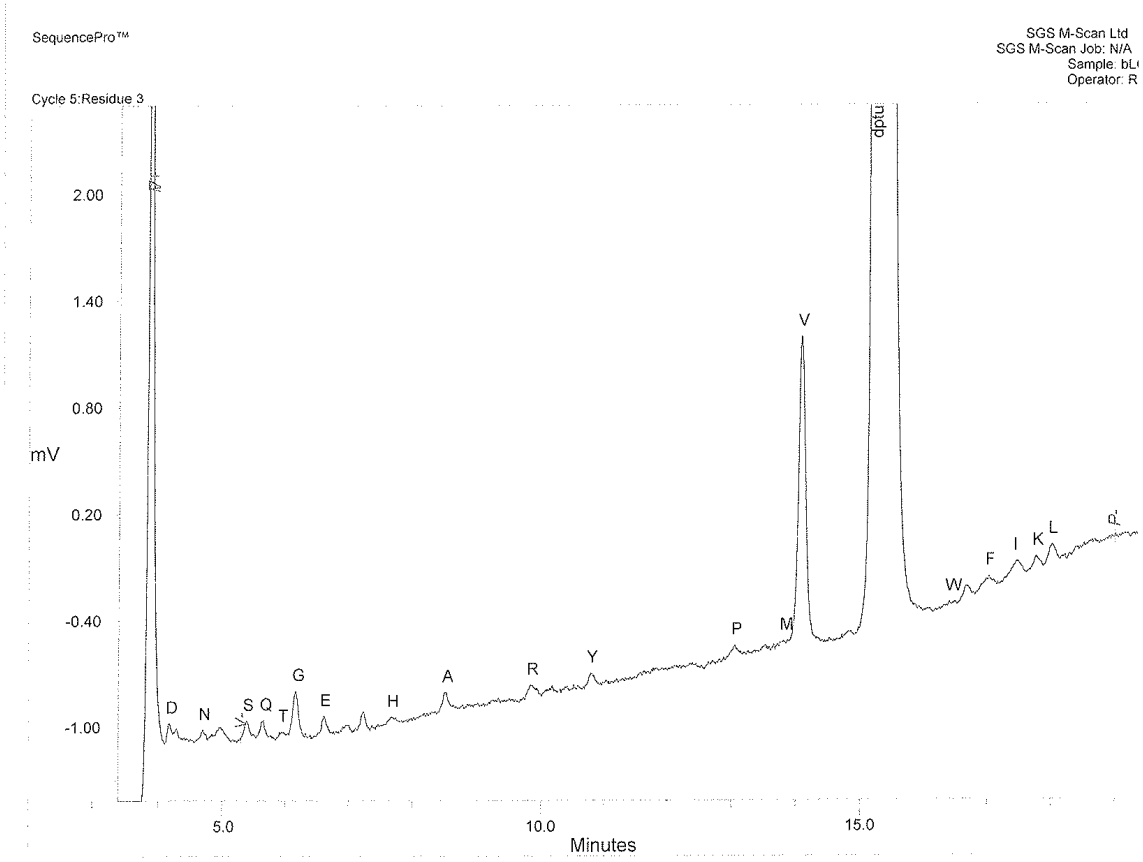
K 29sep11



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.086	0.263
	4.28		0.045	
	4.51		0.017	
N	4.70	4.70	0.063	0.195
	4.84		0.019	
	4.98		0.068	
S	5.39	5.38	0.095	0.448
Q	5.66	5.64	0.098	0.359
T	5.95	5.93	0.044	0.216
G	6.17	6.16	0.267	1.180
E	6.62	6.62	0.110	0.363
	6.96		0.045	
	7.24		0.113	
	7.38		0.030	
H	7.66	7.70	0.028	0.125
	7.91		0.016	
	8.02		0.018	
	8.19		0.021	
	8.36		0.019	
A	8.52	8.51	0.102	0.433
	8.78		0.020	
	8.85		0.022	
	9.01		0.022	
	9.25		0.012	
	9.43		0.016	
R	9.83	9.84	0.082	0.392
	10.18		0.037	
	10.31		0.022	
	10.41		0.033	
	10.52		0.034	
Y	10.79	10.80	0.071	0.299
	11.03		0.026	
	11.20		0.021	
	11.34		0.017	
	11.58		0.025	
	11.63		0.034	
	11.71		0.025	
	11.78		0.039	
	11.93		0.026	
	12.02		0.021	
	12.15		0.024	
	12.20		0.021	
	12.40		0.020	
	12.65		0.024	
	12.82		0.021	
P	13.06	13.06	0.058	0.268
	13.26		0.021	
	13.53		0.032	
	13.66		0.033	
M	13.82	13.79	0.041	0.162
V	14.10	14.10	1.725	7.078
	14.42		0.014	
	14.63		0.014	
	14.87		0.030	
dptu	15.28	15.27	17.939	84.510
	15.47		21.376	
	16.10		0.024	
	16.25		0.019	
W	16.42	16.38	0.030	0.114
	16.50		0.019	
	16.68		0.085	
F	17.04	17.02	0.086	0.376
I	17.48	17.49	0.105	0.603
K	17.78	17.79	0.089	0.258
L	18.03	18.02	0.119	0.536
	18.23		0.033	
	18.30		0.028	
	18.42		0.043	
	18.52		0.047	
	18.69		0.044	
	18.96		0.016	

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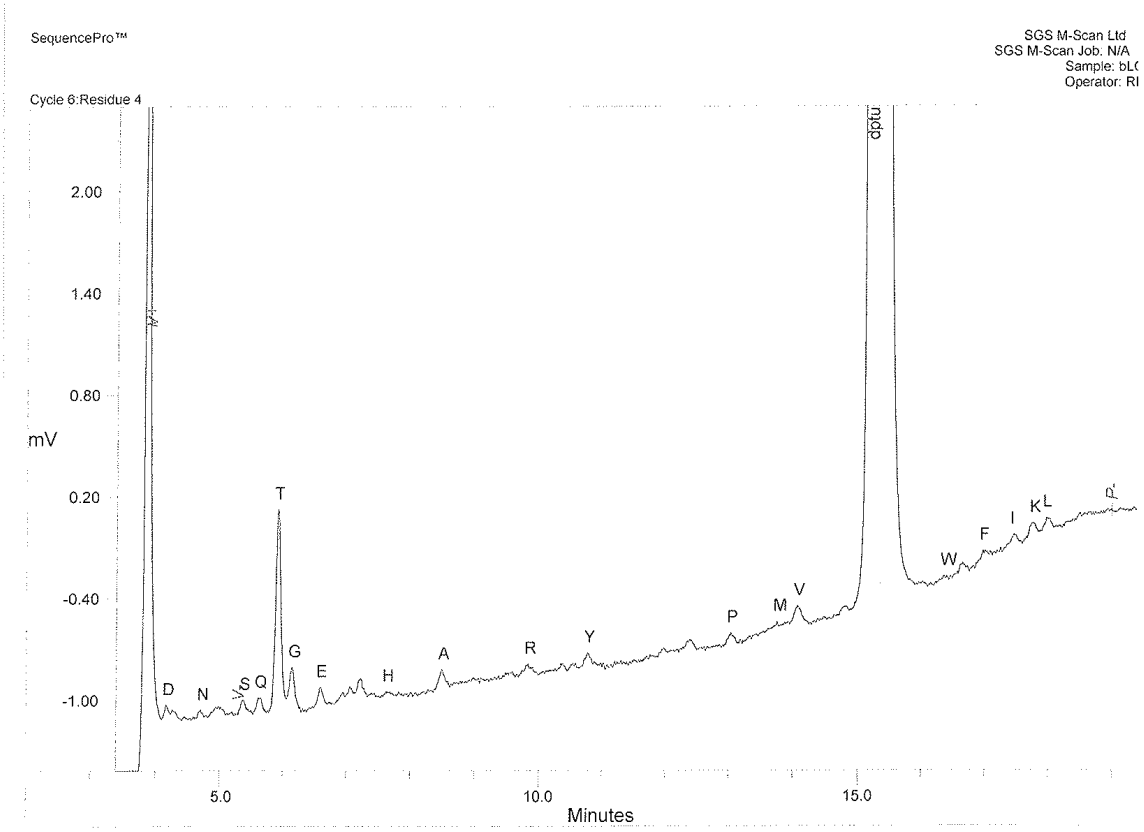

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.070	0.217
	4.26		0.032	
	4.44		0.024	
N	4.70	4.70	0.049	0.153
	4.80		0.014	
	4.98		0.027	
	5.19		0.023	
S	5.38	5.38	0.083	0.392
Q	5.62	5.64	0.098	0.357
T	5.92	5.93	1.193	5.829
G	6.15	6.16	0.253	1.119
	6.32		0.023	
E	6.59	6.62	0.117	0.387
	6.96		0.067	
	7.06		0.093	
	7.23		0.134	
	7.40		0.037	
H	7.63	7.70	0.015	0.069
	7.83		0.026	
	7.92		0.018	
	8.00		0.027	
	8.22		0.018	
A	8.50	8.51	0.118	0.502
	8.69		0.020	
	8.97		0.034	
	9.05		0.029	
	9.20		0.014	
	9.31		0.014	
	9.74		0.028	
R	9.83	9.84	0.044	0.208
	10.07		0.019	
	10.27		0.025	
	10.36		0.050	
	10.55		0.024	
Y	10.78	10.80	0.084	0.354
	10.88		0.027	
	11.09		0.028	
	11.15		0.025	
	11.52		0.025	
	11.72		0.023	
	11.99		0.046	
	12.40		0.072	
	12.58		0.015	
	12.67		0.023	
	12.81		0.012	
P	13.04	13.06	0.078	0.363
	13.18		0.030	
	13.34		0.026	
	13.43		0.021	
	13.54		0.017	
M	13.76	13.79	0.036	0.144
	13.92		0.023	
V	14.07	14.10	0.115	0.470
	14.39		0.025	
	14.48		0.028	
	14.56		0.011	
	14.63		0.017	
	14.83		0.034	
dptu	15.25	15.27	18.558	87.428
	15.45		20.455	
	15.97		0.016	
	16.15		0.019	
W	16.38	16.38	0.037	0.139
	16.66		0.050	
F	16.99	17.02	0.029	0.125
	17.21		0.022	
	17.27		0.032	
I	17.48	17.49	0.079	0.450
K	17.77	17.79	0.100	0.289
L	17.98	18.02	0.102	0.457
	18.18		0.025	
	18.50		0.043	
	18.58		0.031	
	18.67		0.025	
	18.93		0.020	

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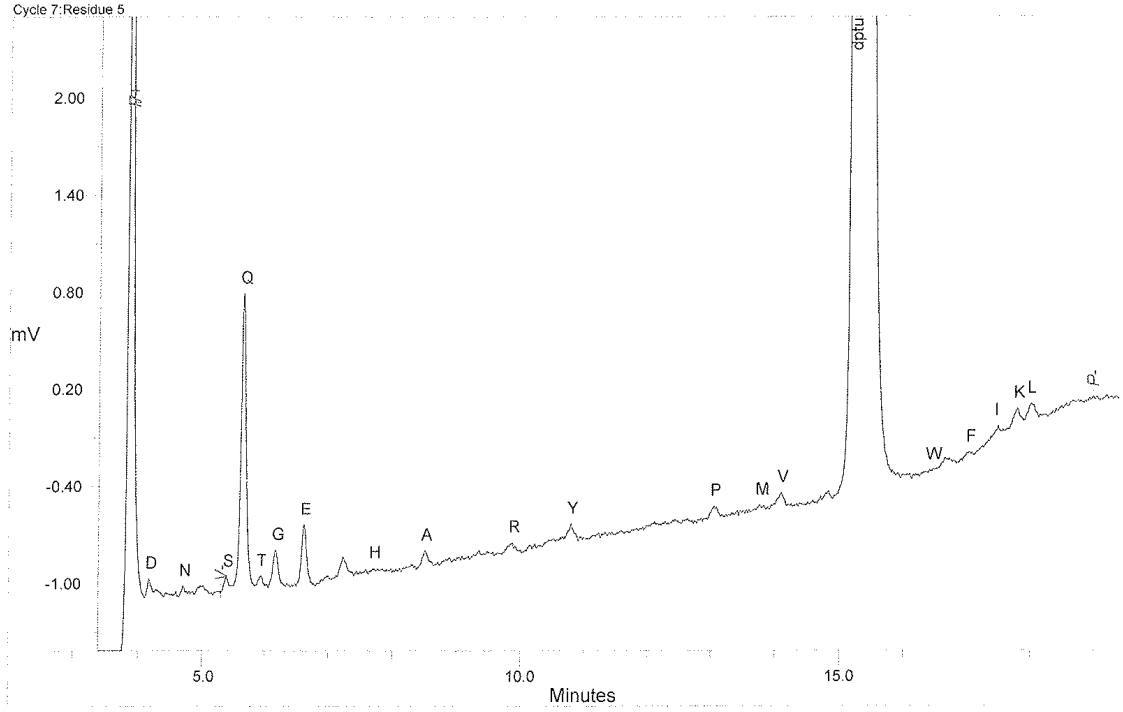
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RI



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.102	0.314
	4.28		0.023	
	4.44		0.024	
	4.59		0.032	
N	4.70	4.70	0.059	0.182
	4.86		0.020	
	4.97		0.055	
	5.27		0.022	
S	5.38	5.38	0.101	0.477
Q	5.64	5.64	1.834	6.697
T	5.92	5.93	0.066	0.321
G	6.16	6.16	0.232	1.024
E	6.61	6.62	0.379	1.257
	6.90		0.035	
	6.97		0.045	
	7.23		0.140	
	7.44		0.036	
	7.58		0.030	
H	7.70	7.70	0.026	0.116
	7.92		0.011	
	8.06		0.017	
	8.31		0.038	
A	8.50	8.51	0.107	0.454
	8.70		0.023	
	8.88		0.031	
	8.97		0.011	
	9.18		0.023	
	9.26		0.032	
	9.35		0.052	
	9.48		0.039	
R	9.86	9.84	0.040	0.193
	10.14		0.039	
	10.23		0.043	
	10.29		0.029	
	10.45		0.049	
	10.53		0.047	
Y	10.80	10.80	0.115	0.486
	10.94		0.023	
	11.14		0.026	
	11.23		0.035	
	11.34		0.031	
	11.45		0.026	
	11.59		0.022	
	11.69		0.016	
	11.83		0.017	
	12.12		0.033	
	12.29		0.024	
	12.44		0.029	
	12.64		0.025	
	12.75		0.018	
	12.85		0.026	
P	13.05	13.06	0.077	0.359
	13.33		0.023	
	13.42		0.032	
	13.55		0.024	
M	13.76	13.79	0.039	0.157
	13.82		0.029	
	13.89		0.016	
V	14.10	14.10	0.090	0.371
	14.41		0.014	
	14.51		0.017	
	14.59		0.022	
	14.71		0.040	
	14.83		0.057	
dptu	15.28	15.27	13.386	63.060
	15.47		18.567	
	15.93		0.022	
	16.01		0.018	
	16.20		0.028	
W	16.43	16.38	0.018	0.069
	16.67		0.037	
F	17.06	17.02	0.033	0.144
I	17.51	17.49	0.096	0.550
K	17.81	17.79	0.134	0.389
L	18.02	18.02	0.115	0.516
	18.42		0.030	
	18.50		0.029	
	18.57		0.035	
	18.68		0.033	
	18.76		0.020	

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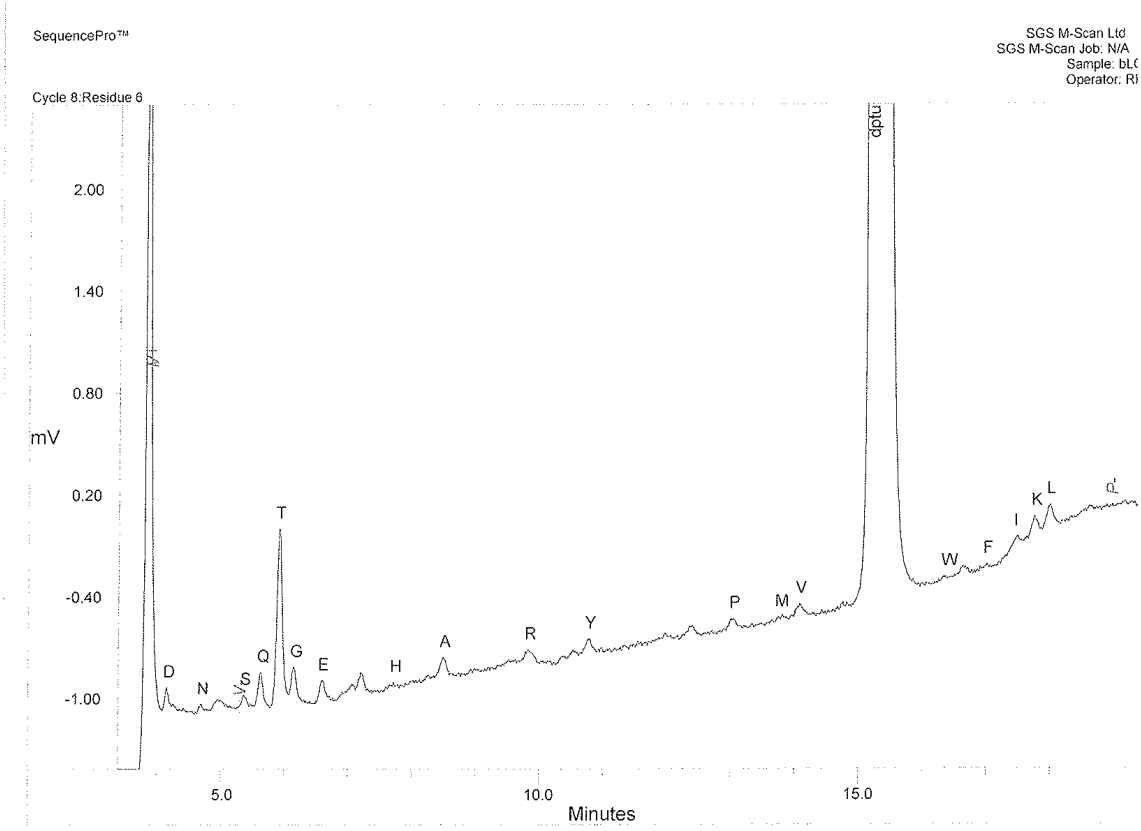

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.127	0.390
	4.42		0.023	
N	4.69	4.70	0.047	0.146
	4.95		0.056	
	5.15		0.015	
S	5.37	5.38	0.086	0.404
Q	5.63	5.64	0.216	0.788
T	5.92	5.93	1.050	5.130
G	6.15	6.16	0.216	0.954
	6.40		0.016	
E	6.59	6.62	0.123	0.408
	6.90		0.043	
	7.08		0.064	
	7.20		0.120	
	7.60		0.021	
	7.65		0.024	
H	7.72	7.70	0.032	0.142
	7.90		0.022	
	8.06		0.026	
	8.13		0.028	
	8.25		0.037	
A	8.49	8.51	0.118	0.499
	8.74		0.022	
	8.93		0.025	
	8.99		0.021	
	9.15		0.016	
	9.21		0.021	
	9.53		0.046	
	9.62		0.041	
R	9.83	9.84	0.090	0.430
	10.05		0.017	
	10.39		0.018	
	10.54		0.053	
Y	10.78	10.80	0.098	0.413
	10.98		0.033	
	11.06		0.015	
	11.19		0.023	
	11.34		0.033	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.54		0.026	
	11.71		0.024	
	11.87		0.025	
	11.98		0.030	
	12.18		0.027	
	12.42		0.060	
	12.65		0.022	
	12.84		0.016	
	12.89		0.024	
P	13.04	13.06	0.077	0.360
	13.21		0.023	
	13.33		0.028	
	13.49		0.024	
	13.65		0.020	
M	13.76	13.79	0.022	0.087
	13.95		0.022	
V	14.08	14.10	0.082	0.337
	14.29		0.017	
	14.43		0.036	
	14.53		0.025	
	14.60		0.020	
	14.68		0.026	
	14.76		0.036	
dptu	15.26	15.27	12.822	60.403
	15.45		19.183	
W	16.38	16.38	0.023	0.085
	16.65		0.036	
	16.92		0.029	
F	17.02	17.02	0.031	0.137
	17.20		0.019	
I	17.50	17.49	0.103	0.589
K	17.77	17.79	0.161	0.465
L	18.02	18.02	0.169	0.759
	18.27		0.025	
	18.56		0.040	
	18.63		0.052	
	18.79		0.028	
	18.91		0.027	

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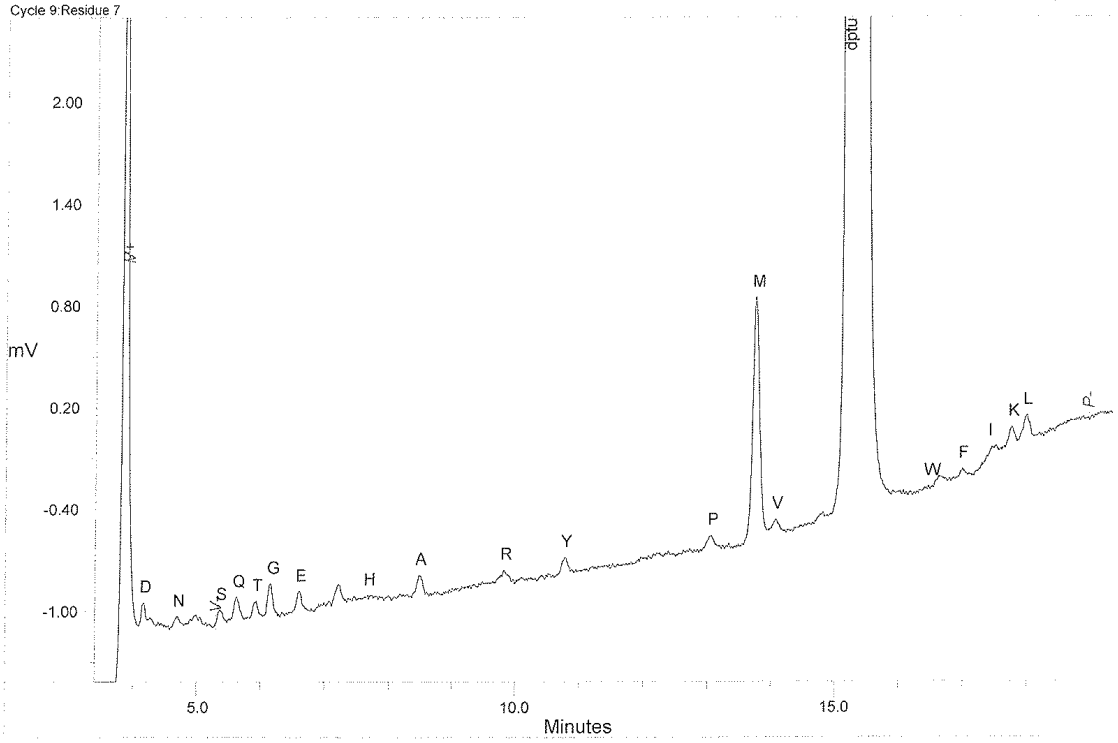
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RI

Cycle 9:Residue 7



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.120	0.368
	4.27		0.029	
N	4.69	4.70	0.066	0.203
	4.99		0.044	
	5.05		0.030	
S	5.36	5.38	0.100	0.470
	5.51		0.042	
Q	5.62	5.64	0.161	0.587
T	5.93	5.93	0.119	0.580
G	6.15	6.16	0.206	0.912
	6.33		0.027	
E	6.60	6.62	0.125	0.414
	6.91		0.026	
	7.06		0.028	
	7.21		0.121	
	7.37		0.034	
	7.45		0.040	
	7.54		0.025	
H	7.66	7.70	0.035	0.157
	7.73		0.031	
	7.80		0.024	
	7.99		0.029	
	8.08		0.019	
	8.18		0.020	
A	8.48	8.51	0.125	0.530
	8.74		0.023	
	8.90		0.016	
	9.01		0.018	
	9.16		0.023	
	9.47		0.018	
	9.65		0.015	
R	9.82	9.84	0.081	0.388
	9.97		0.029	
	10.10		0.031	
	10.25		0.026	
	10.43		0.027	
Y	10.78	10.80	0.095	0.403
	10.95		0.015	
	11.09		0.015	
	11.22		0.026	
	11.47		0.021	
	11.80		0.018	
	11.96		0.024	
	12.21		0.018	
	12.39		0.027	
	12.55		0.013	
P	13.06	13.06	0.093	0.434
	13.24		0.026	
	13.33		0.034	
	13.48		0.020	
M	13.76	13.79	1.443	5.753
V	14.07	14.10	0.097	0.399
	14.28		0.016	
	14.53		0.014	
	14.60		0.014	
	14.82		0.051	
	14.89		0.023	
dptu	15.25	15.27	12.156	57.266
	15.45		21.347	
	15.92		0.021	
	16.09		0.030	
	16.20		0.024	
	16.35		0.017	
W	16.47	16.38	0.023	0.089
	16.64		0.064	
	16.86		0.025	
F	17.00	17.02	0.061	0.268
I	17.46	17.49	0.114	0.654
	17.52		0.105	
K	17.77	17.79	0.165	0.475
L	18.01	18.02	0.183	0.822
	18.20		0.037	
	18.37		0.025	
	18.60		0.021	
	18.92		0.023	

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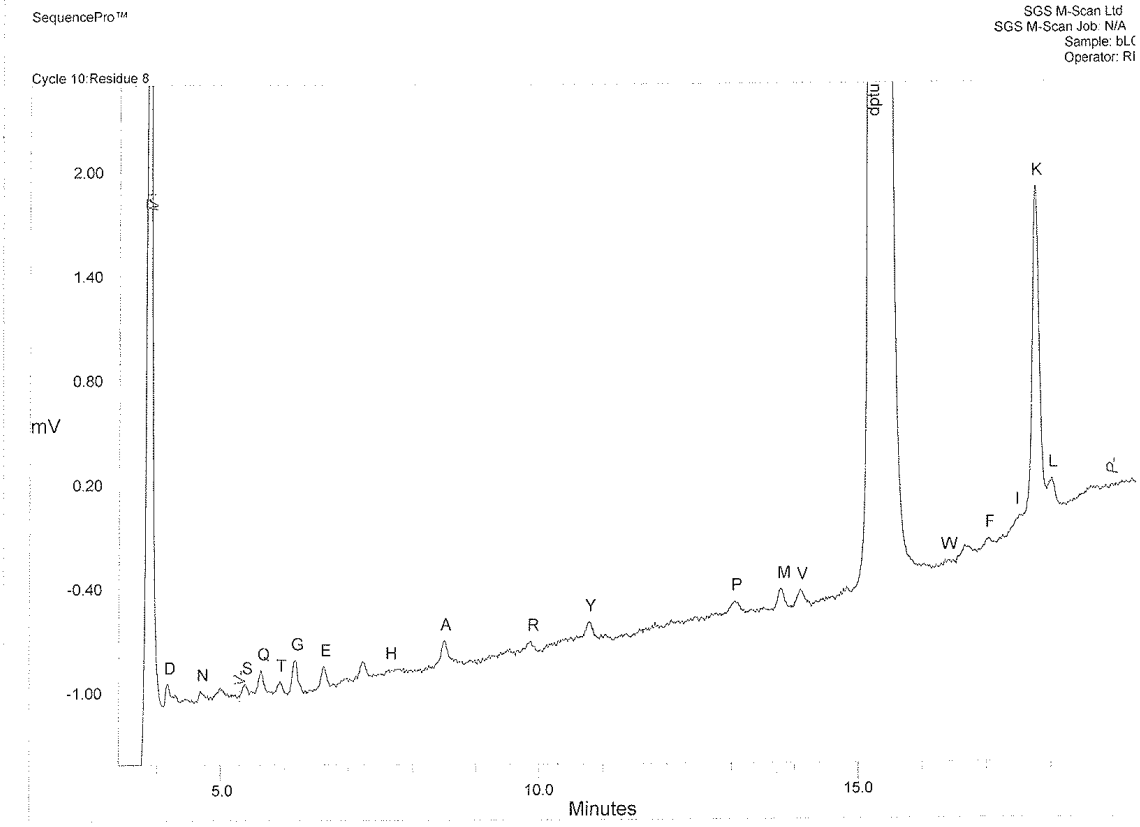
RK 29Sep11



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.105	0.322
	4.44		0.021	
N	4.68	4.70	0.059	0.181
	5.00		0.059	
	5.24		0.023	
S	5.40	5.38	0.073	0.346
	5.49		0.029	
Q	5.63	5.64	0.136	0.498
T	5.93	5.93	0.077	0.379
G	6.15	6.16	0.199	0.879
	6.32		0.021	
	6.38		0.017	
E	6.61	6.62	0.135	0.448
	6.75		0.026	
	6.97		0.033	
	7.11		0.029	
	7.22		0.100	
	7.38		0.016	
H	7.62	7.70	0.037	0.165
	7.75		0.034	
	7.85		0.035	
	8.21		0.018	
A	8.50	8.51	0.147	0.625
	8.67		0.028	
	8.91		0.028	
	8.98		0.026	
	9.11		0.024	
	9.21		0.025	
	9.53		0.031	
	9.66		0.034	
R	9.88	9.84	0.072	0.344
	10.12		0.023	
	10.38		0.012	
	10.50		0.026	
	10.59		0.031	
Y	10.79	10.80	0.107	0.454
	10.98		0.029	
	11.29		0.025	
	11.36		0.027	
	11.66		0.027	
	11.81		0.044	
	12.06		0.028	
	12.17		0.015	
	12.36		0.028	
	12.44		0.035	
	12.53		0.030	
	12.61		0.026	
	12.68		0.037	
	12.76		0.030	
	12.82		0.024	
P	13.06	13.06	0.072	0.335
	13.31		0.025	
M	13.78	13.79	0.123	0.489
V	14.09	14.10	0.089	0.364
	14.33		0.028	
	14.60		0.026	
	14.73		0.040	
	14.82		0.056	
dptu	15.28	15.27	14.300	67.366
	15.46		23.263	
	15.97		0.052	
	16.05		0.050	
	16.23		0.023	
W	16.37	16.38	0.027	0.102
	16.68		0.043	
F	17.05	17.02	0.043	0.186
	17.26		0.027	
I	17.52	17.49	0.088	0.506
K	17.79	17.79	1.924	5.557
L	18.03	18.02	0.172	0.776
	18.51		0.013	
	18.65		0.024	
	18.88		0.024	

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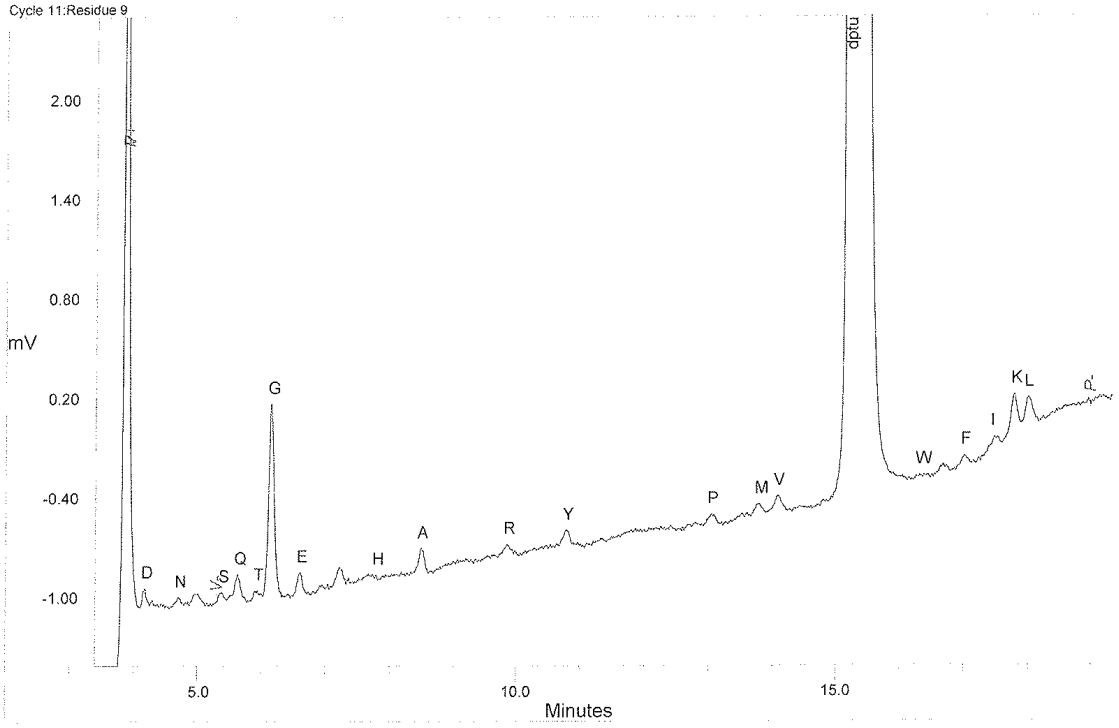
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bL(
Operator: R



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.115	0.352
	4.29		0.035	
N	4.70	4.70	0.058	0.179
	4.97		0.076	
	5.15		0.018	
S	5.39	5.38	0.076	0.359
Q	5.63	5.64	0.176	0.641
T	5.95	5.93	0.021	0.104
G	6.16	6.16	1.164	5.142
	6.42		0.030	
E	6.61	6.62	0.143	0.475
	6.81		0.023	
	6.95		0.044	
	7.04		0.040	
	7.22		0.119	
	7.36		0.036	
	7.51		0.022	
	7.67		0.022	
H	7.78	7.70	0.027	0.121
	7.89		0.017	
	8.18		0.028	
A	8.49	8.51	0.155	0.656
	8.80		0.026	
	8.93		0.025	
	9.01		0.022	
	9.07		0.026	
	9.29		0.025	
	9.55		0.029	
	9.65		0.025	
R	9.86	9.84	0.052	0.249
	10.07		0.017	
	10.15		0.025	
	10.30		0.025	
	10.42		0.022	
	10.48		0.028	
	10.55		0.023	
	10.64		0.021	
Y	10.80	10.80	0.097	0.410
	11.11		0.027	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.33		0.035	
	11.50		0.024	
	11.62		0.017	
	11.80		0.027	
	11.87		0.026	
	12.01		0.016	
	12.07		0.023	
	12.19		0.017	
	12.42		0.030	
	12.55		0.018	
	12.61		0.023	
	12.72		0.037	
	12.80		0.043	
P	13.05	13.06	0.081	0.375
	13.53		0.053	
	13.61		0.053	
M	13.79	13.79	0.095	0.379
V	14.09	14.10	0.121	0.498
	14.28		0.030	
	14.44		0.033	
	14.61		0.023	
	14.81		0.026	
dptu	15.28	15.27	13.877	65.373
	15.47		25.519	
W	16.33	16.38	0.025	0.094
	16.40		0.016	
	16.69		0.033	
F	17.03	17.02	0.072	0.312
	17.24		0.023	
I	17.49	17.49	0.036	0.205
K	17.80	17.79	0.263	0.761
L	18.02	18.02	0.205	0.922
	18.24		0.048	
	18.40		0.048	
	18.61		0.049	
	18.71		0.034	
	18.80		0.030	
	18.88		0.017	

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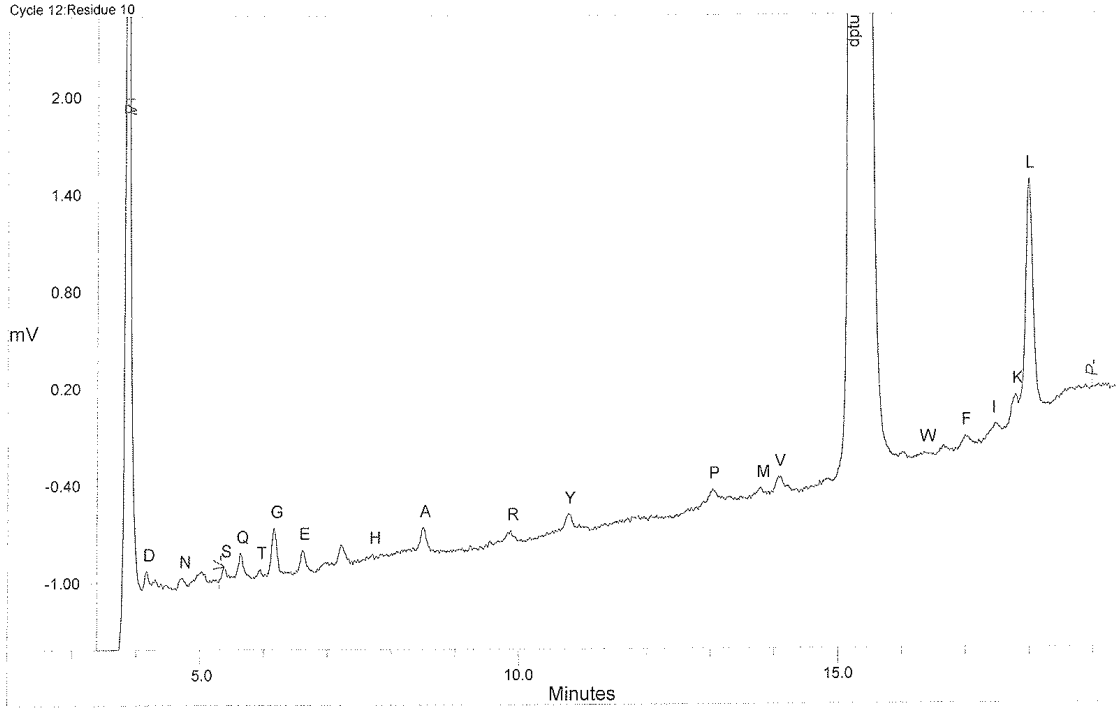
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RI

Cycle 12:Residue 10



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.17	4.19	0.102	0.313
	4.30		0.042	
	4.38		0.025	
	4.47		0.024	
N	4.73	4.70	0.060	0.187
	5.03		0.028	
	5.21		0.018	
S	5.38	5.38	0.068	0.322
Q	5.63	5.64	0.154	0.562
T	5.95	5.93	0.052	0.256
	6.03		0.015	
G	6.16	6.16	0.285	1.258
E	6.61	6.62	0.145	0.481
	6.98		0.015	
	7.07		0.019	
	7.22		0.122	
	7.62		0.029	
H	7.71	7.70	0.037	0.165
	7.86		0.019	
	8.00		0.021	
	8.08		0.029	
	8.20		0.031	
	8.30		0.033	
	8.34		0.038	
A	8.50	8.51	0.152	0.647
	8.73		0.018	
	9.23		0.033	
	9.41		0.023	
	9.48		0.024	
	9.68		0.022	
R	9.88	9.84	0.073	0.351
	10.18		0.021	
	10.25		0.013	
	10.45		0.026	
Y	10.79	10.80	0.110	0.464
	10.95		0.029	
	11.20		0.019	
	11.35		0.026	
	11.53		0.013	
	11.71		0.029	
	11.78		0.034	
	11.88		0.040	
	11.97		0.031	
	12.08		0.030	
	12.38		0.019	
	12.48		0.018	
	12.66		0.031	
	12.80		0.023	
	12.90		0.039	
P	13.04	13.06	0.090	0.417
	13.31		0.026	
	13.49		0.023	
	13.62		0.022	
M	13.79	13.79	0.046	0.184
	13.96		0.016	
V	14.07	14.10	0.086	0.355
	14.35		0.023	
	14.43		0.018	
	14.57		0.021	
	14.71		0.034	
	14.85		0.028	
dptu	15.27	15.27	11.250	53.000
	15.46		23.219	
	16.03		0.035	
	16.32		0.017	
W	16.36	16.38	0.022	0.083
	16.67		0.046	
	16.86		0.020	
F	17.01	17.02	0.059	0.258
	17.22		0.027	
I	17.48	17.49	0.071	0.407
K	17.80	17.79	0.172	0.498
L	18.02	18.02	1.447	6.504
	18.32		0.017	
	18.45		0.038	
	18.58		0.061	
	18.66		0.056	
	18.79		0.047	
	18.90		0.039	

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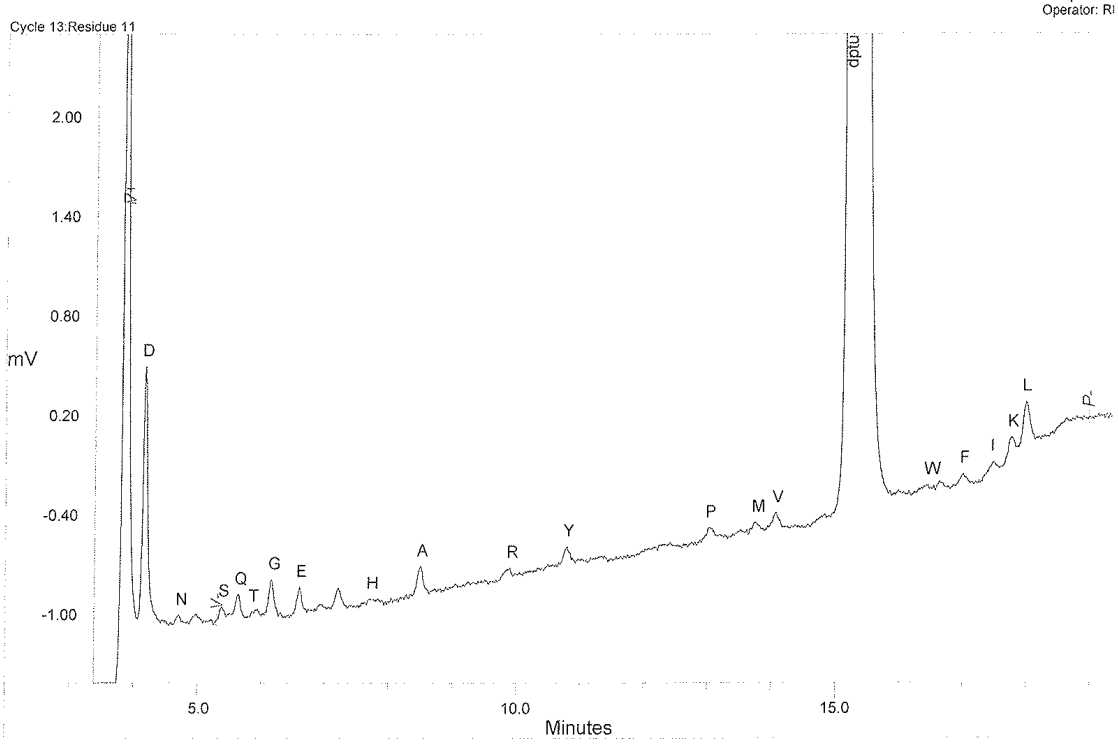
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: RI



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	1.529	4.700
N	4.71	4.70	0.050	0.155
	4.97		0.044	
S	5.39	5.38	0.102	0.482
Q	5.63	5.64	0.153	0.559
T	5.86	5.93	0.020	0.097
G	6.16	6.16	0.226	1.000
	6.31		0.028	
	6.43		0.019	
E	6.60	6.62	0.169	0.561
	6.96		0.042	
	7.22		0.131	
	7.44		0.014	
	7.52		0.013	
	7.64		0.026	
H	7.72	7.70	0.036	0.159
	7.83		0.023	
	8.05		0.035	
	8.14		0.022	
	8.28		0.028	
A	8.50	8.51	0.181	0.768
	8.74		0.032	
	8.81		0.024	
	8.90		0.032	
	9.05		0.038	
	9.25		0.034	
	9.34		0.026	
	9.40		0.032	
	9.50		0.030	
	9.60		0.015	
R	9.90	9.84	0.065	0.311
	9.98		0.025	
	10.05		0.023	
	10.36		0.018	
	10.50		0.034	
	10.63		0.016	
Y	10.80	10.80	0.095	0.400
	10.98		0.016	
	11.17		0.028	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.33		0.020	
	11.57		0.019	
	11.75		0.024	
	11.85		0.013	
	12.03		0.036	
	12.16		0.034	
	12.35		0.036	
	12.43		0.035	
	12.64		0.029	
	12.76		0.029	
P	13.04	13.06	0.094	0.435
	13.20		0.039	
	13.28		0.030	
	13.54		0.040	
	13.63		0.034	
M	13.77	13.79	0.069	0.273
V	14.09	14.10	0.100	0.410
	14.38		0.022	
	14.59		0.019	
	14.85		0.042	
dptu	15.27	15.27	10.195	48.028
	15.45		22.060	
	15.93		0.015	
	16.00		0.027	
	16.16		0.025	
	16.25		0.019	
W	16.46	16.38	0.031	0.116
	16.55		0.030	
	16.64		0.057	
F	17.02	17.02	0.079	0.345
	17.15		0.023	
I	17.51	17.49	0.067	0.381
K	17.78	17.79	0.141	0.409
L	18.01	18.02	0.293	1.318
	18.22		0.031	
	18.65		0.033	
	18.81		0.023	
	18.91		0.023	

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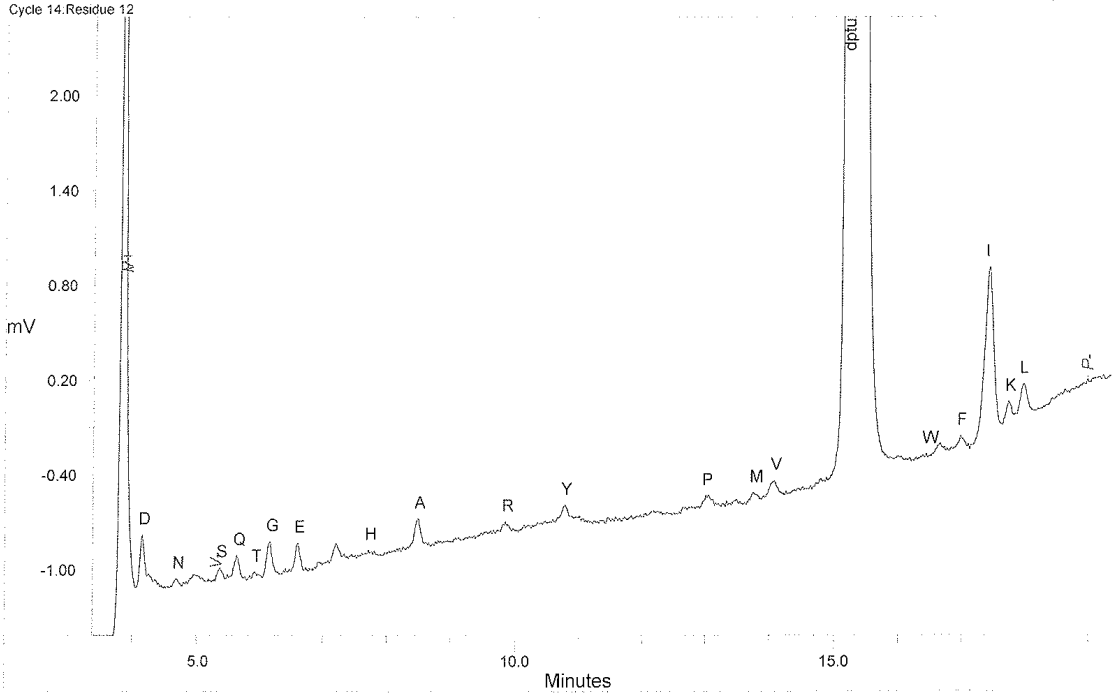
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bLG
Operator: R

Cycle 14:Residue 12



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.15	4.19	0.298	0.917
	4.35		0.022	
N	4.68	4.70	0.054	0.169
	4.97		0.023	
	5.19		0.018	
S	5.37	5.38	0.087	0.408
	5.48		0.035	
Q	5.62	5.64	0.150	0.548
	5.83		0.026	
T	5.92	5.93	0.044	0.217
G	6.15	6.16	0.221	0.978
	6.41		0.038	
E	6.60	6.62	0.189	0.628
	6.72		0.026	
	6.95		0.019	
	7.08		0.023	
	7.20		0.117	
	7.33		0.045	
	7.45		0.031	
H	7.72	7.70	0.035	0.159
	8.04		0.021	
	8.20		0.023	
	8.25		0.029	
A	8.49	8.51	0.183	0.778
	8.80		0.027	
	9.07		0.018	
	9.29		0.019	
	9.47		0.030	
	9.54		0.028	
	9.66		0.026	
	9.72		0.018	
R	9.85	9.84	0.039	0.189
	10.14		0.035	
	10.20		0.025	
	10.35		0.015	
	10.47		0.020	
	10.59		0.023	
Y	10.78	10.80	0.112	0.473
	11.01		0.034	
	11.33		0.024	
	11.45		0.036	
	11.51		0.027	
	11.57		0.036	
	11.71		0.030	
	11.82		0.029	
	11.92		0.015	
	12.05		0.021	
	12.18		0.020	
	12.51		0.013	
	12.68		0.033	
	12.78		0.015	
P	13.01	13.06	0.029	0.134
	13.20		0.026	
	13.28		0.023	
	13.48		0.033	
M	13.75	13.79	0.067	0.268
	13.92		0.029	
V	14.08	14.10	0.115	0.471
	14.39		0.024	
	14.51		0.030	
	14.65		0.026	
	14.73		0.015	
	14.79		0.030	
	14.88		0.020	
dptu	15.25	15.27	10.851	51.120
	15.44		20.670	
	16.00		0.027	
	16.17		0.025	
	16.28		0.020	
W	16.45	16.38	0.027	0.102
	16.66		0.045	
	16.85		0.023	
F	16.99	17.02	0.093	0.404
I	17.47	17.49	1.076	6.157
K	17.76	17.79	0.159	0.461
L	18.00	18.02	0.216	0.969
	18.44		0.021	
	18.64		0.031	
	18.75		0.022	
	18.82		0.022	
	18.94		0.017	

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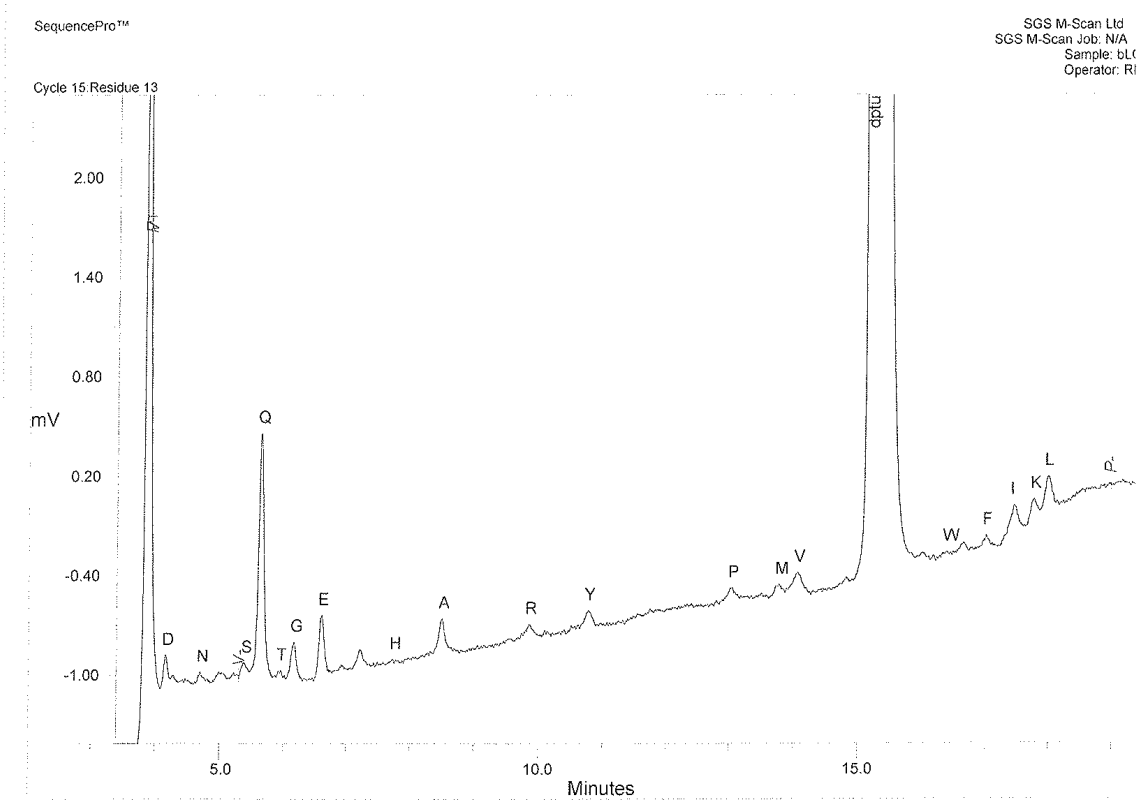

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.175	0.536
	4.28		0.033	
	4.43		0.022	
N	4.70	4.70	0.065	0.202
	4.99		0.057	
	5.22		0.041	
S	5.39	5.38	0.104	0.489
Q	5.63	5.64	1.492	5.447
T	5.95	5.93	0.062	0.302
G	6.16	6.16	0.234	1.034
E	6.60	6.62	0.371	1.229
	6.92		0.042	
	7.06		0.013	
	7.22		0.109	
	7.43		0.027	
	7.49		0.028	
	7.58		0.021	
H	7.74	7.70	0.029	0.129
	7.98		0.024	
	8.21		0.019	
A	8.51	8.51	0.191	0.812
	8.81		0.019	
	9.06		0.027	
	9.16		0.025	
	9.29		0.021	
	9.55		0.041	
	9.75		0.059	
R	9.85	9.84	0.103	0.490
	10.10		0.045	
	10.23		0.026	
	10.40		0.030	
	10.52		0.025	
	10.62		0.023	
Y	10.78	10.80	0.101	0.426
	10.98		0.017	
	11.07		0.024	
	11.17		0.015	
	11.25		0.016	
	11.49		0.033	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.55		0.035	
	11.75		0.038	
	11.90		0.017	
	12.07		0.015	
	12.21		0.020	
	12.34		0.029	
	12.41		0.031	
	12.56		0.020	
	12.67		0.018	
	12.81		0.029	
P	13.05	13.06	0.061	0.283
	13.19		0.018	
	13.36		0.022	
	13.52		0.024	
M	13.79	13.79	0.071	0.284
V	14.09	14.10	0.113	0.462
	14.32		0.022	
	14.45		0.023	
	14.57		0.020	
	14.83		0.041	
dptu	15.27	15.27	10.657	50.204
	15.45		19.900	
	16.00		0.032	
	16.19		0.033	
W	16.40	16.38	0.023	0.086
	16.50		0.024	
	16.67		0.062	
	16.80		0.018	
F	17.03	17.02	0.070	0.305
	17.21		-0.011	
I	17.48	17.49	0.182	1.040
K	17.79	17.79	0.127	0.366
L	18.02	18.02	0.199	0.894
	18.25		0.021	
	18.55		0.048	
	18.62		0.048	
	18.75		0.034	
	18.90		0.029	

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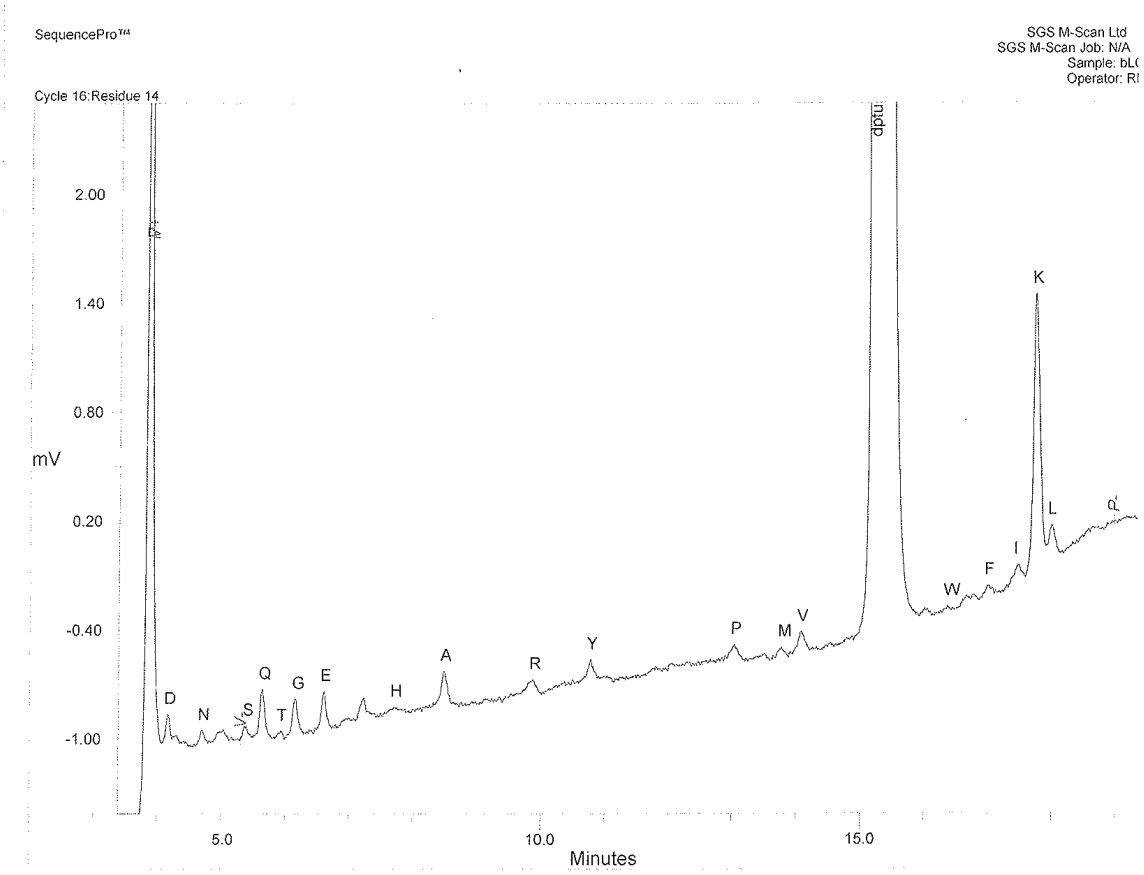

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.16	4.19	0.150	0.461		11.82		0.044	
N	4.69	4.70	0.082	0.253		11.97		0.024	
	5.04		0.066			12.07		0.034	
	5.15		0.018			12.31		0.012	
	5.25		0.011			12.48		0.025	
S	5.39	5.38	0.083	0.390		12.60		0.020	
Q	5.64	5.64	0.281	1.028		12.67		0.021	
T	5.92	5.93	0.047	0.232		12.86		0.030	
G	6.16	6.16	0.209	0.925	P	13.05	13.06	0.096	0.447
E	6.61	6.62	0.215	0.714		13.30		0.020	
	7.00		0.026			13.41		0.029	
	7.24		0.114			13.52		0.045	
	7.50		0.022		M	13.78	13.79	0.066	0.262
H	7.71	7.70	0.030	0.133		13.93		0.028	
	8.01		0.019		V	14.09	14.10	0.120	0.492
	8.09		0.012			14.27		0.017	
	8.27		0.027			14.55		0.030	
A	8.50	8.51	0.193	0.820		14.80		0.028	
	8.76		0.023			14.88		0.030	
	8.82		0.015		dptu	15.27	15.27	10.629	50.074
	8.94		0.017			15.46		23.561	
	9.17		0.022			16.03		0.023	
	9.29		0.018		W	16.38	16.38	0.031	0.117
	9.36		0.032			16.68		0.059	
	9.45		0.033			16.78		0.050	
	9.53		0.043		F	17.02	17.02	0.070	0.308
	9.62		0.040			17.09		0.051	
R	9.88	9.84	0.095	0.454		17.13		0.032	
	10.14		0.023			17.30		0.029	
	10.38		0.032		I	17.48	17.49	0.097	0.557
	10.50		0.027		K	17.78	17.79	1.517	4.384
Y	10.79	10.80	0.116	0.490	L	18.01	18.02	0.179	0.806
	11.00		0.008			18.34		0.026	
	11.25		0.018			18.50		0.018	
	11.52		0.018			18.68		0.017	

Thursday, September 29, 2011 11:26:21

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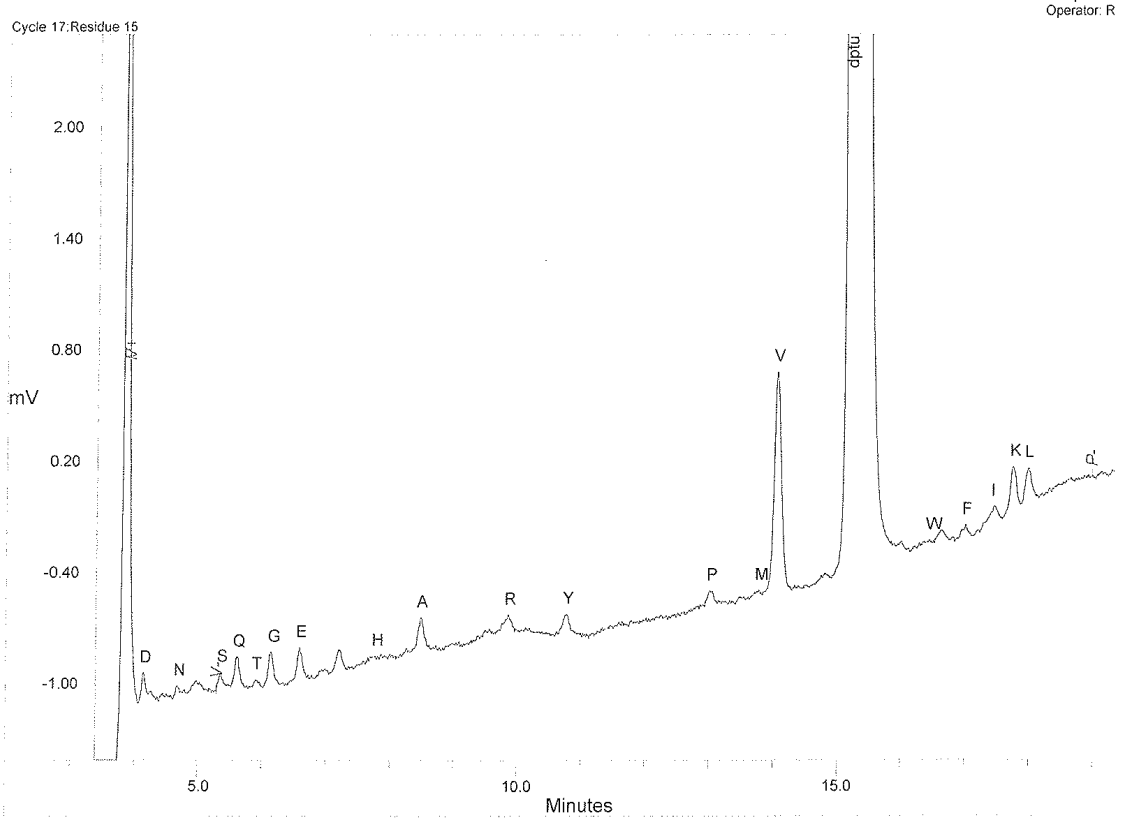
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: N/A
Sample: bL
Operator: R



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.14	4.19	0.142	0.436
	4.28		0.031	
	4.45		0.033	
N	4.68	4.70	0.053	0.165
	4.80		0.020	
	4.98		0.063	
S	5.36	5.38	0.105	0.494
Q	5.60	5.64	0.175	0.638
T	5.90	5.93	0.048	0.233
G	6.15	6.16	0.189	0.836
	6.32		0.016	
E	6.59	6.62	0.178	0.588
	6.94		0.018	
	7.20		0.121	
	7.59		0.022	
H	7.78	7.70	0.022	0.100
	7.86		0.030	
	8.00		0.028	
	8.26		0.049	
A	8.48	8.51	0.202	0.858
	8.72		0.033	
	8.98		0.016	
	9.09		0.025	
	9.28		0.019	
	9.39		0.024	
	9.50		0.025	
R	9.86	9.84	0.100	0.475
	10.14		0.019	
	10.63		0.025	
Y	10.78	10.80	0.117	0.494
	11.19		0.023	
	11.27		0.024	
	11.35		0.030	
	11.47		0.025	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.60		0.025	
	11.80		0.022	
	12.20		0.016	
	12.26		0.020	
	12.47		0.020	
	12.62		0.020	
	12.91		0.026	
P	13.03	13.06	0.091	0.422
	13.15		0.025	
	13.49		0.023	
	13.61		0.014	
M	13.78	13.79	0.041	0.163
V	14.07	14.10	1.199	4.917
	14.34		0.024	
	14.51		0.018	
	14.81		0.044	
dptu	15.25	15.27	9.789	46.116
	15.44		25.023	
	16.02		0.041	
	16.14		0.019	
	16.23		0.021	
	16.30		0.027	
	16.38		0.030	
W	16.47	16.38	0.035	0.133
	16.66		0.074	
	16.84		0.028	
F	17.03	17.02	0.076	0.334
I	17.48	17.49	0.103	0.592
K	17.77	17.79	0.261	0.754
L	18.00	18.02	0.208	0.935
	18.22		0.033	
	18.33		0.036	
	18.67		0.030	
	18.94		0.018	

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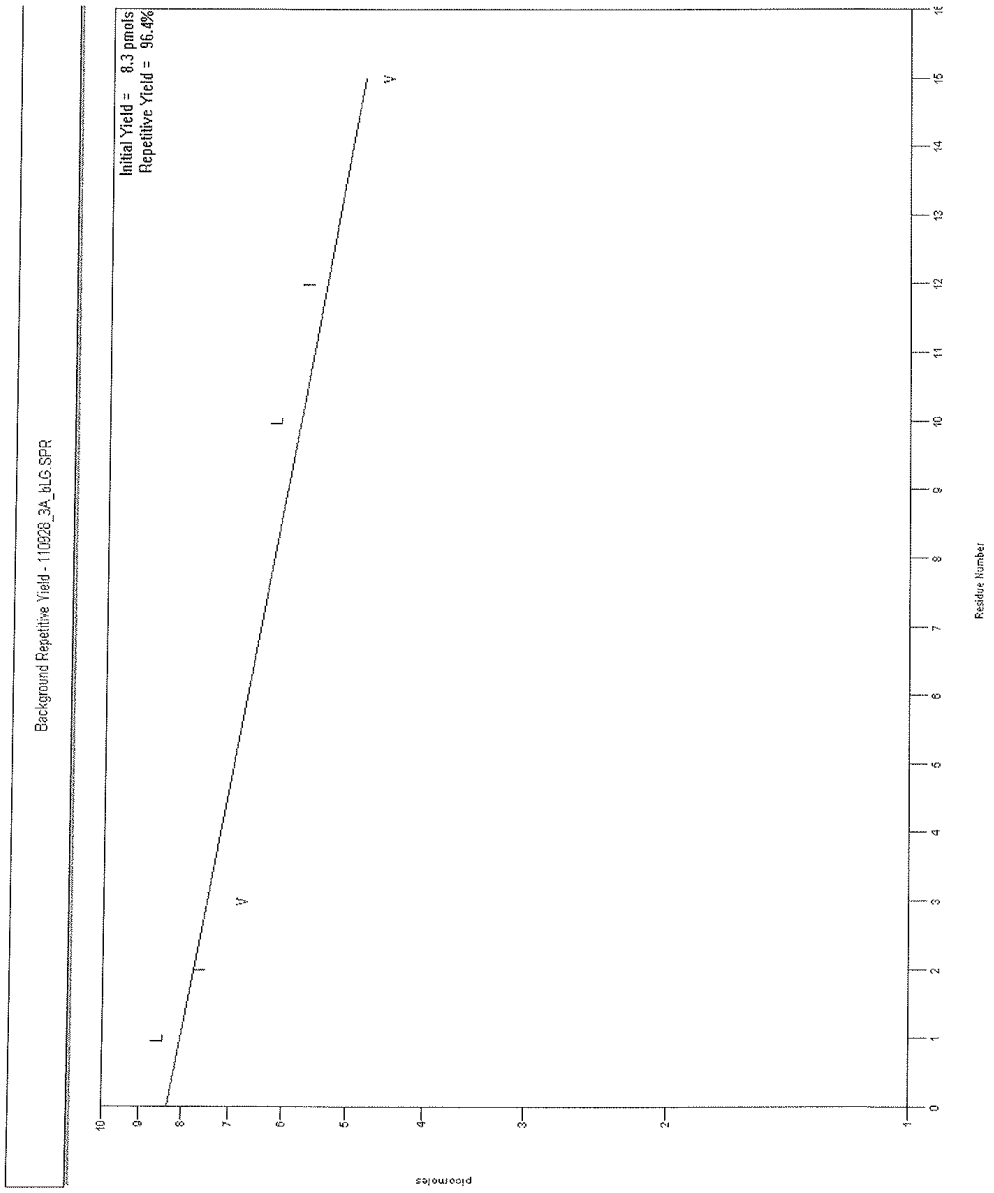
Background Repetitive Yield - 110928_3A_bLG.SPR
Initial Yield = 8.3 pmols
Repetitive Yield = 96.4%
picomoles
Residue Number
L
I
V
L
I
V

Appendix III – Raw Data for 12 Residues of N-terminal Sequencing of SYHT0H2 AVHPPD-03 sample (SGS M-Scan No. 97246)

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97246
Operator: MIM

SAMPLE INFORMATION

Sample Name:	111009_1A_97246	Std Amount:	10.000 pmols
ID Code:		Sample Amount:	0.000 pmols
		Detector Scale:	0.005 AUFS

Comments:

SEQUENCER INFORMATION

Name:	PROCISE	Model Number:	492
Method:	Job22745_sample97246	Cartridge:	A
Operator:	MIM		

CHEMICAL INFORMATION

R1	1105171	03 October, 2011	X3	0	01 September, 2001
R2	1105087	12 September, 2011	PTH Column	G110606031	27 September, 2011
R3	1012198	30 September, 2011	Solvent A	1106619	27 September, 2011
R4	1009128	05 October, 2011	Solvent B	1105351	30 September, 2011
R5	1011047	09 October, 2011	Premix	1104103	27 September, 2011
S1	0	01 September, 2001	Guard Column	0	01 January, 2002
S2	1010693	05 October, 2011	Cartridge Seals	0	01 January, 2002
S3	1106495	05 October, 2011	Glass Fiber Filter	0	01 January, 2002
S4	1105166	05 October, 2011	pth standards	1007028	01 January, 2002
X1	0	01 September, 2001		12345678	01 January, 2002
X2	0	01 September, 2001	Total Cycles Count		01 January, 2002

Monday, October 10, 2011 11:29:58

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MM 10.10.11

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97246
Operator: MIM

ORIGINAL METHOD TEMPLATE: D:\Program Files\AppliedBiosystems\Procise\SequencePro\Methods\Procise.met

CALIBRATION TABLE

COMPONENT	RTIME	RESPONSE	REFERENCE	INTERNAL STD	ABS WND	REL WND
Aspartic Acid	4.07	1.000	---	---	0.20	0.00
Asparagine	4.57	1.000	---	---	0.20	0.00
Serine	5.24	1.000	---	---	0.20	0.00
Glutamine	5.49	1.000	---	---	0.20	0.00
Threonine	5.78	1.000	---	---	0.20	0.00
Glycine	6.00	1.000	---	---	0.20	0.00
Glutamic Acid	6.43	1.000	---	---	0.20	0.00
Histidine	7.65	1.000	---	---	0.20	0.00
Alanine	8.31	1.000	---	---	0.20	0.00
Arginine	9.75	1.000	---	---	0.20	0.00
Tyrosine	10.56	1.000	---	---	0.20	0.00
Proline	12.83	1.000	---	---	0.20	0.00
Methionine	13.54	1.000	---	---	0.20	0.00
Valine	13.86	1.000	---	---	0.20	0.00
dptu	15.04	1.000	---	---	0.20	0.00
Tryptophan	16.13	1.000	---	---	0.20	0.00
Phenylalanine	16.79	1.000	---	---	0.20	0.00
Isoleucine	17.25	1.000	---	---	0.20	0.00
Lysine	17.55	1.000	---	---	0.20	0.00
Leucine	17.78	1.000	---	---	0.20	0.00

GLOBAL INTEGRATION EVENTS

EVENT	TIME	VALUE	EVENT	TIME	VALUE
Peak Detect Off	0.00	---	Valley to Valley Off	5.30	---
Peak Detect On	3.90	---	Peak Detect Off	19.00	---
Valley to Valley On	3.90	---			

INTEGRATION PARAMETERS

PEAK DETECTION PARAMETERS

Bunching Factor	4	Noise Threshold:	0.954 µVolts
Max Peaks	128	Area Threshold:	41.000 µVolts

PEAK SEPARATION CRITERIA		EXPONENTIAL SKIM CRITERIA	
Width Ratio:	0.20	Peak Height Ratio:	5.00
Valley to Peak Ratio:	0.01	Adjusted Height Ratio:	4.00
Tangent Width:	1000.00	Valley Height Ratio:	3.00

SEQUENCE CALLING PARAMETERS

Use Pmol Heights, Allow Negative Background Off, Refine Data On

SEQUENCE SCORING VALUES

Raw Slope 1:	2.00	Raw Yield	1.00
Raw Slope 2:	1.00	Bkgd Yield	1.00
Bkgd Slope 1:	2.00	Lag Yield	1.00
Bkgd Slope 2:	1.00	Rep Yield	1.00
Max Slope:	1.50	Low Yield	1.00
Rule Book:	0.60	Bkgd Sensitivity	1.00
Dev Mult:	3.00		

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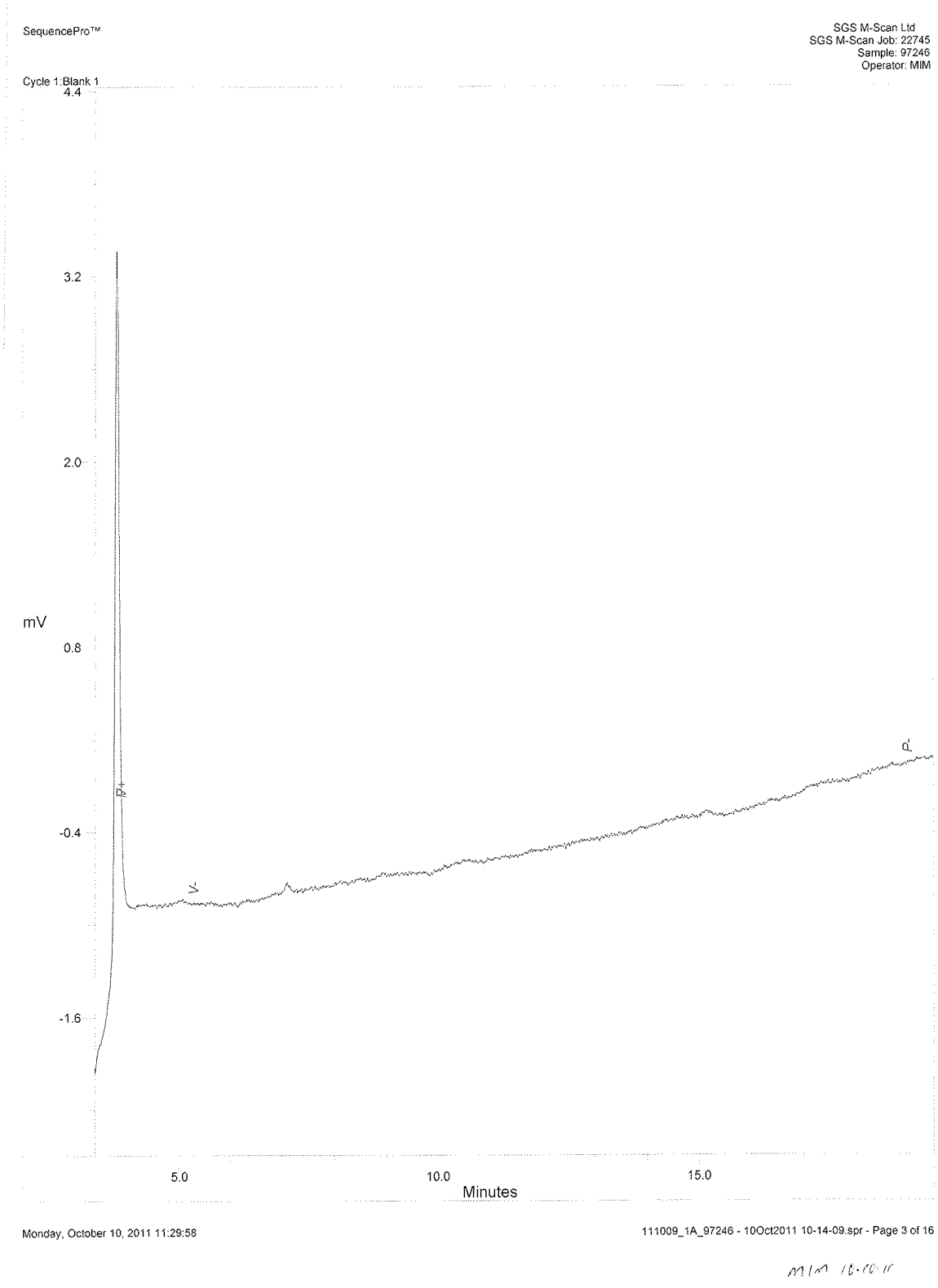
MIM 10.10.11


SequencePro™
SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97246
Operator: MIM
Cycle 1:Blank 1
4.4
3.2
2.0
mV
0.8
-0.4
-1.6
5.0
10.0
15.0
Minutes
Monday, October 10, 2011 11:29:58
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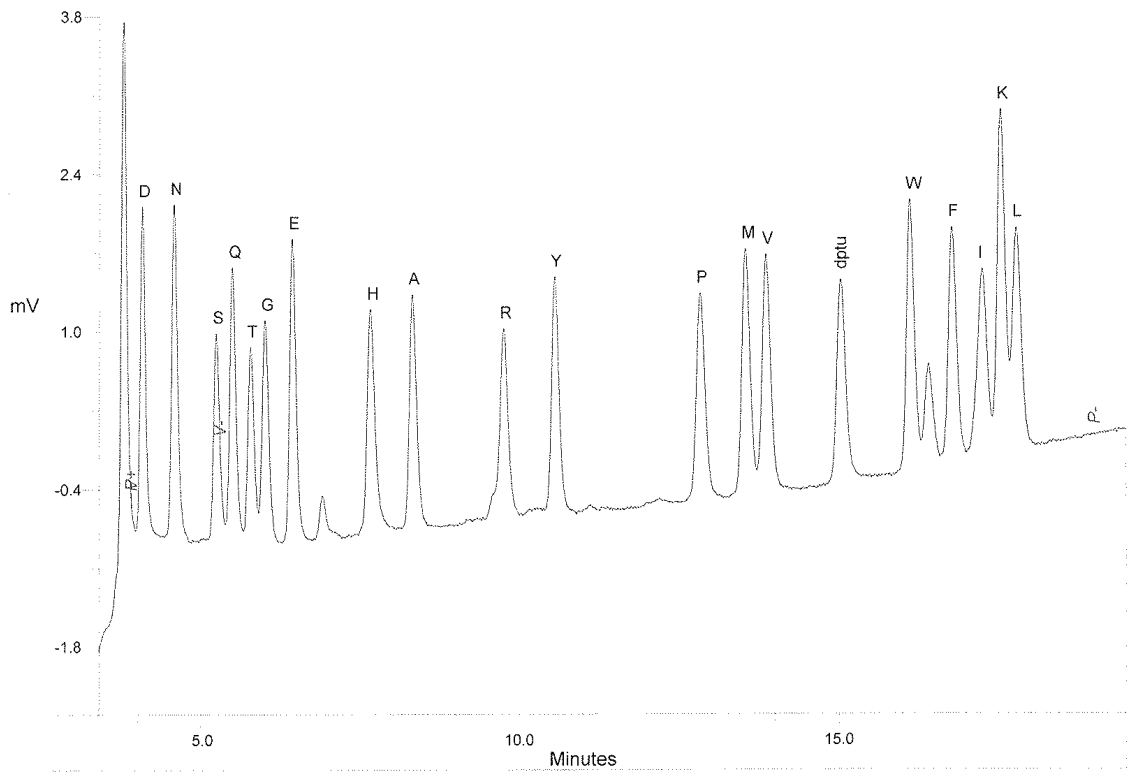
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97246
Operator: MIM

Cycle 2:Standard 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.07	4.07	2.895	10.000		12.00		0.030	
D	4.57	4.57	2.977	10.000		12.20		0.036	
N	4.87		0.025			12.46		0.019	
	5.05		0.034		P	12.83	12.83	1.832	10.000
	5.24	5.24	1.856	10.000	M	13.54	13.54	2.191	10.000
S	5.49	5.49	2.441	10.000	V	13.86	13.86	2.108	10.000
Q	5.78	5.78	1.734	10.000		14.28		0.014	
T	6.00	6.00	1.977	10.000		14.45		0.020	
G	6.43	6.43	2.697	10.000		14.55		0.022	
E	6.90		0.362			14.77		0.026	
	7.32		0.028		dptu	15.04	15.04	1.815	10.000
	7.65	7.65	2.002	10.000		15.39		0.036	
H	8.00		0.040			15.50		0.036	
	8.31	8.31	2.075	10.000		15.58		0.027	
A	8.83		0.010			15.68		0.017	
	9.05		0.024		W	16.13	16.13	2.421	10.000
	9.22		0.043			16.42		0.925	
	9.34		0.038		F	16.79	16.79	2.097	10.000
	9.75	9.75	1.683	10.000	I	17.25	17.25	1.671	10.000
R	10.17		0.031		K	17.55	17.55	3.054	10.000
	10.23		0.018		L	17.78	17.78	1.977	10.000
	10.56	10.56	2.076	10.000		18.30		0.033	
Y	10.94		0.028			18.35		0.034	
	11.12		0.064			18.60		0.027	
	11.29		0.029			18.69		0.028	
	11.62		0.019			18.76		0.014	
	11.70		0.020			18.90		0.019	
	11.76		0.023						

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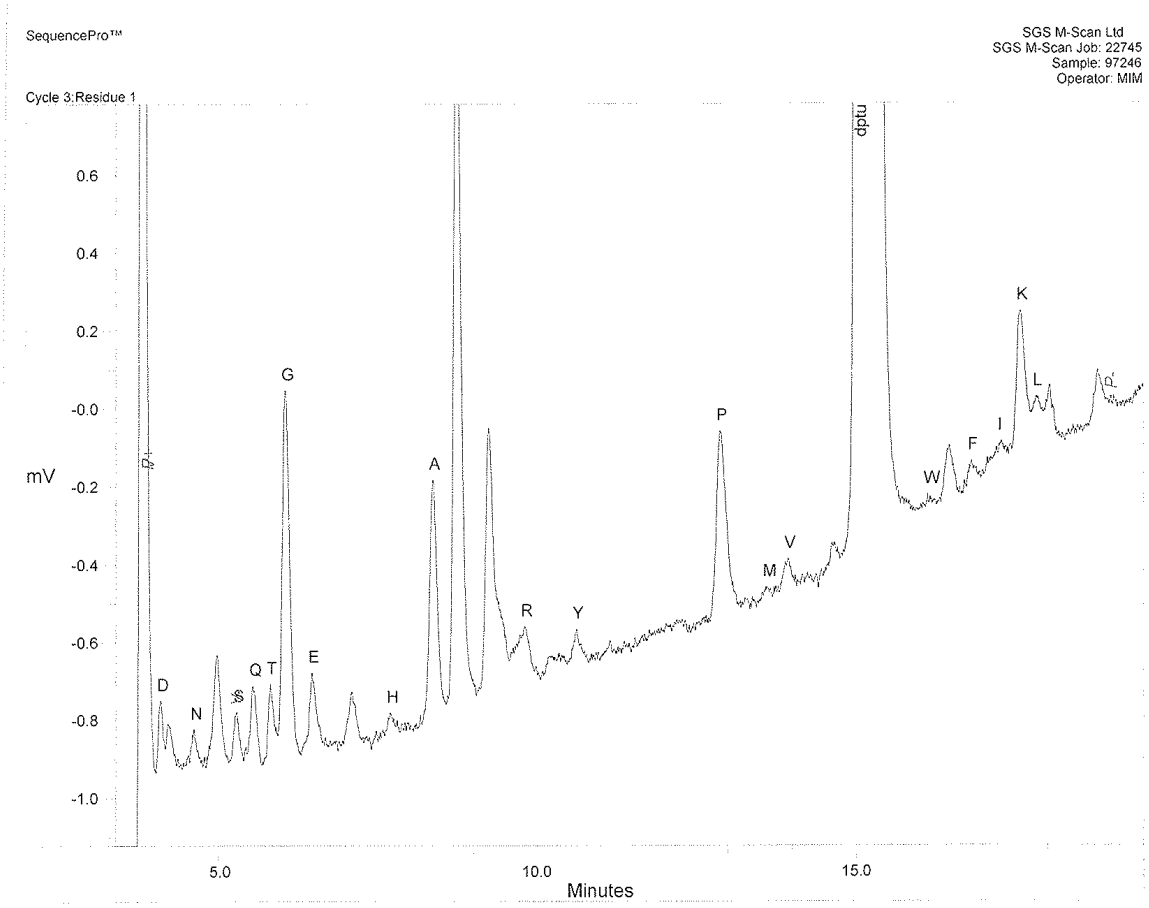

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.07	0.156	0.539		11.67		0.021	
	4.21		0.076			11.78		0.021	
	4.36		0.021			12.04		0.023	
	4.54		0.021			12.20		0.016	
N	4.62	4.57	0.089	0.299		12.33		0.022	
	4.79		0.027			12.47		0.021	
	4.99		0.289			12.56		0.017	
S	5.30	5.24	0.141	0.759		12.66		0.022	
Q	5.55	5.49	0.206	0.842	P	12.88	12.83	0.476	2.599
T	5.83	5.78	0.203	1.170		13.27		0.028	
G	6.05	6.00	0.948	4.795		13.38		0.030	
E	6.47	6.43	0.200	0.743	M	13.61	13.54	0.038	0.172
	6.77		0.015		V	13.94	13.86	0.080	0.377
	7.10		0.137			14.16		0.025	
	7.48		0.035			14.24		0.020	
H	7.70	7.65	0.036	0.181		14.38		0.028	
	7.88		0.020			14.46		0.039	
	7.98		0.020			14.66		0.066	
	8.11		0.017		dptu	15.09	15.04	11.098	61.131
	8.17		0.034			15.27		21.596	
A	8.37	8.31	0.623	3.001		15.73		0.018	
	8.74		1.936			16.06		0.013	
	9.24		0.686		W	16.13	16.13	0.030	0.126
R	9.81	9.75	0.129	0.765		16.48		0.137	
	10.10		0.020		F	16.83	16.79	0.035	0.168
	10.19		0.049			17.01		0.017	
	10.35		0.042		I	17.30	17.25	0.067	0.401
Y	10.62	10.56	0.055	0.264	K	17.59	17.55	0.375	1.228
	10.86		0.021		L	17.83	17.78	0.134	0.677
	11.16		0.044			18.03		0.144	
	11.23		0.021			18.40		0.026	
	11.38		0.032			18.47		0.021	
	11.55		0.030			18.78		0.121	

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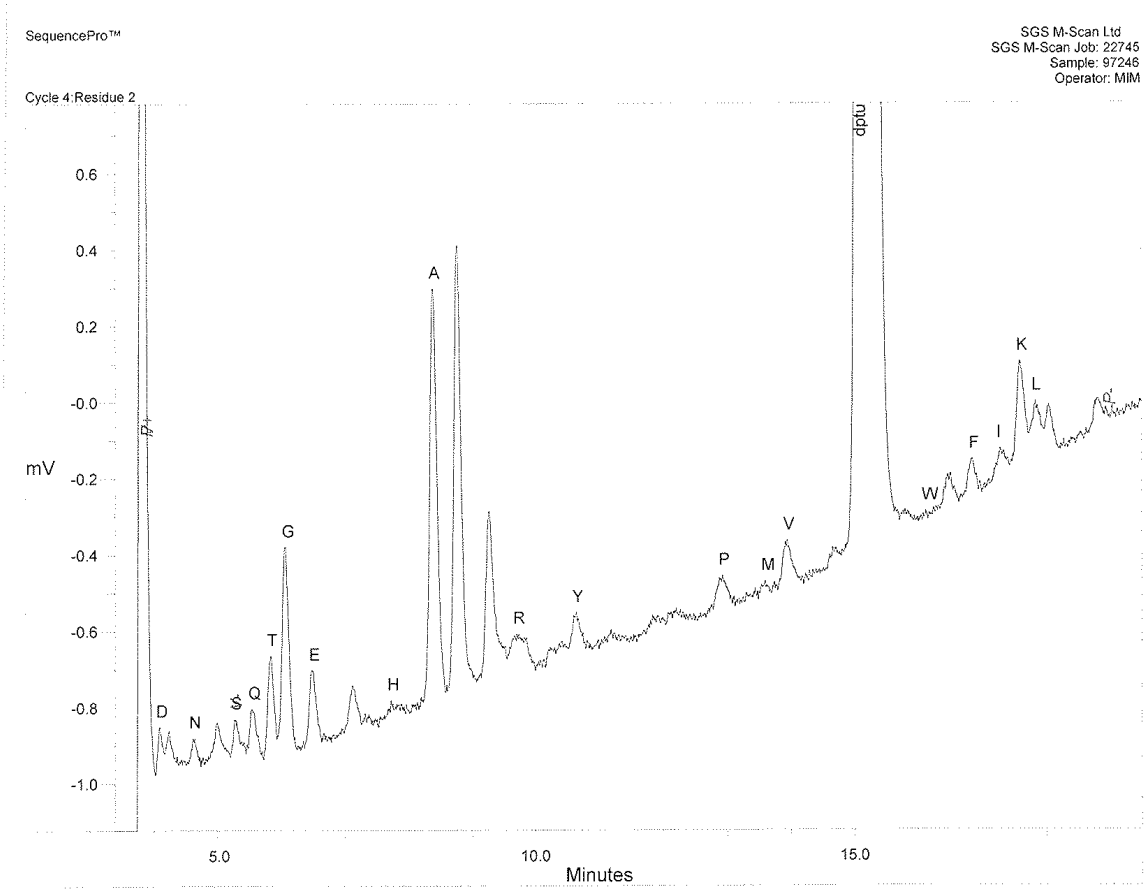

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.07	0.100	0.347
	4.24		0.069	
N	4.61	4.57	0.073	0.245
	4.78		0.020	
	5.00		0.107	
S	5.28	5.24	0.112	0.605
Q	5.55	5.49	0.136	0.556
T	5.84	5.78	0.267	1.540
G	6.07	6.00	0.541	2.737
	6.35		0.022	
E	6.50	6.43	0.198	0.735
	6.67		0.024	
	6.82		0.018	
	7.12		0.116	
	7.32		0.026	
	7.47		0.019	
	7.55		0.026	
H	7.72	7.65	0.050	0.248
	7.88		0.022	
	7.96		0.023	
	8.10		0.018	
	8.13		0.021	
A	8.38	8.31	1.086	5.233
	8.76		1.170	
	9.27		0.403	
R	9.71	9.75	0.041	0.241
	10.07		0.021	
	10.30		0.027	
	10.42		0.020	
Y	10.64	10.56	0.096	0.464
	10.81		0.024	
	10.99		0.025	
	11.03		0.023	
	11.19		0.028	
	11.70		0.022	
	11.84		0.053	
	11.92		0.044	
	12.00		0.031	
	12.13		0.038	
	12.20		0.041	
	12.27		0.032	
P	12.93	12.83	0.079	0.430
	13.11		0.024	
	13.18		0.015	
	13.30		0.018	
	13.44		0.026	
M	13.60	13.54	0.041	0.187
	13.74		0.028	
V	13.94	13.86	0.123	0.582
	14.23		0.025	
	14.39		0.013	
	14.65		0.023	
dptu	15.10	15.04	6.589	36.294
	15.28		10.584	
W	16.13	16.13	0.024	0.101
	16.32		0.014	
	16.52		0.056	
F	16.86	16.79	0.092	0.437
	17.05		0.016	
	17.12		0.016	
I	17.29	17.25	0.030	0.182
K	17.60	17.55	0.268	0.877
L	17.84	17.78	0.150	0.759
	18.04		0.131	
	18.27		0.027	
	18.53		0.025	
	18.63		0.021	
	18.80		0.067	

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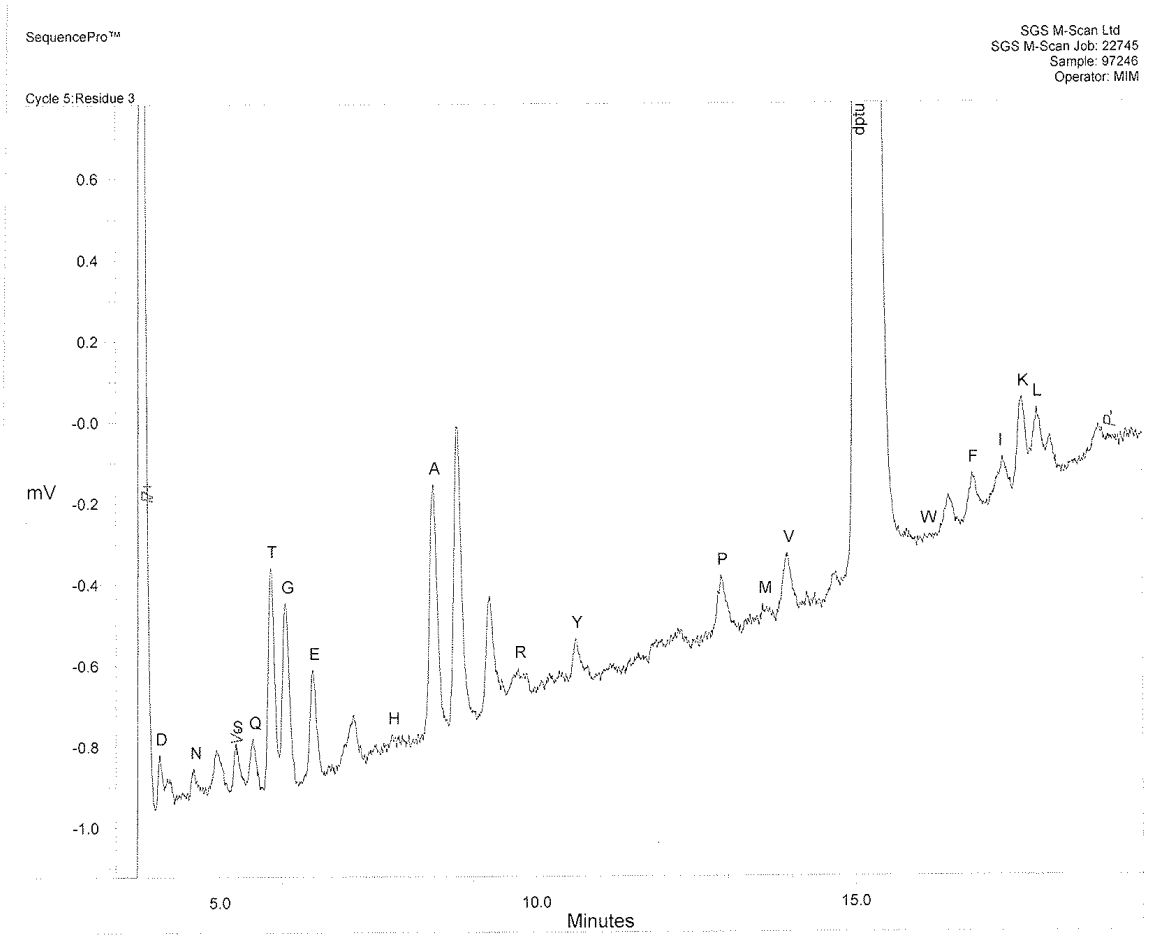

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.08	4.07	0.115	0.396
	4.20		0.040	
	4.35		0.020	
N	4.63	4.57	0.049	0.166
	4.98		0.109	
S	5.28	5.24	0.122	0.657
Q	5.55	5.49	0.129	0.529
T	5.83	5.78	0.546	3.149
G	6.06	6.00	0.455	2.300
E	6.48	6.43	0.273	1.014
	7.13		0.106	
	7.39		0.028	
	7.47		0.033	
H	7.73	7.65	0.022	0.112
	7.83		0.026	
	8.05		0.020	
A	8.38	8.31	0.618	2.978
	8.75		0.726	
	9.27		0.265	
R	9.72	9.75	0.041	0.243
	9.81		0.016	
	10.08		0.026	
	10.21		0.023	
	10.36		0.031	
Y	10.62	10.56	0.108	0.521
	10.81		0.041	
	10.98		0.019	
	11.16		0.016	
	11.37		0.022	
	11.47		0.033	
	11.60		0.039	
	11.68		0.027	
	11.88		0.042	
	11.97		0.030	
	12.11		0.020	
	12.22		0.020	
	12.45		0.019	
	12.66		0.030	
P	12.89	12.83	0.150	0.817
	13.28		0.021	
M	13.55	13.54	0.036	0.166
V	13.93	13.86	0.158	0.751
	14.17		0.024	
	14.23		0.043	
	14.37		0.032	
	14.70		0.056	
dptu	15.10	15.04	7.094	39.076
	15.28		14.584	
W	16.10	16.13	0.021	0.089
	16.27		0.012	
	16.47		0.093	
F	16.84	16.79	0.116	0.554
I	17.31	17.25	0.118	0.707
K	17.60	17.55	0.240	0.787
L	17.84	17.78	0.196	0.993
	18.04		0.112	
	18.17		0.027	
	18.24		0.020	
	18.44		0.018	
	18.51		0.016	
	18.81		0.042	

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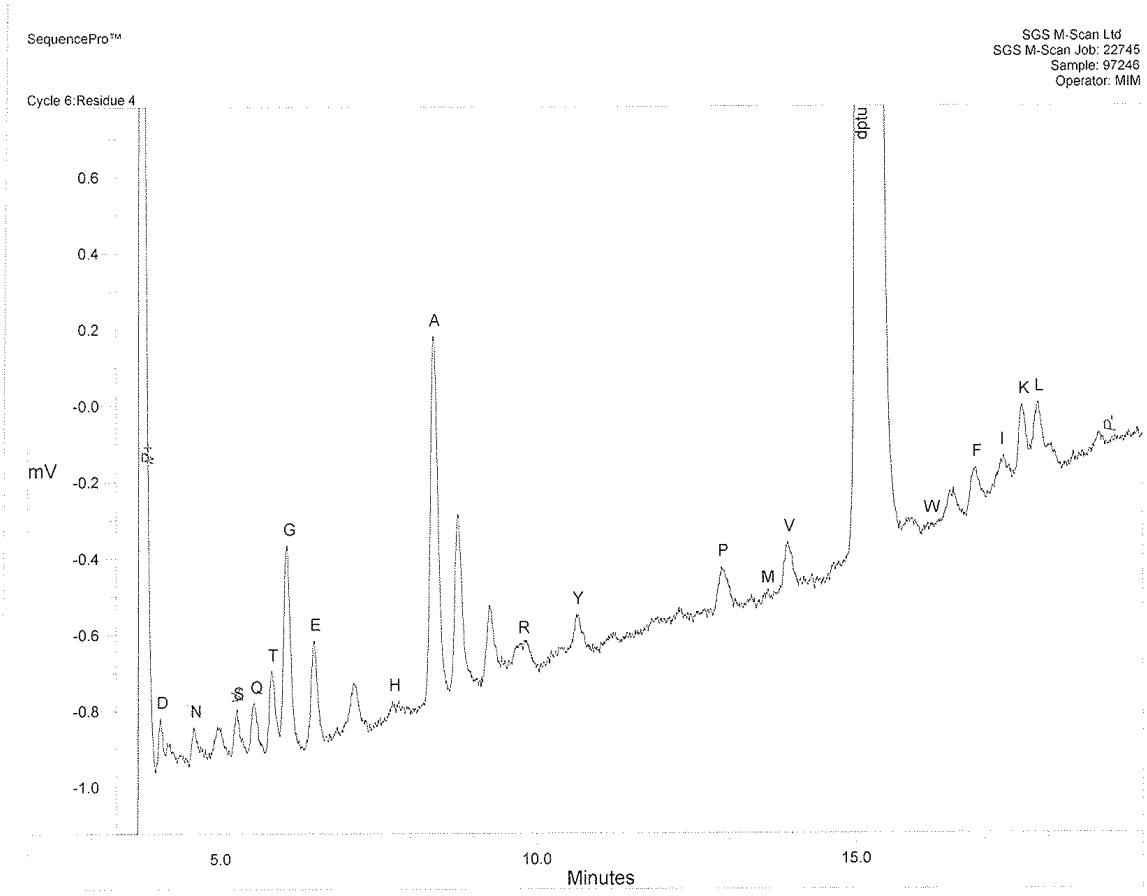

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.07	0.123	0.423
	4.40		0.022	
N	4.61	4.57	0.087	0.292
	4.85		0.017	
	4.98		0.080	
S	5.29	5.24	0.102	0.551
Q	5.56	5.49	0.130	0.534
T	5.83	5.78	0.213	1.229
G	6.07	6.00	0.541	2.737
E	6.49	6.43	0.281	1.044
	6.68		0.018	
	6.85		0.037	
	7.12		0.141	
	7.29		0.036	
	7.37		0.021	
	7.45		0.018	
	7.53		0.023	
H	7.73	7.65	0.043	0.215
	7.83		0.032	
	7.95		0.021	
A	8.37	8.31	0.967	4.659
	8.76		0.466	
	9.26		0.193	
R	9.77	9.75	0.021	0.123
	10.10		0.024	
	10.18		0.031	
	10.34		0.046	
Y	10.62	10.56	0.114	0.548
	11.00		0.028	
	11.19		0.040	
	11.40		0.021	
	11.54		0.021	
	11.72		0.020	
	11.79		0.018	
	12.07		0.020	
	12.14		0.022	
	12.22		0.038	
	12.50		0.028	
	12.63		0.027	
P	12.89	12.83	0.126	0.689
	13.10		0.035	
	13.36		0.040	
	13.49		0.017	
M	13.57	13.54	0.018	0.084
	13.72		0.020	
V	13.93	13.86	0.133	0.630
	14.14		0.020	
	14.32		0.036	
	14.40		0.028	
	14.50		0.017	
	14.65		0.024	
dptu	15.11	15.04	7.162	39.450
	15.28		13.675	
	15.83		0.013	
W	16.13	16.13	0.021	0.089
	16.48		0.031	
F	16.88	16.79	0.106	0.506
	17.06		0.015	
I	17.32	17.25	0.051	0.307
K	17.61	17.55	0.187	0.612
L	17.86	17.78	0.187	0.944
	18.06		0.072	
	18.26		0.017	
	18.41		0.030	
	18.56		0.021	
	18.67		0.018	
	18.82		0.031	

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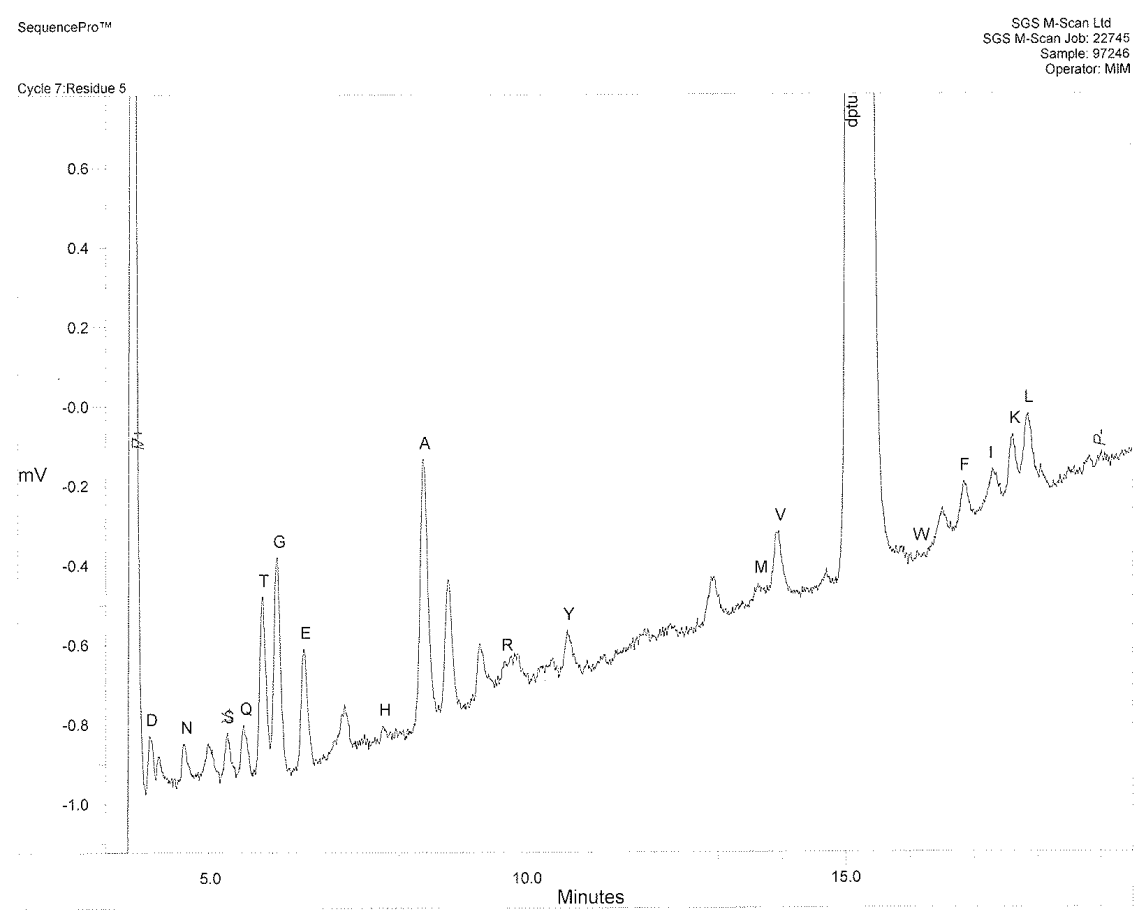

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.08	4.07	0.133	0.461		11.41		0.028	
	4.22		0.061			11.52		0.023	
	4.48		0.024			11.66		0.033	
N	4.63	4.57	0.103	0.345		11.73		0.027	
	4.84		0.021			11.85		0.023	
	5.00		0.097			12.13		0.027	
S	5.29	5.24	0.125	0.672		12.25		0.022	
Q	5.55	5.49	0.141	0.577		12.51		0.025	
T	5.84	5.78	0.458	2.641		12.68		0.031	
G	6.07	6.00	0.552	2.794		12.94		0.109	
E	6.50	6.43	0.304	1.129		13.32		0.021	
	6.69		0.014			13.38		0.023	
	6.81		0.022		M	13.63	13.54	0.052	0.237
	7.14		0.119		V	13.96	13.86	0.163	0.772
	7.45		0.035			14.55		0.016	
H	7.74	7.65	0.042	0.209		14.70		0.048	
	7.86		0.022			14.80		0.016	
	8.13		0.025		dptu	15.12	15.04	7.498	41.302
A	8.38	8.31	0.676	3.259		15.30		14.147	
	8.62		0.026			15.88		0.028	
	8.77		0.330			16.02		0.027	
	9.07		0.015		W	16.12	16.13	0.026	0.110
	9.27		0.121			16.23		0.016	
R	9.66	9.75	0.037	0.222		16.51		0.106	
	9.76		0.029		F	16.85	16.79	0.142	0.679
	10.10		0.025		I	17.30	17.25	0.131	0.787
	10.25		0.039		K	17.63	17.55	0.190	0.622
	10.41		0.053		L	17.87	17.78	0.221	1.119
	10.47		0.033			18.06		0.075	
Y	10.63	10.56	0.089	0.430		18.49		0.026	
	10.95		0.033			18.68		0.019	
	11.15		0.013			18.83		0.039	

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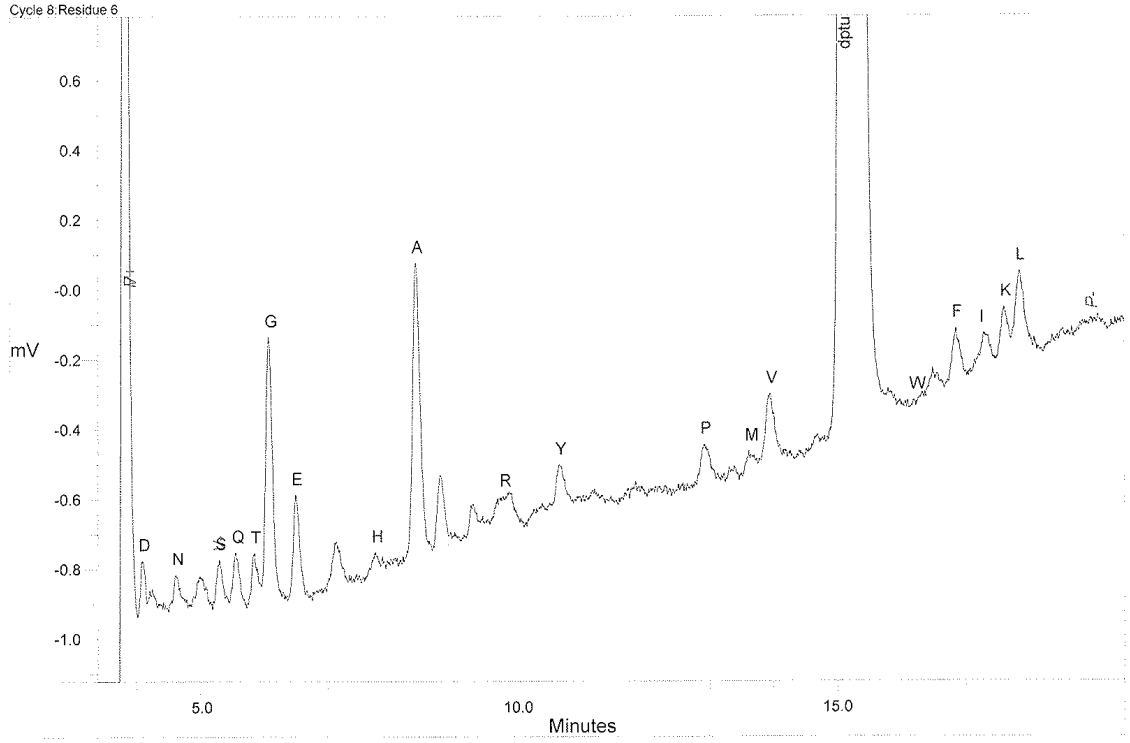
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97246
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.07	0.145	0.500
	4.25		0.048	
N	4.62	4.57	0.102	0.343
	5.00		0.054	
S	5.30	5.24	0.126	0.677
Q	5.55	5.49	0.149	0.612
T	5.85	5.78	0.149	0.859
G	6.06	6.00	0.756	3.824
	6.33		0.012	
E	6.49	6.43	0.304	1.127
	6.67		0.020	
	6.83		0.019	
	6.90		0.016	
	7.13		0.134	
	7.33		0.026	
	7.45		0.019	
	7.55		0.015	
H	7.74	7.65	0.071	0.354
	7.85		0.049	
	7.93		0.030	
	8.09		0.027	
	8.14		0.016	
A	8.38	8.31	0.835	4.027
	8.76		0.207	
	8.93		0.030	
	9.00		0.033	
	9.29		0.096	
	9.44		0.054	
	9.52		0.039	
R	9.76	9.75	0.090	0.538
	9.86		0.098	
	10.24		0.041	
	10.38		0.046	
	10.48		0.039	
Y	10.64	10.56	0.135	0.650
	10.93		0.020	
	11.18		0.038	
	11.30		0.023	
	11.44		0.021	
	11.71		0.040	
	11.82		0.053	
	11.92		0.036	
	12.14		0.019	
	12.30		0.025	
	12.36		0.021	
	12.42		0.019	
	12.57		0.026	
P	12.90	12.83	0.121	0.660
	13.12		0.030	
	13.22		0.027	
	13.30		0.042	
	13.38		0.042	
M	13.61	13.54	0.028	0.130
V	13.95	13.86	0.190	0.900
	14.27		0.019	
	14.40		0.024	
	14.52		0.022	
	14.69		0.042	
	14.80		0.022	
dptu	15.12	15.04	7.823	43.094
	15.29		18.395	
	15.98		0.020	
W	16.19	16.13	0.021	0.088
	16.34		0.027	
	16.49		0.078	
	16.55		0.057	
F	16.85	16.79	0.146	0.699
	17.08		0.017	
I	17.29	17.25	0.098	0.586
K	17.61	17.55	0.135	0.444
L	17.86	17.78	0.211	1.066
	18.08		0.029	
	18.37		0.035	
	18.54		0.039	
	18.68		0.031	
	18.85		0.021	

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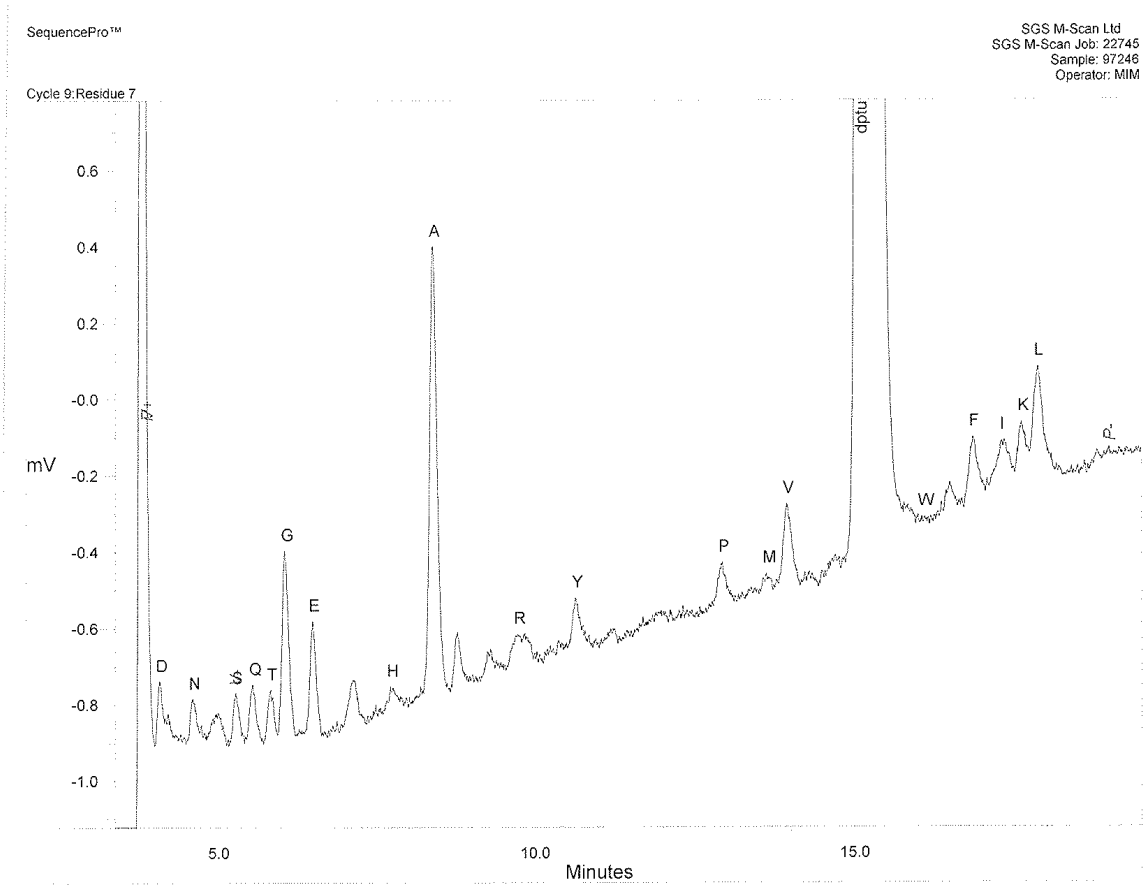

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.07	0.140	0.482
	4.43		0.020	
	4.50		0.017	
N	4.61	4.57	0.105	0.352
	4.75		0.027	
	4.99		0.015	
S	5.29	5.24	0.136	0.735
Q	5.56	5.49	0.158	0.647
T	5.84	5.78	0.140	0.805
G	6.05	6.00	0.499	2.523
	6.28		0.026	
E	6.49	6.43	0.303	1.125
	6.81		0.020	
	6.95		0.021	
	7.13		0.126	
	7.35		0.020	
H	7.71	7.65	0.025	0.124
	7.96		0.025	
	8.19		0.043	
A	8.38	8.31	1.185	5.713
	8.77		0.150	
	9.01		0.022	
	9.29		0.061	
R	9.72	9.75	0.083	0.493
	9.84		0.077	
	10.23		0.035	
	10.36		0.028	
	10.49		0.015	
Y	10.63	10.56	0.105	0.504
	10.95		0.025	
	11.18		0.015	
	11.43		0.026	
	11.50		0.020	
	11.63		0.025	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.68		0.033	
	11.97		0.016	
	12.11		0.031	
	12.25		0.025	
	12.31		0.034	
	12.37		0.021	
	12.53		0.016	
P	12.92	12.83	0.117	0.639
	13.24		0.021	
	13.38		0.018	
M	13.62	13.54	0.051	0.235
	13.78		0.029	
V	13.93	13.86	0.223	1.057
	14.22		0.033	
	14.29		0.030	
	14.48		0.034	
	14.70		0.047	
dptu	15.12	15.04	9.038	49.787
	15.29		26.116	
	16.00		0.023	
W	16.06	16.13	0.016	0.065
	16.31		0.017	
	16.36		0.033	
	16.50		0.061	
	16.68		0.024	
F	16.86	16.79	0.178	0.849
I	17.33	17.25	0.144	0.860
K	17.61	17.55	0.177	0.579
L	17.87	17.78	0.308	1.559
	18.15		0.037	
	18.39		0.026	
	18.49		0.026	
	18.80		0.037	

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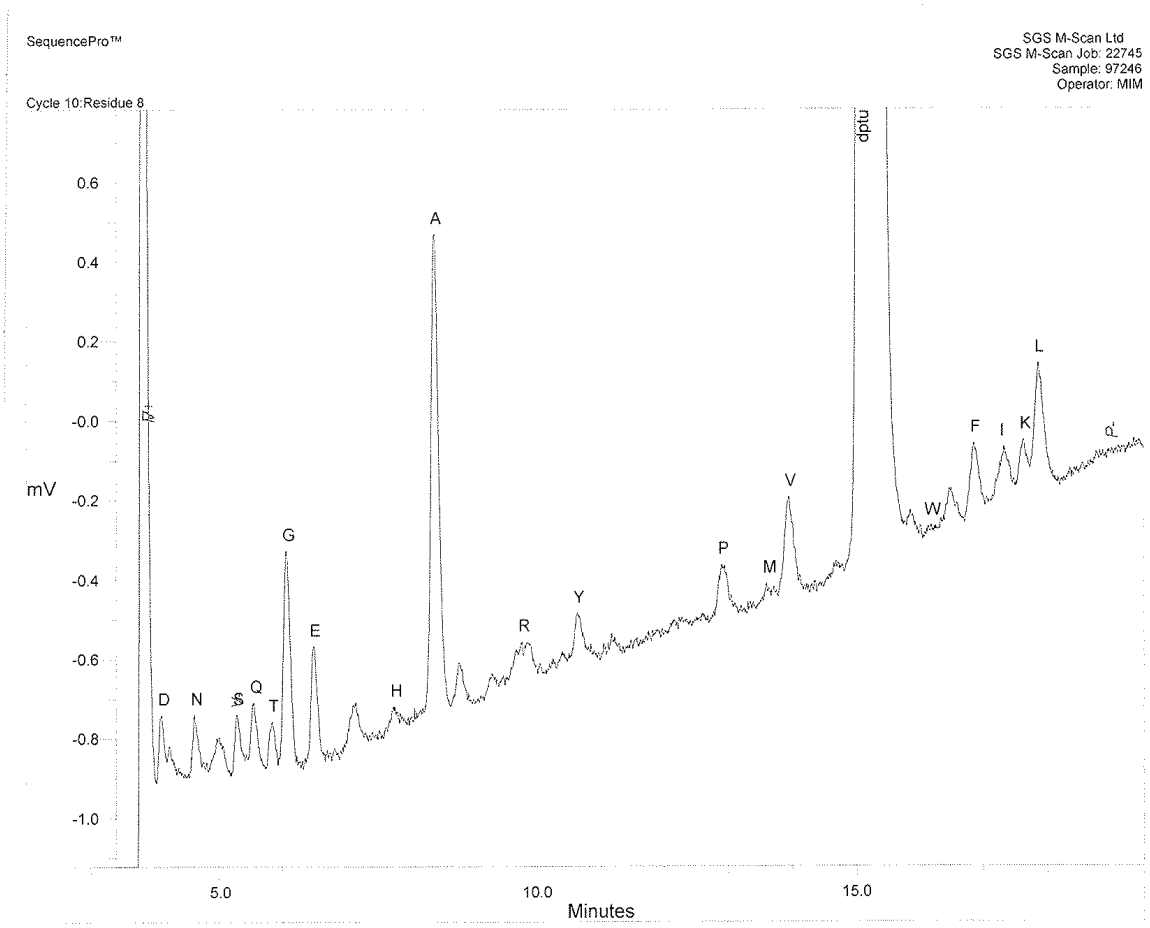

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.07	0.147	0.506
	4.22		0.048	
	4.37		0.018	
N	4.62	4.57	0.148	0.497
	5.01		0.048	
S	5.28	5.24	0.150	0.809
Q	5.55	5.49	0.172	0.707
T	5.84	5.78	0.115	0.661
G	6.06	6.00	0.538	2.720
E	6.49	6.43	0.302	1.120
	6.70		0.032	
	6.82		0.030	
	7.15		0.106	
	7.41		0.021	
H	7.75	7.65	0.054	0.271
	8.07		0.039	
A	8.38	8.31	1.214	5.851
	8.76		0.115	
	9.29		0.029	
	9.46		0.026	
	9.66		0.081	
R	9.76	9.75	0.097	0.575
	9.84		0.090	
	10.05		0.028	
	10.25		0.030	
	10.40		0.033	
Y	10.63	10.56	0.082	0.395
	11.05		0.034	
	11.17		0.031	
	11.42		0.011	
	11.57		0.025	
	11.63		0.016	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.75		0.031	
	11.90		0.024	
	12.16		0.029	
	12.27		0.020	
	12.60		0.027	
P	12.90	12.83	0.022	0.121
	13.20		0.018	
	13.39		0.024	
M	13.60	13.54	0.043	0.196
V	13.94	13.86	0.230	1.091
	14.35		0.029	
	14.42		0.020	
	14.68		0.043	
	14.77		0.023	
dptu	15.12	15.04	8.875	48.887
	15.29		22.702	
	15.85		0.048	
W	16.14	16.13	0.015	0.064
	16.34		0.020	
	16.50		0.061	
F	16.86	16.79	0.181	0.865
	17.06		0.018	
I	17.32	17.25	0.109	0.656
K	17.63	17.55	0.112	0.368
L	17.85	17.78	0.295	1.494
	18.34		0.029	
	18.45		0.022	
	18.55		0.023	
	18.63		0.019	
	18.75		0.033	
	18.81		0.030	

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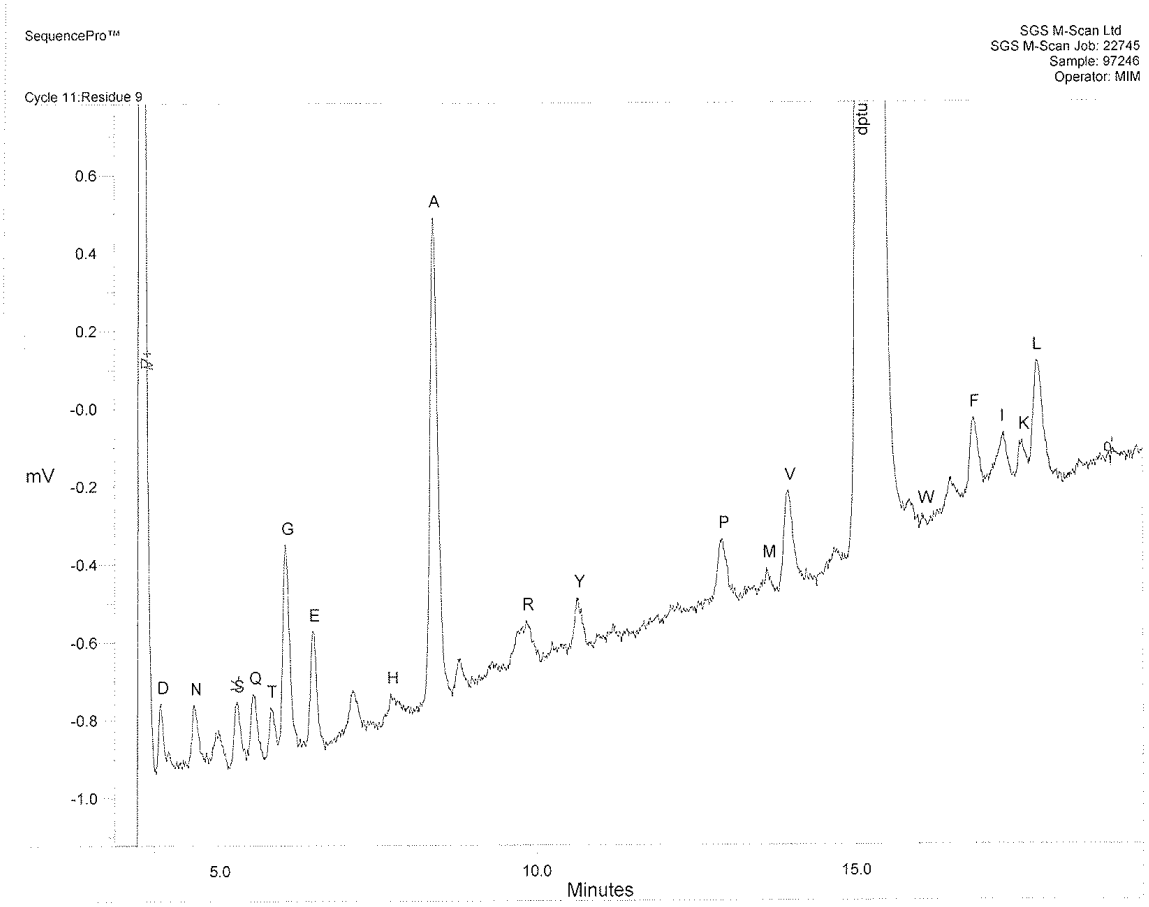

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.07	0.168	0.580
	4.22		0.030	
	4.35		0.020	
N	4.62	4.57	0.157	0.528
	4.82		0.027	
	5.01		0.096	
S	5.30	5.24	0.168	0.907
Q	5.55	5.49	0.178	0.728
T	5.83	5.78	0.130	0.749
G	6.06	6.00	0.532	2.693
	6.36		0.018	
E	6.50	6.43	0.301	1.118
	6.96		0.027	
	7.13		0.110	
	7.36		0.025	
	7.54		0.018	
H	7.72	7.65	0.027	0.134
	7.99		0.018	
	8.13		0.017	
A	8.38	8.31	1.253	6.041
	8.80		0.090	
	8.98		0.030	
	9.31		0.026	
	9.78		0.103	
R	9.83	9.75	0.117	0.697
	10.10		0.023	
	10.24		0.037	
	10.30		0.018	
	10.45		0.020	
Y	10.65	10.56	0.135	0.653
	10.95		0.028	
	11.20		0.036	
	11.42		0.024	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.70		0.032	
	11.80		0.029	
	11.92		0.025	
	12.11		0.044	
	12.23		0.043	
	12.32		0.024	
	12.45		0.021	
	12.55		0.029	
	12.65		0.025	
P	12.93	12.83	0.157	0.855
	13.28		0.033	
	13.43		0.027	
M	13.62	13.54	0.042	0.192
V	13.96	13.86	0.251	1.192
	14.23		0.032	
	14.66		0.061	
dptu	15.13	15.04	8.552	47.105
	15.30		18.655	
	15.85		0.041	
W	16.05	16.13	0.036	0.148
	16.23		0.028	
	16.49		0.090	
F	16.87	16.79	0.208	0.991
	17.08		0.041	
I	17.35	17.25	0.125	0.751
K	17.63	17.55	0.099	0.324
L	17.85	17.78	0.304	1.540
	18.16		0.024	
	18.40		0.019	
	18.50		0.032	
	18.69		0.017	
	18.79		0.013	
	18.88		0.015	

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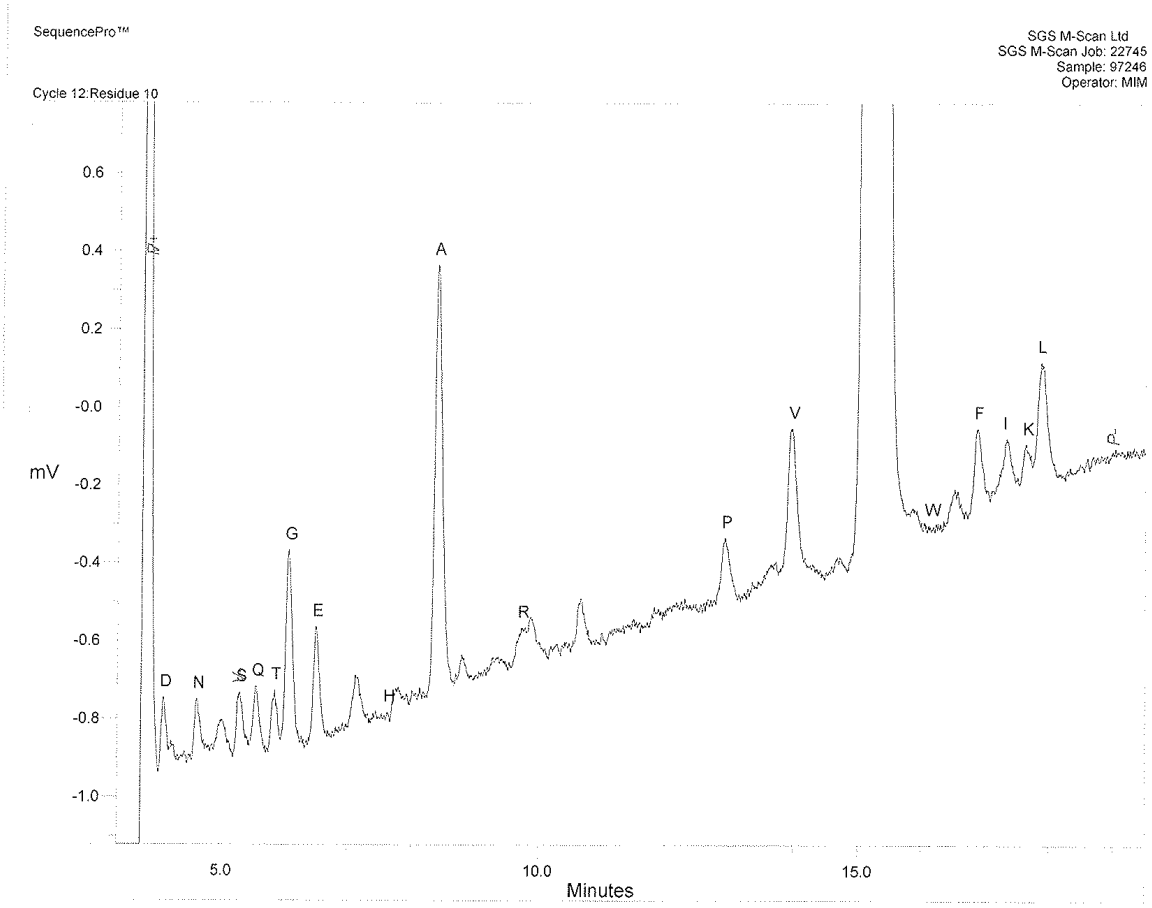

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.11	4.07	0.165	0.569		11.50		0.022	
	4.23		0.033			11.82		0.045	
	4.44		0.030			11.88		0.035	
N	4.64	4.57	0.154	0.519		12.16		0.021	
	5.04		0.090			12.30		0.029	
S	5.32	5.24	0.165	0.889		12.44		0.017	
Q	5.57	5.49	0.174	0.714		12.65		0.030	
T	5.87	5.78	0.156	0.900	P	12.93	12.83	0.172	0.936
G	6.08	6.00	0.504	2.550		13.23		0.019	
E	6.51	6.43	0.298	1.107		13.35		0.036	
	6.83		0.021			13.65		0.050	
	7.13		0.136			13.73		0.048	
	7.44		0.026		V	13.98	13.86	0.363	1.724
H	7.63	7.65	0.024	0.119		14.29		0.016	
	7.80		0.048			14.50		0.017	
	7.92		0.022			14.68		0.021	
	8.01		0.026			15.14		8.781	
	8.13		0.030			15.32		20.331	
	8.19		0.019			15.86		0.030	
A	8.40	8.31	1.100	5.303	W	16.11	16.13	0.018	0.076
	8.79		0.072			16.35		0.021	
	9.06		0.015			16.53		0.033	
	9.28		0.032		F	16.89	16.79	0.211	1.005
	9.36		0.022		I	17.34	17.25	0.128	0.768
R	9.73	9.75	0.025	0.146	K	17.64	17.55	0.103	0.338
	9.88		0.053		L	17.87	17.78	0.305	1.542
	10.20		0.031			18.28		0.017	
	10.29		0.032			18.47		0.024	
	10.41		0.032			18.60		0.034	
	10.67		0.132			18.68		0.029	
	10.90		0.016			18.75		0.018	
	11.27		0.022			18.90		0.019	
	11.40		0.019						

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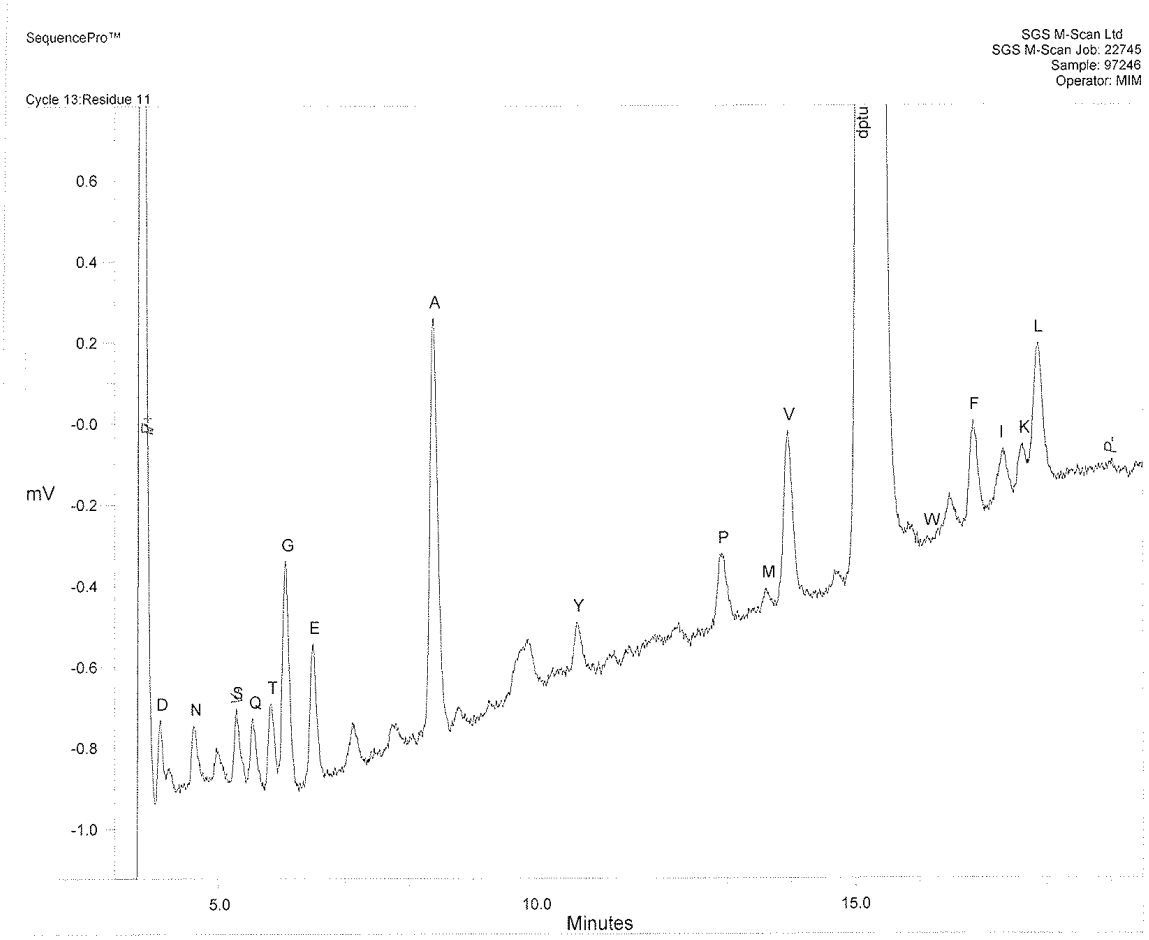

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.07	0.179	0.619
	4.23		0.040	
	4.47		0.017	
N	4.62	4.57	0.142	0.476
	4.98		0.082	
S	5.29	5.24	0.184	0.993
Q	5.55	5.49	0.165	0.677
T	5.84	5.78	0.214	1.235
G	6.06	6.00	0.567	2.870
E	6.50	6.43	0.348	1.291
	6.72		0.021	
	6.85		0.023	
	7.12		0.105	
	7.41		0.028	
	7.46		0.033	
	7.77		0.018	
	8.06		0.028	
	8.19		0.033	
A	8.39	8.31	1.037	4.998
	8.77		0.042	
	9.02		0.025	
	9.13		0.013	
	9.24		0.028	
	9.37		0.021	
	9.87		0.126	
	10.26		0.040	
	10.39		0.032	
	10.48		0.032	
Y	10.63	10.56	0.133	0.640
	10.98		0.032	
	11.15		0.042	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.22		0.045	
	11.46		0.040	
	11.59		0.029	
	11.72		0.023	
	12.24		0.027	
	12.47		0.027	
	12.54		0.027	
	12.68		0.019	
P	12.92	12.83	0.181	0.988
	13.43		0.020	
	13.49		0.018	
M	13.60	13.54	0.056	0.257
V	13.94	13.86	0.413	1.957
	14.24		0.017	
	14.43		0.022	
	14.54		0.018	
	14.67		0.054	
dptu	15.12	15.04	9.218	50.775
	15.30		21.421	
	15.82		0.082	
	15.89		0.070	
	15.97		0.046	
W	16.14	16.13	0.024	0.098
	16.30		0.023	
	16.48		0.087	
F	16.86	16.79	0.249	1.189
I	17.33	17.25	0.140	0.839
K	17.63	17.55	0.127	0.415
L	17.86	17.78	0.355	1.796
	18.32		0.019	
	18.55		0.020	

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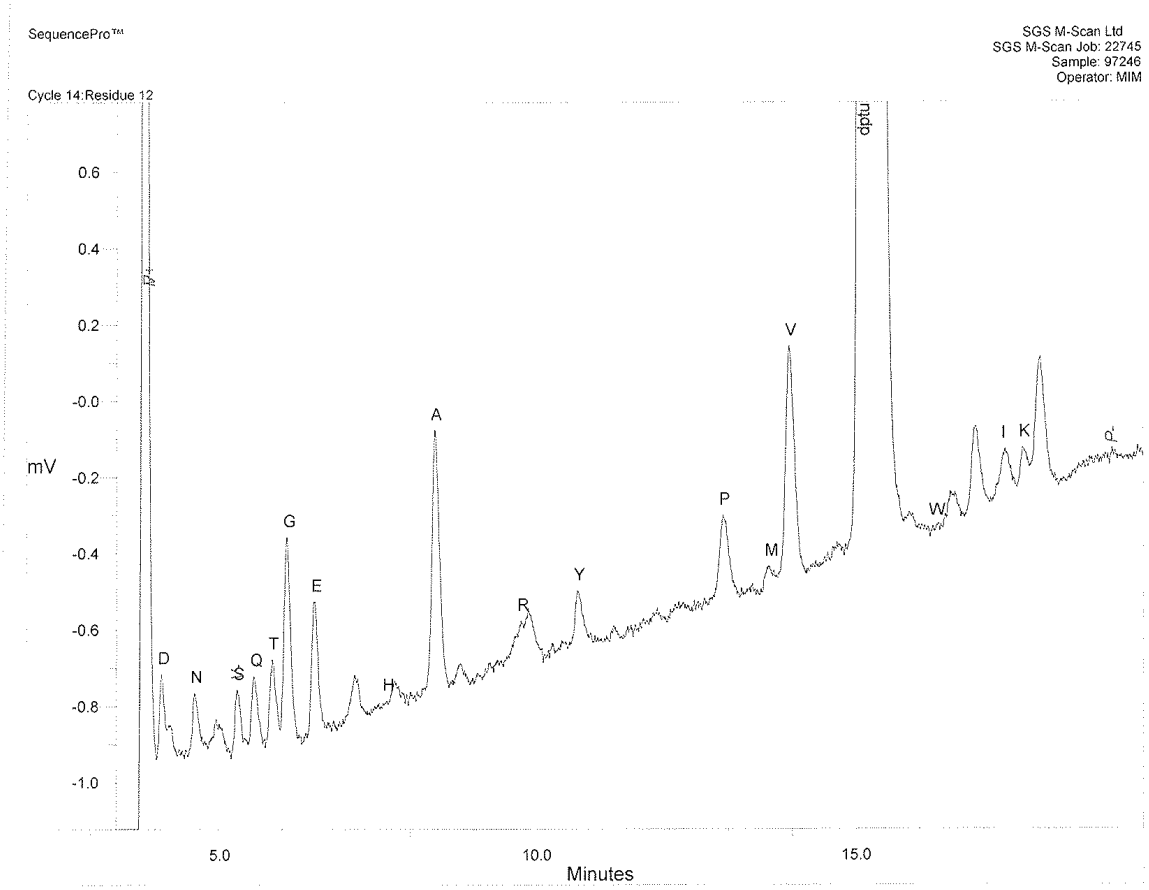

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.11	4.07	0.187	0.645
	4.25		0.038	
	4.38		0.020	
N	4.63	4.57	0.153	0.516
	4.97		0.038	
S	5.30	5.24	0.160	0.861
Q	5.56	5.49	0.183	0.749
T	5.85	5.78	0.229	1.318
G	6.08	6.00	0.536	2.714
	6.38		0.021	
E	6.52	6.43	0.350	1.296
	6.82		0.027	
	6.91		0.020	
	7.13		0.123	
	7.52		0.018	
H	7.63	7.65	0.013	0.066
	7.76		0.053	
	8.01		0.032	
A	8.39	8.31	0.692	3.338
	8.66		0.015	
	8.79		0.039	
	8.98		0.017	
	9.07		0.026	
	9.19		0.032	
	9.27		0.042	
	9.37		0.045	
R	9.75	9.75	0.128	0.758
	9.87		0.156	
	10.24		0.039	
	10.39		0.033	
Y	10.65	10.56	0.141	0.679
	10.91		0.018	
	11.22		0.038	
	11.43		0.018	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.55		0.027	
	11.68		0.021	
	11.82		0.031	
	11.90		0.042	
	11.98		0.018	
	12.18		0.033	
	12.23		0.034	
	12.47		0.028	
	12.63		0.031	
P	12.92	12.83	0.236	1.286
	13.20		0.031	
	13.38		0.034	
M	13.63	13.54	0.066	0.300
V	13.95	13.86	0.619	2.935
	14.32		0.013	
	14.50		0.022	
	14.70		0.046	
dptu	15.13	15.04	8.922	49.144
	15.31		19.530	
	15.84		0.018	
	15.99		0.012	
	16.15		0.021	
W	16.21	16.13	0.025	0.103
	16.30		0.021	
	16.48		0.046	
	16.89		0.222	
I	17.34	17.25	0.105	0.627
K	17.61	17.55	0.102	0.334
	17.89		0.329	
	18.48		0.025	
	18.55		0.023	
	18.78		0.024	
	18.90		0.030	

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Appendix IV – Raw Data for 12 Residues of N-terminal Sequencing of Microbial AVHPPD-03-0209 (SGS M-Scan No. 97248)

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

SAMPLE INFORMATION

Sample Name:	111009_2B_97248	Std Amount:	10.000 pmols
ID Code:		Sample Amount:	0.000 pmols
		Detector Scale:	0.005 AUFS

Comments:

SEQUENCER INFORMATION

Name:	PROCISE	Model Number:	492
Method:	Pulsed liquid PVDF	Cartridge:	B
Operator:	MIM		

CHEMICAL INFORMATION

R1	1105171	03 October, 2011	X3	0	01 September, 2001
R2	1105087	12 September, 2011	PTH Column	G110608031	27 September, 2011
R3	1012198	30 September, 2011	Solvent A	1106619	27 September, 2011
R4	1009128	05 October, 2011	Solvent B	1105351	30 September, 2011
R5	1011047	09 October, 2011	Premix	1104103	27 September, 2011
S1	0	01 September, 2001	Guard Column	0	01 January, 2002
S2	1010693	05 October, 2011	Cartridge Seals	0	01 January, 2002
S3	1106495	05 October, 2011	Glass Fiber Filter	0	01 January, 2002
S4	1105166	05 October, 2011	pth standards	1007028	01 January, 2002
X1	0	01 September, 2001		12345678	01 January, 2002
X2	0	01 September, 2001	Total Cycles Count		01 January, 2002

MIM 10.10.11

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

ORIGINAL METHOD TEMPLATE: D:\Program Files\AppliedBiosystems\Procise\SequencePro\Methods\Procise.met

CALIBRATION TABLE

COMPONENT	RTIME	RESPONSE	REFERENCE	INTERNAL STD	ABS WND	REL WND
Aspartic Acid	4.12	1.000	---	---	0.20	0.00
Asparagine	4.63	1.000	---	---	0.20	0.00
Serine	5.30	1.000	---	---	0.20	0.00
Glutamine	5.56	1.000	---	---	0.20	0.00
Threonine	5.84	1.000	---	---	0.20	0.00
Glycine	6.07	1.000	---	---	0.20	0.00
Glutamic Acid	6.51	1.000	---	---	0.20	0.00
Histidine	7.75	1.000	---	---	0.20	0.00
Alanine	8.39	1.000	---	---	0.20	0.00
Arginine	9.88	1.000	---	---	0.20	0.00
Tyrosine	10.65	1.000	---	---	0.20	0.00
Proline	12.92	1.000	---	---	0.20	0.00
Methionine	13.63	1.000	---	---	0.20	0.00
Valine	13.95	1.000	---	---	0.20	0.00
dptu	15.12	1.000	---	---	0.20	0.00
Tryptophan	16.21	1.000	---	---	0.20	0.00
Phenylalanine	16.86	1.000	---	---	0.20	0.00
Isoleucine	17.33	1.000	---	---	0.20	0.00
Lysine	17.62	1.000	---	---	0.20	0.00
Leucine	17.86	1.000	---	---	0.20	0.00

GLOBAL INTEGRATION EVENTS

EVENT	TIME	VALUE	EVENT	TIME	VALUE
Peak Detect Off	0.00	---	Valley to Valley Off	5.30	---
Valley to Valley On	3.90	---	Peak Detect Off	19.00	---
Peak Detect On	3.90	---			

INTEGRATION PARAMETERS

PEAK DETECTION PARAMETERS

Bunching Factor	4	Noise Threshold:	0.954 µVolts
Max Peaks	128	Area Threshold:	41.000 µVolts

PEAK SEPARATION CRITERIA		EXPONENTIAL SKIM CRITERIA	
Width Ratio:	0.20	Peak Height Ratio:	5.00
Valley to Peak Ratio:	0.01	Adjusted Height Ratio:	4.00
Tangent Width:	1000.00	Valley Height Ratio:	3.00

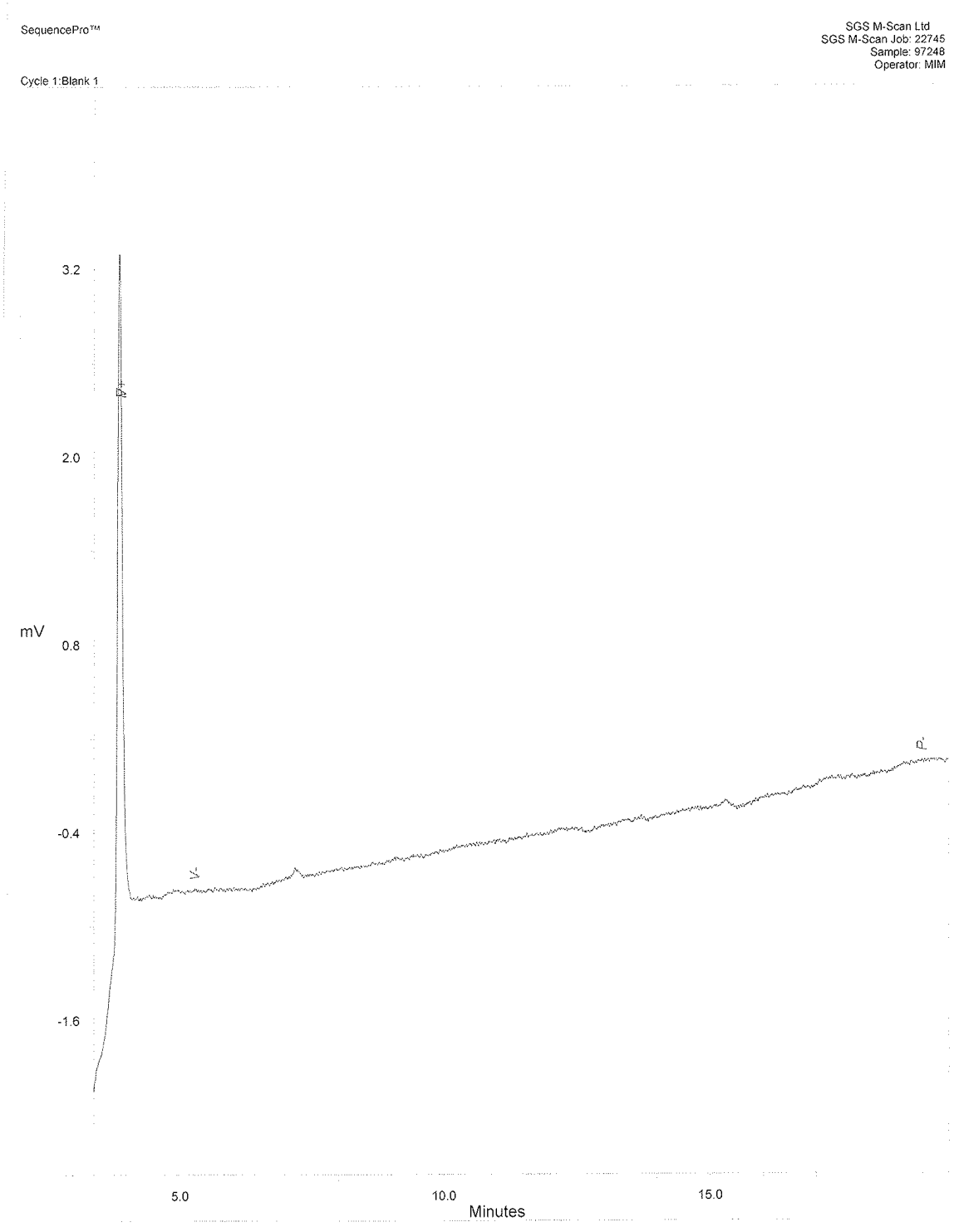
SEQUENCE CALLING PARAMETERS

Use Pmol Heights, Allow Negative Background Off, Refine Data On

SEQUENCE SCORING VALUES

Raw Slope 1:	2.00	Raw Yield	1.00
Raw Slope 2:	1.00	Bkgd Yield	1.00
Bkgd Slope 1:	2.00	Lag Yield	1.00
Bkgd Slope 2:	1.00	Rep Yield	1.00
Max Slope:	1.50	Low Yield	1.00
Rule Book:	0.60	Bkgd Sensitivity	1.00
Dev Mult:	3.00		

MIM 10/10/11


SequencePro™
SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM
Cycle 1:Blank 1
3.2
2.0
mV
0.8
-0.4
-1.6
5.0
10.0
15.0
Minutes
Monday, October 10, 2011 11:28:45
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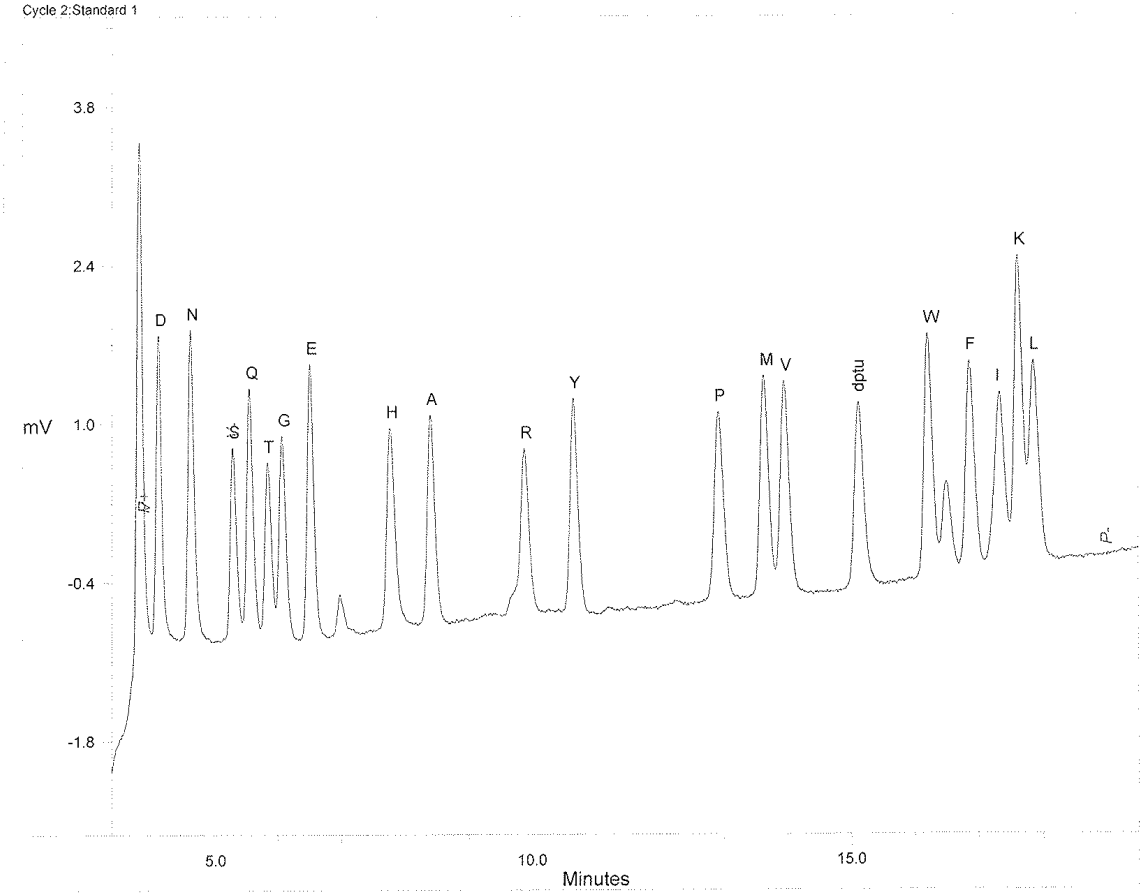
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 2:Standard 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
						11.56		0.021	
D	4.12	4.12	2.643	10.000		11.68		0.022	
N	4.63	4.63	2.735	10.000		12.05		0.033	
	5.07		0.019			12.23		0.053	
S	5.30	5.30	1.714	10.000		12.50		0.018	
Q	5.56	5.56	2.237	10.000		12.55		0.022	
T	5.84	5.84	1.577	10.000	P	12.92	12.92	1.662	10.000
G	6.07	6.07	1.808	10.000		13.30		0.026	
E	6.51	6.51	2.431	10.000	M	13.63	13.63	1.947	10.000
	6.97		0.339		V	13.95	13.95	1.873	10.000
	7.33		0.011			14.47		0.025	
	7.45		0.030			14.63		0.022	
H	7.75	7.75	1.790	10.000		14.78		0.021	
	8.16		0.024		dptu	15.12	15.12	1.651	10.000
A	8.39	8.39	1.861	10.000		15.47		0.026	
	8.75		0.017			15.57		0.024	
	8.82		0.030			15.81		0.030	
	8.96		0.029			15.91		0.036	
	9.09		0.017		W	16.21	16.21	2.160	10.000
	9.25		0.034			16.50		0.825	
	9.35		0.024		F	16.86	16.86	1.854	10.000
R	9.88	9.88	1.454	10.000	I	17.33	17.33	1.530	10.000
	10.26		0.024		K	17.62	17.62	2.707	10.000
	10.42		0.032		L	17.86	17.86	1.759	10.000
Y	10.65	10.65	1.891	10.000		18.32		0.021	
	11.21		0.025			18.43		0.026	
	11.34		0.017			18.73		0.033	
	11.40		0.028			18.90		0.021	
	11.49		0.037						

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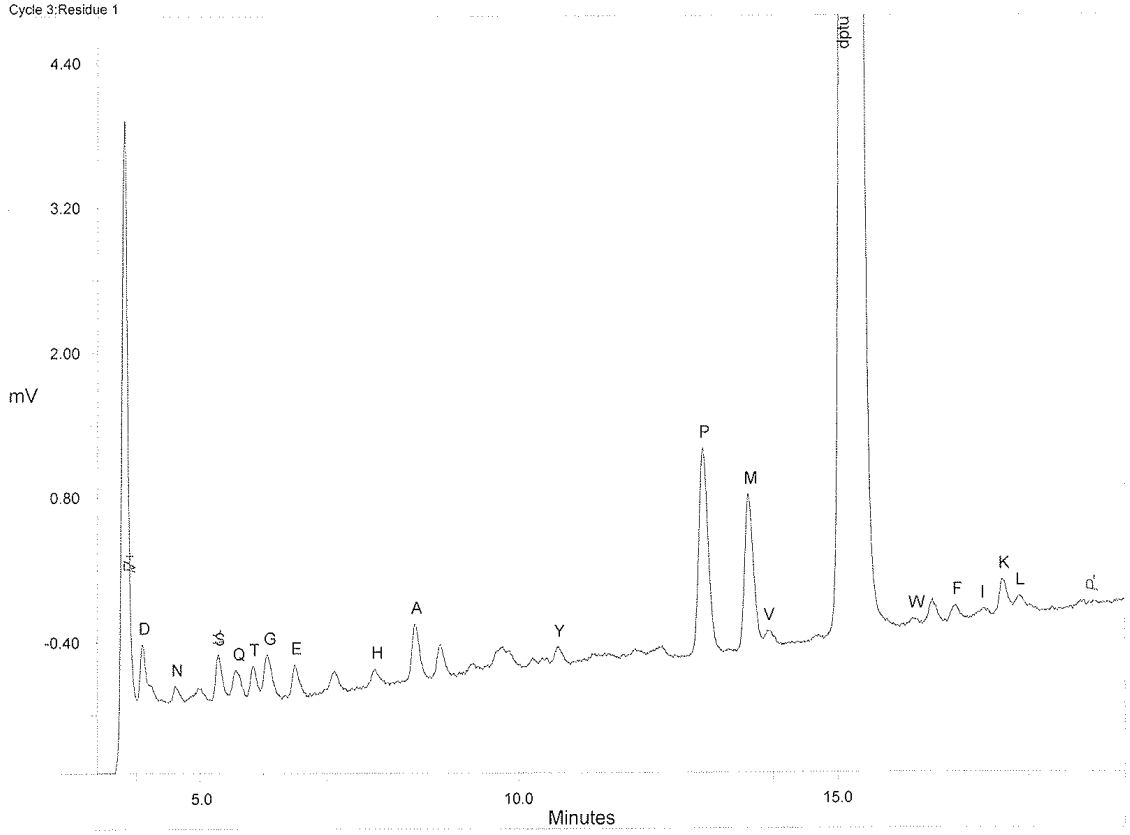
SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 3:Residue 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.12	0.463	1.753		11.43		0.031	
N	4.61	4.63	0.136	0.496		11.65		0.030	
	4.97		0.038			11.85		0.028	
S	5.29	5.30	0.376	2.195		12.13		0.017	
Q	5.58	5.56	0.246	1.101		12.22		0.013	
T	5.84	5.84	0.279	1.769		12.46		0.025	
G	6.07	6.07	0.371	2.051	P	12.91	12.92	1.721	10.357
	6.35		0.024			13.30		0.029	
E	6.50	6.51	0.270	1.113	M	13.61	13.63	1.292	6.638
	6.74		0.022		V	13.91	13.95	0.108	0.577
	6.82		0.025			14.25		0.015	
	7.12		0.166			14.38		0.019	
	7.40		0.025			14.48		0.017	
	7.46		0.028			14.55		0.022	
	7.57		0.026			14.65		0.020	
H	7.76	7.75	0.156	0.869	dptu	15.12	15.12	10.548	63.901
	7.96		0.027			15.29		30.640	
	8.03		0.021		W	16.18	16.21	0.034	0.158
	8.11		0.029			16.32		0.023	
A	8.38	8.39	0.475	2.554		16.50		0.204	
	8.77		0.276		F	16.87	16.86	0.132	0.713
	9.07		0.032		I	17.32	17.33	0.036	0.235
	9.30		0.054		K	17.60	17.62	0.287	1.061
	9.75		0.078		L	17.86	17.86	0.127	0.722
	10.23		0.077			18.03		0.038	
	10.39		0.069			18.26		0.013	
	10.45		0.069			18.30		0.015	
Y	10.63	10.65	0.155	0.822		18.38		0.031	
	10.88		0.021			18.49		0.016	
	11.04		0.026			18.60		0.016	
	11.16		0.024			18.87		0.051	
	11.36		0.032			18.95		0.027	

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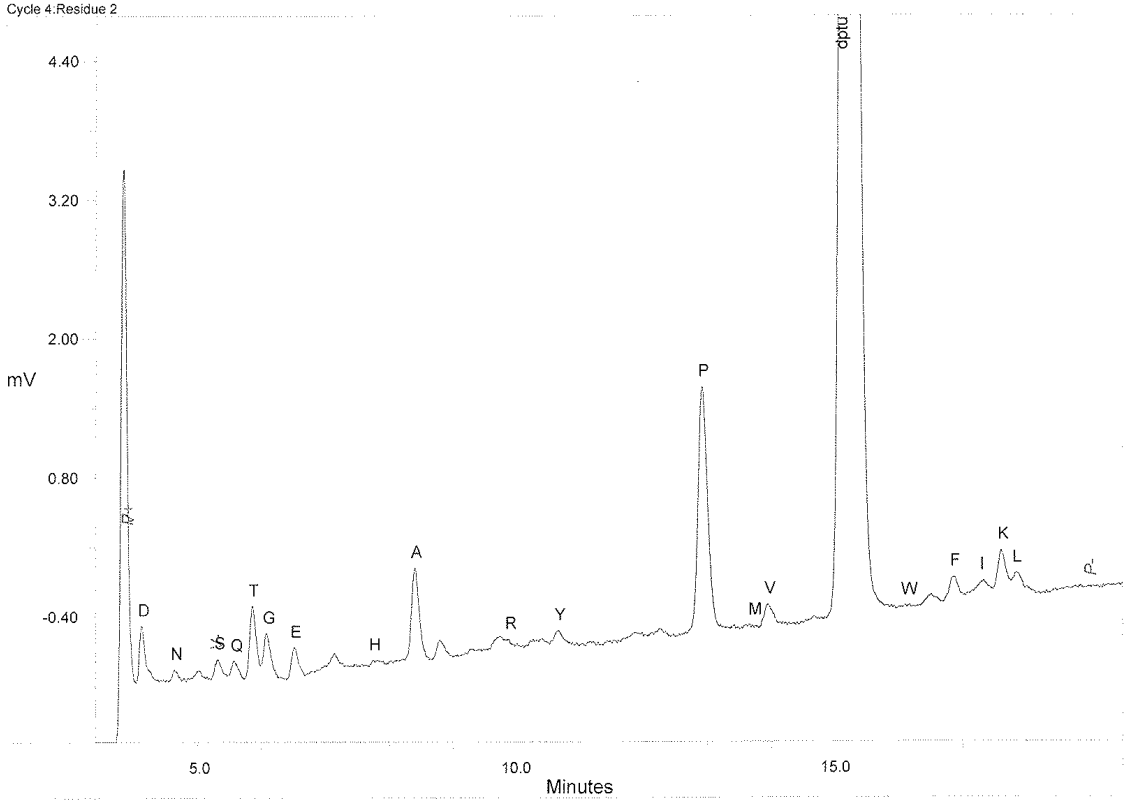
MM 10.10.11

SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 4:Residue 2



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.11	4.12	0.482	1.822		11.29		0.023	
	4.42		0.015			11.43		0.038	
N	4.63	4.63	0.101	0.370		11.53		0.029	
	5.02		0.079			11.87		0.038	
S	5.32	5.30	0.178	1.040		12.14		0.025	
Q	5.58	5.56	0.158	0.706		12.26		0.046	
T	5.86	5.84	0.630	3.993		12.47		0.026	
G	6.08	6.07	0.389	2.153		12.67		0.023	
E	6.52	6.51	0.266	1.093	P	12.92	12.92	2.109	12.690
	6.81		0.030			13.42		0.031	
	6.99		0.052			13.60		0.019	
	7.15		0.135		M	13.70	13.63	0.026	0.134
	7.40		0.014		V	13.95	13.95	0.197	1.051
	7.58		0.024			14.13		0.016	
	7.61		0.017			14.54		0.023	
H	7.74	7.75	0.046	0.259		14.68		0.036	
	7.83		0.030		dptu	15.13	15.12	7.473	45.275
	8.03		0.031			15.30		17.897	
	8.13		0.034			15.89		0.021	
	8.20		0.037			16.02		0.017	
A	8.40	8.39	0.810	4.352	W	16.10	16.21	0.023	0.109
	8.79		0.161			16.18		0.023	
	9.07		0.016			16.32		0.012	
	9.30		0.039			16.52		0.084	
	9.40		0.022		F	16.87	16.86	0.207	1.115
	9.74		0.057		I	17.34	17.33	0.131	0.857
R	9.88	9.88	0.030	0.206	K	17.61	17.62	0.367	1.356
	10.10		0.018		L	17.85	17.86	0.147	0.836
	10.26		0.028			18.19		0.021	
	10.42		0.037			18.26		0.018	
Y	10.67	10.65	0.118	0.627		18.45		0.032	
	10.96		0.025			18.63		0.031	
	11.05		0.026			18.75		0.030	
	11.17		0.021			18.85		0.031	

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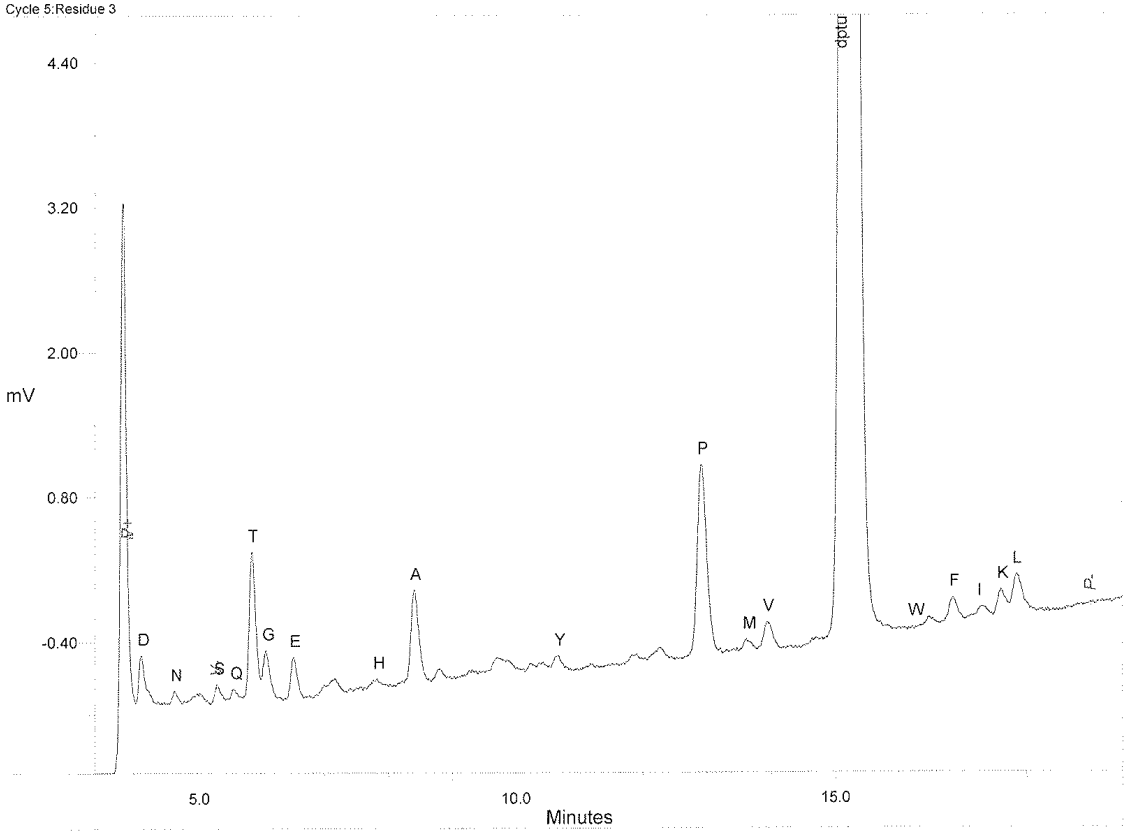
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
						11.60		0.021	
	4.47		0.020			11.82		0.057	
D	4.12	4.12	0.397	1.502		11.90		0.054	
N	4.63	4.63	0.107	0.392		12.04		0.018	
	4.79		0.022			12.26		0.099	
	5.04		0.073			12.91	12.92	1.598	9.614
S	5.32	5.30	0.160	0.932	P	13.21		0.048	
Q	5.58	5.56	0.109	0.489		13.31		0.041	
T	5.85	5.84	1.234	7.825		13.47		0.048	
G	6.08	6.07	0.409	2.263		13.62	13.63	0.113	0.581
E	6.51	6.51	0.351	1.443	M	13.93	13.95	0.240	1.281
	6.76		0.021		V	14.20		0.018	
	6.99		0.089			14.38		0.017	
	7.16		0.127			14.43		0.015	
	7.40		0.034			14.64		0.052	
	7.57		0.033			14.70		0.047	
H	7.82	7.75	0.066	0.368		15.12	15.12	7.308	44.276
	8.21		0.036		dptu	15.30		17.709	
A	8.40	8.39	0.770	4.137		16.02		0.021	
	8.80		0.090			16.22	16.21	0.028	0.129
	9.03		0.023		W	16.48		0.039	
	9.27		0.028			16.86	16.86	0.203	1.095
	9.43		0.024		F	17.30	17.33	0.092	0.602
	9.48		0.020		I	17.61	17.62	0.208	0.770
	9.70		0.038		K	17.85	17.86	0.308	1.749
	10.24		0.068		L	18.25		0.028	
	10.36		0.065			18.34		0.035	
	10.43		0.068			18.42		0.020	
Y	10.67	10.65	0.110	0.580		18.52		0.018	
	10.97		0.019			18.63		0.027	
	11.21		0.040			18.85		0.021	
	11.46		0.023						
	11.51		0.029						

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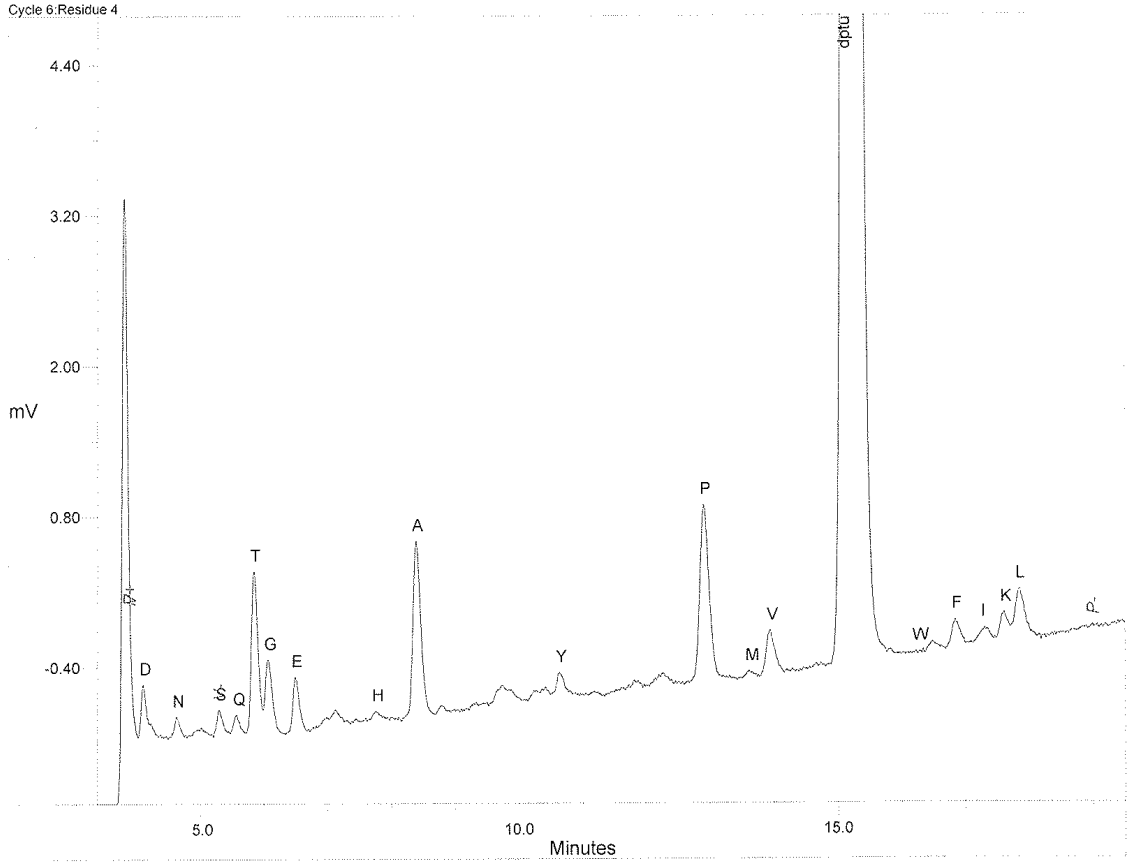
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.12	0.374	1.417		11.65		0.029	
	4.37		0.021			11.74		0.028	
	4.45		0.020			11.82		0.037	
N	4.62	4.63	0.167	0.611		12.28		0.079	
	4.96		0.017			12.47		0.024	
S	5.30	5.30	0.199	1.162		12.66		0.024	
Q	5.57	5.56	0.148	0.662	P	12.91	12.92	1.398	8.413
T	5.84	5.84	1.295	8.213	M	13.62	13.63	0.061	0.312
G	6.06	6.07	0.590	3.262	V	13.95	13.95	0.360	1.924
E	6.50	6.51	0.446	1.834		14.21		0.027	
	6.70		0.020			14.29		0.029	
	6.99		0.094			14.45		0.028	
	7.13		0.137			14.67		0.044	
	7.30		0.035			14.73		0.039	
	7.45		0.026		dptu	15.12	15.12	8.269	50.094
	7.60		0.027			15.29		23.001	
H	7.76	7.75	0.063	0.354		15.98		0.012	
	7.99		0.024		W	16.23	16.21	0.026	0.119
A	8.38	8.39	1.412	7.585		16.28		0.023	
	8.78		0.058			16.49		0.081	
	9.18		0.020		F	16.85	16.86	0.221	1.195
	9.33		0.050		I	17.31	17.33	0.036	0.235
	9.49		0.034		K	17.62	17.62	0.209	0.771
	9.75		0.074		L	17.85	17.86	0.390	2.218
	10.27		0.077			18.25		0.030	
	10.36		0.079			18.40		0.031	
	10.42		0.091			18.53		0.024	
Y	10.64	10.65	0.190	1.003		18.71		0.018	
	11.06		0.016			18.84		0.015	
	11.19		0.021						

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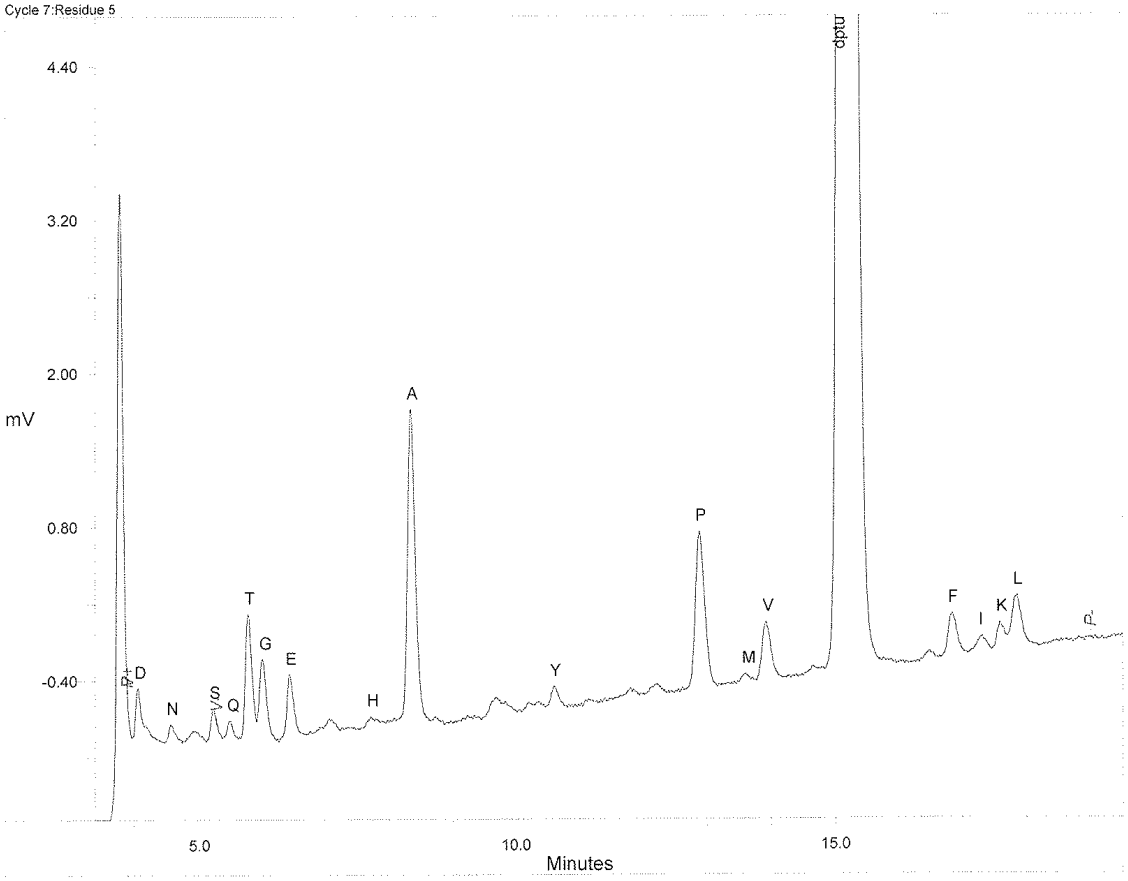
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.06	4.12	0.346	1.310		10.94		0.020	
N	4.57	4.63	0.145	0.531		11.14		0.040	
	4.93		0.040			11.34		0.021	
S	5.25	5.30	0.262	1.528		11.40		0.028	
Q	5.52	5.56	0.165	0.739		11.51		0.013	
T	5.80	5.84	1.000	6.342		11.64		0.011	
G	6.03	6.07	0.644	3.564		11.80		0.040	
E	6.45	6.51	0.511	2.101		12.21		0.059	
	6.78		0.038			12.62		0.038	
	6.96		0.058		P	12.88	12.92	1.236	7.439
	7.08		0.114			13.19		0.017	
	7.28		0.024		M	13.60	13.63	0.060	0.309
	7.37		0.021		V	13.93	13.95	0.456	2.435
H	7.71	7.75	0.057	0.316		14.39		0.027	
	7.98		0.023			14.66		0.041	
	8.07		0.034			14.79		0.016	
A	8.34	8.39	2.428	13.045	dptu	15.10	15.12	7.824	47.402
	8.72		0.035			15.28		19.725	
	8.91		0.018			15.78		0.023	
	8.97		0.022			16.50		0.081	
	9.15		0.022		F	16.84	16.86	0.342	1.845
	9.23		0.028		I	17.32	17.33	0.125	0.816
	9.34		0.033		K	17.59	17.62	0.206	0.759
	9.69		0.094		L	17.86	17.86	0.393	2.234
	10.07		0.020			18.30		0.019	
	10.20		0.070			18.46		0.023	
	10.36		0.068			18.54		0.020	
Y	10.60	10.65	0.166	0.880		18.81		0.029	
	10.84		0.026						

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MIM 18.10.11

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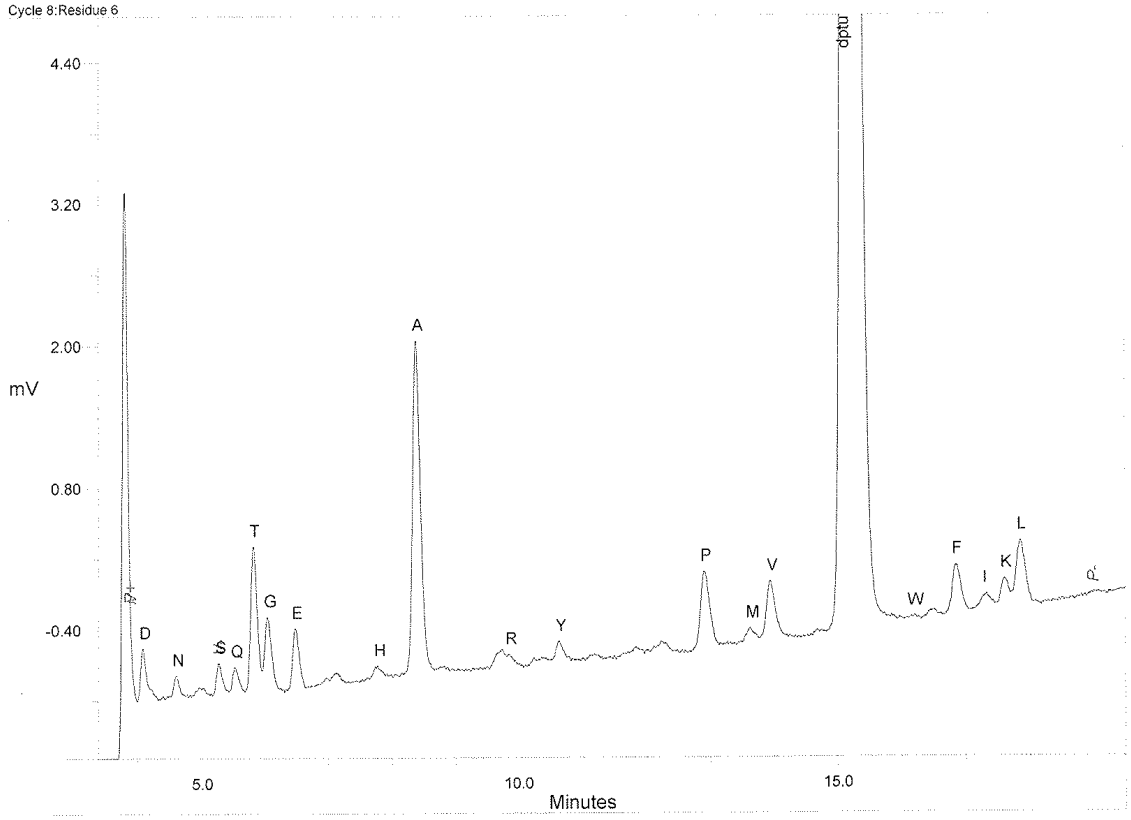
SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 8:Residue 6



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.12	0.443	1.678
	4.40		0.034	
N	4.62	4.63	0.183	0.668
	4.98		0.034	
S	5.29	5.30	0.266	1.554
Q	5.54	5.56	0.218	0.974
T	5.83	5.84	1.220	7.737
G	6.06	6.07	0.616	3.407
E	6.49	6.51	0.531	2.183
	6.98		0.075	
	7.13		0.103	
	7.31		0.021	
	7.42		0.017	
	7.53		0.019	
H	7.77	7.75	0.100	0.559
	7.98		0.023	
	8.06		0.024	
	8.15		0.033	
A	8.38	8.39	2.818	15.138
	8.76		0.012	
	9.16		0.018	
	9.50		0.020	
	9.74		0.152	
R	9.85	9.88	0.101	0.696
	10.10		0.020	
	10.23		0.074	
	10.35		0.072	
Y	10.64	10.65	0.198	1.047
	10.80		0.062	
	10.84		0.042	
	11.02		0.038	
	11.19		0.063	
	11.44		0.031	
	11.50		0.034	
	11.83		0.039	
	12.01		0.035	
	12.11		0.032	
	12.23		0.039	
	12.48		0.022	
	12.56		0.026	
	12.68		0.015	
P	12.90	12.92	0.661	3.979
	13.26		0.025	
	13.48		0.023	
M	13.61	13.63	0.128	0.660
V	13.94	13.95	0.507	2.708
	14.25		0.024	
	14.52		0.015	
	14.67		0.047	
	14.75		0.029	
dptu	15.11	15.12	8.154	49.398
	15.29		20.862	
	15.97		0.027	
	16.10		0.011	
W	16.16	16.21	0.014	0.063
	16.51		0.071	
F	16.85	16.86	0.428	2.307
I	17.33	17.33	0.150	0.982
K	17.60	17.62	0.263	0.970
L	17.86	17.86	0.568	3.229
	18.10		0.025	
	18.28		0.024	
	18.52		0.029	
	18.81		0.027	
	18.88		0.024	

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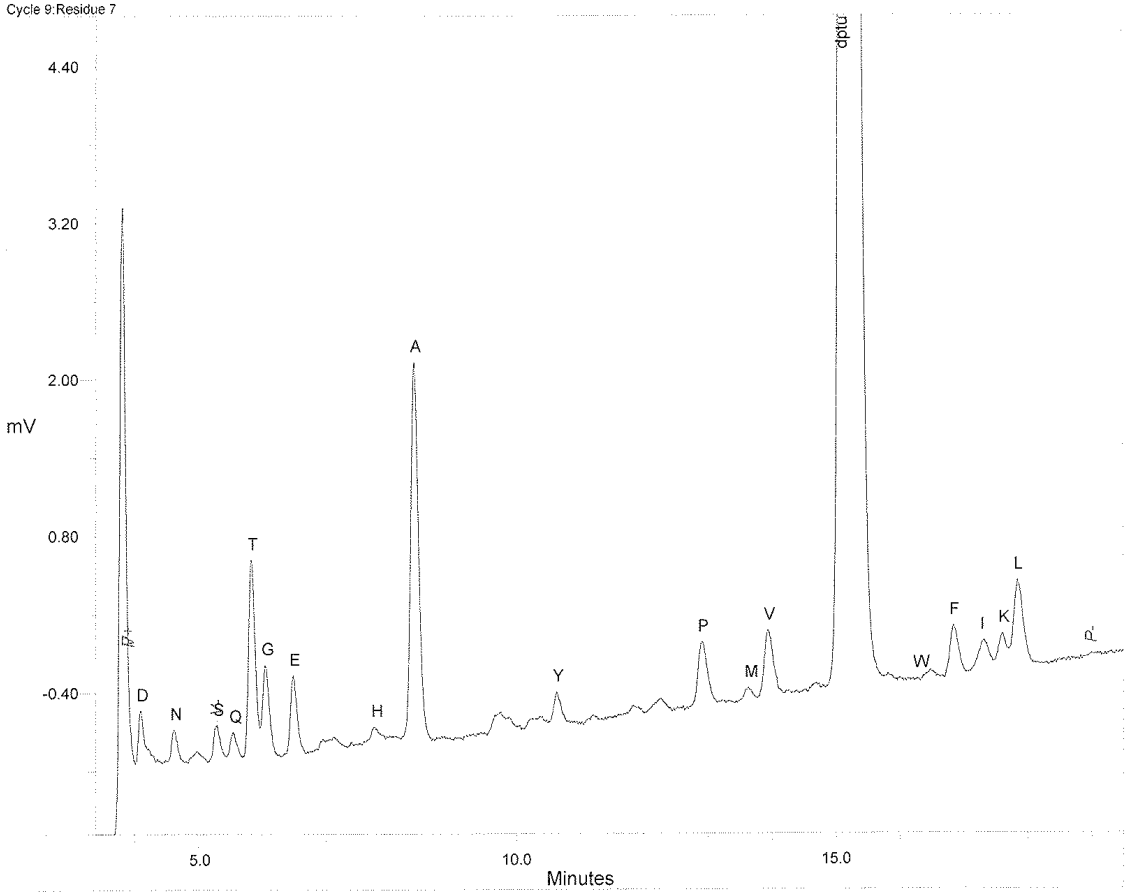
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 9:Residue 7



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.12	0.374	1.414
N	4.62	4.63	0.258	0.943
	4.97		0.087	
S	5.30	5.30	0.274	1.598
Q	5.55	5.56	0.214	0.955
T	5.84	5.84	1.526	9.679
G	6.05	6.07	0.711	3.934
E	6.50	6.51	0.611	2.512
	6.96		0.092	
	7.12		0.102	
	7.41		0.031	
H	7.77	7.75	0.091	0.511
	8.00		0.028	
	8.09		0.028	
A	8.38	8.39	2.896	15.558
	8.86		0.027	
	9.04		0.015	
	9.31		0.027	
	9.44		0.023	
	9.75		0.090	
	10.25		0.075	
	10.38		0.084	
Y	10.63	10.65	0.245	1.297
	11.21		0.060	
	11.33		0.022	
	11.47		0.024	
	11.55		0.015	
	11.83		0.034	
	12.26		0.093	
	12.62		0.036	
P	12.90	12.92	0.493	2.968
	13.17		0.011	
	13.32		0.017	
M	13.63	13.63	0.100	0.513
V	13.93	13.95	0.512	2.736
	14.40		0.027	
	14.53		0.026	
	14.70		0.024	
	14.80		0.020	
dptu	15.12	15.12	8.619	52.214
	15.29		23.304	
	15.82		0.062	
	16.01		0.032	
W	16.28	16.21	0.022	0.103
	16.49		0.064	
	16.67		0.015	
F	16.85	16.86	0.391	2.109
I	17.33	17.33	0.241	1.578
K	17.62	17.62	0.270	0.997
L	17.85	17.86	0.661	3.759
	18.30		0.018	
	18.43		0.025	
	18.48		0.021	
	18.65		0.027	
	18.83		0.028	

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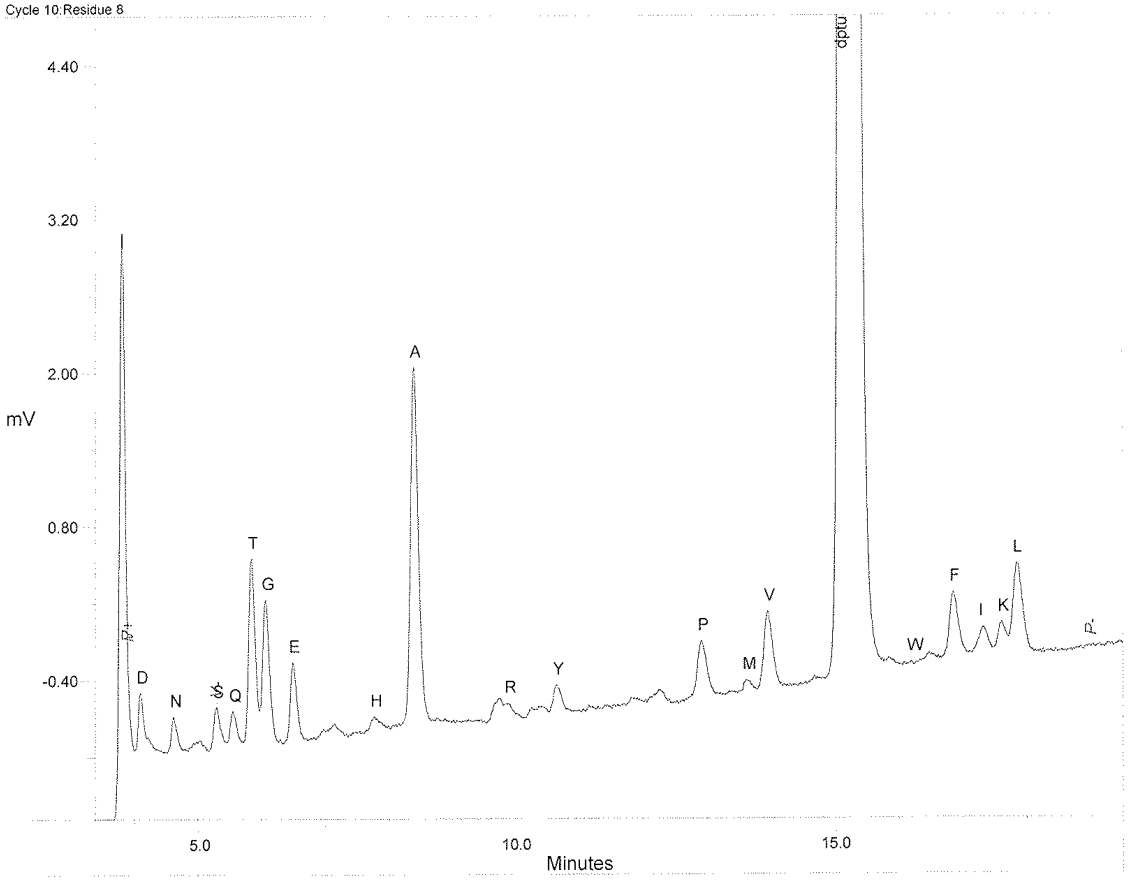
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.12	0.409	1.546
N	4.62	4.63	0.273	0.998
	5.03		0.088	
S	5.30	5.30	0.337	1.965
Q	5.55	5.56	0.292	1.308
T	5.84	5.84	1.463	9.276
G	6.06	6.07	1.126	6.230
E	6.49	6.51	0.621	2.555
	6.77		0.013	
	6.97		0.075	
	7.15		0.107	
	7.49		0.024	
	7.54		0.024	
H	7.77	7.75	0.040	0.226
	8.15		0.032	
A	8.38	8.39	2.791	14.992
	8.88		0.024	
	9.01		0.026	
	9.24		0.023	
	9.74		0.160	
R	9.87	9.88	0.104	0.717
	10.24		0.071	
	10.40		0.071	
Y	10.64	10.65	0.212	1.119
	11.05		0.022	
	11.14		0.044	
	11.29		0.026	
	11.43		0.019	
	11.55		0.024	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.70		0.023	
	11.81		0.042	
	12.04		0.022	
	12.26		0.099	
	12.53		0.024	
	12.60		0.023	
P	12.91	12.92	0.445	2.679
	13.38		0.029	
M	13.60	13.63	0.098	0.503
V	13.94	13.95	0.607	3.242
	14.46		0.018	
	14.53		0.023	
	14.68		0.034	
dptu	15.12	15.12	9.411	57.015
	15.29		26.901	
	15.85		0.060	
	16.10		0.030	
W	16.20	16.21	0.029	0.133
	16.48		0.038	
F	16.86	16.86	0.514	2.774
	17.12		0.031	
I	17.32	17.33	0.224	1.467
K	17.61	17.62	0.252	0.931
L	17.85	17.86	0.704	4.001
	18.25		0.030	
	18.33		0.019	
	18.44		0.024	
	18.68		0.021	
	18.84		0.024	

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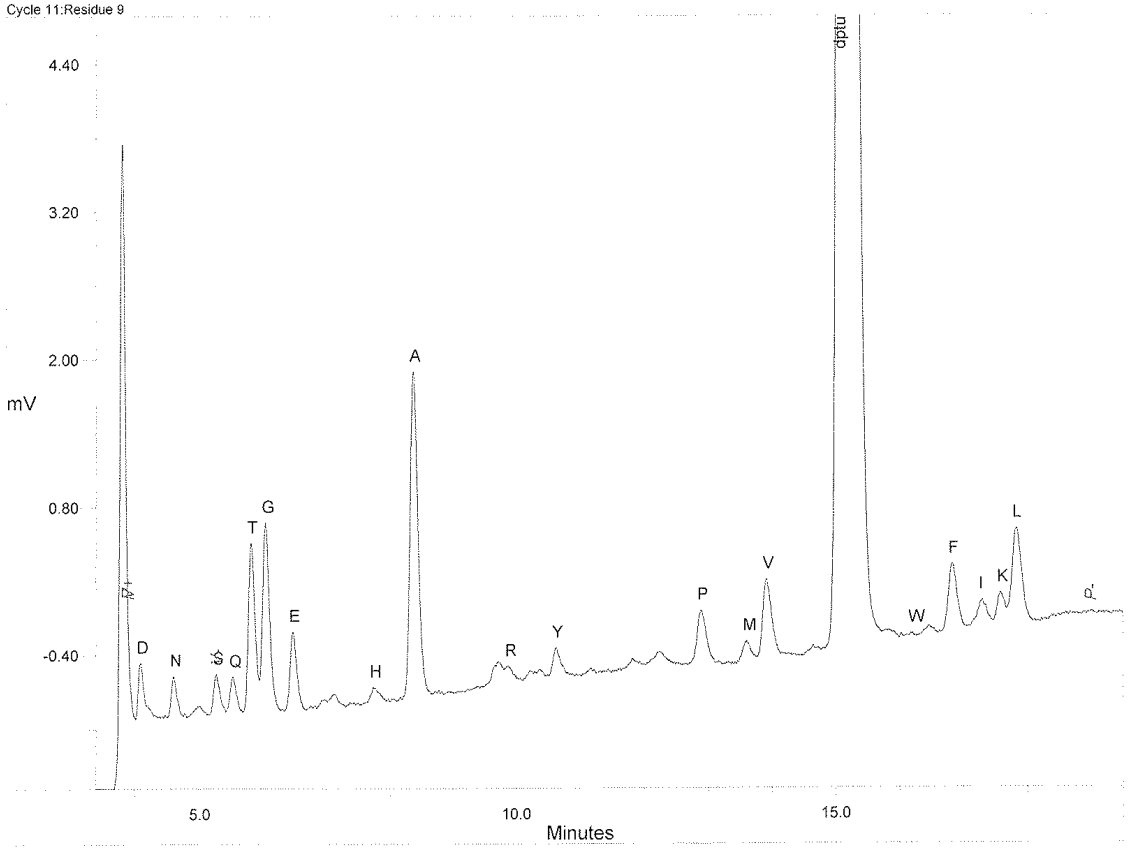
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 11:Residue 9



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.09	4.12	0.410	1.550
	4.49		0.022	
N	4.62	4.63	0.328	1.200
	5.03		0.087	
S	5.30	5.30	0.330	1.928
Q	5.55	5.56	0.294	1.313
T	5.83	5.84	1.373	8.706
G	6.06	6.07	1.536	8.495
E	6.49	6.51	0.646	2.656
	6.77		0.025	
	6.97		0.071	
	7.14		0.109	
	7.38		0.035	
	7.44		0.029	
	7.51		0.018	
H	7.74	7.75	0.135	0.756
	7.97		0.029	
	8.07		0.021	
	8.17		0.027	
A	8.38	8.39	2.653	14.253
	8.72		0.017	
	8.95		0.021	
	9.08		0.015	
	9.22		0.021	
	9.39		0.026	
	9.71		0.178	
R	9.88	9.88	0.119	0.820
	10.22		0.074	
	10.38		0.077	
Y	10.62	10.65	0.235	1.245
	11.17		0.032	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.32		0.021	
	11.45		0.035	
	11.63		0.024	
	11.81		0.093	
	11.93		0.057	
	12.24		0.118	
	12.65		0.014	
P	12.89	12.92	0.448	2.695
	13.17		0.029	
	13.32		0.022	
M	13.60	13.63	0.170	0.876
V	13.92	13.95	0.639	3.412
	14.65		0.037	
dptu	15.10	15.12	9.475	57.400
	15.27		24.719	
	15.79		0.014	
	16.03		0.039	
W	16.21	16.21	0.032	0.148
	16.46		0.073	
F	16.83	16.86	0.548	2.956
I	17.30	17.33	0.225	1.472
K	17.59	17.62	0.264	0.974
L	17.83	17.86	0.774	4.401
	18.28		0.022	
	18.42		0.031	
	18.48		0.027	
	18.60		0.031	
	18.67		0.016	
	18.77		0.013	
	18.91		0.019	

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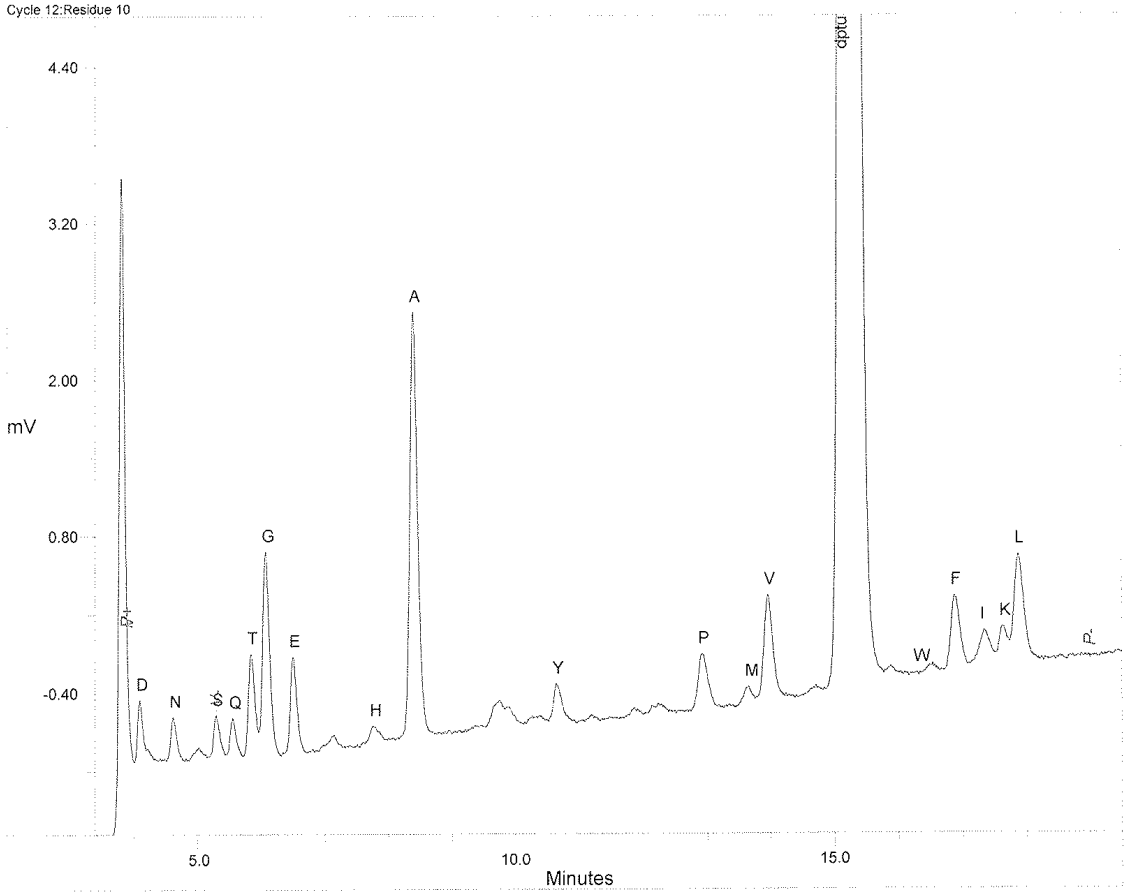
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.12	0.435	1.647
	4.43		0.021	
N	4.63	4.63	0.337	1.232
	5.02		0.099	
S	5.29	5.30	0.341	1.992
Q	5.55	5.56	0.311	1.393
T	5.84	5.84	0.796	5.048
G	6.07	6.07	1.572	8.698
E	6.50	6.51	0.750	3.085
	6.81		0.023	
	6.97		0.047	
	7.14		0.106	
	7.40		0.019	
	7.60		0.035	
H	7.76	7.75	0.134	0.746
A	8.39	8.39	3.258	17.500
	8.89		0.022	
	8.95		0.029	
	9.04		0.019	
	9.20		0.020	
	9.36		0.036	
	9.46		0.025	
	9.75		0.112	
	10.26		0.056	
	10.38		0.060	
Y	10.64	10.65	0.293	1.551
	11.07		0.016	
	11.18		0.037	
	11.35		0.017	
	11.46		0.019	
	11.68		0.013	
	11.85		0.035	
	12.13		0.043	
	12.22		0.029	
	12.55		0.022	
	12.68		0.019	
P	12.92	12.92	0.425	2.560
	13.33		0.026	
	13.40		0.024	
M	13.65	13.63	0.137	0.706
V	13.95	13.95	0.805	4.297
	14.71		0.061	
dptu	15.13	15.12	10.034	60.789
	15.31		26.391	
	15.88		0.066	
W	16.30	16.21	0.025	0.117
	16.53		0.080	
F	16.87	16.86	0.577	3.112
I	17.33	17.33	0.279	1.824
K	17.63	17.62	0.287	1.059
L	17.87	17.86	0.824	4.686
	18.24		0.026	
	18.52		0.033	
	18.73		0.031	
	18.87		0.024	

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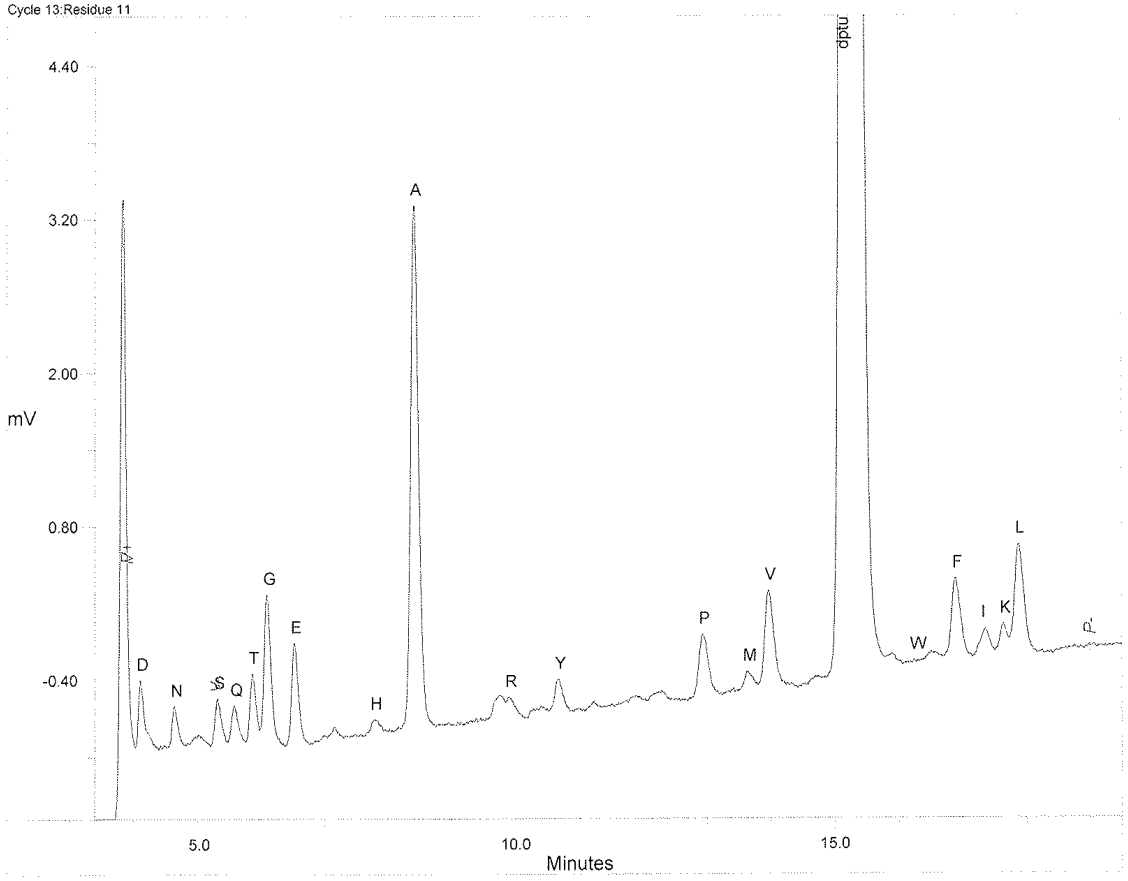
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 13:Residue 11



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.11	4.12	0.530	2.007
	4.48		0.027	
N	4.64	4.63	0.315	1.153
	4.86		0.018	
	5.01		0.081	
S	5.32	5.30	0.372	2.171
Q	5.57	5.56	0.314	1.405
T	5.86	5.84	0.561	3.560
G	6.08	6.07	1.178	6.514
E	6.52	6.51	0.802	3.298
	6.85		0.024	
	6.98		0.054	
	7.15		0.100	
	7.48		0.021	
H	7.78	7.75	0.112	0.625
	8.03		0.024	
	8.17		0.033	
A	8.40	8.39	4.087	21.959
	8.80		0.027	
	8.95		0.029	
	9.38		0.037	
	9.46		0.033	
	9.76		0.180	
R	9.89	9.88	0.150	1.032
	10.27		0.030	
	10.40		0.021	
Y	10.68	10.65	0.250	1.323
	10.95		0.028	
	11.23		0.069	

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	11.50		0.026	
	11.74		0.031	
	11.88		0.058	
	12.03		0.023	
	12.24		0.018	
	12.73		0.022	
P	12.93	12.92	0.489	2.942
	13.33		0.022	
	13.42		0.034	
M	13.63	13.63	0.144	0.741
V	13.97	13.95	0.742	3.960
	14.32		0.023	
	14.74		0.060	
dptu	15.15	15.12	10.396	62.982
	15.32		33.178	
	15.90		0.079	
	16.15		0.014	
W	16.24	16.21	0.023	0.105
	16.51		0.027	
F	16.89	16.86	0.617	3.327
I	17.35	17.33	0.226	1.474
K	17.65	17.62	0.252	0.929
L	17.89	17.86	0.867	4.933
	18.26		0.027	
	18.34		0.018	
	18.48		0.023	
	18.62		0.029	
	18.76		0.034	

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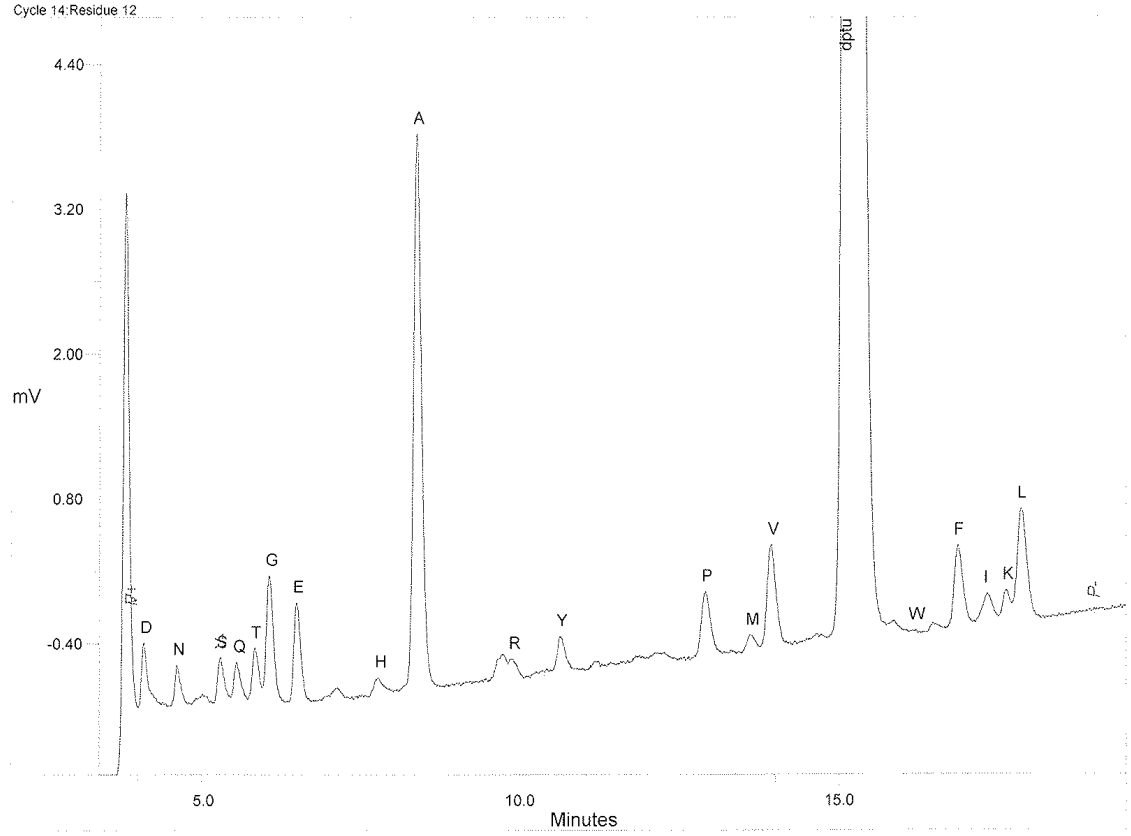
SequencePro™

SGS M-Scan Ltd
SGS M-Scan Job: 22745
Sample: 97248
Operator: MIM

Cycle 14:Residue 12



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.10	4.12	0.529	2.001		11.57		0.017	
	4.39		0.026			11.69		0.020	
N	4.61	4.63	0.335	1.225		11.82		0.019	
	5.02		0.082			12.16		0.051	
S	5.29	5.30	0.379	2.213		12.25		0.054	
Q	5.55	5.56	0.343	1.532		12.58		0.028	
T	5.83	5.84	0.455	2.888		12.65		0.029	
G	6.05	6.07	1.053	5.824	P	12.91	12.92	0.551	3.314
	6.34		0.010			13.20		0.029	
E	6.49	6.51	0.822	3.380		13.31		0.039	
	6.85		0.011			13.43		0.020	
	7.13		0.090		M	13.60	13.63	0.137	0.705
	7.36		0.018		V	13.95	13.95	0.840	4.487
	7.53		0.022			14.32		0.028	
H	7.78	7.75	0.139	0.778		14.66		0.073	
	8.07		0.019			14.71		0.063	
A	8.38	8.39	4.599	24.708	dptu	15.12	15.12	10.213	61.873
	8.88		0.017			15.29		28.339	
	8.94		0.020			15.85		0.094	
	9.05		0.031		W	16.15	16.21	0.017	0.080
	9.22		0.033			16.28		-0.015	
	9.31		0.026			16.46		0.059	
	9.47		0.037		F	16.86	16.86	0.674	3.632
	9.72		0.225		I	17.32	17.33	0.247	1.612
R	9.88	9.88	0.175	1.200	K	17.62	17.62	0.253	0.936
	10.11		0.020		L	17.85	17.86	0.909	5.170
	10.26		0.040			18.28		0.017	
	10.35		0.041			18.35		0.021	
	10.42		0.045			18.45		0.031	
Y	10.63	10.65	0.296	1.565		18.77		0.020	
	11.21		0.056			18.84		0.017	
	11.41		0.032			18.95		0.022	

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