APPENDIX 1 – CLINICAL DETAILS OF HUMAN SERA AND THEIR IgG CONCENTRATIONS DETERMINED USING RADIAL IMMUNODIFFUSION

Identifier	Sex	Age	AA Type	Clinical Status	Other Disorders	Whole Serum IgG Concentration (mg/mL)
Alopecia Areata Patient Sera						
AA1	F	36	Au	R	Vitiligo Hypothyroidism	9.37
AA2	F	47	Au	R	Atopy Eczema	10.30
AA3	F	39	Au	A	Hypothyroidism Eczema	10.30
AA4	F	34	Au	R	Thyroid problem	11.30
AA5	F	50	Au	R	Eczema Asthema	12.90
AA6	F	41	AA	A	None	10.80
AA7	F	25	AT	R	Thyroid problem	8.43
AA8	F	62	AT	A	Hypothyroidism Vitiligo Atopy	8.90
AA9	F	32	Ophiasus	R	Candidiasis	12.90
AA10	M	22	AA	A	N/A	15.70
AA11	M	33	AA	A	N/A	N/A
AA12	M	22	AA	A	N/A	10.80
Normal Sera						
N1	F	24	Normal Healthy			8.43
N2	F	26				13.40
N3	M	27				11.90
N4	M	24				9.37
N5	F	26				16.80
N6	F	28				9.85
N7	M	34				7.98
N8	M	28				11.30
N9	F	32				16.80
N10	F	30				13.40
N11	F	25				8.90
N12	F	19				9.85
N13	M	40				21.20
N14	M	48				9.85
N15	F	44				7.54
N16	M	62				10.80
N17	M	41				11.90

F = Female; M = Male; Au = Alopecia Universalis; AT = Alopecia Totalis; AA = Patchy Alopecia;
R = Regrowth; A = Active;

APPENDIX 2 – CHEMICAL REAGENTS AND BUFFERS

General Use

1 L Phosphate Buffered Saline (PBS)

8 g	sodium chloride (Riedel-de Haën, Germany)
0.2 g	potassium chloride (BDH, UK)
1.44 g	sodium phosphate dibasic (BDH, UK)
0.24 g	potassium phosphate monobasic (BDH, UK)
800 mL	dH_2O

Adjusted to pH 7.4 with hydrochloric acid and sodium hydroxide and volume made up to 1 L with dH_2O

IgG Purification

Binding Buffer A (Millipore Corporation, Massachusetts)

1.5 M	glycine/ sodium hydroxide buffer
3 M	sodium chloride

pH 9.0

Elution Buffer B (Millipore Corporation, Massachusetts)

0.2 M	glycine/hydrochloric acid buffer

pH 2.5

Neutralization Buffer C (Millipore Corporation, Massachusetts)

1 M	Tris/ hydrochloric acid buffer

pH 9.0

2 mL 10% Dextran Sulphate

0.2 g	dextran sulphate (Sigma-Aldrich Company Ltd., Dorset)
2 mL	dH_2O

10 mL 1 M Calcium Chloride

1.11 g *calcium chloride* (Sigma-Aldrich Company Ltd., Dorset)

10 mL dH_2O

50 mL Tris-Buffered Saline (TBS)

0.414 g sodium chloride (Riedel-de Haën, Germany)

0.122 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

50 mL dH_2O

Adjust to pH 7.4 with hydrochloric acid and sodium hydroxide

1D SDS-PAGE

500 mL Separation Gel-Tris buffer

90.8 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

2 g sodium dodecyl sulfate (Sigma-Aldrich Company Ltd., Dorset)

500 mL dH_2O

Adjust to pH 8.8 with hydrochloric acid and sodium hydroxide

500 mL Stacking Gel Tris-Buffer

30.3 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

2 g sodium dodecyl sulfate (Sigma-Aldrich Company Ltd., Dorset)

500 mL dH_2O

Adjust to pH 6.9 with hydrochloric acid and sodium hydroxide

9% Separation Gel

7.2 mL AcrylaFLOWGel (30% solution) (National Diagnostics, Inc., Georgia)

2.92 mL Bis-acrylaFLOWGel (2% solution) (National Diagnostics, Inc., Georgia)

6.25 mL Separation Gel Tris-Buffer

7.63 mL dH_2O

30 µL TEMED (Sigma-Aldrich Company Ltd., Dorset)

100 µL 10% ammonium persulfate (Bio-Rad, Hercules, California)

4.5% Stacking Gel

2.2 mL AcrylaFLOWGel (30% solution) (National Diagnostics, Inc., Georgia)

0.9 mL Bis-acrylaFLOWGel (2% solution) (National Diagnostics, Inc., Georgia)

3.75 mL Stacking Gel Tris-Buffer

8.15 mL dH_2O

30 µL TEMED (Sigma-Aldrich Company Ltd., Dorset)

90 µL 10% ammonium persulfate (Bio-Rad, Hercules, California)

1 L Tank Buffer

3 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

14.4 g glycine (Sigma-Aldrich Company Ltd., Dorset)

1 g sodium dodecyl sulfate (Sigma-Aldrich Company Ltd., Dorset)

1 L dH_2O

Check that pH is within 8.4-8.5. Do NOT adjust pH.

Western Blotting

200 mL 0.1% Coomassie Blue Stain

0.2 g Coomassie Blue (BDH, UK)

100 mL dH_2O

80 mL methanol (Fisher Scientific International Company, Loughborough)

20 mL acetic acid (BDH, UK)

Filtered with 3MM Whatman filter paper before use.

1 L Coomassie Blue Destain Solution

500 mL dH_2O

400 mL methanol (Fisher Scientific International Company, Loughborough)

100 mL acetic acid (BDH, UK)

SimplyBlue SafeStain (Invitrogen Corporation, Paisley)

Ready-to-use solution

200 mL 0.1% Fast Green Stain

0.2 g Fast Green (Flatters & Garnett Ltd., Manchester)

200 mL dH_2O

1 L Fast Green Destain Solution

700 mL methanol (Fisher Scientific International Company, Loughborough)

200 mL acetic acid (BDH, UK)

100 mL dH_2O

100 mL 5% Non-Fat Milk Solution

5 g non-fat milk powder (The Original Marvel, Premier International Foods, UK)

100 mL PBS

500 mL PBS/0.05% Tween 20 Solution

500 mL PBS

500 µL Tween 20 (Sigma-Aldrich Company Ltd., Dorset)

30 mL Chromogen Substrate Solution

15 mg 4-chloro-1-napthol (Sigma-Aldrich Company Ltd., Dorset)

5 mL methanol (Fisher Scientific International Company, Loughborough)

25 mL PBS

100 µL hydrogen peroxide (Sigma-Aldrich Company Ltd., Dorset)

Immunoprecipitation (Without Cross-Linking)

800 mL Tris Saline Azide (TSA) Buffer

1.26 g Tris-Cl (Sigma-Aldrich Company Ltd., Dorset)

6.54 g sodium chloride (Riedel-de Haën, Germany)

0.2 g sodium azide (Sigma-Aldrich Company Ltd., Dorset)

800 mL dH_2O

Adjust to pH 8 with hydrochloric acid and sodium hydroxide.

500 mL Radioimmunoprecipitation Assay (RIPA) Buffer

5 g sodium deoxycholate (Sigma-Aldrich Company Ltd., Dorset)

0.5 g sodium dodecyl sulfate (Sigma-Aldrich Company Ltd., Dorset)

5 mL Triton X-100 (Sigma-Aldrich Company Ltd., Dorset)

500 mL TSA

100 mL Tris-Cl Buffer

0.79 g of Tris-Cl (Sigma-Aldrich Company Ltd., Dorset)

100 mL dH_2O

Adjust to pH 6.8 with hydrochloric acid and sodium hydroxide

25 mL Laemmli's Buffer

2.5 mL glycerol (BDH, UK)

0.185 g EDTA (Sigma-Aldrich Company Ltd., Dorset)

1.5 g sodium dodecyl sulfate (Sigma-Aldrich Company Ltd., Dorset)

0.19 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

Make up to 25 mL with dH_2O

Immunoprecipitation (With Cross-Linking)

10 mL PBS/0.65%Tween 20

10 mL PBS

65 µL Tween 20 (Sigma-Aldrich Company Ltd., Dorset)

Adjust to pH 7.5 with hydrochloric acid and sodium hydroxide

10 mL 200 mM Triethanolamine

265 µL triethanolamine (Sigma-Aldrich Company Ltd., Dorset)

10 mL dH_2O

Adjust to pH 8 with hydrochloric acid and sodium hydroxide

500 µL 20 mM Dimethyl Pimelidate Dihydrochloride (DMP) solution (prepare fresh)

0.0026 g DMP (Sigma-Aldrich Company Ltd., Dorset)

0.5 mL 200 mM triethanolamine

10 mL 50 mM Tris

0.061 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

10 mL dH_2O

Adjust to pH 7.5 with hydrochloric acid and sodium hydroxide

10 mL 50 mM Glycine/0.65% Tween 20

0.038 g glycine (Sigma-Aldrich Company Ltd., Dorset)

65 µL Tween 20 (Sigma-Aldrich Company Ltd., Dorset)

10 mL dH_2O

Adjust to pH 2.7 with hydrochloric acid and sodium hydroxide

25 mL Washing Buffer

2.5 mL glycerol (BDH, UK)

0.185 g EDTA (Sigma-Aldrich Company Ltd., Dorset)

0.19 g Trizma base (Sigma-Aldrich Company Ltd., Dorset)

Make up to 25 mL with dH_2O

2D Gel Electrophoresis

Sample Rehydrating Buffer (volume changes depending on sample volume)

8 M urea (Sigma-Aldrich Company Ltd., Dorset)

2% CHAPS Sigma-Aldrich Company Ltd., Dorset)

0.5% ZOOM Carrier Ampholytes (Invitrogen Corporation, Paisley)

0.002% Bromophenol Blue (Sigma-Aldrich Company Ltd., Dorset)

20 mM dithiothreitol (Sigma-Aldrich Company Ltd., Dorset)

5 mL of Reducing Solution

4.5 mL 1X NuPAGE LDS Sample Buffer (Invitrogen Corporation, Paisley)

0.5 mL NuPAGE Sample Reducing Agent (Invitrogen Corporation, Paisley)

5 mL Alkylating Solution

5 mL 1X NuPAGE LDS Sample Buffer (Invitrogen Corporation, Paisley)

116 mg iodoacetamide (Sigma-Aldrich Company Ltd., Dorset)

500 mL Running Buffer

50 mL Novex Tris-Glycine SDS Running Buffer (10X) (Invitrogen Corporation, Paisley)

450 mL dH_2O

500 mL NuPAGE Transfer Buffer

25 mL NuPAGE Transfer Buffer (20X) (Invitrogen Corporation, Paisley)

475 mL dH_2O

Enhanced Chemiluminescence (ECL)

250 mL 1.5 M Tris Solution

30.28 g Trizma Base (Sigma-Aldrich Company Ltd., Dorset)

250 mL dH_2O

Adjust to pH 8.5 with hydrochloric acid and sodium hydroxide

10 mL 0.09 M p-Coumaric acid Solution

0.148 g p-coumaric acid (Sigma-Aldrich Company Ltd., Dorset)

10 mL dimethyl sulfoxide (Sigma-Aldrich Company Ltd., Dorset)

10 mL 0.25 M Luminol Solution

0.443 g Luminol (Fluka and Riedel-de Haën, Sigma-Aldrich Company Ltd., Buchs SG)

10 mL dimethyl sulfoxide (Sigma-Aldrich Company Ltd., Dorset)

25 mL Chemilunescence Solution

22.32 mL dH_2O
2.5 mL 1.5M Tris solution
56 µL 0.09 M p-coumaric acid solution
125 µL Luminol solution
8 µL hydrogen peroxide (Sigma-Aldrich Company Ltd., Dorset)

250 mL Developer Solution

50 mL GBX Developer (Sigma-Aldrich Company Ltd., Dorset)

200 mL tap water

250 mL Fixer Solution

50 mL	GBX Fixer (Sigma-Aldrich Company Ltd., Dorset)
200 mL	tap water

Liquid Chromatography followed by MALDI-TOF/TOF

Solvent A

2%	ACN (Fisher Scientific International Company, Loughborough)
0.05%	TFA (Fluka and Riedel-de Haën, Sigma-Aldrich Company Ltd., Buchs SG)

Solvent B

80%	ACN (Fisher Scientific International Company, Loughborough)
0.05%	TFA (Fluka and Riedel-de Haën, Sigma-Aldrich Company Ltd., Buchs SG)

48 mL 2:1 Ethanol/Acetone

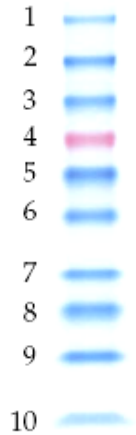
32 mL	ethanol (Fisher Scientific International Company, Loughborough)
16 mL	acetone (Fisher Scientific International Company, Loughborough)

1.2 mL Working Matrix Solution

1.056 mL	2:1 ethanol/acetone
120 µL	saturated HCCA solution of 30% ACN/0.1% TFA
12 µL	100 mM ammonium phosphate monobasic (BDH, UK)
12 µL	10% TFA (Fluka and Riedel-de Haën, Sigma-Aldrich Company Ltd., Buchs SG)

APPENDIX 3 – MOLECULAR WEIGHT LADDERS

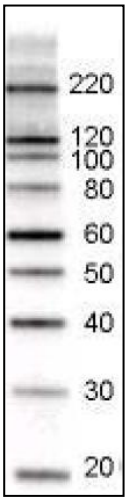
An example of BenchMark Prestained Protein Ladder (Invitrogen Corporation, Paisley) separated on a Novex 4-20% Tris-Glycine gel (in kDa).



Band No.	Average Apparent Molecular Weight
1	~180 kDa
2	~115 kDa
3	~82 kDa
4	**~64 kDa***
5	~49 kDa
6	~37 kDa
7	~26 kDa
8	~19 kDa
9	~15 kDa
10	~6 kDa

*Orientation band (pink in color)

An example of MagicMark XP Western Standard (Invitrogen Corporation, Paisley) separated on a Novex 4-20% Tris-Glycine gel.

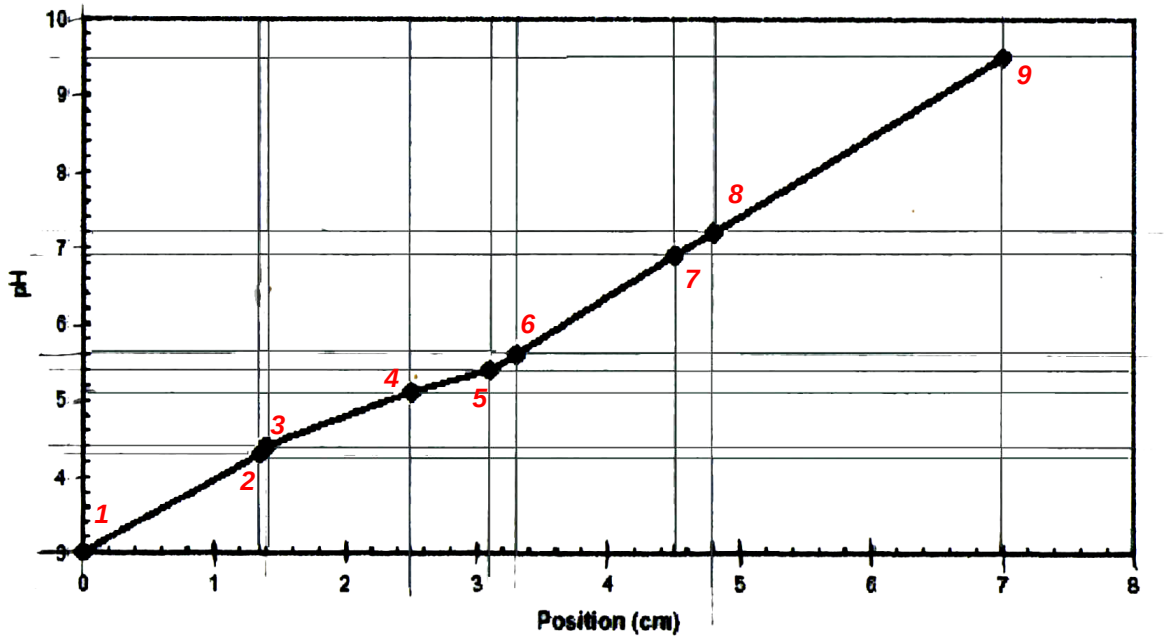


APPENDIX 4 – ANTIBODIES USED IN THIS PROJECT

Antibody Name	Full Description	Commercial Origin
Primary Antibodies		
Rabbit anti-human albumin	Anti-human albumin developed in rabbit IgG fraction of antiserum	Sigma-Aldrich Company Ltd., Dorset
AE1/AE3	Mouse anti-cytokeratin AE1/AE3 monoclonal antibody	Chemicon International, Millipore Corporation, Massachusetts
Trichohyalin	Trichohyalin (N-16)	Santa Cruz Biotechnology Inc., California
Keratin 16	Cytokeratin 16 (C-12)	Santa Cruz Biotechnology Inc., California
Ki67	Ki67 Antigen Ready to Use Liquid Mouse Monoclonal Antibody	Novocastra Laboratories Ltd., Newcastle Upon Tyne
AE15	Mouse anti-trichohyalin AE15 monoclonal antibody	Gift of T. T. Sun from New York University Medical Center
Secondary Antibodies		
Jackson Biotin-SP goat anti-human IgG H+L	Biotin-SP-conjugated AffiniPure Goat Anti-Human IgG (H+L)	Jackson Laboratory, Pennsylvania
Biotin-conjugated bovine anti-rabbit IgG	Bovine anti-rabbit IgG-B, mouse/human adsorbed, Biotin conjugated	Santa Cruz Biotechnology Inc., California
Zymed HRP-conjugated goat anti-human IgG H+L	HRP-Goat Anti-Human IgG (H+L)	Zymed Laboratories, South San Francisco
ICN Biotin-conjugated goat anti-human IgG	Biotin-Conjugated Goat Affinity Purified Antibody To Human IgG (Whole Molecule)	ICN/Cappel biomedicals Inc. (MP Biomedicals), Irvine, CA
ICN Biotin-conjugated goat anti-mouse IgG	Biotin-Conjugated Goat Affinity Purified Antibody To Mouse IgG (Whole Molecule)	ICN/Cappel biomedicals Inc. (MP Biomedicals), Irvine, California
ICN Biotin-conjugated goat anti-rabbit IgG	Biotin-Conjugated Goat Affinity Purified Antibody To Rabbit IgG (Whole Molecule)	ICN/Cappel biomedicals Inc. (MP Biomedicals), Irvine, California
Dako biotinylated goat anti-mouse IgG	Biotinylated goat anti-mouse IgG, ready-to-use, LSAB2 System-HRP	DakoCytomation, Denmark
Alexa 594 donkey anti-mouse IgG	Alexa 594 Donkey anti–mouse IgG (H+L) antibody	Invitrogen Corporation, Paisley
Alexa 488 donkey anti-mouse IgG	Alexa 488 Donkey anti–mouse IgG (H+L) antibody	Invitrogen Corporation, Paisley
FITC goat anti-human IgG	Anti-Human IgG (whole molecule)−FITC antibody produced in goat, IgG fraction of antiserum, buffered aqueous solution	Sigma-Aldrich Company Ltd., Dorset

APPENDIX 5 – GENERATION OF CALIBRATION GRAPHS FOR p*I* CALCULATION OF PROTEIN SEPARATED BY IEF USING ZOOM STRIPS

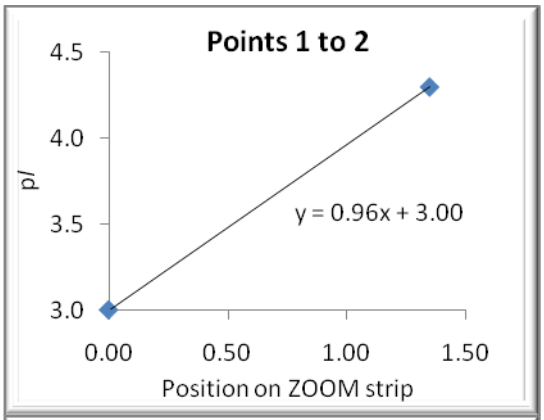
Gradient for a 3-10NL ZOOM® IPG Strip (Invitrogen Corporation, Paisley)

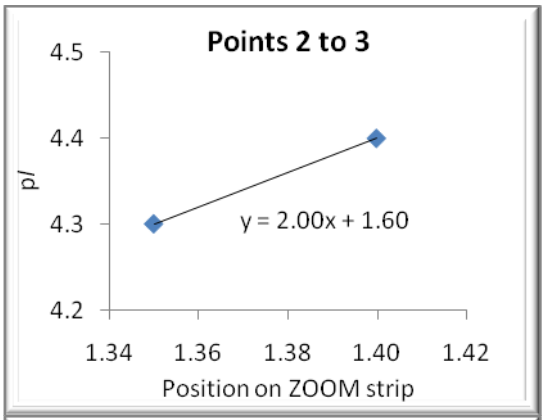


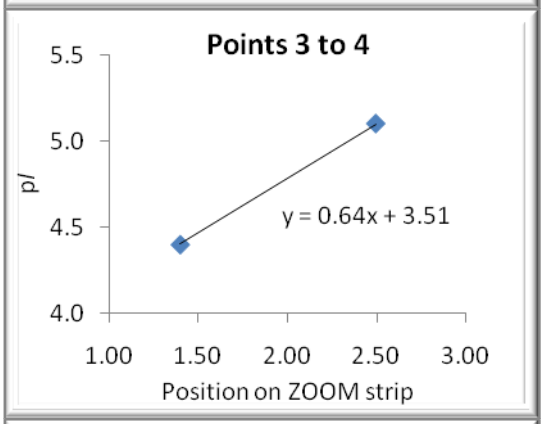
The points 1 to 9 are labelled on the graph. The coordinates (X and Y values) for each of these points were measured and entered in the table below:

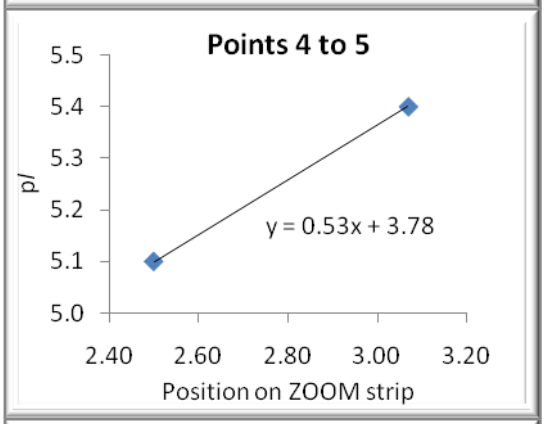
Data Point	1	2	3	4	5	6	7	8	9
X-axis	0.00	1.35	1.40	2.50	3.07	3.30	4.50	4.80	7.00
Y-axis	3.00	4.30	4.40	5.10	5.40	5.60	6.90	7.20	9.50

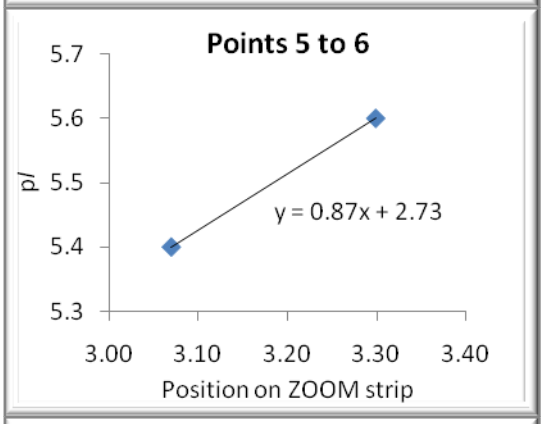
These coordinates were used for producing the calibration graphs (as below) and their linear equations.

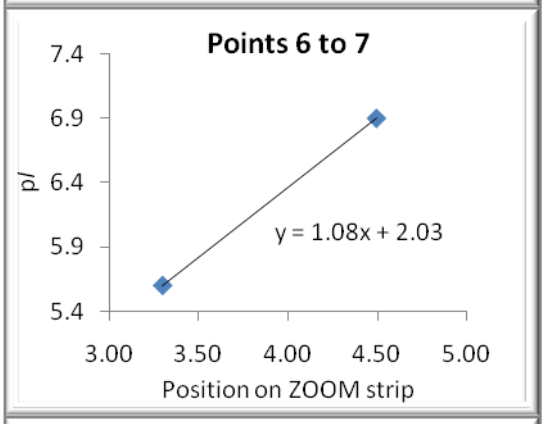

Points 1 to 2
4.5
4.0
3.5
3.0
p/
y = 0.96x + 3.00
0.00
0.50
1.00
1.50
Position on ZOOM strip

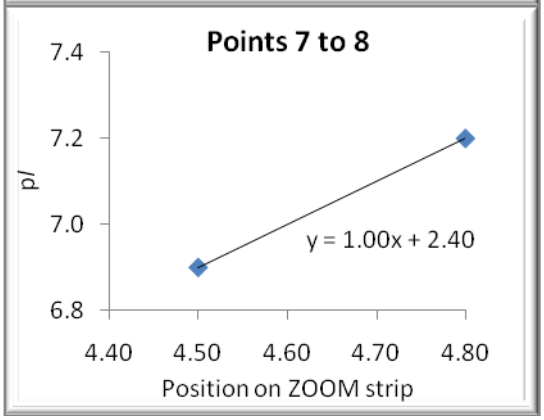

Points 2 to 3
4.5
4.4
4.3
4.2
p/
y = 2.00x + 1.60
1.34
1.36
1.38
1.40
1.42
Position on ZOOM strip

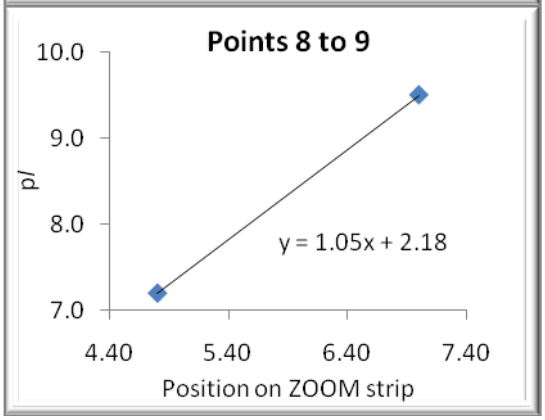

Points 3 to 4
5.5
5.0
4.5
4.0
p/
y = 0.64x + 3.51
1.00
1.50
2.00
2.50
3.00
Position on ZOOM strip


Points 4 to 5
5.5
5.4
5.3
5.2
5.1
5.0
p/
y = 0.53x + 3.78
2.40
2.60
2.80
3.00
3.20
Position on ZOOM strip


Points 5 to 6
5.7
5.6
5.5
5.4
5.3
p/
y = 0.87x + 2.73
3.00
3.10
3.20
3.30
3.40
Position on ZOOM strip


Points 6 to 7
7.4
6.9
6.4
5.9
5.4
p/
y = 1.08x + 2.03
3.00
3.50
4.00
4.50
5.00
Position on ZOOM strip

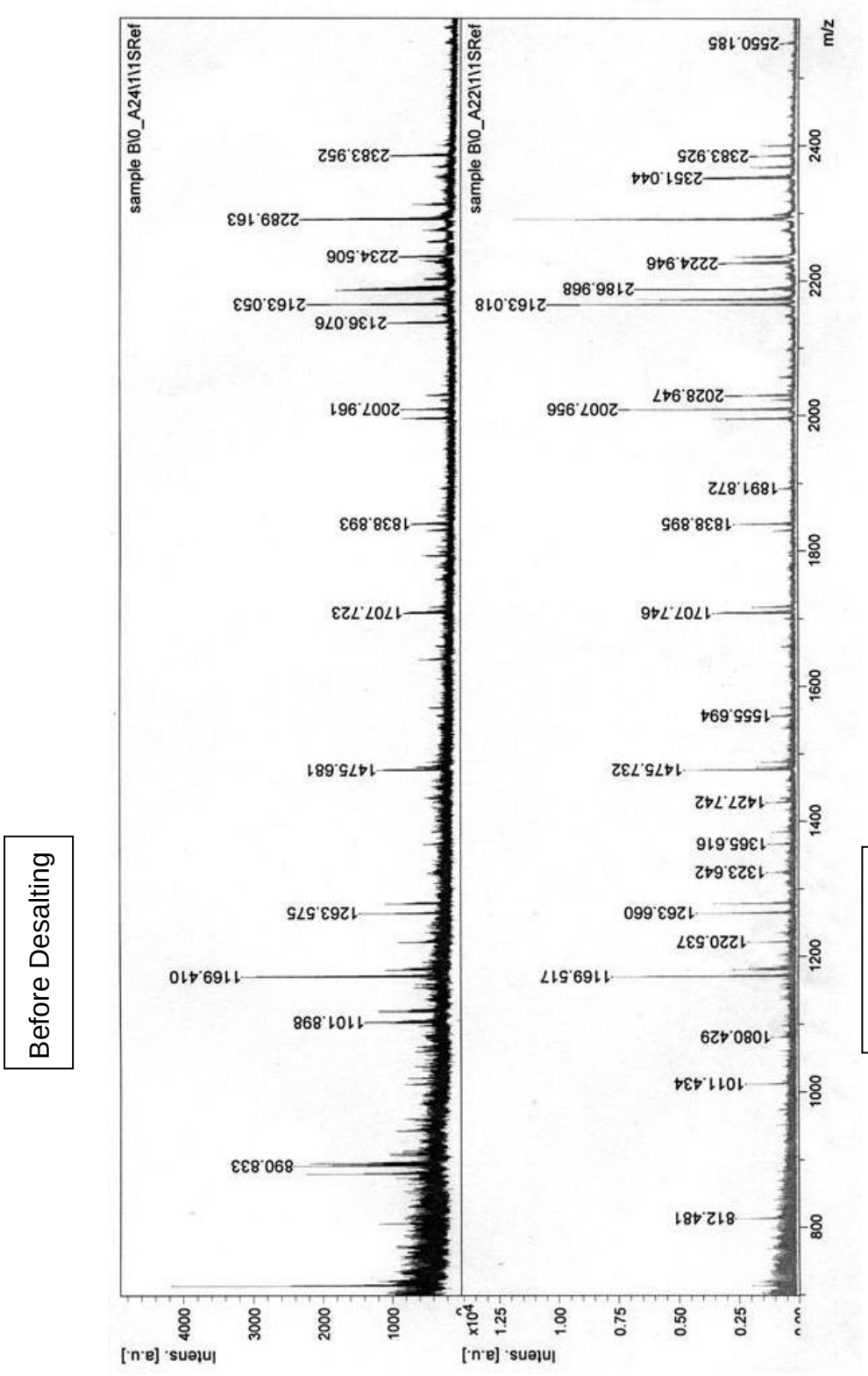

Points 7 to 8
7.4
7.2
7.0
6.8
p/
y = 1.00x + 2.40
4.40
4.50
4.60
4.70
4.80
Position on ZOOM strip


Points 8 to 9
10.0
9.0
8.0
7.0
p/
y = 1.05x + 2.18
4.40
5.40
6.40
7.40
Position on ZOOM strip

APPENDIX 6 – *PEPTIDE CALIBRATION STANDARD II (BRUKER DALTONICS, MASSACHUSETTS) COMPOSITION*

Peptide	Average Ion Mass
Bradykinin 1-7	757.86
Angiotensin II	1047.19
Angiotensin I	1297.49
Substance P	1348.64
Bombesin	1620.86
Renin Substrate	1760.03
ACTH clip 1-17	2094.43
ACTH clip 18-39	2466.68
Somatostatin 28	3149.57

APPENDIX 7 – EXAMPLE MASS SPECTRUM (SPOT B FROM TABLE 6) BEFORE AND AFTER DESALTING



APPENDIX 8 – SEQUENCE HOMOLOGY

Analysed using ClustalW2, EMBL-EBI (http://www.ebi.ac.uk/Tools/clustalw2)
The score represents percentage sequence homology between the two proteins in comparison. An example of one of the sequence alignments is shown at end the end of this appendix.

- Keratin 81 and Keratin 83

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin81	505	2	Keratin83	493	93

- Keratin 81 and 86

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin81	505	2	Keratin86	486	93

- Keratin 83 and Keratin 86

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin83	493	2	Keratin86	486	88

- Keratin 5 and Keratin 6a

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin5	590	2	Keratin6a	564	79

- Keratin 5 and Keratin 6b

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin5	590	2	Keratin6b	564	79

- Keratin 5 and Keratin 6c

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin5	590	2	Keratin6c	564	78

- Keratin 6a and Keratin 6b

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin6a	564	2	Keratin6b	564	98

- Keratin 6a and Keratin 6c

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin6a	564	2	Keratin6c	564	98

- Keratin 6b and Keratin 6c

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	Keratin6b	564	2	Keratin6c	564	99

Sequence Alignment for Keratin 5 and Keratin 6a

```
CLUSTAL 2.0.8 multiple sequence alignment


Keratin5        -MSRQSSVSFRSGGSRSFSTASAITPSVSRTSFTSVSRSGGGGGGGFGRVSLAGACGVGG 59
Keratin6a       MASTSTTIRSHSSSRRGFSANSARLPGVSRSGFSSVSVSRSRGSGGLG-----GACGGAG 55
                  * .:::  :*.. *.**: **  *.***:.*:*** * . *.**:*     **** .*

Keratin5        YGSRSLYNLGGSKRISISTSGGSFRNRFGAGAGGGYGFGGGAGSGFGFGGGAGGGFGLGG 119
Keratin6a       FGSRSLYGLGGSKRISIGGGSCAISGGYGSRAGGSYGFGG-AGSGFGFGGGAGIGFGLGG 114
                :******.*********. .. :: . :*: ***.***** ************ ******

Keratin5        GAGFGGGFGGPGFPVCPPGGIQEVTVNQSLLTPLNLQIDPSIQRVRTEEREQIKTLNNKF 179
Keratin6a       GAGLAGGFGGPGFPVCPPGGIQEVTVNQSLLTPLNLQIDPTIQRVRAEEREQIKTLNNKF 174
                ***:.**************************************:*****:**********

Keratin5        ASFIDKVRFLEQQNKVLDTKWTLLQEQGTKTVRQNLEPLFEQYINNLRRQLDSIVGERGR 239
Keratin6a       ASFIDKVRFLEQQNKVLETKWTLLQEQGTKTVRQNLEPLFEQYINNLRRQLDSIVGERGR 234
                ****************:*********************************************

Keratin5        LDSELRNMQDLVEDFKNKYEDEINKRTTAENEFVMLKKDVDAAYMNKVELEAKVDALMDE 299
Keratin6a       LDSELRGMQDLVEDFKNKYEDEINKRTAAENEFVTLKKDVDAAYMNKVELQAKADTLTDE 294
                ******.********************:****** ***************:**.*:* **

Keratin5        INFMKMFFDAELSQMQTHVSDTSVVLSMDNNRNLDLDSIIAEVKAQYEEIANRSRTEAES 359
Keratin6a       INFLRALYDAELSQMQTHISDTSVVLSMDNNRNLDLDSIIAEVKAQYEEIAQRSRAEAES 354
                ***:: ::***********:**********************************:***:****

Keratin5        WYQTKYEELQQTAGRHGDDLRNTKHEISEMNRMIQRLRAEIDNVKKQCANLQNAIADAEQ 419
Keratin6a       WYQTKYEELQVTAGRHGDDLRNTKQEIAEINRMIQRLRSEIDHVKKQCANLQAAIADAEQ 414
                ********** *************:**:*:********:***:********* *******

Keratin5        RGELALKDARNKLAELEEALQKAKQDMARLLREYQELMNTKLALDVEIATYRKLLEGEEC 479
Keratin6a       RGEMALKDAKNKLEGLEDALQKAKQDLARLLKEYQELMNVKLALDVEIATYRKLLEGEEC 474
                ***:*****:***  **:*********:****:*******.********************

Keratin5        RLSGEGVGPVNISVVTSSVSSGYGSGSGYGGGLGGGLGGGLGGGLAGGSSGSYYSSSSGG 539
Keratin6a       RLNGEGVGQVNISVVQSTVSSGYGGASGVGSGLG----------LGGGSSYSY------- 517
                **.***** ****** *:******..** *.***          *.**** **

Keratin5        VGLGGGLSVGGSGFSASSGRGLGVGFGSGGGSSSSVKFVSTTSSSRKSFKS 590
Keratin6a       ---GSGLGVGG-GFSSSSGRAIGGGLSSVGGGSSTIKYTTTSSSSRKSYKH 564
                   *.**.*** ***:****.:* *:.* **.**::*:.:*:******:*
```

“*” = identical residue

“:” = conserved substitution

“.” = semi-conserved substitution

APPENDIX 9 – ANALYSIS OF EFFECTS OF DIFFERENT CULTURE CONDITIONS ON HAIR SHAFT ELONGATION USING ONE-WAY ANOVA WITH POST-HOC DUNNETT'S TEST

Experiment 1

Control (GPC) vs Control (no serum)	P > 0.05
Control (GPC) vs NaN_3	**P < 0.01**
Control (GPC) vs NaN_3-L	P > 0.05
Control (GPC) vs AA2	P > 0.05
Control (GPC) vs AA7	P > 0.05
Control (GPC) vs N4	P > 0.05
Control (GPC) vs N7	P > 0.05
Control (GPC) vs Anti-THH antibody	**P < 0.05**
Control (GPC) vs Anti-K16 antibody	**P < 0.01**

NaN_3 vs Control (no serum)	**P < 0.01**
NaN_3 vs Control (GPC)	**P < 0.01**
NaN_3 vs NaN_3-L	**P < 0.01**
NaN_3 vs AA2	**P < 0.01**
NaN_3 vs AA7	**P < 0.01**
NaN_3 vs N4	**P < 0.01**
NaN_3 vs N7	**P < 0.01**
NaN_3 vs Anti-THH antibody	P > 0.05
NaN_3 vs Anti-K16 antibody	P > 0.05

NaN_3-L vs Control (no serum)	P > 0.05
NaN_3-L vs Control (GPC)	P > 0.05
NaN_3-L vs NaN_3	**P < 0.01**
NaN_3-L vs AA2	P > 0.05
NaN_3-L vs AA7	P > 0.05
NaN_3-L vs N4	P > 0.05
NaN_3-L vs N7	P > 0.05
NaN_3-L vs Anti-THH antibody	P > 0.05
NaN_3-L vs Anti-K16 antibody	**P < 0.05**

NaN_3 = 1.04mM
NaN_3-L = 0.01mM

Experiment 2

Control (GPC) vs Control (no serum)	P > 0.05
Control (GPC) vs NaN_3	**P < 0.05**
Control (GPC) vs NaN_3-L	P > 0.05
Control (GPC) vs AA1	P > 0.05
Control (GPC) vs AA8	P > 0.05
Control (GPC) vs N1	P > 0.05
Control (GPC) vs N10	P > 0.05
Control (GPC) vs Anti-THH antibody	P > 0.05
Control (GPC) vs Anti-K16 antibody	P > 0.05

NaN_3 vs Control (no serum)	P > 0.05
NaN_3 vs Control (GPC)	**P < 0.05**
NaN_3 vs NaN_3-L	**P < 0.05**
NaN_3 vs AA1	P > 0.05
NaN_3 vs AA8	**P < 0.05**
NaN_3 vs N1	**P < 0.01**
NaN_3 vs N10	P > 0.05
NaN_3 vs Anti-THH antibody	P > 0.05
NaN_3 vs Anti-K16 antibody	P > 0.05

NaN_3-L vs Control (no serum)	P > 0.05
NaN_3-L vs Control (GPC)	P > 0.05
NaN_3-L vs NaN_3	**P < 0.05**
NaN_3-L vs AA1	P > 0.05
NaN_3-L vs AA8	P > 0.05
NaN_3-L vs N1	P > 0.05
NaN_3-L vs N10	P > 0.05
NaN_3-L vs Anti-THH antibody	P > 0.05
NaN_3-L vs Anti-K16 antibody	P > 0.05

NaN_3 = 1.04 mM
NaN_3-L = 0.01 mM