

The susceptibility of disulfide bonds to modification in keratin fibers undergoing tensile stress

Duane P. Harland,^{1,*} Crisan Popescu,^{2,3} Marina Richena,¹ Santanu Deb-Choudhury,¹ Claudia Wichlatz,² Erin Lee,¹ and Jeffrey E. Plowman¹

¹Smart Foods & Bioproducts, AgResearch, Ltd., Lincoln, New Zealand; ²DWI, Aachen, Germany; and ³Kao Germany GmbH, Darmstadt, Germany

ABSTRACT Cysteine residues perform a dual role in mammalian hairs. The majority help stabilize the overall assembly of keratins and their associated proteins, but a proportion of inter-molecular disulfide bonds are assumed to be associated with hair mechanical flexibility. Hair cortical microstructure is hierarchical, with a complex macro-molecular organization resulting in arrays of intermediate filaments at a scale of micrometres. Intermolecular disulfide bonds occur within filaments and between them and the surrounding matrix. Wool fibers provide a good model for studying various contributions of differently situated disulfide bonds to fiber mechanics. Within this context, it is not known if all intermolecular disulfide bonds contribute equally, and, if not, then do the disproportionately involved cysteine residues occur at common locations on proteins? In this study, fibers from Romney sheep were subjected to stretching or to their breaking point under wet or dry conditions to detect, through labeling, disulfide bonds that were broken more often than randomly. We found that some cysteines were labeled more often than randomly and that these vary with fiber water content (water disrupts protein-protein hydrogen bonds). Many of the identified cysteine residues were located close to the terminal ends of keratins (head or tail domains) and keratin-associated proteins. Some cysteines in the head and tail domains of type II keratin K85 were labeled in all experimental conditions. When inter-protein hydrogen bonds were disrupted under wet conditions, disulfide labeling occurred in the head domains of type II keratins, likely affecting keratin-keratin-associated protein interactions, and tail domains of the type I keratins, likely affecting keratin-keratin interactions. In contrast, in dry fibers (containing more protein-protein hydrogen bonding), disulfide labeling was also observed in the central domains of affected keratins. This central “rod” region is associated with keratin-keratin interactions between anti-parallel heterodimers in the tetramer of the intermediate filament.

SIGNIFICANCE The proteomic approach using alkylating labels and mass spectrometry allowed us to identify the location within protein chains (keratins and keratin-associated proteins) of disulfide bonds broken by tensile forces. Stretching the fibers when hydrogen bonds are disrupted affects interprotein interactions with the head domain of keratins with keratin-associated proteins and between the tail domains of keratins. Stretching the fibers when hydrogen bonds are unaffected affects interactions between heterodimers in the tetramers in the coiled domain.

INTRODUCTION

Keratin fibers, from hair and wool, are sophisticated multi-component and multi-scalar materials formed by a developmental process that includes biologically, chemically, and physically driven self-assembly processes (1,2). In addition to medical and subjective interest in human hair (3), there is the potential to biomimetically harness keratin material self-assembly processes to produce high-performing bio-based

materials (e.g., plastic replacements) (4,5). A barrier to efforts in this context is the high level of internal molecular and structural complexity in hair which makes phenotypic expression, such as localized elastic properties, challenging to re-create during material development. Therefore, emerging approaches in reconstituted keratin (6) and other bio-based materials are increasingly turning to recreating mechanically functional aspects of native structure (7). Connecting macroscopic mechanical performance to its causation in molecular organization is termed mechanomics (8). Here, we report on mechanomic experiments investigating the location of mechanically relevant disulfide bonds across the microstructure of wool fibers (ovine hairs).

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*Correspondence: duane.harland@agresearch.co.nz

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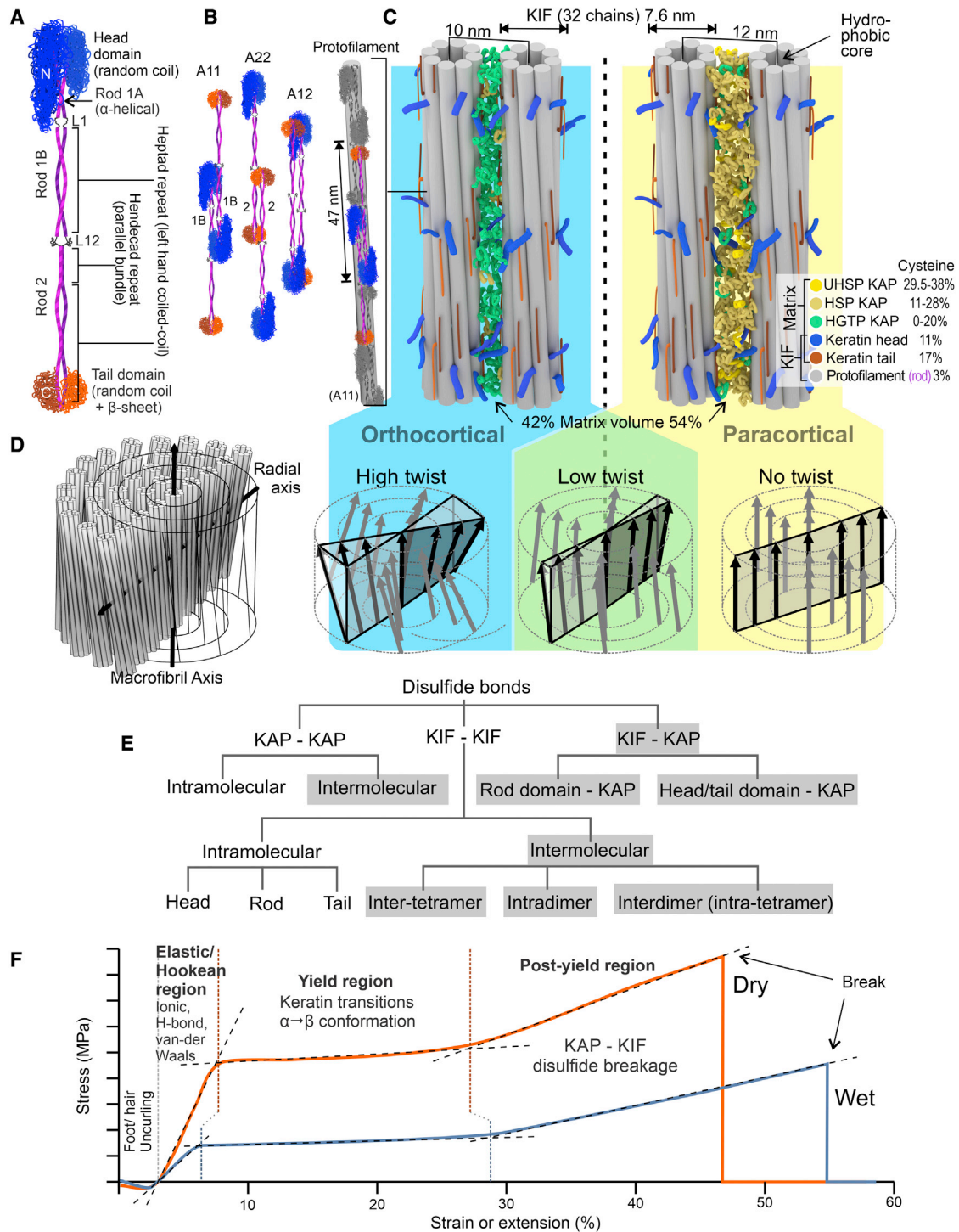


FIGURE 1 Protein structure and mechanical properties of hair cortex. (A) Trichokeratin type I, type II dimer. (B) Variants of dimer relationships in tetramers and one example of a protofilament from end-to-end tetramers, with A¹¹ representing the interaction of rod 1 regions of the heterodimers, A²² the interaction of the rod 2 regions, and A¹² the interaction between rods 1 and 2. (C) Intermediate filament arrangement, relationship with matrix, and differences between orthocortex and paracortex. (D) Example of double-twist macrofibril and variation in twist intensity across cortex. (E) Disulfide bonds between and within key fiber components with inter-structural disulfide bonds highlighted. (F) The tensile behavior of wet versus dry stretched fibers. KIF, keratin intermediate filament; L1 linker 1; N, amino-terminus; C, carboxy-terminus; L12, linker 1,2; HGTP, high glycine-tyrosine protein; HSP, high sulfur protein; UHSP, ultra-high sulfur protein; KAP, keratin-associated protein. To see this figure in color, go online.

Hairs develop within follicles via cellular-driven growth leading to programmed cell death that begins in a chemically reducing environment and progresses through a chemically oxidizing environment, ultimately culminating in hardening, cornification, and dehydration (9,10). Hair-shaft flexibility and its unique response to water are due to a chemical-bond network that stabilizes a multi-scalar organization of keratin and keratin-associated proteins (KAPs). Aside from the ionic and covalent bonds internal to proteins, hair or trichocyte keratins contain an unusually high level of disulfide bonds compared with epithelial keratins (11,12). In addition, the proximity of tightly packed protein chains within hair means that hydrogen bonds also play an important role in modulating mechanical performance (13–15).

During hair growth, the cortex (~90% of the volume of most fibers) undergoes an initial stage in which a progression of type I and II keratins are expressed (16,17) and form heterodimers (Fig. 1 A). The heterodimers associate into tetramers (Fig. 1 B), which then laterally associate into an eight-tetramer ring-like monomer (unit-length filament) and polymerize into keratin intermediate filaments (KIFs) such that the tetramers stack end to end to form protofilaments (Fig. 1 B) (18). Protofilaments form a ring surrounding a hydrophobic core, and it is thought that the cysteine-rich keratin head and tail domains (Fig. 1 A) largely decorate the outside of KIFs, protruding into the surrounding matrix, which is composed of keratin associate proteins (Fig. 1 C), and also project into the center of the protofilament to create a hydrophobic environment there (19). Composite bundles of KIFs and matrix, aligned with the fiber axis, are called macrofibrils (9). Macrofibrils are either crudely nematic (with no twist) or are double-twist structures (20) with inter-macrofibril variation in the intensity of the radial twist (Fig. 1 D) (21,22). All levels of structure are stabilized by disulfide bonds.

The matrix composition varies between macrofibril types. Orthocortical cell macrofibrils have KIFs that are more closely spaced (23), and the matrix is dominated by high glycine-tyrosine (HGT) KAPs, while KIFs in paracortical macrofibrils are more widely spaced and the matrix is dominated by high-sulfur (HS) and ultra-high-sulfur (UHS) KAPs (Fig. 1 C). The molecular and spatial organization of the matrix is poorly understood (24). During hair growth in the follicle, KIFs undergo an internal reorganization in an oxidizing environment, reducing the diameter from ~10 to ~7.5 nm. This transformation lines up cysteine residues to enable intra-KIF disulfide-bond formation (18), and, at about the same time, disulfide bonds between KIFs and KAPs and between KAPs and KAPs also form. The contribution of different classes of inter-protein connections within the disulfide-bond network within the KIF-matrix composite (Fig. 1 E) to the mechanical properties (e.g., tensile strength) of the hair shaft (Fig. 1 F) is the topic of this study.

Our hypothesis is that tensile-induced disulfide-bond breakage is not random and how easily a specific disulfide bond is broken by mechanical stress and how much that bond contributes to the overall fiber mechanics will depend on where it occurs within the keratin-KAP composite (Fig. 1 E). The null hypothesis is that all disulfide bonds contribute equally.

Hydrogen bonds between proteins are another factor affecting tensile behavior. They are more abundant than disulfide bonds in dry keratin fibers (one hydrogen bond and 1/9 disulfide bond per 122 molecular weight of keratin (14)), but the energy required to open a hydrogen bond (12–20 kJ/mol) is much lower than that for a disulfide bond (120–200 kJ/mol). The importance of hydrogen bonds in defining whole-hair-shaft tensile behavior is evident in the difference between the tensile behavior of wet compared with dry single hair fibers (Fig. 1 F). Water disrupts many of the protein-protein hydrogen bonds found in dry hair, and measurements of Young's modulus of longitudinally sectioned hair at various humidities using surface-probe nano-indentation has indicated that macrofibrils, and thus the KIF-KAP composite structure, is important in this context (15). Thus, water is a variable that affects how the proteins and bond network behave under mechanical stress.

Mechanical stresses that are harsh enough to break disulfide bonds may result in thiol-disulfide exchange with nearby cysteines on stress release, but also cysteines can be lost to the bond network through oxidation events.

In this study, we used tensile stress and alkylating agents, which cap thiols resulting from broken disulfide



FIGURE 2 Plastic trays used to incubate wool fibers that had been mounted between plastic anchors in label and wash solution prior to stretching. The lower tray held the mounted hairs in an unstretched state, and the lid secured the anchors while allowing solutions access to the hairs.

bonds, to investigate the mechanical contribution of disulfide bonds in wet and dry contexts. This allowed us to examine differences in the mechanics of hair supported by the disulfide-bond network alone and when additionally reinforced by the hydrogen-bond network.

MATERIALS AND METHODS

Sample origin and preparation

Wool fibers (mean diameter $33.0 \mu\text{m} \pm 6.6 \text{ SD}$, measured by an optical fiber diameter analyzer [OFDA]) from a single Romney sheep were sourced from a farm in Barry's Bay, Canterbury, New Zealand. Staples of wool were de-tipped and scoured (washed) in 0.15% Teric GN9 in distilled water at 60°C and then 40°C , followed by washings in distilled water at 40°C and 60°C for 2 min in each condition (to simulate commercial scouring). The wool was washed with dichloromethane, ethanol, and water to remove dirt and unbound lipids (25). Additional washes with dichloromethane were performed after air drying.

Fiber handling and tensile test conditions

The root-end 30 mm of 700 fibers were individually mounted between pairs of clip-together plastic tabs (Dia-Stron, Andover, UK) used to grip the fibers during stretching. All mounted fibers were placed in specially constructed plastic trays (Fig. 2) in which they could be immersed in solutions of the different alkylating agents and the wash solutions without individual handling of fibers.

Sets of 25 fibers were prepared, and each set was subjected to one of five experimental conditions: two wet conditions (conditions 1a and 1b), two dry conditions (conditions 2a and 2b), and a control that was not stretched but underwent all sample handling steps, as shown in Fig. 2. Conditions 1a, 1b, 2a, and 2b were repeated five times with separate sets of 25 fibers. The control was repeated seven times with separate sets of 25 fibers.

The four experimental conditions (Fig. 3) followed a similar sample-preparation protocol, with differences that facilitated variation in the water state of fibers (wet or dry) and two tensile conditions (stretched, held and relaxed, or stretched to break).

All samples were incubated in two alkylating agent labels at specific steps. Label A was 2 mM iodoacetamide (AppliChem GmbH, Darmstadt, Germany) in a 0.1 M sodium phosphate (Merck, Darmstadt, Germany) buffer and 1 mM EDTA (AppliChem GmbH, Darmstadt, Germany) made up in double-distilled

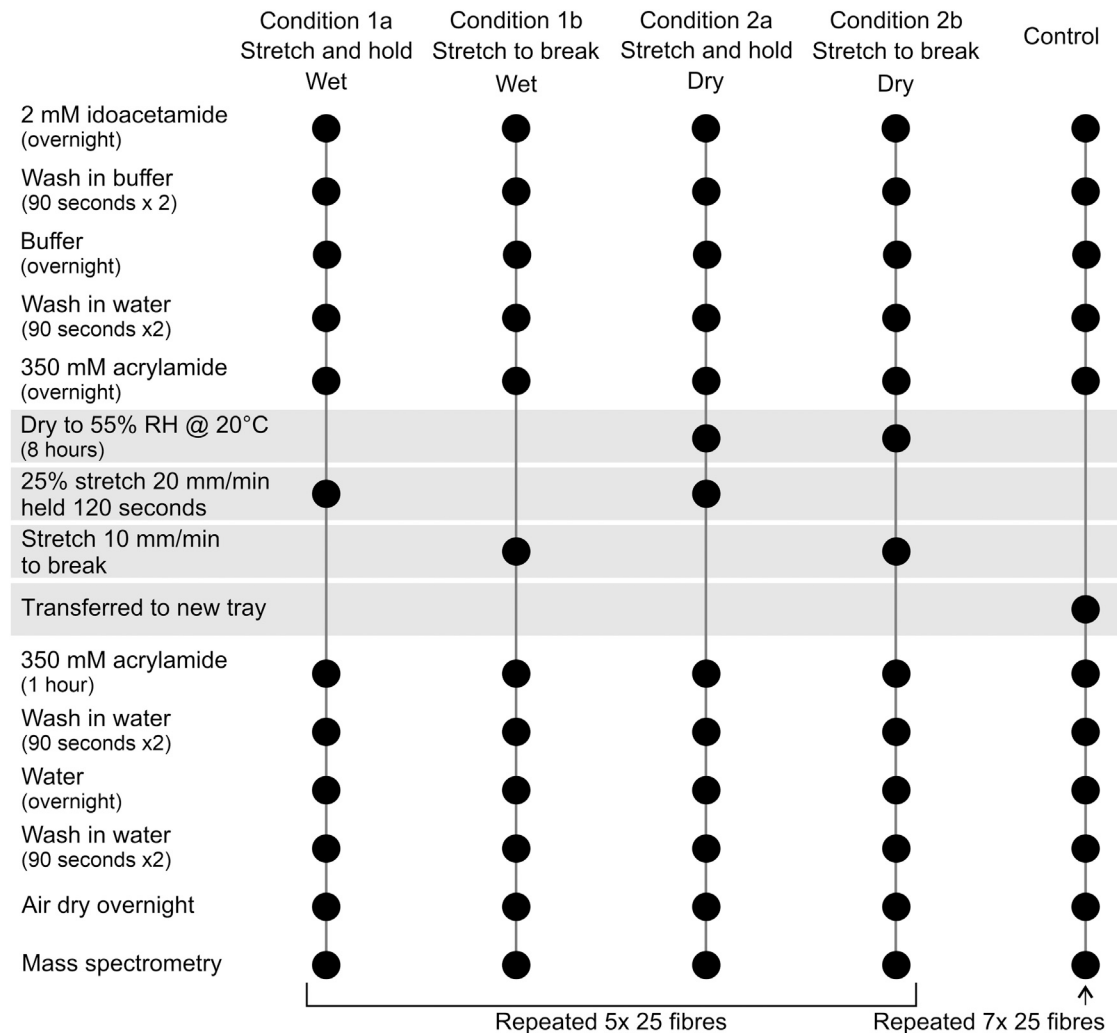


FIGURE 3 Summary of experimental sample protocol for all experimental conditions.

water to pH 7.4. Incubation in label A was carried out in the dark (closed laboratory cupboard). Label A's wash buffer was 1 mM EDTA in 0.1 M sodium phosphate buffer (pH 7.4). Label B was 350 mM acrylamide made up with double-distilled water, with a wash buffer that was double-distilled water. The process and timing for each label and wash is given in Fig. 2.

Tensile measurements were performed using a Dia-Stron Miniature Tensile Tester Model 675 (MTT675) with an attached Fiber Dimensional Analysis Unit Model 765 (FDAS765) (Dia-Stron). Depending on the condition, fibers were either stretched at 20 mm/min to 125% and then held for 120 s before being released or were stretched continuously at 10 mm/min until tensile failure. Samples subjected to wet testing were immersed in 350 mM acrylamide label upon loading into the sample carousel. The carousel's plastic lid was secured to reduce contact between the label and the room atmosphere to minimize evaporation during the testing period.

Following tensile testing, all samples were transferred to a tray and immersed in fresh acrylamide label for 1 h before undergoing a washing process, before being left to air dry overnight. Dry fibers were individually removed from the tray, cut away from the plastic tabs, and accumulated into Eppendorf tubes for further processing by mass spectrometry.

Fiber digestion and mass spectrometry (MS) analysis

Accumulated fibers from each batch of 25 were digested with TPCK-trypsin at an enzyme:substrate ratio of 1:50 in 50 mM ammonium bicarbonate:AcN (9:1) for 18 h at 37°C. The resultant peptide digest was vortexed for 3 h in the presence of Empore disks, which had previously been prewetted with AcN, methanol, and water, following which the peptides were extracted with 75% AcN in 0.1% trifluoroacetic acid. The extracts were dried down on a Centrивap vacuum centrifugal concentrator (Labconco, Kansas City, MI, USA) and reconstituted in 0.1% trifluoroacetic acid.

MS using nanoflow-liquid chromatography and electrospray ionization was performed on a nanoAdvance UPLC coupled to an amaZon speed ETD mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonik, Bremen, Germany). Sample aliquots (2 μ L) were loaded onto a C18AQ nanotrap (Bruker)(2 cm \times 75 μ m, 3 μ m particles, 200 \AA pore size). The trap column was then switched in line with the analytical column

(Bruker Magic C18AQ, 15 cm \times 100 μ m, 3 μ m particles, 200 \AA pore size). The column oven temperature was maintained at 50°C. A gradient from 0% to 40% B in 90 min at a flow rate of 800 nL/min was used for eluting the peptides. Solvent A was liquid chromatography-mass spectrometry (LCMS)-grade water with 0.1% formic acid and LCMS-grade 1% AcN; solvent B was 99% LCMS-grade AcN with 0.1% formic acid.

Automated information-dependent acquisition was performed using TrapControl (version 7.1, Build 83, Bruker, Billerica, MA, USA) software, with an MS survey scan over the range m/z 350–1200 followed by three MS/MS spectra from 50 to 3000 m/z acquired during each cycle of 30 ms duration.

Bioinformatics

Peak lists generated from each MS using nanoflow-liquid chromatography and electrospray ionization run were queried against *Ovis aries* in the NCBI database (August 10, 2015) using the Mascot search engine (v2.4, Matrix Science, London, UK) maintained on an in-house server. The Mascot search parameters included semitrypsin as the proteolytic enzyme with two missed cleavages; error tolerance was set to 0.15 Da for MS and 0.3 Da for MS/MS. Variable modifications used were carbamidomethylation and propionamide. Search results were compiled and analyzed using ProteinScape 3.1.0 (Bruker) with the ProteinExtractor function. Acceptance thresholds for peptide and protein scores were set at 25 and 80, respectively. At least one peptide with a score indicating identity (as calculated by the search engine) was required for protein identification. Results assessed as being true matches were used for statistical analysis.

Statistical analysis

For each treatment condition, the two-sample Binomial exact test was used to compare whether the presence of individually labeled peptides occurred at a greater than random probability. This test was applied to each peptide that was not labeled in at least three of the seven unstretched (control) samples. Given the number of peptides and samples, three or greater occurrences was considered non-random at a confidence level of $p < 0.05$. This result was compared with the peptide's detection probability in five

TABLE 1 Peptides from which consistently labeled cysteines were detected in at least 3 out of 7 control samples (not stretched, 7 repeats)

Sequence	Protein ID	Position	Region
I ¹¹⁶ LDELTLCK	K31	1B-70	
C ⁴² GPCNSYVR	K31	T-42, T-45	tail domain
C ⁴² GPCNSYVR	K31	T-42	tail domain
A ⁷ VPAFSCVSACGPR	K81-like <i>Capra hircus</i>	H-13, H-17	head domain
A ⁷ VPAFSCVSACGPR	K81-like <i>Capra hircus</i>	H-13	head domain
V ⁴⁷⁵ GGSILGCK	K81-like <i>Capra hircus</i>	T-69	tail domain
T ¹ CGFSTVVGSGFGSR	K83	H-2	head domain
C ²⁸ CITAAPYR	K83	H-28, H-29	head domain
C ²⁸ CITAAPYR	K83	H-28	head domain
D ²⁷² LNMDCVVAEIK	K83	2-4	coil 2
T ¹⁶ FSSCSAVAPK	K85	H-20	head domain
S ⁵⁴ VSALGSCGPR	K85	H-61	head domain
D ²³⁰ VDCAYLRL	K85	1B-66	coil 1B
D ²⁸⁴ LNMDCVVAEIK	K85	2-4	coil 2
G ⁴⁴⁹ GVACGGLTYSSTAGR	K85	T-24	tail domain
Q ¹⁶² CCESNLEPLFSGYIETLRR	K86	L1-5	linker 1
P ¹¹⁹ TSCQPAPCR	KAP2.3 var2	22	
P ¹¹⁹ TSCQPAPCR	KAP2.3 var2	27	
P ¹¹⁹ TSCQPAPCR	KAP2.3 var2	22, 27	
L ³⁷ GCGYGCGYGYGSR	KAP6.1	39, 43	
L ³⁷ GCGYGCGYGYGSR	KAP6.1	43	
Y ²⁰ SGSSCGSFPNVLVYR	KAP13.1	25	

treated samples against the detection probability of zero of the seven control samples. This resulted in peptides that were present in at least three treated samples being statistically significant ($p = 0.001, 0.010, \text{ and } 0.045$ for peptides observed in five, four, and three treated samples, respectively).

RESULTS

Some cysteines were consistently labeled in the fibers in the control experiment (no stretching, repeated 7 times) (Tables 1 and S1), though not every cysteine in a multi-cysteine peptide was labeled, in contrast to the situation for the cysteines in the

stretched samples (each condition repeated 5 times). A total of 84 unique sequences/peptides with labels (almost exclusively label A) were identified in all control and experimental samples. Only 43 sequences/peptides were not observed at all in any of 7 control samples, and this formed the basis for statistical comparisons. These peptides came from both keratins and KAPs. Of these peptides, 19 were found consistently in at least three of the five replicates compared with control samples, suggesting that there is a 95% probability that this was not by chance but due to one or more of the tensile treatments applied (Tables 2 and S1–S5).

TABLE 2 Peptides from the keratins and KAPs that contained consistently labeled cysteines and were detected in one or more of the 5 repeats of a treatment condition

Sequence	Significance/conditions				Protein ID	Position	Region	Fig. 8 label
	Wet		Dry (55% RH)					
	1a hold	1b break	2a hold	2b break				
Consistently labeled cysteines irrespective of water and stretch conditions								
A ⁴⁸⁹ SSFSCGSSR	5***	4**	5***	5***	K85	T-65	tail domain	A
C ³¹ CISAAPYR	5***	5***	4**	3*	K85	H-31, H-32	head domain	A
Consistently labeled cysteines only when wet (hydrogen bonds disrupted) and both stretch conditions								
T ¹² GPATTICSSDKFCR	5***	4**	2	2	KAP3.2	19, 25	close to N-terminus	B
S ⁹⁸ SSCSSLSSGSR	4**	3*	2		KAP13.1 var1	102	in the middle (protein is 166 residues long)	B
G ²⁵ CGCGSFR	5***	3*	1		KAP19.5	26, 28	in the middle (protein is 72 residues long)	B
T ³⁸⁰ ITPCISSPCAPAAPCTPCVPR	5***	5***	1	1	K31	T-22, T-27, T-33, T-36	close to C-terminus	B
S ³⁹⁴ RFGPCNTSGC	5***	3*	2	1	K33b	T-37, T-42	C-terminal peptide	B
S ⁵³ VCGGFR	4**	3*	2	2	K83	H-55	head domain	B
V ⁴⁹² GGSSILGCKK	5***	5***	2		K86-like	T-70	C-terminus but one, terminal cysteine	B
Consistently labeled cysteines when wet and stretched and held only								
L ¹²⁵ GYGICGFPVLSSSGGFCQPTFFASR	4**				KAP13.1 var1	130	close to the C-terminus (protein is 166 residues long)	C
T ¹²⁴ FRTSPCC	3*	2			KAP2.3 var3	130, 131	C-terminal peptide	C
T ¹² GPATTICSSDKF	3*		1		KAP3.2	19	close to N-terminus	C
Consistently labeled cysteines only in dry condition (reinforcing hydrogen bonds present) and both stretch conditions								
Y ³⁰⁶ SCQLSQVQSLIVNVESQLAEIR			3*	3*	K31, K33b	2-90	coil 2B	D
Consistently labeled cysteines when dry and stretched and held only								
V ¹⁷⁹ ESLKEELICK			3*	1	K31 var1, K33B	1B-87	coil 1B (seq. number for K31)	E
V ¹⁷⁹ ESLKEELLCLK			3*	1	K34	1B-87	coil 1B	E
Y ³⁰⁶ SCQLNQVQSLISNVESQLAEIR			3*	2	K31, K33b	2-90	coil 2B	E
Consistently labeled cysteines only in stretch and hold condition but both wet and dry								
S ¹²¹ ISTVCQPVGGVSTICQPTCGVSR	5***	2	3*		KAP11.1	126, 136, 140	close to the C-terminus (residue 158)	F
Consistently labeled cysteines only when fiber stretched to break and dry								
A ²¹⁴ DLEAQVESLKEELLCLK			1	3*	K35	1B-87	coil 1B	G
Consistently labeled cysteines in wet (both stretch conditions) and in dry stretched and held but not when dry stretched to break								
C ³⁹⁵ GPCNTFVH	3*	3*	3*	2	K33a	T-33, T-36	tail domain	H

Statistical likelihood is marked as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Of the cysteines that were consistently labeled under all experimental conditions, three were from one type II keratin, K85, with two cysteines, H-31 and H-32, being in the head domain and the other, T-65, in the tail domain (Table 2 A, Fig. 4 B). These three disulfides were consistently labeled irrespective of the extra stabilizing influence of hydrogen bonds or whether the fiber was rapidly stretched to break or was given a longer but less severe stretch.

The effect of wet versus dry conditions

Conditions 1a and 1b were tested under wet conditions (stretch 25% and hold or break) and samples in conditions 2a and 2b were tested at 55% relative humidity (stretch or break). Disulfides that were consistently labeled in the wet state, when hydrogen bonds were disrupted, came from peptides found in both keratins and KAPs (Table 2 B; Fig. 5). What is notable about the keratin data is that all disulfides identified were from the head or tail domains. This included disulfides from the head domain of K83 (H-55) and the tail domains of K31 (T-22, T-27, T-33, and T-35), K33b (T-33 and T-36), and K86-like (T-70). In contrast, disulfides that were consistently labeled in the dry state when reinforcing hydrogen bonds were present were from the coil domains,

specifically cysteines from Coil 1B in K31 var1 and K34 (1B-87), and coil 2B in K31 and K33b (2B-90) (Table 2 D and E; Fig. 6 A–C).

The effect of hold at 25% versus break

A number of cysteines were observed to be labeled when stretched to 25% but not when stretched to break. Among them were three cysteines in KAP11.1, where disulfide labeling occurred under both wet and dry conditions (Table 2 F; Fig. 6 D). In a further three KAPs, specifically KAP3.2, KAP2.3 var 3, and KAP13.1 var 1, cysteines were only labeled when stretched to 25% in the wet state (Table 2 C; Fig. 7). In contrast, stretching to 25% under dry conditions only labeled cysteines in the coil domains of keratins, specifically K31, K33b, and K34 (Table 2 E).

Other conditions

Two disulfide-linked cysteines in the tail domain of K33a were labeled in all conditions except when stretched to break under dry conditions (Table 2 H). One cysteine in coil 1B in K35 was only labeled when the fiber was stretched to break in the dry state (Table 2 G).

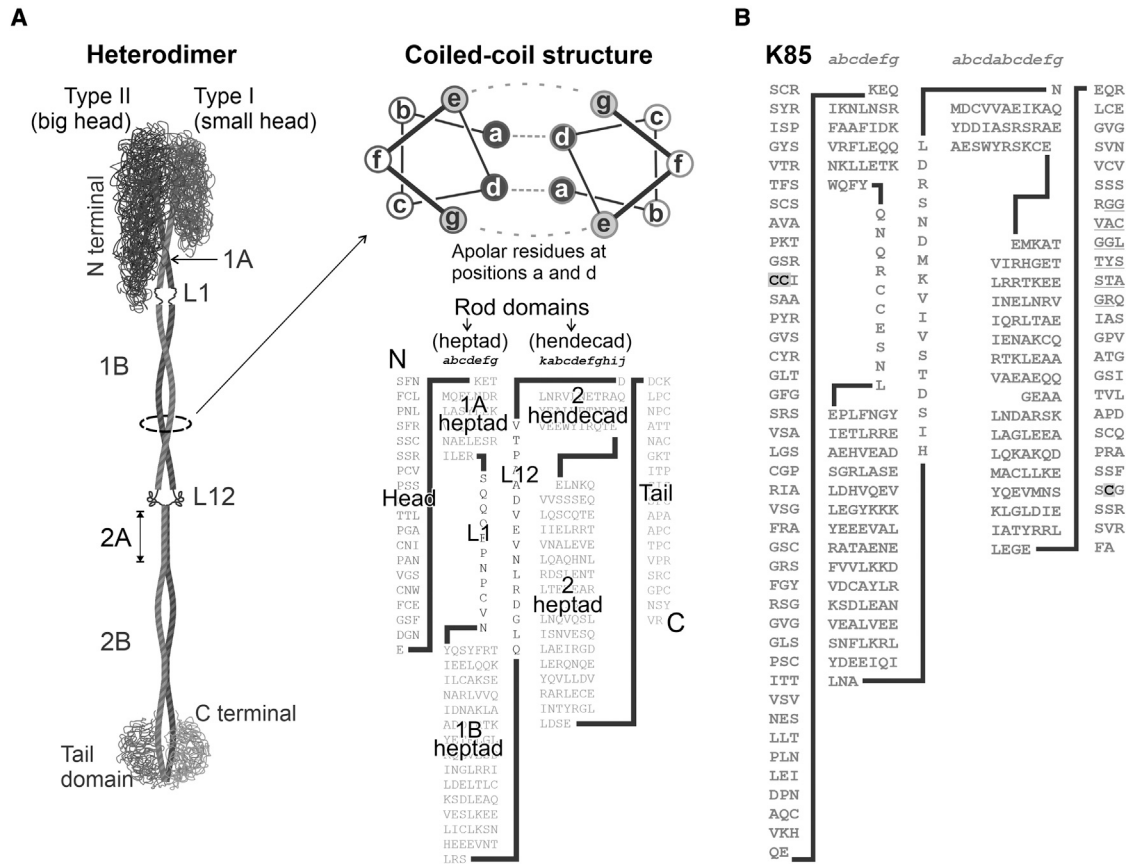


FIGURE 4 Trichokeratin intermediate filament heterodimers. (A) Key to relate sequence results to dimer structure. (B) K85 indicating consistently labeled disulfides highlighted and in bold.

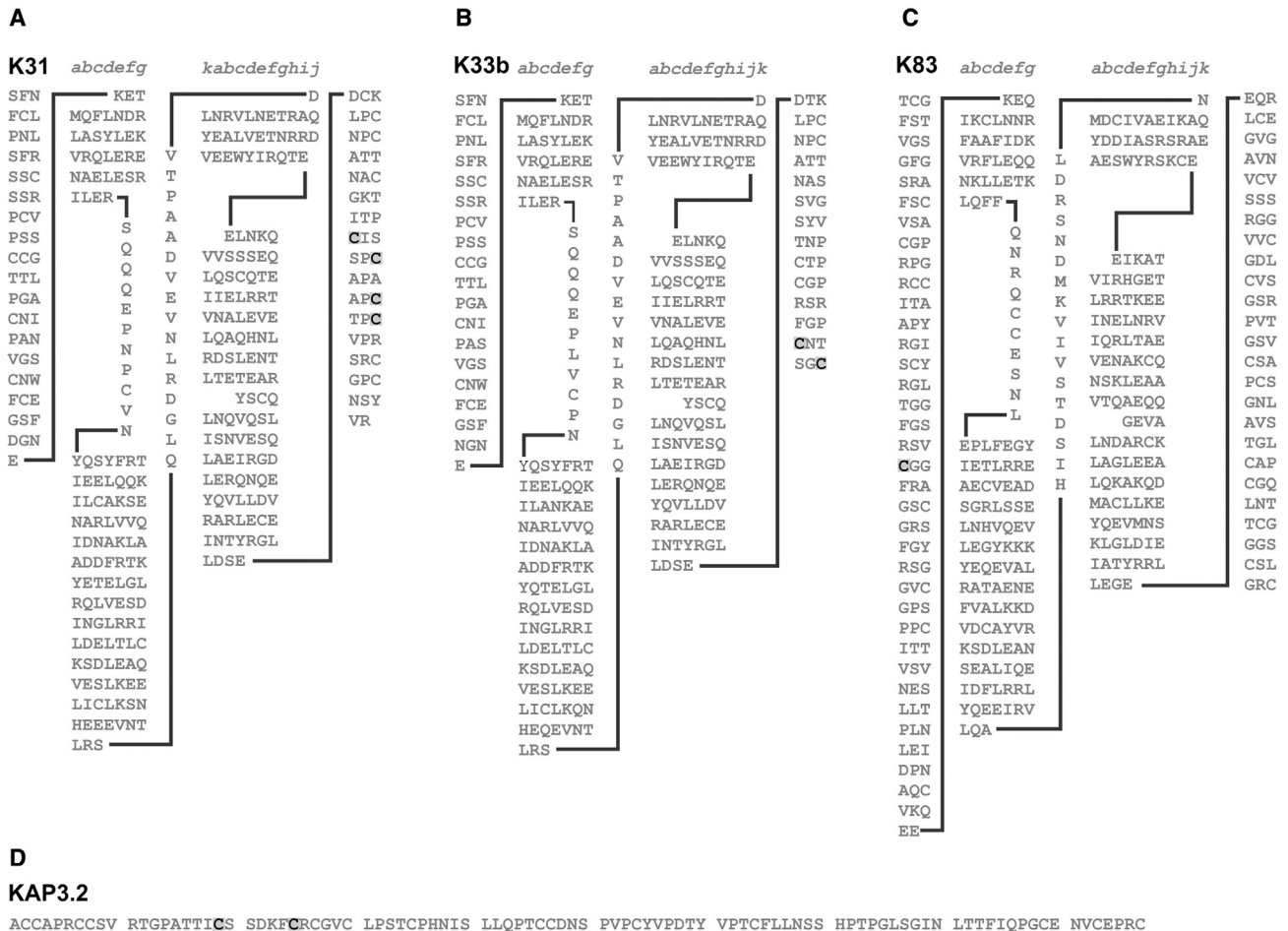


FIGURE 5 Some keratins and KAPs in which labeling occurred consistently in wet conditions only. (A) K31. (B) K33b. (C) K83. (D) KAP 3.2. Labeled cysteines are highlighted and in bold.

DISCUSSION

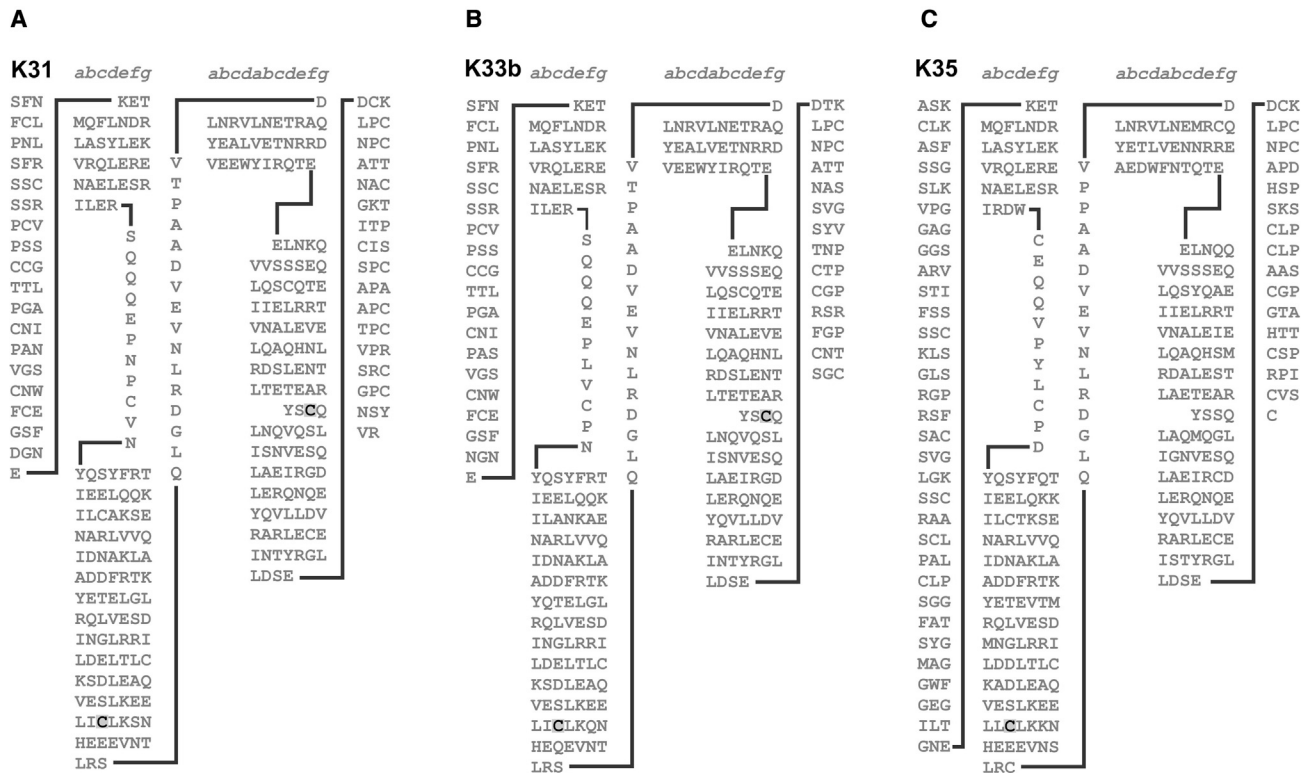
Disulfide labeling during tensile stress is not random

The key finding of this study is that disulfide labeling in wool fibers during the process of stretching or breaking the fiber is not random but disproportionately affects the cysteines at specific sites on keratins and KAPs (Fig. 8). Our method identifies this disproportionality against the background of random disulfide labeling that is also likely occurring within stressed fibers. Effectively, it identifies those cysteines that form weak points in the disulfide-bond network that are readily accessible to water. Conversely, labeling of the cysteines in the hydrophobic core of the 8 + 0 KIF is less likely to occur because of water-accessibility issues (19). Although the individual bonds themselves are no weaker at these points, the local molecular organization (intra- and inter-keratin/KAP quaternary structure) probably generates a disproportionate effect of strain at the molecular level at these points.

Non-random cysteine labeling was found in the control samples that had not undergone tensile stress. These may represent free thiols naturally present in the wool sample used or could be a result of single-fiber handling during experiments (handling steps occurred with controls, and only stretching was omitted). We consider that handling is the most likely explanation because in the control, only single labels were typically detected on peptides with multiple cysteines, and two previous studies using similar labeling chemistry but excluding most of the single-fiber handling steps (26,27) did not detect free thiols.

Keratin 85's head and tail domains contain key disulfides

The disulfides that showed consistent labeling under all stretching conditions were on K85 (Table 2 A; Fig. 4 B), which is found in both the cortex and cuticle, and is the first type II keratin to be expressed in the follicle (28). As these cysteines were found on either the head or tail domain, all would potentially be positioned to interact



D
KAP11.1
 SYSCSTRNCS SRRIGGEYTV PVVTVSSPDA DCLSGIYLP SFOQTGSWLLD HCQETCCEPT VCQSTCYQPT PCVSSPVRVT SRQTTVCVSSP CSTTCSRPLT
 FISSGCQPLS GVSTVCKPVR SISTV**C**QPVG GVSTI**C**QPT**C** GVSRTYQQSC VSSCRRIC

FIGURE 6 Keratins that show consistent labeling in the dry treatments and KAP11.1, which was labeled only in fiber stretch and holds conditions both in aqueous and dry conditions. (A) K31. (B) K33b. (C) K35, (D) KAP11.1. Labeled cysteines are highlighted and in bold.

with KAPs, though some theories hold that cysteines in the tail domain are involved in keratin-keratin crosslinking (29). In the early stages of follicle development, keratin-KAP interaction is most likely to be between K85 and KAP11.1, the expression of the latter starting in the mid-upper bulb region of the follicle (30). In the case of KAP11.1, three potential sites of disulfide bonding have been identified at cysteines 126, 136, and 140 (Table 2 F; Fig. 6 D). In addition to this, KAP interactions are also possible between KAP13.1 and KAP8.1 with K85. With the expression of keratin K35 starting around the

same time as K85, followed shortly after by K31, these are also likely candidates for interactions with KAP11.1, KAP13.1, and KAP8.1.

Keratin 35 is an outlier

We identified a cysteine residue in the 1B rod domain of K35 that was more readily labeled than random but only under dry conditions (i.e., hydrogen-bond reinforcement) and only when fibers were stretched to break (Table 2 G; Fig. 6 C). While we do not understand

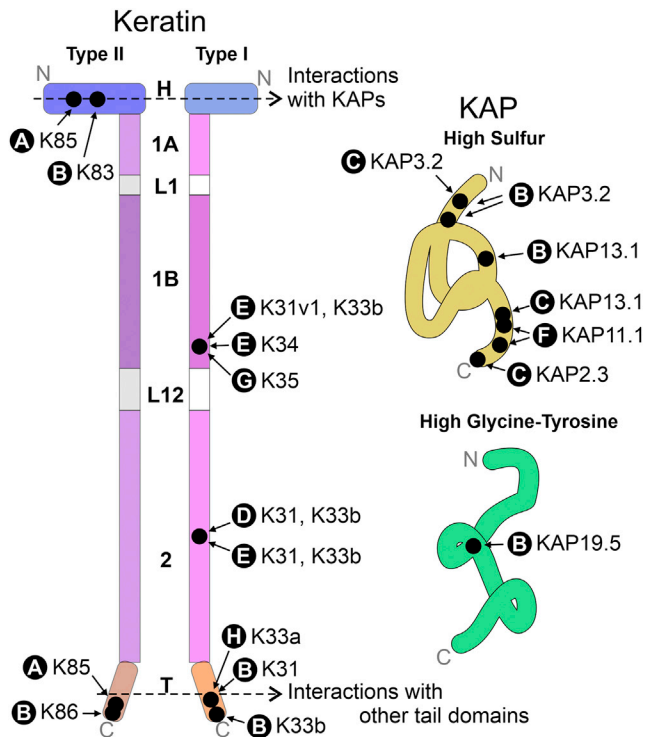
KAP2.3

TGSCCGPTFS SLSCGGGCLQ PCCYRDPCCC RVPVSCQTVSR PVTFVPRCTR PICEPCRRPV CDDPCSLQEG CCRPITCCPT SCQAVVCRPC CWATTCCQPV
 SVQCPCCRPT SCQPAPCSRT TCRTFRTSP**C** **C**

KAP3.2

ACCAPRCCSV RTGPATTI**C**S SDKFCRCGVC LPSTCPHNIS LLQPTCCDNS PVPCYVPTY VPTCFLNNS HPTPGLSGIN LTTFIQPGCE NVCEPRC

FIGURE 7 KAPs with cysteines labeled when stretched for an extended period under water.



Consistently labelled cysteines detected in:

- A** all conditions
- B** wet broken or stretch & held
- C** wet stretch & held only
- D** dry broken or stretch & held
- E** dry stretch & held only
- F** wet or dry stretch & held only
- G** wet stretched to break only
- H** wet or dry stretched & held or wet broken, but not when dry broken

FIGURE 8 Summary of locations of consistently labeled cysteines under all conditions on generic keratin and KAP structures. Letters for test conditions as in Table 2. Colors and domain names as in Figs. 1 and 4 A. To see this figure in color, go online.

how this relates to either fiber mechanics or hair growth, it is worth noting that K35 is unusual in other ways. During fiber growth in the follicle bulb, K35 is the first type I keratin expressed in both the cortical and cuticular cell compartments (28,31), and its transient expression leads to a lower abundance than other type I keratins (32).

Disproportionately affected molecular locations change with water state

When stretching is carried out under wet conditions, in which hydrogen bonding had been disrupted, the cysteines disproportionately affected by tensile stress were those from the head domain of the type II keratin K83 and the tail do-

main of the type I keratins K31 and K33b (Table 2 B; Fig. 5). This is consistent with previous observations in the context of chemical accessibility. Of particular interest is the X-Pro-Cys repeat sequence in the type I keratin K31. This has previously been identified as a potential site of interaction between tail domains of keratins (26,29). In the case of the type II keratins, the cysteines in the longer head domains appear to be more accessible to reduction and alkylation than those in the shorter head domains of the type I keratins (26). This suggests that the head domains of the type II keratins, via their cysteine residues, are primarily involved in keratin-KAP interactions. This may be because these cysteine residues are more exposed to water in the fiber than those in the shorter head domains of the type I keratins.

Among the potential candidates for crosslinking with these keratins are the HS proteins KAP3.2 and KAP13.1 var1 and the HGT protein KAP19.5 (Table 2 B; Fig. 7), all of which were labeled under the same stretching conditions. Interestingly, the same cysteine in KAP 3.2 that had high accessibility to chemical damage (27) was also identified here (Fig. 7).

In contrast, when stretching to 25% extension was carried out in dry conditions, when the protein molecular structure is reinforced by protein-protein hydrogen bonds, the only cysteines consistently labeled were those coming from the 1B and 2 rod regions of the type I keratins (Table 2 D, and E; Fig. 6 A and B). In the case of these cysteines, 1B-87 in coil 1B of K33 var1 and K34 has been shown to be involved in crosslinking to the cysteine L1-9 of a type I keratin in mouse type I trichocyte keratins (33). Such a crosslink would be consistent with an A¹¹ alignment of the heterodimers in the tetramer (26). Likewise, cysteine 2-90 in coil 2 of K31 and K33b has been shown to crosslink to cysteine 2-90 in another type I human in epithelial keratins and consistent with an A²² alignment in the tetramer (34). All of these cysteines have previously been shown to be readily accessible to reducing and alkylating agents (26). Thus, it would appear that stretching carried out under these conditions results in the breakage of disulfide bonds between the heterodimers in the tetramer.

KAP11.1 stands out from the many other KAPs

One protein that showed interesting behavior was KAP11.1 because three of its disulfide bonds, involving cysteines at positions 126, 136, and 140, were only labeled when tensioned to 25% for 2 min when either wet or dry and not when stretched to break for 10 min under the same conditions (Table 2 F; Fig. 6 D). One of the reasons for conducting the two different treatments, tensioning or stretched to break, was to determine whether the tensioning process would allow more disulfide bonds to break and reform if the stress was spread more evenly across the structure, i.e., which disulfide bonds are the most pliable. This would appear to be the case for the disulfide bonds involving these cysteines in KAP11.1.

CONCLUSIONS

This study on the effect on keratins in the cortex of intact wool fibers subject to stretching to break under wet or dry conditions has revealed the following:

- Our primary conclusion is that tensile stress breaks some disulfide bonds more readily than others and that which disulfide bonds and where they are located within keratins and KAPs depends on the rate of strain and the water state of the hair.
- Cysteines in the head and tail domain of K85 are disrupted under all stretching conditions.
- Stretching when hydrogen-bond disruption occurs, under wet conditions, results in the labeling of cysteines in the head domain of type II keratins most likely to be involved in keratin-KAP interactions, possibly because these are more exposed to water in the fiber.
- Stretching when hydrogen-bond disruption occurs, under wet conditions, results in the labeling of interactions in the tail domain of type I keratins most likely to be involved in keratin-keratin interactions.
- Stretching when the structure is maintained by hydrogen bonds, under dry conditions, was confined to the rod domains and primarily affects cysteines involved in interactions between heterodimers in the tetramers of the intermediate filament consistent with A₁₁ and A₂₂ alignments.
- In the early stages of follicle development, keratin-KAP interactions are most likely to be between K85 and two proteins, KAP11.1 and KAP13.1.

SUPPORTING MATERIAL

Supporting material can be found online at <https://doi.org/10.1016/j.bj.2022.04.029>.

AUTHOR CONTRIBUTIONS

D.P.H., S.D.-C., and J.E.P. conceived the experiment. C.W., E.L., C.P., and D.P.H. carried out the fiber-stretching and sample-processing stages. S.D.-C. and J.E.P. carried out the MS stage. Data analysis and interpretation involved D.P.H., C.P., M.R., S.D.-C., and J.E.P. The manuscript was drafted by J.E.P. and D.P.H. with contributions, review, and edits by C.P., M.R. and S.D.-C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental information

**The susceptibility of disulfide bonds to modification in keratin fibers
undergoing tensile stress**

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Supplementary file for

The susceptibility of disulfide bonds to modification in keratin fibres undergoing tensile stress

Duane P. Harland¹, Crisan Popescu^{2,3}, Marina Richena¹, Santanu Deb-Choudhury¹, Claudia Wichlatz², Erin Lee¹ and Jeffrey E. Plowman¹

¹ AgResearch, 1365 Springs Road, Lincoln 7674, New Zealand

² DWI, Forckenbeckstrasse 50, D-52056, Aachen, Germany

³ Kao Germany GmbH, Pfungstaedterstrasse 92-100, D-6427, Darmstadt, Germany

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Data tables of peptides for each sample

Supplementary Table 1. Controls

Supplementary Table 1

Control 1			
Sequence	Modifications	Accessions	Proteins
PTSCQPAPCR	Carbamidomethyl: 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 164652864	keratin 31 [Ovis aries]
SLCGSGYGCGSR	Carbamidomethyl: 9	gi 224555998	keratin associated protein 6 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 125117	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
LGCYGCYGYGYSR	Carbamidomethyl: 7	gi 224555998	keratin associated protein 6 [Ovis aries]
TGCFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPR	Carbamidomethyl: 7	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
ILDELTLCCK	Carbamidomethyl: 8	gi 312283590; gi 164652864; gi 426238003	keratin 33B [Ovis aries] keratin 31 [Ovis aries] PREDICTED: keratin 34 [Ovis aries]
DLNMDCVVAEIK	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DLNMDCIVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]

QCCESNLEPLFSGYIETLRR	Carbamidomethyl: 2	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
Control 2			
Sequence	Modifications	Accessions	Proteins
EAE <u>C</u> VEADSGR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
TYQ <u>Q</u> S <u>C</u> VSSCR	Carbamidomethyl: 6	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
LG <u>C</u> GSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
<u>C</u> GP <u>C</u> NSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
TGPAT <u>T</u> <u>C</u> SSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
AF <u>S</u> <u>C</u> VSA <u>C</u> GPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
VGG <u>S</u> ILG <u>C</u> K	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
<u>C</u> CITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
<u>C</u> CITAAPYR	Carbamidomethyl: 1	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
GGVA <u>C</u> GGLTYSSTAGR	Carbamidomethyl: 5	gi 246276; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
DVD <u>C</u> AYLR	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 246276; gi 164652870	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
LG <u>C</u> GYG <u>C</u> GYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
LGCGY <u>G</u> CGYGYGSR	Carbamidomethyl: 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
L <u>C</u> EGVGAVNVCVSSSR	Carbamidomethyl: 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
ILDEL <u>T</u> L <u>C</u> K	Carbamidomethyl: 8	gi 999000013; gi 312283590; gi 426238003	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries] PREDICTED: keratin 34 [Ovis aries]
YSGSS <u>C</u> GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]

DLNMD <u>C</u> IVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
Q <u>C</u> CESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
Q <u>C</u> CESNLEPLFSGYIETLRR	Carbamidomethyl: 2	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
SDLEAQVESLKEELL <u>C</u> LK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
SDLEAQVESLKEELI <u>C</u> LK	Carbamidomethyl: 16	gi 999000013; gi 312283590	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries]
Control 3			
Sequence	Modifications	Accessions	Proteins
<u>C</u> PSSFGR	Carbamidomethyl: 1	gi 999000016	KAP 19.3 (tentative) cs39 [Ovis aries]
TGPATTI <u>C</u> SSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
SLCGSGYG <u>C</u> GSR	Carbamidomethyl: 9	gi 32329266	high-glycine tyrosine keratin type II.6 [Capra hircus]
SVSALG <u>S</u> CGR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGG <u>S</u> ILG <u>C</u> K	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
<u>C</u> CITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
<u>C</u> CITAAPYR	Carbamidomethyl: 1	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
AF <u>S</u> CVSACGPR	Carbamidomethyl: 4	gi 312283588; gi 1308	keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
GGVA <u>C</u> GGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DVD <u>C</u> AYLR	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 125117	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
LG <u>C</u> GYG <u>C</u> GYGYSR	Carbamidomethyl: 3, 7	gi 32329266	high-glycine tyrosine keratin type II.6 [Capra hircus]
LG <u>C</u> GYGCGYGYGYSR	Carbamidomethyl: 3	gi 32329266	high-glycine tyrosine keratin type II.6 [Capra hircus]
QDMA <u>C</u> LLK	Carbamidomethyl: 5	gi 312283584; gi 312283588; gi 1308; gi 125117	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II

			keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
T_CGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCV_SACGPR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
L_CEGVGAVNVCVSSSR	Carbamidomethyl: 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
AVPAFSCV_SACGPR	Carbamidomethyl: 7	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
ILDELT_LCK	Carbamidomethyl: 8	gi 312283590; gi 999000013; gi 999000014; gi 426238003	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries] PREDICTED: keratin 34 [Ovis aries]
YSGSS_CGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Control 4			
Sequence	Modifications	Accessions	Proteins
PTSCQPAP_CPR	Carbamidomethyl: 4, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
PTSCQPAP_CPR	Carbamidomethyl: 4	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
PTSCQPAP_CPR	Carbamidomethyl: 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
TYQQSCVSS_CPR	Carbamidomethyl: 10	gi 328905561	keratin-associated protein KAP11-1 [Ovis aries]
LG_CGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
C_GPCNSYVR	Carbamidomethyl: 1	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SVSALGSC_GPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSS_CSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
VGG_SILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
C_CITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
C_CITAAPYR	Carbamidomethyl: 1	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
LE_CINTYR	Carbamidomethyl: 3	gi 999000013; gi 312283592	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33 [Ovis aries]

AFSCVSAACGPR	Carbamidomethyl: 4	gi 1308; gi 312283588	hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
ARLECEINTYR	Carbamidomethyl: 5	gi 999000013; gi 312283592	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33 [Ovis aries]
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 246276	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
AVPAFSCVSAACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LCEGVGSVNVCVSSSR	Carbamidomethyl: 2	gi 1308; gi 246276	hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
ILDELTLCCK	Carbamidomethyl: 8	gi 999000013; gi 312283590; gi 309323371; gi 312283592	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries] keratin 34 [Ovis aries] keratin 33 [Ovis aries]
SQQQEPLVCPNYQSYFR	Carbamidomethyl: 9	gi 999000013; gi 312283590; gi 312283592	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries] keratin 33 [Ovis aries]
DLNMDCVVAEIK	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DLNMDCIVAIEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELLCLK	Carbamidomethyl: 16	gi 309323371	keratin 34 [Ovis aries]
SDLEAQVESLKEELICLK	Carbamidomethyl: 16	gi 999000013; gi 312283590	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries]
Control 5			
Sequence	Modifications	Accessions	Proteins
PTSCQPAPCR	Carbamidomethyl: 4, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
VGGGILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283588; gi 312283584; gi 1308	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1	gi 312283588; gi 312283584; gi 1308	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]

GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
DVDCAYLR	Carbamidomethyl: 4	gi 312283584; gi 246276; gi 1308; gi 164652870	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 85 [Ovis aries]
QDMA ⁺ CLLK	Carbamidomethyl: 5	gi 312283588; gi 312283584; gi 246276; gi 1308; gi 164652870	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 85 [Ovis aries]
T ⁺ CGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
ILDELT ⁺ LCK	Carbamidomethyl: 8	gi 312283590; gi 999000013; gi 426238003; gi 125091	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries] PREDICTED: keratin 34 [Ovis aries] RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
YSGSS ⁺ CGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
SQQQEPLV ⁺ C ⁺ PNYQSYFR	Carbamidomethyl: 9	gi 312283590; gi 999000013; gi 426238003; gi 125091	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries] PREDICTED: keratin 34 [Ovis aries] RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
DLNMD ⁺ C ⁺ VVAEIK	Carbamidomethyl: 6	gi 246276; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
DLNMD ⁺ C ⁺ VAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELI ⁺ CLK	Carbamidomethyl: 16	gi 312283590; gi 999000013	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SDLEAQVESLKEELL ⁺ CLK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
Control 6			
Sequence	Modifications	Accessions	Proteins
PTSCQPAP ⁺ CR	Carbamidomethyl: 4, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
TFSS ⁺ CSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

VGG <u>S</u> ILG <u>C</u> K	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
<u>C</u> CITAA <u>P</u> Y <u>R</u>	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
LE <u>C</u> EINT <u>Y</u> R	Carbamidomethyl: 3	gi 164652864; gi 999000014	keratin 31 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
DVD <u>C</u> AY <u>L</u> R	Carbamidomethyl: 4	gi 312283584; gi 246276; gi 1308	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries]
QDMA <u>C</u> LL <u>K</u>	Carbamidomethyl: 5	gi 312283584; gi 246276; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
T <u>C</u> GFSTVGS <u>G</u> FG <u>S</u> R	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPA <u>F</u> SC <u>V</u> SAC <u>G</u> PR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
ILDELT <u>L</u> C <u>K</u>	Carbamidomethyl: 8	gi 312283590; gi 164652864; gi 426238003; gi 999000014	keratin 33B [Ovis aries] keratin 31 [Ovis aries] PREDICTED: keratin 34 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
YSG <u>S</u> CGSS <u>F</u> PSNL <u>V</u> Y <u>R</u>	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
SQQE <u>P</u> LV <u>C</u> PNYQ <u>S</u> Y <u>F</u> R	Carbamidomethyl: 9	gi 312283590; gi 164652864; gi 426238003; gi 999000014	keratin 33B [Ovis aries] keratin 31 [Ovis aries] PREDICTED: keratin 34 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
DLNMD <u>C</u> VVA <u>E</u> IK	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DLNMD <u>C</u> IVAE <u>I</u> K	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELI <u>C</u> L <u>K</u>	Carbamidomethyl: 16	gi 312283590; gi 164652864	keratin 33B [Ovis aries] keratin 31 [Ovis aries]
SDLEAQVESLKEELL <u>C</u> L <u>K</u>	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
Y <u>S</u> Q <u>L</u> NQVQSLIVSVESQLAE <u>I</u> R	Carbamidomethyl: 3	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
Control 7			
Sequence	Modifications	Accessions	Proteins
PT <u>S</u> Q <u>P</u> AP <u>C</u> R	Carbamidomethyl: 4, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
PT <u>S</u> Q <u>P</u> AP <u>C</u> R	Carbamidomethyl: 4	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]

PTSCQPAPCR	Carbamidomethyl: 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
TGPATTI_CSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIII B2
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVD_CAYVR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
VGGSilGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CCITAAPYR	Carbamidomethyl: 2	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 999000013; gi 999000014	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
TQTVVSSPCQTSCYRPR	Carbamidomethyl: 13	gi 999000030; gi 426217167	KAP 13.1 (tentative) cs39 [Ovis aries] PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
DVD_CAYLR	Carbamidomethyl: 4	gi 312283584; gi 246276; gi 1308; gi 164652870	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 85 [Ovis aries]
GGVACGGLTYSTAGR	Carbamidomethyl: 5	gi 246276; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
LGCGYG_CGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
LPSNPCATTNASSNFCR	Carbamidomethyl: 6, 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
LGCGYG_CGYGYGSR	Carbamidomethyl: 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
QDMA_CLLK	Carbamidomethyl: 5	gi 312283584; gi 246276; gi 312283588; gi 1308; gi 164652870	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 83 [Ovis aries] hair type II keratin

			intermediate filament protein [Ovis aries] keratin 85 [Ovis aries]
T_CGFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
L_CEGVGSVNVCVSSSR	Carbamidomethyl: 2	gi 246276; gi 1308; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 85 [Ovis aries]
L_CEGVGAVNVCVSSSR	Carbamidomethyl: 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
AVPAFSCVSA_CGPR	Carbamidomethyl: 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
ILDELT_CK	Carbamidomethyl: 8	gi 312283590; gi 999000013; gi 426238003; gi 999000014	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries] PREDICTED: keratin 34 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
YSGSS_CGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
SGQSLSFQPT_CGSGFY	Carbamidomethyl: 11	gi 999000030; gi 426217167	KAP 13.1 (tentative) cs39 [Ovis aries] PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
DLNMD_CVVAEIK	Carbamidomethyl: 6	gi 246276; gi 164652870	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] keratin 85 [Ovis aries]
PLTFISSG_CQPLSGVSTVCKPVR	Carbamidomethyl: 9	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
DLNMD_CIVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELI_CLK	Carbamidomethyl: 16	gi 312283590; gi 999000013	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SDLEAQVESLKEELL_CLK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
Q_CCESNLEPLFSGYIETLR	Carbamidomethyl: 2	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]

Supplementary Table 2. Sample 1a

Supplementary Table 2

Sample 1a 25% stretch -1			
Sequence	Modifications	Accessions	Proteins
ASSFSC_GSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

GCGGGSFR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TFRTSPCC	Carbamidomethyl: 7, 8	gi 999000042	KAP 2.3 var3 (tentative) cs39 [Ovis aries]
TGPATTI_CSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
VGGSILGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSPCQTSYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
TGPATTI_CSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
TCGFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]

LCEGVGAVNVCVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
SISTVCQPVGGVSTICQPTCGVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
YSGSSCGSSFFSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITPCISSPCAPAAPCTPCVPR	Carbamidomethyl: 5, 10, 16, 19	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
SGQSLSFQPTCGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Sample 1a 25% stretch -2			
Sequence	Modifications	Accessions	Proteins
EAECAEADSGR	Carbamidomethyl: 4	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SSSCSSLSSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
GCGCGSFR	Carbamidomethyl: 2, 4	gi 999000015; gi 999000016	KAP 19.5 (tentative) cs39 [Ovis aries] KAP 19.3 (tentative) cs39 [Ovis aries]
CPSSFGR	Carbamidomethyl: 1	gi 999000016	KAP 19.3 (tentative) cs39 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SRCGPCNSYVR	Carbamidomethyl: 3, 6	gi 57164221	keratin, type I microfibrillar 48 kDa, component 8C-1 [Ovis aries]
VGGSILGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 57164221	keratin, type I microfibrillar 48 kDa, component 8C-1 [Ovis aries]
LGCSSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TGPATTICSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIIIB2
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
TRCGPCNTFVH	Carbamidomethyl: 3, 6	gi 125091	RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSilGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 125091	RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSPCQTSQYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIIIB2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 125117	hair keratin type II intermediate filament [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
TGPATTICSSDKF	Carbamidomethyl: 8	gi 229307	keratin SCMKIIIIB2
TCGFSTVSGGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LCEGVGAVNVCVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
SISTVCQPVGGVSTICQPTCGVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
YSGSSCGSSFPNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITPCISSPCAPAAPCTPCVPR	Carbamidomethyl: 5, 10, 16, 19	gi 57164221	keratin, type I microfibrillar 48 kDa, component 8C-1 [Ovis aries]
SGQSLSFQPTCGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
SQQQEPLLCPNYQSYFR	Carbamidomethyl: 9	gi 309323371	keratin 34 [Ovis aries]
QCCESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGYGICGFPVLSSGSGFCQPTFFASR	Carbamidomethyl: 6, 18	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]

Sample 1a 25% stretch -3			
Sequence	Modifications	Accessions	Proteins
SSSCSSLSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
GCGCGSFR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRCGPCNSVVR	Carbamidomethyl: 3, 6	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
VSSCCGSVCSDQSCGR	Carbamidomethyl: 4, 5, 9, 14	gi 426237981	PREDICTED: keratin-associated protein 4-8-like isoform 2 [Ovis aries]
TYQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
TGPATTICSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
CGPCNSVVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
CCQSVCCQSPCR	Carbamidomethyl: 1, 2, 6, 7, 11	gi 426237981	PREDICTED: keratin-associated protein 4-8-like isoform 2 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSA CGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSPCQTSCYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGCGYCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]

TCGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSA_CGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LCEGVGAVNV_CVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
SISTV_CQPVG_VSTIC_QPT_CGVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
YSGSS_CGSSFP_NLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITP_CISSP_CAPAAP_CTP_CVPR	Carbamidomethyl: 5, 10, 16, 19	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SGQSLSFQPT_CGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
LGYGIC_GFPVLSSGSGF_CQPTFFASR	Carbamidomethyl: 6, 18	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
Sample 1a 25% stretch -4			
Sequence	Modifications	Accessions	Proteins
EAE_CAEADSGR	Carbamidomethyl: 4	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SSS_CSSLSSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASSF_CGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
G_CG_CGSR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TYQQ_CVSSCR	Carbamidomethyl: 6, 10	gi 328905561	keratin-associated protein KAP11-1 [Ovis aries]
RLG_CGSGFR	Carbamidomethyl: 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SVC_GGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TFRTSP_C_C	Carbamidomethyl: 7, 8	gi 999000042	KAP 2.3 var3 (tentative) cs39 [Ovis aries]
VGG_SILG_CCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
_CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
TGPATTI_CSSDK	Carbamidomethyl: 8	gi 999000024	KAP 3.2 (tentative) cs39 [Ovis aries]
LG_CGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGPCNTSG_C	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TQTVVSSPQCTSCYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 125090; gi 999000014	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 999000024	KAP 3.2 (tentative) cs39 [Ovis aries]
GGVACGGLTYSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 125117	hair keratin type II intermediate filament [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
VGGSILGCK	Propionamide: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TGPATTICSSDKF	Carbamidomethyl: 8	gi 999000024	KAP 3.2 (tentative) cs39 [Ovis aries]
T_CGFSTVSGSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SISTVCQPVGGVSTICQPTCGVSR	Carbamidomethyl: 6, 16, 20	gi 328905561	keratin-associated protein KAP11-1 [Ovis aries]
TGSCCGPTFSSLS_CGGGCLQPR	Carbamidomethyl: 4, 5, 14, 18	gi 999000042	KAP 2.3 var3 (tentative) cs39 [Ovis aries]
YSGSSCGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITPCISSPCAPAAPCTPCVPR	Carbamidomethyl: 5, 10, 16, 19	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
QIASGPVATGGSSITVLAPDSCQPR	Carbamidomethyl: 21	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

SGQSLSFQPTCGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
DLNMDCVVAEIK	Propionamide: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
QCCESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGYGICGFPVLSSGSGFCQPTFFASR	Carbamidomethyl: 6, 18	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
Sample 1a 25% stretch -5			
Sequence	Modifications	Accessions	Proteins
SSSCSSLSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
GCGCGSFR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TYQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
TFRTSPCC	Carbamidomethyl: 7, 8	gi 999000042	KAP 2.3 var3 (tentative) cs39 [Ovis aries]
TGPATTICSSDK	Carbamidomethyl: 8	gi 165917	BIIB2 high sulfur keratin [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 164652864	keratin 31 [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
VGGILGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
VGGILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSAAGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]

CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTI_CSSDKF_C	Carbamidomethyl: 8, 14	gi 165917	BIIB2 high sulfur keratin [Ovis aries]
DVD_CAYLR	Carbamidomethyl: 4	gi 312283584; gi 246276	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGGYGC_GYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
TGPATTI_CSSDKF	Carbamidomethyl: 8	gi 165917	BIIB2 high sulfur keratin [Ovis aries]
T_CGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
CCITAAPYRGIS_CYR	Carbamidomethyl: 1, 2, 13	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
L_CEGVGSVNV_CVSSSR	Carbamidomethyl: 2, 11	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
AVPAF_CVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TGSCC_GPTFSSLS_CGGG_C_LQPR	Carbamidomethyl: 4, 5, 14, 18	gi 999000042	KAP 2.3 var3 (tentative) cs39 [Ovis aries]
SISTV_CQPVG_VSTI_CQPT_CGVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
TITP_CISSP_CAPAAP_CTP_CVPR	Carbamidomethyl: 5, 10, 16, 19	gi 164652864	keratin 31 [Ovis aries]
YSGSS_CGSSFP_NLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
QIASGPVATGGSITV_LAPD_S_CQPR	Carbamidomethyl: 21	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SGQSLSF_QPT_C_GSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Q_CCESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGYGIC_GFPVLSSGSGF_CQPTFFASR	Carbamidomethyl: 6, 18	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]

Supplementary Table 3. Sample 1b

Supplementary Table 3

Sample 1b stretch to break -1			
Sequence	Modifications	Accessions	Proteins
SSS_CSSLSSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]

ASSFSCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
GCGCGSFR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SRGPGCNSYVR	Carbamidomethyl: 3, 6	gi 164652864	keratin 31 [Ovis aries]
TFRTSPCC	Carbamidomethyl: 7, 8	gi 125644	RecName: Full=Keratin, high-sulfur matrix protein, IIIA3 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 164652864	keratin 31 [Ovis aries]
VGGSilGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
VGGSilGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSPCQTSYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMIII B2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TGCFSTVSGSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
LCEGVGAVNVCVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]

SISTV <u>C</u> QPVGGVSTI <u>C</u> QPT <u>C</u> GVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
ILDELTL <u>L</u> CK	Carbamidomethyl: 8	gi 164652864; gi 312283590	keratin 31 [Ovis aries] keratin 33B [Ovis aries]
TG <u>S</u> CCGPTFSSLS <u>C</u> GGG <u>L</u> QPR	Carbamidomethyl: 4, 5, 14, 18	gi 125644	RecName: Full=Keratin, high-sulfur matrix protein, IIIA3 [Ovis aries]
YSGSS <u>C</u> GSFFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITP <u>C</u> ISSP <u>C</u> APAAP <u>C</u> TP <u>C</u> VPR	Carbamidomethyl: 5, 10, 16, 19	gi 164652864	keratin 31 [Ovis aries]
SGQSLSFQPT <u>C</u> SGGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Q <u>C</u> CESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
Sample 1b stretch to break -2			
Sequence	Modifications	Accessions	Proteins
SVC <u>G</u> GFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
<u>C</u> GPC <u>N</u> SYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
TGPATTI <u>C</u> SSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
VGG <u>S</u> ILG <u>C</u> CK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SRFGPC <u>N</u> TS <u>G</u> C	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
LG <u>C</u> SGGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TFSS <u>C</u> SAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SVSALG <u>S</u> CGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SL <u>C</u> GGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
<u>C</u> CISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
AF <u>S</u> CVSA <u>C</u> GPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
VGG <u>S</u> ILG <u>C</u> CK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
<u>C</u> CITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSP <u>C</u> QTS <u>C</u> YRPR	Carbamidomethyl: 9, 13	gi 426217167	PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
TGPATTI <u>C</u> SSDK <u>F</u> CR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2

LGC ^u GYG ^u CGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
T ^u CGFSTVGS ^u SGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAF ^u SC ^u VAC ^u GPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TITPC ^u ISSPC ^u APAAP ^u CTPC ^u VPR	Carbamidomethyl: 5, 10, 16, 19	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SGQSL ^u SFQPT ^u CGSGFY	Carbamidomethyl: 11	gi 426217167	PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
Sample 1b stretch to break -3			
Sequence	Modifications	Accessions	Proteins
EAE ^u CAEAD ^u SGR	Carbamidomethyl: 4	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SS ^u SS ^u SS ^u SS ^u SGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASS ^u F ^u CG ^u SSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
G ^u CG ^u GS ^u FR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TYQ ^u Q ^u SC ^u VSS ^u CR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SV ^u CG ^u GF ^u R	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
C ^u GPC ^u NS ^u YV ^u R	Carbamidomethyl: 1, 4	gi 164652864	keratin 31 [Ovis aries]
LG ^u CG ^u SG ^u FR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
VGG ^u SILG ^u C ^u KK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TFSS ^u CAVAP ^u K	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SVSALG ^u SC ^u GPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SL ^u CG ^u SGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
C ^u C ^u SAAPY ^u R	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
C ^u GPC ^u NTFV ^u H	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
VGG ^u SILG ^u C ^u K	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
GGVAC ^u GG ^u GLTYSS ^u TAG ^u R	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

DVD <u>C</u> AYLR	Carbamidomethyl: 4	gi 312283584; gi 125117	hair keratin type II intermediate filament [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
LGC <u>G</u> YG <u>C</u> GYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
<u>C</u> CJTAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
T <u>C</u> GFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAF <u>S</u> C <u>V</u> SAC <u>G</u> PRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SISTV <u>C</u> QPVG <u>V</u> STI <u>C</u> QPT <u>C</u> GVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
YSGSS <u>C</u> GSSFPNSLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITP <u>C</u> ISSP <u>C</u> APAAP <u>C</u> TP <u>C</u> VPR	Carbamidomethyl: 5, 10, 16, 19	gi 164652864	keratin 31 [Ovis aries]
QIASGPVATGGSSITVLAPD <u>S</u> CQPR	Carbamidomethyl: 21	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SGQSLSFQPT <u>C</u> SGSFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Sample 1b stretch to break -4			
Sequence	Modifications	Accessions	Proteins
ASSF <u>S</u> CGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
G <u>C</u> G <u>C</u> GSFR	Carbamidomethyl: 2, 4	gi 999000015; gi 999000016	KAP 19.5 (tentative) cs39 [Ovis aries] KAP 19.3 (tentative) cs39 [Ovis aries]
<u>C</u> PSSFGR	Carbamidomethyl: 1	gi 999000016	KAP 19.3 (tentative) cs39 [Ovis aries]
SR <u>C</u> GP <u>C</u> NSYVR	Carbamidomethyl: 3, 6	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
TFRTSP <u>C</u> C	Carbamidomethyl: 7, 8	gi 125644	RecName: Full=Keratin, high-sulfur matrix protein, IIIA3 [Ovis aries]
<u>C</u> GP <u>C</u> NSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
VGG <u>S</u> ILG <u>C</u> KK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
L <u>G</u> C <u>G</u> SGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TGPATTI <u>C</u> SSDK	Carbamidomethyl: 8	gi 229307	keratin SCMIIIIB2
SRFGP <u>C</u> NTSG <u>C</u>	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
TFSS <u>C</u> SAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SVSALG <u>S</u> CGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5

SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSilGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 125091	RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
AFSCVSA_CGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TQTVVSSP_CQTSCYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TGPATTI_CSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
LG_CGYG_CGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AVPAFSCVSA_CGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
T_CGFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
ILDELT_LCK	Carbamidomethyl: 8	gi 312283590; gi 999000013; gi 125091	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries] RecName: Full=Keratin, type I microfibrillar, 47.6 kDa; AltName: Full=Low-sulfur keratin
TGSCCGPTFSSLS_CGGGCLQPR	Carbamidomethyl: 4, 5, 14, 18	gi 125644	RecName: Full=Keratin, high-sulfur matrix protein, IIIA3 [Ovis aries]
YSGSS_CGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITPCISSPCAPAAP_CTP_CVPR	Carbamidomethyl: 5, 10, 16, 19	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SGQSLSFQPT_CGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
DLNMD_CVVAEIK	Propionamide: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DLNMD_CIVAIEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
Sample 1b stretch to break -5			
Sequence	Modifications	Accessions	Proteins
SSSC_SSSLSSGSR	Carbamidomethyl: 4	gi 426219157	PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
ASSFSC_GSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

GISCYR	Carbamidomethyl: 4	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTI_CSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIIB2
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
VGGSILGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTI_CSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIIB2
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
DVD_CAYLR	Carbamidomethyl: 4	gi 312283584; gi 246276	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
T_CGFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
YSGSS_CGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITP_CISSPCAPAAPCTPCVPR	Carbamidomethyl: 5, 10, 16, 19	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
QIASGPVATGGSITVLAPDSCQPR	Carbamidomethyl: 21	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SGQSLSFQPT_CGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]

Supplementary Table 4 - Sample 2a

Supplementary Table 4

Sample 2a 25% stretch -1			
Sequence	Modifications	Accessions	Proteins
EAECAEADSGR	Carbamidomethyl: 4	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
PTSCQPAPCR	Carbamidomethyl: 4, 9	gi 999000040	KAP 2.3 var1 (tentative) cs39 [Ovis aries]
CPSSFGR	Carbamidomethyl: 1	gi 999000016	KAP 19.3 (tentative) cs39 [Ovis aries]
TYQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283588; gi 312283584; gi 1308	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CTRPICEPCR	Carbamidomethyl: 1, 6, 9	gi 999000040	KAP 2.3 var1 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAAYVR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSAAGPR	Carbamidomethyl: 4, 8	gi 312283588; gi 1308	keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283588; gi 312283584; gi 1308	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
TQTVVSSPQTSYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
GGVACGGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 246276	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-

			filament protein component 5 [sheep, wool, Peptide, 503 aa]
ARLE <u>CE</u> INTYR	Carbamidomethyl: 5	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
LPSNP <u>C</u> ATTNASSNF <u>CR</u>	Carbamidomethyl: 6, 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
T <u>CG</u> FSTVGS <u>G</u> FGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
L <u>CE</u> GVGSVNV <u>C</u> VSSSR	Carbamidomethyl: 2, 11	gi 1308; gi 246276	hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
L <u>CE</u> GVGAVNV <u>C</u> VSSSR	Carbamidomethyl: 2, 11	gi 312283588; gi 312283584	keratin 83 [Ovis aries] hair keratin type II intermediate filament [Ovis aries]
AVPAF <u>S</u> <u>C</u> VSAC <u>G</u> PR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
SISTV <u>C</u> QPVG <u>V</u> STI <u>C</u> QPT <u>C</u> GVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
ILDELTL <u>C</u> K	Carbamidomethyl: 8	gi 999000013; gi 312283590; gi 426238003	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries] PREDICTED: keratin 34 [Ovis aries]
YSGSS <u>C</u> GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
VESLKEELL <u>C</u> LK	Carbamidomethyl: 10	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
VESLKEELI <u>C</u> LK	Carbamidomethyl: 10	gi 999000013; gi 312283590	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries]
SGQSLSFQPT <u>C</u> GSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
DLNMD <u>C</u> VVAEIK	Propionamide: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DLNMD <u>C</u> IVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELL <u>C</u> LK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
SDLEAQVESLKEELI <u>C</u> LK	Carbamidomethyl: 16	gi 999000013; gi 312283590	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries]
Y <u>S</u> QNLNQVQSLISNVESQLAEIR	Carbamidomethyl: 3	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
Y <u>S</u> QLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]
Sample 2a 25% stretch -2			
Sequence	Modifications	Accessions	Proteins
SS <u>S</u> CSLSSGSR	Carbamidomethyl: 4	gi 359801728	keratin-associated protein KAP13-3 [Ovis aries]
ASSF <u>S</u> CGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

GCGGGSFR	Carbamidomethyl: 2, 4	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
TGPATTI_CSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
VGGSILGCKK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AFSCVSA_CGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
VGGSILGCK	Propionamide: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1; Propionamide: 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 125090; gi 999000014	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
DVD_CAYLR	Carbamidomethyl: 4	gi 246276; gi 312283584	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair keratin type II intermediate filament [Ovis aries]
TGPATTI_CSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGCGYG_CGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]

TGPATTI <u>C</u> SSDKF	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
T <u>C</u> GFSTVGS <u>G</u> FGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
SISTV <u>C</u> QPVGGVSTI <u>C</u> QPT <u>C</u> GVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
ILDELTL <u>C</u> K	Carbamidomethyl: 8	gi 312283590; gi 125090; gi 426238003; gi 999000014	keratin 33B [Ovis aries] RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin PREDICTED: keratin 34 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
YSGSS <u>C</u> GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITP <u>C</u> ISSP <u>C</u> APAAP <u>C</u> TP <u>C</u> VPR	Carbamidomethyl: 5, 10, 16, 19	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
QIASGPVATGGISITVLAPD <u>S</u> CQPR	Carbamidomethyl: 21	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SGQSLSFQPT <u>C</u> GSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
<u>C</u> CESNLEPLFNGYIETLRR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
Q <u>C</u> CESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
Sample 2a 25% stretch -3			
Sequence	Modifications	Accessions	Proteins
SSS <u>C</u> SSLSSGSR	Carbamidomethyl: 4	gi 999000017	KAP 13.1 var1 (tentative) cs39 [Ovis aries]
ASSF <u>S</u> C <u>G</u> SSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
GIS <u>C</u> YR	Carbamidomethyl: 4	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TYQQS <u>C</u> VSS <u>C</u> R	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SVC <u>G</u> GFR	Carbamidomethyl: 3	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
<u>C</u> G <u>P</u> CNSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
TGPATTI <u>C</u> SSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
VGG <u>S</u> ILG <u>C</u> KK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
LG <u>C</u> GSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SRFGP <u>C</u> NTSG <u>C</u>	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]

SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TRCGPCNTFVH	Carbamidomethyl: 3, 6	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
VGGILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
LGCYGCYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
VGGILGCK	Propionamide: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
TCGFSTVSGFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSACGPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYRGISCYR	Carbamidomethyl: 1, 2, 13	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
RCGFSSFY	Carbamidomethyl: 2	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
SISTVCQPVGGVSTICQPTCGVSR	Carbamidomethyl: 6, 16, 20	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SGQSLFQPTCGSGFY	Carbamidomethyl: 11	gi 426217167	PREDICTED: keratin-associated protein 13-1-like [Ovis aries]
Sample 2a 25% stretch -4			
Sequence	Modifications	Accessions	Proteins
EAECAEADSGR	Carbamidomethyl: 4	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
ASSFCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

TYQQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAYVR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
AFSCVSACGPR	Carbamidomethyl: 4, 8	gi 1308; gi 312283588	hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 125090	RecName: Full=Keratin, type I microfibillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
DVDCAYLR	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 246276	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
ARLECEINTYR	Carbamidomethyl: 5	gi 125090	RecName: Full=Keratin, type I microfibillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
LCEGVGSVNVCVSSSR	Carbamidomethyl: 2, 11	gi 1308; gi 246276	hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TCGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
LCEGVGAVNVCVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]

QDMA <u>C</u> LLK	Carbamidomethyl: 5	gi 312283584; gi 1308; gi 312283588; gi 246276	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
ILDELT <u>L</u> CK	Carbamidomethyl: 8	gi 125090; gi 312283590; gi 426238003	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries] PREDICTED: keratin 34 [Ovis aries]
YSGSS <u>C</u> GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
VESLKEEL <u>I</u> CLK	Carbamidomethyl: 10	gi 125090; gi 312283590	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries]
VESLKEEL <u>L</u> CLK	Carbamidomethyl: 10	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
DLNMD <u>C</u> VVAEIK	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
Q <u>C</u> CESNLEPLFSGYIETLR	Carbamidomethyl: 2, 3	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
SDLEAQVESLKEEL <u>I</u> CLK	Carbamidomethyl: 16	gi 125090; gi 312283590	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries]
SDLEAQVESLKEEL <u>L</u> CLK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
Y <u>S</u> QLNQVQSLISNVESQLAEIR	Carbamidomethyl: 3	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
Y <u>S</u> QLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]
Sample 2a 25% stretch -5			
Sequence	Modifications	Accessions	Proteins
ASSF <u>S</u> CGSSR	Carbamidomethyl: 6	gi 125117; gi 164652870	RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]
EAE <u>C</u> VEADSGR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
TYQQ <u>S</u> CVSS <u>C</u> R	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
<u>C</u> TRPI <u>C</u> EP <u>C</u> R	Carbamidomethyl: 1, 6, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
SS <u>C</u> SSRP <u>C</u> VPSS <u>C</u> R	Carbamidomethyl: 3, 8, 13	gi 309323371	keratin 34 [Ovis aries]
LG <u>C</u> GSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]

CGPCNSYVR	Carbamidomethyl: 1, 4	gi 164652864	keratin 31 [Ovis aries]
SLGSGYGCGR	Carbamidomethyl: 3, 9	gi 224555998	keratin associated protein 6 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
AFSCVSACGPR	Carbamidomethyl: 4, 8	gi 312283588; gi 1308	keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588; gi 1308	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 164652864; gi 999000014	keratin 31 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
ARLECEINTYR	Carbamidomethyl: 5	gi 164652864; gi 999000014	keratin 31 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117; gi 164652870	RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]
DVDCAYLR	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 125117; gi 164652870	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]
LGCGYGCYGYGSR	Carbamidomethyl: 3, 7	gi 224555998	keratin associated protein 6 [Ovis aries]
LPSNPCATTNASSNFCR	Carbamidomethyl: 6, 16	gi 309323371	keratin 34 [Ovis aries]
NLEIDPNAQCVK	Carbamidomethyl: 10	gi 312283584; gi 312283588; gi 1308; gi 125117	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
QDMACLK	Carbamidomethyl: 5	gi 312283584; gi 312283588; gi 1308; gi 125117; gi 164652870	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]

T_CGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSA_CGPR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
C_DSDILPLR	Carbamidomethyl: 1	gi 999000051	Leucine Rich Repeat Containing 15 Partial (tentative) cs39 [Ovis aries]
L_CEGVGAVNV_CVSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
ILDELT_LCK	Carbamidomethyl: 8	gi 164652864; gi 309323371; gi 312283590; gi 999000014	keratin 31 [Ovis aries] keratin 34 [Ovis aries] keratin 33B [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
ILDDLTL_LCK	Carbamidomethyl: 8	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
YSGSS_CGSSFPNLYVR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
VESLKEELI_CCLK	Carbamidomethyl: 10	gi 164652864; gi 312283590; gi 999000014	keratin 31 [Ovis aries] keratin 33B [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
VESLKEELL_CCLK	Carbamidomethyl: 10	gi 309323371; gi 168693435	keratin 34 [Ovis aries] keratin, type I cuticular Ha5 [Ovis aries]
PLTFISSG_CQPLSGVSTV_CKPVVR	Carbamidomethyl: 9, 19	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
SGQSLSFQPT_CGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
DLNMD_CVVAEIK	Carbamidomethyl: 6	gi 125117; gi 164652870	RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]
DLNMD_CIVAIEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
Q_CCESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
C_CCESNLEPLFNGYIETLR	Carbamidomethyl: 1, 2	gi 125117; gi 164652870	RecName: Full=Keratin, type II microfibrillar, component 5 keratin 85 [Ovis aries]
Q_CCESNLEPLFSGYIETLR	Carbamidomethyl: 2, 3	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
Q_CCESNLEPLFEGYIETLR	Carbamidomethyl: 2, 3	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELI_CCLK	Carbamidomethyl: 16	gi 164652864; gi 312283590	keratin 31 [Ovis aries] keratin 33B [Ovis aries]
SDLEAQVESLKEELL_CCLK	Carbamidomethyl: 16	gi 309323371	keratin 34 [Ovis aries]
ADLEAQVESLKEELL_CCLK	Carbamidomethyl: 16	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
YSCQLNQVQSLISNVESQLAEIR	Carbamidomethyl: 3	gi 164652864	keratin 31 [Ovis aries]
YSCQLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]

Supplementary Table 5 - Sample 2b

Supplementary Table 5

Sample 2b stretch to break -1			
Sequence	Modifications	Accessions	Proteins
ASSFSCGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
VGGSLGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
LGCYGCYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
TGCFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
SGQSLFQPTCGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Sample 2b stretch to break -2			
Sequence	Modifications	Accessions	Proteins
ASSFSCGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
TGPATTICSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
SRFGPCNTSGC	Carbamidomethyl: 6, 11	gi 312283590	keratin 33B [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
TRCGPCNTFVH	Carbamidomethyl: 3, 6	gi 312283592	keratin 33 [Ovis aries]

TFSSCSAVAPK	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
SLCGSGYGYGSR	Carbamidomethyl: 3	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 312283592	keratin 33 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1; Propionamide: 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
TCGFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
CCITAAPYRGISCYR	Carbamidomethyl: 1, 2, 13	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
YSGSSCGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
SGQSLSFQPTCGSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
Sample 2b stretch to break -3			
Sequence	Modifications	Accessions	Proteins
EAECAEADSGR	Carbamidomethyl: 4	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
CPSSFGR	Carbamidomethyl: 1	gi 999000016	KAP 19.3 (tentative) cs39 [Ovis aries]
EAEQVEADSGR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]

CCISAAPYR	Carbamidomethyl: 1, 2	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 125117	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5
LPSNPCATTNASSNFCR	Carbamidomethyl: 6, 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
QDMACLK	Carbamidomethyl: 5	gi 312283584; gi 1308; gi 125117; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] RecName: Full=Keratin, type II microfibrillar, component 5 keratin 83 [Ovis aries]
TGCFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
ILDELTCLK	Carbamidomethyl: 8	gi 426238003; gi 312283590; gi 999000013	PREDICTED: keratin 34 [Ovis aries] keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
YSGSSCGSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
DLNMDCVVAEIK	Carbamidomethyl: 6	gi 125117	RecName: Full=Keratin, type II microfibrillar, component 5
DLNMDCIVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELCLK	Carbamidomethyl: 16	gi 312283590; gi 999000013	keratin 33B [Ovis aries] IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SDLEAQVESLKEELLCLK	Carbamidomethyl: 16	gi 426238003	PREDICTED: keratin 34 [Ovis aries]
ADLEAQVESLKEELLCLK	Carbamidomethyl: 16	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
YSCQLNQVQSLISNVESQLAEIR	Carbamidomethyl: 3	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
YSCQLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]
Sample 2b stretch to break -4			
Sequence	Modifications	Accessions	Proteins
EAECAEADSGR	Carbamidomethyl: 4	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
ASSFSCGSSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

PTSCQPAPCR	Carbamidomethyl: 4, 9	gi 999000041	KAP 2.3 var2 (tentative) cs39 [Ovis aries]
EAECEADSGR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
SSCSSRPCVPSSCR	Carbamidomethyl: 3, 8, 13	gi 309323371	keratin 34 [Ovis aries]
LGCGSGFR	Carbamidomethyl: 3	gi 999000015	KAP 19.5 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAAYVR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
AFSCVSACGPRPGR	Carbamidomethyl: 4, 8	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
VGGSilGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CGPCNTFVH	Carbamidomethyl: 1, 4	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
TQTVVSSPCQTSYRPR	Carbamidomethyl: 9, 13	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
AFSCVSACGPR	Carbamidomethyl: 4, 8	gi 1308; gi 312283588	hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 999000013; gi 999000014	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
GGVACGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 246276; gi 1308	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries]
ARLECEINTYR	Carbamidomethyl: 5	gi 999000013; gi 999000014	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
QDMACLKK	Carbamidomethyl: 5	gi 312283584; gi 246276; gi 1308; gi 312283588	hair keratin type II intermediate filament [Ovis aries] type II intermediate-filament protein

			component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries] keratin 83 [Ovis aries]
AVPAFSCVSA ^C GPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
T ^C GFSTVGS ^G FGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSCVSA ^C GPR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
L ^C EGVGSVNV ^C VSSSR	Carbamidomethyl: 2, 11	gi 246276; gi 1308	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] hair type II keratin intermediate filament protein [Ovis aries]
L ^C EGVGAVNV ^C VSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
ILDDLTL ^C K	Carbamidomethyl: 8	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
ILDELT ^C K	Carbamidomethyl: 8	gi 999000013; gi 312283590; gi 309323371; gi 999000014	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries] keratin 34 [Ovis aries] IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
YSGSS ^C GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
TITPC ^I SSPC ^A AP ^A CTPC ^V PR	Carbamidomethyl: 5, 10, 16, 19	gi 999000013	IFP Type I K31 var1 (tentative) cs39 [Ovis aries]
SQQQEPLL ^C PNYQSYFR	Carbamidomethyl: 9	gi 309323371	keratin 34 [Ovis aries]
DLNMD ^C VVAEIK	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DLNMD ^C VVAEIK	Propionamide: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
QCCESNLEPLFEGYIETLRR	Carbamidomethyl: 2, 3	gi 312283588	keratin 83 [Ovis aries]
SDLEAQVESLKEELL ^C CLK	Carbamidomethyl: 16	gi 309323371	keratin 34 [Ovis aries]
SDLEAQVESLKEELI ^C CLK	Carbamidomethyl: 16	gi 999000013; gi 312283590	IFP Type I K31 var1 (tentative) cs39 [Ovis aries] keratin 33B [Ovis aries]
ADLEAQVESLKEELL ^C CLK	Carbamidomethyl: 16	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
YSCQLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]
YSCQLNQVQSLIVSVESQLAEIR	Carbamidomethyl: 3	gi 999000014	IFP Type I K33a var1 (tentative) cs39 [Ovis aries]
Sample 2b stretch to break -5			
Sequence	Modifications	Accessions	Proteins
ASSFSC ^G SSR	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]

EAEQVEADSGR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
SVCGGFR	Carbamidomethyl: 3	gi 312283584; gi 312283588; gi 1308; gi 426226636	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] PREDICTED: keratin, type II cuticular Hb3-like [Ovis aries]
TYQSCVSSCR	Carbamidomethyl: 6, 10	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
CGPCNSYVR	Carbamidomethyl: 1, 4	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
TGPATTICSSDK	Carbamidomethyl: 8	gi 229307	keratin SCMKIIB2
SVSALGSCGPR	Carbamidomethyl: 8	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
TFSSCSAVAPK	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DVDCAAYVR	Carbamidomethyl: 4	gi 312283588	keratin 83 [Ovis aries]
VGGSILGCK	Carbamidomethyl: 8	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
CCISAAPYR	Carbamidomethyl: 1, 2	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
CCITAAPYR	Carbamidomethyl: 1, 2	gi 312283584; gi 312283588; gi 1308; gi 426226636	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] PREDICTED: keratin, type II cuticular Hb3-like [Ovis aries]
AFSCVSACGPR	Carbamidomethyl: 4, 8	gi 312283588; gi 1308; gi 426226636	keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] PREDICTED: keratin, type II cuticular Hb3-like [Ovis aries]
LECEINTYR	Carbamidomethyl: 3	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
TGPATTICSSDKFCR	Carbamidomethyl: 8, 14	gi 229307	keratin SCMKIIB2
GGVACGGGLTYSSTAGR	Carbamidomethyl: 5	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
ARLECEINTYR	Carbamidomethyl: 5	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
DVDCAYLRL	Carbamidomethyl: 4	gi 312283584; gi 1308; gi 246276	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-

			filament protein component 5 [sheep, wool, Peptide, 503 aa]
LGCGYGCGYGYGSR	Carbamidomethyl: 3, 7	gi 999000021	KAP 6.1 (tentative) cs39 [Ovis aries]
SFSA _C SVGLGK	Carbamidomethyl: 5	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
QDMA _C LLK	Carbamidomethyl: 5	gi 312283584; gi 312283588; gi 1308; gi 246276; gi 426226636	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries] type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa] PREDICTED: keratin, type II cuticular Hb3-like [Ovis aries]
AVPAFSC _C VSA _C GPRPGR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
T _C GFSTVGSFGSR	Carbamidomethyl: 2	gi 312283588	keratin 83 [Ovis aries]
AVPAFSC _C VSA _C GPR	Carbamidomethyl: 7, 11	gi 312283584	hair keratin type II intermediate filament [Ovis aries]
L _C EGVGAVNV _C VSSSR	Carbamidomethyl: 2, 11	gi 312283584; gi 312283588	hair keratin type II intermediate filament [Ovis aries] keratin 83 [Ovis aries]
RILDELTL _C K	Carbamidomethyl: 9	gi 125090; gi 312283590; gi 309323371	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries] keratin 34 [Ovis aries]
ILDDLTL _C K	Carbamidomethyl: 8	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
ILDELTL _C K	Carbamidomethyl: 8	gi 125090; gi 312283590; gi 309323371	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries] keratin 34 [Ovis aries]
YSGSS _C GSSFPSNLVYR	Carbamidomethyl: 6	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
VESLKEELI _C LK	Carbamidomethyl: 10	gi 125090; gi 312283590	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries]
VESLKEELL _C LK	Carbamidomethyl: 10	gi 309323371; gi 168693435	keratin 34 [Ovis aries] keratin, type I cuticular Ha5 [Ovis aries]
SGQSLSFQPT _C GSGFY	Carbamidomethyl: 11	gi 999000030	KAP 13.1 (tentative) cs39 [Ovis aries]
PLTFISSG _C QPLSGVSTV _C KPVR	Carbamidomethyl: 9, 19	gi 999000018	KAP 11.1 (tentative) cs39 [Ovis aries]
DLNMD _C VVAEIK	Carbamidomethyl: 6	gi 246276	type II intermediate-filament protein component 5 [sheep, wool, Peptide, 503 aa]
DLNMD _C IVAEIK	Carbamidomethyl: 6	gi 312283588	keratin 83 [Ovis aries]

QCCESNLEPLFSGYIETLRR	Carbamidomethyl: 2, 3	gi 312283584; gi 1308	hair keratin type II intermediate filament [Ovis aries] hair type II keratin intermediate filament protein [Ovis aries]
SDLEAQVESLKEELI ¹⁶ CLK	Carbamidomethyl: 16	gi 125090; gi 312283590	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin keratin 33B [Ovis aries]
SDLEAQVESLKEELL ¹⁶ CLK	Carbamidomethyl: 16	gi 309323371	keratin 34 [Ovis aries]
QCCESNLEPLFEGYIETLR	Carbamidomethyl: 2, 3	gi 312283588	keratin 83 [Ovis aries]
ADLEAQVESLKEELL ¹⁶ CLK	Carbamidomethyl: 16	gi 168693435	keratin, type I cuticular Ha5 [Ovis aries]
YSCQLNQVQSLISNVESQLAEIR	Carbamidomethyl: 3	gi 125090	RecName: Full=Keratin, type I microfibrillar 48 kDa, component 8C-1; AltName: Full=Low-sulfur keratin
YSCQLSQVQSLIVNVESQLAEIR	Carbamidomethyl: 3	gi 312283590	keratin 33B [Ovis aries]

Coloured version of summary Table 2

Peptides from which consistently labelled cysteines were detected in one or more of the 5 repeats of a treatment condition. Green shading indicates a statistical likelihood (*, P<0.05; ** P<0.01; *** P<0.001) that labelling is a consistent effect of the treatment. Identified proteins from which peptides arise are highlighted as either keratins (blue) or KAPs (orange).

Supplementary Table 6

Sequence	Significance/conditions				Protein ID	Position	Region
	Wet		Dry (55% RH)				
	1a hold	1b break	2a hold	2b break			
A. Cysteines consistently labelled irrespective of conditions							
A ⁴⁸⁹ SSFSCGSSR	5***	4**	5***	5***	K85	T-65	Tail domain
C ³¹ CISAAPYR	5***	5***	4**	3*	K85	H-31, H-32	Head domain
B. Cysteines consistently labelled when wet (hydrogen bonds disrupted)							
T ¹² GPATTICSSDKFCR	5***	4**	2	2	KAP3.2	19, 25	Close to N-terminus
S ⁹⁸ SSCSSLSSGSR	4**	3*	2		KAP13.1 var1	102	In the middle (protein is 166 residues long)
G ²⁵ CGCGSFR	5***	3*	1		KAP19.5	26, 28	In the middle (protein is 72 residues long)
T ³⁸⁰ ITPCISSPCAPAAPCTPCVPR	5***	5***	1	1	K31	T-22, T-27, T-33, T-36	Close to C-terminus
S ³⁹⁴ RFGPCNTSGC	5***	3*	2	1	K33b	T-37, T-42	C-terminal peptide
S ⁵³ VCGGFR	4**	3*	2	2	K83	H-55	Head domain
V ⁴⁹² GGSilGCKK	5***	5***	2		K86-like	T-70	C-terminus but one, terminal cysteine
C. Cysteines consistently labelled when wet and stretched and held only							
L ¹²⁵ GYGICGFPVLSSGSGFCQPTFFASR	4**				KAP13.1 var1	130	Close to the C-terminus (protein is 166 residues long)
T ¹²⁴ FRTSPCC	3*	2			KAP2.3 var3	130, 131	C-terminal peptide
T ¹² GPATTICSSDKF	3*		1		KAP3.2	19	Close to N-terminus
D. Cysteines consistently labelled only in dry condition (reinforcing hydrogen bonds present)							
Y ³⁰⁶ SCQLSQVQLIVNVESQLAEIR			3*	3*	K31, K33b	2-90	Coil 2B
E. Cysteines consistently labelled when dry and stretched and held only							
V ¹⁷⁹ ESLKEELICLK			3*	1	K31 var1, K33b	1B-87	Coil 1B (Seq. number for K31)
V ¹⁷⁹ ESLKEELLCLK			3*	1	K34	1B-87	Coil 1B
Y ³⁰⁶ SCQLNQVQLISNVESQLAEIR			3*	2	K31, K33b	2-90	Coil 2B
F. Cysteines consistently labelled on prolonged stretching irrespective of water content							
S ¹²¹ ISTVCQPVGGVSTICQPTCGVSR	5***	2	3*		KAP11.1	126, 136, 140	Close to the C-terminus (residue 158)
G. Cysteines consistently labelled only when fibre stretched to break and reinforced by hydrogen bonds							
A ²¹⁴ DLEAQVESLKEELLCLK			1	3*	K35	1B-87	Coil 1B
H. Cysteines consistently labelled when fibre was wet or stretched and held, but not when stretched to break dry							
C ³⁹⁵ GPCNTFVH	3*	3*	3*	2	K33a	T-33, T-36	Tail domain

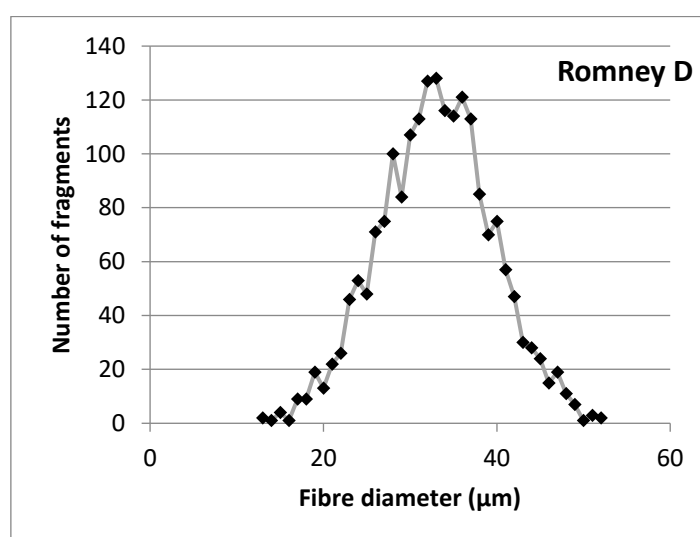
Fibre diameter distribution and other characteristics measured by OFDA method

Romney breed, Barry's Bay Sheep D was selected from wool from 10 sheep in the same flock because of its well-defined narrow distribution of fibre diameters when measured using the industry standard Optical Fibre Diameter Analyser (OFDA) method (Supplementary Supplementary Figure 1 and Supplementary Supplementary Table 7).

The table below contains measurement categories used in the wool industry in New Zealand. The key values for this study are the average fibre diameter (MICRON), standard deviation of the fibre diameter distribution (SD) and the curliness of fibres (CURVE) expressed in degrees of fibre curvature per millimetre.

Supplementary Table 7. OFDA data for wool sample used in this study

SAMPLE ID	Breed	MICRON	SD	CV	CEM	CF	SF	CURVE	BULK
D	Romney	33.02	6.6	19.9	11.48	34.5	31.84	45.9	25.71



Supplementary Figure 1. OFDA diameter distribution of wool fibres from wool sample used in study.

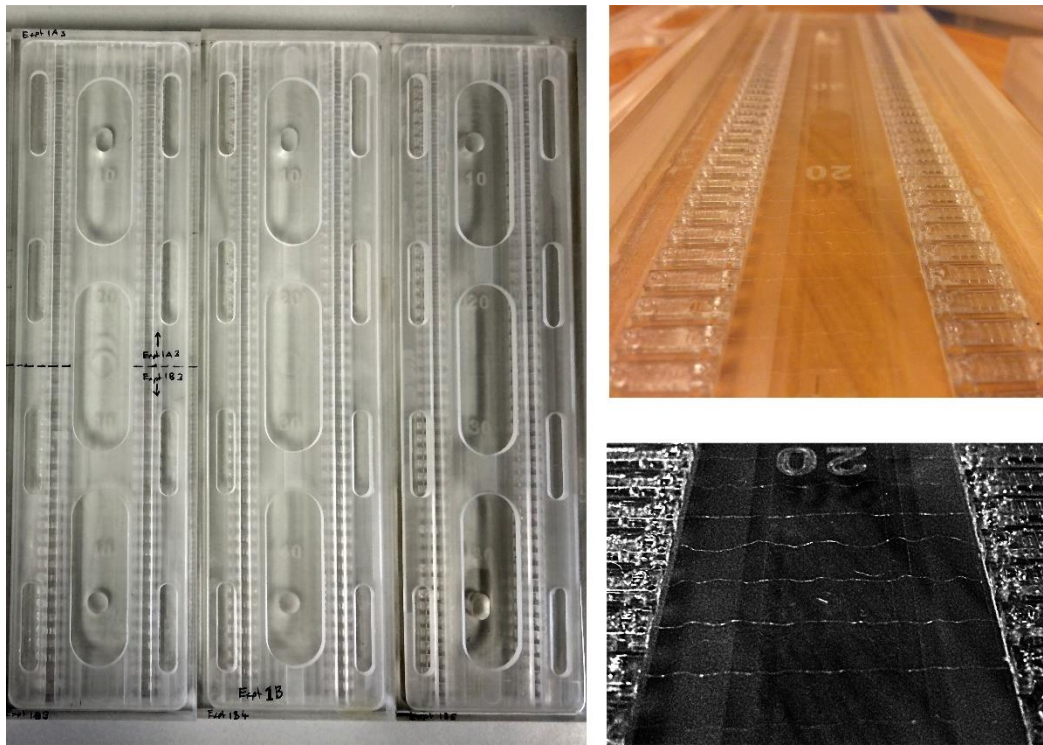
Tensile breakage of single fibres from same sample

Although single fibres were stretched and broken during the study (wet, dry but always with alkylating reagents present), the data from those tests were not reliable because the samples were mounted between plastic clips but stretched in an instrument designed for single fibres stabilised by metal crimps.

Reference data for the tensile properties of a select group of fibres from the Romney D sample is presented here. Two groups of fibres were tested, one containing 50 fibres and effectively a larger replicate of the groups used in the study ($n = 25$) and a second set of 12 fibres which were selected in the same way as other fibres but for which the cross-section of the break surface was measured optically.

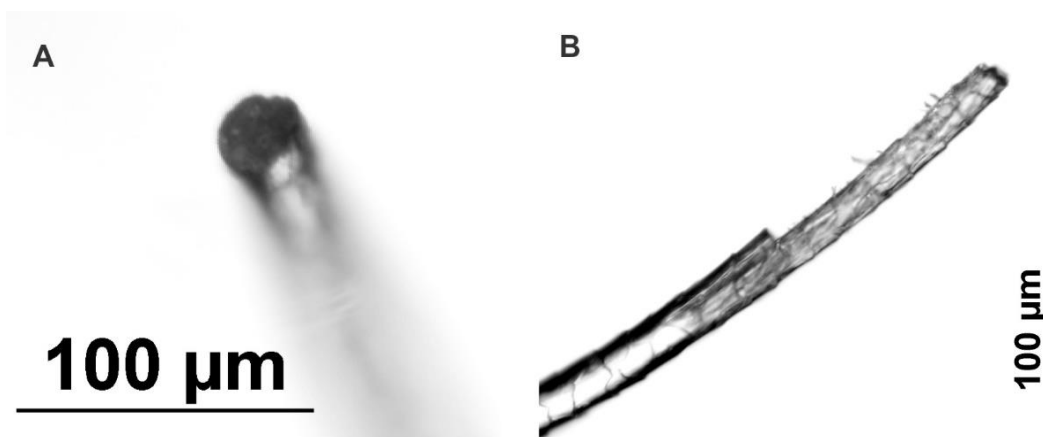
The root-end 30 mm of 50 fibres were individually mounted between pairs of clip-together plastic tabs (Dia-Stron, UK) used to grip the fibres during stretching and further secured

using UV cure glue (Dymax 3094-adhesive) (see main article for details). All the mounted fibres were placed in specially constructed plastic trays (Main article Figure 2 and Supplementary Supplementary Figure 2).



Supplementary Figure 2. Picture of the trays used in the study and insets images showing the arrangement of individual wool fibres.

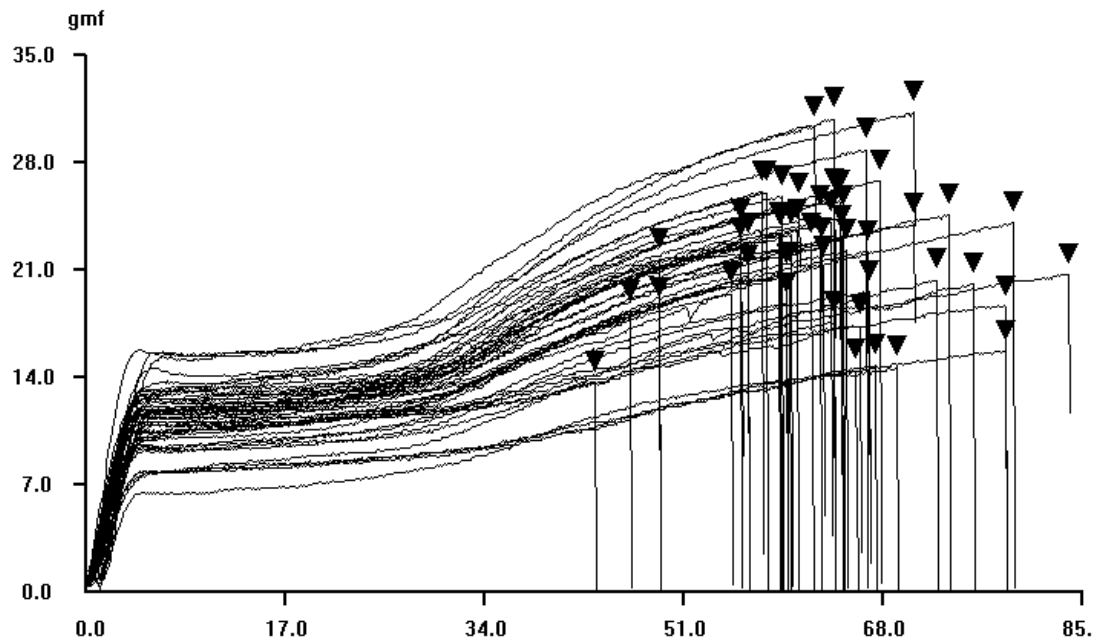
For 12 of the fibres, the broken fibre ends were recovered and imaged end-on under a microscope (Zeiss Standard 14 with an Olympus DP12 digital camera) (Supplementary Figure 3) and the cross-sectional area (CSA) of the break measured (Olympus AnalySIS version 5) along with mean diameter (from 360 diameter measurements taken at 1 degree angles) and aspect ratio (the maximum diameter divided by minimum diameter).



Supplementary Figure 3. Examples of break types. A. Oblique view of clean transverse break. B. Step break, common in dry condition.

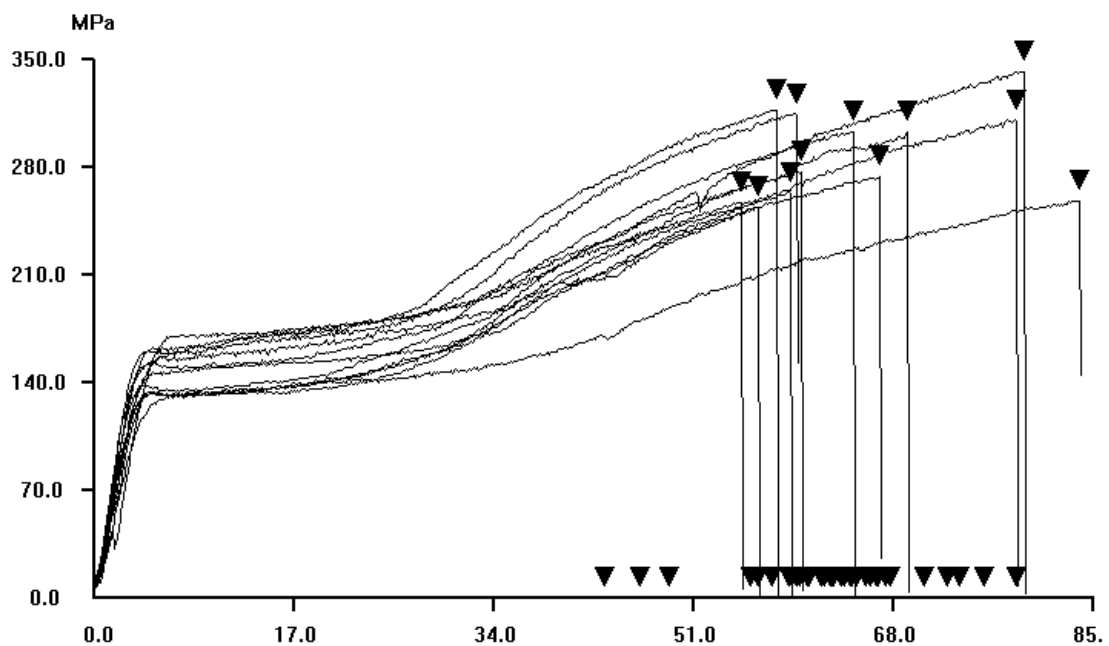
Tensile tests were carried out to failure for each fibre (Dia Stron, UK, LEX820 linear extensometer with an ALS1500 automatic loading system). Data is plotted all fibres (Supplementary Supplementary Figure 4) and as stress/strain plots for the 12 fibres with Cross Sectional area of break surface data (Supplementary Supplementary Figure 5), and all data is summarised (Supplementary Supplementary Table 8).

Description: Slot 1 : LEX Extension Method, Cycle: 1 Romney D



Supplementary Figure 4. Tensile plots of all 50 wool fibres.

Description: Slot 1 : LEX Extension Method, Cycle: 1 Romney D



Supplementary Figure 5. Tensile stress/strain plots for the 12 in which cross-section at break was measured.

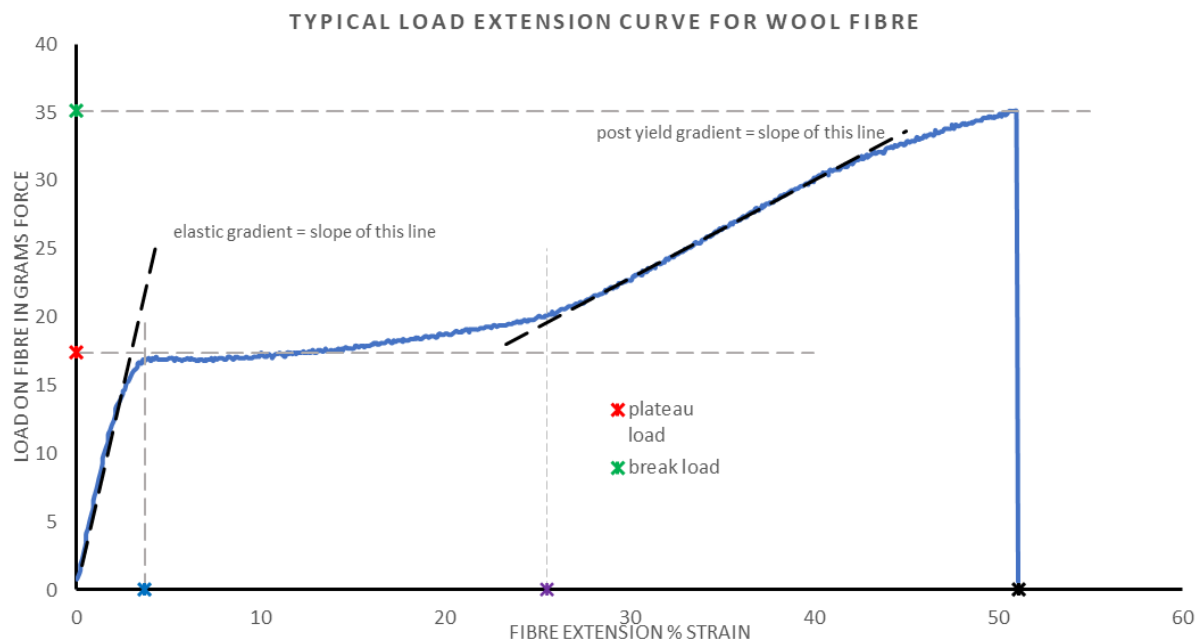
Supplementary Table 8. Tensile data summary from Dia Stron for Romney D with CSA for 12 samples. Note that properties dependent on CSA have only 12 data points whereas others have 50.

	CROSS-SECTIONAL AREA	ELASTIC_EMOD	ELASTIC_GRADIENT	ELASTIC_EXT	PLATEAU_LOAD	PLATEAU_STRESS	LOAD_15%	STRESS_15%	WORK_15%
	sq. microns	N/m2(Pa)	gmf/mm	% Strain	gmf	gmf/sq micron	gmf	gmf/sq micron	Joules
Min:	476.4	2.51E+09	35.4	3.26	7.32	0.0143	6.8	0.0137	3.52E-05
Max:	864.9	4.38E+09	94.5	5.57	16.9	0.019	15.8	0.0177	9.07E-05
Median:	764.55	3.55E+09	64.45	4.13	12.5	0.0162	11.95	0.01565	0.000063
Mean:	713.275	3.56E+09	65.248	4.206	12.2748	0.016325	11.76	0.015633	6.22E-05
Std. Dev.:	128.7361	5.52E+08	12.66066	0.552626	2.066367	0.001647	1.919263	0.001606	1.05E-05
data count	12	12	50	50	50	12	50	12	50

LOAD_25%	STRESS_25%	WORK_25%	YIELD_EXT	POSTYIELD_GRADIENT	BREAK_EXT	BREAK_LOAD	BREAK_STRESS	TOTAL_WORK	TOUGHNESS
gmf	gmf/sq micron	Joules	% Strain	gmf/mm	% Strain	gmf	gmf/sq micron	Joules	Joules/m3
7.65	0.0146	6.53E-05	23.89	3.33	43.49	13.8	0.026	0.000182	94000000
17.1	0.0186	0.000162	44.23	9.97	83.87	31.3	0.035	0.000619	1.81E+08
12.9	0.0167	0.000115	33.73	6.465	63.3	22.65	0.02965	0.000404	1.25E+08
12.646	0.016792	0.000113	33.6606	6.2196	63.3988	22.282	0.029592	0.000398	1.3E+08
2.058825	0.001517	1.9E-05	4.129862	1.497167	8.146724	4.071187	0.002973	9.02E-05	26865298
50	12	50	50	50	50	50	12	50	12

Plot below defines the properties in Supplementary Supplementary Table 8, the most useful of which are listed below in Supplementary Supplementary Figure 6

1. Elastic gradient
2. Post yield gradient
3. Plateau load (or stress if fibre CSA is known)
4. Break load (or stress if fibre CSA is known)
5. Elastic extent (or stress if fibre CSA is known)
6. Break extension (or stress if fibre CSA is known)
7. Yield extent



Supplementary Figure 6. Example data from a typical tensile extension test for a single wool fibre.