

CLAIMS

1.- ~~_____~~ A method for measuring the amount of dystrophin protein in a ~~protein sample isolated from a~~ biological sample comprising:

(i) contacting the ~~protein sample~~ with a ~~first~~ protease, forming a protein digest comprising dystrophin peptides;

(ii) applying the protein digest to ~~a~~ an affinity matrix comprising antibodies, or antigen binding fragments thereof, specific for at least one dystrophin peptide-affinity reagent, wherein the;

(iii) ~~eluting dystrophin peptides in the protein digest are bound to the dystrophin peptide affinity reagent, and eluting the bound dystrophin peptides~~ matrix, forming a dystrophin peptide-enriched sample containing endogenous dystrophin peptides, engineered dystrophin peptides, or endogenous dystrophin peptides and engineered dystrophin peptides; and

(~~iii~~iv) performing liquid-chromatography-mass spectrometry (LC/MS) on the said dystrophin peptide-enriched sample.

2.- ~~_____~~ The method ~~as set forth in of~~ claim 1, ~~wherein~~ further comprising lysing the biological sample ~~is from a human patient having Duchenne's muscular dystrophy (DMD), and the human patient having DMD has been treated with a gene therapy encoding an engineered dystrophin protein in a lysis buffer.~~

3.- ~~_____~~ The method ~~as set forth in claim 1 or of~~ claim 2, wherein the endogenous dystrophin protein has an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:242 lysis buffer is a RIPA buffer comprising a detergent.

4.- ~~_____~~ The method ~~as set forth in of~~ any one of claims 1 to 3, ~~wherein the dystrophin protein being measured is an engineered dystrophin protein.~~

5. ~~The method as set forth in any one of claims 1 to 4, wherein the engineered dystrophin protein has an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:243, SEQ ID NO:244, or SEQ ID NO:245.~~

6. ~~The method as set forth in any one of claims 1 to 5, wherein the engineered dystrophin protein has an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:243.~~

- ~~7. The method as set forth in any one of claims 1 to 6, wherein the engineered dystrophin protein has an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:243.~~
- ~~8. The method as set forth in any one of claims 1 to 7, wherein the protein sample further comprises a metabolically labeled exogenous dystrophin reference protein added to the protein sample.~~
- ~~9. The methods as set forth in claim 8, wherein the exogenous dystrophin reference protein has an amino acid sequence that shares at least 95% identity with the engineered dystrophin protein.~~
- ~~10. The method as set forth in any one of claim 1 to 9, wherein digestion of both the endogenous dystrophin protein and the engineered dystrophin protein by the first protease yields a common dystrophin peptide that is present in both an endogenous dystrophin protein and an engineered dystrophin protein.~~
- ~~11. The method as set forth in claim 10, wherein the common dystrophin peptide has the amino acid sequence of SLEGSDDAVLLQR (SEQ ID NO:179) or LLQVAVEDR (SEQ ID NO:200).~~
- ~~12. The method as set forth in any one of claim 1 to 11, wherein the engineered dystrophin peptide comprises a contiguous amino acid sequence that is present in the engineered dystrophin protein and not present in the endogenous dystrophin protein.~~
- ~~13. The method as set forth in any one of claim 1 to 12, wherein the engineered dystrophin-specific peptide has an amino acid sequence of SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, or SEQ ID NO:241.~~
- ~~14. The method as set forth in any one of claim 1 to 13, wherein the engineered dystrophin-specific peptide has the amino acid sequence of LEMPSSLMLEVPTHR (SEQ ID NO:236).~~
- ~~15. The method as set forth in any one claim 1 to 14, wherein further comprising precipitating proteins in the biological sample is homogenized in a lysis buffer.~~
- ~~16. The method as set forth in any one of claim 15, wherein the lysis buffer includes a detergent.~~
- ~~17. The method as set forth in any one of claim 15 to 16, wherein the detergent is SDS.~~

~~18. The method as set forth in any one of claim 1 to 17, wherein the lysis buffer is a RIPA buffer, and RIPA buffer contains 10 to 50 mM Tris-HCl, 100 to 200 mM NaCl, 0.5 to 1.0% NP40, 1 to 2% sodium deoxycholate and 0.1% SDS.~~

~~19. The method as set forth in any one of claim 1 to 18, wherein isolating the protein component of a biological sample includes contacting a homogenized biological sample with an organic solvent forming a protein precipitate.~~

~~205. The method as set forth in of claim 194, wherein the organic solvent is selected from the group consisting of acetic acid, acetone, acetonitrile, dimethylformamide, dimethyl sulfoxide, dioxane, ethanol, isopropanol, methanol, 1-propanol, or and tetrahydrofuran, or a combination thereof.~~

~~216. The method as set forth in of any one of claims 1-20 to 5, further comprising solubilizing the protein precipitate with a solution comprising trypsin and a chaotropic agent.~~

~~7. The method of any one of claims 1 to 6, wherein the first said protease is provided in a protease buffer, and trypsin.~~

~~8. The method of any one of claims 1 to 7, further comprising reduction and alkylation of the protease buffer solubilized protein protein precipitate.~~

~~9. The method of any one of claims 1 to 8, further comprising quantifying the amount of dystrophin protein in the sample relative to a reference.~~

~~10. The method of any one of claims 1 to 9, wherein said dystrophin protein comprises at least a chaotropic agent, organic solvent and buffer salts, and preferably the amino acid sequence of any one of SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, or SEQ ID NO:245.~~

~~11. The method of any one of claims 1 to 10, wherein said dystrophin peptide-enriched sample comprises about 0.8M urea and about 10% acetonitrile in phosphate buffered saline a peptide consisting of the amino acid sequence of any one of SEQ ID NO:1 to SEQ ID NO:241.~~

~~22-12. The method as set forth in of any one of claim claims 1 to 2111, wherein the said dystrophin peptide affinity reagent binds at least one dystrophin-enriched sample comprises a peptide in the peptide sample.~~

~~23. The method as set forth in consisting of the amino acid sequence of any one of SEQ ID NO:235 to SEQ ID NO:241.~~

~~13. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in the amino acid sequence each of full length wild type human dystrophin protein and that of an engineered dystrophin protein.~~

~~14. The method of claim 1 to 2213, wherein said peptide amino acid sequence consists of SLEGSDDAVLLQR (SEQ ID NO:179) or LLQVAVEDR (SEQ ID NO:200).~~

15. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in the bound dystrophin peptides are from an endogenous amino acid sequence of full length wild type human dystrophin protein, but not that of an engineered dystrophin protein, and/or.

16. The method of claim 15, wherein said peptide amino acid sequence consists of ILADLEEENR (SEQ ID NO:224).

17. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in an exogenous dystrophin reference protein.

24. The method as set forth in any one of claim 1 to 23, wherein the dystrophin peptide affinity column binds a common dystrophin peptide that is present in both the endogenous dystrophin protein and the engineered dystrophin protein.

25. The method as set forth in any one of claim 1 to 24, wherein the but not that of full length wild type human dystrophin peptide affinity reagent comprises protein.

18. The method of claim 17, wherein said peptide amino acid sequence consists of LEMPSSLMLEVPTHR (SEQ ID NO:236).

19. The method of any one of claims 1 to 18, wherein said biological sample is a sample of skeletal muscle obtained from a human diagnosed with Duchenne's muscular dystrophy (DMD).

20. The method of claim 19, wherein said human with DMD is treated with a gene therapy vector expressing an anti-engineered dystrophin peptide antibody.

26. The method as set forth in any one of claim 1 to 25, wherein the anti-dystrophin peptide antibody is raised against a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, or SEQ ID NO:241.

~~27. The method as set forth in any one of claim 1 to 26, wherein the anti-dystrophin peptide antibody is raised against a peptide comprising the amino acid sequence of SEQ ID NO:236.~~

~~28. The method as set forth in any one of claim 1 to 27, wherein the anti-dystrophin peptide antibody is raised against a peptide comprising the amino acid sequence of LLQVAVEDR (SEQ ID NO:200).~~

~~29. The method as set forth in any one of claim 1 to 28, wherein the anti-dystrophin peptide antibody is a polyclonal antibody.~~

~~30. An anti-dystrophin peptide antibody raised against a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, or SEQ ID NO:241.~~

~~31. A peptide protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, or SEQ ID NO:241 of SEQ ID NO:243.~~