SUPPORTING INFORMATION

A Library Approach to Rapidly Discover Photoaffinity Probes of the mRNA Decapping Enzyme DcpS

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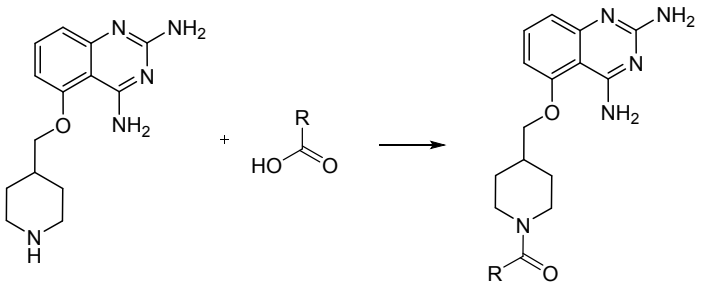
Materials and Methods

Materials

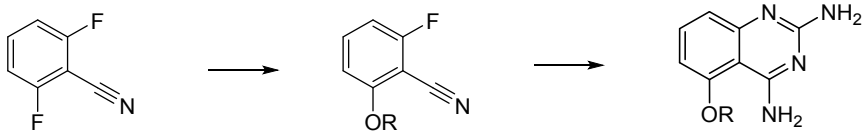
The DcpS antibody (for Western blot) was obtained from Abcam (Cambridge, MA). Other reagents were obtained from Thermo Fisher Scientific (Waltham, MA), unless otherwise noted.

Library synthesis - amides



To a mixture of the amine (200 μmol) and acid (200 μmol, 1.0 eq) in DMSO were added DIEA (600 μmol, 2.0 eq) and HATU (0.2 M in DMSO, 200 μmol, 1.0 eq). The samples were shaken for 16 hours at 30°C and purified by preparative HPLC (Phenomenex Gemini C18 250×21.2mm×8μm; mobile phase: acetonitrile-NH_4OH, pH10) to give the final products. Compounds were confirmed by LC-MS as being >95% pure.

Ethers



All ethers were prepared using the general procedure described below for the synthesis of probes 1 and 2 (alcohol displacement of the fluorine in 2,6-difluorobenzonitrile, followed by cyclisation to the diaminoquinazoline using guanidine carbonate).

Preparation of compound 1 4-[(2,4-diaminoquinazolin-5-yl)oxy]phenyl}[4-(prop-2-yn-1-yloxy)phenyl]methanone

A mixture of (4-hydroxyphenyl)(4-(prop-2-ynyloxy)phenyl)methanone (100 mg, 0.40 mmol) in DMF (5 mL) was cooled in an ice-water bath and treated with NaH (19 mg, 0.48 mmol, 60% in mineral oil). The mixture was stirred at 50°C for 0.5 h at which time 2,6-difluorobenzonitrile (110 mg, 0.81 mmol) was

added. The resulting mixture was stirred at 50°C for 17 h and then quenched with water (2 drops). After evaporation of solvent under vacuum, the desired product, 2-fluoro-6-(4-(4-(prop-2-ynyloxy)benzoyl)phenoxy)benzonitrile (120 mg, 81%), was isolated as a brown solid by flash chromatography on silica gel eluting with 17% to 50% EtOAc in petroleum ether. Then a mixture of 2-fluoro-6-(4-(4-(prop-2-ynyloxy)benzoyl)phenoxy)benzonitrile (50 mg, 0.14 mmol) and guanidine carbonate (48 mg, 0.27 mmol) in N,N-dimethylacetamide (2 mL) was stirred at 140°C for 4 h. The reaction mixture was filtered and purified by preparative HPLC (Phenomenex Gemini c18 250*21.2mm*8μm; mobile phase: from 24 % MeCN in water to 44 % MeCN in water) to afford PF-06743608 (4.4 mg, 8%) as a green solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.996-7.975 (d, 2H), 7.774-7.713 (m, 4H), 7.157-7.135 (d, 3 H), 6.953 (s, 1 H), 6.155-6.085 (m, 1H), 4.935-4.930 (s, 1H), 3.655 (s, 1H) ppm. MS (ESI+) calcd for $C_{24}H_{18}N_4O_3$ [M+H]: 410.43, found: 411.0.

Preparation of compound 2 ((4-((2,4-diaminoquinazolin-5-yloxy)methyl)phenyl)(4-(prop-2-ynyloxy)phenyl)methanone)

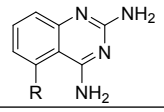
A mixture of (4-(hydroxymethyl)phenyl)(4-(prop-2-ynyloxy)phenyl)methanone (150 mg, 0.56 mmol) in THF (8 mL) was cooled in an ice-water bath and treated with NaH (27 mg, 0.68 mmol, 60% in mineral oil). The mixture was stirred at room temperature for 0.5 h at which time 2,6-difluorobenzonitrile (157 mg, 1.13 mmol) was added. The resulting mixture was stirred at room temperature for 17 h and then quenched with water (3 drops). After evaporation of solvent under vacuum, the desired product, 2-fluoro-6-(4-(4-(prop-2-ynyloxy)benzoyl)benzyloxy)benzonitrile (110 mg, 51%), was isolated as a yellow solid by flash chromatography on silica gel eluting with a gradient of 17% to 50% EtOAc in petroleum ether. Then a mixture of 2-fluoro-6-(4-(4-(prop-2-ynyloxy)benzoyl)benzyloxy)benzonitrile (50 mg, 0.13 mmol) and guanidine carbonate (47 mg, 0.26 mmol) in N,N-dimethylacetamide (2 mL) was stirred at 140°C for 4 h. The reaction mixture was filtered and purified by preparative HPLC (DIKMA Diamonsil(2) C18 200*20mm*5μm; mobile phase: from 25 % MeCN in water (0.225%FA) to 45 % MeCN in water (0.225%FA) to afford PF-06743608 (5.9 mg, 11%) as a yellow solid. ^{1}H NMR (400 MHz, acetone-d6) δ 8.342 (s, 1H), 7.831-7.724 (m, 4H), 7.744-7.724 (d, 2H), 7.456-7.436 (t, 1 H), 6.989-6.968 (d, 1 H), 6.799-6.779 (d, 1H), 5.497 (s, 1H), 4.921-4.916 (d, 2H) ppm. MS (ESI+) calcd for $C_{25}H_{20}N_4O_3$ [M+H]: 425.46, found: 425.0.

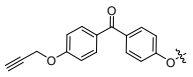
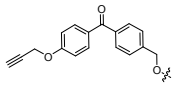
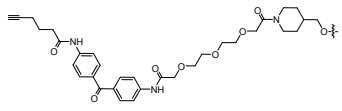
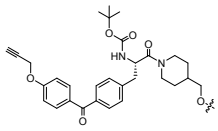
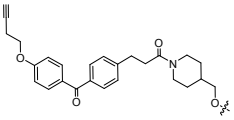
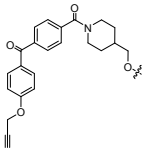
DcpS inhibition assay

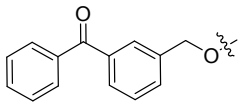
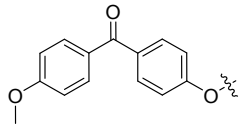
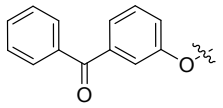
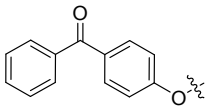
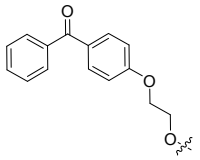
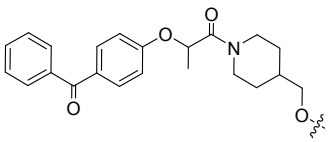
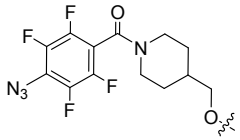
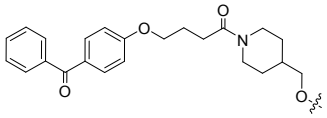
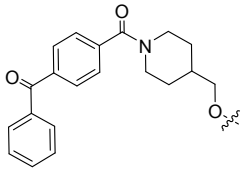
The DcpS enzyme inhibition assay was performed similar to described previously.[1] Briefly, 0.05 nM human DcpS recombinant enzyme was incubated with 50 nM biotinylated m^7GpppN mRNA cap substrate with varying concentrations of the inhibitors in the decapping buffer (50 mM Tris, 20 mM

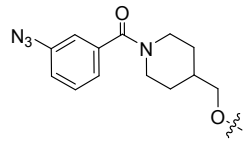
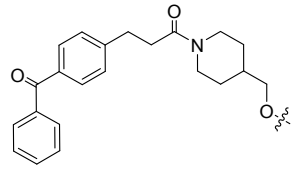
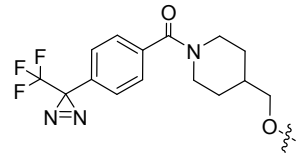
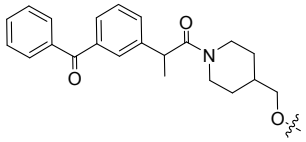
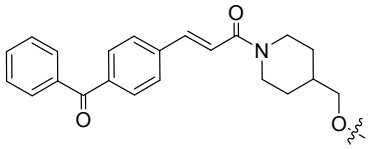
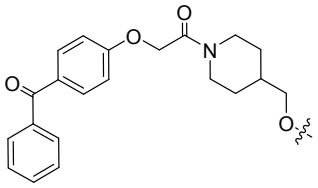
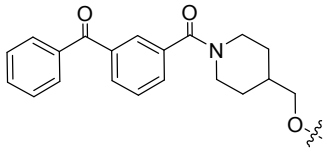
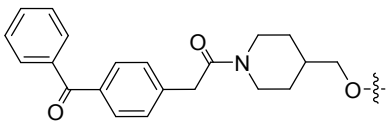
$MgCl_2$, 60 mM $(NH_4)_2SO_4$, 2 mM DTT, and 0.5 mg/mL BSA, pH 7.9). After quenching the reaction with an excess of a DcpS inhibitor compound, the biotinylated ADP product was captured by streptavidin-coated plates, and subsequently detected using ADP2 antibody (BellBrook Labs #2051), HRP-conjugated goat anti-mouse IgG (Invitrogen # 62-6520) and TMB Ultra ELISA substrate (Pierce #34028). The absorbance at 450 nm was measured in a Perkin Elmer Envision plate reader.

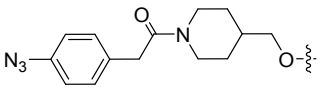
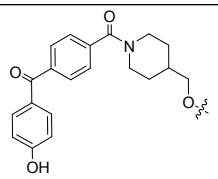
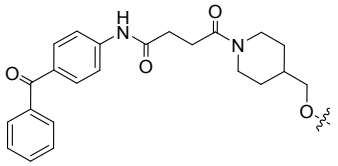
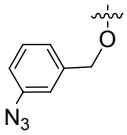
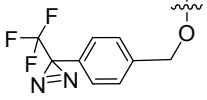
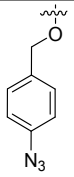
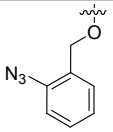
Table S1: Library compounds and their DcpS activities



Compound	R	DcpS IC_{50} (nM)	Note
1		5560	
2		6.0	
3		27	
4		7.6	
5		12	
6		0.29	

7		0.038
8		639
9		0.87
10		>4.9 x 10^3
11		1.5
12		12
13		0.21
14		2.6
15		6.0

16		0.41
17		2.3
18		2.3
19		3.1
20		0.46
21		5.2
22		1.9
23		4.7

24		0.32
25		<0.10
26		1.8
27		0.25
28		1.4
29		0.74
30		2.1

Intact protein mass spectra of DcpS after PAL

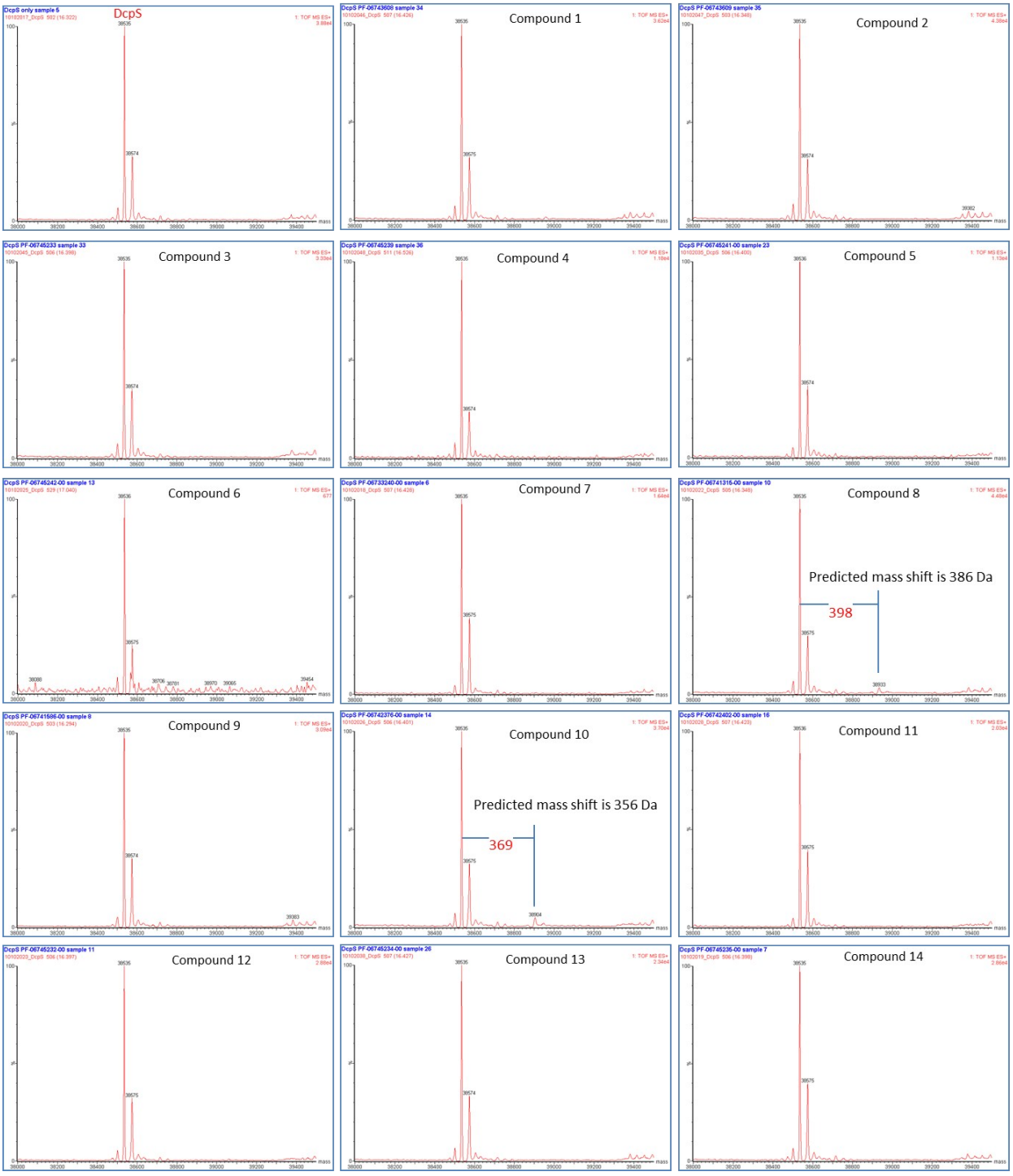


Fig. S1 Intact protein mass spectra of DcpS after PAL by compounds 1-14. No mass shift was observed for compounds 1-7, 9, 11-14. A mass shift was seen for compounds 8 and 10, but does not match the theoretical mass addition.

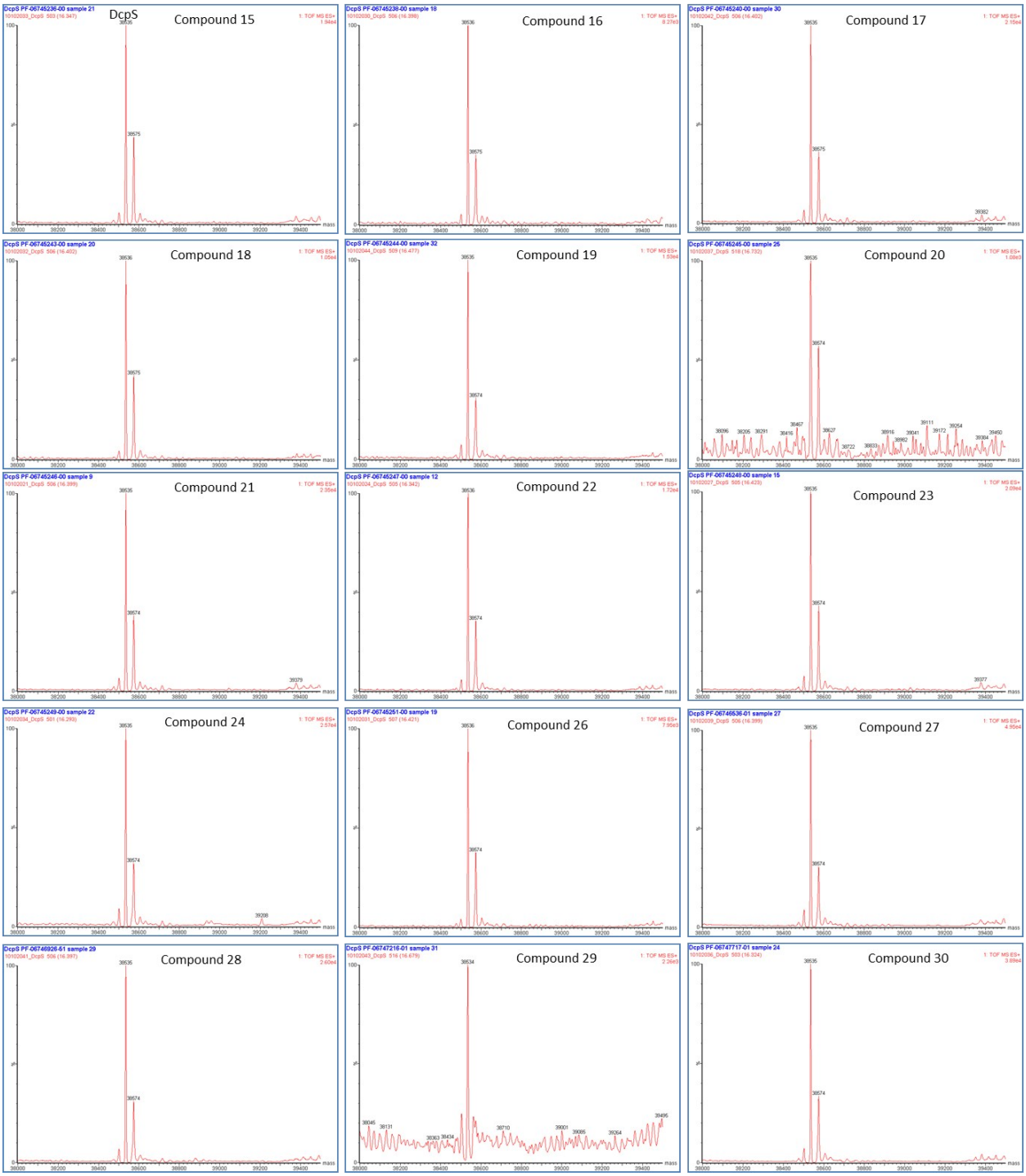


Fig. S2 Intact protein mass spectra of DcpS after PAL by compounds 15-30. No mass addition was observed. Compound 25 caused the precipitation of DcpS protein and loss of protein signals (not shown).

Photolabelling and streptavidin enrichment

1 μM DcpS was incubated with 10 μM probe in 50 mM HEPES buffer (pH 7.5) at room temperature for 1 hour (sample volume 50 μL). After UV irradiation at 365 nm (or no irradiation) at 4°C for 30 min, 38.2 μL of 4% SDS in HEPES buffer (pH 7.5) was added to each sample, followed by the addition of 1.9 μL of 4 mM biotin azide (Life Technologies), 2 μL of 50 mM $CuSO_4$, 5.9 μL TBTA in DMSO:t-BuOH (1:5), and 2 μL of 50 mM TCEP (final concentrations: 1.5% SDS, 75 μM biotin azide, 1 mM CuSO4, 5% t-BuOH and 1 mM TCEP). The click reaction was carried out at room temperature for 2 hours. Then 1 mL 6 M urea in TBST (50 mM Tris, 150 mM NaCl, 0.2% tween-20, pH 7.5) was added to the reaction mixture to reduce SDS to 0.2%, and then incubated with 180 μL high-capacity streptavidin agarose (Thermo Fisher) at 4°C overnight. After wash thrice with 300 μL 4 M urea in TTBS, the protein was eluted with 100 μL of LDS sample buffer (Life Technologies). The eluates were analyzed by immunoblot using DcpS antibody (Abcam) after SDS-PAGE.

References

1. J. P. Van Meerbeke, R. M. Gibbs, H. L. Plasterer, W. Miao, Z. Feng, M. Y. Lin, A. A. Rucki, C. D. Wee, B. Xia, S. Sharma, V. Jacques, D. K. Li, L. Pellizzoni, J. R. Rusche, C. P. Ko and C. J. Sumner, *Human molecular genetics*, 2013, **22**, 4074-4083.