

Supplementary Table 1: Fluorophores evaluated for sequencing, with vendors (TF, ThermoFisher).

Fluorophore	Source
Alexa Fluor 405	TF; Cat # A30000
Dylite 405	TF; Cat # 46400
Hilyte 405	AnaSpec; Cat # AS-89317-1
Atto 425	Sigma; Cat # 16805-1MG-F
Lucifer Yellow	In-house synthesis
Hilyte 488	AnaSpec; Cat # AS-81161-1
Alexa Fluor 488	TF; Cat # A20000
Atto 488	Sigma; Cat # 41698-1MG-F
Dylight 488	TF; Cat # 46402
c-Rhodamine 110	AnaSpec; product discontinued
BODIPY FL	TF; Cat # D2184
Cy3	Lumiprobe; Cat # 11020
Alexa Fluor 555	TF; Cat # A20009
Rhodamine-B	In-house synthesis
Tetramethylrhodamine	TF; Cat # 46406
Rhodamine-6G	TF; Cat #C6127
Cy5	Lumiprobe; Cat # 13020
BODIPY 650	TF; Cat # D10001
Hilyte 647	AnaSpec; Cat # AS-81256
Alexa Fluor 647	TF; Cat # A37473
Atto 647	Atto-tec; Cat # AD 647-31
Atto 647N	Atto-tec; Cat # AD 647N-31
Atto 655	Atto-tec; Cat # AD 655-31

Alexa Fluor 680	TF; Cat # A20008
Janelia Fluor 549, SE	Tocris; Cat # 6147
Si-Rhodamine	In-house synthesis

Supplementary Table 2: Automated Edman degradation

	Protocol step	Reagents	Incubation time (min) System A	Incubation time (min) System B
Step 1	Pump wash	Water	wash	wash
Step 2	Methanol wash	Methanol	wash	wash
Step 3	Free basing solution	1. Acetonitrile, pyridine, triethylamine, water (10:3:2:1 v/v) 2. Acetonitrile, triethylamine (2:1 v/v)	wash	5
Step 4	Mock or Edman solution	100% acetonitrile or acetonitrile, phenylisothiocyanate (9:1 v/v)	30	20
Step 5	Free basing solution	1. Acetonitrile, pyridine, triethylamine, water (10:3:2:1 v/v) 2. Acetonitrile, triethylamine (2:1 v/v)	wash	NA
Step 6	Ethylacetate/ACN wash	Ethylacetate (A) or acetonitrile (B)	wash	wash
Step 7	Cleavage solution	100% trifluoroacetic acid	30	15
Step 8	Ethylacetate wash	Ethylacetate	wash	wash
Step 9	Pump wash	Water	wash	wash
Step 10	Methanol wash	Methanol	wash	wash
Step 11	Oxygen scavenging solution	Trolox in methanol (1mM)	10	5.5