Supplementary Method 1 - Protein biotinylation

For the immobilization on SA beads, target proteins need to be biotinylated. In general, protein biotinylation can be done either enzymatically or chemically. We routinely use the chemical biotinylation procedure, adapted from the Biotin-NHS manufacturer`s instructions, as described herein. Before and after biotinylation, standard protein quality control assays such as SDS-PAGE and FPLC analysis should be performed. The success of the biotinylation reaction can be assessed using a bandshift assay or, for some proteins, MS analysis.

▲ **CRITICAL** Whenever possible, handle proteins at 4°C or on ice. For storage, snap freeze using liquid nitrogen and place at -80 °C.

1| Dissolve 1 mg of protein in 1 ml protein buffer, such as PBS.

▲ **CRITICAL STEP** Amine-containing buffers like Tris can not be used as protein buffer since amine would react with the biotinylation reagent.

2| Prepare a 10 mM solution of EZ-Link NHS-LC-Biotin (Thermo Fisher Scientific).

▲ **CRITICAL STEP** This solution should be prepared immediately before use. Alternatively, 100 mM stock solutions may be prepared using water-free DMSO and protection with argon.

3| Calculate the molar amount of protein. Add a 20-fold molar excess of EZ-Link NHS-LC-Biotin.

4| Incubate at 4 °C for 3 h.

5| Add Tris-HCl 1 M pH 7.4 to a 10-fold molar excess of Tris.

6| Incubate at 4°C for 1 h.

7| Purify biotinylated protein using a PD-10 column (GE Healthcare) according to the manufacturer`s instructions. Collect fractions separately.

8| Measure OD_{280} of all fractions using the Nanodrop spectrophotometer. Pool protein-containing fractions.

Supplementary Data 1 - Protocol Status Report

Laboratory information

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Run information

Protocol name	150623_DECL_selection_1_plates1+2
Execution started	23.06.2015 08:53:20+02:00
Executing user	admin
Total warnings	0
Total errors	0
Description	

Instrument parameters

Instrument name	KingFisher
Instrument version	1.3.3
Instrument serial number	700-596

SW Parameters

Run software version	BindIt Software 3.1 for KingFisher Instruments
Current software version	BindIt Software 3.1 for KingFisher Instruments

Run Log

23.06.2015 08:53:20+02:00 150623_DECL_selection_1_plates1+2: started
23.06.2015 08:53:23+02:00 Tip1: started
23.06.2015 08:53:23+02:00 Mix plate1 A beads: started
23.06.2015 09:00:00+02:00 Mix plate1 A beads: completed
23.06.2015 09:00:00+02:00 Mix plate1 B protein: started
23.06.2015 09:31:57+02:00 Mix plate1 B protein: completed
23.06.2015 09:31:57+02:00 Mix plate1 C wash1: started
23.06.2015 09:37:13+02:00 Mix plate1 C wash1: completed
23.06.2015 09:37:13+02:00 Mix plate1 D wash2: started
23.06.2015 09:42:29+02:00 Mix plate1 D wash2: completed
23.06.2015 09:42:29+02:00 Mix plate1 E wash3: started
23.06.2015 09:47:45+02:00 Mix plate1 E wash3: completed
23.06.2015 09:47:45+02:00 Mix plate1 F library: started
23.06.2015 10:49:41+02:00 Mix plate1 F library: completed
23.06.2015 10:49:41+02:00 Mix plate1 G wash1: started
23.06.2015 10:52:27+02:00 Mix plate1 G wash1: completed
23.06.2015 10:52:27+02:00 Mix plate1 H wash2: started
23.06.2015 10:55:12+02:00 Mix plate1 H wash2: completed
23.06.2015 10:55:12+02:00 Mix plate2 A wash3: started
23.06.2015 10:59:06+02:00 Mix plate2 A wash3: completed
23.06.2015 10:59:07+02:00 Mix plate2 B wash4: started
23.06.2015 11:01:52+02:00 Mix plate2 B wash4: completed
23.06.2015 11:01:52+02:00 Mix plate2 C wash5: started
23.06.2015 11:04:38+02:00 Mix plate2 C wash5: completed
23.06.2015 11:04:38+02:00 Mix plate2 D elution: started
23.06.2015 11:08:13+02:00 Mix plate2 D elution: completed
23.06.2015 11:08:13+02:00 Tip1: completed
23.06.2015 11:08:17+02:00 150623_DECL_selection_1_plates1+2: completed

Steps Data

150623_DECL_selection_1_plates1+2

Instrument type	KingFisher
Kit name	
Description	

Tip1

Tip	KingFisher tip comb

Mix plate1 A beads

Plate	DECL plate 1 (A) - beads, PBS
Beginning of step:	
Precollect	No
Release beads	No
Mixing/pause parameters:	
Pause for manual handling	No
1. Mixing time [hh:mm:ss]	00:00:30
1. Mixing speed	Bottom mix
2. Mixing time [hh:mm:ss]	00:00:30
2. Mixing speed	Medium
Loop count	5
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 B protein

Plate	DECL plate 1 (B) - protein, PBS
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:30:00
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 C wash1

Plate	DECL plate 1 (C) - wash1, PBST-Biotin
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:03:00
Mixing speed	Medium

...Mix plate1 C wash1

End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 D wash2

Plate	DECL plate 1 (D) - wash2, PBST-Biotin
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:03:00
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 E wash3

Plate	DECL plate 1 (E) - wash3, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:03:00
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 F library

Plate	DECL plate 1 (F) - library, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	01:00:00
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 G wash1

Plate	DECL plate 1 (G) - wash1, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:00:30
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate1 H wash2

Plate	DECL plate 1 (H) - wash2, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:00:30
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate2 A wash3

Plate	DECL plate 2 (A) - wash3, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:00:30
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate2 B wash4

Plate	DECL plate 2 (B) - wash4, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	

...Mix plate2 B wash4

Pause for manual handling	No
Mixing time [hh:mm:ss]	00:00:30
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate2 C wash5

Plate	DECL plate 2 (C) - wash5, PBST
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:00:30
Mixing speed	Medium
End of step:	
Postmix	No
Collect count	5
Collect time [s]	10

Mix plate2 D elution

Plate	DECL plate 2 (D) - elution, Tris
Beginning of step:	
Precollect	No
Release time [hh:mm:ss]	00:00:30
Release speed	Medium
Mixing/pause parameters:	
Pause for manual handling	No
Mixing time [hh:mm:ss]	00:03:00
Mixing speed	Medium
End of step:	
Postmix	No
Collect beads	No

Layout Data

Contents

DECL plate 1

Tray/plate type KingFisher plate 200 ul

A (beads, PBS)

Reagents:

Name	beads, PBS
Volume [µl]	100
Type	Reagent

B (protein, PBS)

Reagents:

Name	protein, PBS
Volume [µl]	100
Type	Reagent

C (wash1, PBST-Biotin)

Reagents:

Name	PBST-Biotin
Volume [µl]	200
Type	Reagent

D (wash2, PBST-Biotin)

Reagents:

Name	PBST-Biotin
Volume [µl]	200
Type	Reagent

E (wash3, PBST)

Reagents:

Name	PBST
Volume [µl]	200
Type	Reagent

F (library, PBST)

Reagents:

Name	library, PBST
Volume [µl]	100
Type	Reagent

G (wash1, PBST)

Reagents:

Name	PBST
Volume [µl]	200
Type	Reagent

H (wash2, PBST)

Reagents:

Name	PBST
Volume [µl]	200

...H (wash2, PBST)

Type	Reagent

DECL plate 2

Tray/plate type	KingFisher plate 200 ul

A (wash3, PBST)

Reagents:

Name	PBST
Volume [µl]	200
Type	Reagent

B (wash4, PBST)

Reagents:

Name	PBST
Volume [µl]	200
Type	Reagent

C (wash5, PBST)

Reagents:

Name	PBST
Volume [µl]	200
Type	Reagent

D (elution, Tris)

Reagents:

Name	Tris
Volume [µl]	100
Type	Reagent

E

Reagents	<empty>

F

Reagents	<empty>

G

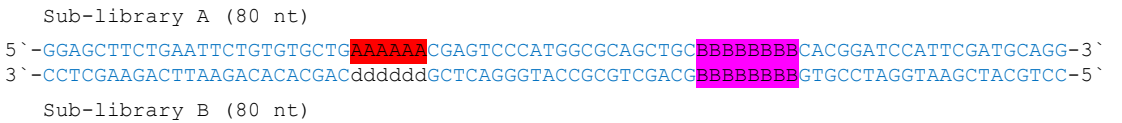
Reagents	<empty>

H

Reagents	<empty>

Supplementary Note

ESAC library design



ESAC two-step PCR encoding for Illumina sequencing

PCR 1

PCR2

```
IlluminaPCR2a (58 nt)     5`-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3`
IlluminaPCR2b (63 nt)     5`-CAAGCAGAAGACGGCATACGAGAT333333GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3`
```

The C++ program defines the selection and library codes with the symbols x,y,z,$:

Code 3 at the 3` end of the library is the indexed code of the TruSeq adapter. It is used for filtering the Illumina data. This is performed by the Illumina software; our C++ program does not use code 3.

Supplementary Method 2 - Ethanol precipitation

The PCR 2 amplification product is purified by ethanol precipitation after the gel extraction procedure. The ethanol precipitation removes any remaining impurities carried over from the agarose gel and guarantees optimal quality of the DNA solution, which is required for HTDS.

1| Pipet the DNA to be purified into a new 2.0 ml reaction tube. Usually, the DNA is present in 40 µl Buffer NE (5 mM Tris-HCl, pH 8.5).
▲ **CRITICAL** 2.0 ml reaction tubes are preferred over 1.5 ml reaction tubes as after the centrifugation steps, it is easier to remove the ethanol from a 2.0 ml tube without impairing the DNA pellet.

2| Add 0.1 volume of 3 M sodium acetate pH 4.7. Mix thoroughly.

3| Add 3 volumes of 99% Ethanol. Mix thoroughly.

4| Incubate at -20 °C for ≥1 h.

5| Centrifuge at 4°C and ≥14.000 g for ≥20 min.

6| Decant the supernatant carefully.
▲ **CRITICAL** Pipet very slowly. It is normal to have a very faint or no visible pellet. Avoid touching the area where the pellet is expected with the pipet tip.

7| Add 0.5 ml 85% ethanol.

8| Centrifuge at 4°C and ≥14.000 g for ≥20 min.

9| Decant the supernatant carefully.

10| Air dry pellet.
▲ **CRITICAL** Make sure no ethanol is left in the tube.

11| Resuspend the DNA in 20 µl Buffer NE (5 mM Tris-HCl, pH 8.5).