User Guide

YOUSEQ

NGS Library Clean-up kit

Magnetic bead-based cleaning kit for NGS libraries

Version 1.2

Kit contents

Bead clean pack

	Cap colour	Size
YouSeq beads		5 ml
Wash buffer*		80 ml*
Elution buffer		10 ml

*Add 64 ml of molecular biology grade ethanol to the supplied diluent to make the wash buffer ready to use.

Other items to be supplied by the user

Essential

- Magnetic rack
- Pipettes and general laboratory equipment

Introduction and kit principles

The YouSeq bead clean-up kit uses magnetic beads that bind to your DNA Library. A magnet then pulls the beads to one side so that all contaminants and artefacts can be washed away. A couple of washes completes the cleaning process then a simple elution step washes the DNA library off the beads in to solution.

The kit can be used to remove either small and/or large (or both) unwanted fragments.

Size selection – Principles.

You can remove unwanted DNA fragments of a specific size by simply adding more or less beads. The ratio of beads vs volume of sample dictates the size of DNA that will be captured.

Size selection – removing small fragments.

Ration beads /Sample	Your Library Size bp
0.45	600-900
0.5	400-600
0.6	300-400
0.65	200-300
0.9	150-200
1.2	100-150

Size selection – removing large fragments.

You can remove unwanted large DNA fragments by capturing them smaller fragments on the beads and collect the supernatant fraction instead

Ration beads /Sample	Your Library Size bp
0.4	2000-4000
0.45	1000-2000
0.5	700-1000
0.55	500-700
0.6	450-500
0.65	350-450
0.7	300-350

e.g. if a 0.9x ratio is required on 20 µl PCR reaction

- 0.9 x 20 = 18
- So, 18 µl beads needs to be added during the clean-up

Example protocol to remove small fragments

E.g. a 0.8x bead ratio was required.

Bring the beads to room temperature by removing from fridge 30 minutes before use

Bind

- Mix beads well until suspension is homogeneous
- Add **16 µL*** YouSeq NGS Beads to 20µl of your NGS Library and mix well
- Incubate for 3 mins at room temp
- Place on magnet and wait until liquid is clear
- Remove supernatant

1st wash

- Leave your tube on the magnet

***N.B.** ensure the wash buffer has had correct volume of ethanol added*

- Add **200 µL** wash buffer
- Incubate for 30s
- Remove and discard the supernatant carefully

2nd wash

- Repeat as per 1st wash
- Ensure all remainder ethanol has been removed

Dry beads

- Leave until noticeably dry (cracking) on the magnet ***** (should be around 10 minutes)

***N.B.** if you notice any residual buffer on the sides of the tubes, carefully remove with pipette.

Elute

- Add **20 µL** Elution buffer
- Remove your tube from the magnet and mix well
- Incubate for **5 min** at RT
- Place on magnet and wait until liquid is clear

DNA is in the solution.

*This example is for removing DNA fragments of around 250bp and smaller. See tables above for precise volumes/ratios to use for your particular experiment

Example protocol to remove large fragments

E.g. a 0.45x bead ratio was required.

Bring the beads to room temperature by removing from fridge 30 minutes before use

Bind

- Mix beads well until suspension is homogeneous
- Add 9µL** YouSeq NGS Beads to 20µl of your NGS Library and mix well
- Incubate for 10 mins at room temp
- Place on magnet and wait until liquid is clear
- Remove supernatant

This step can be repeated to improve the stringency of the clean-up if necessary

Your size selected Library is in the supernatant.

** This example is for removing DNA fragments of around 1000bp and above. See tables above for precise volumes/ratios to use for your particular experiment

Example protocol for Dual Size Selection

E.g. all products between 0.45x – 1.0x bead ratios

Bring the beads to room temperature by removing from fridge 30 minutes before use

Bind large fragments

- Mix beads well until suspension is homogeneous
- Add **9 µL** YouSeq NGS Beads to 20 µL of your NGS Library and mix well
- Incubate for 3 mins at room temp
- Place on magnet and wait until liquid is clear
- Transfer 25 µL supernatant to fresh tube

Bind small fragments

- Mix beads well until suspension is homogeneous
- Add **25 µL** YouSeq NGS Beads to 25 µL of your NGS Library and mix well
- Incubate for 3 mins at room temp
- Place on magnet and wait until liquid is clear
- Remove the supernatant

1st wash

- Leave your tube on the magnet

***N.B.** ensure the wash buffer has had correct volume of ethanol added*

- Add **200 µL** wash buffer
- Incubate for 30s
- Remove and discard the supernatant carefully

2nd wash

- Repeat as per 1st wash
- Ensure all remainder ethanol has been removed

Dry beads

- Leave until noticeably dry (cracking) on the magnet ***** (should be around 10 minutes)

***N.B.** if you notice any residual buffer on the sides of the tubes, carefully remove with pipette.

Elute

- Add **20 µL** Elution buffer
- Remove your tube from the magnet and mix well
- Incubate for **5 min** at RT
- Place on magnet and wait until liquid is clear

DNA is in the solution.