User Guide

YOUSEQ

NGS Library Clean-up kit

Magnetic bead-based cleaning kit for NGS libraries

Version 1.0

Kit contents

Bead clean pack

	Cap colour	Size
YouSeq beads		6ml
Wash buffer*		80ml*
Elution buffer		10ml

*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 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.

e.g. add 12µl beads to a 20µl Library Ration is **0.8**.

e.g. add 22µl beads to a 20µl Library Ration is **1.2**

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

Example protocol to remove small fragments

Bind

Mix beads well until suspension is homogeneous
Add 16µL* 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

1st wash

Leave your tube on the magnet
Add 200µL wash buffer
Incubate for 30s
Remove supernatant carefully

2nd wash

Repeat as per 1st wash

Dry beads

Leave for 5min at RT

Elute

Remove your tube from the magnet
Add 60µL Elution buffer 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

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

Tips and tricks to make life easy

Drying step

Remove as much ethanol as you can (without disturbing the clump of beads) before the bead drying step. It is sometimes useful to centrifuge your tube, return it to the magnet, and then remove the final few microliters.

If the pellet is not dried sufficiently then remaining ethanol may affect your processes downstream. If you over-dry the clump it is difficult to resuspend the pellet in the next step.

The ideal, precise bead drying time, will depend on the temperature in your lab. Inspect the pellet of beads by eye. As soon as you can see no visible liquid, but the bead clump still has a wet sheen to it this is the ideal time to proceed to the elution step. If the pellet has taken a cracked appearance you will need to increase the elution time as the bead has become too dry.