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Bead-based normalization for NGS

Tomasz Suchan[1]

[1]W. Szafer Institute of Botany, Polish Academy of Sciences

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Tomasz Suchan
W. Szafer Institute of Botany, Polish Academy of Sciences

ABSTRACT

Bead-based normalization protocol for genomic libraries based on Hosomichi et al. (2014). It uses diluted AMPure or other SPRI beads solution in order to bind a small amount of DNA from each sample.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hosomichi, K., Mitsunaga, S., Nagasaki, H., & Inoue, I. (2014). A Bead-based Normalization for Uniform Sequencing depth (BeNUS) protocol for multi-samples sequencing exemplified by HLA-B. *BMC genomics*, *15*(1), 645.

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Hosomichi, K., Mitsunaga, S., Nagasaki, H., & Inoue, I. (2014). A Bead-based Normalization for Uniform Sequencing depth (BeNUS) protocol for multi-samples sequencing exemplified by HLA-B. *BMC genomics*, *15*(1), 645.

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MATERIALS

NAME	CATALOG #	VENDOR
Agencourt AmPure XP beads	A63880	
PEG-8000		
Isopropanol		
NaCl		
Water, nuclease free		
1 M Tris-HCl pH 8.0		
80% Ethanol		
0.5 M EDTA pH 8.0		

Preparation

1 Prepare 2.5 M solution of NaCl:

- place a 50 ml Falcon tube on the scale and tare
- add 14.61 g of NaCl to the tube
- add water to 40 ml
- dissolve NaCl
- fill up the tube with water to 50 ml and mix

2 Prepare 50% solution of PEG-8000:
- place a 50 ml Falcon tube on the scale and tare
- add 12.5 g of PEG-8000 to the tube
- add 14 ml of nuclease-free water
- close the tube, and dissolve PEG by mixing vigorously, preferably on an orbital shaker with heating
- fill up the tube with water to 25 ml and mix gently by flipping the tube to avoid bubbles

3 Prepare a solution of 20% PEG, 2.5 M NaCl in TE buffer by mixing:
- 5 ml of 5 M NaCl
- 0.83 ml of molecular-grade water
- 100 ul of 1 M Tris
- 20 ul of EDTA
- 50 uL of 10% Tween 10
- 4 ml of 50% PEG-8000

4 Dilute AMPure or homemade SeraMag beads 20-fold in the above solution (for instance, add 10 ul of AMPure to 190 ul of the solution).

Normalization

5 Add 20 ul of DNA library to the tube.

6 Add 20 ul of the diluted beads.

7 Add 20 ul of isopropanol.

8 Mix well by pipetting.

9 Incubate for 5 minutes at room temperature.

00:05:00 DNA binding

10 Place the tube on the magnetic stand and wait until the beads separate.

11 With the tube still on the magnet, remove and discard the supernatant.

12 Add 10 ul of 80% ethanol and incubate for 30 sec. at room temperature.

00:00:30 EtOH wash

13 Carefully remove all the ethanol and discard it.

14 Repeat steps 13 and 14.

15 Leave the tube on the magnetic rack and let the beads dry. Do not overdry the beads (the pellet should not be shiny anymore but there should not be cracks on it).

16 Add 20 ul of molecular-grade water on the beads. Make sure that the beads are wet before removing the tube from the magnet!

17 Remove from the magnet and mix the beads with water by pipetting.

18 Incubate 10 minutes at room temperature.
00:10:00 DNA elution