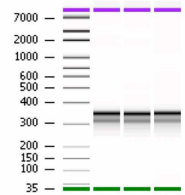


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🌐 Phage Display Library Prep Method

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol was designed to be used after the Scaled High Throughput Vacuum PhIP Protocol or the Scaled Moderate Throughput Multichannel PhIP Protocol. The immunoprecipitated phage targets are prepared for sequencing through a two PCR amplification rounds which first amplify the peptide target and second, add on UMIs.

Materials

✕ Phusion High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0530L**

✕ Agencourt AmPure XP beads **Catalog #A63880**

✕ Qubit dsDNA HS Assay kit **Thermo Fisher Scientific Catalog #Q32854**

✕ Bioanalyzer chips and reagents (DNA High Sensitivity kit) **Agilent Technologies**



Protocol materials

⊗ Bioanalyzer chips and reagents (DNA High Sensitivity kit) **Agilent Technologies**

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Lysis

- 1 Dilute phage lysis product in water at a 1:4 ratio. Phage lysis should be used either immediately after IP or within 1 week of being stored at  4 °C .
 - NOTE: we have done several tests and noticed that diluting the phage lysate 1:4 in water results in the most product at the end. Other dilutions ranging from 1:2 to 1:16 also worked. Using undiluted lysate also works, however there is the possibility that too much input can inhibit the PCR.
- 2 Heat lysate in preparation for PCR at  70 °C for  00:15:00 (can be done on the PCR machine if aliquoted into PCR tubes or plates). Keep on ice afterwards.

15m

PCR 1- Amplifying Insert

15m

- 3  Phusion High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0530L**
 1. Add the following reagents. Reaction volumes of  50 µL ,  25 µL or  12.5 µL all work.
 2. Multiply the number of samples by either the  50 µL ,  25 µL or  12.5 µL reaction coefficients to get the total volumes of each reagent to add to the master mix.
 3. Make master mix and aliquot into a new PCR plate.
 4. Add pre-heated phage lysis to PCR I master mix.

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	A	B	C	D
	COMPONENT	50uL REACTION	25uL REACTION	12.5uL REACTION
	Nuclease-free water	34.5	16.25	8.125
	5X Phusion HF or GC Buffer	10	5	2.5
	10 mM dNTPs	1	0.5	0.25
	10 uM Forward Primer	1	0.5	0.25
	10 uM Reverse Primer	1	0.5	0.25
	Polymerase	0.5	0.25	0.125



	A	B	C	D
	Template DNA (lysis)	2	2	1
	Total amount of Master Mix	48	23	11.5

5 Program PCR Machine for the following steps:

	A	B	C
	STEP	TEMP	TIME
	Initial Denaturation	98°C	30 seconds
	13-16 Cycles	98°C	5 seconds
		70°C	20 seconds
		72°C	15 seconds
	Final Extension	72°C	2 minutes
	Hold	10°C	

PCR 2- Adding on UMI

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Phusion High-Fidelity DNA Polymerase - 500 units **New England**
Biolabs Catalog #M0530L

1. Multiply number of samples by either the  50 µL ,  25 µL or  12.5 µL reaction number coefficients to get total volume of each reagent to add to master mix.
2. Combine the following reagents (except barcode primers and PCR I product) to make PCR II master mix, then aliquot master mix volume into new PCR plate
3. Next, add barcode primers and PCR I product, making sure to keep track of which barcode IDs corresponds to which sample well.

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A	B	C	D
COMPONENT	50uL REACTION	25uL REACTION	12.5uL REACTION
Nuclease-free water	33.5	16.25	8.125
5X Phusion HF or GC Buffer	10	5	2.5
10 mM dNTPs	1	0.5	0.25
Polymerase	0.5	0.25	0.125
5 uM Forward and Reverse Primer Barcodes	4	2	1
Template DNA (product of PCR I)	1	1	0.5
Total amount of Master Mix	46	22	11

8 Program the PCR machine for the following steps:

A	B	C
STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
5 Cycles	98°C	5 seconds
	70°C	20 seconds
	72°C	15 seconds
Final Extension	72°C	2 minutes
Hold	10°C	

Pool and Bead Clean



- 9 Amplified samples are now uniquely labeled and are very similar in concentration and library size, so equal volume of each sample can be pooled together. If there is concern that samples are dissimilar, skip this step, bead clean, BioAnalyze and Qubit each sample individually. However, in our experience it is easier to pool first and then bead clean everything together prior to the QC check via BioAnalyzer and Qubit.

If pooling, it is recommend to pool anywhere between  5 μ L -  10 μ L of each sample together by using a multichannel and combining all of the rows into a reservoir.

- NOTE: It is also recommended to pool each plate separately from other plates. This allows us to protect individual plates from phage contamination.

10

 Agencourt AmPure XP beads **Catalog #A63880**

52m 30s

*Allow beads to sit in  Room temperature for  00:30:00 prior

*Ratio of beads changes based on application and library size.

1. Determine the total volume of the pool. (i.e. 100uL)
2. Use Agencourt AmPure XP Beads 0.9x ratio of beads-to-total volume of sample.
Prepare 80% EtOH.
3. Add 0.9x (i.e. 90uL) beads of room temperature AmPure Beads to pool. Mix well by pipetting up and down gently.
4. Pulse spin the tube but do not spin down beads. Incubate for  00:05:00 at  Room temperature .
5. Place samples on magnetic rack and incubate for  00:05:00 on the rack.
6. Remove supernatant without disturbing the beads.
7. Add 2x original volume of 80% EtOH or enough to submerge bead pellet while on the magnetic rack. Incubate at  Room temperature for  00:00:30 then remove the supernatant.
8. Repeat EtOH wash (step 7) for a total of 2 times.
9. Air dry the beads for  00:05:00 while on the magnetic rack.
10. Remove tube from magnetic rack. Elute DNA from beads in desired volume of 0.1x TE Buffer, 10mM Tris-HCl, or Nuclease free water plus  3 μ L of dead volume.
11. Vortex to mix. Pulse spin tubes and incubate for  00:02:00 at  Room temperature off the magnetic rack.
12. Place on magnetic rack until solution is clear and bead pellet has formed ~  00:05:00 .

13. Remove desired volume of eluant and transfer to a clean nuclease-free PCR tube. Do not disturb the bead pellet.

Quantify and Qualify Library

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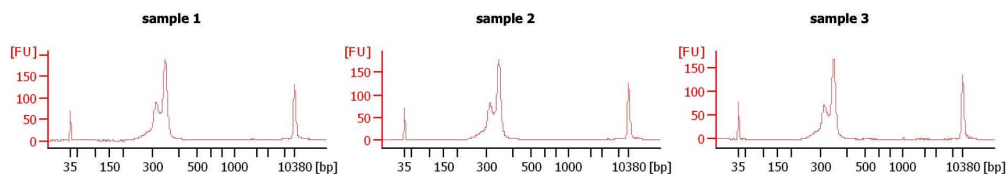
Qubit dsDNA HS Assay kit **Thermo Fisher Scientific Catalog #Q32854**

Bioanalyzer chips and reagents (DNA High Sensitivity kit) **Agilent Technologies**

1. Qubit the samples for accurate concentration. BioAnalyzer also gives the concentration of sample but in practice this number not as accurate as the Qubit result. The Qubit is our gold standard for confirming DNA concentration for dilution prior to using the BioAnalyzer as to not overload it.
2. BioAnalyze or TapeStation library pools.
3. If the library size looks as expected, pool separate plate pools together at equal concentration or at desired ratio for read depth. Final libraries can be stored at

4 °C

-20 °C .



Expected result of library on a BioAnalyzer, it is normal to see these two peaks, the first peak should be smaller than the second. Make sure to confirm that the size of the main peak is the predicted size based on insert and primer scheme.