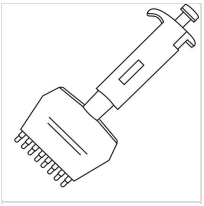

Mar 14, 2022

Scaled Moderate Throughput Multichannel PhIP Protocol

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This protocol was developed as a semi-high throughput technique to perform phage display immunopreceipitation. Here we describe all the steps: from isolating antibodies from a complex matrix of patient sample, washing the sample and amplify targets through two rounds of selection.

Sabrina A Mann, Sara Vazquez, Lillian M Khan, Jayant Rajan, Caleigh Mandel-Brehm, Joseph Derisi 2022. Scaled Moderate Throughput Multichannel PhIP Protocol. **protocols.io** https://protocols.io/view/scaled-moderate-throughput-multichannel-phip-proto-bwwdpfa6

phage display, immunoprecipitation, phage

protocol ,

Jul 26, 2021

Mar 14, 2022

51877

Equipment Needed and Part Numbers:

- Plate Sealer- Applied Biosystems Cat No: 43-331-83
- Foil seals- Bio-Rad Cat No: MSF1001
- Gas permeable seal- 4titude Cat No: 1149R80
- Round 96 well 2ml deep well plate- Genesee Science Cat No: 22-485
- Square 96 well 2ml deep well plate- VWR Cat No: 75870-796
- 12 span 1000uL multichannel pipet

Reagents Needed:

- Protein A/G Beads
- 1xTBS-Tween
- BSA Fraction V
- LB-Carb
- BLT5403
- Phage Display Library
- RIPA
  -10mM Tris-HCL (pH 7.4)
  -1.0% Triton X
  -0.1% SDS
  -140mM NaCl
- TNP40
  -10mM Tris-HCl (pH 7.4)
  -140mM NaCl
  -0.1% NP40 (Tergitol)
- SM Buffer
  -50mM Tris-HCl (pH 7.4)
  -100mM NaCl
  -10mM MgSO4+7h2O anhydrous (powder)

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Before getting started you will need a titered phage display library, patient samples and all required materials mentioned in this protocol.

Day 1- Blocking Plates

1 Make blocking solution to prevent sample and library from binding to plates. This step also helps with movement of protein A/G beads later on.

Blocking solution (make fresh for every experiment) :
3.0% BSA Fraction V
1x TBS-Tween
Mix thoroughly and filter through a 0.22um filter

2 Aliquot blocking solution into **Round-bottom 96 well 2ml deep well plates** according to the number of wells and plates needed for experiment for both rounds of IPs. Seal tightly with foil seal.
Example: If running 4 plates worth of samples (384 samples including controls), block a total of 8 plates, 4 for each round.

3 Incubate plates at **4 °C** inverting overhead or rocking on platform **Overnight** or for a minimum of **04:00:00** at **Room temperature** . Solution can block for several days at **4 °C** . 4h

Day 1- Setting up IPs

4 After plates have sufficiently blocked, unseal plates and discard all blocking solution contained in one set of IP plates by dumping the contents into the sink and gently tapping on clean paper towels. Continue blocking the other set of plates for round two of IP.

5 Add **500 µL** of phage library per well. If a canary well is desired (to control for phage contamination of reagents during the protocol) add SM buffer to the canary well instead of the phage library.

6 Add sample to library. Amounts will vary depending on sample type and concentration of IgG in sample:

1 µL of serum OR plasma in 1x Storage Buffer

10-20 µL of CSF in 1x Storage Buffer

1 µg of Commercial Antibody (for positive control, recommend to put one of these on each

plate randomized location.

An optional but recommended sample type is also an AG control well with no sample to control for nonspecific binding of phage library to A/G beads. We Recommend putting 1-2 of these on each plate in a random location.

7 Seal plate tightly (should be able to see outline of the individual wells) with foil seal and plastic plate sealer tool. Then, incubate by inverting overhead or on rocker platform at **4 °C** **Overnight** (minimum of 6 hours, maximum of 18 hours)

8 On a clean, phage-free lab bench, prepare an overnight starter culture of E.coli (BLT5403) as a working stock from a frozen glycerol stock in LB-Carb.

Day 2- Preparing AG Beads

9 **Prepare A/G Beads:**
NOTE: handle the beads gently using wide bore tips if possible to prevent damaging the beads.

1. Fully re-suspend beads by gently flipping by hand or placing on overhead rotator.
2. Calculate amount of beads needed. For the peptidome library we use **10 µL** of A and **10 µL** of G beads per reaction however some prefer to use **20 µL** of A and **20 µL** of G per reaction to increase the capture of IgG and IgA.
3. Mix packed Protein A and Protein G beads in a 1:1 ratio.

10 Aliquot amount of beads needed into a container able to hold more twice the volume of beads. 5m
We typically use a 5mL eppendorf tube or 15mL conical tube. Add 1x bead volume of cold TNP40 and mix overhead for **00:05:00** at **4 °C** or until fully resuspended. Place on magnetic rack until beads fully pelleted.

11 Remove supernatant and immediately add cold TNP40 again. Mix overhead again. Repeat this wash step a total of three times to fully remove the solution that the beads were stored in.

12 On the final wash step, elute the beads in enough TNP40 for the number of reactions needed. From experience, eluting in **25 µL** per reaction works well to form a nice droplet with the widebore tips when adding beads to the peptidome and sample.

Day 2- Adding Beads to Sample Bound to Library Targets 2m

13 1. Spin plates from overnight incubation at **800 rpm** for **00:02:00** to remove liquid off the seal. 2m

2. Gently remove seal.

14 1h
1. Put washed A/G beads into a reservoir container.
2. Add A/G beads to sample/library plate with wide bore tips using a multichannel quickly, making sure the beads stay resuspended and do not settle on the bottom of the reservoir.
3. Seal plate with foil seal.
4. Overhead mix for **01:00:00** at **4 °C**

NOTE: Don't exceed 1 hour, more time will increase nonspecific binding. (50min-1hr of bead binding is okay; start first group of plates at 50 mins)

15 Simultaneously, prepare new E.coli culture by growing 1-3mL of the overnight culture in enough LB-Carb (0.5mL for each sample).

Day 2- Immunoprecipitation Washes 1m

16 2m
NOTES:
- Keep RIPA on ice to keep samples cool during washes
- Plate spins should be at **800 rpm** for **00:01:00** on a tabletop centrifuge, as we have found that this is the minimum amount of time and speed required to remove liquid from the top of seal. This may vary by centrifuge.
- After hour is up, spin 2 plates (first set if doing more than 2 plates total) at **800 rpm** **00:01:00**

17 17m
1. Place plate magnet on top of a firm ice bucket and plate on top of magnet. Wait 1-2 minutes for all of the beads to collect on magnet and liquid to clarify. Remove foil seal to verify that supernatant is clear.
2. Using a 12-channel P1000 multichannel, aspirate the supernatant from each, discarding the tips.
3. Using a second multichannel, add **500 µL** of RIPA from a reservoir (empty tip box works great) to that row. Don't let the beads stay dry for too long.
4. Repeat with each rows.
5. Seal plate very well with foil seal and sealer
6. Take plate off magnet and invert a few times till beads are resuspended and place on rocker at **4 °C** for **00:07:00** to **00:10:00** on the rocker so liquid is moving freely in each well.
7. If doing more than one set of plates, begin on the next set of two plates while the first pair incubates on the rocker.

18 34m 20s
1. After **00:07:00** to **00:10:00** of incubation, spin plates at **800 rpm** for **00:00:20** , and place on magnet to let beads collect while carefully removing the seal

2. Aspirate **500 µL** of RIPA from one row of the plate and add **500 µL** of RIPA to same row. Repeat for all rows.
3. Seal plate very well with foil seal and sealer
4. Take plate off magnet and invert a few times till beads are resuspended and place on rocker at **4 °C** for **00:07:00** to **00:10:00** .

19 Repeat step18 three more times for a total of 5 washes.
NOTES:
- Each addition of RIPA counts as a wash.
- Aim for no more than 2 hours for the 5 washes such that the sample is not in RIPA for too long.
- When doing multiple plates, batch them in pairs for easy centrifugation. i.e. six plates would be done in three pairs at a time.

Day 2- Lysis/Amplification

20
1. After final/5th wash, remove supernatant and add 150uL LB-Carb to each well.
2. Using a multichannel, mix one row until all beads resuspended and transfer to a new **Square 96 well 2ml deep well plate**. Discard tips.
3. Repeat with fresh tips for each row.
4. Add **500 µL** E.coli at OD600 0.3-0.6 to the bead/LB mixture.

NOTES: If doing multiple plates, try to add the E. coli to the LB around the same time so lysis occurs simultaneously.

21
1. Seal with a **gas permeable seal**, and incubate in a shaker incubator for 1-2 hrs.

NOTES:
- Catch the clarification as soon as possible!
- Compare lysis to a canary well (can be on a completely separate plate or tube) which should remain cloudy while the wells containing phage library clarify when on magnet to pellet beads.
- Once the wells look clear, proceed immediately to next step. Do not let it incubate for longer than necessary, as this will decrease phage titers.

22 Add **5.0 Molarity (M)** Sodium chloride **P212121** to each well for a final molarity of **0.5 Molarity (M)** in the clarified E. coli culture (volume changes with amount of lysate, normally around 60-65uL).

Day 3- Preparing for Round 2 30m

23 30m
1. Spin plate at **4 °C** for **00:30:00** at **3220 rcf** (or max speed) to remove E. coli cell debris.
2. While plate is spinning, empty out blocking solution from second round of plates.
3. After spinning, use the Integra or a multichannel to transfer **500 µL** from the top of the wells, avoiding the cell debris at the bottom, to the previously blocked plates.

NOTE: Save Round 1 lysates and store at **4 °C** as a backup in case round 2 goes poorly or if you wish to sequence Round 1.

24

1. Add samples in same volume and configuration as done in starting Round 1 to plates.
2. Repeat IP one more time for a total of 2 rounds of immunoprecipitation. One can do additional rounds of IP for more selection if desired.
3. Store final lysate short term at **4 °C** or long term at **-20 °C**
4. To prepare samples for sequencing, follow with protocol below:

Phage Display Library Prep Method
by Sabrina Mann,
Chan Zuckerberg Biohub

PREVIEW RUN