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IgA Metagenomic Immunoglobulin Sequencing (MIG-Seq)

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Protocol status: Working

We use this protocol and it's working

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Abstract

IgA, the most highly produced human antibody, is continually secreted into the gut to shape the intestinal microbiota. Methodological limitations have critically hindered defining which microbial strains are targeted by IgA and why. Here, we develop a new technique, Metagenomic Immunoglobulin Sequencing (MIG-Seq), and use it to determine IgA coating levels for thousands of gut microbiome strains in healthy humans. We find that microbes associated with both health and disease have higher levels of coating, and that microbial genes are highly predictive of IgA binding levels, with mucus degradation genes especially correlated with high binding. We find a significant reduction in replication rates among microbes bound by IgA, and demonstrate that IgA binding is more correlated with host immune status than traditional microbial abundance measures. This study introduces a powerful technique for assessing strain-level IgA binding in human stool, paving the way for deeper understanding of IgA-based host-microbe interactions.

Protocol materials

 SYBR™ Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO **Invitrogen - Thermo Fisher Catalog #S7563**

Step 21

 IgA Antibody, anti-human, APC **Miltenyi Biotec Catalog #130-113-472** Step 9

 Normal Mouse Serum **Jackson ImmunoResearch Laboratories, Inc. Catalog #015-000-120** Step 9

 EasySep™ APC Positive Selection Kit II **STEMCELL Technologies Inc. Catalog #17681** In 2 steps

Preparation of fecal samples

10m

- 1 Weigh out  300 mg of thawed  human fecal sample in 2 mL centrifuge tube
- 2 Vigorously vortex and mix samples via pipetting to ensure homogenization
- 3 Rehydrate sample with  1.25 mL cold  1X PBS (Phosphate-buffered saline).
Incubate  00:05:00 on ice
- 4 Centrifuge samples at  500 x g, 00:15:00
- 5 Following centrifugation, filter the clarified supernatant (~1mL) through a 70 µm filter
- 6 Remove and retain  100 µL of filtered supernatent for use as the "unsorted" or "native" fraction for subsequent metagenomic sequencing. Keep this fraction on ice for remainder of protocol.
- 7 Divide the remaining supernatant (~900 uL) into 2 separate wells of a 96 deep-well plate. These wells will be treated as individual samples throughout the remainder of the protocol, and the two samples will be combined at the end of the protocol to increase sample biomass.

Antibody staining

10m

- 8 Prepare the following staining buffer in PBS:
 - 3% Fetal Bovine Serum
 - 0.5% Sodium Azide
 - 1 mM EDTAKeep staining buffer on ice
- 9 Prepare the following antibody master-mix in the staining buffer:
 - 1:30 dilution of IgA Antibody, anti-human, APC **Miltenyi Biotec Catalog #130-113-472**

- 1:50 dilution of

 Normal Mouse Serum **Jackson ImmunoResearch Laboratories, Inc.** Catalog #015-000-120

10 Centrifuge the 96 deep-well plate at  5000 x g, 00:05:00

5m

11 Discard supernatant and resuspend pellets in  100 µL antibody master-mix. Incubate on ice for  00:30:00

30m

12 Wash samples with  1 mL cold staining buffer. Centrifuge  5000 x g, 00:05:00

5m

13 Discard supernatant and resuspend pellet in  200 µL staining buffer

14 Retain  20 µL of the resuspend pellet to act as a stained, unsorted control for Flow Cytometry analysis to establish background to calculate “Native IgA binding percentage”

Magnetic cell separation

35m

15 Stain the remaining  180 µL with  20 µL of EasySep APC Selection Cocktail

15m

 EasySep™ APC Positive Selection Kit II **STEMCELL Technologies**
Inc. Catalog #17681

for  00:15:00 at room temperature

16 Add  22 µL of EasySep Dextran RapidSpheres 50100

10m

 EasySep™ APC Positive Selection Kit II **STEMCELL Technologies**
Inc. Catalog #17681

and incubate for  00:10:00 at room temperature

17 Place samples on

5m

Equipment

MO BIO PowerMag Magnetic Separator

NAME

MO BIO PowerMag Magnetic Separator

BRAND

27400

SKU

for  00:05:00 to allow for magnetic separation.

- 18 Carefully discard unbound supernatent while on the magnet. Then remove 96-well plate from magnet and wash with  200 µL of staining buffer.
- 19 Repeat steps 17 and 18 an additional 2 times, for a total of 3  00:05:00 magnetic enrichments and washes. 5m
- 20 After the final resuspension, combine the duplicate wells (established in Step 7) to establish the "IgA positive" fraction

Flow cytometry

- 21 Prepare tubes for flow cytometry by combining  5 µL of sample,  20 µL of counting beads, and a 1:10,000 dilution of  SYBR™ Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO **Invitrogen - Thermo Fisher Catalog #S7563** into staining buffer for a final volume of  200 µL
- 21.1 Tubes should be made using 1) unstained native sample (step 6), 2) stained native sample (from step 14), and 3) stained IgA+ samples (from step 20)
- 22 Analyze cells on a flow cytometer

Equipment

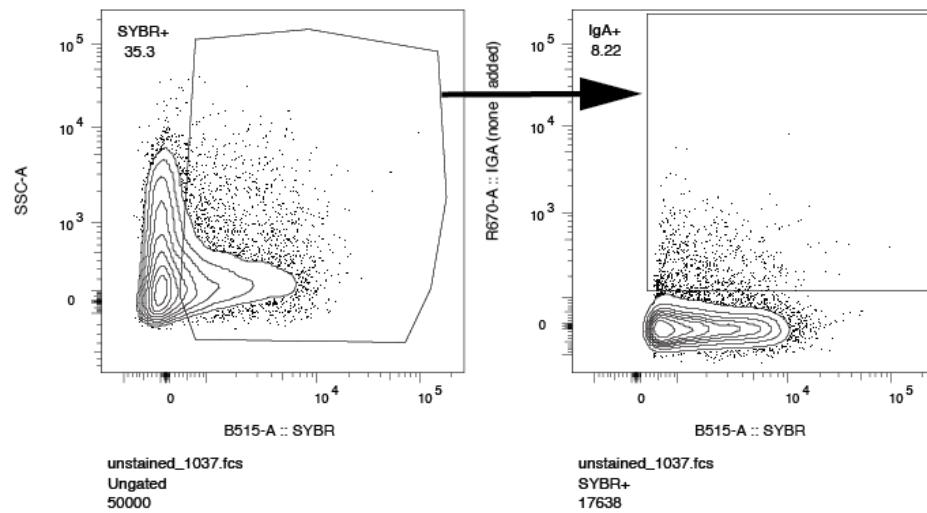
FACSymphony™ A5 Cell Analyzer NAME

BD BRAND

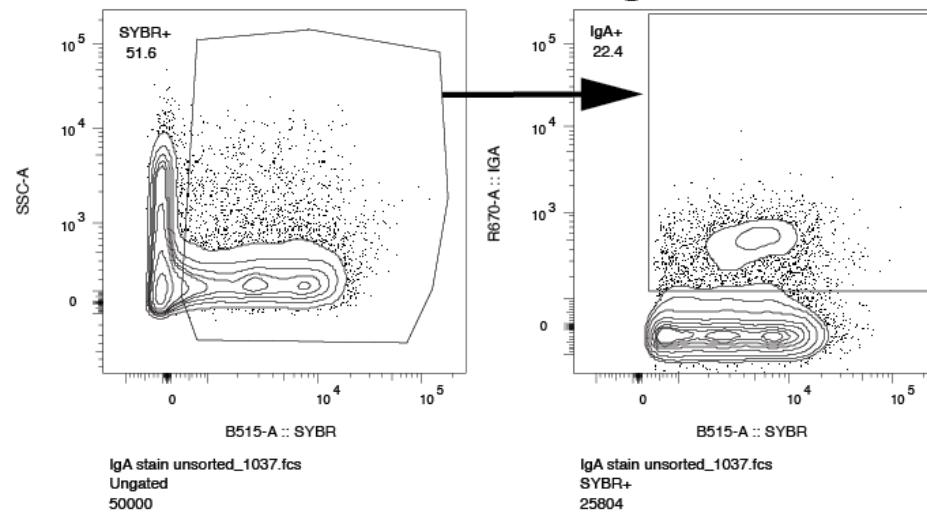
A5 SKU

22.1 Refer to this image to establish flow cytometry gates:

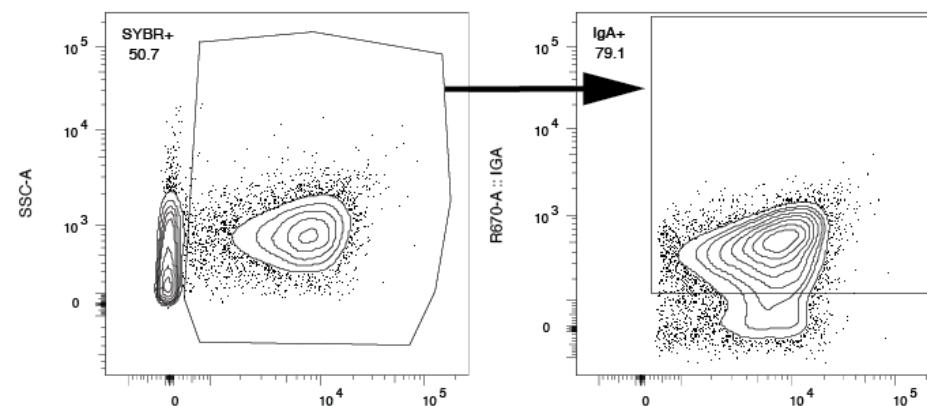
A) Unstained (with SYBR, no IgA)



B) Stained with SYBR + IgA, unsorted



C) Stained with SYBR + IgA, sorted



B515-A :: SYBR

IgA+ sort_1037.fcs
Ungated
50000

B515-A :: SYBR

IgA+ sort_1037.fcs
SYBR+
25350

Supplemental Figure S6. Gating strategy for MiG-Seq protocol. The preliminary gate to identify IgA-positive cells was SSC and SYBR Green, with SYBR-positive gate drawn based on preliminary experiments using a sample without SYBR green (SYBR stains all bacteria). SYBR-positive cells were then analyzed for IgA staining, with an IgA-positive gate drawn based on a sample without IgA staining. Representative flow plots are shown for a single sample (#1037) that is unstained (**A**), staining and unsorted (**B**), and stained and sorted (**C**).

- 23 For each sample, the native “IgA fraction” is calculated as the number of IgA+ cells in the native sample (b) minus the number of IgA+ cells in the unstained control (a). Samples in which the unstained control (a) had a higher number of IgA+ cells than the native stained sample (b), or the native stained sample (b) had a higher number of IgA+ cells than the IgA+ sample (c), should be considered to have “failed QC” and be removed from subsequent analysis.