

Profiling Serum Antibodies with a Pan Allergen Phage Library Identifies Key Wheat Allergy Epitopes

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Method Article

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Abstract

Allergic reactions occur when IgE molecules become crosslinked by antigens such as food proteins. The physiologic consequences range from mild to life-threatening. Here we create the 'AllerScan' bacteriophage display library for efficiently profiling allergic antibodies to characterize binding specificities associated with known allergens. AllerScan enables sensitive and unbiased characterization of circulating IgE and IgG antibody reactivities against thousands of allergenic proteins from hundreds of organisms at peptide resolution. Using AllerScan to analyze food allergy reactivities, we identify robust anti-wheat IgE reactivities in wheat allergic patients but not in wheat-sensitized individuals. Meanwhile, a wheat allergen epitope, alpha purothionin, elicits dominant IgE responses among allergic patients but frequent IgG responses among sensitized and non-allergic patients; a double-blind placebo-controlled trial shows that alpha purothionin reactivity, among others, is strongly modulated by oral immunotherapy. Our proof-of-principle results thus suggest that AllerScan to be a high-throughput platform for unbiased analysis of allergic antibody fine specificities.

Introduction

Reagents

Equipment

Procedure

1. Construct AllerScan phage display library
 - a. Download all protein sequences from Uniprot database included in the Allergome and collapsed on 90% sequence identity
 - b. Design peptide sequences 56 amino acids in length that overlap by 28, to tile through all representative allergen proteins.
 - c. Next, reverse-translate these sequences into DNA nucleotide sequences optimized for *E. coli* expression and added adapter sequences to both the 5' and 3' ends.
 - d. Construct/purchase these 200 nucleotide sequences.
 - e. PCR amplify oligonucleotide library and restriction clone into the T7FNS2 vector backbone.
 - f. Package resulting library via the T7 Select Packaging Kit (EMD Millipore) and expand for use as a PhIP-Seq library.

IgG screening of the AllerScan library

1. Quantify IgG serum concentrations via ELISA (Southern Biotech).
2. Add two mg of IgG to 1 mL of AllerScan library at 1.9×10^9 pfu for each reaction.
3. Rotate serum and phage library overnight at 4°C
4. Add 20 mL of protein A and 20 mL of protein G coated magnetic beads (Invitrogen, 10002D and 10004D) to each reaction
5. Rotate an additional 4hr at 4°C.
6. Wash beads three times in 0.1% NP-40 and then resuspend in a Herculase II Polymerase (Agilent cat # 600679) PCR master mix.
7. Perform 20 cycle PCR.
8. Add two mL of each reaction to new PCR reactions containing sample specific barcoding primers.
9. Perform an additional 20 cycles of PCR.
10. Pool final amplified product and submit for sequencing using an Illumina NovaSeq 6000 or NextSeq 500.

IgG screening of the AllerScan library

1. Quantify IgE serum concentrations via ELISA (Southern Biotech).
2. Add 100 ug of IgE to 1 mL of AllerScan library at 1.9×10^9 pfu for each reaction.
3. Rotate serum and phage library overnight at 4°C
- 4.a. For each reaction, coat 10 ug of Streptavidin-bound M280 magnetic beads (ThermoFisher - 11205D) with biotinylated omalizumab (Genentech, CA) - follow manufacturer guidelines
- 4.b. Mix 10 ug of omalizumab coated Streptavidin-bound M280 magnetic beads with each reaction
5. Rotate an additional 4hr at 4°C.

6. Wash beads three times in 0.01% PBST and then resuspend in a Herculase II Polymerase (Agilent cat # 600679) PCR master mix.
7. Perform 20 cycle PCR.
8. Add two mL of each reaction to new PCR reactions containing sample specific barcoding primers.
9. Perform an additional 20 cycles of PCR.
10. Pool final amplified product and submit for sequencing using an Illumina NovaSeq 6000 or NextSeq 500.