nature portfolio

https://doi.org/10.1038/s41596-022-00766-8

Supplementary information

PepSeq: a fully in vitro platform for highly multiplexed serology using customizable DNA-barcoded peptide libraries

In the format provided by the authors and unedited

Supplementary Information

Supplementary Methods

Synthesis and purification of puromycin adapter

Production of the puromycin adapter according to this protocol requires standard oligonucleotide synthesis chemistry. Alternatively, the adapter can be sourced from the City of Hope Core Facility - DNA Synthesis Laboratory, or from a commercial custom chemical synthesis company with similar capabilities. A typical synthetic yield at the scale described in this protocol is 150-180 mg, which is sufficient for ≥50 PAL reactions.

The oligonucleotide has the following structure and should be synthesized from the 3'-end to 5'-end, on 89.0 µmol scale in a 6.3 mL reactor, using standard phosphoramidite chemistry on an automated DNA/RNA Synthesizer (eg the OligoPilot10 plus from GE Company).

Reagents

- dA(Bz) Phosphoramidite (Thermo Fisher, cat. no. 27-2030-05)
- dC(Ac) Phosphoramidite (Thermo Fisher, cat. no. 29-1727-07)
- dG(ibu) Phosphoramidite (Thermo Fisher, cat. no. 27-1734-04)
- T-Phosphoramidite (Thermo Fisher, cat. no. 27-1736-05)
- 3'-DMT-deoxy Cytidine (n-acetyl)-5'-CED phosphoramidite (ChemGenes, ANP-4675)
- 3'-DMT-Thymidine 5'-CED phosphoramidite (ChemGenes, ANP4674)
- 3'-DMT-deoxy Adenosine (n-bz) 5'-phosphoramidite (ChemGenes, ANP-4671)
- 3'-DMT deoxy Guanosine 5'-CED phosphoramidite (ChemGenes, ANP 4673)
- Puromycin-CPG Support (Store at room temperature; Glen Research, cat. No.20-4040-10)
- 5-Me-dC Brancher Phosphoramidite (Glen Research, cat. No.10-1018-02)
- Spacer Phosphoramidite 18 (Glen Research, cat. No. 10-1918-02)
- Chemical Phosphorylation Reagent (CPR) (Glen Research, cat. No.10-1900-02)
- Source30Q resin from GE
- PRP-1 resin column from Hamilton

Equipment

- SpeedVac
- Automated DNA/RNA Synthesizer OligoPilot10 plus from GE

- AKTA Purifier

Synthesis of puromycin adapter

(i). Begin the synthesis from the puromycin support (1.95 g, 32 umole/g loading, scale 62 umole) and couple standard DNA phosphoramidites (3.0 eq) for 4min each.

(ii). Couple reversed DNA phosphoramidites (3.0 eq) for 3.5min, using 0.5 M ETT in acetonitrile as an activator (Phosphoramidite-activator ratio, 3:2.)

(iii). Double couple Spacer Phosphoramidite 18 (eq. 3.1), concentration 100 mM for 8.0min.

(iv). Double couple 5-Me-dC Brancher Phosphoramidite (eq. 3.2), concentration 100 mM for 7.0min.

(v). Double couple chemical Phosphorylation Reagent (eq. 3.0), concentration 100 mM for 5.0 min. After the phosphorylation is complete, remove the DMT group and cap the synthesis in order to terminate this branch of the oligonucleotide.

(vi). In order to continue the synthesis of the second branch of the oligonucleotide (the reversed branch) the Levulinyl protecting group of the Brancher had to be removed [21]. To achieve this, connect the reactor on the synthesizer to two 50 mL disposable syringes. Fill the inlet syringe with the 25 mL Lev deprotection mixture, 0.5 M hydrazine hydrate in pyridine: acetic acid (1:1). Pass the solution back and forth through the reactor for 25 minutes.

(vii). Rinse the support three times with 15 mL pyridine: acetic acid (1:1) and then three times with 25 mL acetonitrile.

(viii). Dry the support with an argon stream for 10min prior to the synthesis of the second, the reversed branch.

(ix). After the synthesis of the second branch is complete, treat the support in the reactor with a solution of 20% DEA in acetonitrile for 20min with a flow of 2.5 mL/min.

(x). Wash with acetonitrile for 3min, flow 20 mL/min.

(xi). Dry with the flow of argon and treat with 70 mL of solution of AMA (methylamine 33 wt. % in absolute ethanol - conc. Ammonia – water, 2:1:1) for 5hrs at RT.

(xii). After the cleavage/deprotection is complete, remove the support by filtration and wash the cake with 3x10 mL of water. Combine washes and filtrate, and evaporate under vacuum to achieve a dry residue.

Purification of puromycin adapter

(i). Purify the crude puromycin adapter on the anion exchange resin. Use resin such as SOURCE30Q (GE Healthcare), in Buffer A: 50 mM phosphate buffer, pH 12 and Buffer B: 50 mM phosphate buffer, 2 M NaCl, pH12. Collected fractions are analyzed by analytical PAGE for FLP.

(ii).Analyze fractions containing FLP by HPLC and desalt those containing 85+% FLP by Ion-Paired chromatography on the PRP-1 resin column from Hamilton, using 5 mM TBAA buffers pH 7.1. Pool fractions, concentrate in a Speedvac, and aliquot/precipitate into IPA in presence of sodium chloride in order to remove the TBAA.

Plate washing using multichannel pipettes

Timing ~2 h active time

(i). After completion of the incubation (step 103), quickly spin down the plate and place onto a plate magnet. Allow the beads to collect. Remove and discard the supernatant.

(ii). Remove the plate from the magnet and add 100 µL of 1X PBST to each well. Place onto the magnet and discard the supernatant. Repeat this wash step two more times, first using 120 µL and then 140 µL of 1X PBST.

(iii). After the final wash, remove the plate from the magnet and resuspend beads in 60 µL of 1X PBST. Transfer resuspended beads to a new LoBind plate.

(iv). Place the plate onto a magnet and discard the supernatant. Remove plate from the magnet and add 100 µL of 1X PBST to each well. Place onto the magnet and discard the supernatant. Repeat plate wash seven more times, increasing the volume of PBST by 10 µL for each subsequent wash.

▲ **CRITICAL STEP** It is critical to remove all remaining PBST during the final wash. We recommend quickly spinning the plate in a centrifuge prior to removing residual PBST with a 10 µL multichannel pipette.

(v). Remove plate from magnet and resuspend beads in 30 µL of ultrapure water. Pipette up and down ten times to ensure sufficient resuspension of beads and spin down the plate for 30 seconds so there are little to no beads adherent to the well wall.

(vi). Transfer the plate to a thermocycler and heat at 95℃ for five minutes to elute the final product off of the beads.

(vii). Spin the plate down and place on the magnet. Allow the beads to collect and transfer the final eluted product to a new full-skirted LoBind plate

Bead clean up using multichannel pipettes

Timing: ~25 minutes of active time and 20 minutes of incubation time

(i). Equilibrate Agencourt AMPure XP beads to RT, heat Tris-Tween at 50℃, and prepare ~50 mL fresh 80% (vol/vol) EtOH per 96-well LoBind plate of indexed product.

(ii). Distribute 27 µL of AMPure beads to each well of the PCR plate containing indexed antibody assay samples (from step 109).

(iii). Pipette up and down ten times to mix. Incubate at RT for five minutes.

(iv). Place the plate onto a magnet and allow the solution to clear for five minutes. Discard the supernatant.

(v). Without removing the plate from the magnet, wash the beads with 200 µL 80% (vol/vol) EtOH. Incubate beads for 30 seconds. Carefully discard the EtOH without disrupting the beads. Repeat the wash once more for a total of two washes.

(vi). Be sure to remove all residual EtOH after the second wash using a 10 µL pipette.

(vii). Let the beads air dry at RT for five minutes.

▲ **CRITICAL STEP** It is important not to extend the drying time past five minutes. Extending the time will cause the magnetic beads to crack, which can cause significant loss of product.

(viii). Remove the plate from the magnet and resuspend the magnetic beads bound to PCR product with 15 µL of pre-warmed Tris-Ttween. Ensure each well is properly mixed by pipetting up and down ten times.

(ix). Incubate at RT for two minutes.

(x). Place plate onto magnet and allow beads to collect for two minutes.

(xi). Carefully transfer eluate to a new skirted LoBind 96-well plate.

Supplementary Information Table 1. Oligonucleotide sequences used in protocol.

Sequence name	**Nucleotide sequence (5' to 3')**
DNA template	CCTATACTTCCAAGGCGCA:variable_region:GGTGACTCTCTGTCTTGGC
DNA amplification primers	
DNA amplification primer (Forward)	GCGAATTAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGGGA GAAAACCTATACTTCCAAGGCGCA
DNA amplification primer (Reverse)	AGCTCCTGCTGCATTTCCGTTCAGCAGACGCAGCAGCCAAGACAGAGAGTCACC
Sequencing primers	
Forward indexing primer	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGA TCTNNNNNNNNNNNNBBBBBBBBBBCCTATACTTCCAAGGCGCA
Reverse indexing primer	CAAGCAGAAGACGGCATACGAGATBBBBBBBBBGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTGCCAAGACAGAGAGTCACC
Standards	
TEV oligonucleotide standard	GGGCTTAAGTATAAGGAGGAAAAAATATGGGAGAAAACCTATACTTCCAAGGCGC AGGTGACTCTCTGTCTTGGCTGCTGCGTCTGCTGAACGGAAATGCAGCAGGAGCT GGGCTTAAGTATAAGGAGGAAAAAATATGGGTATGGCATACCG

*B=barcode sequence, N=randomer sequence