**Supplementary Methods**

**Reagents**

* Span 80, Tween 80, Triton X-100, and mineral oil and oligonucleotides were obtained from Sigma-Aldrich, Bangalore, India.
* PfuUltra II Fusion HS DNA polymerase was from Agilent Technologies, Santa Clara, US.
* BSA (Fraction V), dNTPs were obtained from Roche, Mannheim, Germany.
* All other standard chemicals were obtained from Affymetrix, CA, USA.
* Plasmid pVCMTBLIB41F136006 used as template for ePCR for optimization of extraction protocol, plasmids encoding single chain Fvs [scFv(s)], namely HuAb1184 and HuAb1190 used as template for the amplification of scFv encoding DNA for cloning, and vector pVMAKHuscFvclo002 used for cloning scFv(s) were available in the laboratory.
* Different DNA purification kits namely, QIAquick PCR purification Kit (Qiagen; Cat no. 28106 and Column Lot no. 154038931, Buffer Lot no. 157011841), PureLink Quick PCR purification kit (Thermo Fisher Scientific; Cat no. K310001 and Lot no. 00654132), Monarch PCR and DNA Cleanup kit (NEB; Cat no. T1030S and Lot no. 10020890), DNA Clean & Concentrator – 5 (Zymo Research; Cat no. D4003 and Lot no. ZRC203516), QuickClean II PCR extraction kit (GenScript; Cat no. L00419-50 and Lot no. C70011806), QIAquick Gel extraction kit (Qiagen; Cat no. 28706 and Column Lot no. 154038931, Buffer Lot no. 148019900), GenElute PCR Clean-Up Kit (Sigma-Aldrich; Cat no. NA1020-1KT and Lot no. SLBW1595), NucleoSpin Gel and PCR Clean-up (Macherey-Nagel; Cat no. 740609.50 and Lot no. 1812/004), NucleoTraPCR (Macherey-Nagel; Cat no. 740587 and Lot no. 1904/001), Wizard SV Gel and PCR Clean-Up System (Promega; Cat no. A9281 and Lot no. 0000354719), GeneJET PCR purification kit (Thermo scientific; Cat no. K0701 and Lot no. 00705184) were from respective manufacturers.

**Methods**

**Optimization of protocol for the extraction and purification of PCR products after ePCR**

The efficiency of different protocols for extraction and purification of PCR products after ePCR was tested using plasmid pVCMTBLIB41F136006 as a template DNA, which carries a 389 bp DNA fragment flanked by known adapter sequences (L and K).

Aqueous PCR was set up in a volume of 350 µl containing 10 mg/ml BSA, 200 µM dNTPs, 105 pmoles each of 5’ primer L3-s and 3’ primer K2-s, 4 x 109 template DNA molecules and 8.4 U of PfuUltra II Fusion HS polymerase in 1 x PfuUltra II Fusion HS polymerase buffer. 300 µl aqueous PCR mixture was added drop-wise over a period of 2 min to 600 µl oil-surfactant mixture (4.5 % Span 80 (v/v), 0.4 % Tween 80 (v/v) and 0.05 % Triton X-100 (v/v) in mineral oil) with constant stirring using a 2 x 6 mm magnetic flea (Tarsons, India) at 1000 rpm at RT in a 1.8 ml round bottom cryo tube (Nunc, Thermo Fisher Scientific, USA). The stirring was continued for additional 5 min after complete addition of the aqueous PCR mix and the emulsion was divided (~ 100 µl) into nine 0.2 ml PCR tubes and overlaid with 30 µl mineral oil. The emulsion containing tubes (ePCR) were subjected to thermocycling for 30 cycles as per manufacturer’s instructions for the use of PfuUltra II Fusion HS polymerase. The contents of three tubes, each containing 100 µl emulsion were individually processed for purification using three methods as described below:

**Method 1 (Conventional method, Spin + DEE + Column)**

1. The ePCR from each PCR tube (100 µl + 30 µl overlaid mineral oil) was transferred to 1.5 ml microfuge tube and centrifuged at 13,000g for 5 min at RT.
2. The top oil layer was carefully removed, 70 µl of 0.1 x TE was added to increase the volume, and the emulsion was broken by addition of 1 ml water-saturated diethyl ether (DEE) followed by vortexing. The suspension was centrifuged at 12,000 rpm for 1 min at RT, and the top layer of DEE was removed. This step was repeated twice, and leftover DEE was removed using a centrifugal evaporator.
3. The PCR products were purified from aqueous layer (~ 100 µl) using QIAquick PCR purification kit as per manufacturer’s protocol and eluted in 40 µl EB.

**Method 2 (Spin + Column):**

1. The ePCR from each PCR tube (100 µl + 30 µl overlaid mineral oil) was transferred to 1.5 ml microfuge tube and centrifuged at 13,000g for 5 min at RT.
2. The top oil layer was carefully removed, 70 µl of 0.1 x TE was added to increase the volume.
3. 500 µl of QIA PB purification buffer (5 x volume) was added to each tube and emulsion was broken by vortexing for 1 min.
4. The suspension (emulsification oil + aqueous layer) was centrifuged at 12,000 rpm for 1 min at RT and purified using QIAquick PCR purification kit as described above.

**Method 3 (only Column; Quick ePCR extraction protocol):**

1. The ePCR from each PCR tube (100 µl + 30 µl overlaid mineral oil) was transferred to 1.5 ml microfuge tube and 500 µl of QIA PB purification buffer (5 x volume) was directly added to each tube and emulsion was broken by vortexing for 1 min.
2. The suspension (emulsification oil + aqueous layer) was centrifuged at 12,000 rpm for 1 min at RT and purified using QIAquick PCR purification kit as described above.

The purified ePCR products obtained using three different methods were analyzed using agarose gel electrophoresis and quantitated using Nanodrop 2000c spectrophotometer and Qubit Fluorometer 2.0 (Thermo Fisher Scientific, USA) using dsDNA HS kit.

**Cloning of DNA fragments amplified using ePCR or cPCR**

Plasmids encoding single chain Fvs [scFv(s)], namely HuAb1184 and HuAb1190 were used as template for the amplification of scFv encoding DNA using 5’ primer PelBclo-51 and 3’ primer HuJGclo34. For this, aqueous PCR mixture was prepared in a volume of 200 µl containing 10 mg/ml BSA, 200 µM dNTPs, 60 pmoles each of 5’ primer PelBclo-51 and 3’ primer HuJGclo34, 2.4 x 109 template DNA molecules, and 4.8 U of PfuUltra II Fusion HS polymerase in 1 x PfuUltra II Fusion HS polymerase buffer. The PCR mixture was emulsified in 400 µl oil-surfactant mix as described above and divided as 50 µl emulsion into 0.2 ml PCR tubes and overlaid with 30 µl mineral oil. Thermocycling was performed for 30 cycles and the ePCR products were purified using Quick ePCR extraction protocol. Simultaneously, the DNA fragments encoding scFv were amplified using cPCR and purified using QIAquick PCR purification kit. The ePCR and cPCR products were quantified using dsDNA BR quantification kit with Qubit Fluorometer (version 2.0) and approximately 2 µg product was subjected to T4 DNA polymerase treatment in the presence of dTTP to generate 4 base overhangs at 5’ ends of the DNA fragments as described before [15]. The T4 DNA polymerase-treated products were purified and ligated to BsaI-digested vector pVMAKHuscFvclo002 as described before [15]. The ligation mix was electroporated in E. coli TOP10F’ and electroporation efficiency was calculated. For each construct, six random colonies were screened using colony PCR followed by DNA sequencing to determine the number of recombinants with correct sequences.

**Next-generation sequencing of DNA libraries purified using Quick ePCR extraction protocol**

The single-stranded phage DNA sample was obtained by boiling phage particles captured on Streptavidin (SA)-magnetic beads after panning a phage-displayed gene-fragment library, MTBLIB42C02, on different antibodies following panning methods as described before [19, 20]. The extracted DNA (without any purification) was used as a template in the three-step PCR-based strategy for preparation of dual-indexed NGS libraries. For the initial pre-amplification of template, cPCR was set up in a volume of 50 µl as described above using primers, which annealed completely to the sequences flanking the target. The reactions were subjected to 13 cycles of cPCR followed by dilution of the reaction by two-fold using fresh 50 µl PCR mix (containing all components except template). Approximately 80 µl of diluted cPCR product was emulsified in 160 µl oil-surfactant mix as described above using the same primers as used for cPCR and divided into three 0.2 ml PCR tubes (80 µl aliquots) and overlaid with 20 µl mineral oil and subjected to 30 cycles of ePCR. The first step ePCR products were purified using Quick ePCR extraction protocol and quantified using dsDNA HS quantification kit with Qubit Fluorometer. This ePCR product was employed as template for the final step of ePCR using primers carrying Illumina adapter sequences for preparation of dual-indexed NGS libraries. For this, aqueous PCR was set up in a volume of 100 µl and approximately 80 µl was emulsified in 160 µl oil-surfactant mix as described above and subjected to 30 cycles of ePCR. The ePCR products were purified using ‘Quick ePCR extraction protocol’ and quantified using dsDNA HS quantification kit with Qubit Fluorometer, and average library size was determined using High Sensitivity DNA kit on 2100 Bioanalyzer (Agilent, CA, USA) as per the manufacturer’s instructions. Since these were low-diversity amplicon libraries carrying same 30-35 bp adapter sequence at 5’ and 3’ end of each DNA molecule, they were loaded on the flow cell at a low concentration i.e. 6 pM along with 15 % PhiX as internal control. The libraries were subjected to sequencing on MiSeq Nano v2 reagent kit (MS-103-1003, Illumina) for 2 x 250 cycles of paired-end sequencing. The run statistics were viewed using Illumina Sequencing Analysis Viewer Software (v2.4.5).

**Evaluation of Quick ePCR extraction protocol using commercially available kits**

The emulsion PCR (22 x 100 µl) was setup as described above in the optimization section. Eleven commercially available kits were analyzed and for each the extraction was performed in duplicates (two tubes of 100 µl ePCR per kit). The ePCR from each PCR tube (100 µl + 30 µl overlaid mineral oil) was transferred to 1.5 ml microfuge tube and different volumes of binding buffers were added as per manufacturer’s protocol for different kits. The emulsion was broken by vortexing for 1 min and the suspension (emulsification oil + aqueous layer) was centrifuged at 12,000 rpm for 1 min at RT. The suspension was loaded on the columns and purified as per manufacturer’s protocol. The ePCR products were eluted in 40 ul elution buffer provided by respective manufacturers.

The purified ePCR products were analyzed using agarose gel electrophoresis and quantitated using Nanodrop 2000c spectrophotometer and Qubit Fluorometer 2.0 (Thermo Fisher Scientific, USA) using dsDNA HS kit.