



| | PART 1: START OF SELECTION ROUNDS KIT VERSION 1.0, MARCH 2013. SEE ALSO DETAILED VERSION OF THIS PROTOCOL. |
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| Α | COUNTERSELECTION (OPTIONAL) |
| 1 | 1. Counterselection (optional step) Start with 1 - 3 μg purified dsDNA from the previous selection round. (First SELEX round: 10 - 50 μg ssDNA library). Mix dsDNA with 50 - 100 μl (10x] SELEX buffer (=1/10 final volume) and fill up with nuclease-free water to 500 - 1000 μl final volume. Denature dsDNA at 94°C for 3 min, then put immediately on ice. Add to magnetic beads without immobilized target structure. Incubate 1 h (round 1) or 15 min to 30 min (starting from round 2) at RT or 37°C. Capture magnetic beads with a magnet. Remove supernatant for use in the further selection process. |
| В | APTAMER-TARGET BINDING |
| 2 | 2. Binding Immobilization: Bind target structure to beads (see separate protocol). Start with 1 - 3 µg purified dsDNA from counterselection step or from the previous selection round (First SELEX round: 10 - 50 µg ssDNA library). Mix DNA (in water or elution buffer) with 50 - 100 µl [10x] SELEX buffer (=1/10 final volume) and fill up with nuclease-free water to 500 - 1000 µl final volume. Denature DNA at 94°C for 3 min, then put immediately on ice (Round 2 and later). Add precooled DNA or ssDNA to magnetic beads with immobilized target structure Amount of beads: Round 1: 10fold- (max. 200 µl), later rounds: Ifold binding capacity amount. Add 500 µl 1x SELEX Buffer. Incubate approx. 1 h (round 1) or 15- 30 min (round 2 and later). Mix gently to prevent sedimentation of beads (gentle shaking or occasional pipetting). Incubation temperature: Room temperature for analyses, 37°C for in-vivo targets |
| С | APTAMER-TARGET WASHING |
| 3 | 3. Washing → Round 1: 1 x with 1 ml SELEX Buffer Round 2: 2 x with 500 µl - 1 ml SELEX Buffer Additional rounds: Variable, roughly one additional washing step per additional selection round. → Varying and adjusting the selection stringency: Adjust DNA / RNA and magnetic beads amounts, binding time, number of washing steps, binding temperature and salt conditions. |
| D | APTAMER-TARGET ELUTION |
| 4 | 4. Elution (final volume 100 µl, choose one of the following alternate methods) → Direct PCR from DNA bound to beads Introduce beads directly into PCR reactions without elution. Applicable for 1 µM beads only. Heat Heat beads in dest. H₂O + 2 mM EDTA to 70°C - 94°C for complete denaturation. Fix beads to one side of tube using a magnetic device, quickly (!) pipet the supernatant off. Transfer supernatant without any beads to a new reaction tube. Precipitate DNA or purify on DNA spin columns. Heat + SDS (2 % [w/v]) Incubate beads in 2 % [w/v] SDS and heat to 70°C - 94°C. Fix beads to one side of tube using a magnetic device, quickly (!) pipet the supernatant off. Transfer supernatant without any beads to a new reaction tube. Precipitate DNA or purify on DNA spin columns. Heat + SDS (2 % [w/v]) Incubate beads in 2 % [w/v] SDS and heat to 70°C - 94°C. Fix beads to one side of tube using a magnetic device, quickly (!) pipet the supernatant off. Transfer supernatant without any beads to a new reaction tube. Precipitate DNA or purify on DNA spin columns. Elute DNA with Binding Buffer. Add 400 µl of orange-colored Orange-DX buffer and purify on DNA spin columns. Competitive elution with either natural ligand of immobilized target for specific displacement or excess amount of free target. The latter approach may fail to enrich high affinity binders. |

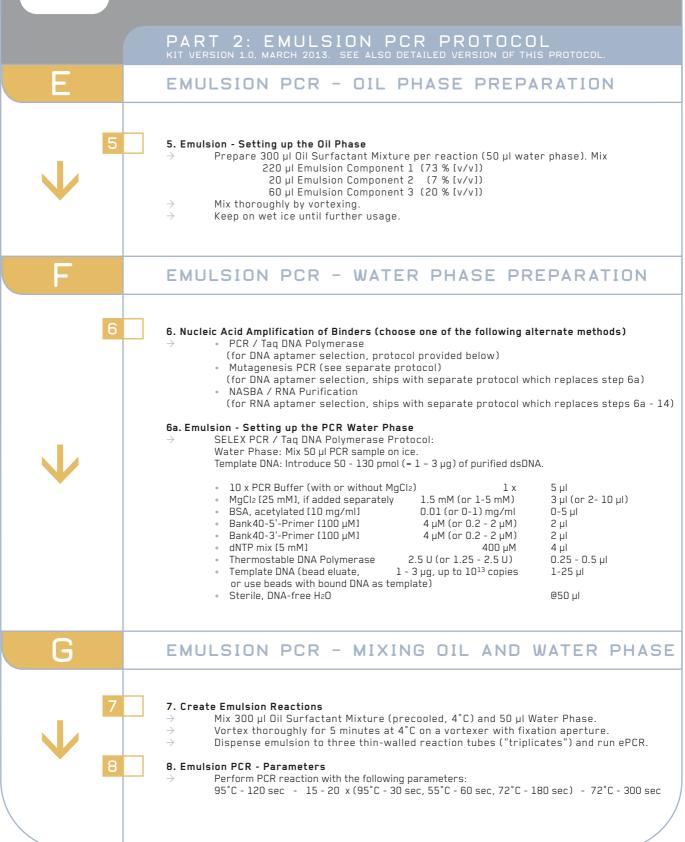
The latter approach may fail to enrich high affinity binders. For each selection round, keep backups of 50 % of (non-amplified) bead eluate.

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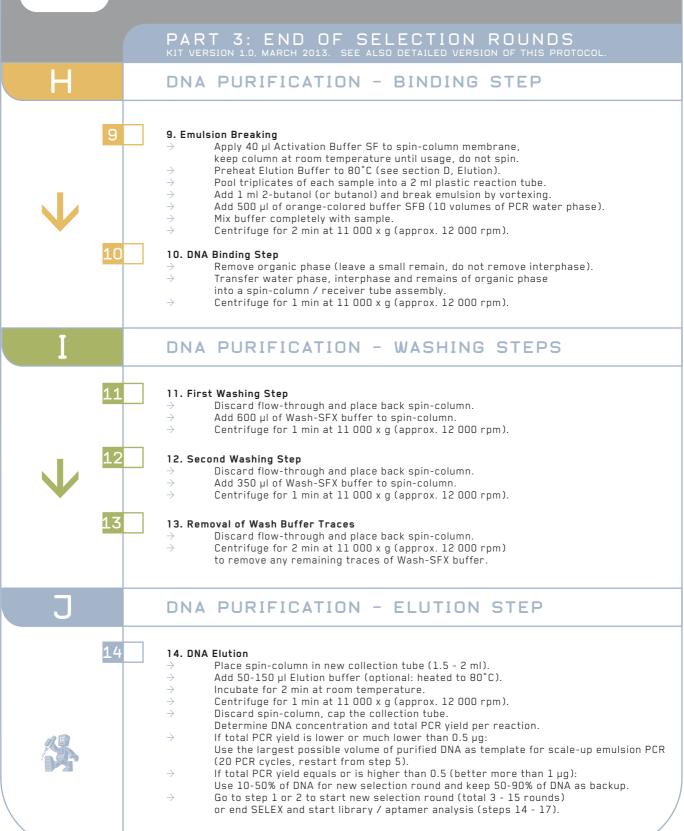


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PART 4: LIBRARY AND APTAMER ANALYSIS KIT VERSION 1.0, MARCH 2013. SEE ALSO DETAILED VERSION OF THIS PROTOCOL. K DIVERSITY STANDARD PCR 15. Diversity Standard PCR amplification (non-emulsified PCR) 15 10 x PCR Buffer B (with 1.5 mM MqCl₂) 10 µl 1 x Bank40-5'-Primer [100 μM] lμM lμl Bank40-3'-Primer [100 µM] lμM lμl dNTP mix [5 mM] 400 µM 8 µI Thermostable DNA Polymerase [5U/µl] 2.5 U 0.5 µl 0.5 µg, up to 1013 copies Template DNA Sterile, DNA-free H2O @100 µl 16 16. Diversity Standard PCR - Parameters Perform non-emulsified PCR reaction with the following parameters: 95°C - 120 sec - 2 - 4 x (95°C - 30 sec, 59°C - 30 sec, 72°C - 90 sec) - 72°C - 300 sec DIVE ASSAY 17a. DiVE Assay (alternate method to DiStRO assay) 17 Transfer 200 ng aliguots of standard or sample to separate plastic reaction tubes. Duplicates: Four tubes per sample. Assay: S1 Nuclease treatment, control: untreated. Add [5x] S1-Nuclease buffer to assay and control tubes (final concentration: 1x). Mix thoroughly. Keep final volumes identical and as low as possible. Final volume : _ Denature and reanneal both the assay and the control tubes: Denaturation: 3 min at 98°C Reannealing: 5 min at 65°C Add 1 μ l S1-Nuclease [1 U/ μ l] to the assay tube, but not to the control tube.

- Incubate for 30 min at 65°C.
 Add 2 µI EDTA [0.5 M, pH 8.0] (final concentration: 2 mM).
- Analyse 20-40 ng equivalent assay and control tubes side-by-side on 2-3 % agarose gels.

DIStro Assay

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17b. DiStRO Assay (alternate method to DiVE assay) 17 Mix the following components: 300 ng of each diversity-standard or DNA-sample + 1 µI DA-buffer (10x) + 1 µl SYBR-Green (1:1000 in nuclease free H2O) Fill up to 10 µl with nuclease free H2O Real-Time-Cycler Program: Denaturation: 2 min at 95°C Annealing: 180 min at 76°C (or 2°C less than calculated Tm) Take one measurement per minute. Remelting: Cool to 20°C, then heat by incrementing + 0,5°C per 7 sec to 98°C. Take one measurement per increment, i.e. per 7 sec. FLAA ASSAY (LIBRARY AND CLONE ANALYSIS) 18. FLAA Assay 18 Binder Assay: Bind biotinylated target to streptavidin-coated microtiterplate wells. Add a 10-fold excess of target in relation to the denoted binding capacity. Incubate 1 h at room temperature. Negative control: Add 2 µl Biotin [5 mM]. Incubate for 10 min at room temperature. Wash plate twice with 250 µl SELEX buffer [1x]. Dilute an appropriate amount of binder DNA in 50 µl SELEX Buffer [1x]. Amount : _____ Heat denature the DNA solution (95°C, 2 min) and immediately cool down on ice. Transfer the precooled DNA solution to the target coated microtiter plate. Incubate over night at 4°C or for one to two hours at room temperature. Discard the DNA supernatant. Wash plate immediately before measurement with 100 μl binding buffer [1x]. Add 50 µl Oligreen (or Picogreen) (1:500 in binding buffer). Measure twice after 9 min. (or after 4 min.): Ex. 485 nm, em. 527 nm.

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