The first cycle of SELEX

Negative selection:

1. Take the beads from 4 degrees celsius and vortex for 15-20 seconds.
2. Take 10microlitres out of it and put it in an Eppendorf (1.5ml)
3. The 1.5 ml tube is set over a magnetic separator.
4. After the beads were settled/pmicrolitresled to one side, pipette out the buffer.
5. Add 100microlitres of the 1X selex buffer, vortex for 1-2 seconds, put it over the magnetic separator and remove the buffer.
6. Repeat the washing step 2 more times.
7. Add 190microlitres of selex buffer and repeat the washing - one time only.
8. Remove the buffer from the beads and add the DNA library.
   (58microlitres DNA + 10 microlitres 10x PBS +10 microlitres MgCl2 + 22 microlitres water)
9. Vortex the beads for 1-2 seconds and incubate them for 1 hour
10. vortex occasionally in every 10-15 min

Positive selection:

1. Take 150microlitres of the beads in the 1.5ml Eppendorf and wash it with 190 microlitres of 1x PBS buffer as did for negative selection beads three times
2. Add 190 microlitres of 1x PBS buffer and incubate it for 10 minutes
3. Remove the buffer by removing the beads using a magnetic separator
4. Incubation with protein.
   (Protein mixture: 10 micrograms of protein + 50 microlitres of PBS + 10 microlitres water)
5. Vortex for 1-2 seconds and incubate at room temperature for 10 mins.
6. After 10 minutes, incubate at 4 degrees/ice for 45 minutes
7. Gently vortex the tube every 10 to 15 mins.
8. After 45 mins of incubation, the beads were removed using a magnetic separator, and the buffer was removed.
   (proteins immobilised)
9. Remove the supernatant, add 190 microlitres of the 1x PBS buffer vortex for 1-2 sec, remove the supernatant, and repeat it twice.

Incubation of immobilised protein-coated beads with DNA:

1. Now transfer the DNA supernatant from negative selection beads to the protein-immobilised beads.
2. Incubate the DNA library with the beads for 4 hours at room temperature and occasionally vortex for 1-2 seconds.
3. After incubation, remove the supernatant and store it separately.

4. We have the beads with protein and DNA with them. -these are the selex beads.

RECIPE FOR 25 MICROLITRES OF PCR REACTION:

10x buffer	2.5 microlitre
2.5miliomolar dNTPs	1.5 microlitre
10 micromolar Forward primer	1.25 microlitre
10 micromolar Reverse primer	1.25 microlitre
Taq polymerase	0.25 microlitre
DNA Library	1.12 microlitre
Total	25 microlitre

Details of PCR machine :

STEP	TEMP at degree Celcius	TIME
Initial denaturation	95 degrees	30 seconds
7 cycles	Denaturation 95 degrees Annealing 61 degrees extension 68 degrees	15 seconds 15 seconds 5 seconds
Final extension	68 degrees	5 minutes