

# Fusion BioLabs DNA Aptamer Library Kit (Magnetic beads based -SELEX module) Manual

SKU# DAL-01: DNA Aptamer Library Kit

## Product Overview

Fusion BioLabs offers a validated **DNA Aptamer Library Kit** for your own DNA aptamer development. This kit has enough ssDNA library oligos and primers for your primary library construction for 1st round SELEX, and secondary library construction for the iterative SELEX of 2-4 independent DNA aptamer development projects depends on your SELEX strategies. With customizable features and robust performance, our DNA Aptamer Library Kit is designed for omitting your library construction optimization and thus facilitating your DNA aptamer development.

## Key Features

- **High Efficiency:** Library and primer oligos designed for specific and efficient amplification of DNA library for [DNA aptamer SELEX](#) using either [Capture SELEX platform](#) or traditional magnetic bead-based SELEX platform.
- **Flexibility and versatility:** For single-stranded DNA (ssDNA) generation, the kit could be used either
  - 1) Upgrading SELEX technology by using lambda exonuclease digestion or
  - 2) Regular strand separation with streptavidin-coated magnetic beads and alkaline denaturation.

## Kit contents

The following components are included in the Kit.

Component	Quantity
Vial 1: ssDNA Library Oligos	30 $\mu$ l, 100 $\mu$ M
Vial 2: Forward Primer	30 $\mu$ l, 100 $\mu$ M
Vial 3: 5'-FAM Labeled Forward Primer	30 $\mu$ l, 100 $\mu$ M
Vial 4: Reverse Primer	30 $\mu$ l, 100 $\mu$ M
Vial 5: 5'-Phosphorylated Reverse Primer	30 $\mu$ l, 100 $\mu$ M
Vial 6: 5'-biotinylated reverse primer	30 $\mu$ l, 100 $\mu$ M

- Store at -20°; reagents are guaranteed stable for 12 months when properly stored.

## ssDNA library pool Structure

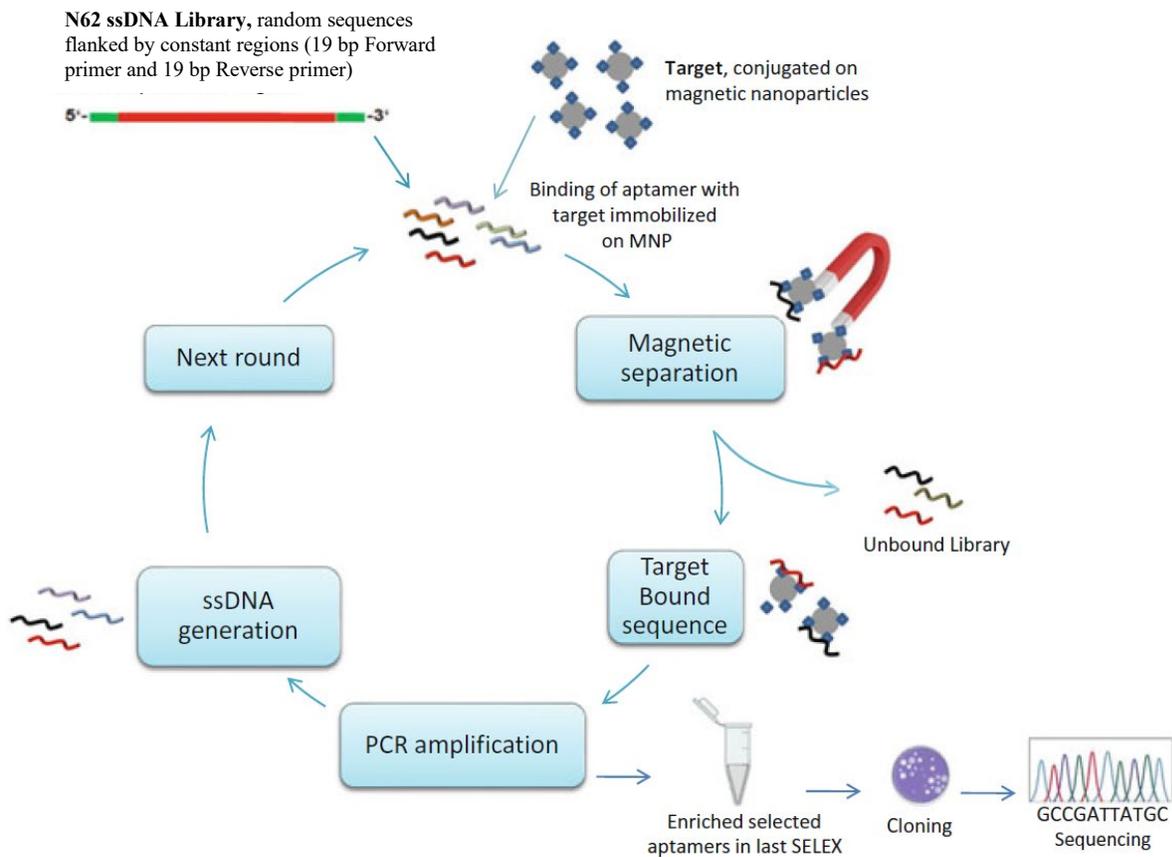
**F**<sub>19</sub> (19 bp Forward primer) + **N**<sub>62</sub> (62 bp random sequence) + **R**<sub>19</sub> (19 bp Reverse primer)



## Kit Usage

- Primary library construction for 1st round SELEX.
- Secondary library construction for the iterative SELEX.

Flowchart of magnetic beads based-SELEX technology to find the candidate DNA aptamer.



## Protocols

In this protocol, the magnetic beads based-SELEX technology was used to select the aptamer bound to your target *in vitro*. The entire selection process includes 10-16 rounds, which contains steps listed as follows.

### 1. Target protein conjugated on magnetic beads

- Weight out 3.0 mg conjugate magnetic nanoparticles (MNP) beads (for protein and peptide targets, we suggest using Dynabeads™ M-270 Epoxy from Thermo Fisher) and suspend and wash the beads three times with 1ml 0.1 M sodium phosphate buffer, pH 7.4 (0.019 M NaH<sub>2</sub>PO<sub>4</sub>, 0.081 M Na<sub>2</sub>HPO<sub>4</sub>). Make a homogeneous suspension of the washed beads in the same buffer.
- Add the calculated amount of target protein/peptide solution to the bead suspension. Mix the suspension well before adding the calculated ammonium sulfate stock solution (3 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>).



- Incubate for 16-24 h at 37°C with slow tilt rotation.
  - Place the tube on the magnet for 2 min for magnetic separation. Carefully turn the magnet (with the tube in place) upside-down twice, to ensure collection of any beads that might remain in the cap. Remove the supernatant.
  - Wash the coated beads four times with Phosphate buffered saline (PBS), pH 7.4 with 0.1% BSA or 0.5% Tween-20.
  - Resuspend the coated beads to the desired concentration (e.g. 6.0 µg/µl for 500 µl) in PBS with 0.1% BSA.
- \*Uncoated MNP beads for counter-selection were produced in a similar manner without a target.

## 2. SELEX for the first round

- Dilute up to 30 µl (first round 1~3 nmol, the continual rounds with 90-100 µl enriched) of the ssDNA library (Vial 1) to a new DNase/RNase-free 2.0 ml centrifuge tube in final 450 µl PBS-T (PBS with 0.5% Tween-20).
- Denature at 95°C for 5 minutes and then left on ice to cool for 15 minutes.
- Add 50 µl of target-coated MNP beads to the ssDNA tube and 50 µl 1 mg/ml BSA (molecular grade), and vortex gently.
- Incubate the mixture at room temperature with an EOE (ender-over-ender) mixer for 30-60 minutes.
- Separate MNP beads with magnetic rack.
- Wash the MNP beads 10—15 times with 1 ml PBS-T.
- Resuspend the washed MNP beads in 50 µl PCR-grades water.

## 3. Enriched pool amplification and purification

- PCR setup and PCR protocol

Component	Amount	PCR Protocol	
2 x PCR Master Mix	200 µl	Initial denaturation 94°C for 2 min	
Enriched ssDNA pool	50 µl	15-20 PCR cycles	Denature 94°C for 30 sec
Forward Primer (10 µM)*	8 µl		Anneal 51°C for 30 sec
Reverse primer (10 µM)*	8 µl		Extend 72°C for 90 sec
Water, nuclease-free	134 µl	Final Extension 72°C for 10 min	
*The primer combination: 1) If preparing ssDNA enrichment library via streptavidin-coated magnetic beads, using Vial 2 forward primer and Vial 6 reverse primer; 2) If preparing ssDNA enrichment library via lambda exonuclease digestion, using Vial 2 forward primer and Vial 5 reverse primer; 2) If monitoring the SELEX enrichment progress using real-time PCR or fluorescent ELISA, the forward primer should be Vial 3.		Hold 4°C, indefinitely	
		Note: Recommended for our PCR condition. Optimization maybe needed.	

- Using your favorite methods to purify the enriched dsDNA pool. An ethanol precipitation method is followed. To perform ethanol precipitation for single-stranded DNA (ssDNA), add 1/10th volume of 3 M sodium acetate (pH 5.2) and 2 to 2.5 volumes of ice-cold absolute ethanol to the DNA sample, mix, and incubate at -20°C for at least one hour or overnight. Centrifuge the sample to pellet the DNA, and then wash the pellet with 70-80% ethanol to remove salts, and dry the pellet before re-dissolving it.

## 4. Preparation of ssDNA enriched library for the next round SELEX

1. Take 200 µl for first round and 100 µl for the continual rounds of Dynabeads M-280 streptavidin-coated magnetic beads suspension.



2. Wash three times with a 1× B & W buffer (5 mM Tris-HCl, 0.5 mM EDTA, 1 M NaCl, 20 mM KCl, 5 mM MgCl<sub>2</sub>, 0.1% BSA, 0.05% Tween-20. Adjust pH to 7.5.).
3. Incubate the biotinylated PCR products with pre-washed beads in an EOE mixer for 30 min at room temperature.
4. Add 50 µl of a freshly prepared 150 mM NaOH solution and incubate at room temperature for 5 min.
5. Neutralize the eluted ssDNA with 50 µl HCl (150 mM).
6. Dilute up to 100 µl of the above enriched ssDNA library in final 450 µl PBS-T.
7. Denature at 95°C for 5 minutes and then left on ice to cool for 15 minutes.
8. Add 50 µl of uncoated MNP beads to the ssDNA tube and 50 µl 1 mg/ml BSA (molecular grade), and vortex gently.
9. Incubate the mixture at room temperature with an EOE (ender-over-ender) mixer for 30-60 minutes.
10. Separate MNP beads with magnetic rack.
11. Collect the supernatant for the next round of positive SELEX following the procedure listed on **Step 3 of 2. SELEX for the first round.**

## 5. Monitoring the enrichment progress of SELEX

- Generally 12-16 SELEX rounds is enough to get high specificity and affinity DNA aptamer candidates.
- Use 4 µl of the fraction you collected in counter-elution and target-binding elution.
- Measure the amount of FAM labelled ssDNA pool in each round sample according to the **Supplementary protocol 1**.
- Calculate the ratio of the amount of ssDNA pool eluted in the target binding step compared to the background elution step.
- The expected results: ratio should be increased from ~1 (round 1) up to 5-20. If reached the platform. SELEX process could be terminated.

## 6. Cloning and sequencing the candidate DNA aptamers

- After the SELEX was completed, do the final PCR amplification according to the Step of PCR setup and PCR protocol, but using the unmodified forward primer and reverse primer pair from Vial 2 and Vial 4.
- Purify the PCR amplification products (final ssDNA pool) using your favorite methods.
- For Sanger sequencing: cloning purified PCR fragments into your TA cloning compatible sequencing vector. We generally pick up 96 clones to identify the unique DNA aptamer candidates.
- For high-throughput sequencing (HTS) or next-generation sequencing (NGS): send your purified PCR fragments to your service provider. We recommend using Illumina Miseq platform.

## 7. Affinity characteristics of aptamer candidates

Aptamer candidates with high enrichment, low free energy level and the large difference in the secondary structure will be selected for binding assay. All experiments should be carried out under dark conditions.

- Synthesize the aptamer candidates with 5'-end labelled with FAM.
- Add individual aptamer candidate with varying concentration (e.g. 10-500 nM) to a specific amount of target-coated MNP beads or uncoated MNP beads according to the strategy of the SELEX procedure.
- Incubate the mixture at room temperature for 30 min under EOE mild rotation.
- Separate beads with magnetic rack and collect the supernatant to measure the amount of 5'-FAM-labelled candidate individually.
- The nonlinear fitting formula will be employed to determine the  $K_D$  of an aptamer candidate to its target. We recommend using GraphPad Prism Software to simplify the calculation.



## Supplement protocol 1 how to measure the amount of FAM labelled single stranded oligos

You can measure the concentration of FAM-labeled single-strand oligonucleotides (ssDNA) using a combination of UV absorbance and fluorescence spectroscopy. Because both the nucleic acid and the FAM dye absorb light, it is important to correct for the contribution of the dye to get an accurate measurement of the oligo concentration.

### Method 1: Using a NanoDrop with the MicroArray module

For fluorescently labeled oligos, the MicroArray module in NanoDrop software can automatically correct for the dye's absorbance.

1. **Select the "MicroArray" module** and the "Oligo" option for your sample type.
2. **Measure the absorbance** of your sample. The instrument will measure both the absorbance of the FAM dye (at its maximum wavelength,  $\lambda_{max}$ , around 494 nm) and the absorbance of the nucleic acid (at 260 nm).
3. **The software will correct the A260 reading** using a correction factor and an oligo-specific extinction coefficient, providing a more accurate concentration value for your labeled oligo.

### Method 2: Calculating concentration manually

If you do not have software with an automatic correction feature, you can calculate the concentration manually using the Beer-Lambert Law, factoring in the dye's contribution to the A260 reading. This requires knowing the extinction coefficients for both the FAM dye and the unlabeled oligo.

#### Steps for manual calculation

1. **Measure absorbance:** Use a spectrophotometer to measure the absorbance of your sample at two wavelengths:
  - $A_{260}$ : To measure the concentration of the oligo bases.
  - $A_{494}$ : To measure the concentration of the FAM dye.
2. **Look up or calculate extinction coefficients:**
  - $\epsilon^{oligo/260}$ : The extinction coefficient of your specific, unlabeled oligo at 260 nm. This can be calculated using online tools like IDT's OligoAnalyzer.
  - $\epsilon^{FAM/260}$ : The extinction coefficient of FAM at 260 nm (approx.  $9,000 \text{ M}^{-1}\text{cm}^{-1}$ ).
  - $\epsilon^{FAM/494}$ : The extinction coefficient of FAM at its maximum absorption wavelength (494 nm, approx.  $75,000 \text{ M}^{-1}\text{cm}^{-1}$ ).
3. **Calculate dye concentration:** Using the  $A_{494}$  reading, calculate the molar concentration of the FAM dye:



- $C_{FAM} = A_{494} / \epsilon^{FAM/494}$

4. **Calculate oligo concentration:** Use the FAM concentration to subtract the dye's contribution from the total  $A_{260}$  reading, then solve for the oligo concentration:

- $A^{total/260} = A^{oligo/260} + A^{FAM/260}$

- $A^{oligo/260} = A^{total/260} - (C_{FAM} \cdot \epsilon^{FAM/260})$

- $C_{oligo} = A^{oligo/260} / \epsilon^{oligo/260}$

### Method 3: Using a fluorometer

For greater sensitivity, especially with low-concentration samples, you can use a fluorometer with a standard curve.

1. **Prepare standards:** Create a series of dilutions of a stock FAM-labeled oligo with a known concentration.
2. **Generate a standard curve:** Measure the fluorescence of the standards at FAM's excitation (494 nm) and emission (520 nm) wavelengths.
3. **Measure your sample:** Measure the fluorescence of your unknown sample.
4. **Determine concentration:** Compare the sample's fluorescence to the standard curve to determine its concentration.

Tips for accurate measurement

- **Remove excess dye:** If your oligo was labeled in-house, ensure you have removed any free dye that may remain from the labeling reaction. Free dye can significantly alter your readings.
- **Use calibrated equipment:** Regardless of the method, ensure that all equipment especially pipettes, are properly calibrated to avoid measurement errors.
- **Mix thoroughly:** Before taking any measurement, ensure that your sample is completely mixed and homogeneous.
- **Run controls:** For fluorescence methods, include blank controls to account for any background fluorescence from the buffer or plate.