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eGene™ Prep Kit Quick Start

1. **Template DNA:**
   ▹ Resuspend the lyophilized DNA fragment to 10 ng/µL with 0.1 TE buffer
   1.1 Convert DNA concentration to molarity using the following formula:

$$GOI\ (nM) = \frac{Concentration\ (ng\ /\ \mu L)}{[\ Length\ (bp)\ x\ 617.96\ g\ /\ mol\ /\ bp\ ] + 36.04\ g\ /\ mol} \times 1{,}000{,}000$$

   1.2 Prepare fresh 10 µL of the GOI template normalized to 2 nM using the following formula:

$$Volume\ GOI\ (\mu L) = \frac{2\ (Target\ Molar\ Concentration\ in\ nM)\ X\ 10\ (nuclease\text{-}free\ water\ [NFW]\ in\ \mu L)}{Obtained\ Molar\ Concentration\ (nM) - 2\ (Target\ Molar\ Concentration\ in\ nM)}$$

2. **Control Template DNA at 2 nM:**
   ▹ Add 1 µL of 10x Control Template to 9 µL of nuclease-free water

3. **PCR reaction mix + 1 negative control + 1 positive control:**

Reagent	Reaction volume
Nuclease free water (NFW)	19 µL
2X high fidelity PCR mastermix	30 µL
GOI at 2 nM, or water (negative control), or Control Template (positive control)	1 µL
Primer Mix	10 µL
Final volume (maximum recommended volume per reaction)	**60 µL**

4. **PCR assembly - thermocycler parameters**

PCR step	Temperature	Time	Number of cycles
Pre-incubation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	27
Annealing	60°C	20 seconds	
Elongation	72°C *	30 seconds per kb **	
Final elongation	72°C *	2 minutes	1

*** Elongation temperature: to be based on high fidelity PCR mastermix manufacturer's recommendation.**
**** Elongation time: to be based on high fidelity PCR mastermix manufacturer's recommendation but should not be less than 30 seconds per kb of the final eGene construct.**

5. **Evaluation of the amplified DNA by agarose gel electrophoresis**
   ▹ Prepare a 1% (w/v) agarose gel with a DNA gel stain in 1x TAE or 1x TBE buffer.
   ▹ Using new wells (or PCR tubes), prepare 12 µL samples for loading on the gel.

6x gel loading dye	Nuclease free water	eGene DNA construct
2 µL	8 µL	2µL

▹ Load 10 µL of PCR products + control on the gel along with an appropriate ladder.

6. PCR purification

▹ Clean the PCR reactions using the DNA purification method of your choice.

Important note: The samples must be eluted using the supplied eGene Elution Buffer (50 µL) to make them compatible with the eProtein Discovery cartridge.

7. DNA quantification using fluorescent method

▹ Measure the DNA concentration (ng/µL). Blanks must be done using the eGene Elution Buffer provided in the kit.

▹ Determine the length of the eGene using the formula and table below:
eGene Length (*bp*) = *GOI Length* (*bp*) + *Primer Mix Length* (*bp*)

Primer Mix	Total length of the flanks (bp)
P17-Strep	1134
CUSF-Strep	1302
FH8-Strep	1239
TRX-Strep	1359
ZZ-Strep	1383
SUMO-Strep	1341
SNUT-Strep	1476
-Strep only	990

▹ Calculate the molar concentration (nM) of the eGene constructs using the formula in point 1.1 of the protocol.

Note: If the DNA concentration is lower than 5 nM, please refer to the reamplification protocol in the troubleshooting section of the user manual.

8. eGene constructs normalization to 5nM

Determine the volume of eGene constructs to add to 60 µL of eGene Elution Buffer using the following formula:

$$\textit{Volume eGene (µL)} = \frac{\textit{5 (Target Molar Concentration in nM) X 60 (eGene Elution Buffer in µL)}}{\textit{Obtained Molar Concentration (nM) – 5 (Target Molar Concentration in nM)}}$$

9. Final gel QC

It is recommended to run 2 µL of the samples on a 1% agarose gel post PCR clean-up.

10. Storage of the eGene constructs

Purified and normalized eGene constructs can be stored at -20°C short term (days) or -80°C for long term.

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