

T4 ligation protocol for oligonucleotide containing compounds and analysis by MACS® Matchmaker

Scope

This protocol allows simple and fast ligation of oligonucleotide containing compounds (as used in DNA-encoded libraries) to the complementary strands oligonucleotide (5'Phosphor adapter) used for focal molography. After the ligation, no purification is needed and direct immobilization of the ligated compounds on the surface of a MACS® Matchmaker sensor chips is possible.

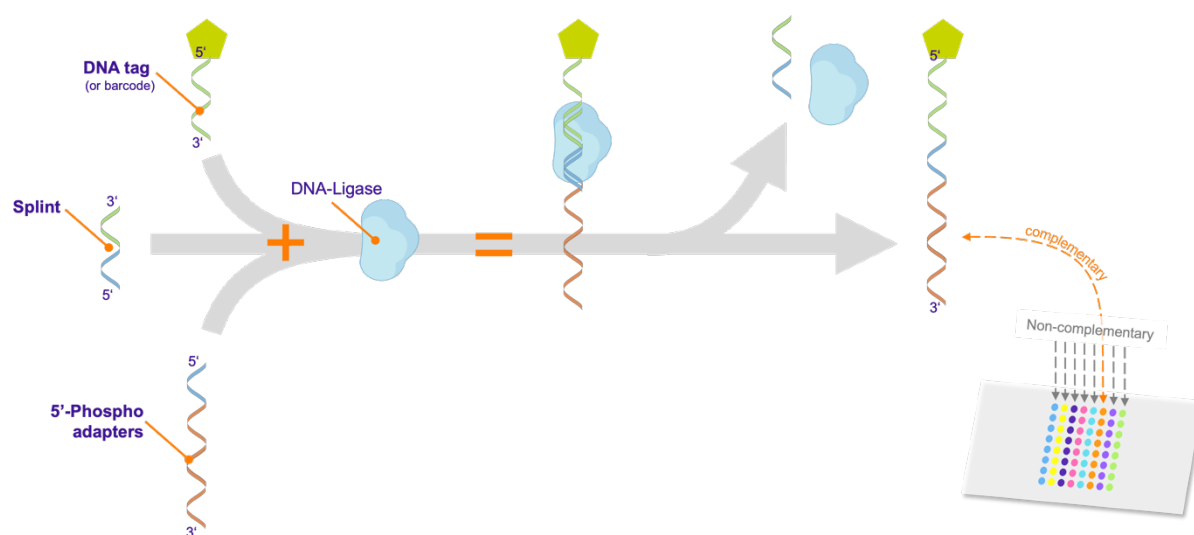


Figure 1: Overview of the DNA ligation process described in this protocol. The final ligated compound is complementary to a specific mologram sequence predefined by the sensor chip anticipated for the experiment.

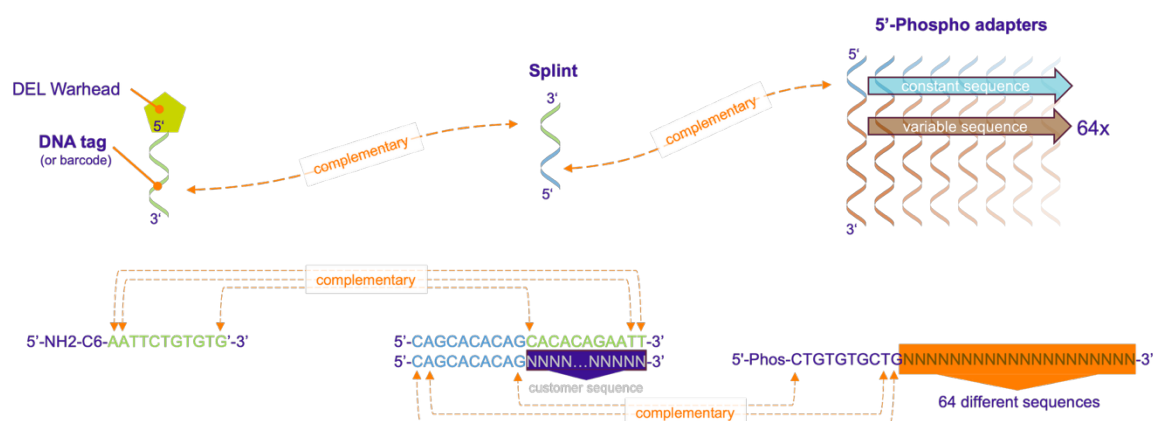


Figure 2: **Left:** The DNA tag or barcode is attached to the small molecule warhead from the DNA-encoded library hit. The DNA tag can be used for convenient immobilization on the ready to use multiplexed oligonucleotide chip for the MACS® Matchmaker. **Middle:** The splint is a single stranded oligonucleotide capable of bridging parts of the DNA tagged warhead and the complementary oligonucleotide. Since the customer is in possession of his DNA tag sequence information and the constant sequence of the complementary oligonucleotides he can purchase the splint at any suitable vendor of his choice. **Right:** Different single stranded 5'-phospho adapters which are complementary to the commercial sensor chips of lino Biotech are available for DNA directed immobilization. The upper sequence is constant and used as an overlap for the splint ligation. The lower sequence has 64 varying sequences in respect to the available prefucionalized complementary sensor chips.

Material

1. **T4 ligase 30 U/μL (5000 U)** (<https://www.thermofisher.com/order/catalog/product/EL0011>)
 2. **T4 DNA Ligase Buffer 10x** (<https://www.thermofisher.com/order/catalog/product/EL0011>)
 3. **50% PEG Solution** (<https://www.thermofisher.com/order/catalog/product/EL0011>)
 4. **Nuclease free water (NFW)**
 5. **5' Phospho-oligonucleotide** - provided by lino Biotech
 6. **Splint oligonucleotide** – ordered by customer
 7. **DNA tagged compound** – provided by customer
- Thermal incubator, centrifuge

Ligation Protocol

The reaction is by default done in a **final volume of 80 μL**. This can be adjustable; however, all reagents should be scaled accordingly. Importantly, the molar ratio between **5'-Phospho adapter : Splint : DNA tagged compound** needs to be **1 : 1.5 : 2**.

Step 1

- 1 nmol of 5' Phospho oligonucleotide
- 1.5 nmol of Splint oligonucleotide
- 2 nmol of DNA tagged compound

Mix all together, spin down and incubate at thermal incubator at 70 °C for 5 min, no agitation. This denatures all secondary structures of DNA molecules and allow better T4 ligation. Leave for 30 min at RT to cool down.

Step 2

- 8 μL of T4 DNA Ligase Buffer 10x (final concentration 1x)
- 8 μL of 50% PEG Solution
- 1 μL T4 ligase
- Nuclease free water (NFW) up to 80 μL

Mix all together, spin down and incubate at thermal incubator at 37 °C for 2 hours, no agitation. Alternatively, the sample can be incubated over night at 22 °C.

Step 3

Inactivation of T4 ligase by 70 °C for 5 min at thermal incubator, no agitation.

The reaction mixture can be directly injected on the sensor chip without further purification. If you are using multiplex chips, you can also combine multiple ligation mixtures, e.g. with phospho-oligo 1, 2, 3, and 4. Like this, only one immobilization step is needed, the DEL-compounds will be immobilized only to complementary molograms.

In general, the ligation yield is estimated to be around 80 %. The presence of free 5'-phospho oligonucleotide is estimated to be around 10 %. Even though this oligonucleotide competes with the ligated oligonucleotide tagged substance during the immobilization, it is acceptable in such a low concentration.

Example of T4 ligation calculations: Your oligonucleotides are at stock concentration of 100 μM. Then 1 nmol corresponds to 10 μL, 1.5 nmol to 15 μL and 2 nmol to 20 μL. This would sum up to 45 μL. Accounting for 8 μL T4 ligation buffer, 8 μL PEG solution and 1 μL T4 ligase we have total volume of 62 μL. We need to add 18 μL nuclease free water to complete reaction mixture.

DEL hit validations by MACS® Matchmaker

Material

- Analyte (defined by customer)
- Running buffer – usually PBS-T pH 7.4 (PBS with 0.05 % Tween 20)
- Regeneration solution – usually 3M Guanidine with 125 mM NaOH
- T4 ligation mixture $\approx$ 200 nM – usually immobilization – 10 min @ 10 μ L/min

Ligation yield is 80%, thus we can roughly estimate the concentration of ligated DNA tagged ligand. For example, if we initially used 1 nmol of 5'-phospho adapter for the reaction (10 μ L at 100 μ M) in the reaction volume of 80 μ L, this means that the concentration of 5'-phospho adapter is 12.5 μ M. Therefore, 80 % is 10 μ M. This can be considered as stock concentration of the DNA tagged ligand. We just need to calculate how much of stock we need to prepare 200 nM solution of T4 ligation mixture.

Experimental design (example):

1. **Baseline**
 - 1.1. running buffer (200 μ L/min for 5 min),
 - 1.2. 3M Gua 125 mM NaOH (400 μ L/min for 0.5 min)
 - 1.3. running buffer (200 μ L/min for 5 min)
2. **Immobilization of T4 mixture**
 - 2.1. 200 nM - 6 μ L/min for 10 min
 - 2.2. running buffer (200 μ L/min for 5 min)
3. **Analyte screening/binding –**
 - 3.1. at least 5 concentrations for singly cycle kinetics experiments (SCK)
 - 3.2. association 30 μ L/min for 2 min
 - 3.3. dissociation running buffer (200 μ L/min for 2 min)
 - 3.4. final dissociation depends on the affinity
4. **Regeneration**
 - 4.1. 3M Guanidin + 125 mM NaOH (400 μ L/min for 0.5 min)
 - 4.2. running buffer (200 μ L/min for 5 min)
 - 4.3. 3M Gua 125 mM NaOH (400 μ L/min for 0.5 min)
 - 4.4. running buffer (200 μ L/min for 5 min)



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