How to order Fusion Primers

Amplicon sequencing primers for 454 technologies

Just follow the instructions below for successful amplicon sequencing!

Layout of fusion primers

- Each amplicon requires 2 fusion primers on A-side and B-side for sequencing (see image below).
- One of the primers should contain the A-side sequence and the other one the B-side sequence (**see bases in blue**).
- One of the fusion primer must contain a MID (multiplex identifier) sequence. Adding a MID on both ends maintains flexibility for bidirectional sequencing (see **green** and **red** brackets).
- Each fusion primer must have some specific 20 – 25 bases which are complementary to the ends of the amplicon (see **black** end of the primer).

1x A-side primer (A)

5'-CGTATCGCCTCCCTCGCGCCATCAG-(MID)-forward-specific-sequence-3'

1x B-side primer (B)

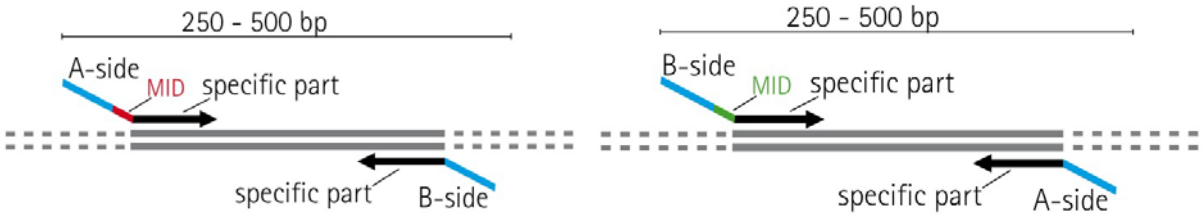
5'-CTATGCGCCTTGCCAGCCCGCTCAG-(MID)-reverse-specific-sequence-3'

Uni- and bidirectional sequencing of amplicons

Unidirectional sequencing

- Sequencing is either performed on the forward (upper) or reverse (lower) strand of the amplicon using an A-side **or** B-side primer. Below is an example of a **forward strand** sequencing using the **B-side primer** (see Figure 1 right)
  - The B-side primer is designed containing the B-side sequence (blue), a MID and a specific sequence that is complementary to the **forward** strand
  - The A-side primer must contain the A-side (blue) and a sequence complementary to the **reverse** strand.
  - Including a MID sequence in both the A-side and B-side primers (see fig. 2) allows greater flexibility for future projects in case the same PCR pool needs to be re-sequenced.
- All strands of the amplicons in one PCR pool will only be sequenced using the A- **or** the B-side primers.

Figure 1 - MID only on one side of the amplicon (sufficient for unidirectional sequencing)



Bidirectional sequencing

- Sequencing is performed in parallel on both the forward (upper) and reverse (lower) strands of the amplicon using both the A-side **and** B-side primers (see fig. 2).
- Primers must contain a MID to identify the strands and facilitate sequencing from both ends of the amplicon.
- Both primers of an amplicon can contain the **same MID** to differentiate between different amplicons in single PCR pool.
- The primers of an amplicon must contain **different MIDs** to differentiate between the forward and reverse strand in a single PCR pool.

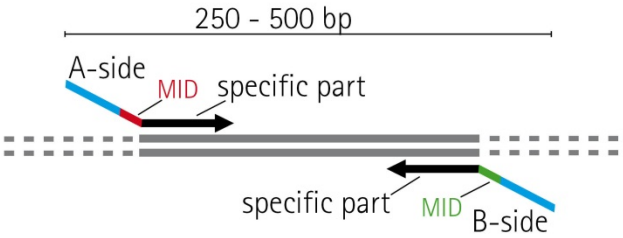
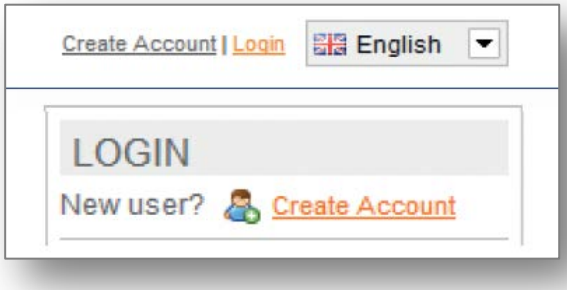


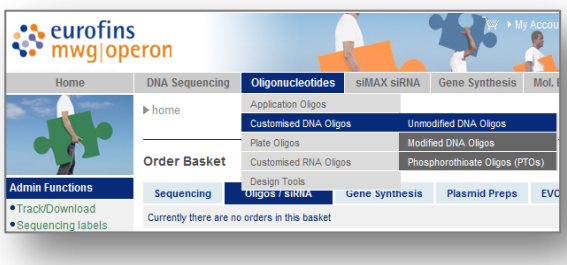
Figure 2 - MID on both sides of the amplicon (mandatory for bidirectional sequencing)
Upper strand = Forward sequence, lower strand = reverse sequence.

How to order Fusion Primers in ECOM



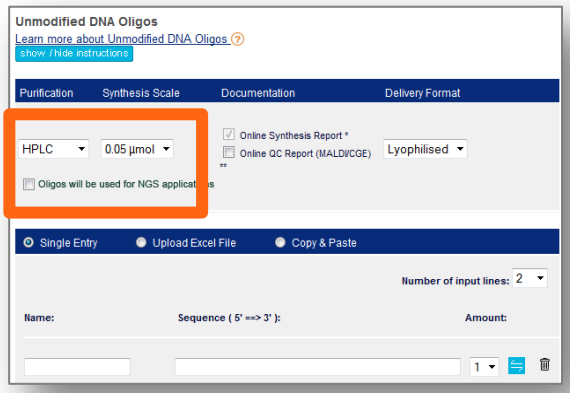
Login to Ecom

Login to your secure online account at **eurofinsgenomics.com**. If you don't have an account, simply create a new account by clicking **Create Account**.



Access the order page

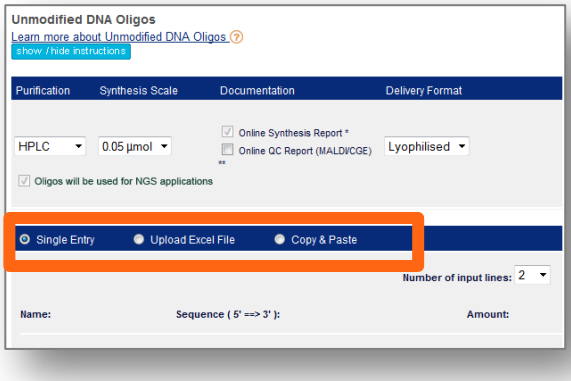
Select **Unmodified DNA Oligos** from the Customised DNA Oligos menu under the main Oligonucleotides drop-down menu.



Select purification & synthesis scale

Select the purification **HPLC** and the synthesis scale **0.05 µmol** in the drop-down menus.

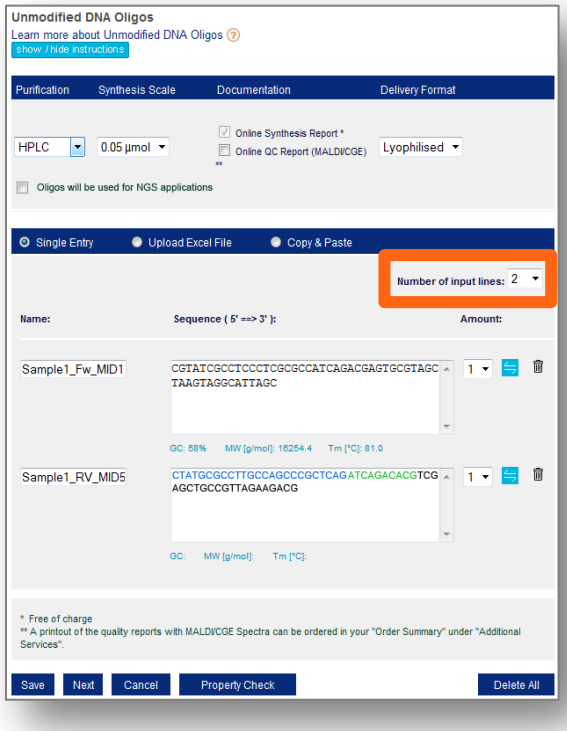
Click on checkbox "**Oligos will be used for NGS applications"** that appears after selecting the HPLC as purification method



Select data entry style

Select "**Single Entry**" or "**Copy & Paste**" if your PCR pool only contains a few number of amplicons.

Select "**Upload Excel File**" for PCR pools with numerous amplicons. Fusion Primer sequences are uploaded in Ecom from your excel file.



Enter Fusion Primer sequence using "Single Entry" style

Select the number of input lines depending on the number of amplicons in your PCR pool.

Enter the name of the sequence and the complete sequence consisting of the A- or B-key sequence (2nd input field in blue), the MID sequence (green) and the specific sequence (black).

Sequences will not be coloured in Ecom (see input line 1).

A list of 48 individual MID sequences for successful primer design and primer ordering is available at the end of this document

List of 48 individual MID sequences for bar-coded Fusion Primer:

MID-01 ACGAGTGCGT	MID-19 TGTACTACTC	MID-35 CAGTAGACGT
MID-02 ACGCTCGACA	MID-20 ACGACTACAG	MID-36 CGACGTGACT
MID-03 AGACGCACTC	MID-21 CGTAGACTAG	MID-37 TACACACACT
MID-04 AGCACTGTAG	MID-22 TACGAGTATG	MID-38 TACACGTGAT
MID-05 ATCAGACACG	MID-23 TACTCTCGTG	MID-39 TACAGATCGT
MID-06 ATATCGCGAG	MID-24 TAGAGACGAG	MID-40 TACGCTGTCT
MID-07 CGTGTCTCTA	MID-25 TCGTCGCTCG	MID-41 TAGTGTAGAT
MID-08 CTCGCGTGTC	MID-26 ACATACGCGT	MID-42 TCGATCACGT
MID-10 TCTCTATGCG	MID-27 ACGCGAGTAT	MID-43 TCGCACTAGT
MID-11 TGATACGTCT	MID-28 ACTACTATGT	MID-44 TCTAGCGACT
MID-13 CATAGTAGTG	MID-29 ACTGTACAGT	MID-45 TCTATACTAT
MID-14 CGAGAGATAC	MID-30 AGACTATACT	MID-46 TGACGTATGT
MID-15 ATACGACGTA	MID-31 AGCGTCGTCT	MID-47 TGTGAGTAGT
MID-16 TCACGTACTA	MID-32 AGTACGCTAT	MID-48 ACAGTATATA
MID-17 CGTCTAGTAC	MID-33 ATAGAGTACT	MID-49 ACGCGATCGA
MID-18 TCTACGTAGC	MID-34 CACGCTACGT	MID-50 ACTAGCAGTA

(Sequence of 10 bases that have a sequence-error optimized design)

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