RAPOligoDesigner Quick Guide Manual

RAPOligoDesigner designs tiling oligos to pull down a set of transcripts by direct hybridization. All oligos include a primer pair on the ends for amplification. Run the program at the command line like this:

java -jar <JVM arguments (optional)> RAPOligoDesigner.jar <program arguments>

Basic parameters

-tfa <String> Fasta file of target transcripts (required)
Transcript sequences to design probes against, in fasta format.

-pl <int> Probe length (required)
Length of probes not including primers. Full length of oligo is probe length + 2 * primer length.

-ps <int> Probe step size (required)
Distance between probe starts on transcript. For example, 120bp probes tiled end to end would have a step size of 120. 120bp probes tiled evenly at 8x coverage would have a step size of 15.

-prl <int> Primer length (required)
Length of each primer.

-tm <double> Optimal primer Tm (required)
Optimal Tm for PCR amplification of oligos using the primer pair. Note that if the Tm is incompatible with the primer length, the program will fail.

-p3 <String> Full path to primer3core executable (required)
File system path to primer3_core executable provided along with this program. This executable is needed to design primer pairs.

-o <String> Output file prefix (required)
Prefix for output files.

Low complexity filter for probes

This optional filter removes probes with too much low complexity sequence.

-aplc <boolean> Apply probe low complexity filter: remove probes with low complexity sequence (required)
“true” if including filter, “false” otherwise.

Poly base filter for probes

This filter removes probes with repeated single nucleotides.

-appb <boolean> Apply probe poly base filter: remove probes with strings of single nucleotides (required)

“true” if including filter, “false” otherwise.

-ppbb <String> Bases for probe poly base filter, e.g. ACGT (default=ACGT)
Bases to include with filter, e.g. “ACGT”, “CG”, “AGT”

-ppbl <int> For probe poly base filter, length of windows to check for too many single nucleotides (default=15)
Size of windows to check. For example, default is to remove probes with a 15bp window containing 12 of the same nucleotide.

-ppbc <int> For probe poly base filter, max number of repeats of single base within window (default=12)
Max number of occurrences of single nucleotide in window. For example, default is to remove probes with a 15bp window containing 12 of the same nucleotide.

Poly base filter for primers

This filter removes primers with repeated single nucleotides.

-aprpb <boolean> Apply primer poly base filter: remove primers with strings of single nucleotides (required)
“true” if including filter, “false” otherwise.

-prpbb <String> Bases for primer poly base filter, e.g. ACGT (default=ACGT)
Bases to include with filter, e.g. “ACGT”, “CG”, “AGT”

-prpbl <int> For primer poly base filter, length of windows to check for too many single nucleotides (default=15)
Size of windows to check. For example, default is to remove probes with a 15bp window containing 12 of the same nucleotide.

-prpbc <int> For primer poly base filter, max number of repeats of single base within window (default=12)
Max number of occurrences of single nucleotide in window. For example, default is to remove probes with a 15bp window containing 12 of the same nucleotide.

Repeat filter for probes

This filter removes probes with too many repeat masked bases. Only works if target sequences are repeat masked with either lower case or Ns.

-apr <boolean> Apply probe repeat filter: remove probes with repeat masked bases (required)
“true” if including filter, “false” otherwise.

-prmp <double> For probe repeat filter, max percentage of repeat masked bases (default=0.07)
Max acceptable percentage of probe that is repeat masked.

-prlo <boolean> For probe repeat filter, lower case bases count as repeats (default=false)
"true" if repeat masked bases are indicated by lower case letters.

-prn <boolean> For probe repeat filter, Ns count as repeats (default=false)
"true" if repeat masked bases are indicated by Ns.

Output

The program writes several output files:

- **<prefix>_full_design.out** Comprehensive table with one line per oligo and all information about the oligo
- **<prefix>_filter_results.out** Count of probes removed by each filter
- **<prefix>_primers.out** Primers to amplify oligos
- **<prefix>_oligos.fa** Oligo sequences in fasta format, one sequence per oligo
- **<prefix>_oligos.out** Simple list of oligo sequences, one line per oligo
- **<prefix>_probes.fa** Probe sequences (no primers) in fasta format

<prefix>_probes.out Simple list of probe sequences