A. Overall structure of Edesign core program

Control Edesign core
primer3_boulder_main

Read input parameters and files
read_boulder

Design primers and internal probes and
provide functions for other programs
libprimer3

Output in a user-friendly web format
format_output

Output in a computer-friendly format
print_boulder

Calculate $T_M$ with
complementary sequence
oligotm

Perform old-style alignments
dpal

Perform thermodynamic
alignments
thal

B. Workflow of primer and internal probe design in libprimer3

choose_primers()

1. Generate primer list
make_detection_primer_lists()

2. Generate internal probe list
make_internal_oligo_list()

3. Select the combinations
choose_pair_or_triple()

Evaluate the combinations

Filter primer pairs and calculate primer-pair complementarity
characterize_pair()

Filter internal probes and calculate primer-probe complementarity
choose_internal_oligo()

Evaluate single primer or probe

Calculate all oligonucleotide features for any direction of
single primer or probe
calcl_and_check_oligo_features

Calculate the penalty score for
each single primer or probe
p_obj_fn

S1 Fig. Schematic structure of Edesign core program.
(A) Overall structure of Edesign core program. (B) Workflow of primer and internal probe design run by
choose_primers() in libprimer3.