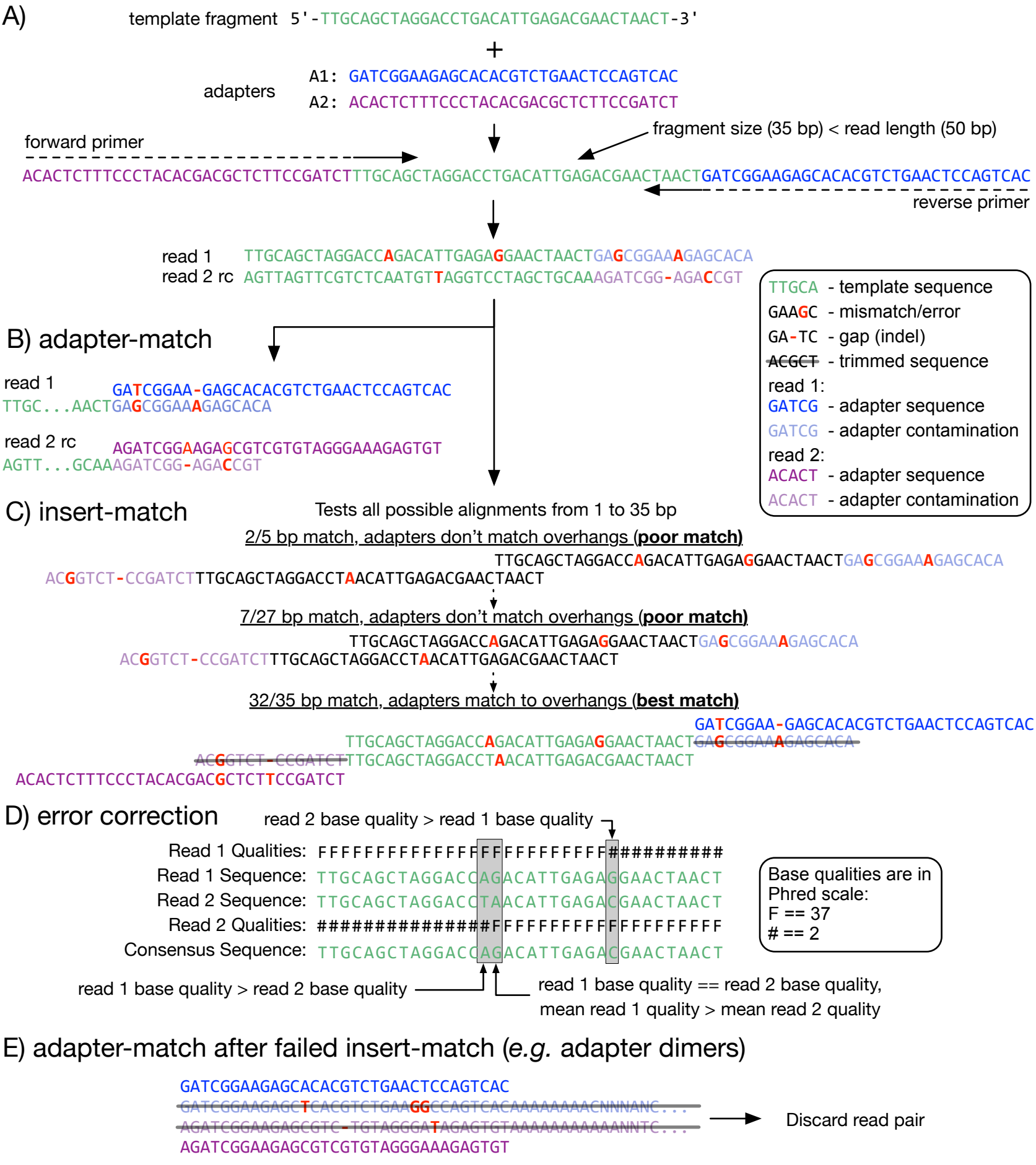
A)
template fragment 5'-TTGCAGCTAGGACCTGACATTGAGACGAACTAACT-3'
+
adapters
A1: GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
A2: ACACTCTTTCCCTACACGACGCTCTTCCGATCT
forward primer
fragment size (35 bp) < read length (50 bp)
ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGCAGCTAGGACCTGACATTGAGACGAACTAACTGATCGGAAGAGCACACGTCTGAACTCCAGTCAC
reverse primer
read 1 TTGCAGCTAGGACCAGACATTGAGAGGAACTAACTGAGCGGAAAGAGCACA
read 2 rc AGTTAGTTCGTCTCAATGTTAGGTCCTAGCTGCAAAGATCGG-AGACCGT
TTGCA - template sequence
GAAGC - mismatch/error
GA-TC - gap (indel)
ACGCT - trimmed sequence
read 1:
GATCG - adapter sequence
GATCG - adapter contamination
read 2:
ACACT - adapter sequence
ACACT - adapter contamination
B) adapter-match
read 1
GATCGGAA-GAGCACACGTCTGAACTCCAGTCAC
TTGC...AACTGAGCGGAAAGAGCACA
read 2 rc
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
AGTT...GCAAAGATCGG-AGACCGT
C) insert-match
Tests all possible alignments from 1 to 35 bp
2/5 bp match, adapters don't match overhangs (poor match)
TTGCAGCTAGGACCAGACATTGAGAGGAACTAACTGAGCGGAAAGAGCACA
ACGGTCT-CCGATCTTTGCAGCTAGGACCTAACATTGAGACGAACTAACT
7/27 bp match, adapters don't match overhangs (poor match)
TTGCAGCTAGGACCAGACATTGAGAGGAACTAACTGAGCGGAAAGAGCACA
ACGGTCT-CCGATCTTTGCAGCTAGGACCTAACATTGAGACGAACTAACT
32/35 bp match, adapters match to overhangs (best match)
GATCGGAA-GAGCACACGTCTGAACTCCAGTCAC
TTGCAGCTAGGACCAGACATTGAGAGGAACTAACTGAGCGGAAAGAGCACA
ACGGTCT-CCGATCTTTGCAGCTAGGACCTAACATTGAGACGAACTAACT
ACACTCTTTCCCTACACGACGCTCTTCCGATCT
D) error correction
read 2 base quality > read 1 base quality
Read 1 Qualities: FFFFFFFFFFFFFFFFFFFFFFFFF##########
Read 1 Sequence: TTGCAGCTAGGACCAGACATTGAGAGGAACTAACT
Read 2 Sequence: TTGCAGCTAGGACCTAACATTGAGACGAACTAACT
Read 2 Qualities: ##############FFFFFFFFFFFFFFFFFFF
Consensus Sequence: TTGCAGCTAGGACCAGACATTGAGACGAACTAACT
Base qualities are in Phred scale:
F == 37
# == 2
read 1 base quality > read 2 base quality
read 1 base quality == read 2 base quality, mean read 1 quality > mean read 2 quality
E) adapter-match after failed insert-match (e.g. adapter dimers)
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
GATCGGAAGAGCTCACGTCTGAAGGCCAGTCACAAAAAAAACNNNANC...
AGATCGGAAGAGCGTC-TGTAGGGATAGAGTGTAAAAAAAAAAAANNTC...
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
Discard read pair