454 - all flavors

If this page isn't formatted well on your screen, try shrinking the side bar on the left side.

**ONLY Rapid adaptor sequences are strongly recommended as of June 2011.** Using standard or Titanium adaptor sequences may halt your project while we accumulate enough samples with these adaptors to fill a picotiter plate region because they are incompatible with the Rapid library design. Standard and Titanium libraries require different emPCR kits, but can be mixed upon sequencing. Titanium and Rapid libraries use the same emPCR kit, but cannot be mixed upon sequencing.

**Canonical 454 library design as of Nov. 2011 (all 5’-3’):**

- `<emPCR primer site A>` `<sequencing primer site A>`
- `<4-base Key>`
- `<MID/Barcode>`
- `<template - gDNA, RNA, amplicon, whatever>`
- `<B-side bits>`

The required 4-base “key” sequences are immediately before the MID sequences (chosen to barcode samples).

**Library types - Rapid (current), Titanium (mid-2010), and Standard (mid-2009)**

- `<B-side bits>`

of Rapid Libraries are quite different from Standard or Titanium Libraries.

- Standard & Titanium library `<B-side bits>` have
  - `<emPCR primer site B>`
- Rapid library `<B-side bits>` are:
  - `<rev. comp. of MID/Barcode>`
  - `<rev. comp of 4-base Key>`
  - `<partial RC of sequencing primer site A>` `<emPCR primer site B>`

**Rapid adaptor sequences: Key sequence: GACT**

These are Y-adaptor designs, meaning that the 3’ end of the “A” oligo is hybridized with the 5’ end of the “B” oligo, and the resulting “Y” molecule ligates to both sides of the template. Note that this is NOT a blunt-end ligation but sticky-end with a single “A” base overhang on the 3’ end of the template molecules.

The "A" adaptor (with RLMID 22):

```
5' - 6FAM-C*C*A* T*CT CAT CCC TGC GTG TCT CCG ACG ACT CGA CGA G*T*A* C*T
```

The "B" adaptor (with RLMID 22):

```
5' Phos-G*T*A* C*TI GTC GIG TCG ICG TCT CTC AAG GCA CAC AGG GGA* T*A*G* G
```

The Y adaptor after annealing, A adaptor on top, PS markings removed for clarity, showing region of homology:
Note that the Rapid and Titanium A-side adaptors are identical up to their key sequences:

A-side (shown 5'-3'; sequencing reactions start from here)
CCA TCT CAT CCC TGC GTG TCT CCG AC - Titanium
CCA TCT CAT CCC TGC GTG TCT CCG AC - Rapid
The B-side (shown 3' to 5') diverges earlier:
GGA TAG GGG ACA CAC GGA ACC GTC AGA GT - Titanium (3'-5')
GGA TAG GGG ACA CAC GGA ACT CTC TGC IG - Rapid (3'-5')

Rapid adaptor MID (barcode) sequences and numbers (note that the MID and it's reverse compliment are given since the software must trim the MID from both ends of the sequence due to the nature of the Y-adaptor):

When designing your own adaptors or other sequences, remember the FIRST sequence should be the one immediately following the KEY sequence. For example, for RL1, your adaptor sequence should be:
CCATCTCATCCCGTGCCTCTCCGACGACTACACGACGACT (blue is the KEY, red is the MID/barcode)

mid = "RL1", "ACACGACGACT", 1, "AGTCGTGGTGT";
mid = "RL2", "ACACGTAGTAT", 1, "ATACTAGGTGT";
mid = "RL3", "ACACTACTCGT", 1, "ACGAGTGGTGT";
mid = "RL4", "ACGACACGTAT", 1, "ATACCGTGGCGT";
mid = "RL5", "ACGAGTAGACT", 1, "ATGCTACGCGT";
mid = "RL6", "ACGCGTCTACTG", 1, "ACTAGAGCGGT";
mid = "RL7", "ACGTACACACT", 1, "AGTGTGTGCGT";
mid = "RL8", "ACGTACTGTGT", 1, "ACACGATCCGT";
mid = "RL9", "ACGTAGATCGT", 1, "ACGATCTGCGT";
mid = "RL10", "ACTACGTCTCT", 1, "AGAGACGGAGT";
mid = "RL11", "ACTATAAGGT", 1, "ACTGTAAGACT";
mid = "RL12", "ACTCGCGTCTGT", 1, "ACGACGAGGT";
mid = "RL13", "AGACTCGACGT", 1, "ACGTCGACTGT";
mid = "RL14", "AGTACGAGATG", 1, "ACTCTGGGACT";
mid = "RL15", "AGTACTACTAT", 1, "ATAGTGGACT";
mid = "RL16", "AGTACGCGTCT", 1, "AGACGTCGACT";
mid = "RL17", "AGTGTACGACT", 1, "AGTGTGACGT";
mid = "RL18", "AGTATAGTAGT", 1, "ACTACTAGACT";
mid = "RL19", "ATAGTATACGT", 1, "ACGTATAGTAT";
mid = "RL20", "CAGTACGTACT", 1, "AGTACGTGCTG";
mid = "RL21", "CGACGACGTAC", 1, "ACGCGTGGTCG";
mid = "RL22", "CGACGAGGACT", 1, "AGTACGGGACT";
mid = "RL23", "CGTACGACGCT", 1, "AGTAGTGGTCG";
mid = "RL24", "CTACGTCGATG", 1, "ACTCGACGGG";
mid = "RL25", "CTACGTAGCAGT", 1, "ACGTACGCTG";
mid = "RL26", "GTACGACGTAT", 1, "AGTACGCTGAC";
mid = "RL27", "GTACGTACGTAT", 1, "ATACGCTAGAC";
mid = "RL81", "CAGCGTACTGT", 1, "ACAGTAGGCTG";
mid = "RL82", "CAGTCTCTAGT", 1, "ACTAGAGGCTG";
mid = "RL83", "CATAGTGCGCT", 1, "ACGAGCAGGCT";
mid = "RL84", "CGAGACACTAT", 1, "ATAGTGTGTCG";
mid = "RL85", "CGAGAGTGTCT", 1, "ACACACTGTCG";
mid = "RL86", "CGAGTCATCGT", 1, "ACGATGAGTCG";
mid = "RL87", "CGATCGTATAT", 1, "ATATACGGTCG";
mid = "RL88", "CGCAGTACGCT", 1, "AGCGTACGGCG";
mid = "RL89", "CGCGATCGTAT", 1, "ACGATGGGACG";
mid = "RL90", "CGCGCTATACT", 1, "AGCTATAGGCG";
mid = "RL91", "CGTACAGATAT", 1, "ATATCTGGACG";
mid = "RL92", "CGTAGCTCCTC", 1, "AGAGACGGAG";
mid = "RL93", "CGTATAGTGCT", 1, "AGCACTAGGAC";
mid = "RL94", "CGTCAAGGACT", 1, "AGTCAGGACTG";
mid = "RL95", "CGTCGCAAGTAG", 1, "ACACCTGGGAC";
mid = "RL96", "CGTCTCAGCAT", 1, "ATACGATGGAC";
mid = "RL97", "CGTGACTCAGT", 1, "ACTGACAGGTC";
mid = "RL98", "CTACGATACAT", 1, "ATGCTGTGATG";
mid = "RL99", "CTAGACAGACT", 1, "AGTCTGAGGTC";
mid = "RL100", "CTAGCAGCATG", 1, "AGTCTGAGGTC";
mid = "RL101", "CTAGTACCTAT", 1, "ATGAGTATAGG";
mid = "RL102", "CTATATGTGCT", 1, "ACGAGAGGTC";
mid = "RL103", "CTATGCTGACT", 1, "AGTCTGTGAGG";
mid = "RL104", "CTATGTAGAGT", 1, "ACTCTACGTCG";
mid = "RL105", "CTGACGTACAT", 1, "ATGAGCTGGAG";
mid = "RL106", "CTGCAGTGCTC", 1, "AGAGACGGAGC";
mid = "RL107", "CTGTCGATCTC", 1, "ACTCCAGGACG";
mid = "RL108", "CTTCAGGATCT", 1, "AGGCTCPCCAT";
mid = "RL109", "CTTCGATGCTC", 1, "ACGAGAAGGTC";
mid = "RL110", "CTTGACAAGGCT", 1, "ACGAGGAGGCT";
mid = "RL111", "GACGCTGCTCAGT", 1, "AGCAGAGGGT";
mid = "RL112", "GACGTCAGTCG", 1, "AGTCATCGGCT";
mid = "RL113", "GACTAGCTAGT", 1, "ACTAGCTGGTC";
mid = "RL114", "GAGACGTCCCT", 1, "AGGCAAGCCTCC";
mid = "RL115", "GAGAGACGCT", 1, "ACGCTGCTCCT";
mid = "RL116", "GCGTAGACTAC", 1, "ATAGCTGCGGC";
mid = "RL117", "GCCTCGCTCCT", 1, "AGACGACCCGC";
mid = "RL118", "GCTCTCTACGT", 1, "ACGTAAGGGAC";
mid = "RL119", "GTACACTGATAT", 1, "ATACAGGGGTAC";
mid = "RL120", "GTACGGGACAT", 1, "ATGTCGGGTAC";
mid = "RL121", "GTACTATAGAT", 1, "ATCTATGGTAC";
mid = "RL122", "GTACTGAGTCT", 1, "AGACTCGGTAC";
mid = "RL123", "GTAGCTAGCTC", 1, "ACAGTGAGTAC";
mid = "RL124", "GTAGTACCTCT", 1, "ACAGTGAGTAC";
mid = "RL125", "GTAGTACCTCT", 1, "ACAGTGAGTAC";
mid = "RL126", "GTATACTAGAT", 1, "ACATAGGTGCAC";
mid = "RL127", "GTTCAGTCCTC", 1, "ACGAGAGGAC";
mid = "RL128", "GTTCGAGGCCGT", 1, "ACGAGGAGGCT";
mid = "RL129", "GTTCGAGAAGGCT", 1, "ACTACGACGGC";
mid = "RL130", "GTTCAGGACTC", 1, "AGATAGTGAC";
mid = "RL131", "GTGTCACGACCT", 1, "AGTCTAGGAC";
mid = "RL132", "GTGTGATCCTG", 1, "ACGATACGAC";
Titanium fragment adaptor sequences: Key sequence: TCAG (shown WITH MID)

(asterisks (*) indicate phosphorothioate bases)
Adaptor A (both top and bottom strands shown, with MID 1):

5' - C*C*A*T*CTCATCCCTGCGTGT C T C CGAC

TCAG

ACGAGT*G*C*G*T

3' - A*G*A*G*GCTG

ATGC

TGCTCA*C*G*C*A

Adaptor B (both top and bottom strands shown):

5' - BioTEG/C*C*T*A*TCCCCTGTGTGCCTT G G C AGTC*T*C*A*G

3' - A*C*C*G*TCAG*A*G*T*C

Titanium fragment MID (barcode) sequences and numbers:

mid = "MID1", "ACGAGTGCGR", 2;
mid = "MID2", "ACGCTCGACA", 2;
mid = "MID3", "AGACCGCAGC", 2;
mid = "MID4", "AGCAGTGCAG", 2;
mid = "MID5", "ATCAGACACG", 2;
mid = "MID6", "ATCGCGCGG", 2;
mid = "MID7", "CGTGTCCTCT", 2;
mid = "MID8", "CTCGGCTGTC", 2;
mid = "MID9", "TAGTATCAGC", 2;
mid = "MID10", "TCTCTATGCG", 2;
mid = "MID11", "TGATACGTCT", 2;
mid = "MID12", "TACTGAGCTA", 2;
mid = "MID13", "CATAGTAGTG", 2;
mid = "MID14", "CGAGAGATAC", 2;

More are available - contact the GSAF or Roche for more information, or use RL MIDs.

Standard fragment library adaptor sequences: Key sequence: TCAG (with NO MID /Barcode)
Adaptor A:

5' - CGTATCGCCTCCCTCGCGCCA

TCAG

- (MID)
3’ - GCATAGCGGAGGGAGCGCGGT
- (MID)

Adaptor B:
5’ - CTATGCGCCTTGCCAGCCCGCTCAG-(MID)

3’ - GATACGCGGAACGGTCGGGCGAGTC-(MID)

These are the oldest 454 designs and are no longer recommended for new projects

Note that in oligo sequences, the *** means a phosphorothioate base.