Strategy for indexed, library-free Illumina sequencing of MIP-derived amplicons
The example below is based on the first MIP sequence described in Supplementary File 1 of Turner et al, 2009.

Annotated sequence of MIP precursor synthesized on programmable microarray (arrows indicate cut sites for nicking endonucleases):

				flanking seq 1	*targeting arm 1*	*common linker*	*targeting arm 2*	*flanking seq 2*
				Nt.AlwI ↓				↓ ***Nb.BsrDI***
DIP2C	**10**	**317139**	**317262**	**AGGACCGGATCAACTaagtcgtgagcttgcagcttCTTCAGCTTCCCGATATCCGACGGTAGTGTgcaggctgtcattactccacCATTGCGTGAACCGA**				

Sequence of MIP released by nicking endonucleases:

5′-aagtcgtgagcttgcagcttCTTCAGCTTCCCGATATCCGACGGTAGTGTgcaggctgtcattactccac-3′

Turner primers for inverse PCR and direct sequencing of amplicons, and alignment with Illumina flow cell oligos:

```
Capture_Slxa_Fwd_Amp:                    5′-AATGATACGGCGACCACCGAGCACGATCCGACGGTAGTGT
Flow cell oligo 'A': 5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3
Capture Seq sequencing primer:                          5′-CCGAGCACGATCCGACGGTAGTGT

Capture_Slxa_Rev_Amp:                    5'-CAAGCAGAAGACGGCATACGACCGTAATCGGGAAGCTGAAG
Flow cell oligo 'B':          5 -PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-3
```

Strategy for inverse PCR from linker region:

```
Capture_Slxa_Fwd_Amp: 5′-AATGATACGGCGACCACCGAGCACG \
                                                   ATCCGACGGTAGTGT
                5′-aagtcgtgagcttgcagcttCTTCAGCTTCCCGATATCCGACGGTAGTGTgcaggctgtcattactccac-3′
                                   GAAGTCGAAGGGCTA \
                                                    ATGCCAGCATACGGCAGAAGACGAAC-5′ Capture_Slxa_Rev_Amp
```

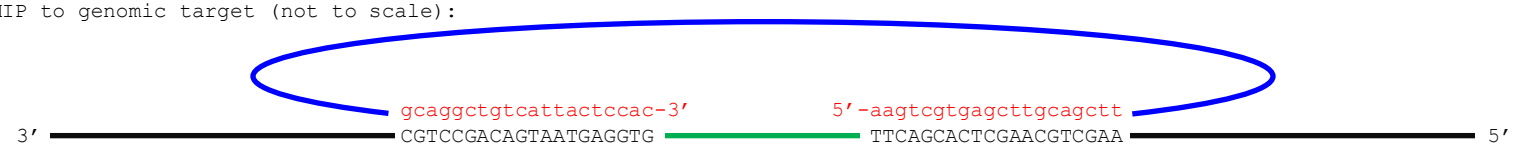
For indexing, incorporate sequence ID tag (purple) into reverse PCR primer between linker-specific sequence and flow cell annealing sequence:

```
Indexing_Rev_Amp:                        5'-CAAGCAGAAGACGGCATACGACCGTAFEDCBAATCGGGAAGCTGAAG
Flow cell oligo 'B':          5 -PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-3
```

Inverse PCR from linker region:

```
Capture_Slxa_Fwd_Amp: 5′-AATGATACGGCGACCACCGAGCACG \
                                                   ATCCGACGGTAGTGT
                5′-aagtcgtgagcttgcagcttCTTCAGCTTCCCGATATCCGACGGTAGTGTgcaggctgtcattactccac-3′
                                   GAAGTCGAAGGGCTA \
                                                    ABCDEFATGCCAGCATACGGCAGAAGACGAAC-5′ Indexing_Rev_Amp
```

Annealing of MIP to genomic target (not to scale):



Extension/ligation to incorporate captured sequence (green):

Inverse PCR reaction:

Products of inverse PCR:

Cluster amplification, denaturation, annealing to oligo B:

Extension of oligo B:

Denaturation, sequencing with Capture seq primer:

2nd sequencing reaction with indexing primer: