## Supplemental Table S1. RRNA and snRNA fragments detected in unique RNA band of extracellular miRNAs

**(Figure 1, D and E, star)**

### Table S1A

**Hs 5.8S ribosomal RNA**

```
1                   GACTCTTAGC GGTGGATCAC TCGGCTCGTG CGTCGATGAAG AACGCAGCTAG
51                  CTGCGAGAAT TAATGTGAAT TGCAGGACAC ATTGATCATCG ACACTTCGAAC
101                 GCACTTGCGG CCCCGGGTTC CTCCCGGGGC TACGCCTGTCT GAGCGTCGCTT
2_B1                                     CTCCCGGGGC TACGCCTGTCT GAGCGTCGC
1_B2                                     CTCCCGGGGC TACGCCTGTCT GAGCGTCGC
1_D2                                     CTCCCGGGGC TACGCCTGTCT GAGCGTCGC
2_E2                                   C CTCCCGGGGC TACGCCTGTCT GAGCGTCGCT
```

### Table S1B

**Hs U1A small nuclear RNA (RNU1A)**

```
1                   ATACTTACCT GGCAGGGGAG ATACCATGAT CACGAAGGTG GTTTTCCCAG
2_B3                                GAG ATACCATGAT CACGAAGGTG GTTTTCCCAG
1_E3                                GAG ATACCATGAT CACGAAGGTG GTTTTCCCAG

51                  GGCGAGGCTT ATCCATTGCA CTCCGGATGT GCTGACCCCT GCGATTTCCC
2_B3                GGCGAGGC
1_E3                GGCGAGGC

101                 CAAATGTGGG AAACTCGACT GCATAATTTG TGGTAGTGGG GGACTGCGTT
151                 CGCGCTTTCC CCTG
```

## Supplemental Table S2. RNA Subpopulation Enriched in the Extracellular Space

**(D1, D2, D3: cellular sequences, others, extracellular.)**

```
1-5035 Homo sapiens 28S ribosomal RNA (LOC100008589), NR_003287
1     CGCGACCTCA GATCAGACGT GGCGACCCGC TGAATTTAAG CATATTAGTC AGCGGAGGAA AAGAAACTAA CCAGGATTCC CTCAGTAACG GCGAGTGAAC
mir-923                                          TATTTGTC AGCGGAGGAA AAGAAACTAA CCAGGATTCC CTCAGTAATG GCGAGTG
G2                                                                                                     TAACG GCGAGTGAAC
D3                                                                                                     TAACG GCGAGTGAAC
C3                                                                                                  AGGTAACG GCGAGTGAAC
D1                                                                                                          TGAAC
G1                                                                                                          TGAAC
E1                                                                                                          TAGGT

101   AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGCGGGGCGC GGGACATGTG GCGTACGGAA GACCCGCTCC CCGGCGCCGC TCGTGGGGGG CCCAAGTCCT
G2    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
D3    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
C3    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
D1    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
G1    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
C2    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
E1    AGGGAAGAGC CCAGCGCCGA ATCCCCGCCC CGC
B3                                    GGCGGGGCGC GGGACATGTG GCGTACGGAA GACCCGC
D2                                    AGGGGGGCGC GGGACATGTG GCGTACGGAA GACCCGC

 201  TCTGATCGAG GCCCAGCCCG TGGACGGTGT GAGGCCGGTA GCGGCCGGCG CGCGCCCGGG TCTTCCCGGA GTCGGGTTGC TTGGGAATGC AGCCCAAAGC
 301  GGGTGGTAAA CTCCATCTAA GGCTAAATAC CGGCACGAGA CCGATAGTCA ACAAGTACCG TAAGGGAAAG TTGAAAAGAA CTTTGAAGAG AGAGTTCAAG
 401  AGGGCGTGAA ACCGTTAAGA GGTAAACGGG TGGGGTCCGC GCAGTCCGCC CGGAGGATTC AACCCGGCGG CGGGTCCGGC CGTGTCGGCG GCCCGGCGGA
```

```
 501  TCTTTCCCGC CCCCCGTTCC TCCCGACCCC TCCACCCGCC CTCCCTTCCC CCGCCGCCCC TCCTCCTCCT CCCCGGAGGG GGCGGGCTCC GGCGGGTGCG
 601  GGGGTGGGCG GGCGGGGCCG GGGGTGGGGT CGGCGGGGGA CCGTCCCCCG ACCGGCGACC GGCCGCCGCC GGGCGCATTT CCACCGCGGC GGTGCGCCGC
 701  GACCGGCTCC GGGACGGCTG GGAAGGCCCG GCGGGGAAGG TGGCTCGGGG GGCCCCGTCC GTCCGTCCGT CCTCCTCCTC CCCCGTCTCC GCCCCCCGGC
A2    GACCGGCTCC GGGACGGCTG GGAAGGCCCG GCGGGGAAGG T
C2    GACCGGCTCC GGGACGGCTG GGAAGGCCCG GCGGGGAAGG T
E2                                                                                          TCCTCCTC CCCCGTCTCC GCCCCCCGGC

 801  CCCGCGTCCT CCCTCGGGAG GGCGCGCGGG TCGGGGCGGC GGCGGCGGCG GCGGTGGCGG CGGCGGCGGG GGCGGCGGGA CCGAAACCCC CCCCGAGTGT
E2    CCCGCGTCCT CCCT

 901  TACAGCCCCC CCGGCAGCAG CACTCGCCGA ATCCCGGGGC CGAGGGAGCG AGACCCGTCG CCGCGCTCTC CCCCCTCCCG GCGCCCACCC CCGCGGGGAA
G2                                                 CGAGGGAGCG AGACCCGTCG CCGCGCT

1001  TCCCCCGCGA GGGGGGTCTC CCCCGCGGGG GCGCGCCGGC GTCTCCTCGT GGGGGGGCCG GGCCACCCCT CCCACGGCGC GACCGCTCTC CCACCCCTCC
1101  TCCCCGCGCC CCCGCCCCGG CGACGGGGGG GGTGCCGCGC GCGGGTCGGG GGGCGGGGCG GACTGTCCCC AGTGCGCCCC GGGCGGGTCG CGCCGTCGGG
1201  CCCGGGGGAG GTTCTCTCGG GGCCACGCGC GCGTCCCCCG AAGAGGGGGA CGGCGGAGCG AGCGCACGGG GTCGGCGGCG ACGTCGGCTA CCCACCCGAC
1301  CCGTCTTGAA ACACGGACCA AGGAGTCTAA CACGTGCGCG AGTCGGGGGC TCGCACGAAA GCCGCCGTGG CGCAATGAAG GTGAAGGCCG GCGCGCTCGC
1401  CGGCCGAGGT GGGATCCCGA GGCCTCTCCA GTCCGCCGAG GGCGCACCAC CGGCCCGTCT CGCCCGCCGC GCCGGGGAGG TGGAGCACGA GCGCACGTGT
1501  TAGGACCCGA AAGATGGTGA ACTATGCCTG GGCAGGGCGA AGCCAGAGGA AACTCTGGTG GAGGTCCGTA GCGGTCCTGA CGTGCAAATC GGTCGTCCGA
1601  CCTGGGTATA GGGGCGAAAG ACTAATCGAA CCATCTAGTA GCTGGTTCCC TCCGAAGTTT CCCTCAGGAT AGCTGGCGCT CTCGCAGACC CGACGCACCC
1701  CCGCCACGCA GTTTTATCCG GTAAAGCGAA TGATTAGAGG TCTTGGGGCC GAAACGATCT CAACCTATTC TCAAACTTTA AATGGGTAAG AAGCCCGGCT
1801  CGCTGGCGTG GAGCCGGGCG TGGAATGCGA GTGCCTAGTG GGCCACTTTT GGTAAGCAGA ACTGGCGCTG CGGGATGAAC CGAACGCCGG GTTAAGGCGC
1901  CCGATGCCGA CGCTCATCAG ACCCCAGAAA AGGTGTTGGT TGATATAGAC AGCAGGACGG TGGCCATGGA AGTCGGAATC CGCTAAGGAG TGTGTAACAA
2001  CTCACCTGCC GAATCAACTA GCCCTGAAAA TGGATGGCGC TGGAGCGTCG GGCCCATACC CGGCCGTCGC CGGCAGTCGA GAGTGGACGG GAGCGGCGGG
2101  GGCGGCGCGC GCGCGCGCGC GTGTGGTGTG CGTCGGAGGG CGGCGGCGGC GGCGGCGGCG GGGGTGTGGG GTCCTTCCCC CGCCCCCCCC CCCACGCCTC
C3                                TG CGTCGGAGGG CGGCGGCGGC GGCGGCGGCG GGGGT
2A                                TG CGTCGGAGGG CGGCGGCGGC GGCGGCGGCG GGGGT

2201  CTCCCCTCCT CCCGCCCACG CCCCGCTCCC CGCCCCCGGA GCCCCGCGGA CGCTACGCCG CGACGAGTAG GAGGGCCGCT GCGGTGAGCC TTGAAGCCTA
2301  GGGCGCGGGC CCGGGTGGAG CCGCCGCAGG TGCAGATCTT GGTGGTAGTA GCAAATATTC AAACGAGAAC TTTGAAGGCC GAAGTGGAGA AGGGTTCCAT
2401   GTGAACAGCA GTTGAACATG GGTCAGTCGG TCCTGAGAGA TGGGCGAGCG CCGTTCCGAA GGGACGGGCG ATGGCCTCCG TTGCCCTCGG CCGATCGAAA
2501  GGGAGTCGGG TTCAGATCCC CGAATCCGGA GTGGCGGAGA TGGGCGCCGC GAGGCGTCCA GTGCGGTAAC GCGACCGATC CCGGAGAAGC CGGCGGGAGC
2601  CCCGGGGAGA GTTCTCTTTT CTTTGTGAAG GGCAGGGCGC CCTGGAATGG GTTCGCCCCG AGAGAGGGGC CCGTGCCTTG GAAAGCGTCG CGGTTCCGGC
G3                                          GT CCTGGAATGG GTTC

2701  GGCGTCCGGT GAGCTCTCGC TGGCCCTTGA AAATCCGGGG GAGAGGGTGT AAATCTCGCG CCGGGCCGTA CCCATATCCG CAGCAGGTCT CCAAGGTGAA
2801  CAGCCTCTGG CATGTTGGAA CAATGTAGGT AAGGGAAGTC GGCAAGCCGG ATCCGTAACT TCGGGATAAG GATTGGCTCT AAGGGCTGGG TCGGTCGGGC
2901  TGGGGCGCGA AGCGGGGCTG GGCGCGCGCC GCGGCTGGAC GAGGCGCGCG CCCCCCCCAC GCCCGGGGCA CCCCCCTCGC GGCCCTCCCC CGCCCCACCC
3001  GCGCGCGCCG CTCGCTCCCT CCCCACCCCG CGCCCTCTCT CTCTCTCTCT CCCCCGCTCC CCGTCCTCCC CCCTCCCCGG GGGAGCGCCG CGTGGGGGCG
3101  CGGCGGGGGG AGAAGGGTCG GGGCGGCAGG GGCCGCGCGG CGGCCGCCGG GGCGGCCGGC GGGGGCAGGT CCCCGCGAGG GGGGCCCCGG GGACCCGGGG
3201  GGCCGGCGGC GGCGCGGACT CTGGACGCGA GCCGGGCCCT TCCCGTGGAT CGCCCCAGCT GCGGCGGGCG TCGCGGCCGC CCCCGGGGAG CCCGGCGGCG
3301  GCGCGGCGCG CCCCCCACCC CCACCCCACG TCTCGGTCGC GCGCGCGTCC GCTGGGGGCG GGAGCGGTCG GGCGGCGGCG GTCGGCGGGC GGCGGGGCGG
3401  GGCGGTTCGT CCCCCCGCCC TACCCCCCCG GCCCCGTCCG CCCCCCGTTC CCCCCTCCTC CTCGGCGCGC GGCGGCGGCG GCGGCAGGCG GCGGAGGGGC
3501  CGCGGGCCGG TCCCCCCCGC CGGGTCCGCC CCCGGGGCCG CGGTTCCGCG CGCGCCTCGC CTCGGCCGGC GCCTAGCAGC CGACTTAGAA CTGGTGCGGA
3601  CCAGGGGAAT CCGACTGTTT AATTAAAACA AAGCATCGCG AAGGCCCGCG GCGGGTGTTG ACGCGATGTG ATTTCTGCCC AGTGCTCTGA ATGTCAAAGT
3701  GAAGAAATTC AATGAAGCGC GGGTAAACGG CGGGAGTAAC TATGACTCTC TTAAGGTAGC CAAATGCCTC GTCATCTAAT TAGTGACGCG CATGAATGGA
3801  TGAACGAGAT TCCCACTGTC CCTACCTACT ATCCAGCGAA ACCACAGCCA AGGGAACGGG CTTGGCGGAA TCAGCGGGGA AAGAAGACCC TGTTGAGCTT
3901  GACTCTAGTC TGGCACGGTG AAGAGACATG AGAGGTGTAG AATAAGTGGG AGGCCCCCGG CGCCCCCCCG GTGTCCCCGC GAGGGGCCCG GGGCGGGGTC
4001  CGCGGCCCTG CGGGCCGCCG GTGAAATACC ACTACTCTGA TCGTTTTTTC ACTGACCCGG TGAGGCGGGG GGGCGAGCCC GAGGGGCTCT CGCTTCTGGC
4101  GCCAAGCGCC CGCCCGGCCG GGCGCGACCC GCTCCGGGGA CAGTGCCAGG TGGGGAGTTT GACTGGGGCG GTACACCTGT CAAACGGTAA CGCAGGTGTC
H2                                                    CAGG TGGGGAGTTT GACTGGGGCG GTACACCTGT CAAACGGT
A3                                                    CAGG TGGGGAGTTT GACTGGGGCG GTACACCTGT CAAACGGT

4201  CTAAGGCGAG CTCAGGGAGG ACAGAAACCT CCCGTGGAGC AGAAGGGCAA AAGCTCGCTT GATCTTGATT TTCAGTACGA ATACAGACCG TGAAAGCGGG
4301  GCCTCACGAT CCTTCTGACC TTTTGGGTTT TAAGCAGGAG GTGTCAGAAA AGTTACCACA GGGATAACTG GCTTGTGGCG GCCAAGCGTT CATAGCGACG
4401  TCGCTTTTTG ATCCTTCGAT GTCGGCTCTT CCTATCATTG TGAAGCAGAA TTCGCCAAGC GTTGGATTGT TCACCCACTA ATAGGGAACG TGAGCTGGGT
4501  TTAGACCGTC GTGAGACAGG TTAGTTTTAC CCTACTGATG ATGTGTTGTT GCCATGGTAA TCCTGCTCAG TACGAGAGGA ACCGCAGGTT CAGACATTTG
4601  GTGTATGTGC TTGGCTGAGG AGCCAATGGG GCGAAGCTAC CATCTGTGGG ATTATGACTG AACGCCTCTA AGTCAGAATC CCGCCCAGGC GAACGATACG
4701  GCAGCGCCGC GGAGCCTCGG TTGGCCTCGG ATAGCCGGTC CCCCGCCTGT CCCCGCCGGC GGGCCGCCCC CCCCTCCACG CGCCCCGCCG CGGGAGGGCG
4801  CGTGCCCCGC CGCGCGCCGG GACCGGGGTC CGGTGCGGAG TGCCCTTCGT CCTGGGAAAC GGGGCGCGGC CGGAAAGGCG GCCGCCCCCT CGCCCGTCAC
4901  GCACCGCACG TTCGTGGGGA ACCTGGCGCT AAACCATTCG TAGACGACCT GCTTCTGGGT CGGGGTTTCG TACGTAGCAG AGCAGCTCCC TCGCTGCGAT
5001  CTATTGAAAG TCAGCCCTCG ACACAAGGGT TTGTC
```

# Supplemental Table S3 Oligonucleotides used for Mature miRNA Quantitation

| | primers for linker ligation only | for linker ligation and stem-loops | for stem loops only | sequence | Stem looped primer |
|---|---|---|---|---|---|
| let-7c | | hsa-let7c_15 | | TGAGGTAGTAGGTTG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACC |
| let-7f | hsa-let7f_14 | | | TGAGGTAGTAGATTG | N/A |
| let-7g | | hsa-let7g_16 | | TGAGGTAGTAGTTTGT | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACT |
| miR-107 | | hsa-miR107_15 | | AGCAGCATTGTACAG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtgatag |
| miR-1246 | | hsa-miR1246_16 | | AATGGATTTTTGGAGC | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCTG |
| miR-1275 | hsa-miR-1275_14 | | | GTGGGGGAGAGGCT | N/A |
| miR-1275 | | | hsa-miR-1275_15* | GTGGGGGGAGAGGCT | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGACA |
| miR-148a | | | miR-148a_15 | tcagtgcactacagaa | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacaaa |
| miR-149* | | hsa-miR149*_15 | | AGGGAGGGACGGGGG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTCC |
| miR-16 | | hsa-miR16_14 | | TAGCAGCACGTAAA | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcgcca |
| miR-1915 | hsa-miR1915_15 | | | CCCCAGGGCGACGCG | N/A |
| miR-196a | | | miR-196a_15 | taggtagtttcatgt | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcccaa |
| miR-200b | | | miR-200b_15 | taatactgcctggta | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcatca |
| miR-200c | hsa-miR200c_14 | | | TAATACTGCCGGGT | N/A |
| miR-21 | | hsa-mir21_15 | | TAGCTTATCAGACTG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcaaca |
| miR-210 | | | miR-210_15 | ctgtgcgtgtgacag | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtcagcc |
| miR-22 | hsa-miR-22_14 | | | AAGCTGCCAGTTGA | N/A |
| miR-23a | | hsa-miR23a_14 | | ATCACATTGCCAGG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGAA |
| miR-27b | | hsa-miR27b_14-5 | | CAGTGGCTAAGTTC | N/A |
| miR-30c | hsa-miR30c_15 | | | TGTAAACATCCTACA | N/A |
| miR-335 | | | miR-335_15 | tcaagagcaataacg | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacattt |
| miR-345 | hsa-miR345_15 | | | GCTGACTCCTAGTCC | N/A |
| miR-373 | | | miR-373_15 | gaagtgcttcgattt | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacacc |
| miR-451 | | hsa-miR451_16 | | AAACCGTTACCATTAC | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACT |
| miR-483-5P | | hsa-miR483-5p_15 | | AAGACGGGAGGAAAG | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTCC |
| miR-520c | | | miR-520c_15 | aaagtgcttcctttt | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACaccctc |
| miR-638 | hsa-miR638_15 | | | AGGGATCGCGGGCGG | N/A |
| miR-720 | | hsa-miR720_15 | | TCTCGCTGGGGCCTC | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGA |
| miR-99a | | hsa-miR100_15 | | AACCCGTAGATCCGA | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACcacaa |
| MODBAN | 3'modban_rcrtPCR | | | ATTGATGGTGCCTACAG | |

## Supplemental Table S4 Stem Looped Primers used for Simultaneous Analyses of Multiple Transcripts

STEM-LOOPED PRIMER AMPLIFICATION

| hsa- | MIRNA SEQUENCE | PRIMER NAME | PRIMER SEQUENCE |
|---|---|---|---|
| miR-16 | UAGCAGCACGUAAAUA*UUGGCG* | hsa-miR-16-14 | TAGCAGCACGTAAA |
| | | miR-16_14-loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*CGCCAA* |
| miR-1246 | AAUGGAUUUUUGG*AGC*AGG | 1246_loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*CCTGCT* |
| | | 1246_16 | AATGGATTTTTGGAGC |
| miR-720 | UCUCGCUGGGG*CCUC*CA | 720_loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*TGGAGG* |
| | | 720_14 | CTCGCTGGGGCCTC |
| miR-451 | AAACCGUUACCAUUAC*UGAGUU* | 451_loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*AACTCA* |
| | | hsa-miR451_15 | AAACCGTTACCATTA |
| let-7g | UGAGGUAGUAGUUUGU*ACAGUU* | let-7g-loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*AACTGT* |
| | | hsa-let-7g-16 | TGAGGTAGTAGTTTGT |
| miR-99A (miR-100) | AACCCGUAGAUCCGAU*CUUGUG* | miR-100-loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*CACAAG* |
| | | 100-15 | AACCCGTAGATCCGA |
| miR-210 | CUGUGCGUGUGACAGC*GGCUGA* | miR-210-loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*TCAGCC* |
| | | miR-210_15 | CTGTGCGTGTGACAG |
| internal loading control: | | | |
| INT-RNA | GGAAAGGGGAACCCCUGUGUGU | INT_RNA-loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*ACACAC* |
| | | INT_15 | GGAAAGGGGAACCCC |
| | | INT_RNA | GGGGGGAAAAACCCCUUUUUCC |
| miR-1275 | GUGGGGGAGAG*GCU*G*UC* | 1275_loli | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC*GACAGC* |
| | | hsa-miR-1275_15* | GTGGGGGGAGAGGCT |
| lolirev | | loli_rev | GTGCAGGGTCCGAGGT |

## Supplemental Table S5. Confirmed sequences of qPCR products quantified using linker ligated primers

| | |
|---|---|
| **hsa-miR-221** | **AGCUACAUUGUCUGCTGGGUUUC** |
| 1_B05_271599_ECRC | AGCTACATTGTCTGCTGGGTTTC |
| 1_F03_271602_CCRC | AGCTACATTGTCTGCTGGGTTTC |
| 1_B04_271597_EA | AGCTACATTGTCTGCTGGGTTTC |
| 1_F02_271600_CA | AGCTACATTGTCTGCTGGGTT**A**C |
| 1_F04_271598_EBRC | AGCTACATTGTCTGCTGGGTTTC |
| | |
| **hsa-miR-222** | **AGCUACAUCUGGCUACUGGGU** |
| 1_C04_271603_EA | AGCTACATCTGGCTACTGGGT |
| 1_G04_271604_EB | AGCTACATCTGGCTACTGGGT |
| 1_G02_271606_CA | AGCTACATCTGGCTACTGGGT |
| 1_C03_271607_CB | AGCTACATCTGGCTACTGGGT |
| 1_G03_271608_CC | AGCTACATCTGGCTACTGGGT |
| | |
| **hsa-miR-100** | **AACCCGUAGAUCCGAACUUGUG** |
| **hsa-miR-99a** | **AACCCGUAGAUCCGAUCUUGUG** |
| 1_A04_271609_EA | AACCCGTAGATCCGA**T**CTTGT |
| 1_E03_271614_CC | AACCCGTAGATCCGA**T**CTTGTG |
| 1_E02_271612_CA | AACCCGTAGATCCGA**T**CTTGTG |
| 1_A03_271613_CB | AACCCGTAGATCCGA**T**CTTGT |
| 1_A05_271611_EC | AACCCGTAGATCCGA**T**CTTGTG |
| | |
| **hsa-miR-720** | **UCUCGCUGGGGCCUCCA** |
| 1_D04_271615_EA | T**A**TCGCTGGGGCCTCCA |
| 1_D05_271617_EC | TCTCGCTGGGGCCTCCA |
| 1_H04_271616_EB | TCTCGCTGGGGCCTCCA |
| 1_D03_271619_CB | TCTCGCTGGGGCCTCCA |
| 1_H02_271618_CArc | TCTCGCTGGGGCCTCCA |

**Confirmed sequences for looped miRs**

| | |
|---|---|
| **hsa-miR-16** | **UAGCAGCACGUAAAUAUUGGCG** |
| 1_A09_2742 | TAGCAGCACGTAAATATTGGCG |
| 1_B09_2742 | TAGCAGCACGTAAATATTGGCG |
| 1_H11_2742 | TAGCAGCACGTAAATATTGGCG |
| | |
| **hsa-miR-1246** | **AAUGGAUUUUUGGAGCAGG** |
| 1_E07_2742rc | AATGGATTTTTGGAGCAGG |
| 1_F07_2742rc | AATGGATTTTTGGAGCAGG |
| 1_G07_2742rc | AATGGATTTTTGGAGCAGG |
| 1_H07_2742 | AATGGATTTTTGGAGCAGG |
| | |
| **hsa-miR-720** | **UCUCGCUGGGGCCUCCA** |
| 1_H10_2742 | TCTCGCTGGGGCCTCCA |
| 1_B11_2742 | TCTCGCTGGGGCCTCCA |
| 1_C11_2742 | TCTCGCTGGGGCCTCCA |
| | |
| **hsa-miR-451** | **AAACCGUUACCAUUACUGAGUU** |
| 1_B10_2742 rc | AAACCGTTACCATTACTGAG-- |
| 1_A10_2742 rc | AAACCGTTACCATTACTGAGTT |
| 1_C10_2742 | AAACCGTTACCATTACTGAGTT |
| | |
| **hsa-let-7g** | **UGAGGUAGUAGUUUGUACAGUU** |
| 1_D11_2742 | TGAGGTAGTAGTTTGTACAGTT |
| 1_F11_2742 | TGAGGTAGTAGTTTGTACAGTT |

| | |
|---|---|
| **>hsa-miR-1275** | **GUGGGGGAGAGGCUGUC** |
| 1_E08_2742 Trc | GTGGGGGAGAGGCTGTC |
| 1_C08_2742 Lrc | GTGGGGGAGAGGCTGTC |
| 1_H08_2742 U | GTGGGGGAGAGGCTGTC |
| 1_A08_2742 L | GTGGGGGAGAGGCTGA- |

**Supplemental Table S6. Primers for Quantifying pre-miRNAs and Other Immature miRNAs**

| RNA | | name | sequence |
|---|---|---|---|
| pre-let-7f | 5' | let7Fm-1Fm | GATTGTATAGTTGTGGGGTAGTG |
| pre-let-7f | 3' | let7Rm-1Rm | GGGAAGGCAATAGATTGTATAG |
| pre-let-7g | 5' | let7gFm | GTAGTAGTTTGTACAGTTTGAGGGT |
| pre-let-7g | 3' | let7gRm | GGCAGTGGCCTGTACAGT |
| pre-let-7c | 5' | let7cFm | TTGAGGTAGTAGGTTGTATGGTT |
| pre-let-7c | 3' | let7cRm | GGAAAGCTAGAAGGTTGTACAG |
| pre-let-7c | pri-S | Let-7c-US1 | TGAAGCAACATTGGAAGC |
| pre-let-7c | pri-L | Let-7c-US2 | TCTATATCCTTGCCAAGCC |
| pre-miR-100 | 5' | miR-100Fm | AACCCGTAGATCCGAACTTG |
| pre-miR-100 | 3' | miR-100Rm | TACCTATAGATACAAGCTTGTGCG |
| pre-miR-1246 | 5' | hsa-miR1246_19 | TTGAATGGATTTTTGGAGC |
| pre-miR-1246 | 3' | 1246_matrev_22 | ATTGCTAGCCTATGGATTGATT |
| pre-miR-1275 | 5' | hsa-miR-1275_15* | GTGGGGGGAGAGGCT |
| pre-miR-1275 | 3' | 1275_mat rev | TCCCTCTGCCTTGGG |
| pre-miR-149* | 5' | 149*_matfor | GCCGGCGCCCGAGCT |
| pre-miR-149* | 3' | 149*_matrev | GCCCCCGTCCCTCCC |
| pre-miR-155 | 5' | miR-155Fm | GTTAATGCTAATCGTGATAGGG |
| pre-miR-155 | 3' | miR-155Rm | GCTAATATGTAGGAGTCAGTTGGA |
| pre-miR-16 | 5' | miR-016-1Fmm | GCAGCACGTAAATATTGGCGT |
| pre-miR-16 | 3' | miR-016-1Rm | CAGCAGCACAGTTAATACTGGAGA |
| pre-miR-1915 | 5' | 1915_matfor | TGAGAGGCCGCACCT |
| pre-miR-1915 | 3' | 1915_matrev_13 | CGCGTCGCCCTGG |
| pre-miR-200c | 5' | miR-200cFm | CTCGTCTTACCCAGCAGTGT |
| pre-miR-200c | 3' | miR-200cRm2 | CATCATTACCCGGCAGTATTAG |
| pre-miR-200c | pri-S | miR-200c US1 | GAAGCTGCCTGACCCAAG |
| pre-miR-200c | pri-L | miR-200c US2 | CAGGGATCTGCAGCTTTTC |
| pre-miR-221 | 5' | miR-221Fm | CCTGGCATACAATGTAGATTTCTG |
| pre-miR-221 | 3' | miR-221Rm | AAACCCAGCAGACAATGTAGCT |
| pre-miR-221 | pri-S | miR-221 US1 | AAATAGTATGTGAGAATTACTTGCAAGC |
| pre-miR-221 | pri-L | miR-221 US2 | GAAATTTTGTTGGTAGTAGGTAAGTCC |
| pre-miR-222 | 5' | miR-222(p)Fm | CCCCAGAAGGCAAAGGAT |
| pre-miR-222 | 3' | miR-222(p)Rm | CTCTCTCAGGACACTGAAGCAG |
| pre-miR-23a | 5' | miR-023aFm | CTGGGGTTCCTGGGGAT |
| pre-miR-23a | 3' | miR-023aRm | TGGTAATCCCTGGCAATGTG |
| pre-miR-27 | 5' | miR-027a,bFm | GCAGGGCTTAGCTGCTTG |
| pre-miR-27 | 3' | miR-027a,bRm | GGCGGAACTTAGCCACTGT |
| pre-miR-30c | 5' | miR-030c-1,2Fm | TGTGTAAACATCCTACACTCTCAG |
| pre-miR-30c | 3' | miR-030c-1,2Rm | GAGTAAACAACCCTCTCCCA |
| pre-miR-451 | 5' | hsa-miR-451_21 | CAAGGAAACCGTTACCATTAC |
| pre-miR-451 | 3' | 451_mat rev | TCTGGGTATAGCAAGAGAACC |
| pre-miR-638 | 5' | 638_13 | AGGGATCGCGGGC |
| pre-miR-638 | 3' | 638_matrev | AGCGCCGCAGTTACG |
| pre-pre-miR-21 | 5' | miR-021Fm | GCTTATCAGACTGATGTTGACTG |
| pre-pre-miR-21 | 3' | miR-021Rm | CAGCCCATCGACTGGTG |
| pre-pre-miR-21 | pri-S | miR-021 US1 | ATCTCCATGGCTGTACCACC |
| pre-pre-miR-21 | pri-L | miR-021 US2 | TCAAATCCTGCCTGACTGTC |

# Supplemental Table S7 Sequences of Immature miRNAs Detected

```
           1                                                                                                                  110
hsa-mir-221UGAACAUCCA GGUCUGGGGC AUGAACCUGG CAUACAAUGU AGAUUUCUGU GUUCGUUAGG CAACAGCUAC AUUGUCUGCU GGGUUUCAGG CUACCUGGAA ACAUGUUCUC
Cmim221C_1                            CCTGG CATACAATGT AGATTTCTGT GTTCGTTAGG CAACAGCTAC ATTGTCTGCT GGGTTT
Cmim221B_1                            CCTGG CATACAATGT AGATTTCTGT GTTCGTTAGG CAACAGCTAC ATTGTCTGCT GGGTTT
Emrcm221C_                            CCTGG CATACAATGT AGATTTCTGT GTTCGTTAGG CAACAGCTAC ATTGTCTGCT GGGTTT
Crcim221A_                            CCTGG CATACAATGT AGATTTCTGT GTTCGTTAGG CAACAGCTAC ATTGTCTGCT GGGTTT
Emim221A_1                            CCTGG CATACAATGT AGATTTCTGT GTTCGTTAGG CAACAGCTAC ATTGTCTGCT GGGTTT
Emim221B_1                                                    CGCGT CGCCCTGGGC AGAAATCTAC ATTGTATGCC AGG

           1                                                                  72
hsa-mir-21     UGUCGGGUAG CUUAUCAGAC UGAUGUUGAC UGUUGAAUCU CAUGGCAACA CCAGUCGAUG GGCUGUCUGA CA
Cmim21B_16              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG
Crcim21C_1              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG
Ercim21B_1              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG
Crcim21A_1              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG
Ercim21C_1              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG
Ercim21A_1              G CTTATCAGAC TGATGTTGAC TGTTGAATCT CATGGCAACA CCAGTCGATG GGCTG


           1                                                          73
hsa-miR-23a    GGCCGGCUG GGGUUCCUGG GGAUGGGAUU UGCUUCCUGU CACAAAUCAC AUUGCCAGGG AUUUCCAACC GACC
CC23a_186            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA
EC23a_183            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA
EB23a_182            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA
EA23a_181            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA
CB23a_185            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA
CA23a_184            CTG GGGTTCCTGG GGATGGGATT TGCTTCCTGT CACAAATCAC ATTGCCAGGG ATTACCA

           1                                                                                                          90
hsa-mir-161    GUCAGCAGUG CCUUAGCAGC ACGUAAAUAU UGGCGUUAAG AUUCUAAAAU UAUCUCCAGU AUUAACUGUG CUGCUGAAGU AAGGUUGAC
Crcim16C_1                   GCAGC ACGTAAATAT TGGCGTTAAG ATTCTAAAAT TATCTCCAGT ATTAACTGTG CTGCTG
Cfcim16B_1*                  GCAGC ACGTAAATAT TGGCGTTAAG ATTCTAAAAT TATCTCCAGT ATTAACTGTG CTGCTG
Efcim16A_1*                  GCAGC ACGTAAATAT TGGCGTTAAG ATTCTAAAAT TATCTCCAGT ATTAACTGTG CTGCTG
Efcim16B_1*                  GCAGC ACGTAAATAT TGGCGTTAAG ATTCTAAAAT TATCTCCAGT ATTAACTGTG CTGCTG
Cfcim16A_1*                  GCAGC ACGTAAATAT TGGCGTTAAG ATTCTAAAAT TATCTCCAGT ATTAACTGTG CTGCTG


miR-200c

immature     CAGGGAUCU GCAGCUUUUC CGCAGGGAUC CUGGGCCUGA AGCUGCCUGA CCCAAGGUGG GCGGGCUGGG CGGGGGCCCU CGUCUUACCC AGCAGUGUUU GGGUGCGGUU GGGAGUCUCU AAUACUGCCG
                  GGUAAUGAUG GAGG
EfiUS2C      CAGGGATCT GCAGCTTTTC CGCAGGGATC CTGGGCCTGA AGCTGCCTGA CCCAAGGTGG GCGGGCTGGG CGGGGGCCCT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
EriUS2B      CAGGGATCT GCAGCTTTTC CGCAGGGATC CTGGGCCTGA AGCTGCCTGA CCCAAGGTGG GCGGGCTGGG CGGGGGCCCT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
CriUS1B                                          TGA AGCTGCCTGA CCCAAGGTGG GCGGGCTGGG CGGGGGCCCT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
CfiUS1C                                           GA AGCTGCCTGA CCCAAGGTGG GCGGGCTGGG CGGGGGCCCT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
CfiUS1A                                           GA AGCTGCCTGA CCCAAGGTGG GCGGGCTGGG CGGGGGCCCT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
Cri200cC                                                                                                CT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
Cri200cA                                                                                                CT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC
Efi200cA_                                                                                               CT CGTCTTACCC AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT
                  GGTAATGATG AC   Efi200cB_                                                                                                 CT CGACTTACCC
                  AGCAGTGTTT GGGTGCGGTT GGGAGTCTCT AATACTGCCT GGTAATGATG AC


           1                                                                                  84
hsa-let-7g   AGGCUGAGGU AGUAGUUUGU ACAGUUUGAG GGUCUAUGAU ACCACCCGGU ACAGGAGAUA ACUGUACAGG CCACUGCCUU GCCA
Crcim7gC_1           GT AGTAGTTTGT ACAGTTTGAG GGTCTATGAT ACCACCCGGT ACAGGAGATA ACTGTACAGG CCACTGCC
Crcim7gB_1           GT AGTAGTTTGT ACAGTTTGAG GGTCTATGAT ACCACCCGGT ACAGGAGATA ACTGTACAGG CCACTGCC
Emim7gA_15           GT AGTAGTTTGT ACAGTTTGAG GGTCTATGAT ACCACCCGGT ACAGGAGATA ACTGTACAGG CCACTGCC
Cmim7gA_16           GT AGTAGTTTGT ACAGTTTGAG GGTCTATGAT ACCACCCGGT ACAGGAGATA ACTGTACAGG CCACTGCC
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**Supplementary Figures**

**Supplemental Figure S1. Some Diagnostic miRNAs are Mostly Retained.**

Indicated miRNAs were amplified from RNA collected from cells and the conditioned media of MCF7 cells using looped primers, and separated by native PAGE. **A** miRNAs in which the cellular (c), but not the extracellular (x) RNA population contained the indicated miRNAs. In each case of the cellular sample, the main band (arrow) was excised, cloned and sequenced and found to be the expected amplified product. In the case of extracellular samples the main bands, all of which migrated differently (star), had no resemblance to the miRNA to be amplified as determined by sequencing. **B** miRNAs in which the released miRNA population contained a band of much lower abundance than the retained population.

**Supplemental Figure S2.** Lack of Correlation between Primary Transcript and Release Rate of MiRNAs. Released/retained miRNAs (x/c) as evaluated in Figure 1 are plotted according to chromosomal location (chromosomes 1-X) from top to bottom. Micro-RNA clusters are indicated in color. Note that for cluster miR-200c-141, which is located in a single intron (1), 30 times more of its encoded miR-200c molecules than miR-141 molecules were released from cells (**Figure 2A,** and data not shown) than retained, indicating that the extent of miRNA release is not determined at the primary-miRNA level. In support of this idea, within the other miR-200 cluster, which encodes miR-200a, miR-200b and miR-429 (1), more than half of miR-429 and miR-200b molecules are released, whereas most of miR-200a is retained. Therefore, the extracellular accumulation of mature miRNAs is regulated at levels other than the primary transcript abundance. MicroRNAs contained in particular clusters are indicated by red or green colored bars.

**Supplemental Figure S3. MiR-1246 is a Reliable Indicator of Body Fluids Conditioned by Mammary Epithelia. A** tumor growth of xenografted cells of mice used for assessment of miRNA abundance in blood plasma as indicated in Figure 4A. The tumor size is presented as the product of the

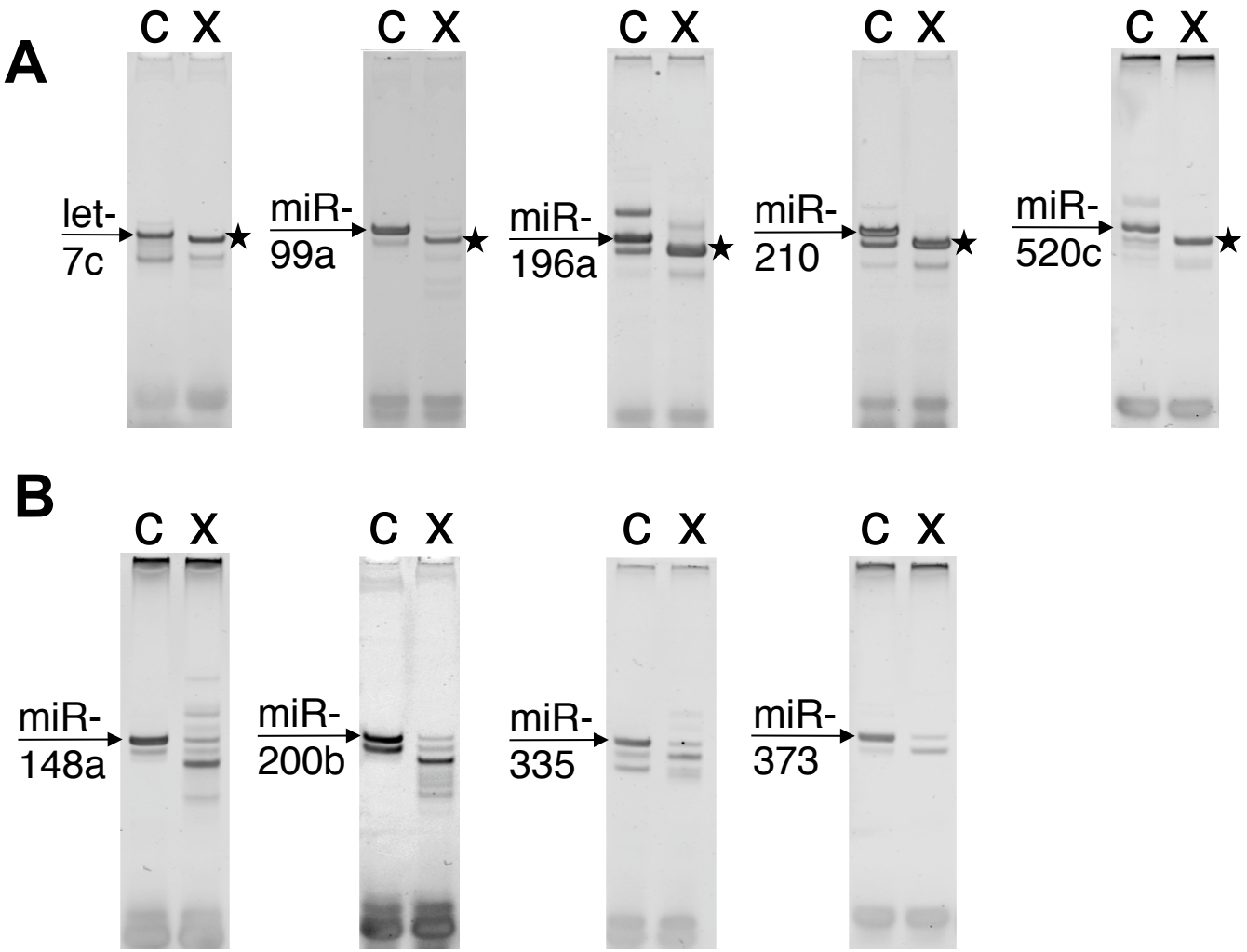
3 diameters. Data presented in Figure 6 are from bleeds at day 30 (arrow). **B** PAGE of end-point PCR of miR-1246 of bleeds of MCF7-1 and MDA-MB-231.

**Supplemental Figure S4. Fetal Bovine Serum Does Not Interfere With Extracellular MiRNA Assessment. A** Relative abundance of indicated miRNAs in calf serum (CS) and fetal bovine serum (FBS), normalized to INT-RNA**.** Note the absence of miR-451 from FBS, but that CS contains measurable levels of *Bos taurus* miR-451, which differs from hsa-miR-451 by a single terminal nucleotide (2). **B** End-point PCR using stem-loop primers on extracellular (x) and cellular (c) miR-451 of indicated breast cancer cell lines grown in complete FBS, or in FBS depleted of microvesicles (FBS S100). **C** Ratio of miRNAs in c and x of cells grown in complete FBS and FBS depleted of microvesicles (FBS S100). **D.** MiRNAs measured as in Supplemental Figure S4C, by qRT-PCR using linker-ligation. Error bars indicate standard deviation. A is an average of 2 experiments, C and E are averages of 3 independent experiments.

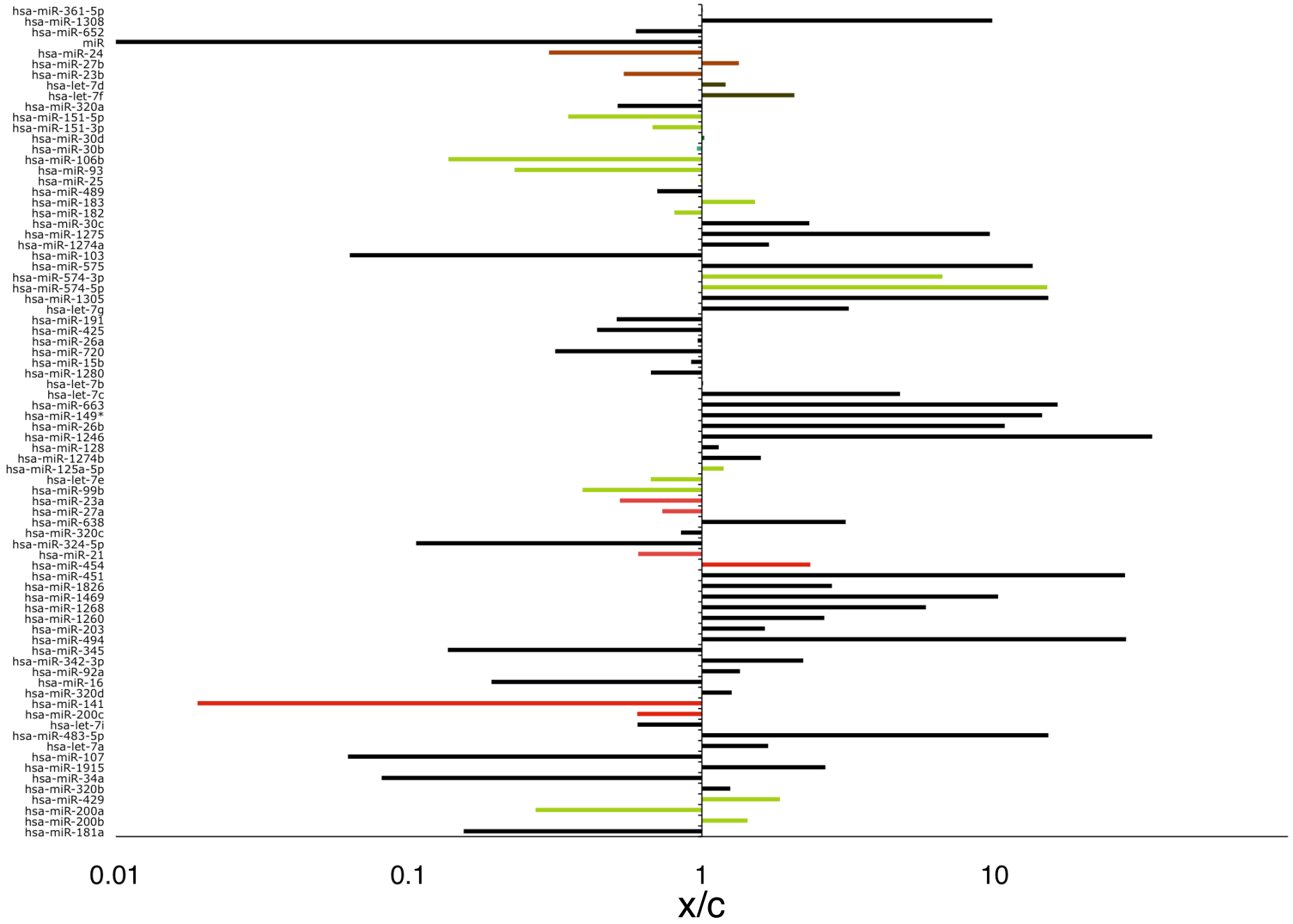
**Supplemental Figure S5. Template-independent amplification products at low template concentrations.** Mature miR-16 was assessed by the stem-loop-primer protocol on 5-fold serial dilutions of a synthetic DNA construct reflecting the expected product of miR-16.

**References of Supplementary Material**

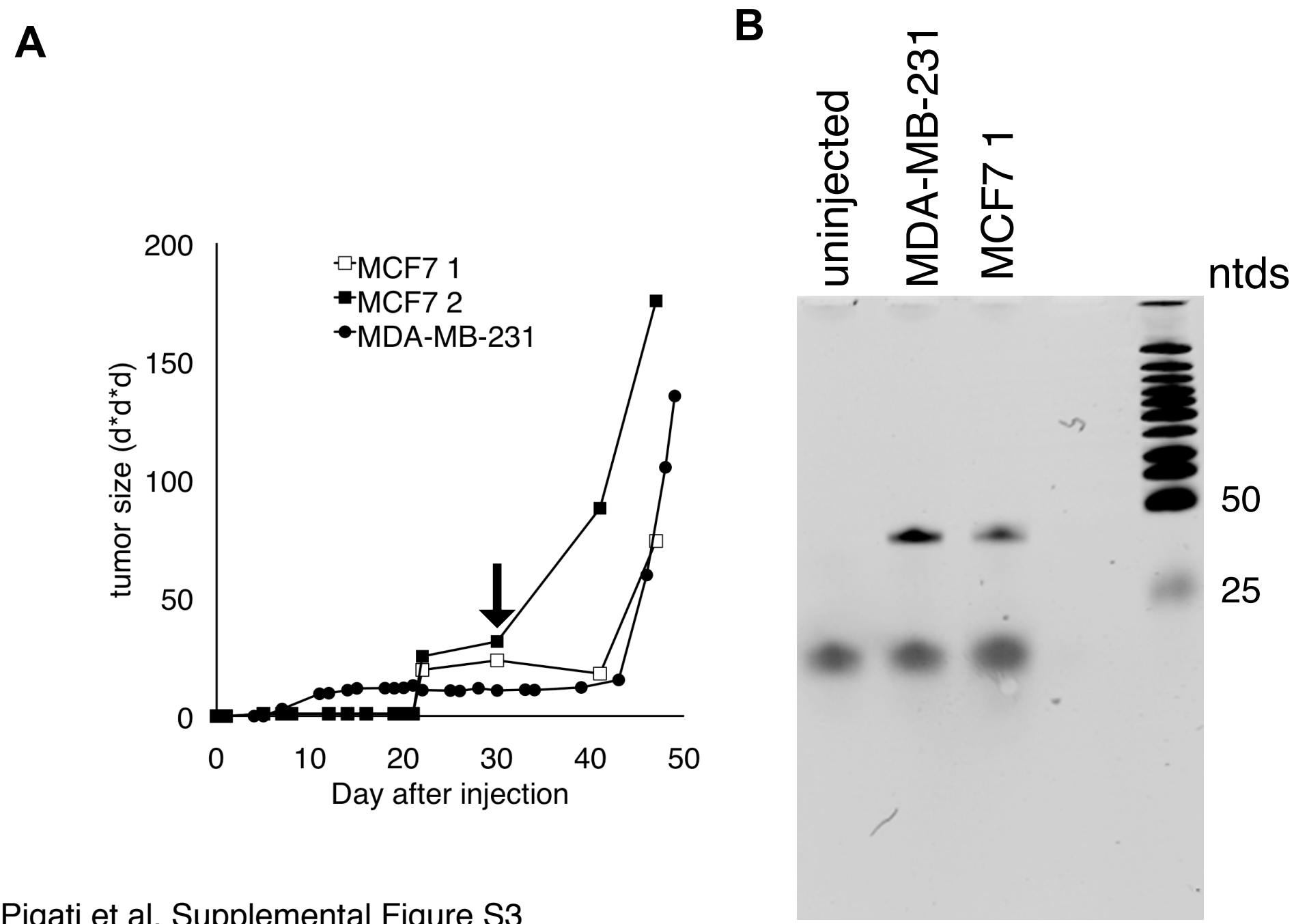
1. Bracken CP, Gregory PA, Kolesnikoff N, Bert AG, Wang J, Shannon MF, Goodall GJ. A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res. 2008 Oct 1;68(19):7846-54.
2. Long JE, Chen HX. Identification and characteristics of cattle microRNAs by homology searching and small RNA cloning. Biochem Genet. 2009 Jun;47(5-6):329-43.




Pigati et al. Supplemental Figure S1




Pigati et al. Supplemental Figure S2

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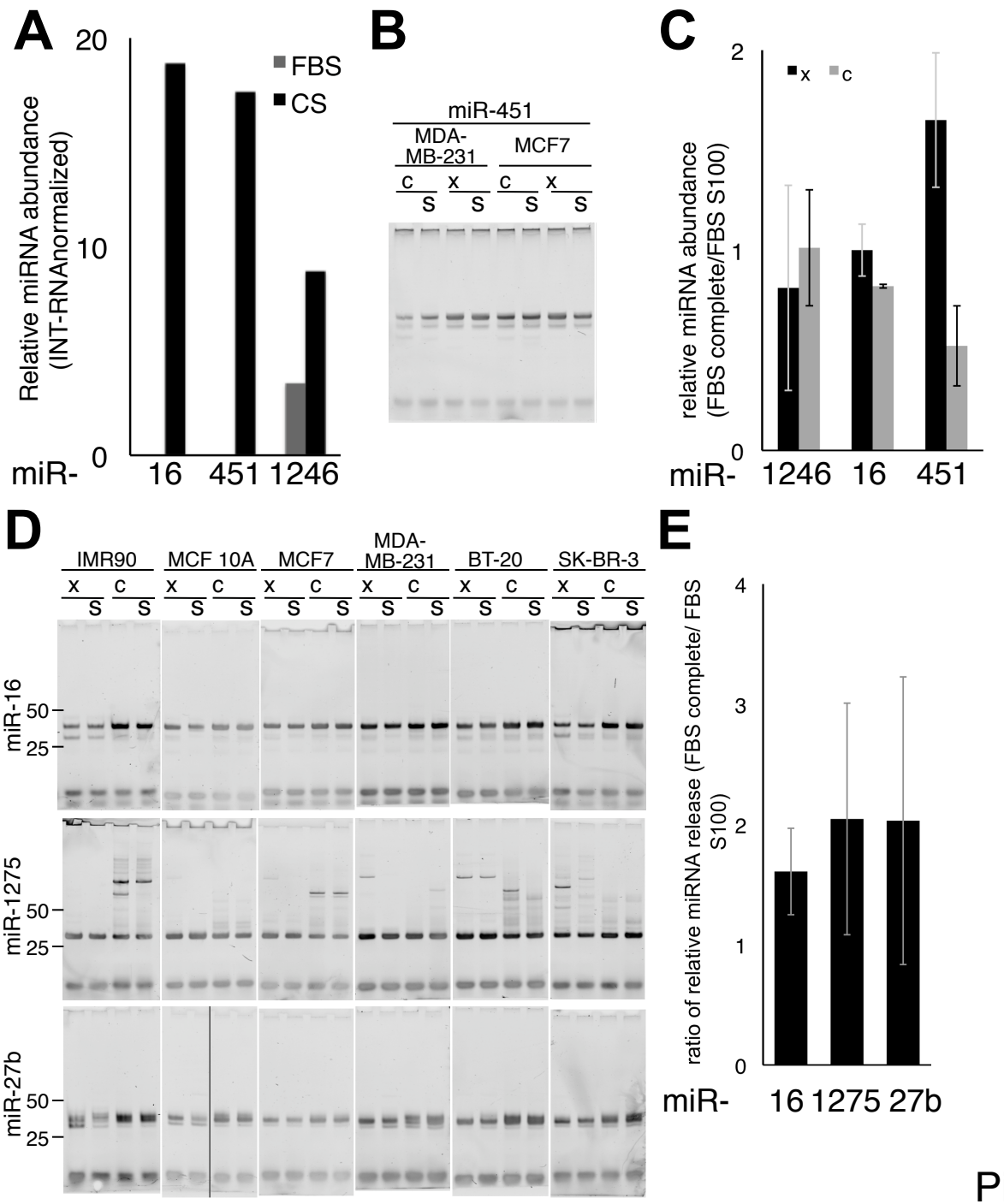
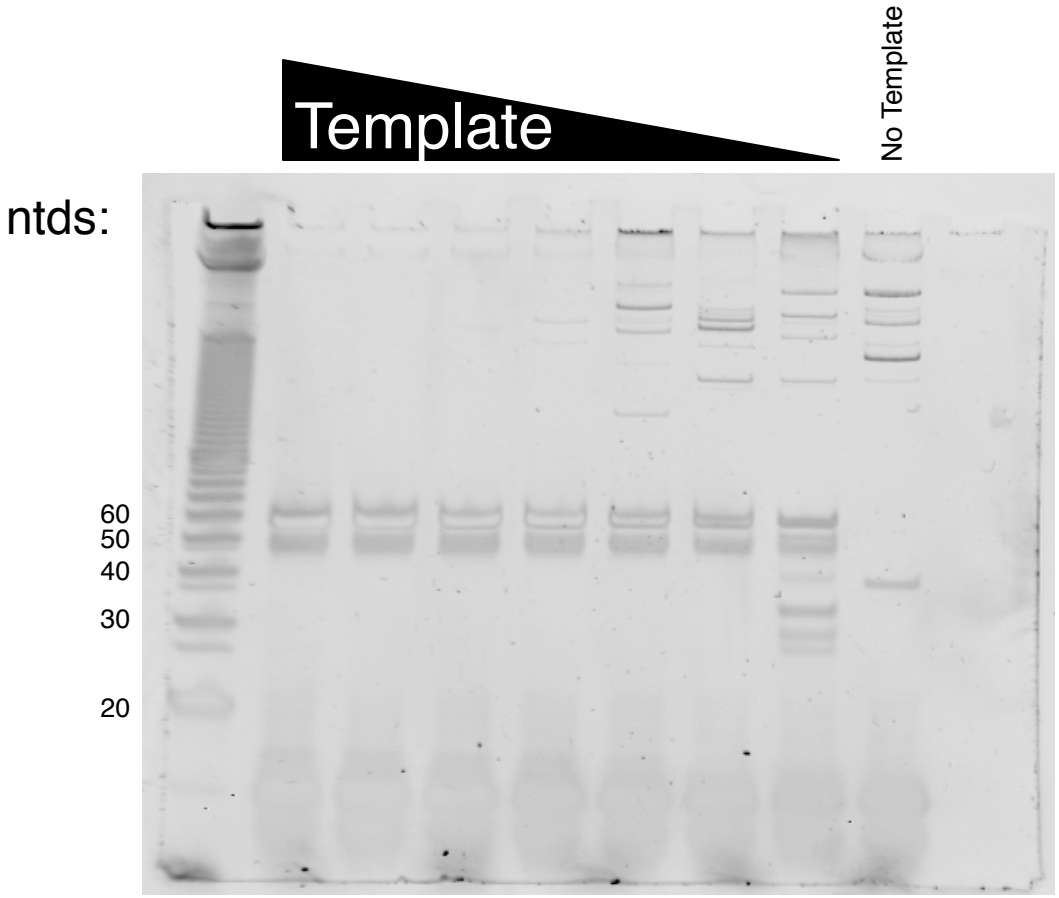



Pigati et al. Supplemental Figure S4




Pigati et al. Supplemental Figure S5