20200113RPF实验记录

样品信息

| NUM | SAMPLE | | index | | index |
|-----|-----------------------|-----|-------|-----|-------|
| 1 | CHO-1(From Zeng lab) | 1-T | 21 | 1-R | 29 |
| 2 | CHO-2(From Zeng lab) | 2-T | 22 | 2-R | 30 |
| 3 | Delta-B(From wangtao) | 3-T | 23 | 3-R | 31 |
| 4 | WT(From wangtao) | 4-T | 24 | 4-R | 32 |
| 5 | Col-O(DMSO) | 5-T | 25 | 5-R | 33 |
| 6 | Col-O(SL) | 6-T | 26 | 6-R | 34 |
| 7 | d14(DMSO) | 7-T | 27 | 7-R | 35 |
| 8 | d14(SL) | 8-T | 28 | 8-R | 36 |

Rnase1 用量

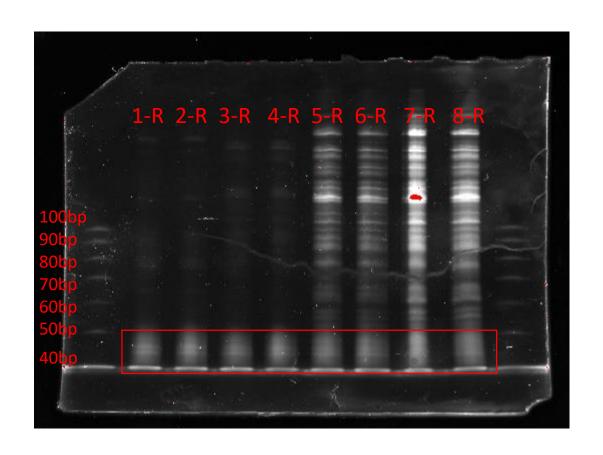
| NUM | A260 | 1/10 Lysisbuffer A260 | V | U | V(Rnase1) |
|-----|-------|-----------------------|------|------|-----------|
| 1-R | 3.945 | 0.5 | 0.13 | 58.5 | 2.6199225 |
| 2-R | 3.753 | 0.5 | 0.13 | 58.5 | 2.4739065 |
| 3-R | 5.318 | 0.5 | 0.13 | 58.5 | 3.664089 |
| 4-R | 4.646 | 0.5 | 0.13 | 58.5 | 3.153033 |
| 5-R | 3.418 | 0.5 | 0.13 | 58.5 | 2.219139 |
| 6-R | 3.279 | 0.5 | 0.13 | 58.5 | 2.1134295 |
| 7-R | 3.871 | 0.5 | 0.13 | 58.5 | 2.5636455 |
| 8-R | 4.498 | 0.5 | 0.13 | 58.5 | 3.040479 |

ribocop

| ribocop | | | | |
|---------|----------|--------|------------|---------|
| num | C(ng/ul) | V (ul) | input (ng) | after C |
| 1-T | 648.7 | 1.5 | 1000 | 26.8 |
| 2-T | 424.4 | 2.4 | 1000 | 29.6 |
| 3-T | 905.1 | 1.1 | 1000 | 38.6 |
| 4-T | 857.9 | 1.2 | 1000 | 41.3 |
| 5-T | 219 | 4.5 | 1000 | 54.3 |
| 6-T | 126.5 | 7.9 | 1000 | 55.5 |
| 7-T | 574.5 | 1.7 | 1000 | 60.6 |
| 8-T | 347.1 | 2.9 | 1000 | 63.8 |
| 1-R | 23.3 | ~15 | all in | 19.1 |
| 2-R | 18.9 | ~15 | all in | 11.8 |
| 3-R | 15.6 | ~15 | all in | 19.2 |
| 4-R | 25.3 | ~15 | all in | 11.2 |
| 5-R | 30.8 | ~15 | all in | 26.1 |
| 6-R | 30.1 | ~15 | all in | 26.9 |
| 7-R | 61 | ~15 | all in | 42.3 |
| 8-R | 45.7 | ~15 | all in | 34.4 |

15%胶图 (RPF)

初始15%胶浓度不对,切胶50bp以下全部片段回收

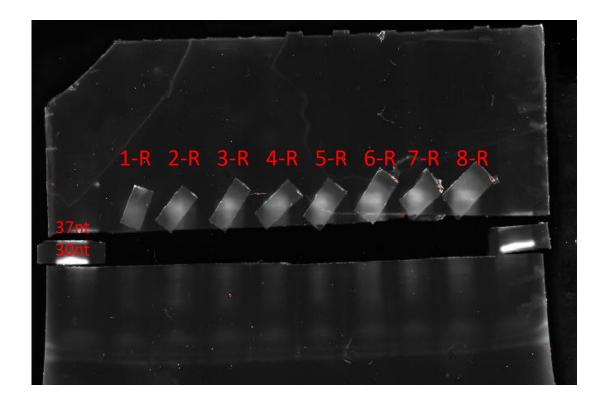


15%胶图

RPF

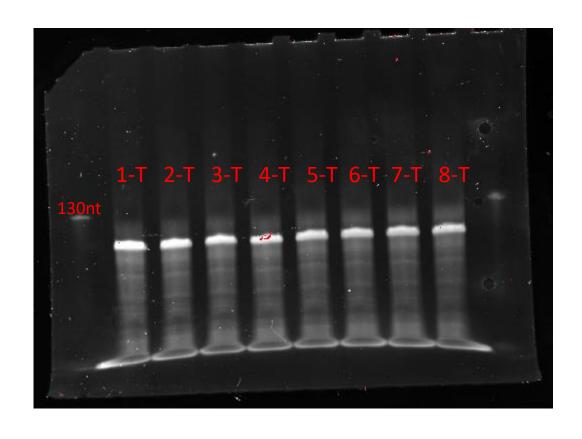


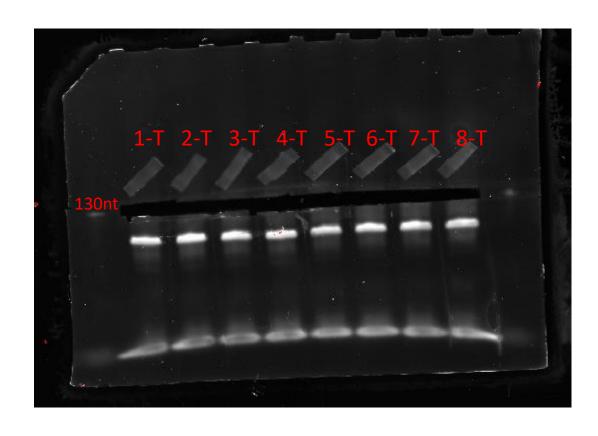
RPF-CUT



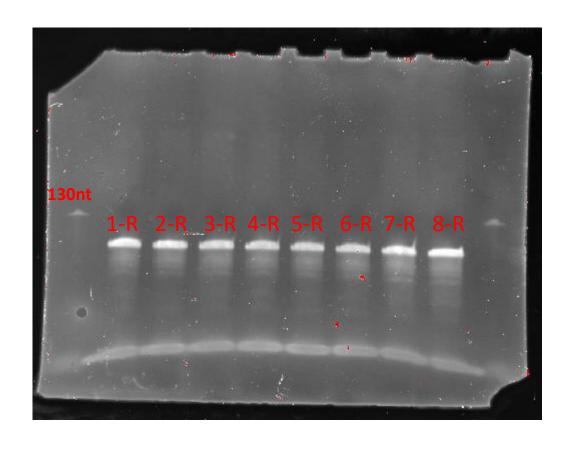
Total cDNA

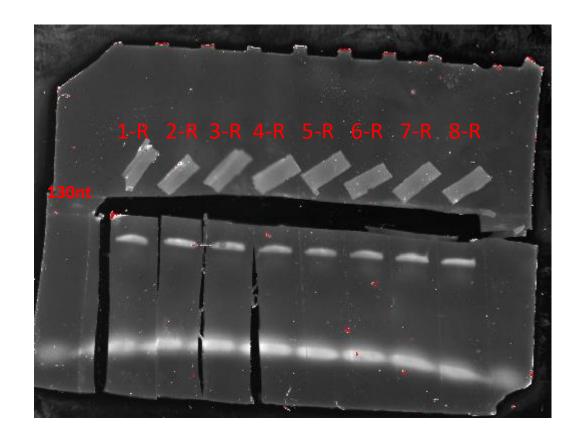
Total cDNA-CUT





RPF cDNA

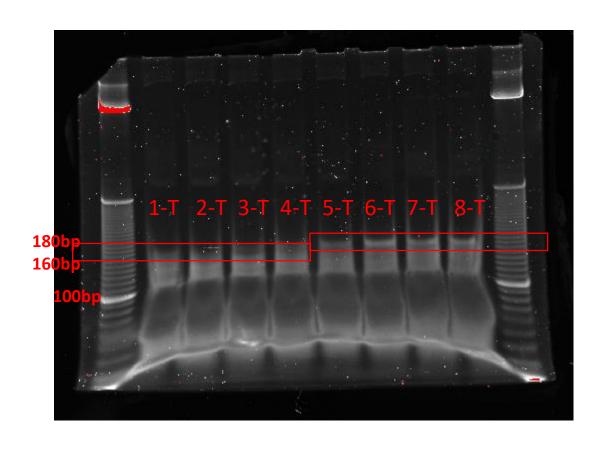


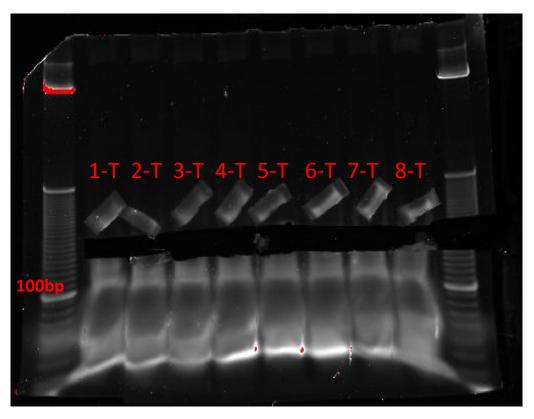


RPF cDNA-CUT

Total PCR

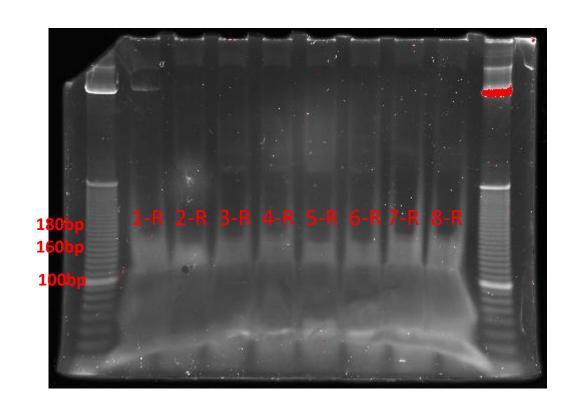


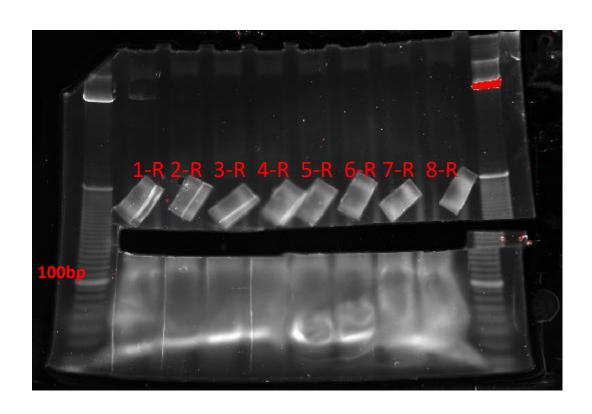




RPF PCR

RPF PCR-CUT





优化PCR体系

| | 原体系50ul | 优化后50ul | 优化后20ul |
|-------------------|---------|---------|---------|
| 模板 | 5 | 2.5 | 1 |
| FWD PRIMER | 2 | 2 | 0.8 |
| INDEX PRIMER | 2 | 2 | 0.8 |
| NF water | 16 | 18.5 | 7.4 |
| Phision HIFI MMIX | 25 | 25 | 10 |
| Cycle | 11 | 9 | 9 |