**结核分枝杆菌耐药检测分析流程准确性测试方案**

1. **项目简介**

结核分枝杆菌耐药检测技术是利用二代测序技术对结核菌进行测序，并对结核分枝杆菌样本中多个基因、位点的耐药变异的检测，现需要对流程中结核分枝杆菌鉴定的结果准确性进行评估。

1. **评估方法**

下载网上（NCBI）数据库中的参考序列，模拟出结核分枝杆菌（mycobacterium tuberculosis，MTB），非结核分枝杆菌（Nontuberculosis mycobacteria, NTM）和其他细菌或病毒的数据，进行多次重复测试，最终得到测试结果。若测试结果与预期结果偏差大于5%的阈值，则认为该样本的单次测试不合格。

1. **测试数据**
   1. 在NCBI上搜索并下载MTB样本，NTM样本，10种其他病原菌物种的全基因组序列。
   2. 将下载序列进行预处理，将不符合条件的序列进行剔除，筛选条件如下：非全长序列，非DNA序列，非MTB或NTM物种等。
2. **测试方法**
   1. 将预处理后的序列随机打断成fastq格式。需要模拟两种平台的数据：BGISEQ-500数据为PE100，样本的错误率为10%，BGISEQ-50平台数据为SE50，样本错误率为10%。每个fastq数据量为1G，再根据如下条件按比例混合。
   2. 打断后的样本分为纯MTB样本（173份），纯NTM样本（17份），MTB与NTM混合样本（21份）和交叉感染样本（10份）四大类，MTB样本与NTM样本见附录一及附录二，混合样本方案如下：

|  |  |  |
| --- | --- | --- |
| 混合样本比列 | | |
| 样本混合方案 | MTB\_5 reads(%) | NTM\_5 reads(%) |
| MTB\_5：NTM\_5 | 1 | 99 |
| MTB\_5：NTM\_5 | 5 | 95 |
| MTB\_5：NTM\_5 | 10 | 90 |
| MTB\_5：NTM\_5 | 15 | 85 |
| MTB\_5：NTM\_5 | 20 | 80 |
| MTB\_5：NTM\_5 | 25 | 75 |
| MTB\_5：NTM\_5 | 30 | 70 |
| MTB\_5：NTM\_5 | 35 | 65 |
| MTB\_5：NTM\_5 | 40 | 60 |
| MTB\_5：NTM\_5 | 45 | 55 |
| MTB\_5：NTM\_5 | 50 | 50 |
| MTB\_5：NTM\_5 | 55 | 45 |
| MTB\_5：NTM\_5 | 60 | 40 |
| MTB\_5：NTM\_5 | 65 | 35 |
| MTB\_5：NTM\_5 | 70 | 30 |
| MTB\_5：NTM\_5 | 75 | 25 |
| MTB\_5：NTM\_5 | 80 | 20 |
| MTB\_5：NTM\_5 | 85 | 15 |
| MTB\_5：NTM\_5 | 90 | 10 |
| MTB\_5：NTM\_5 | 95 | 5 |
| MTB\_5：NTM\_5 | 99 | 1 |

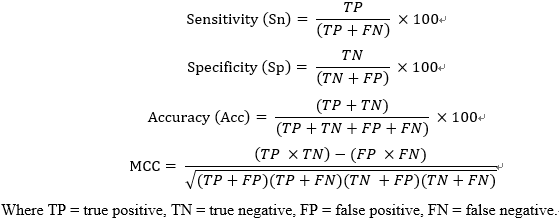
* 1. 交叉反应测试：使用交叉反应的病原菌样品，按10:90的比例分别与上述纯菌样品，混合感染样品再次混合，进行鉴定分析。病原菌样本见附录三。混合方案如下：

|  |  |  |
| --- | --- | --- |
| 交叉反应样本比列 | | |
| 样本混合方案 | S \*reads(%) | MTB\_5 reads(%) |
| S1：MTB\_5 | 10 | 90 |
| S2：MTB\_5 | 10 | 90 |
| S3：MTB\_5 | 10 | 90 |
| S4：MTB\_5 | 10 | 90 |
| S5：MTB\_5 | 10 | 90 |
| S6：MTB\_5 | 10 | 90 |
| S7：MTB\_5 | 10 | 90 |
| S8：MTB\_5 | 10 | 90 |
| S9：MTB\_5 | 10 | 90 |
| S10：MTB\_5 | 10 | 90 |

* 1. 得到两个平台下的模拟样本，通过流程中比对模块进行数据分析，鉴定是否为MTB样本（比对上的uniq reads数比例与真实比例的误差为±5%则为有效），得到实际鉴定的结果。

1. **评估方法**

评估两个平台下本次鉴定结果的敏感性(Sn)、特异性(Sp)、准确性（Acc）和马修斯相关系数(MCC),统计方式见下方计算公式。



将检测结果绘制ROC曲线，选择检测灵敏度和特异性最佳的cutoff值；

将交叉反应样品与纯菌样品和混合感染样品的结果进行比较，确定干扰菌种的影响，要求鉴定结果一致；

**六、结果**

**纯TB与NTM样本测试结果：**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | 总样本量 | 合格样本 | 不合格样本（<95%） | 平均比对率 | 合格率（%） |
| 纯TB（PE） | 173 | 173 | 0 | 99.32 | 100 |
| 纯TB（SE） | 173 | 0 | 173 | 89.81 | 0 |
| 纯NTM（PE） | 17 | 17 | 0 | 99.55 | 100 |
| 纯NTM（SE） | 17 | 0 | 17 | 94.81 | 0 |

解释：PE样本，纯合的TB和NTM模拟样本可以得到较高的比对率（99%以上），合格率为100%；SE样本，纯合的TB和NTM模拟样本平均比对率均低于95%，因此全部判定为不合格。

**混合样本检测结果（1）SE数据：**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 样本编号 | 混合比例 | TB比对率 | NTM比对率 | Unknown比对率 | 合格与否 |
| barcode\_1 | 1:99 | 0.95% | 89.48% | 9.57% | 不合格 |
| barcode\_2 | 5:95 | 4.52% | 85.90% | 9.58% | 不合格 |
| barcode\_3 | 10:90 | 8.99% | 81.37% | 9.64% | 不合格 |
| barcode\_4 | 15:85 | 13.46% | 76.87% | 9.67% | 不合格 |
| barcode\_5 | 20:80 | 17.91% | 72.35% | 9.74% | 不合格 |
| barcode\_6 | 25:75 | 22.39% | 67.86% | 9.75% | 不合格 |
| barcode\_7 | 30:70 | 26.86% | 63.36% | 9.78% | 不合格 |
| barcode\_8 | 35:65 | 31.33% | 58.85% | 9.82% | 不合格 |
| barcode\_9 | 40:60 | 35.81% | 54.34% | 9.85% | 不合格 |
| barcode\_10 | 45:55 | 40.24% | 49.86% | 9.90% | 不合格 |
| barcode\_11 | 50:50 | 44.73% | 45.35% | 9.92% | 不合格 |
| barcode\_12 | 55:45 | 49.21% | 40.86% | 9.93% | 不合格 |
| barcode\_13 | 60:40 | 53.65% | 36.36% | 9.99% | 不合格 |
| barcode\_14 | 65:35 | 58.11% | 31.83% | 10.06% | 不合格 |
| barcode\_15 | 70:30 | 62.57% | 27.35% | 10.09% | 不合格 |
| barcode\_16 | 75:25 | 67.04% | 22.84% | 10.11% | 不合格 |
| barcode\_17 | 80:20 | 71.52% | 18.35% | 10.14% | 不合格 |
| barcode\_18 | 85:15 | 75.98% | 13.84% | 10.18% | 不合格 |
| barcode\_19 | 90:10 | 80.45% | 9.34% | 10.22% | 不合格 |
| barcode\_20 | 95:5 | 84.92% | 4.82% | 10.26% | 不合格 |
| barcode\_21 | 99:1 | 0.95% | 89.49% | 9.56% | 不合格 |

**混合样本测试结果（2）PE数据**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 样本编号 | 混合比例 | TB比对率 | NTM比对率 | Unknown比对率 | 合格与否 |
| barcode\_1 | 1:99 | 1.00% | 98.55% | 0.45% | 合格 |
| barcode\_2 | 5:95 | 4.96% | 94.58% | 0.46% | 合格 |
| barcode\_3 | 10:90 | 9.91% | 89.59% | 0.49% | 合格 |
| barcode\_4 | 15:85 | 14.86% | 84.62% | 0.52% | 合格 |
| barcode\_5 | 20:80 | 19.81% | 79.64% | 0.55% | 合格 |
| barcode\_6 | 25:75 | 24.77% | 74.67% | 0.57% | 合格 |
| barcode\_7 | 30:70 | 29.71% | 69.69% | 0.59% | 合格 |
| barcode\_8 | 35:65 | 34.67% | 64.72% | 0.62% | 合格 |
| barcode\_9 | 40:60 | 39.61% | 59.74% | 0.65% | 合格 |
| barcode\_10 | 45:55 | 44.56% | 54.77% | 0.67% | 合格 |
| barcode\_11 | 50:50 | 49.51% | 49.79% | 0.70% | 合格 |
| barcode\_12 | 55:45 | 54.46% | 44.82% | 0.73% | 合格 |
| barcode\_13 | 60:40 | 59.41% | 39.85% | 0.74% | 合格 |
| barcode\_14 | 65:35 | 64.36% | 34.87% | 0.76% | 合格 |
| barcode\_15 | 70:30 | 69.30% | 29.90% | 0.80% | 合格 |
| barcode\_16 | 75:25 | 74.25% | 24.93% | 0.82% | 合格 |
| barcode\_17 | 80:20 | 79.20% | 19.96% | 0.84% | 合格 |
| barcode\_18 | 85:15 | 84.14% | 14.99% | 0.87% | 合格 |
| barcode\_19 | 90:10 | 89.09% | 10.01% | 0.90% | 合格 |
| barcode\_20 | 95:5 | 94.03% | 5.04% | 0.92% | 合格 |
| barcode\_21 | 99:1 | 1.00% | 98.55% | 0.45% | 合格 |

总体看符合比例趋势，PE数据可以精确的检测出1%比例的TB数据，全部与预期比列一致。而SE数据结果则有较大的偏差，会引入较高的unknown rate（约10%），因此全部被判定为不合格。

**交叉样本检测结果（1）SE数据：**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Barcode |  | MTB\_rate | NTM\_rate | unknown\_rate | 是否合格 |
| S1：MTB\_5 | 10:90 | 80.45% | 0.30% | 19.25% | 不合格 |
| S2：MTB\_5 | 10:90 | 80.45% | 0.29% | 19.26% | 不合格 |
| S3：MTB\_5 | 10:90 | 80.44% | 0.29% | 19.27% | 不合格 |
| S4：MTB\_5 | 10:90 | 80.45% | 0.49% | 19.07% | 不合格 |
| S5：MTB\_5 | 10:90 | 80.43% | 0.30% | 19.27% | 不合格 |
| S6：MTB\_5 | 10:90 | 80.42% | 0.29% | 19.29% | 不合格 |
| S7：MTB\_5 | 10:90 | 80.46% | 0.29% | 19.26% | 不合格 |
| S8：MTB\_5 | 10:90 | 80.42% | 0.30% | 19.28% | 不合格 |
| S9：MTB\_5 | 10:90 | 80.50% | 0.29% | 19.21% | 不合格 |
| S10：MTB\_5 | 10:90 | 80.45% | 0.29% | 19.26% | 不合格 |

**交叉样本检测结果（2）PE数据：**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Barcode |  | MTB\_rate | NTM\_rate | unknown\_rate | 是否合格 |
| S1：MTB\_5 | 10:90 | 89.08% | 0.09% | 10.83% | 合格 |
| S2：MTB\_5 | 10:90 | 89.09% | 0.08% | 10.84% | 合格 |
| S3：MTB\_5 | 10:90 | 89.11% | 0.09% | 10.80% | 合格 |
| S4：MTB\_5 | 10:90 | 89.11% | 0.67% | 10.22% | 合格 |
| S5：MTB\_5 | 10:90 | 89.09% | 0.11% | 10.79% | 合格 |
| S6：MTB\_5 | 10:90 | 89.09% | 0.08% | 10.83% | 合格 |
| S7：MTB\_5 | 10:90 | 89.10% | 0.06% | 10.84% | 合格 |
| S8：MTB\_5 | 10:90 | 89.09% | 0.08% | 10.83% | 合格 |
| S9：MTB\_5 | 10:90 | 89.19% | 0.07% | 10.74% | 合格 |
| S10：MTB\_5 | 10:90 | 89.10% | 0.07% | 10.84% | 合格 |

**汇总：**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | 总样本量 | 合格样本 | 不合格样本 | 平均比对率 | 合格率 |
| 交叉样本（SE） | 10 | 0 | 10 | 0 | 0% |
| 交叉样本（PE） | 10 | 10 | 0 | 0 | 100% |

对于交叉样本，PE数据可以较为准确的检测到与预期一致的比对率（10:90），而SE数据在unknown比对率上有较大偏差，因此被判定为不合格。

公式计算（全部样本汇总）

1. PE数据：

阳性样本数量：TP = 173+17+21+10 = 221

阴性样本数量：FN = 0

=

2）SE数据：

阳性样本数量：TP = 0

阴性样本数量： FN = 173+17+21+10 = 221

=

3）无FP，FN，暂不计算其他公式。

**结论：**

对于PE数据。流程可以得到很好的比对率，对于纯合的TB和NTM样本具有很好的比对率（99%），对于混合样本也可以精确的检测到1%的比例。

对于SE数据：比对率较低，会影响到混合样本的检测，在真实的临床样本中，大多数情况都是混合样本，因此不建议使用SE测序方法。

**讨论**

从结论可以看出，PE数据对于TB的鉴定有比较准确的比对率，推荐使用PE测序方法，另外，本文测试了比对率的准确性，并没有做变异检测的比较，由于模拟数据无法模拟真实突变，因此无法给出突变结果检测的准确性结论。

**附录一：**

|  |  |
| --- | --- |
| 纯TB样本 | |
| 样本编号 | 纯MTB样本（174） |
| MTB\_1 | >NC\_002945.4 Mycobacterium bovis AF2122/97 genome assembly, chromosome: Mycobacterium\_bovis\_AF2122/97 |
| MTB\_2 | >NC\_003888.3 Streptomyces coelicolor A3(2) chromosome, complete genome |
| MTB\_3 | >NC\_000962.3 Mycobacterium tuberculosis H37Rv, complete genome |
| MTB\_4 | >NZ\_AP018035.1 Mycobacterium tuberculosis DNA, complete genome, strain: HN-321 |
| MTB\_5 | >NZ\_AP018034.1 Mycobacterium tuberculosis DNA, complete genome, strain: HN-205 |
| MTB\_6 | >NZ\_AP018036.1 Mycobacterium tuberculosis DNA, complete genome, strain: HN-506 |
| MTB\_7 | >NZ\_AP018033.1 Mycobacterium tuberculosis DNA, complete genome, strain: HN-024 |
| MTB\_8 | >NZ\_CP020381.2 Mycobacterium tuberculosis strain MTB1, complete genome |
| MTB\_9 | >NZ\_CP022014.1 Mycobacterium tuberculosis strain MTB2 chromosome, complete genome |
| MTB\_10 | >NZ\_CP017920.1 Mycobacterium tuberculosis strain TB282 chromosome, complete genome |
| MTB\_11 | >NZ\_CP012506.2 Mycobacterium tuberculosis strain SCAID 187.0 chromosome, complete genome |
| MTB\_12 | >NZ\_CP007809.1 Mycobacterium tuberculosis strain KIT87190, complete genome |
| MTB\_13 | >NZ\_CP025600.1 Mycobacterium tuberculosis strain 77-11 chromosome, complete genome |
| MTB\_14 | >NZ\_CP025599.1 Mycobacterium tuberculosis strain GG-45-11 chromosome, complete genome |
| MTB\_15 | >NZ\_CP025602.1 Mycobacterium tuberculosis strain GG-109-10 chromosome, complete genome |
| MTB\_16 | >NZ\_CP025606.1 Mycobacterium tuberculosis strain GG-137-10 chromosome, complete genome |
| MTB\_17 | >NZ\_CP025596.1 Mycobacterium tuberculosis strain GG-27-11 chromosome, complete genome |
| MTB\_18 | >NZ\_CP025603.1 Mycobacterium tuberculosis strain GG-121-10 chromosome, complete genome |
| MTB\_19 | >NZ\_CP025607.1 Mycobacterium tuberculosis strain GG-186-10 chromosome, complete genome |
| MTB\_20 | >NZ\_CP025598.1 Mycobacterium tuberculosis strain GG-37-11 chromosome, complete genome |
| MTB\_21 | >NZ\_CP025608.1 Mycobacterium tuberculosis strain GG-229-10 chromosome, complete genome |
| MTB\_22 | >NZ\_CP025605.1 Mycobacterium tuberculosis strain GG-134-11 chromosome, complete genome |
| MTB\_23 | >NZ\_CP025595.1 Mycobacterium tuberculosis strain GG-20-11 chromosome, complete genome |
| MTB\_24 | >NZ\_CP025593.1 Mycobacterium tuberculosis strain GG-111-10 chromosome, complete genome |
| MTB\_25 | >NZ\_CP025604.1 Mycobacterium tuberculosis strain GG-129-11 chromosome, complete genome |
| MTB\_26 | >NZ\_CP025594.1 Mycobacterium tuberculosis strain GG-5-10 chromosome, complete genome |
| MTB\_27 | >NZ\_CP025601.1 Mycobacterium tuberculosis strain GG-90-10 chromosome, complete genome |
| MTB\_28 | >NZ\_CP025597.1 Mycobacterium tuberculosis strain 36-11 chromosome, complete genome |
| MTB\_29 | >NZ\_CP023631.1 Mycobacterium tuberculosis strain TBDM1506 chromosome, complete genome |
| MTB\_30 | >NZ\_CP023636.1 Mycobacterium tuberculosis strain TBDM2699 chromosome, complete genome |
| MTB\_31 | >NZ\_CP023626.1 Mycobacterium tuberculosis strain MDRMA1565 chromosome, complete genome |
| MTB\_32 | >NZ\_CP023621.1 Mycobacterium tuberculosis strain LN2900 chromosome, complete genome |
| MTB\_33 | >NZ\_CP023616.1 Mycobacterium tuberculosis strain LN3668 chromosome, complete genome |
| MTB\_34 | >NZ\_CP023611.1 Mycobacterium tuberculosis strain LN763 chromosome, complete genome |
| MTB\_35 | >NZ\_CP023610.1 Mycobacterium tuberculosis strain LN317 chromosome, complete genome |
| MTB\_36 | >NZ\_CP023606.1 Mycobacterium tuberculosis strain LE103 chromosome, complete genome |
| MTB\_37 | >NZ\_CP023605.1 Mycobacterium tuberculosis strain LE79 chromosome, complete genome |
| MTB\_38 | >NZ\_CP023601.1 Mycobacterium tuberculosis strain CSV9577 chromosome, complete genome |
| MTB\_39 | >NZ\_CP023600.1 Mycobacterium tuberculosis strain CSV3611 chromosome, complete genome |
| MTB\_40 | >NZ\_CP023596.1 Mycobacterium tuberculosis strain SLM063 chromosome, complete genome |
| MTB\_41 | >NZ\_CP023595.1 Mycobacterium tuberculosis strain SLM060 chromosome, complete genome |
| MTB\_42 | >NZ\_CP023590.1 Mycobacterium tuberculosis strain TBV5362 chromosome, complete genome |
| MTB\_43 | >NZ\_CP023585.1 Mycobacterium tuberculosis strain MDRDM1098 chromosome, complete genome |
| MTB\_44 | >NZ\_CP023639.1 Mycobacterium tuberculosis strain TBV4768 chromosome, complete genome |
| MTB\_45 | >NZ\_CP023580.1 Mycobacterium tuberculosis strain LN180 chromosome, complete genome |
| MTB\_46 | >NZ\_CP023638.1 Mycobacterium tuberculosis strain TBV4766 chromosome, complete genome |
| MTB\_47 | >NZ\_CP023575.1 Mycobacterium tuberculosis strain CSV5769 chromosome, complete genome |
| MTB\_48 | >NZ\_CP023634.1 Mycobacterium tuberculosis strain TBDM2487 chromosome, complete genome |
| MTB\_49 | >NZ\_CP023633.1 Mycobacterium tuberculosis strain TBDM2444 chromosome, complete genome |
| MTB\_50 | >NZ\_CP023629.1 Mycobacterium tuberculosis strain MDRMA2260 chromosome, complete genome |
| MTB\_51 | >NZ\_CP023628.1 Mycobacterium tuberculosis strain MDRMA2082 chromosome, complete genome |
| MTB\_52 | >NZ\_CP023623.1 Mycobacterium tuberculosis strain MDRMA203 chromosome, complete genome |
| MTB\_53 | >NZ\_CP023618.1 Mycobacterium tuberculosis strain LN3695 chromosome, complete genome |
| MTB\_54 | >NZ\_CP023637.1 Mycobacterium tuberculosis strain TBDM2717 chromosome, complete genome |
| MTB\_55 | >NZ\_CP023613.1 Mycobacterium tuberculosis strain LN3584 chromosome, complete genome |
| MTB\_56 | >NZ\_CP023608.1 Mycobacterium tuberculosis strain LE410 chromosome, complete genome |
| MTB\_57 | >NZ\_CP023632.1 Mycobacterium tuberculosis strain TBDM2189 chromosome, complete genome |
| MTB\_58 | >NZ\_CP023603.1 Mycobacterium tuberculosis strain LE63 chromosome, complete genome |
| MTB\_59 | >NZ\_CP023627.1 Mycobacterium tuberculosis strain MDRMA2019 chromosome, complete genome |
| MTB\_60 | >NZ\_CP023598.1 Mycobacterium tuberculosis strain SLM100 chromosome, complete genome |
| MTB\_61 | >NZ\_CP023622.1 Mycobacterium tuberculosis strain MDRDM827 chromosome, complete genome |
| MTB\_62 | >NZ\_CP023593.1 Mycobacterium tuberculosis strain SLM040 chromosome, complete genome |
| MTB\_63 | >NZ\_CP023617.1 Mycobacterium tuberculosis strain LN3672 chromosome, complete genome |
| MTB\_64 | >NZ\_CP023588.1 Mycobacterium tuberculosis strain TBDM425 chromosome, complete genome |
| MTB\_65 | >NZ\_CP023612.1 Mycobacterium tuberculosis strain LN2978 chromosome, complete genome |
| MTB\_66 | >NZ\_CP023583.1 Mycobacterium tuberculosis strain MDRDM260 chromosome, complete genome |
| MTB\_67 | >NZ\_CP023607.1 Mycobacterium tuberculosis strain LE371 chromosome, complete genome |
| MTB\_68 | >NZ\_CP023578.1 Mycobacterium tuberculosis strain LE486 chromosome, complete genome |
| MTB\_69 | >NZ\_CP023602.1 Mycobacterium tuberculosis strain LE13 chromosome, complete genome |
| MTB\_70 | >NZ\_CP023577.1 Mycobacterium tuberculosis strain CSV11678 chromosome, complete genome |
| MTB\_71 | >NZ\_CP023597.1 Mycobacterium tuberculosis strain SLM088 chromosome, complete genome |
| MTB\_72 | >NZ\_CP023573.1 Mycobacterium tuberculosis strain CSV4519 chromosome, complete genome |
| MTB\_73 | >NZ\_CP023592.1 Mycobacterium tuberculosis strain SLM036 chromosome, complete genome |
| MTB\_74 | >NZ\_CP023591.1 Mycobacterium tuberculosis strain TBV5365 chromosome, complete genome |
| MTB\_75 | >NZ\_CP023587.1 Mycobacterium tuberculosis strain ME1473 chromosome, complete genome |
| MTB\_76 | >NZ\_CP023586.1 Mycobacterium tuberculosis strain MDRMA2491 chromosome, complete genome |
| MTB\_77 | >NZ\_CP023582.1 Mycobacterium tuberculosis strain LN3756 chromosome, complete genome |
| MTB\_78 | >NZ\_CP023581.1 Mycobacterium tuberculosis strain LN2358 chromosome, complete genome |
| MTB\_79 | >NZ\_CP023576.1 Mycobacterium tuberculosis strain CSV10399 chromosome, complete genome |
| MTB\_80 | >NZ\_CP023640.1 Mycobacterium tuberculosis strain TBV4952 chromosome, complete genome |
| MTB\_81 | >NZ\_CP023635.1 Mycobacterium tuberculosis strain TBDM2489 chromosome, complete genome |
| MTB\_82 | >NZ\_CP023630.1 Mycobacterium tuberculosis strain MDRMA2441 chromosome, complete genome |
| MTB\_83 | >NZ\_CP023609.1 Mycobacterium tuberculosis strain LN55 chromosome, complete genome |
| MTB\_84 | >NZ\_CP023625.1 Mycobacterium tuberculosis strain MDRMA863 chromosome, complete genome |
| MTB\_85 | >NZ\_CP023604.1 Mycobacterium tuberculosis strain LE76 chromosome, complete genome |
| MTB\_86 | >NZ\_CP023599.1 Mycobacterium tuberculosis strain CSV383 chromosome, complete genome |
| MTB\_87 | >NZ\_CP023624.1 Mycobacterium tuberculosis strain MDRMA701 chromosome, complete genome |
| MTB\_88 | >NZ\_CP023620.1 Mycobacterium tuberculosis strain LN1856 chromosome, complete genome |
| MTB\_89 | >NZ\_CP023594.1 Mycobacterium tuberculosis strain SLM056 chromosome, complete genome |
| MTB\_90 | >NZ\_CP023619.1 Mycobacterium tuberculosis strain LN1100 chromosome, complete genome |
| MTB\_91 | >NZ\_CP023589.1 Mycobacterium tuberculosis strain TBV5000 chromosome, complete genome |
| MTB\_92 | >NZ\_CP023615.1 Mycobacterium tuberculosis strain LN3589 chromosome, complete genome |
| MTB\_93 | >NZ\_CP023584.1 Mycobacterium tuberculosis strain MDRDM627 chromosome, complete genome |
| MTB\_94 | >NZ\_CP023614.1 Mycobacterium tuberculosis strain LN3588 chromosome, complete genome |
| MTB\_95 | >NZ\_CP023579.1 Mycobacterium tuberculosis strain LE492 chromosome, complete genome |
| MTB\_96 | >NZ\_CP023574.1 Mycobacterium tuberculosis strain CSV4644 chromosome, complete genome |
| MTB\_97 | >NZ\_AP017901.1 Mycobacterium tuberculosis DNA, complete genome, strain: NCGM946K2 |
| MTB\_98 | >NZ\_CP018778.1 Mycobacterium tuberculosis strain DK9897, complete genome |
| MTB\_99 | >NZ\_CP011510.1 Mycobacterium tuberculosis strain Beijing, complete genome |
| MTB\_100 | >NZ\_CP016794.1 Mycobacterium tuberculosis strain SCAID 320.0 chromosome, complete genome |
| MTB\_101 | >NZ\_CP010330.1 Mycobacterium tuberculosis strain F28, complete genome |
| MTB\_102 | >NZ\_CP010337.1 Mycobacterium tuberculosis strain 22115, complete genome |
| MTB\_103 | >NZ\_CP010339.1 Mycobacterium tuberculosis strain 22103, complete genome |
| MTB\_104 | >NZ\_CP009100.1 Mycobacterium tuberculosis strain ZMC13-264, complete genome |
| MTB\_105 | >NZ\_CP009101.1 Mycobacterium tuberculosis strain ZMC13-88, complete genome |
| MTB\_106 | >NZ\_CP009426.1 Mycobacterium tuberculosis strain 96075, complete genome |
| MTB\_107 | >NZ\_CP009427.1 Mycobacterium tuberculosis strain 96121, complete genome |
| MTB\_108 | >NZ\_CP017596.1 Mycobacterium tuberculosis strain Beijing/391 chromosome, complete genome |
| MTB\_109 | >NZ\_CP017595.1 Mycobacterium tuberculosis strain Beijing-like/38774 chromosome, complete genome |
| MTB\_110 | >NZ\_CP017598.1 Mycobacterium tuberculosis strain Beijing-like/1104 chromosome, complete genome |
| MTB\_111 | >NZ\_CP017593.1 Mycobacterium tuberculosis strain Beijing-like/35049 chromosome, complete genome |
| MTB\_112 | >NZ\_CP017597.1 Mycobacterium tuberculosis strain Beijing-like/50148 chromosome, complete genome |
| MTB\_113 | >NZ\_CP017594.1 Mycobacterium tuberculosis strain Beijing-like/36918 chromosome, complete genome |
| MTB\_114 | >NZ\_CP013475.1 Mycobacterium tuberculosis strain 1458, complete genome |
| MTB\_115 | >NZ\_CP018301.1 Mycobacterium tuberculosis strain I0002801-4, complete genome |
| MTB\_116 | >NZ\_CP018304.1 Mycobacterium tuberculosis strain M0002959-6, complete genome |
| MTB\_117 | >NZ\_CP018300.1 Mycobacterium tuberculosis strain I0002353-6, complete genome |
| MTB\_118 | >NZ\_CP018305.1 Mycobacterium tuberculosis strain M0018684-2, complete genome |
| MTB\_119 | >NZ\_CP018303.1 Mycobacterium tuberculosis strain I0004241-1, complete genome |
| MTB\_120 | >NZ\_CP018302.1 Mycobacterium tuberculosis strain I0004000-1, complete genome |
| MTB\_121 | >NZ\_CP016888.1 Mycobacterium tuberculosis strain SCAID 252.0 chromosome, complete genome |
| MTB\_122 | >NZ\_CP010968.1 Mycobacterium tuberculosis strain PR10 genome |
| MTB\_123 | >NZ\_CP010895.1 Mycobacterium tuberculosis strain PR08 genome |
| MTB\_124 | >NZ\_KK338747.1 Mycobacterium tuberculosis strain MAL010075 adPaI-supercont1.1, whole genome shotgun sequence |
| MTB\_125 | >NZ\_CM007646.1 Mycobacterium tuberculosis strain 410 chromosome, whole genome shotgun sequence |
| MTB\_126 | >NZ\_CM007645.1 Mycobacterium tuberculosis strain 6548 chromosome, whole genome shotgun sequence |
| MTB\_127 | >NZ\_MNBY01000001.1 Mycobacterium tuberculosis strain TB284 Contig1, whole genome shotgun sequence |
| MTB\_128 | >NZ\_MRGR01000001.1 Mycobacterium tuberculosis strain M0002521-4 M0002521-4\_contig1, whole genome shotgun sequence |
| MTB\_129 | >NZ\_MRJE01000001.1 Mycobacterium tuberculosis strain M0021686-2 M0021686-2\_contig1, whole genome shotgun sequence |
| MTB\_130 | >NZ\_MRIY01000001.1 Mycobacterium tuberculosis strain M0022470-0 M0022470-0\_contig1, whole genome shotgun sequence |
| MTB\_131 | >NZ\_MRIT01000001.1 Mycobacterium tuberculosis strain M0014870-1 M0014870-1\_contig1, whole genome shotgun sequence |
| MTB\_132 | >NZ\_MRII01000001.1 Mycobacterium tuberculosis strain M0004757-2 M0004757-2\_contig1, whole genome shotgun sequence |
| MTB\_133 | >NZ\_MRJF01000001.1 Mycobacterium tuberculosis strain M0000956-4 M0000956-4\_contig1, whole genome shotgun sequence |
| MTB\_134 | >NZ\_MRIK01000001.1 Mycobacterium tuberculosis strain M0005676-3 M0005676-3\_contig1, whole genome shotgun sequence |
| MTB\_135 | >NZ\_MRGW01000001.1 Mycobacterium tuberculosis strain M0012810-9 M0012810-9\_contig1, whole genome shotgun sequence |
| MTB\_136 | >NZ\_MRGJ01000001.1 Mycobacterium tuberculosis strain M0013032-9 M0013032-9\_contig1, whole genome shotgun sequence |
| MTB\_137 | >NZ\_MRGH01000001.1 Mycobacterium tuberculosis strain M0006465-0 M0006465-0\_contig1, whole genome shotgun sequence |
| MTB\_138 | >NZ\_MRGK01000001.1 Mycobacterium tuberculosis strain M0014892-5 M0014892-5\_contig1, whole genome shotgun sequence |
| MTB\_139 | >NZ\_MRGP01000001.1 Mycobacterium tuberculosis strain M0001638-7 M0001638-7\_contig1, whole genome shotgun sequence |
| MTB\_140 | >NZ\_MRHA01000001.1 Mycobacterium tuberculosis strain M0006024-5 M0006024-5\_contig1, whole genome shotgun sequence |
| MTB\_141 | >NZ\_MRGN01000001.1 Mycobacterium tuberculosis strain M0000827-7 M0000827-7\_contig1, whole genome shotgun sequence |
| MTB\_142 | >NZ\_MRGS01000003.1 Mycobacterium tuberculosis strain M0002998-4 M0002998-4\_contig3, whole genome shotgun sequence |
| MTB\_143 | >NZ\_MRGI01000001.1 Mycobacterium tuberculosis strain M0009182-8 M0009182-8\_contig1, whole genome shotgun sequence |
| MTB\_144 | >NZ\_NARI00000000.1 Mycobacterium tuberculosis strain MTB9, whole genome shotgun sequencing project |
| MTB\_145 | >NZ\_NARL00000000.1 Mycobacterium tuberculosis strain MTB2, whole genome shotgun sequencing project |
| MTB\_146 | >NZ\_NARJ00000000.1 Mycobacterium tuberculosis strain MTB8, whole genome shotgun sequencing project |
| MTB\_147 | >NZ\_NARM00000000.1 Mycobacterium tuberculosis strain MTB1, whole genome shotgun sequencing project |
| MTB\_148 | >NZ\_MQGD01000001.1 Mycobacterium tuberculosis strain I0001224-0 I0001224-0\_contig1, whole genome shotgun sequence |
| MTB\_149 | >NZ\_MMXV01000001.1 Mycobacterium tuberculosis strain 01-R1599 01-R1599\_contig1, whole genome shotgun sequence |
| MTB\_150 | >NZ\_MNAA01000001.1 Mycobacterium tuberculosis strain 01-R0685 01-R0685\_contig1, whole genome shotgun sequence |
| MTB\_151 | >NZ\_MMVS01000002.1 Mycobacterium tuberculosis strain 04-R0275 04-R0275\_contig2, whole genome shotgun sequence |
| MTB\_152 | >NZ\_MMYL01000001.1 Mycobacterium tuberculosis strain 02-R0990 02-R0990\_contig1, whole genome shotgun sequence |
| MTB\_153 | >NZ\_MMOL01000001.1 Mycobacterium tuberculosis strain 99-R893 99-R893\_contig1, whole genome shotgun sequence |
| MTB\_154 | >NZ\_MMPA01000001.1 Mycobacterium tuberculosis strain 01-R0272 01-R0272\_contig1, whole genome shotgun sequence |
| MTB\_155 | >NZ\_MMOU01000001.1 Mycobacterium tuberculosis strain 01-R0953 01-R0953\_contig1, whole genome shotgun sequence |
| MTB\_156 | >NZ\_MMBM01000003.1 Mycobacterium tuberculosis strain I0003938-3 I0003938-3\_contig3, whole genome shotgun sequence |
| MTB\_157 | >NZ\_MMAF01000001.1 Mycobacterium tuberculosis strain I0004557-0 I0004557-0\_contig1, whole genome shotgun sequence |
| MTB\_158 | >NZ\_MMBZ01000001.1 Mycobacterium tuberculosis strain I0002987-1 I0002987-1\_contig1, whole genome shotgun sequence |
| MTB\_159 | >NZ\_MMMH01000001.1 Mycobacterium tuberculosis strain I0005760-9 I0005760-9\_contig1, whole genome shotgun sequence |
| MTB\_160 | >NZ\_MMCW01000001.1 Mycobacterium tuberculosis strain I0003229-7 I0003229-7\_contig1, whole genome shotgun sequence |
| MTB\_161 | >NZ\_MMOE01000001.1 Mycobacterium tuberculosis strain M0002202-1 M0002202-1\_contig1, whole genome shotgun sequence |
| MTB\_162 | >NZ\_MMNZ01000001.1 Mycobacterium tuberculosis strain M0003875-3 M0003875-3\_contig1, whole genome shotgun sequence |
| MTB\_163 | >NZ\_MMNX01000001.1 Mycobacterium tuberculosis strain M0005016-2 M0005016-2\_contig1, whole genome shotgun sequence |
| MTB\_164 | >NZ\_MMHJ01000001.1 Mycobacterium tuberculosis strain 02-R0325 02-R0325\_contig1, whole genome shotgun sequence |
| MTB\_165 | >NZ\_MMNU01000001.1 Mycobacterium tuberculosis strain M0006397-5 M0006397-5\_contig1, whole genome shotgun sequence |
| MTB\_166 | >NZ\_MMLS01000001.1 Mycobacterium tuberculosis strain I0003088-7 I0003088-7\_contig1, whole genome shotgun sequence |
| MTB\_167 | >NZ\_MMNG01000003.1 Mycobacterium tuberculosis strain M0021577-3 M0021577-3\_contig3, whole genome shotgun sequence |
| MTB\_168 | >NZ\_MMHA01000001.1 Mycobacterium tuberculosis strain 01-R0240 01-R0240\_contig1, whole genome shotgun sequence |
| MTB\_169 | >NZ\_MMHD01000001.1 Mycobacterium tuberculosis strain 01-R1556 01-R1556\_contig1, whole genome shotgun sequence |
| MTB\_170 | >NZ\_MMGE01000001.1 Mycobacterium tuberculosis strain 02-R0020 02-R0020\_contig1, whole genome shotgun sequence |
| MTB\_171 | >NZ\_MMNF01000001.1 Mycobacterium tuberculosis strain M0021672-2 M0021672-2\_contig1, whole genome shotgun sequence |
| MTB\_172 | >NZ\_MMMP01000001.1 Mycobacterium tuberculosis strain M0014296-9 M0014296-9\_contig1, whole genome shotgun sequence |
| MTB\_173 | >NZ\_LXWG00000000.1 Mycobacterium tuberculosis strain NZ494, whole genome shotgun sequencing project |
| MTB\_174 | >NZ\_LUDZ00000000.1 Mycobacterium tuberculosis strain SIT745/EAI1-MYS, whole genome shotgun sequencing project |

**附录二：**

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| 纯NTM样本（17） | |
| 样本编号 | 纯NTM样本 |
| NTM\_5 | >NC\_022663.1 Mycobacterium kansasii ATCC 12478, complete genome |
| NTM\_6 | >NZ\_HG917972.2 Mycobacterium marinum E11 main chromosome genome |
| NTM\_7 | >NZ\_LQPX01000034.1 Mycobacterium terrae strain CIP 104321 CONTIG\_4, whole genome shotgun sequence |
| NTM\_8 | >NZ\_LQPZ01000008.1 Mycobacterium triviale strain DSM 44153 CONTIG\_16, whole genome shotgun sequence |
| NTM\_9 | >NZ\_MDUB01000418.1 Mycobacterium ulcerans strain S4018 CONT697, whole genome shotgun sequence |
| NTM\_10 | >NZ\_LQOY01000081.1 Mycobacterium gordonae strain DSM 44160 CONTIG\_171, whole genome shotgun sequence |
| NTM\_11 | >NZ\_LQQB01000146.1 Mycobacterium xenopi strain DSM 43995 CONTIG\_99, whole genome shotgun sequence |
| NTM\_12 | >NZ\_AGAQ01000220.1 Mycobacterium avium subsp. avium Env 77 MAVIUM-ENV77-CONTIG220, whole genome shotgun sequence |
| NTM\_13 | >NZ\_MVIJ01000001.1 Mycobacterium scrofulaceum strain DSM 43992 NODE\_1\_LENGTH\_467512\_COV\_40.3479, whole genome shotgun sequence |
| NTM\_14 | >NZ\_LQPW01000031.1 Mycobacterium szulgai strain DSM 44166 CONTIG\_126, whole genome shotgun sequence |
| NTM\_15 | >NZ\_CP007220.1 Mycobacterium chelonae CCUG 47445, complete genome |
| NTM\_16 | >NZ\_CP011269.1 Mycobacterium fortuitum strain CT6, complete genome |
| NTM\_17 | >NC\_008596.1 Mycobacterium smegmatis str. MC2 155 chromosome, complete genome |
| NTM\_18 | >NC\_010397.1 Mycobacterium abscessus chromosome, complete sequence |
| NTM\_19 | >NZ\_AZYN01000324.1 Mycobacterium gastri 'Wayne' CONTIG324, whole genome shotgun sequence |
| NTM\_20 | >NC\_016946.1 Mycobacterium intracellulare ATCC 13950, complete genome |
| NTM\_21 | >NZ\_CP014475.1 Mycobacterium phlei strain CCUG 21000, complete genome |

**附录三：交叉反应原物种**

|  |  |
| --- | --- |
| 交叉反应物种 | |
| 样本编号 | 样本 |
| S1 | >NC\_003098.1 Streptococcus pneumoniae R6 chromosome, complete genome |
| S2 | >NC\_007795.1 Staphylococcus aureus subsp. aureus NCTC 8325 chromosome, complete genome |
| S3 | >NC\_000907.1 Haemophilus influenzae Rd KW20 chromosome, complete genome |
| S4 | >NZ\_BAED01000083.1 Gordonia amarae NBRC 15530, whole genome shotgun sequence |
| S5 | >NC\_002516.2 Pseudomonas aeruginosa PAO1 chromosome, complete genome |
| S6 | >NC\_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome |
| S7 | >NC\_032089.1 Candida albicans SC5314 chromosome 1 sequence |
| S8 | >NC\_004461.1 Staphylococcus epidermidis ATCC 12228 chromosome, complete genome |
| S9 | >BCLC01000001.1 Cryptococcus sp. JCM 24511 DNA, scaffold: scaffold\_0, strain: JCM 24511, whole genome shotgun sequence |
| S10 | >NC\_007373.1 Influenza A virus (A/New York/392/2004(H3N2)) segment 1, complete sequence |
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