

### C.3 Example `drprg` prediction report

Figure C.6 is an example of the JSON file output from `drprg predict` (see Section 4.3.2). This JSON file shows the resistance prediction for each drug and the supporting evidence. It has had some drugs removed for brevity.

### C.4 Adjustment of default `mykrobe` Nanopore settings

During the process of gathering the resistance prediction results in Section 4.5 and Section 4.4, we investigated whether the `mykrobe` default parameters for Nanopore are optimal. Since they were calibrated on only five samples [44]. In addition, we look at the impact of changing the default Illumina expected error rate (0.05) to the reported 0.001 [228].

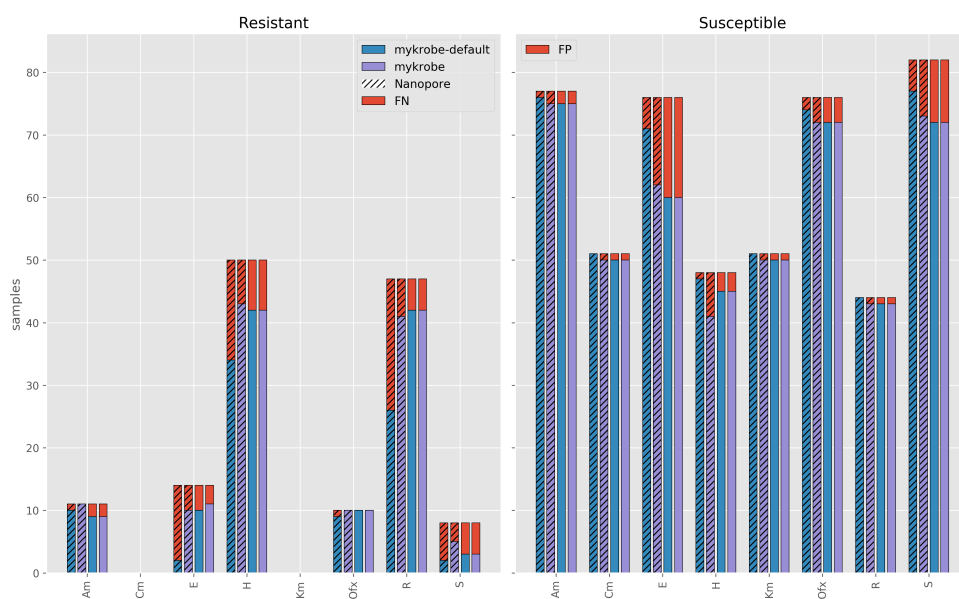
The Nanopore preset in `mykrobe` sets the expected error rate to 0.15, the ploidy model to haploid, and ignores calls below the 90% confidence threshold (as judged by simulating confidence scores from those present in the data) [44]. Instead, we disable this preset and change the expected error rate to 0.08, leave the ploidy as haploid (avoiding minor resistance calls), and turn off the confidence threshold simulations.

The results of these parameter changes are shown in Figure C.7 and summarised in Table C.2 for concordance with phenotype (see Section 4.5). In addition, as culture-based phenotypes are not available for all drugs and samples, we compare the concordance of Nanopore predictions with Illumina in Figure C.8 and Table C.3.

When comparing the WGS concordance with culture-based phenotypes, these results reveal that the default Nanopore settings for `mykrobe` lead to a much higher number of missed resistance (FN) classifications for all drugs when compared to the adjusted settings. However, the default settings do lead to fewer false-positive predictions for all drugs. Additionally, the adjusted Illumina error rate led to one FN being recovered (classified TP) for ethambutol and unchanged classifications for everything else.

Nanopore concordance with Illumina predictions shows default settings lead to more FN calls than with adjusted settings, especially for the first-line drugs ethambutol, isoniazid, and rifampicin. The adjusted settings for `mykrobe` led to quite similar predictions between Nanopore and Illumina. Additionally, they lead to much less Nanopore missed resistance calls but more false positives. However, missed resistance

## Supporting work for Chapter 4



**Fig. C.7:** Number of resistant (left) and susceptible (right) culture-based drug susceptibility phenotypes correctly identified by mykrobe with default (blue) or adjusted (purple) settings. Nanopore data is indicated by diagonal stripes, with Illumina having no stripes. The red bars indicate missed (FN) or incorrect (FP) predictions. The x-axis shows the drugs with available phenotype data. E - ethambutol; H - isoniazid; R - rifampicin; S - streptomycin; Km - kanamycin; Am - amikacin; Ofx - ofloxacin; Cm - capreomycin.

is generally deemed a "worse" error, and as such, for the work in [Chapter 4](#) we chose to use these adjusted parameters for mykrobe.

## C.4 Adjustment of default mykrobe Nanopore settings

Drug	Technology	mykrobe	FN(R)	FP(S)	FNR(95% CI)	FPR(95% CI)	PPV(95% CI)	NPV(95% CI)
Amikacin	Nanopore	mykrobe-default	1(11)	1(77)	9.1% (1.6-37.7%)	1.3% (0.2-7.0%)	90.9% (62.3-98.4%)	98.7% (93.0-99.8%)
		mykrobe	0(11)	2(77)	0.0% (0.0-25.9%)	2.6% (0.7-9.0%)	84.6% (57.8-95.7%)	100.0% (95.1-100.0%)
	Illumina	mykrobe-default	2(11)	2(77)	18.2% (5.1-47.7%)	2.6% (0.7-9.0%)	81.8% (52.3-94.9%)	97.4% (91.0-99.3%)
		mykrobe	2(11)	2(77)	18.2% (5.1-47.7%)	2.6% (0.7-9.0%)	81.8% (52.3-94.9%)	97.4% (91.0-99.3%)
Capreomycin	Nanopore	mykrobe-default	0(0)	0(51)	-	0.0% (0.0-7.0%)	-	100.0% (93.0-100.0%)
		mykrobe	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
	Illumina	mykrobe-default	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
		mykrobe	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
Ethambutol	Nanopore	mykrobe-default	12(14)	5(76)	85.7% (60.1-96.0%)	<b>6.6%</b> (2.8-14.5%)	28.6% (8.2-64.1%)	85.5% (76.4-91.5%)
		mykrobe	4(14)	14(76)	<b>28.6%</b> (11.7-54.6%)	18.4% (11.3-28.6%)	41.7% (24.5-61.2%)	93.9% (85.4-97.6%)
	Illumina	mykrobe-default	4(14)	16(76)	28.6% (11.7-54.6%)	21.1% (13.4-31.5%)	38.5% (22.4-57.5%)	93.8% (85.0-97.5%)
		mykrobe	3(14)	16(76)	21.4% (7.6-47.6%)	21.1% (13.4-31.5%)	40.7% (24.5-59.3%)	95.2% (86.9-98.4%)
Isoniazid	Nanopore	mykrobe-default	16(50)	1(48)	32.0% (20.8-45.8%)	<b>2.1%</b> (0.4-10.9%)	97.1% (85.5-99.5%)	74.6% (62.7-83.7%)
		mykrobe	7(50)	7(48)	<b>14.0%</b> (7.0-26.2%)	14.6% (7.2-27.2%)	86.0% (73.8-93.0%)	85.4% (72.8-92.8%)
	Illumina	mykrobe-default	8(50)	3(48)	16.0% (8.3-28.5%)	6.2% (2.1-16.8%)	93.3% (82.1-97.7%)	84.9% (72.9-92.1%)
		mykrobe	8(50)	3(48)	16.0% (8.3-28.5%)	6.2% (2.1-16.8%)	93.3% (82.1-97.7%)	84.9% (72.9-92.1%)
Kanamycin	Nanopore	mykrobe-default	0(0)	0(51)	-	0.0% (0.0-7.0%)	-	100.0% (93.0-100.0%)
		mykrobe	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
	Illumina	mykrobe-default	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
		mykrobe	0(0)	1(51)	-	2.0% (0.3-10.3%)	0.0% (0.0-79.3%)	100.0% (92.9-100.0%)
Ofloxacin	Nanopore	mykrobe-default	1(10)	2(76)	10.0% (1.8-40.4%)	2.6% (0.7-9.1%)	81.8% (52.3-94.9%)	98.7% (92.8-99.8%)
		mykrobe	0(10)	4(76)	0.0% (-0.0-27.8%)	5.3% (2.1-12.8%)	71.4% (45.4-88.3%)	100.0% (94.9-100.0%)
	Illumina	mykrobe-default	0(10)	4(76)	0.0% (-0.0-27.8%)	5.3% (2.1-12.8%)	71.4% (45.4-88.3%)	100.0% (94.9-100.0%)
		mykrobe	0(10)	4(76)	0.0% (-0.0-27.8%)	5.3% (2.1-12.8%)	71.4% (45.4-88.3%)	100.0% (94.9-100.0%)
Rifampicin	Nanopore	mykrobe-default	21(47)	0(44)	44.7% (31.4-58.8%)	0.0% (0.0-8.0%)	100.0% (87.1-100.0%)	67.7% (55.6-77.8%)
		mykrobe	6(47)	1(44)	<b>12.8%</b> (6.0-25.2%)	2.3% (0.4-11.8%)	97.6% (87.7-99.6%)	87.8% (75.8-94.3%)
	Illumina	mykrobe-default	5(47)	1(44)	10.6% (4.6-22.6%)	2.3% (0.4-11.8%)	97.7% (87.9-99.6%)	89.6% (77.8-95.5%)
		mykrobe	5(47)	1(44)	10.6% (4.6-22.6%)	2.3% (0.4-11.8%)	97.7% (87.9-99.6%)	89.6% (77.8-95.5%)
Streptomycin	Nanopore	mykrobe-default	6(8)	5(82)	75.0% (40.9-92.9%)	<b>6.1%</b> (2.6-13.5%)	28.6% (8.2-64.1%)	92.8% (85.1-96.6%)
		mykrobe	3(8)	9(82)	<b>37.5%</b> (13.7-69.4%)	11.0% (5.9-19.6%)	35.7% (16.3-61.2%)	96.1% (89.0-98.6%)
	Illumina	mykrobe-default	5(8)	10(82)	62.5% (30.6-86.3%)	12.2% (6.8-21.0%)	23.1% (8.2-50.3%)	93.5% (85.7-97.2%)
		mykrobe	5(8)	10(82)	62.5% (30.6-86.3%)	12.2% (6.8-21.0%)	23.1% (8.2-50.3%)	93.5% (85.7-97.2%)

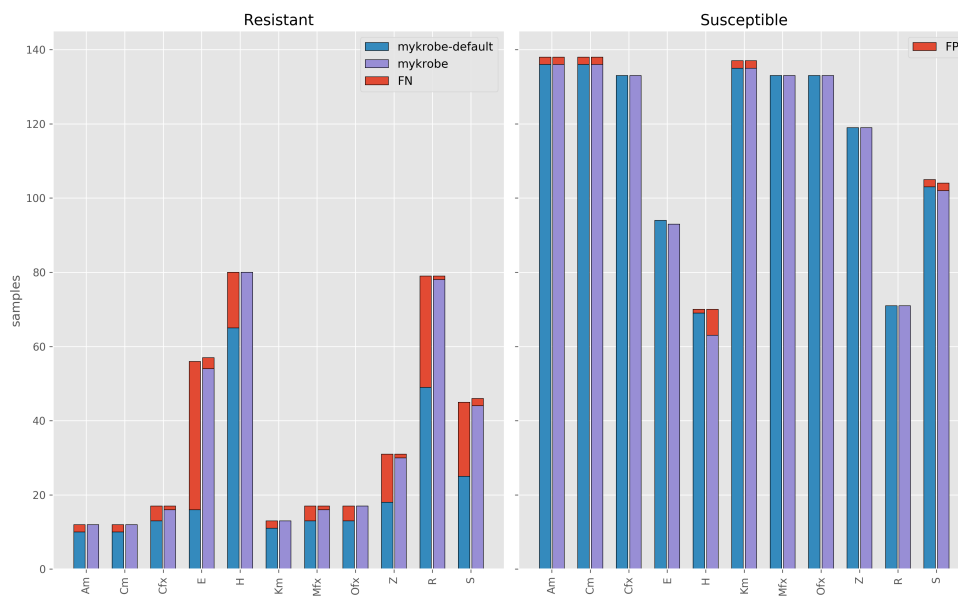
**Table C.2:** Comparison of WGS drug resistance predictions with culture-based phenotype. For this comparison, we assume the culture-based phenotype is correct and evaluate mykrobe with default and adjusted settings for Illumina and Nanopore resistance predictions accordingly. Pyrazinamide and Moxifloxacin are excluded as phenotype information is only available for one sample. Bold text is used to highlight differences of note. FN=false negative; R=number of resistant samples; FP=false positive; S=number of susceptible samples; FNR=false negative rate; FPR=false positive rate; PPV=positive predictive value; NPV=negative predictive value; CI=Wilson score confidence interval

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Drug	Tool	FN(R)	FP(S)	FNR(95% CI)	FPR(95% CI)	PPV(95% CI)	NPV(95% CI)
Amikacin	mykrobe-default	2(12)	2(138)	16.7% (4.7-44.8%)	1.4% (0.4-5.1%)	83.3% (55.2-95.3%)	98.6% (94.9-99.6%)
	mykrobe	0(12)	2(138)	<b>0.0% (0.0-24.2%)</b>	1.4% (0.4-5.1%)	85.7% (60.1-96.0%)	100.0% (97.3-100.0%)
Capreomycin	mykrobe-default	2(12)	2(138)	16.7% (4.7-44.8%)	1.4% (0.4-5.1%)	83.3% (55.2-95.3%)	98.6% (94.9-99.6%)
	mykrobe	0(12)	2(138)	<b>0.0% (0.0-24.2%)</b>	1.4% (0.4-5.1%)	85.7% (60.1-96.0%)	100.0% (97.3-100.0%)
Ciprofloxacin	mykrobe-default	4(17)	0(133)	23.5% (9.6-47.3%)	0.0% (0.0-2.8%)	100.0% (77.2-100.0%)	97.1% (92.7-98.9%)
	mykrobe	1(17)	0(133)	<b>5.9% (1.0-27.0%)</b>	0.0% (0.0-2.8%)	100.0% (80.6-100.0%)	99.3% (95.9-99.9%)
Ethambutol	mykrobe-default	40(56)	0(94)	71.4% (58.5-81.6%)	0.0% (0.0-3.9%)	100.0% (80.6-100.0%)	70.1% (61.9-77.2%)
	mykrobe	3(57)	0(93)	<b>5.3% (1.8-14.4%)</b>	0.0% (0.0-4.0%)	100.0% (93.4-100.0%)	96.9% (91.2-98.9%)
Isoniazid	mykrobe-default	15(80)	1(70)	18.8% (11.7-28.7%)	<b>1.4% (0.3-7.7%)</b>	98.5% (91.9-99.7%)	82.1% (72.6-88.9%)
	mykrobe	0(80)	7(70)	<b>0.0% (0.0-4.6%)</b>	10.0% (4.9-19.2%)	92.0% (84.3-96.0%)	100.0% (94.3-100.0%)
Kanamycin	mykrobe-default	2(13)	2(137)	15.4% (4.3-42.2%)	1.5% (0.4-5.2%)	84.6% (57.8-95.7%)	98.5% (94.8-99.6%)
	mykrobe	0(13)	2(137)	<b>0.0% (0.0-22.8%)</b>	1.5% (0.4-5.2%)	86.7% (62.1-96.3%)	100.0% (97.2-100.0%)
Moxifloxacin	mykrobe-default	4(17)	0(133)	23.5% (9.6-47.3%)	0.0% (0.0-2.8%)	100.0% (77.2-100.0%)	97.1% (92.7-98.9%)
	mykrobe	1(17)	0(133)	<b>5.9% (1.0-27.0%)</b>	0.0% (0.0-2.8%)	100.0% (80.6-100.0%)	99.3% (95.9-99.9%)
Ofloxacin	mykrobe-default	4(17)	0(133)	23.5% (9.6-47.3%)	0.0% (0.0-2.8%)	100.0% (77.2-100.0%)	97.1% (92.7-98.9%)
	mykrobe	0(17)	0(133)	<b>0.0% (0.0-18.4%)</b>	0.0% (0.0-2.8%)	100.0% (81.6-100.0%)	100.0% (97.2-100.0%)
Pyrazinamide	mykrobe-default	13(31)	0(119)	41.9% (26.4-59.2%)	0.0% (0.0-3.1%)	100.0% (82.4-100.0%)	90.2% (83.9-94.2%)
	mykrobe	1(31)	0(119)	<b>3.2% (0.6-16.2%)</b>	0.0% (0.0-3.1%)	100.0% (88.6-100.0%)	99.2% (95.4-99.9%)
Rifampicin	mykrobe-default	30(79)	0(71)	38.0% (28.1-49.0%)	0.0% (0.0-5.1%)	100.0% (92.7-100.0%)	70.3% (60.8-78.3%)
	mykrobe	1(79)	0(71)	<b>1.3% (0.2-6.8%)</b>	0.0% (0.0-5.1%)	100.0% (95.3-100.0%)	98.6% (92.5-99.8%)
Streptomycin	mykrobe-default	20(45)	2(105)	44.4% (30.9-58.8%)	1.9% (0.5-6.7%)	92.6% (76.6-97.9%)	83.7% (76.2-89.2%)
	mykrobe	2(46)	2(104)	<b>4.3% (1.2-14.5%)</b>	1.9% (0.5-6.7%)	95.7% (85.5-98.8%)	98.1% (93.3-99.5%)

**Table C.3:** Comparison of Nanopore drug resistance predictions concordance with Illumina predictions. For this comparison, we assume the mykrobe resistance prediction from Illumina data is correct and evaluate the Nanopore prediction accordingly. mykrobe-default and mykrobe indicates mykrobe with default or adjusted settings, respectively. Bold text is used to highlight differences of note. FN=false negative; R=number of resistant samples; FP=false positive; S=number of susceptible samples; FNR=false negative rate; FPR=false positive rate; PPV=positive predictive value; NPV=negative predictive value; CI=Wilson score confidence interval

## C.4 Adjustment of default mykrobe Nanopore settings



**Fig. C.8:** Number of resistant (left) and susceptible (right) Illumina WGS-based drug susceptibility phenotypes correctly identified using Nanopore data. Nanopore predictions for mykrobe with default (blue) and adjusted (purple) settings are compared to those from Illumina with the same settings. The red bars indicate missed (FN) or incorrect (FP) predictions. The x-axis shows the drugs with available phenotype data. E - ethambutol; H - isoniazid; Z - pyrazinamide; R - rifampicin; S - streptomycin; Km - kanamycin; Am - amikacin; Ofx - ofloxacin; Cm - capreomycin; Mfx - moxifloxacin.