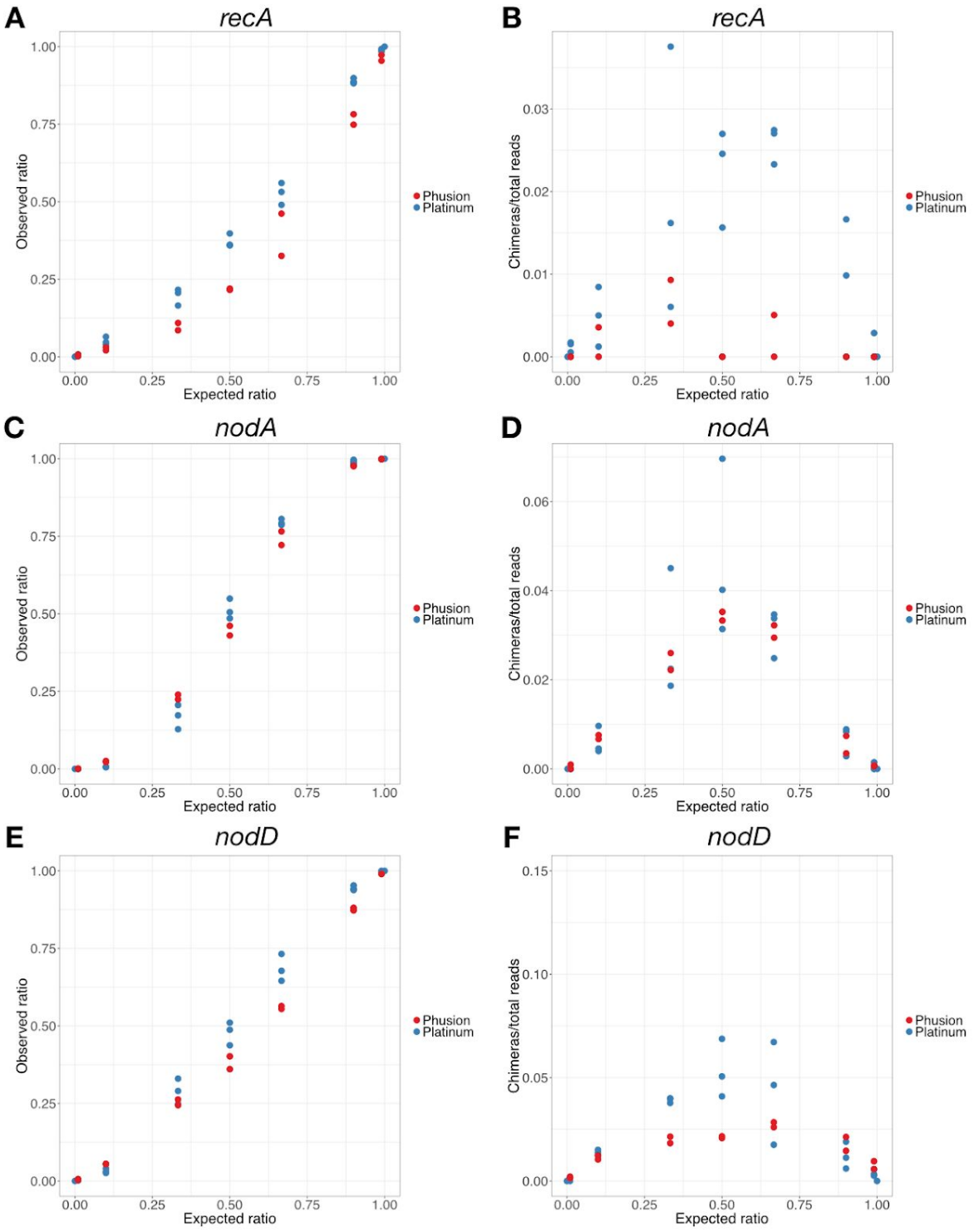
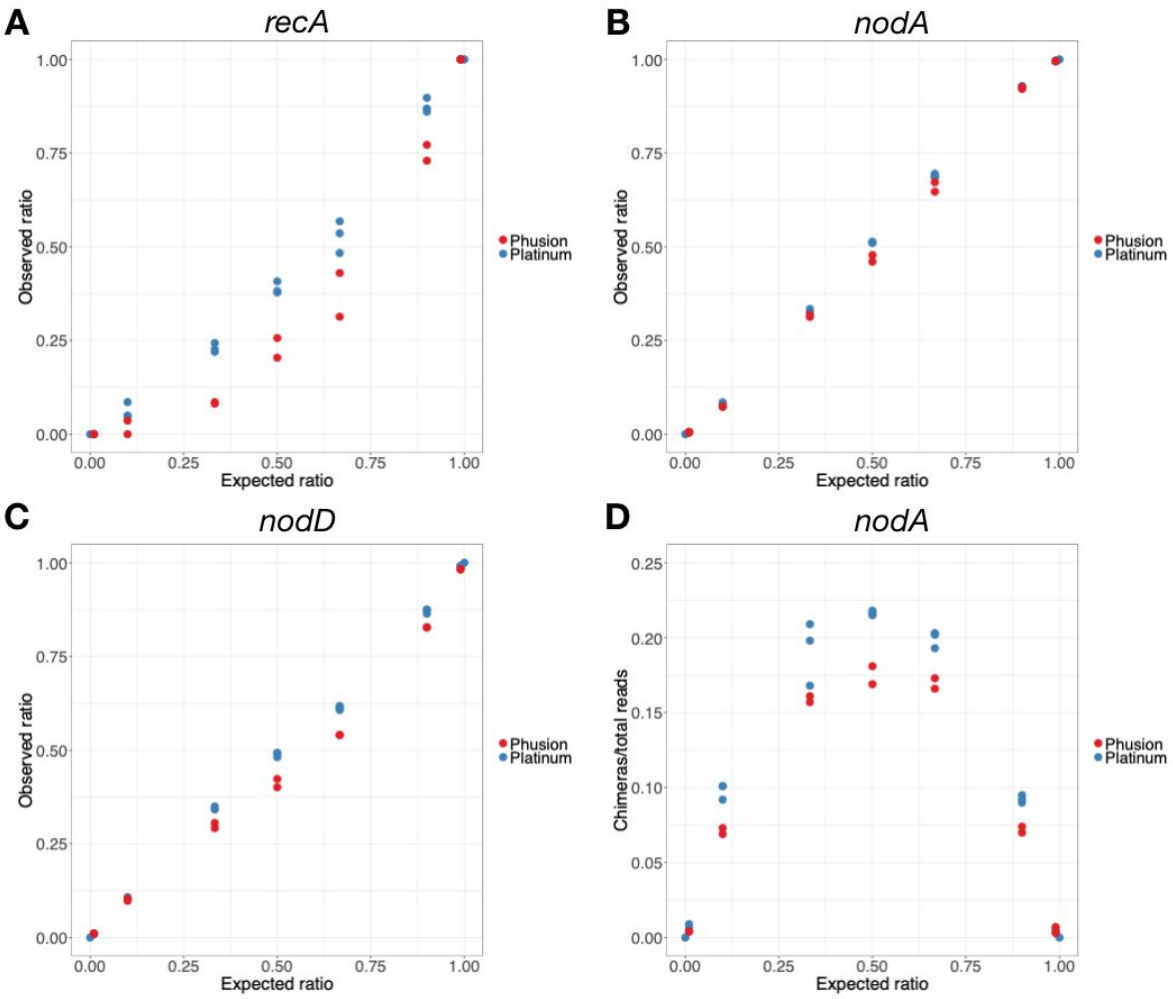
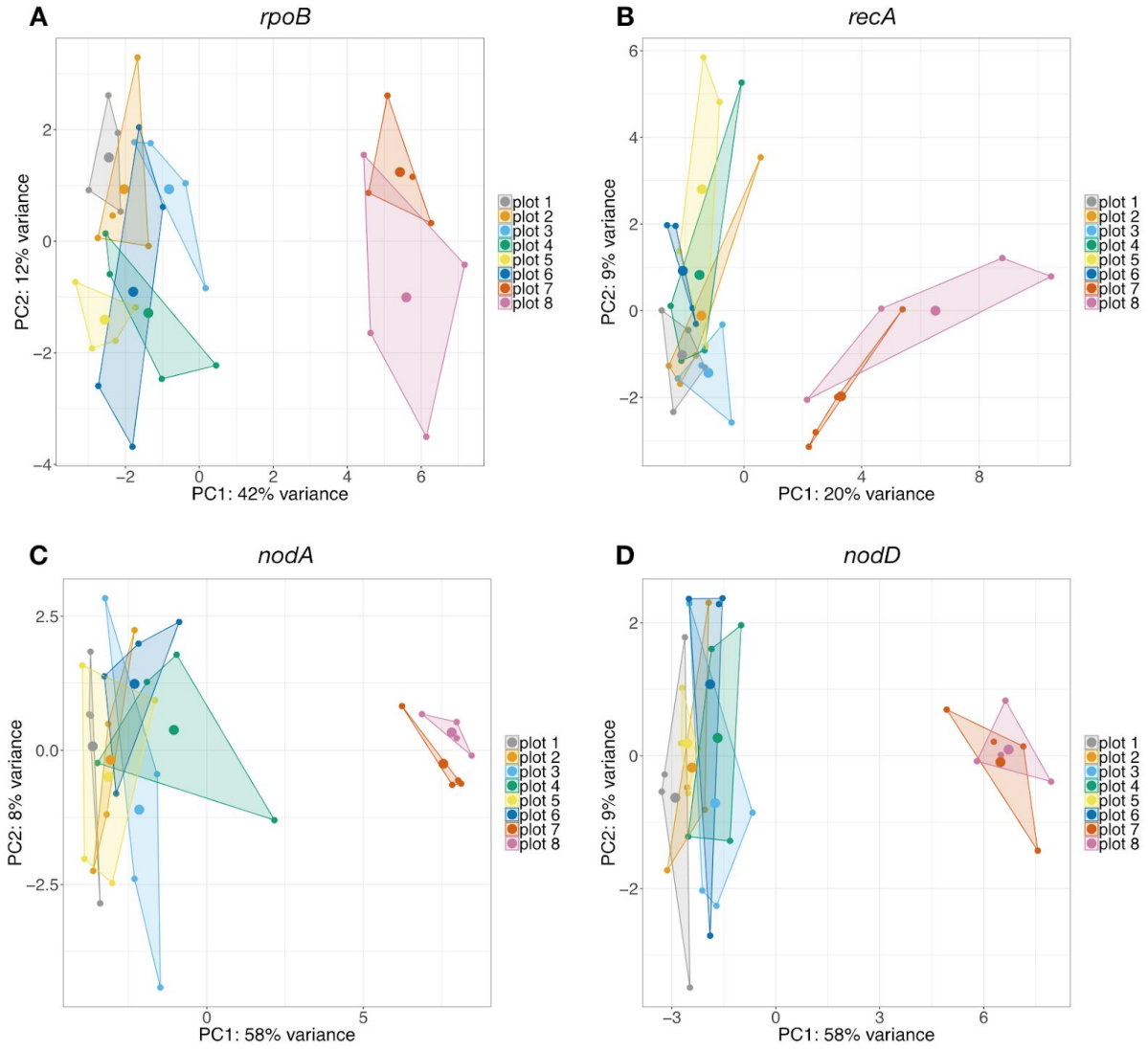


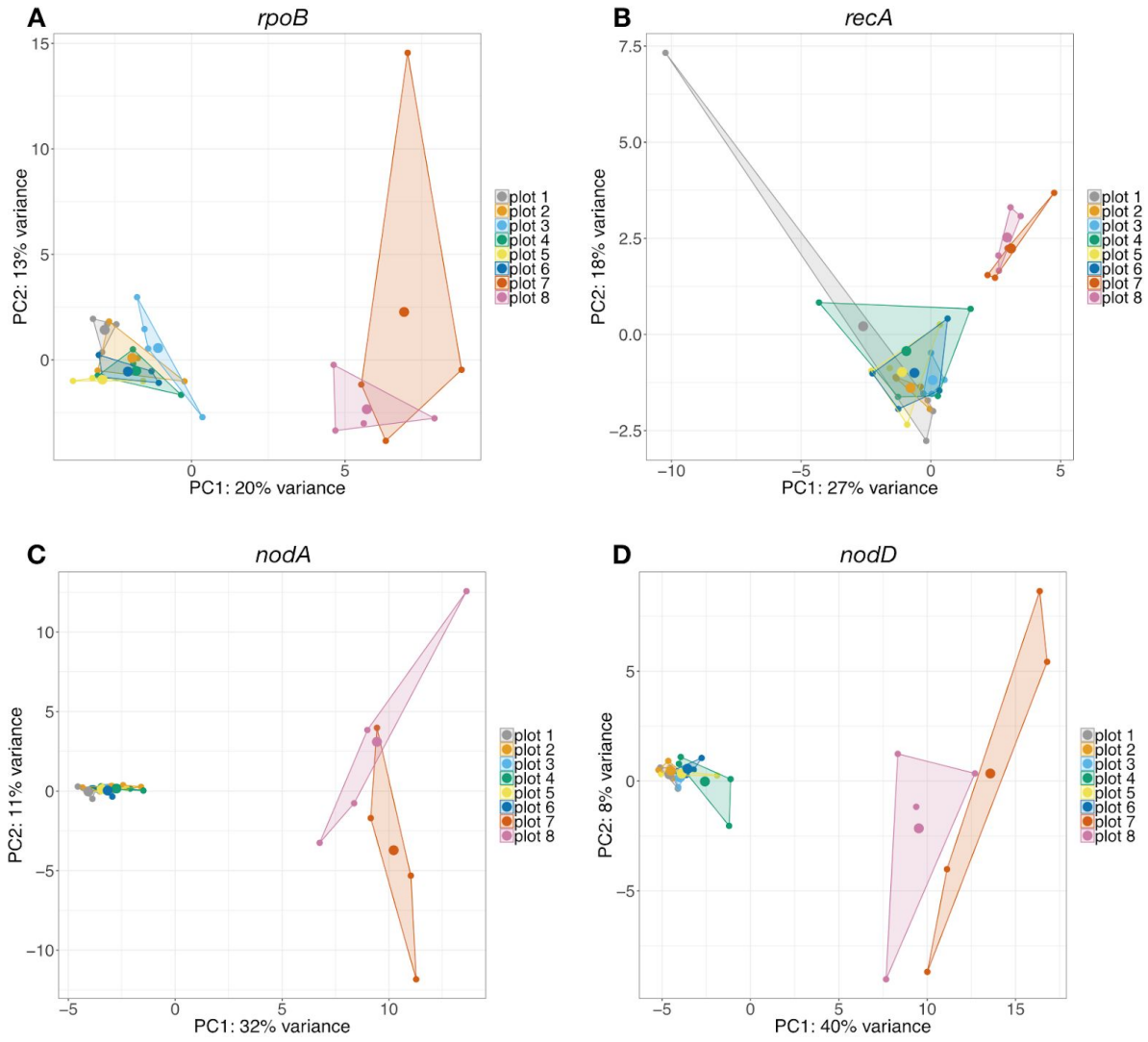
Supplementary Figure 1. UMI clustering performance on DNA mixtures. **A, C,** and **D:** Observed ratio of SM3/SM170C versus the expected ratio for **A:** *recA*, Pearson correlation for Phusion=0.9491334 and Platinum=0.9861944, **C:** *nodA*, Pearson correlation for Phusion=0.9911029 and Platinum=0.9830184, **D:** *nodD*, Pearson correlation for Phusion=0.9917471 and Platinum=0.9964255. **B, D,** and **F:** Proportion of chimeras compared to total reads for each assessed expected ratio for **B:** *recA*, **D:** *nodA*, **F:** *nodD*.



Supplementary Figure 2. Amplicon clustering performance on DNA mixtures. **A, B,** and **C:** Observed ratio of SM3/SM170C versus the expected ratio for **A:** *recA*, Pearson correlation for Phusion=0.9436273 and Platinum= 0.9873055, **B:** *nodA*, Pearson correlation for Phusion=0.9983637 and Platinum=0.9985405, **C:** *nodD*, Pearson correlation for Phusion=0.993404 and Platinum=0.9995299. **D:** Proportion of chimeras compared to total reads for each assessed expected ratio for *nodA*.



Supplementary Figure 3. Individual Principal Components Analysis of four *Rhizobium leguminosarum* biovar *trifolii* genes clustered by UMI. DNA was isolated from 100 nodules from four points on each individual plot. **A:** *rpoB,* **B:** *recA,* **C:** *nodA* and **D:** *nodD*.



Supplementary Figure 4. Individual Principal Components Analysis of four *Rhizobium leguminosarum* biovar *trifolii* genes clustered by amplicon. **A:** *rpoB,* **B:** *recA,* **C:** *nodA* and **D:** *nodD*. DNA was isolated from 100 nodules from four points on each individual plot.

Supplementary Table 1. Synthetic community mixes sample design.

SampleID	Strain	Community percentage (%)	Strain	Community percentage (%)
A1	SM3	100	SM170C	0
A2	SM170C	100	SM3	0
B1	SM3	50	SM170C	50
B2	SM3	50	SM170C	50
B3	SM3	50	SM170C	50
C1	SM3	66.6	SM170C	33.3
C2	SM3	66.6	SM170C	33.3
C3	SM3	66.6	SM170C	33.3
D1	SM3	90	SM170C	10
D2	SM3	90	SM170C	10
D3	SM3	90	SM170C	10
E1	SM3	99	SM170C	1
E2	SM3	99	SM170C	1
E3	SM3	99	SM170C	1
F1	SM170C	66.6	SM3	33.3
F2	SM170C	66.6	SM3	33.3
F3	SM170C	66.6	SM3	33.3
G1	SM170C	90	SM3	10
G2	SM170C	90	SM3	10
G3	SM170C	90	SM3	10
H1	SM170C	99	SM3	1
H2	SM170C	99	SM3	1
H3	SM170C	99	SM3	1

Supplementary Table 2. Primer sequences for QQAD PCR.

Primer-type	Primer name	Sequence
Forward gene specific inner primer	QQMf1-rpoB-Inner	AGATGTGTATAAGAGACAG-A–NNNHNNNWNNNH-GyTCGCAGTGGTGGATGTT
	QQMf1-recA-Inner	AGATGTGTATAAGAGACAG-A–NNNHNNNWNNNH-CGAGAATGTTGTCGAGATyGAGACGA
	QQMf1-nodAt-Inner	AGATGTGTATAAGAGACAG-A–NNNHNNNWNNNH-CCGGATCTsGAGGGGCT
	QQMf1-nodDt-Inner	AGATGTGTATAAGAGACAG-A–NNNHNNNWNNNH-ATGCGTTTTAAGGGmyTGGATCT
Forward universal outer primer	QQMf1-Outer	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-A
Reverse gene specific primer	QQMr1-rpoB	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCGTCTTCRAGGAACGGCAT
	QQMr1-recA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTATCGGTGATTTCRAGCGCCTG
	QQMr1-nodAt	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACACTGCANCCGTTTCGTTCGATCAATGA
	QQMr1-nodDt	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCRCCGGTCAGATTCCGC

Supplementary Table 3. QQAD PCR reaction mixture for Platinum Taq DNA polymerase. **recA* is used as an example gene

	PCR recipe for 25ul reaction	Initial concentration	Final concentration	
Water	15.9			
buffer	2.5	10	1	
$MgCl_2$	1.5	50	3	mM
dNTP	0.5	10	0.2	mM
QQMf1-*recA** (specific gene inner primer)	1	0.1	0.004	uM
QQMf1 (universal outer primer)	1	10	0.4	uM
QQMr1-*recA** (this is the full length reverse primer specific to recA – no inner primer)	1	10	0.4	uM
Platinum HS TAQ	0.1	5	0.02	Units/ul
Template DNA	1.5			

Supplementary Table 4. QQAD PCR programme for Platinum Taq polymerase (approximately 2 hours 20 mins total).

Temperature (°C)	Time (seconds)	Cycles
95	180	
94	30	
70	300	2
72	120	
94	30	
70	60	30
72	60	
72	600	
4	hold	

Supplementary Table 5. Nextera XT indexing PCR reaction mixture for Phusion High-Fidelity Taq proof-reading DNA polymerase.

	PCR recipe for 50ul reaction
Water	23.5
Buffer	10
dNTP	1
XT index S	5
XT index N	5
Phusion	0.5
DNA	5

Supplementary Table 6. Nextera XT index PCR programme.

Temperature (°C)	Time (seconds)	Cycles
95	180	
95	30	
55	30	10
72	30	
72	300	
4	hold	