**Optional: Splitting sequences into ITS and LSU**
Look for LSU (28S) primers sequences in your sequence to see where the ITS sequence ends and LSU sequence starts

- LR0R (forward): ACCCGCTGAACTTAAGC
- LR5 (reverse): TCCTGAGGGAAACTTCG

Common alternates (same region, different amplicon lengths):

- LR3: CCGTGTTTCAAGACGGG (pairs with LR0R for a shorter D1–D2)
- LR7: TACTACCACCAAGATCT (pairs with 5.8S/5.8SR or LR0R for longer reads)

**Or simply align sequences with UGene**
Copy your txt/fasta file that has all sequences in it
For one align all ITS files and remove areas with LSU files
For the other remove all areas with ITS sequences and only keep area with LSU files
Export as FASTA files

CHECK WITH CHAT GPT TO MAKE SURE FILE LOOKS GOOD AND THERE ARE NO DUPLICATES OR DYSFUNCTIONAL CHARACTERS (**periods, hyphens, colons and >**)

**Concatenate files and create .nex file**

Get ChatGPT to create a concatenated file

**2) Add MrBayes block to .nex file**
Append a standard block (adjust the charset ranges to your partition file if they differ):

**Brandon's block:**

```
;
END;

begin mrbayes;
   charset ITS = 1-762;
   charset LSU = 763-2793;
   partition by_gene = 2: ITS, LSU;
   set partition = by_gene;

   lset applyto=(1) nst=6 rates=invgamma;
   lset applyto=(2) nst=6 rates=invgamma;

   unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
   prset ratepr=variable;

   mcmc ngen=2000000 nchains=4 printfreq=1000 samplefreq=1000 diagnfreq=1000 savebrlens=yes;

   sump burnin=500;    [25% of 2000 samples]
   sumt burnin=500;
end;
```

**ChatGPT's block**

```
;
END;

begin mrbayes;
   charset ITS = 1-797;
   charset LSU = 798-2186;
   partition by_gene = 2: ITS, LSU;
```

```
    set partition = by_gene;

    lset applyto=(1) nst=6 rates=invgamma;
    lset applyto=(2) nst=6 rates=invgamma;

    unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
    prset ratepr=variable;

    mcmc ngen=2000000 nchains=4 printfreq=1000 samplefreq=1000 diagnfreq=1000 savebrlens=yes;
sump burninfrac=0.25;
sumt burninfrac=0.25;
contype=allcompat;
end;
```

CHECK WITH CHAT GPT TO MAKE SURE THIS BLOCK IS GOOD

**Run sequence in Terminal**

cd ~/Desktop (or wherever the file is)

then
mb concat.nex (or whatever the file name is)

**Watch the run**

- MrBayes prints **average standard deviation of split frequencies** (ASDSF).
    - **< 0.05** = pretty good convergence, 0.01 is very good
    - You may stop earlier if it drops quickly, or run longer (ngen=2000000 etc.) if it lingers above 0.05.

**Results**

- concat.nex.p → parameter log
- concat.nex.t → sampled trees
- concat.nex.con.tre → consensus tree with posterior probabilities

**Convert .nex file into a file RaxMLGUI can handle**
Ask ChatGPT
**Run RaxMLGUI**

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**ASDOSFs**
Inocybe albofusca:
Inocybe communis: 0.01
Inocybe fissurans:
Mallocybe diabolica: