团队成员和任务分配

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Wei	Lei	极端微生物数据模型测试
Li hui	Tang	极端微生物数据收集
Jia ying	Xie	Wiki 网页制作
Tan	Liu	实验
Jing wen	Liu	实验
Xiao jia	Fang	实验
Lang	Liang	海报制作
Rong	He	ppt 制作以及成果展示

```python
from download_acc import downloadFromAcc
import os,threading

"""
Multi threaded download of extreme microbial assembly data
"""

def execDownload(dir, refseq):
    command = "python download_acc.py %s %s"%(dir, refseq)
    os.system(command)

def selectMkdir(path):
    if os.path.exists(path):
        return False
    os.system("mkdir %s"%(path))
    return True

def start_down(task_pool):
    while len(task_pool)>0:
        refseq, down_dir = task_pool.pop(0)
        path = "%s/%s.zip"%(down_dir, refseq)
        if os.path.exists(path):continue
        command = "python download_acc.py %s %s"%(down_dir, refseq)
        print(command)
        os.system(command)
        print("##"*10)
        print("# %d"%(len(task_pool)))
        print("##"*10)

def main():
    f = open("refseqs.txt")
    task_pool = []
    work_dir = "download_report_zip"
    for line in f:
        refseq = line.strip()
        task_pool.append((refseq, work_dir))
    task_pool = task_pool
    thread_core = 4
    thread_list = []
```

```python
import os,sys,re,json

def load_json(file_path):
    f = open(file_path, "rb")
    c = f.read().decode("UTF-8")
    return json.loads(c)

def save_json(file_path:str, text:dict):
    f = open(file_path, "wb")
    text = json.dumps(text)
    text_bit = text.encode("UTF-8")
    f.write(text_bit)
    f.close()

def exec_command(command):
    #print(command)
    pip = os.popen(command)
    cont = pip.buffer.read()
    text = cont.decode(encoding='utf8')
    return text

def combine_result(c_):
    ids_ = re.findall(">> (.+?) " ,c_)
    gene_string = ", ".join(ids_)
    return gene_string

def extract_seq(file_name, tagets):
    f_ = open(file_name,"r")
    c_ = f_.read()
    m_ = re.findall(">( +?) ( +)\n([^>]+)" c_)
```

```
f train(args):
  model_name = args.model
  optiaml_label = args.optimal_label
  test_rate = args.test_rate
  # -------------------------
  codon_svm = CodonSVM(model_name, optiaml_label, test_rate)
  df = pd.read_csv(args.data,index_col=0,sep="\t")
  X = df.iloc[:,0:-1].values
  Y = df.iloc[:,-1].values
  codon_svm.train(X,Y)

f predict(args):
  model_name = args.model
  optiaml_label = args.optimal_label
  test_rate = args.test_rate
  proba = args.proba
  out = args.out
  assert out!="empty","predict mode must point --out"
  # -------------------------
  codon_svm = CodonSVM(model_name, optiaml_label, test_rate)
  df = pd.read_csv(args.data,index_col=0,sep="\t")
  X = df.iloc[:,0:-1].values
  Y = df.iloc[:,-1].values
  print("> Predict start......")
```

```
        results.append(temp_)
    return results

def loadProtAddr(path,**cond):
    summary_table = {k:0 for k in cond}
    table = load_json(path)
    targeted_refseq = set()
    for refseq in table:
        for k,v in cond.items():
            if table[refseq]["pred"][k]:
                continue
            if table[refseq][k]<v[0] or table[refseq][k]>=v[1]:
                continue
            summary_table[k]+=1
            targeted_refseq.add(refseq)
    prot_pool={}
    summary_table["total"] = len(targeted_refseq)
    for refseq in targeted_refseq:
        prot_pool[refseq] = table[refseq]
        # for k,v in cond.items():
        #     print(table[refseq][k],end="-")
        # print()
    return prot_pool,summary_table

def loadProtTable(path):
    f = open(path)
    c = f.read()
    m  = re.findall(">(.+?) .+([^>]+)",c)
```

```
def saveFasta(file_handle, genes_string, table):
    for gene in genes_string.split(", "):
        try:
            seq = table[gene]
        except:
            print("->",genes_string)
            exit()
        line = ">%s\n%s\n"%(gene, seq)
        file_handle.write(line)

def main():
    work_dir = sys.argv[1]
    hmm_name = sys.argv[2]
    meta_index_table = "bio_model/iExtreme/json/meta_index_tabl
    prot_pool, summary_table = loadProtAddr(meta_index_table,te
    # summary file
    targeted_genes_table_name = "hmm_search.tsv"
    targeted_genes_table_path = "%s/%s"%(work_dir, targeted_gen
    targeted_genes_table_file = open(targeted_genes_table_path,
    # fasta file
    targeted_genes_fasta_name = "hmm_search.fasta"
    targeted_genes_fasta_path = "%s/%s"%(work_dir, targeted_gen
    targeted_genes_fasta_file = open(targeted_genes_fasta_path,
    # start
    cur_i = 0
```

```
    pred_df.to_csv(out,index=None)
    print("> Predict complete")
    print("> Predict data save to ",out,end="\n\n")

ef main():
    args = getArg()
    # Receiving parameter verification
    # Is the model file complete
    model_files_path = {
        "expH":"data/ccdon_freq_selected_pH_less5.tsv",
        "exsalt":"data/ccdon_freq_selected_salinity.tsv",
        "exheat":"data/ccdon_freq_selected_temp_large70.tsv
    }
    assert os.path.exists(args.data), "%s not exists"%(args
    if args.model_type=="train":
        train(args)
    elif args.model_type=="predict":
        predict(args)
    elif args.model_type=="predict_all":
        predict_all(args)

f __name__=="__main__":
    main()
```

```
)
ggsave("struct_cluster_filter_total.pdf",plot = p, dpi = 300, wi


# TM division similarity drawing
p = pheatmap(df_struct_blast,
             border_color = "gray",
             na_col = "black",
             cluster_rows = F,
             cluster_cols = F,
             main = paste(method_cluster, " struct>=0.8 sequence
             clustering_method = "average",
             #annotation_row = color_ori,
             #annotation_col = color_ori,
             cellwidth = 10, cellheight = 10,
             #legend_breaks=seq(0,1,0.2), breaks=bk,
             color = color
)
ggsave("struct_div_simi_total.pdf",plot = p, dpi = 300, width =
```

```
cur_i = 0
total_num = len(prot_pool)
for refseq in prot_pool:
    prot_file = prot_pool[refseq]["protein"]
    prot_to_seq_table = loadProtTable(prot_file)
    c = r"hmmsearch -E 0.00001 --noali %s %s"%(hmm_name,pr
    temp_out = exec_command(c)
    targeted_genes = combine_result(temp_out)
    if not targeted_genes:
        cur_i += 1
        continue
    line = "%s\t%s\n"%(refseq, targeted_genes)
    targeted_genes_table_file.write(line)
    # save fasta
    saveFasta(targeted_genes_fasta_file, targeted_genes, p
    cur_i += 1
    print("\r[%d/%d]"%(cur_i, total_num),end="")
print()
print("-"*10,"summary","-"*10)
print(summary_table)

__name__=="__main__":
 main()
```

```
            count_table[ccodon] += 1
            total_num += 1

or ccodon in count_table:
    count_table[ccodon] /= total_num

eturn count_table


ain():
Prediction data JSON index file
ndex_table = utils.load_json("index_table.js
 codon combine
rint(len(index_table))
ombine_num = 2
codon_table = ccodon.main(combine_num)
ask_pool = []
```

```
import re,sys,os
import zipfile

def zipDir(dirpath, outFullName):
    """
    Compress the specified folder
    """
    zip = zipfile.ZipFile(outFullName, "w", zipfi
    for filename in os.listdir(dirpath):
        if filename == "seq.zip":
            continue
        zip.write(dirpath+"\\"+filename, filename)
    zip.close()

def create_db(file_name):
    f_ = open(file_name,"r")
    c_ = f_.read()
    m_ = re.findall(">(.+?) (.+)\n([^>]+)",c_)
    map_ = {}
    for id_, info, seq in m_:
        #id_ = re.sub("\||:","-",id_)
        map_[id_]={"info": info, "seq":seq}
```

```
    return len(s)

ef main():
    # summary.tsv data-addr tmp_dir
    summary_file_name = sys.argv[1]
    data_path = sys.argv[2]
    tmp_dir = sys.argv[3]
    input_dir = tmp_dir
    out_file = input_dir+"/seq.zip"
    f = open(summary_file_name, "r")
    seq_len_f = open(tmp_dir+"/seq_len.tsv", "w")
    for line in f:
        line = line.replace("\n","")
        arr = line.split("\t")
        if int(arr[1])==0:
            continue
        gene_id_arr = arr[2].split(", ")
        db_path = data_path+"/%s/protein.faa"%(ar
```

```python
#python3
import zipfile
import os,re,sys

"""
extract pdb from alphafold2 predictions
"""

def get_files(path="./"):
    return os.listdir(path)

def extract_pdb(name, path):
    zip = zipfile.ZipFile(name, "r")
    for f in zip.namelist():
        if is_my_pdb(f):
            zip.extract(f, path=path)

def is_my_pdb(name):
    m = re.search(".+?rank_001_.+?.pdb", name)
    if not m:
        return False
    return True

def is_zip(name):
    m = re.search("zip$", name)
```

```r
keep_loc = (df_mat >= struct_threshold) & (df_blast<=blast_threshold)
df_struct_blast = df_mat/df_blast
inf_loc = df_struct_blast==Inf
df_struct_blast[inf_loc] = max(df_struct_blast[!inf_loc])

df_struct_filted = df_mat[,]
df_struct_filted[!keep_loc] = NA
df_struct_blast[!keep_loc] = NA

code = row.names(df_struct_filted)
code_my_row = df_struct_filted[code=="3>H100Sno_homo",]
code[!is.na(code_my_row)]

# Structural filtering drawing
p = pheatmap(df_struct_filted,
             border_color = "gray",
             na_col = "black",
             cluster_rows = F,
             cluster_cols = F,
             main = paste(method_cluster, " struct>=0.8 sequence<=",blast_
             clustering_method = "average",
             #annotation_row = color_ori,
             #annotation_col = color_ori,
             cellwidth = 10, cellheight = 10,
             #legend_breaks=seq(0,1,0.2), breaks=bk,
             color = color
)
ggsave("struct_cluster_filter_total.pdf",plot = p, dpi = 300, width = size
```

```
save_df_path = "ccdon_freq.tsv"
save_df_f = open(save_df_path, "w")
head = ["refseq"]+ccodon_table+["label"]
line = "\t".join(head)+"\n"
save_df_f.write(line)
for refseq in index_table:
    # if cur_i>=5:
    #     exit()
    cur_i += 1
    label = "-1" #index_table[refseq]["lab
    cds_path = "%s"%(index_table[refseq]["
    if not os.path.exists(cds_path):
        # print(cds_path)
        continue
    cds_table = utils.getSeqTable(cds_path
    if len(cds_table)==0:
        print(cds_path)
        continue
```

```
def __init__(self, model_name, optimal_label,test_rat
    self.model_name = model_name
    self.model = svm.SVC()
    self.optimal_label = optimal_label
    self.optimal_model = None
    self.mean = None
    self.var = None
    self.isstandard = False
    self.seed_pool = self.loadSeedPool()
    self.test_rate = test_rate
    self.model_save_path = 'optimal/%s.pickle'%(self.
    self.param = {
        'kernel':  ["rbf","poly","sigmoid"],#
        'C':  [1, 5, 10, 20, 40, 80,120],
        "gamma" : [0, 0.0001, 0.001, 0.1, 1, 10]
    }
    self.scoring = {
        "my_rule": "accuracy"
    }
    self.seed = 6294 # random seed
    self.optimal_seed = None
    self.model_grid = GridSearchCV(self.model, self.p
                                   refit="my_rule",

def saveOptimalModel(self,optimal_data):
    with open(self.model_save_path, 'wb') as f:
```

```bash
#!/bin/bash

# get current work path
current_dir=$(pwd)

# define input/out dir
input_dir="${current_dir}/input"
output_dir="${current_dir}/output"
rm -rf ${output_dir}/* #输出文件清零

# scan input dir to read seq
for input_file in "${input_dir}"/*
do
    # output file name
    filename=$(basename "${input_file}")
    output_file="${output_dir}/${filename}"

    # use alphafold2 to predict
    colabfold_batch --zip "${input_file}" "${output_file}"

    # tips
    echo "已完成 ${input_file} 的结构预测，结果位于 ${output_file}。"

done

```

```python
#python3
import os,re
import threading

"""
Generate structure comparison matrix
"""

def code_num(pdb_file):
    f = open("name_code.tsv", "w")
    index = 1
    for pdb in pdb_file:
        m = re.search("(.+?\.1)_", pdb)
        gene_id = ""
        if m:
            gene_id = m.group(1)
        line = "%s\t%d\t%s\n"%(gene_id, index, pdb)
        f.write(line)
        index += 1

def get_pdb(path="./"):
    return os.listdir(path)

def exec_usglign(pdb1, pdb2):
    #usalign pdb1.pdb pdb2.pdb -outfmt 2
    command = "usalign %s %s -outfmt 2"%(pdb1, pdb2)
    return os.popen(command).read()
```

```
    cds_table = utils.getSeqTable(cds_path)
    if len(cds_table)==0:
        print(cds_path)
        continue
    # print(cds_table)
    # print(len(cds_table))
    # exit()
    tmp = (refseq, cds_table, label)
    # task_pool.append(tmp)
    ##########################
    # start
    ##########################
    table = ccodonCount(cds_table, ccodon_table,
    print("\r[%d/%d]"%(cur_i, len(index_table)),e
    freq_list = list(table.values())
    freq_list = list(map(str,freq_list))
    tmp = [refseq]+freq_list+[label]
    line = "\t".join(tmp)+"\n"
    save_df_f.write(line)
```

```
if not self.isstandard:
    self.mean = np.mean(x,axis=0)
    self.var = np.var(x,axis=0)
    self.isstandard = True
return (x-self.mean)/self.var**0.5

loadSeedPool(self):
f = open("seed_pool.txt","r")
pool = f.readlines()
pool = [int(seed.replace("\n","")) for seed in pool]
return pool

paramOptimal(self,x,y):
"""
Model parameter optimization
"""
# spliting of training/testing data
std_x = self.standard(x)
x_train, x_test, y_train, y_test = sklearn.model_selection.t
# parameter optimization
print("> Start to train and optimalize model......")
res_grid = self.model_grid.fit(x_train, y_train)
# Save optimized parameters and models
grid_record = res_grid.cv_results_
best_index = res_grid.best_index
```

```
def save_table(path, head, table):
    f_ = open(path, "w")
    L = len(table)
    line = ""+"\t"+"\t".join(head)+"\n"
    f_.write(line)
    for i in range(L):
        line = "%s\t"%(head[i])+"\t".join(table[i])+"\n"
        f_.write(line)

def rename_files(dir):
    files = get_pdb(dir)
    if not re.search("unrelaxed_",files[0]):
        return
    new_files = [re.sub("_{1,}unrelaxed_.+","",file)+".pdb"
    for i,j in zip(files, new_files):
        command = "rename %s\%s %s"%(dir, i, j)
        print(command)
        os.system(command)


def main():
    work_dir = "pdb_pool"
    ref_dir = "ref_pdb"
    tag = "Alo"
    ref_pdb = get_pdb(ref_dir)
    cani_pdb = get_pdb(work_dir)
    pdb_pool = ref_pdb + cani_pdb
    code_num(pdb_pool)
    L = len(pdb_pool)
    L ref = len(ref pdb)
```

```
x_train, x_test, y_train, y_test = sklearn.model_selection.train_
model.fit(x_train,y_train)
seed_pred = model.predict(x_test)
seed_report = classification_report(y_test, seed_pred,output_dict
f1_score = seed_report[self.optimal_label]["f1-score"]
f1_score_list.append(f1_score)
print("\r                                    ",end="")
print("\r%s\t%s\t%.3f"%(cur_i,seed,f1_score),end="")
t()
_df = pd.DataFrame({"seed":self.seed_pool,"f1":f1_score_list})
_df = seed_df.sort_values(by='f1', ascending=False)
th = seed_df.shape[0]
an_i = length//2
l_seed = seed_df.iloc[median_i,0]
mal_f1 = seed_df.iloc[median_i,1]
t("> Optimal seed=%s, optiaml f1=%.3f"%(optimal_seed, optimal_f1)
t("> Max f1=%.3f, min f1=%.3f, avearge f1=%.3f"%(seed_df["f1"].ma
.optimal_seed = optimal_seed
rn optimal_seed

lModelTest(self,x,y,best_param,best_seed):

l model testing. Optimal parameters and optimal seeds
```

```
for i in range(L):
    for j in range(i,L):
        if i==j:
            dis_table[i][j] = "1"
            continue
        f1 = pdb_pool[i]
        f2 = pdb_pool[j]
        if i<L_ref:
            work_dir_1 = ref_dir
        else:
            work_dir_1 = work_dir
        if j<L_ref:
            work_dir_2 = ref_dir
        else:
            work_dir_2 = work_dir
        out = exec_usglign(work_dir_1+"/"+f1, work_dir
        tm1 = parse_TM(out, 2)
        tm2 = parse_TM(out, 3)
        dis_table[i][j] = str(tm1)
        dis_table[j][i] = str(tm1)
        print("\r[%d/%d]"%(index, L*L/2),end="")
        index += 1
save_table("result_pdb_%s_total.txt"%(tag), pdb_pool,d
print(dis_table)
```

```
method_cluster = "average"
my_loc = c(1:22)
blast_threshold = 0.15
struct_threshold = 0.8

head_df = read.csv("order_code.txt",sep=",")
df_struct = read.csv("order_struct.csv",sep=",",row.names = 1)
df_blast = read.csv("df_ident.csv",sep=",",header = F)
df_blast = (df_blast-min(df_blast))/(max(df_blast)-min(df_blast))

row = dim(df_struct)[1]
col = dim(df_struct)[2]
df_mat = as.matrix(df_struct[1:row,1:col])
colnames(df_mat) =  row.names(df_mat)
colnames(df_blast) =  row.names(df_mat) #
row.names(df_blast) = row.names(df_mat) #

color=colorRampPalette(c("#4575B4","#E0F3F8","#FFFFBF","#FEE090","#FC8D59"
p = pheatmap(df_mat,
             border_color = "gray",
             cluster_rows = F,
             cluster_cols = F,
             main = paste("struct ",method_cluster,sep=""),
             clustering_method = "centroid",
             #annotation_row = color_ori,
             #annotation_col = color_ori,
             cellwidth = 10, cellheight = 10,
             #legend_breaks=seq(0,1,0.2), breaks=bk,
             color = color
```

```
"""
Step 1: Parameter optimization
Step 2: Seed optimization
Step 3: Final Model
"""
#------------------------------
# step1: Parameter optimization
optimal_param = self.paramOptimal(x,y)
best_param_index = optimal_param["grid"]["my_b
best_param = optimal_param["grid"]["params"][b
print("> Model param optimization complete")
sleep(3)
print("> Best param is",best_param)
sleep(3)

#-------------------------
# step2: Seed optimization
print("> Start to optimal seed[%d] for label %
sleep(3)
best_seed = self.optimalSeed(x,y,best_param)

#------------------------------
# step3 Final Model
print("> Final model test.....")
```

2024

No.

Date.

1/2

1. pJU75e-GFP11 挑菌摇过夜.
2. 枯草-DPE-F246Y、pJU75e-GFP11 PCR → 回收.
3. 枯草-F246Y、pJU75e-GFP11 转 Trans 10.
4.

1/8

1. F246Y 10个克隆 摸 IPTG 浓度
2. PCR R28a

1/9

1. DPE/F246Y 400mL 25°C、1.5mM IPTG 诱导过夜.
2. 耐盐 DPE 1/2/3 PCR

{ 201 dpe bb F/R
5782bp 60°C Tm

{ 201 salt dpE F/R
923bp 55°C Tm

{ 202 salt dpe F/R
910 60°C Tm

{ 203 salt dpE F/R
847bp 57°C Tm.

3. pET28a-2N3-DPE-F246Y mut 重转 Rosetta → 涂 M9 (阿拉伯糖 20μL)

No.
Date.

1/10

1. pET-28a-IN3-DPE 耐盐送测。
2. [redacted] DPE/F246Y 55°C, 60°C, 70°C 催化.

1/11

1. 枯草 DPE 提质粒 200ng/ul，明天送之前提前送测序.
2. DPE/F246Y 65°C 催化.

1/12

1. DPE/F246Y 催化温度 结果做图.
2. F246Y (245-251) (28a) 提质粒 → 转 Rosetta → 涂 Mg (1mM
   zpTG +20g/L Allulose).

1/15

1. 耐盐 DPE 1/2/3 转 Rosetta (催化条件不一样, 可能更能耐
   高浓度阿洛酮糖) → 找江博要文献(✓)
2. pET28a-F246Y (245-251) 挑菌稀释涂布 LB 平板.
3. DPE/F246Y 缓冲液 + 果糖 375g/L.

1/16

1. DPE/F246 55℃、60℃、65℃ 2、4、3、6h 取样
2. 耐盐 DPE 至 Rosetta.

1/29

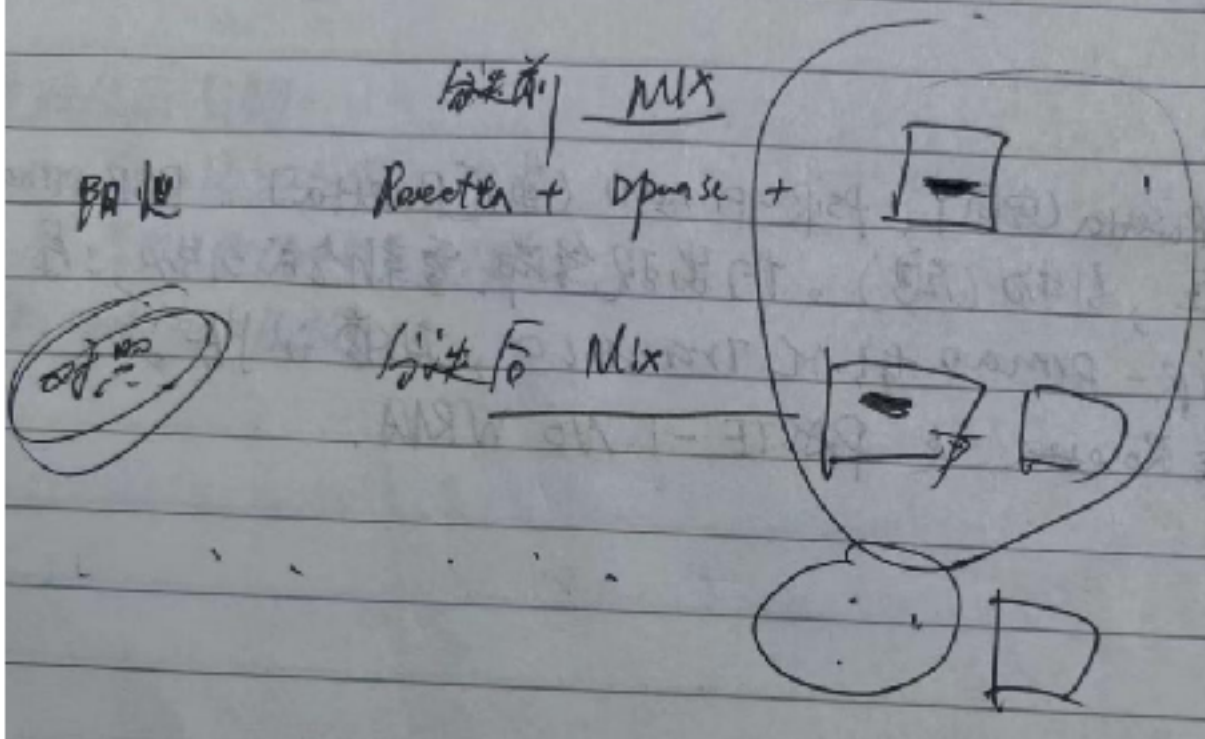
1. 用 Rosetta (空菌)、PsiR-prmaD (菌)[Rosetta]、PsiR-prmaD 质粒，引物 (质)。均出现条带。重新合成引物，导 PsiR-prmaD 转化 Trans10，补菌液测序。
2. 搭 Rosetta & PASTE-1 NO WRNA.

Date

28a - ZN3 - F946Y - S129R (Rosetta) (DNSA)
-A127R (Rosetta XD15A)

gex - F946Y (DNSA)

7A克隆.



No.
Date.

1.30.

1. F246Y催化 OD600 = 2、5、10、20、30. 60°C催化.
F246Y催化 钴酶子 & 锰酶子 0、0.4、0.6、1.6 mM.
2. Rosetta空菌、pSiR-prmaD菌、pgex6p1-F241Y质粒.

*Y2-gexdpe F/R
Rosetta空菌 ⊗ pSiR-prmaD ⊗
pgex6p1-2N3-DpE ✓

11.8
*Y2-prmaD-F/R
Rosetta空菌 ⊗ pSiR-prmaD ✓
pgex6p1-2N3-DpE ⊗

1.24 Y2-D500F/R
rosetta ✓ pSiR-prmaD ✓
pgex6p1-2N3-DpE ✓

各引物全部都
氨苄增出条带：非常不好.

明天增加一个 pSiR-prmaD菌(之前划线的)：
尝试扩增一下筛选出来的菌株！

~~结果：所有条带都变淡~~

No.
Date.

24.

1. 微流控筛选

阳性对照、

筛选前加菌

RosetHa + Dpease → 单带目的条带

对照 ✗

筛选后的Mix菌液 → 单带目的条带

筛选后的单克隆 → 如果有目的单带，分选没问题，

如果无条带，可能分选出现了问题（前提对照都有带）

结果：筛选前菌株用Yeast-gexdpE-FR能产出单一目的条带，筛选后菌株无单一条带。

解决：将筛选后的Mix菌液提质粒扩增，并转化到DH5α（用Amp的板子涂布）

No.

Date.

26.

1. G211V 揣菌.
2. 激相送测.
3. 噬菌改造
4. 分选后MK菌液做无缝克隆，消目涂带 Seamless clone 到一个管里上，涂板测序.
5. 分选后的菌提质粒 PCR. 送 PCR 产物去质粒完测序

27

1. 噬菌体效价.

Sp-77 10^{-4} $[176\times4)\times10^4\times10^3 = 7.04\times10^9$

Sp-PpE 10^{-4}. $163\times4\times10^4\times10^3 = 6.52\times10^9$

(10^{-4}) $169\times4\times10^4\times10^3 = 6.7\times10^9$

No.

Date.

噬菌体富集&做玻王.

取 10ml Sp-dpe 噬菌体

↓

OD_{600} = 0.4-0.6 175e (Str, Amp)

↓ 过夜 37°C, 220rpm.

12000rpm, 1min, 离心, 取上清.

↓ 稀释

ddH2O 10^{-2} / 10^{-4} / ~~10^{-6}~~ (对照)

↓

取稀释后噬菌体 1ml, 加入至 200ml 175e (OD_{600}=0.4-0.6)

侵染 10min

↓

做玻王.

※ Attention:

取 LB 液体至 60°C 加热保温, 加入 LB 固体保温 (做玻王前用无膜的 50ml 离心管装 0.8% LB 固体, 方便混匀.

2/17 ...

No.

Date.

2/18

1. 分选菌株 ~~Seate Seam~~ Seamless clone 引物设计(✓)
2. 7p-Kana突变 和 MLV-F155 突变 PCR. (✓)
55°C 58°C

2/19

1. 分选Mix菌株 PCR [28a骨架 + DPE mix 片段] (✓) (回收胶条 保存)
2. 7p-Kana mut / MLV-Y155 转化 Top10 (✓)

2/20

1. 7p-Kana mut 转化 Top10 / 分选Mix(28a骨架) 转化 Top10 (✓)
2. 噬菌体 PCR 12_SpdpE F/R 50°C / 72°C, 20S. ×
取上清 52°C / 72°C 20S.

2/21

1. 分选Mix mix-F/R PCR, 条带正确,送测。
2. 接 sp-dpE.

No.

Date.

2/22.

1. TP- iSrB 50°C, 72°C 25s. 转化D15d.

2. 先在CM板子上涂布转化. → 排单克隆于CM/Kana双抗平板筛选。E

2. pGex6p1-ZN3-DPE mutation library 分选一轮 涂板.
(10^{-3} / 10^{-4})

3. 噬菌体做双打(失败).

2/23.

1. TP-ISrB 送测.

2. pGex6p1-ZN3-DPE mutation library 排单克隆. → Fe-GexdpE F/R

3. 分选MIX 用 Mix引物 PCR送测?看看.

off gated
ON - gated
E1

2/26 — 3/1

No.

Date.

2/26.

1. TP—TAA 五不P. 62°C. 4061bp. (60°C)(1min).
2. Mix分选 Yz_gexdpe_F/R. 61°C. 1063bp.
3. 噬菌体 ~~添加~~ 侵染.

↓

98°C, 3min, 98°C, 3s, 52°C、55°C、58°C、30s、72°C, 10s.

2/28.

1. 转接 175e → 富集 sp-DPE (10⁻⁴)
2. TP—TAA 五不P.
3. ML(前) Yz_gexdpe_F/R, 送测.
4. Sp-DPE total PCR (991bp) ✓

2/29.

1. 噬菌致王 PCR 55°C 送测 ✓
2. TP—TAA 摇菌, 明天送测. ✓
3. 摇 pet28a 空质粒菌株 ✓

3.1

✓1. 分选 DPEase (mix) 摇菌 提质粒.
2. DPEase (mix) 构建 PCR → 回收 → 无缝克隆 → 转化 ~~(挑板)~~ (Top10)

✓3. TP—TAA 菌液 平板送测序

4. Sp-dpe 纯化紫胶.

No.

Date.

模板：TP-TAA-nlsrB / lsrBMaRTαRNA，(pUC19 Amp)

3.6_TP-TAAF/R 55°C 72bp.

3.6_nlsrB_Marabb.R/F 64°C 5300bp.

↓ pUC19-lsrBMaRTαRNA-~~nlsrB-αRNA~~-Kana(TAA)

↓ 修复TAA的点突变.

3.7_ATGF/R 64°C 5381bp.

↓ pUC19-lsrBMaRTαRNA-~~nlsrB-αRNA~~-Kana(ATG)

模板：pGex6p1 / pUC19-lsrBMaRTαRNA-Kana(ATG)

3.6_nlsrBαRNA19R/F 61°C 2801bp.

3.1_Gexbb_lsrBF/R 62°C 4200bp.

pGex6p1-lsrBMaRTαRNA-nlsrBαRNA-Kana(ATG)

314—318.

No.

Date.

314

1. 分选DPEase (pet28a) 摇菌.

2. PCR 验证 { 2.29 Mix F4 1437bp
2.22 mix R2 61°C

315

1. TP-TAA isrB { 1.29 - isrB-Kana-R 58°C 转化DH5α
(CmR) 2.26 - isrB-Kana-F 4061bp

2. 分选DPEase Mix送测 (擎科)

3. pet28a-6His-ELN (Short) { 3.4.-ELN F/R Ta 72°C 639bp
3.4-28a ELN bb. 60°C 5100bp

4. 摇菌给微流控

316.

1. TP-TAA-ωRNA-isrB & Pet28a-ELN (Short) 送测.

2. DPEase/Ed40Y/Mix 8/9/18/19 诱导 1mM IPTG, 25°C, 220rpm

317

1. Mix 8/9/18/19 摇菌 提质粒.

2. ~~[illegible]~~ pUC19-IsrB MaRTωRNA-Kana (TAA) PCR 转 DH5α(amp)
gexbb-IsrB PCR.

3/11 - 3/15

No.
Date

3/12

1. DPEase 分选 8/9/18/19 诱导 1.5mM 2h 16, 25°C, 过夜.
2. PJ(15e 活化的菌体. (OD600 ≈ 0.6), PCR total
3. ELN-Short 转 BL21 / Rosetta (DE3)

3/13.

1. DPEase 分选 SDSPAGE ✓ (8-1/9-5/18-1/19-4)
   DPEase 催化果糖. X (明天做)
2. ELN-Short 诱导. 活化. (下午).
3. PJ(15e 做双互. (阴、阳对/. 10^{-2}、10^{-4}、原).
4. TP-CURNA-ISrb-kana(7AA) / pet28a-DhaB-ELN(short) 保录中.

3/14

1. 噬菌体 sp-dpe 效价: 115 × 4 × 10^4 × 10^3 = 4.6 × 10^9.
2. ELN-short 诱导. 1mM 2h 16, 37°C, 过夜放
3. DPEase 催化 (明天溶相)
4. Sp-dpe PCR

3/15.

1. ELN-short SDS-PAGE.
2. 噬菌体 PCR

No.

Date.

pet28a - circle

3.18 - circbb - F/R 58°C 5577bp

3.18 _ circF/R 57°C 3006bp

pgex6p1 - ZN3 - DPE

3.18 _ dpe266 F/R 61°C 3006bp

3.18 _ dpe266bb - F/R 61°C 4120 bp

pet28a - PhaB _ ELN

{3.18 _ 28a ECNbb - R 66°C 5386bp
{3.4 _ 28a - ECNbb - F

{3.18 _ ELN F 75°C 660bp
{3.4 - ELN R

3/18 - 3/22

No.
Date.

3/18.
1. PhaB-ELN(short) 引物设计,

3/19.
1. circle / Dpease PCR → 回收 → 酶连 → DH5α. } 送测.
2. ELN PCR 、 pgex6p1-ntsrB-crRNA-kana(酶切) }

3/20.
1. 活化 PSiR-prmaD.
2. DPEase / circle 无缝克隆 → DH5α. 明天转化.

3/21
1. 配阿洛酮糖: 果糖 1g/L, 0.5 mM IPTG., 37°C 培养箱.

3/22
1. 转化 PSiR-prmaD - 8/9/18/19 / pgex6p-DPE 菌P/PCR.
2.

3/25 → 3/29

3/25.

1. ~~将 psiR-pmnao-8/9/18/19 / pgex6p1 - 0.5mM 2p1h, 1g/L果糖,活化~~
~~过夜(摇?不摇?)~~

2. 将 pgex6p1-dpe (264N) & pet28a-circle (250N) 菌活化,周二摇菌

3. ~~pet28a-DnaB-ELNshort) 转化 BL21 & Rosetta.~~

4. 摇易错 DPE (Amp).

3/26.

1. psiR-pmnao ~~做感受态~~ (20mL) 了感受态做好!
S1030-175e

2. pet28a-DnaB (10.ang) -ELNShort 转 BL21 & Rosetta (Kana)

3. sp-dpe 做验证. [3rd spdpe 5 [redacted] x109]

3. 8/9/18/19 PCR 换 pgex6p1 骨架.

3/27.

1. HYER 无缝克隆 → DH5α.

2. 8/9/18/19 / Error DPE / 264 DPE 转 PSIR-pmnao

3. PSIR-pmnao / TADR 重做感受态

No.

Date.

** HYER 扩增后无缝克隆. 转化 DH5α. (结果 较低 效率差(!)
用 JD Error PCR 后的产物 直接无缝克隆 效果好一些 但菌也没有很多
PCR 没有一个条带正确

No.

Date.

3/28.

1. HYER PCR.（无条带→引物设在了载体基因上了）

2. 18/19 转化 PCR.（✓ 有条带）

3. DnaB(10.9μ)-ELN short 诱导.

4. 活化 pSIK-pmnoD / TADR.

3/29.

1. pSIR 荧光.（19）.

2. DnaB-ELN short SDS-PAGE（未见明显表达条带）→ GST表达

3. HYER PCR.（1-4菌 5-8无菌）

4. 电转（TADR-HYER）

5. GHK?

No.

Date.

4/10

1. pSIR-pirmaD 换色 Fru/Allu + IPTG. (看是否有蓝白斑 ✓
2. pJC175e. 划线 ✓
3. 环RNA W1 Day3. ✓
4. DJE 荧光涂板 ✓
5. GHK 活化 → 诱导.
6. pUC19-ccdl 测序 ✓

4/11

1. pJC175e 挑菌活化 (10Y) 6mL ✓ → 加 spdpe → OD变化 ✓
2. 环RNA 7P W1 Day4 ✓
3. ~~GHK SDS-PAGE.~~ GHK → 活化 ✓ → 诱导 ✓.
4. sp-dpe 第一轮 原液 验证PCR.

4/12

1. GHK SDS-PAGE ✓ → 保种 ✓
2. pgex6p1-ELN short 转化 DH5α, 送测 ✓
3. 环RNA 7P Y1 Day5 ✓
4. 老 sp-dpe 第一轮 (10² 次方). ✓
5. BL21 KO 活化 0.1mM IPTG. ✓
6. pJC175e + spdpe 培养 看 OD. ✓

174-18.

No.

Date.

4/18.

1. 环RNA PCR(NGS) 5cycle → 10cycle → 15cycle (下周二给江粤琪)

2. Spdpe 做致斑 ✓

3. 17477 / TP 送测 ✓
(贴了2个)

4. pgex6p1-nZsrB-Marathon RT 1mM 2p1h, 30°C 诱导 ✓

5. 筛选 DPEase (10^{-6}) 涂板 ✓

4/19

1. Spdpe 噬菌体效价: $125 \times 12 \times 10^2 \times 10^3 = 1.5 \times 10^8$.

2. pET28a-GTTP41-ELN PCR

3. 筛选 DPE PCR.

4. Spdpe 包噬菌体 (10^4).

4/22 — 4/26.

No.
Date.

4/22

1. pgex6p1-ELN Short 可重化 BL21 & Rosetta ✓
2. DPE(修改) Y2-gexdpe F/R 验证(排摩尽结 有带, 但条带非常弱, 明天用今天加重新PCR)
3. Spdpe 修改 验证. ✓
4. 活化 TADR / BL21(K.O.) / BL21(DE3). ✓

4/23.

1. DPE(修改) Y2_gexdpe 5°C PCR ✓
2. pgex6p_-ELN Short 排菌 → 诱导
3. Spdpe 排摩 验证. 63×10²×10³×4=2.5×10⁷. , PCR ✓
4. BL21(K.O) & BL21 电转. K/Amp¹ / K/Amp1 cmR ✗
5. 活化 puc19-HYER-ccdB & MazF. ✓

4/24

1. pgex6p1-ELN Short 诱导 37°C, 1mM 2pTG. ✓
✓2. 提质粒 puc19-HYER-ccdB-MazF / TP-Kana(重新A) / pgex6p1-nzsrB-Marathon RT-aRNA-1Kana 扩3倍加.
✓3. BL21(K.O) & BL21(DE3) 电转. 共4个
K/Amp¹ ② K/Amp/cmR ② (先活化(复苏) 加1mM 2pTG.)

4. DPE 突变 活化.

No.

Date.

4/25

1. Spdpe-第四轮包被 ✓
2. pUC19-HYER-ccdB-MazF (7ADR) 挑菌.
3. DPE 分选 PCR 菌液 α 质粒 (没有), 但其它对照正确.
4. pgex6p1-ELNShort SDS-PAGE. (没表达)
5. Amp. A/K/Cm. (2) A/Cm (2)
(2) K/Cm (2) Cm (2) ✓

4/26.

1. DPE 分选 转化DH5α 16, 挑菌 PCR 也无条带.
2. TP circle 3/5intron PCR
3. DPE 4-9 PCR.
4. BL21 & BL21(K.O) TP-Kana / pgex6p1-n25rB-MarathonRT-ωRNA 转化.
涂 Kana 板. (~~100mg/mL~~) 50mg/mL
8. ~~20.~~ .

4/28 - 4/30.

No.

Date.

4/28.

1. aRNA 敲除单克隆活化 + IPTG，过夜，涂布 kana 平板。
   Amp/ CmR/ Kana. 2个. Kana/ CmR 2个.
2. 两 pet28a-circle/5 intron (v5av) 测序 验证 突变.
   (2个 Kana).
3. 果糖 10g/L + IPTG. 37. (A/Kam). 16个.
4. DPEase 4-9 (6个 Amp) (1个 CmR) -7p.
5. 2个 Amp/CmR. → DPEase (Rosetta)
6. Arac-let-puc18 (Amp) / puc19-Marf-ccdB-lacZ (Amp) 活化.

4/29

1. pGEX6p1-ELN short WB. ✓
2. 果糖 10g/L + IPTG (A/ Kana) 2个板.
3. 送测
4. Arac-let-puc18 (下载) / puc19-Marf-ccdB-lacZ OD600
5. spdpe 做酶.

4/30.

1. spdep 测活性，用稀释/原液富集后, PCR. → 有条带，测序对.
   做酶前，富集一下.
2. Arac-LetB (~~Kana~~ CmR.) 摇一下.

5月.

1. 设计突变引物. DPE ✓
2. psiR-pmab- 转化 Rosetta. ✓
3. DPE 9 重新 PCR → 转化 DH5α. ✓
4. 5pdpe 1g/L / 20g/L ~~MIX~~ 做验证. $0/10^{-1}/10^{-2}$ ✓

5/11°.

1. 5pdpe PCR ✓ 送测.
2. D6/7/8 转 Rosetta ✓
3. DPEase 微 PCR. ✓ 条带很淡

No.

Date.

5/18.

1. DPE 4/6/7/8 诱导 ~~10mM IPTG,~~ 0.5mM, 37°C ✓
2. DPE 突变 motif. 转化 涂板 (0.15d) ✓ → 送测.
3. DPE(突变) 涂 Amp / CmR (24板).
4. 构建 Mfp5.
5. pet28a-circle-3/5intron 25-N PCR：条带对，但有杂带

5/17

1. DPE(突变) 涂 Amp / CmR / Kana 板 ✓
2. 扩 pgex6p1-n25rB-MarathonRT. 诱导 → SDS PAGE ✓
3. 构建 Mfp5 2copy
4. DPE 4/6/7/8 纯化果糖 ✓

No.

Date.

{ DPE-9杆112 测序正确.
174T7- 1 2 3
pUC19-MazF T7

AHSN885 ✓

L → XL

衣：175/92A 180/96A
身高 175-182 180-187
体重 72-80 78-86
净胸围 98-100. 98-104

Spdpe 用低浓度果糖色液时，加入20g/L葡萄糖去让菌消耗，减少果糖被消耗而可能性

Mfp5 { 引物减量
Tm 7a 降低

5/20
5/20
1. BL
2. Pf
3. 17
4. 纯
5. N
6. D

5/21
1. S
2.
3.
4.
5.

5/
1.
2.
3.
4.
5.

5/20.

1. BL21(DE3) / 7ADR 划线 ✓
2. PPE M4If 突变 活化 → 提质粒 ✓
3. 175e → 富集 Spdpe 20ml ✓
4. 微 DPEase (Amp) 活化 → 涂板(Amp)
5. MaSp4A1 涂布 Kana板 ✓
6. DPE9 转化 Rosetta ✓

5/21

1. Spdpe 做双酶切 ✓
2. BL21 / 7ADR 活化 ✓
3. MaSp4A1 挑菌活化 ✓
4. pet28a-circle 150N 活化 ✓ + 0.1mM IPTG / 0.5% L-Arac
5. 微流控 PPE/PSIR separate ② ✓

5/22

1. Spdpe 稀释给药 (10^4) $3.8\times10^4\times10^3 = 2.4\times10^9$.
2. 把 174T7-CcdB-MaSF 活化在 BL21 & 7ADR (电转) (+)(-) L-Arac
3. 验证 175e ✓
4. 微 DPEase 扩菌 PCR (有条带)
5. DPE9 / MaSp MaPs 37°C 活 ✓
   1.5mM 1mM

6/11 — 6/14.

No.

Date.

6/11

1. TADR 细胞验证 ✓
2. pet28a-DPE 12/13 跑胶 ✓
3. DPE 13/15/5/18 构建 ✓
4. TADR ~~[illegible]~~ 张学礼 ✓

6/12

1. 提 PSIR-prmaD 质粒 → 转化 Rosetta ✓
2. 保种 MG1655 / W3110 ✓
3. pet28a-circle-250N (TADR) / pet28a-circle-2XL (TADR)

诱化 → ⊕ L-Arac 0.5%   0.1%、0.5%、1%、2%.

⊖ L-Arac

⊕ IPTG 0.1mM   0.05mM、0.1mM、0.5mM、1mM

⊖ IPTG.

实验组 + 对照组 ✓

⑩ ④

4. DPE 13/15/5/18 转 DH5α. ✓

DPE 11/12/16 转 Rosetta. ✓

6/11-6/21

6/17

1. pPE11/12/16 诱导. SDS-PAGE 验证(无表达)
2. pPE 5/8/14/15 摇菌.
3. pSiR-prrnab 阿洛酮糖验证 20g/L (Kana/ CmR 30g/L + 2p7a)
4. pPE (Amp/CmR) 5.0g/L + 2p7a 长沙板子.
摇菌. 40mL.
4mL.

6/18.

1. 提 pPE5/8/14/15 质粒 → 转 Rosetta ✓
2. pge(xp)-pPE 37°C 催化(明天结)
3. 噬菌数量. 5g/L 10g/L、20g/L ✓
4. pPE11/12/16 WB ✓
5. 保了割出看白 4 蛋丝看看.
6 J23119 PCR 环P.

6/19

1. S1030 ~~[illegible]~~ 双抗 → J23119.
2. pge(xp)-pPE 37°C
3. 噬菌体: 6.17 : 5g/L: $36\times8\times10^3\times10^4=2.8\times10^9$.
10g/L: $80\times8\times10^3\times10^4=6.4\times10^9$
20g/L: $150\times8\times10^3\times10^4=1.2\times10^{10}$.

4. pPE 5/8/14/15 诱导, 摇菌.

No.
Date.

6/20

1. DPE 5/8/14/15 测序 ✓.
2. DPE 13 回收 → 无缝克隆 → 转化 DH5α (Kana)
3. 口腔菌体 PCR. (5孔 无条带) (全是野生型 空载).
4. JZ3119 PCR.

6/21

1. DPE 5/8/14/15 未测出条带.
2. DPE 13 无缝克隆. → 转化 DH5α.
3. JZ3119 菌落 PCR.

6/24 - 6/28

No.

Date.

6/24

1. 4 Amp/cmR. (液滴前、后未培养、培养未分、分)
2. 28a 骨架 PCR ✓ → 无缝克隆 → 转化 (MH5α)
3. S1030 (J23119) 涂板 ✓
4. AP + WT / Spdpe (Error PCR) + 聚糖 / 阿门 ... 糖.

6/25

1. pet28a - circle WZ ~~AA~~ NGS PCR 10 cycle 回收. ✓
2. AP + WT / Spdpe (Error PCR) ✓
3. ~~J23119 转化 S1030~~ → J23119 PCR. ✓
4. S1030 挑菌 ✓
5. 175e.

6/26

1. 噬菌体做斑 ✓
2. J23119 转 S1030 ✓
3. DPE11-16 / 518 PCR ✓.
4. circRNA - 引物截短 PCR 验证 ✓

7/1 — 7/4

No. Results

Date.

7/1

1. 噬菌体效价：5μL = 48×10³×10⁴ = 4.8×10⁸

10μL = 160×10³×10⁴ = 1.6×10⁹

20μL = 432×10³×10⁴ = 4.3×10⁹.

7/2

1. Spdpe WT (175ε活化) 噬菌体效价约 10⁷.

2. Yz-circle R1/F1 可以P出环状 RNA.

3. Ap+WT Fru/All. 10μL 活化.

4. DPE 518/11-16 ~~[illegible]~~ ~~[illegible]~~ Top10.

7/3

1. DPE 518/11-16 T7 PCR.

2. pet28a-circle-10nt knokout. 摇菌.

7/4.

1. 20nt/60nt/10nt ~~[illegible]~~ 20nt 回收效果差！
试试新设计一条引物

2. PSiR-pmao-2ns-DPE 转化 DH5α.

3. Ap+WT-fru/All

4. Pet28a-circle 100nt 质粒提取.

5. 5.21/5.4 噬菌体 PCR，明天送测.

7/8 - 7/12.

No.
Date.

7/8.
1. DPE 578111-16 活化✓
2. pSIR-pmnaD-2N3-DPE 100nt 180nt 活化✓ → 提质粒✓
3. 20nt PCR → 回收✓ 无菌室除
4. 消噬菌体菌 PCR 看双峰是否.
5. 活化 TADR✓
6. 需钠弧菌保菌✓

7/9.
1. TADR 转接 → 电转感受态 → 电转 60、80、100nt TADR✓
2. DPE 518111116 诱导 30℃, 1.5mM 2h✓
3. pSIR-pmnaD-2N3-DPE 转化 Rosetta✓
4. J23119 PCR 环 → 回收 → 转化 Dot5α S1039.

7/10. 5-2/8-2/11-1/12-3/13-3/14-2/
1. DPE SDS-PAGE✓ → 挑表达的菌株保存(冷藏) 15-1/16-4.
2. pSIR-pmnaD2N3-DPE(Rosetta) 活化✓
3. 17477-J23119 PCR 验证✓
4. 25g/L NaCl LB. → 摇 活菌(VP)✓

No.
Date.

7/11.

1. pSiR-prmap-ZN3-DpE 诱导, 30°C, 1.5mM IPTG. ✓
2. VP保菌前|溶血菌 ✓
3. pet28a-circle 60、80、100nA (K.O) + 无催化对照 + W2D3对照.
   0.5% 阿拉伯糖 + ~~0.5~~ 0.1mM IPTG.
4. J23119 PCR 跑架 → 回收 → 无缝克隆 → 转化 S1030.
   重新PCR 提质粒重新PCR. ....

7/12.

1. pSiR-prmap-ZN3-DpE SDS-PAGE P3D 2/2Ha-DpE. ✓
2. 催化果糖 (下周再做)
3. J23119 提质粒 ✓
4. nZSrB 转化 BL21 (K.O) ① / BL21 + E7p-kana ④
   ② BL21
   nZSrB-mut 转化 BL21 (K.O) ③
   7p-kana 转化 BL21 ⑤

7/22-7/26.

7/22.

1. Spdpe PCR. ✓ 7.19 Spdpe 数值: 200 × 8 × 10³ × 10⁴ = 1.6 × 10¹⁰

2. HYER 无缝克隆. ✓

3. S1030 电转. ✓

4. 264N PCR. 重新设计引物. ✓

7/23.

1. S1030 电转感受态 ⟶ 电转 HYER (4) ✓ ⟶ 涂板 (amp) ✓

2. DPE 11/12 / 对照 30°C、1.5 mM IPTG 诱导. ✓

3. NJsrB PCR ✓

4. 接 5/8/13-16 175E ✓

5. 264N PCR. ✓ ⟶ 回收 ✓ ⟶ DpnI ✓ ⟶ 无缝克隆 ⟶ 转 DH5α ✓

7/24.

1. pet28a-psiR-prnaD-ZN3-DPE264N 转化 DH5α. ✓

2. DPE 5/8/13-16 诱导 30°C-1.5 mM IPTG ✓

3. 1747-J23119-HYER-C-M S1030 验证 PCR 17-1747-F/R ✓

还是有骨架自连的情况, 整体长克隆子还是少, 但比 250N 少

Date

Error PCR 流程.(...回收)

1. PCR骨架 → DpnI → 回收(纯胶)
2. 巢式 PCR 目的片段.
   ⓐ. 用外引物将含目的片段的序列扩增出来 → DpnI...
   ⓑ 用此序列片段作为模板进行目的片段的易错 PCR.

模板浓度	1ng /10 ng	(30μl)推荐
2× Error PCR mix	15μl	
10× $MnCl_2$	3μl	
ddH_2O	补至30μl	
引物各	1μl.	

扩增后，检测 PCR 情况，条带弥散则 PCR 不好，需调...
回收浓度在1ng – 30ng 之间. 低于 10ng 需富集扩增
回收时转速约 5000 rpm.
ⓒ. 继续易错 PCR.(用回收的产物作模板).
模板浓度 1ng / 10ng.
其余都一样，退火的温度可以先摸 60°C / 65°C 看...
增情况再调整.
ⓓ 继续ⓒ步骤直至 PCR 扩增条带弥散无法再...
错.

No.

Date.

1/25.

1. pet28a-psiR-primaD-ZN3-DpE PCR鉴定.
2. 5/8/B11-16 DpE 储化.
3. primaD-ZN3-DpE 提质粒.

34.	0.29ml	9.21
46.	0.22ml	9.28
32.6A	0.31ml	9.69.
25.	0.4ml	9.6
40.	0.25	9.75

7/29 — 8/12.

No.

Date.

7.29

1. PSiR-prmaD-ZN3-DPE264N 摇菌 ✓→ 保种 ✓→ 提质粒 ✓.
2. 17477-HYER 摇菌 → 保种 → 提质粒
3. 17477-BN250N 摇菌 → 保种 → 提质粒
4. N25rB Kana 验证，只有 Kana 信息，无 N25rB 的菌 涂在 Amp 板上看是否有 Amp 抗性 ✓ 有菌了……
5. 活化 Rosetta (DE3) ✓
6. N25rB 摇菌 → 保菌 ✓ → 提质粒.

7/30.

1. PSiR-prmaD-ZN3-DPE264N 电转 Rosetta ✓
2. N25rB PCR. Kana ✓　N25rB (条带很浅)
3. ~~N25rB 模板 稀释到 1ng/10ng~~
3. Spdpe 校正 10g/L ~~松~~ ✓ = 重新富集+做校正(校)
20g/L ✓ =

7/31

1. PSiR-prmaD-ZN3-DPE264N 涂板 50ul (Kana/cmR) ✓
2. MyrK1 16S PCR → 测序 ✓
3. 构建 prmaD-Amy-ZN3 28a-ZN3-Amy → 无阳克隆 ✓.
4. N25rB ER PCR ⟶ 目的条带 ✓ → ER 3/4/5
⟶ 骨架 ✓ 1.

No.

Date.

long. mg

7.. 3ml 3ml

2g/L XX =

8g/L X X = 2g/L X 100 ml

25ml =

M9-淀粉 CaCl2 / MgSO4 / VB. 按平行之一加入

8g/L 淀粉 25ml → 2g/L.

No.

Date.

8/1

1. M2SrB 片段 BR3/BR4/BR5 Error PCR ✓

模10ng / 1ng 45°C 每个片段约 600bp—500bp.

2. ~~pSiR prrnaD-264N~~

pSiR-prrnaD-ZN3-OPE264N PCR验证 ✓

3. prrnaD-ZN3-Amy / pet28a-ZN3 Amy 转化DH5α ✓

4. Amylase 易错第一轮. ✓

8/2

1. prrnaD-ZN3-AMY / pet28a-ZN3-Amy PCR验证 → 明天送测

2. 倒 M9-淀粉平板. 十 HY的 Amy菌.

3. 回收 17477-ccdB-MazF → 转化 +F因子/ -F因子的质粒至 BL21 (DE3) → 涂板 ✓

4. 大肠 Amylase 摇菌, 稀释至 10^{-4}/10^{-5} 各涂 100ul 至 M9-淀粉 → 平板. 37°C 至周一.

8/5 - 8/9

No.

Date.

8/5.

1. 17477-ccdB-MazF NotI 37℃ 1h
2. 17477-ccdB-MazF PCR验证.没条带.
   4.12-174ccdB-R + Yz-17477-F 未有条带.

且菌株奇怪，硬！像霉菌.

3. N25rB菌(Amp) PCR验证 Kana-F2/R2 ✓→送测序
4. 摇17477-ccdB-MazF菌.
5. ~~摇Masp4A1~~

8/6.

1. 17477-ccdB-MazF 提质粒、切胶. ✓
2. S1030 化转感态、制作 → 转17477-ccdB-MazF（明天）✓
3. N25rB/Amylase 易错PCR 二轮 → 回收 ✓
3. 葡萄糖异构酶 065-4 GI4.(Amp) 转化Rosetta ✓
   GI2 (Amp).
4. pRnaD-Amy / 28a-Amy 摇菌 ✓
   2 4/6

No.

Date.

8/7

1. G12 转 Rosetta. ✓
2. 17477 +F / -F 转化 BL21 / S1030. (6个板)
   NotI ✓
3. G14 活化 (跟 G12 一起).
4. PCR n25rB 骨架 & Amy 骨架. ⟶ 回收 ⟶ 无缝克隆
5. prmap / pet28a-ZN3-Amylase 提质粒 ✓ ✓
6. pet28a-16fyx-Masp 2copy 无缝克隆 ⟶ 转 DH5α.
7. 回收 200 易错片段 扩 1 管. 10 个循环. ✓

8/8

1. G12 / G14 Rosetta 诱导 30°C, 0.5mM IPTG.
2. prmap / 28a-ZN3-Amylase 骨架回收 ⟶ 无缝克隆 ⟶ 转化 DH5α
   n25rB.
3. 17477-HIER (ER mut) - CcaB-Mu2F 转化 BL21 (DE3) ✓.

8/9.

1. G12 / G14 跑胶 ✓.
2. prmap / 28a Amylase 转化 DH5α ✓.
3. 划板. pet28a-MaSp2-16fyx ✓ / pgex6p1-Spider 3 ✓.

No.

Date.

8/12 ✓

1. G12/G14 摇菌 → 保种 ✓
2. Mfps-16fgx-2copy 提质粒 → 转化 BL21 / Rosetta ✓
3. prmaD-2N3-Amylase 涂布 SH板 ✓
4. pet28a-pSIR-prmaD-2N3-ppE 摇菌. X菌加 sensor的 给那

8/13

1. Mfps-16fgx-2copy BL21/Rosetta 活化 → 30℃, 0.5mM IPTG 诱导 ✓
2. G21/G23 转 Rosetta. ✓
3. N2SrB 构建. 无缝克隆 ✓
4. prmaD-2N3-Amylase PCR 验证 (没有)重新无缝克隆了.
5. 构建 prmaD-pSIR-2N3-ppE (ER-PCR) → 无缝克隆中
6. HYER 验证 有杂带, 找, 重新 PCR. (重新 PCR 上面的杂带没有)

8/14

1. G21/G23 转 Rosetta ✓
2. N2SrB PCR 验证 ✓
3. prmaD-2N3-Amylase 转化 DH5α / prmaD-2N3-ppE 转 DH5α ✓
3. prmaD-2N3-Amylase 挑菌摇起来 PCR, 加上重设引物看看
4. HYER 跑胶. ✓
5. Mfps / 2Mfps PCR. 没有 2copy. 重构.

No.
Date.

8/15

1. G22/G24 活化 ✓
2. pet28a-PSIR-prmab 2w)-OPE ER PCR 验证 ✓
3. prmab-2w)-Amylase 验证. ✓
4. Mfp5-16fgx-optimized 2copy 重构→P骨架.

8/16.

1. ~~G22/24~~ Mfp5 16fgx-optimized-2copy 转化 DH5d.
2. HYER Nested PCR 着着.

8/19—8/23.

No.
Date.

8/19.

1. 活化 J二[illegible]酸菌 / BL21(DE3) TP-TAA.
2. [illegible] psir-prmap-2N3-DPE ER ⟶ 提质粒 ✓
3. prmap-2N3-Amylase ⟶ 提质粒 ✓
4. N2SrB ⟶ 提质粒 ✓
5. Mfp5-2copy 转化 DH5α. (Kana)

8/20

1. psiR-prmap-2N3-DPE ER 转化 Rosetta ✓
2. pJOE-gpl-N2SrB-MarathonRT 和R3 ER 转化 BL21(K) TP. ✓
3. gpl PCR 验证 ✓
4. Mfp5-16Egx 2copy PCR 验证 ✓
5. J二酸 / 甲醇 Nissle, 做化转感受态

8/21

1. 甲醇 Nissle 做化转感受态 ⟶ 转化 frdA (Amp), CarAKR (Amp)、SthA(Str)、IG22 (Amp)、腈氨酸 (Amp).
2. 活化 N2SrB.
3. 活化 pet28a-psiR-prmap-2N3-DPE ER.

8/22
1. G21 转化 Rosetta.
2. pgex6p1-N25rB-MarathonRT BR3 ER. 菌液转接 A/C.
0.5mM IPTG 诱导, 30°C 过夜.
3. 甲醇 Nissle 诱导. 0.5mM IPTG, 30°C 诱导过夜.
4. G23 30°C, 0.5mM IPTG 诱导.
5. pirnab- ZN3-Amylase M4 淀粉 稀释 10⁻⁶, 涂布.

8/23
1. pgex6p1-N25rB-MarathonRT BR3 ER. 洗涤 → 转接 5%/10%
LB A/C/K; 直接 30°C 培养 0.5mM IPTG.
2. 甲醇 Nissle 诱导. 0.5mM IPTG. 30°C 诱导过夜.
3. G23 30°C, mM 诱导.
4. HYER NF3/R3. PCR NF1/ ccdB-R / 菌液 X

9.5 第1轮 ①
9.6 第1轮 ②
9.7 第1轮 ③

11:30 做完ARTP，涂板上
到第二天 相同时间 OD600
首次 0.876×2
之后 0.138
0.876×2 − 0.138
= 1.752 − 0.138 = 1.614

WB800 −ARTP − COBA 平板 涂板

TB 5ml + 2.5μl Cm (WX) + 100μl 甘油
TB 5ml + 2.5μl Cm (WX) + 100μl 甘油

9.17

9.17 淀粉酶 gene

WB800 枯草芽孢 1 ×4
淀粉酶F 1 ×4
淀粉酶R 1 ×4
MIX 25μl ×4
ddH2O 22μl ×4

58℃、60℃、62℃、64℃

枯草质粒骨架
LH质粒 0.5μl ×2 = 1
枯草骨架F 1 ×4 = 4
枯草骨架R 1 ×4 = 4
Mix 25 ×4 = 100
ddH2O 22 ×4 = 88

58℃ 60℃ 65℃ 65℃

Takara DL10000 marker　DNA琼脂糖凝胶电泳

做胶回收

xp binding buffer
spw wash buffer

5μl ddH2O 洗脱

Seamless cloning Master Mix 10μl
枯草质粒骨架 ~~5μl~~ 3
淀粉酶片段 6μl 5
ddH2O ~~[illegible]~~ 2
50℃ 1h

~~5×CE Ⅱ buffer 4μl~~

实验者：　记录者：　复核者：　审阅者：
~~Exnase Ⅱ 2μl~~
第　页
骨架 3　37℃ 30min.

上海　月　　天气：　　温度：　℃　相对湿度：　%

实验名称：　　　　日期：　年　月　日

9.25

pTet-SfGFP-WB800　转化->25Cm 4个　转化平板 4个

pHT01-淀粉酶800　转化->25Cm 4个　转化平板 4个

挑单克隆子 10ul 于PCR管中，98℃，10min

取 0.5ul DNA 作为模板

DNA 2.5ul

F 0.5ul

R 0.5ul

Mix 10ul

ddH₂O 8.5ul

SfGFP

F 枯草-825sfGFP F

R 枯草-825sfGFP R

淀粉酶

F 10-C3-26 F

R 10-C3-36 R

9.26

接菌乳杆菌 抑菌实验 10:30 放入37℃摇床 200rpm

测乳杆菌 OD600

+ LB+Amp + 1% 受测菌(LB+Amp)

~~1.5ml 乳杆菌~~

13.5ml 大肠 + 10ml 乳杆菌

17.5ml 大肠 + 10ml RMS

抑菌实验 OD600 无明显下降

LB+Cm25 pTet-SfGFP-WB800 单克隆①②③

LB+Amp100 pTet-SfGFP-DH5α

Tet 40mg/L = 89nM

89×2=178nM

96孔板做实验

DH5α　DH5α　LB　①　②　③　LB

Tet

Tet

Tet

实验者：　　记录者：　　复核者：　　审阅者：

9.26 实验名称：

pHT01-欧拉蛋白-WB800

Cm25 单1. 单2. 单3

Cm10 1. 2. 3

涂板 LB+Cm25 15:00 37℃

9.27 500ul → 14ml TB+Cm25

37℃ 220rpm

留1ml 未诱导样 18:00 + 14ul 1M IPTG

37℃ 诱导过夜

9.28 离心，取上清 倒掉

160ul上清 + 40ul buffer

marker	单①	单②	单①	单②	单③	①	②	①	②	③
	未	未	+	+	+	未	未	+	+	+

(Cm25 over 单 lanes; Cm10 over numbered lanes)

9.26 R2810 - pET28a-Gl9-9

涂板 LB+Kan

9.27 1ml → 150ml LB+Kan

37℃ 200rpm

9.28 12000rpm, 5min 收集菌体

9.28 pHT01-SfGFP 构建

pHT01-Ang①-C3-D 质粒 0.5×2

pHT01-SfGFP骨架 F/R: F 1ul ×4, R 1ul ×4, MIX 25ul ×4, ddH₂O 22.5ul ×4

骨架有条带

~~[struck]~~

pMA-SfGFP 质粒 0.5×2

pHT01-SfGFP基因 F/R: F 1ul ×4, R 1ul ×4, MIX 25ul ×4, ddH₂O 22.5ul ×4

片段无条带

换 新提的 pMA-SfGFP 质粒

狄苗 9.

pHT01-sfGFP构建

T01-AmyQ-C3-36质粒 0.5×2

GFP骨架 F 1ul ×4

F/R R 1ul ×4

MIx 25ul ×4 =100

ddH2O 22ul ×4 =88

pET-sfGFP质粒 0.5×2

pET-sfGFP F 1ul ×4

骨架R 1ul ×4

Mix 25ul ×4

ddH2O 22ul ×4

退火 60°C

2× Rapid Taq M

95°C 3min

3cycle { 95°C 30s / 60°C 30s / 72°C 1min }

72°C 5min

骨架 PCR

T01-淀粉酶-WB800 Cm25平板 单1.2.3 活化 LB+Cm 25

37°C

Cloning Master Mix 10ul

骨架 3

片段 7

~~ddH2O~~

5×CE II buffer

Exnase II

骨架

片段

ddH

审阅者

复核者:

记录者 ... 11.5 Min

上接　　页　　　　　　　　天气：　　　温度：　℃　相对湿度：

9.26 实验名称：　　　　　　　　　　　　　　　日期：　年　月

pHT01-鲸扣钙-WB800

Cm25 单1、单2、单3

Cm10 1、2、3

涂培 LB+Cm25 15:00 37℃

9.27 300ul → 14ml TB+Cm25

37℃ 220rpm

留1ml未诱导样 18:00 + 14ul IMIPTG

9.28 37℃诱导过夜

离心，取上清倒掉

160ul上清 + 40ul buffer

marker	Cm25					Cm10				
	单①	单②	单①	单②	单③	①	②	①	②	③
	未	未	+	+	+	未	未	+	+	+

9.26 Rosetta-pET28a-GFP-9

涂培 LB+Kan

9.27 1ml → 150ml LB+Kan

37℃ 200rpm

9.28 12000rpm, 5min 收集菌体

9.28 pHT01-SfGFP 构建

pHT01-AmyQ-C3-加质粒 0.5×2

pHT01-SfGFP骨架 F 1ul ×4

F/R R 1ul ×4

MIX 25ul ×4

ddH2O 22.5ul ×4

骨架有杂带

~~[illegible]~~

pTac-SfGFP质粒 0.5×2

pHT01-SfGFP插入 F 1ul ×4

F/R R 1ul ×4

MIX 25ul ×4

ddH2O 22.5ul ×4

序列无杂带

换 新模板 pTac-SfGFP质粒

实验者：　　　记录者：　　　复核者：　　　审阅者：

苏和 9.??

2x Rapid Taq Master Mix
缓冲液

95°C 3min
30cycle { 95°C 30s
60°C 30s
72°C 1min }
72°C 5min

骨架 PCR 失败

10.7. pHT01-淀粉酶-WB800 Cm25平板 单1.2.3 活化 LB+Cm 25
14:30 37°C

5x CE II buffer
Exnase II
骨架
片段

Seamless Cloning Master Mix 10ul
骨架 3
片段 7

50°C 45Min

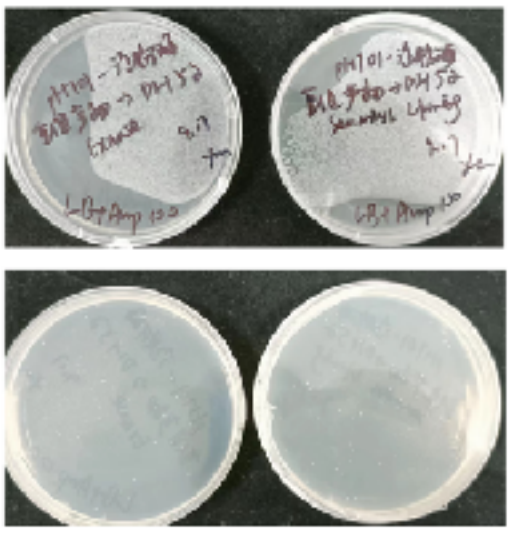
实验者： 记录者： 复核者： 审阅者

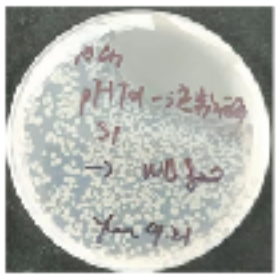
第 页

→ 转 连接产物 → DH5α

载体构建
pHT01-淀粉酶

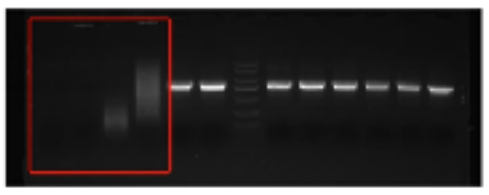
同时用两种方法进行片段连接诺唯赞Exnase和生工的Seamlessing cloning
各挑2个单克隆测序，S1测序结果正确。

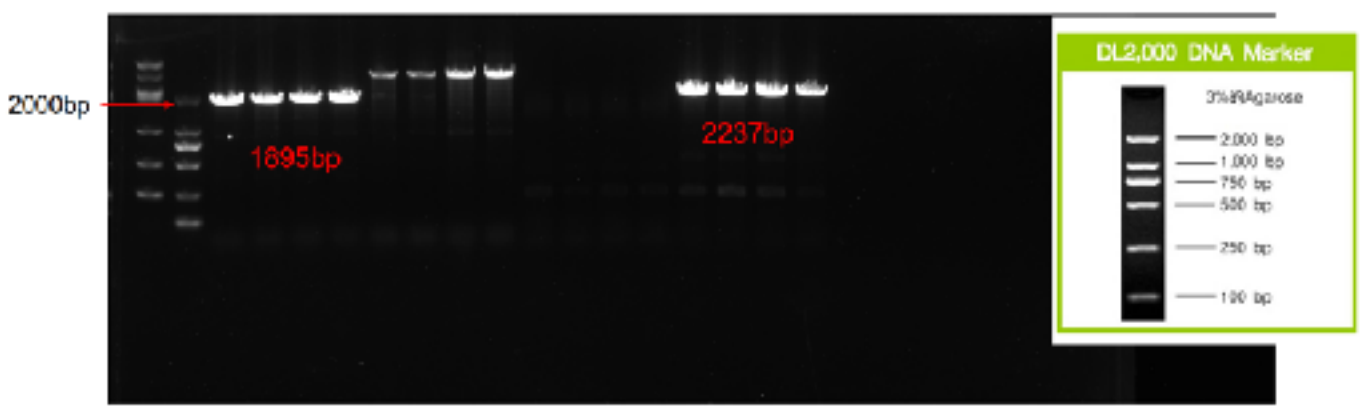




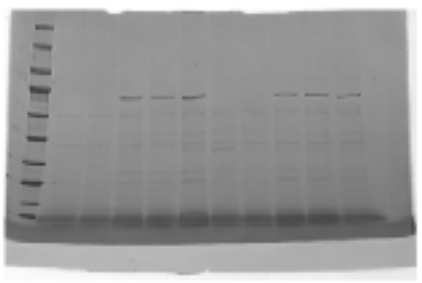
10μL 220ng/μL pHT01-淀粉酶质粒 转化500 μL WB800感受态
37℃，220rpm液培2.5h，5000rpm离心1min,吸取400μL上清，
剩100μL重悬，涂板LB+10mM Cm

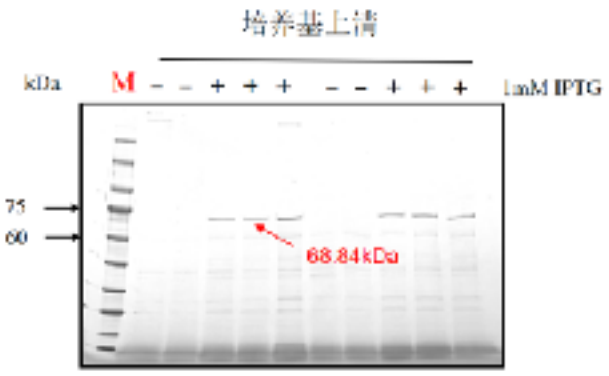
挑4个单克隆，菌液PCR测序



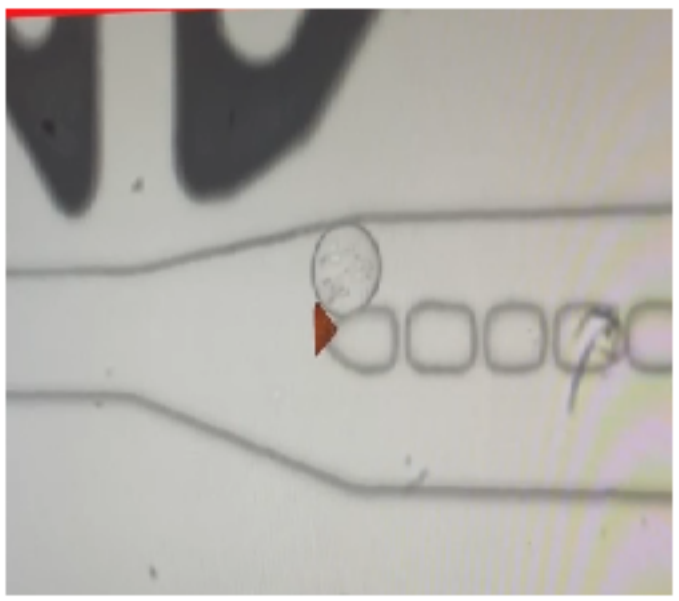

pTet-sfGFP-WB800单克隆
pHT01-淀粉酶-WB800单克隆
2000bp
1695bp
2237bp
DL2,000 DNA Marker
3%琼脂糖
2,000 bp
1,000 bp
750 bp
500 bp
250 bp
100 bp

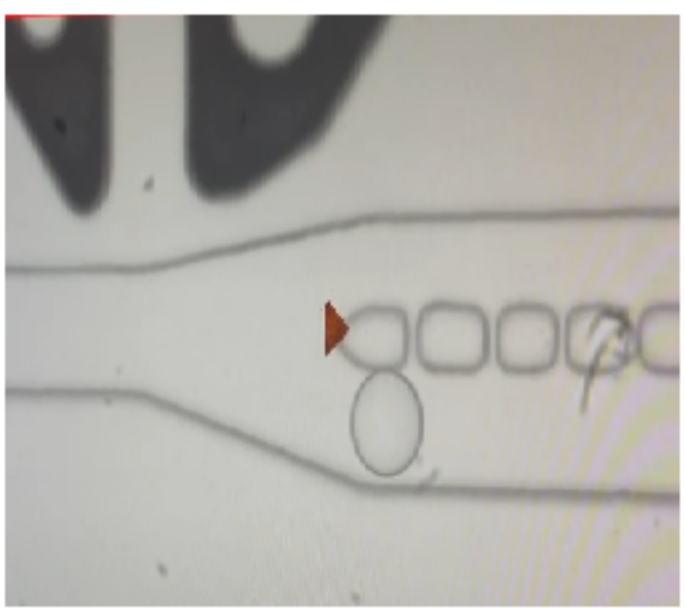
pHT01-淀粉酶-WB800蛋白诱导表达

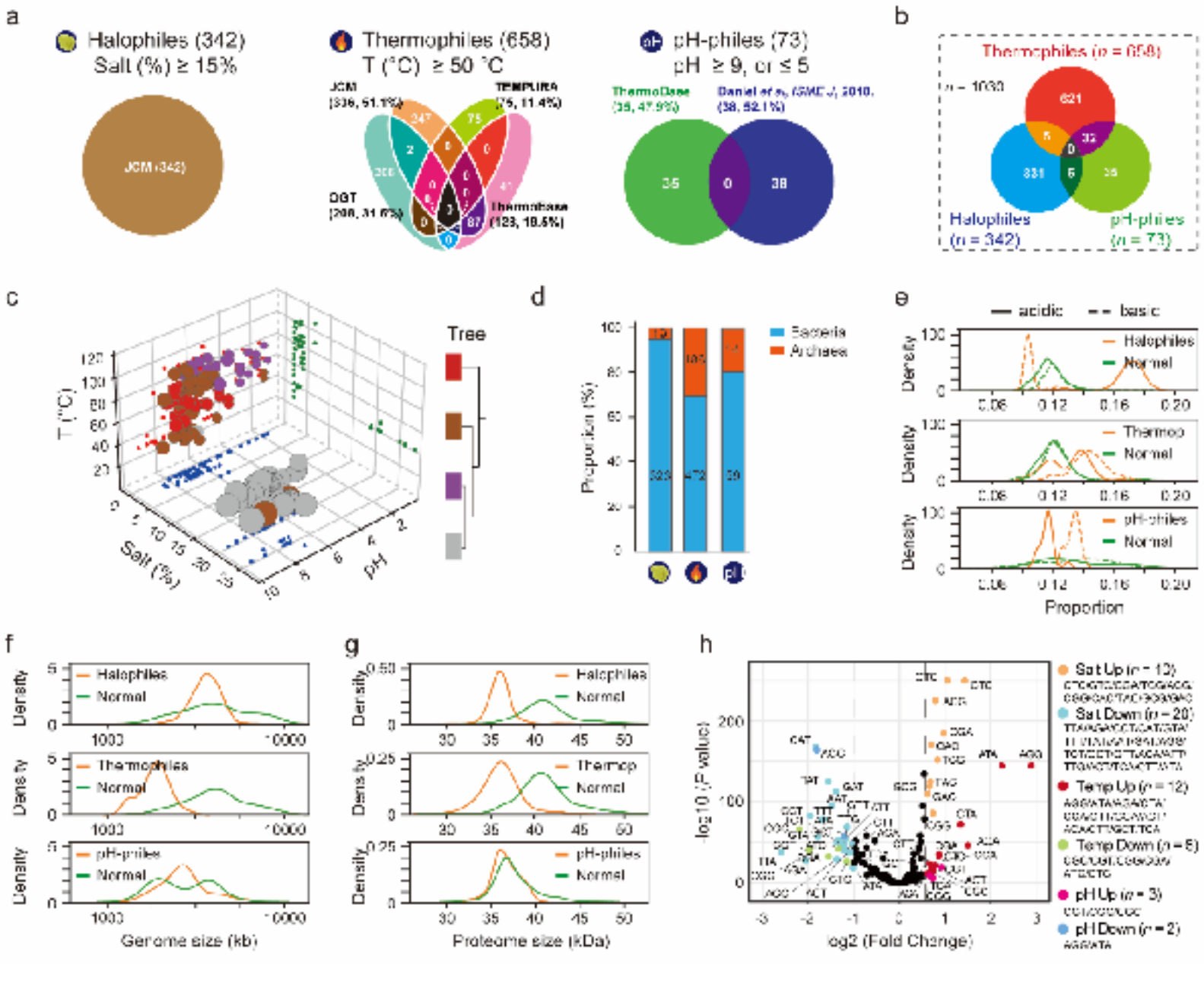


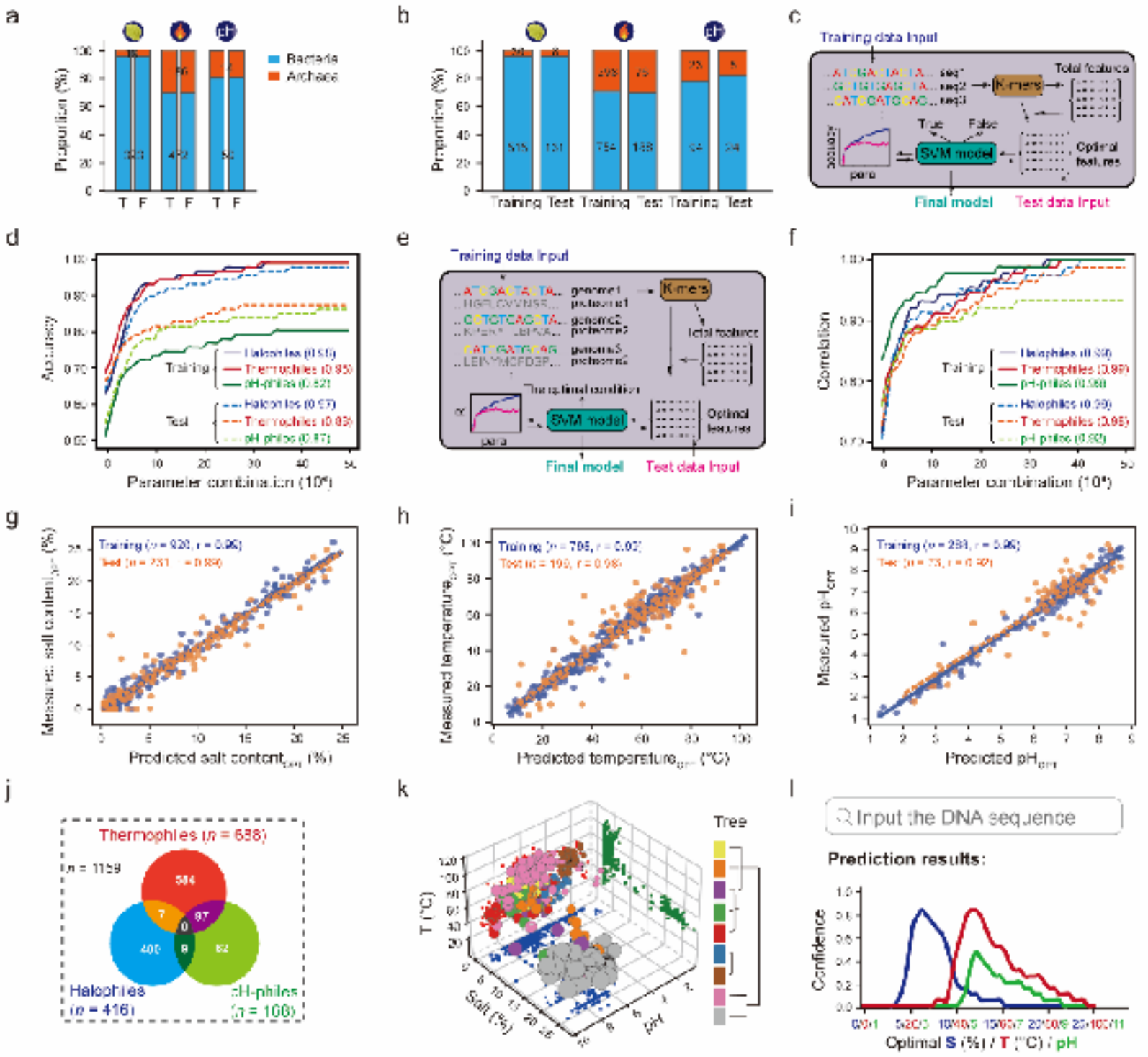


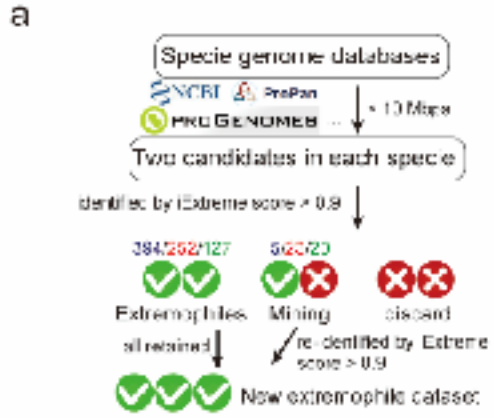
pHT01-淀粉酶-WB800

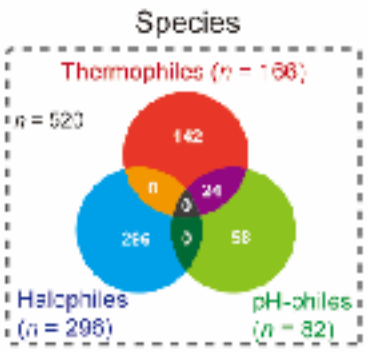


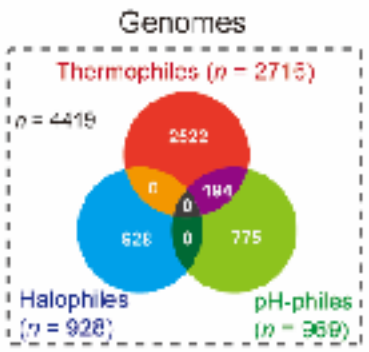


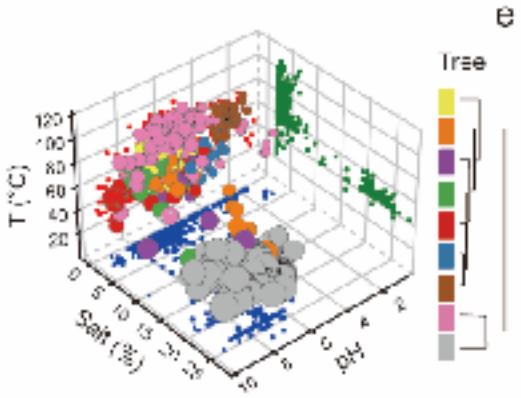

a
Halophiles (342)
Salt (%) ≥ 15%
JCM (342)
Thermophiles (658)
T (°C) ≥ 50 °C
JCM
(336, 51.1%)
TEMPURA
(75, 11.4%)
DGT
(208, 31.6%)
Thermohasa
(123, 18.5%)
pH-philes (73)
pH ≥ 9, or ≤ 5
ThermoDesa
(35, 47.9%)
Daniel et al. (SMC J, 2010,
(38, 52.1%)
b
Thermophiles (n = 658)
n = 1030
621
331
38
Halophiles
(n = 342)
pH-philes
(n = 73)
c
T (°C)
Salt (%)
pH
Tree
d
Proportion (%)
Bacteria
Archaea
e
acidic
basic
Halophiles
Normal
Thermo
pH-philes
Density
Proportion
f
Halophiles
Normal
Thermophiles
pH-philes
Density
Genome size (kb)
g
Thermop
Proteome size (kDa)
h
-log10 (P value)
log2 (Fold Change)
Sal Up (n = 12)
Sal Down (n = 20)
Temp Up (n = 12)
Temp Down (n = 6)
pH Up (n = 3)
pH Down (n = 2)

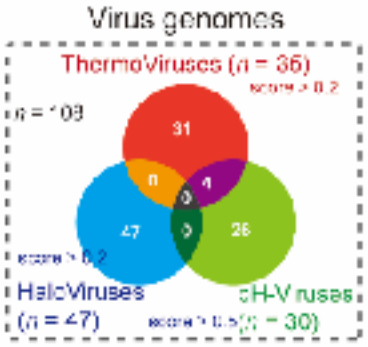

a
Proportion (%)
Bacteria
Archaea
T F T F T F
b
Proportion (%)
Training Test Training Test Training Test
c
Training data input
Kmers
Total features
True
False
SVM model
Optimal features
Final model
Test data input
d
Accuracy
Training
Test
Parameter combination (10⁴)
e
Training data input
Kmers
Total features
SVM model
Optimal features
Final model
Test data input
f
Correlation
Training
Test
Parameter combination (10⁴)
g
Predicted salt content (%)
h
Measured temperature (°C)
Predicted temperature (°C)
i
Measured pH
Predicted pH
j
Thermophiles (n = 638)
n = 1159
584
7
97
0
400
9
82
Halophiles
(n = 416)
pH-philes
(n = 100)
k
Tree
T (°C)
Salt (%)
pH
l
Input the DNA sequence
Prediction results:
Confidence
Optimal S (%) / T (°C) / pH

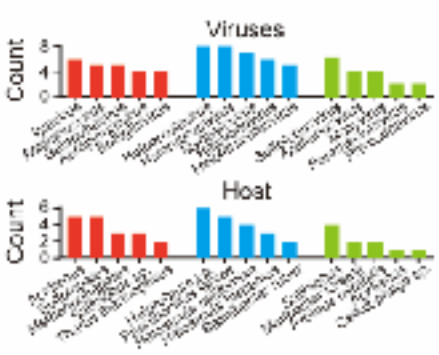

Specie genome databases
NCBI
PROGENOMES
Two candidates in each specie
identified by iExtreme score > 0.9
Extremophiles
discard
all retained
re-identified by Extreme score > 0.9
New extremophile species

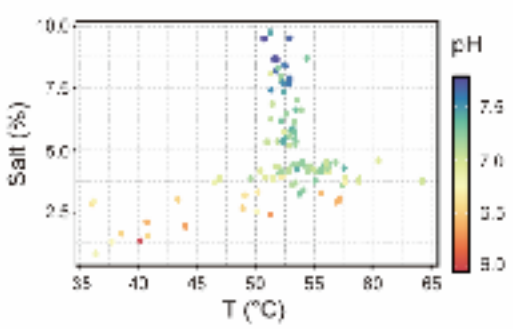

Species
Thermophiles (n = 166)
n = 523
142
24
266
58
Halophiles (n = 298)
pH-philes (n = 82)

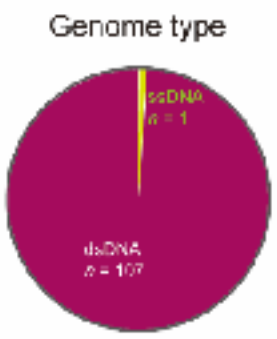

Genomes
Thermophiles (n = 2715)
n = 4419
2522
194
626
775
Halophiles (n = 926)
pH-philes (n = 969)

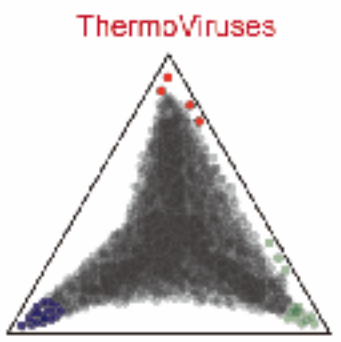

Tree
T (°C)
Salt (%)
pH

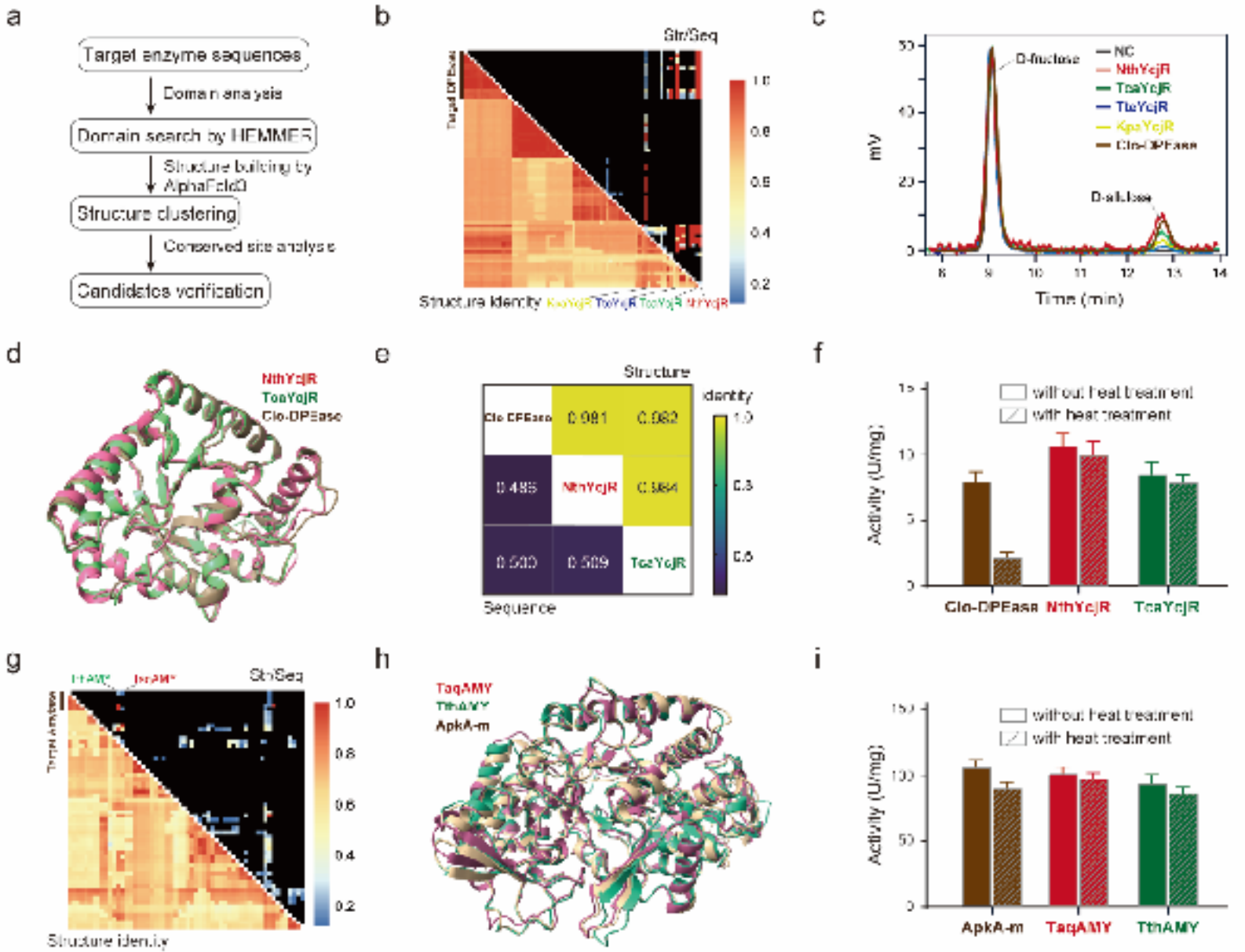

Virus genomes
ThermoViruses (n = 36)
n = 108
31
47
26
HaloViruses (n = 47)
pH-Viruses (n = 30)


Viruses
Count
Host
Count


Salt (%)
T (°C)
pH


Genome type
ssDNA
n = 1
dsDNA
n = 107


ThermoViruses
HaloViruses
pH-Viruses


a
Target enzyme sequences
Domain analysis
Domain search by HEMMER
Structure building by AlphaFold2
Structure clustering
Conserved site analysis
Candidates verification
b
Str/Seq
Structure identity
c
D-fructose
D-allulose
NC
NthYcjR
TcaYcjR
TtaYcjR
KpaYcjR
Clo-DPEase
mV
Time (min)
d
NthYcjR
TcaYcjR
Clo-DPEase
e
Structure
Identity
Clo-DPEase
0.991
0.982
0.485
NthYcjR
0.984
0.503
0.509
TcaYcjR
Sequence
f
without heat treatment
with heat treatment
Activity (U/mg)
Clo-DPEase
NthYcjR
TcaYcjR
g
Str/Seq
Structure identity
h
TaqAMY
TthAMY
ApkA-m
i
without heat treatment
with heat treatment
Activity (U/mg)
ApkA-m
TaqAMY
TthAMY