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**FAKULTET ELEKTROTEHNIKE I RAČUNARSTVA**

Bioinformatika

**Projekt**

**Improving Bloom Filter Performance on Sequence Data Using k-merBloom Filters**

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# Uvod

Ovaj rad bavi se problemom pohranjivanja velikog broja k-mera. K-meri su kraći podnizovi zadane sekvence jednake duljine. Sekvencu želimo podijeliti na više dijelova, odnosno k-mera, jer algoritmi zasnovani na upotrebi k-mera uvelike poboljšavaju usporedbu sekvenci [1].

Sekvenca zbog svoje veličine može generirati stotine milijuna k-mera. Pohranjivanje tako velikog broja k-mera predstavlja veliki problem te se često za pohranjivanje koristi Bloomov filter, prostorno učinkovita podatkovna struktura koja se koristi za ispitivanje članstva elemenata u skupu. Učinkovitost se ostvaruje nauštrub male vjerojatnosti krivog odgovora, naime moguće je da se za neki element tvrdi da on jest član skupa iako on to nije (obrat ne vrijedi). Takav rezultat naziva se lažno pozitivni rezultat [2].

U ovom radu su opisani različiti algoritmi kojima se nastoji unaprijediti upotreba običnog Bloomovog filtera. Želi se smanjiti broj lažno pozitivnih rezultata, veličina korištene memorije te vrijeme potrebno za dobivanje rezultata. Originalna implementacija algoritma nalazi se na poveznici <https://github.com/Kingsford-Group/kbf>, a opisana je u [1]. Za Bloomov filter koristila se već postojeća implementacija dostupna na poveznici [https://github.com/mavam/libbf](https://l.facebook.com/l.php?u=https%3A%2F%2Fgithub.com%2Fmavam%2Flibbf&h=ATMBHJjeByDW8dwgPa5ub-USq0lqNEtVlCJXEphcj9h98XBIZe3ldvbowFo0v4wnqlG6qOkjwye5w1YpJPNeHzXIRMBK8hPpiybGRDIMurjWyy6rcMo6tXzFEJheZHuuyOExOelgYwEhOg).

# Algoritmi za smanjenje broja lažno pozitivnih rezultata

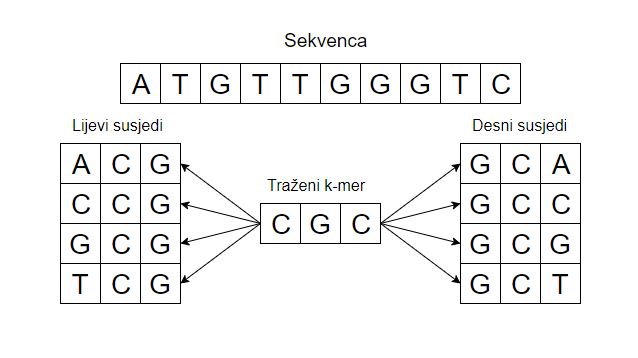
Prilikom testiranja Bloomovog filtera na postojanje nekog k-mera u skupu, on može vratiti pozitivan ili negativan rezultat. U slučaju negativnog rezultata, zbog same definicije Bloomovog filtera sigurni smo da se traženi k-mer ne nalazi u početnom skupu. Međutim, u slučaju pozitivnog rezultata, k-mer se može nalaziti u početnom skupu, ali i ne mora, jer se može raditi i o lažno pozitivnom rezultatu. Međutim, zbog načina na koji nastaju k-meri, ispitivanjem susjedstva možemo smanjiti vjerojatnost pojave lažno pozitivnih rezultata. Upravo to je osnovna ideja sljedećih algoritama.

## Jednostrani k-mer Bloomov filter

U ovom algoritmu stvara se k-mer Bloomov filter koji provjerava postojanje svih mogućih susjeda za trenutni k-mer. Ukoliko pretraga Bloomovog filtera za ni jedan od susjeda ne vrati pozitivnu vrijednost tada se sa sigurnošću može reći da je k-mer bio lažno pozitivan. Ukoliko pretraga susjeda vrati pozitivan rezultat ne možemo tvrditi da je traženi k-mer sigurno pozitivan jer je i susjedni k-mer kao i traženi mogao biti lažno pozitivan.

Za svaki k-mer se stvara 8 susjeda, baze A, C, T, G dodaju se s lijeve ili desne strane.

Primjer:



Slika 1. Primjer jednostranog filtera

Ukoliko Bloomov filter na Slici 1. vrati pozitivan rezultat te ukoliko ni jedan od njegovih susjeda ne vrati lažno pozitivan rezultat za traženi k-mer se sa sigurnošću može reći da nije sadržan u sekvenci.

## Dvostrani k-mer Bloomov filter

Ovaj algoritam vodi se sličnom idejom kao i prethodno opisani. Dvostrani k-mer Bloomov filter proširuje pravilo jednostranog k-mer Bloomov filter o postojanju susjeda. U jednostranom filteru očekujemo da barem jedan od osam kombinacija susjeda vrati pozitivan rezultat dok u dvostranom filteru očekujemo da sa svake strane zadanog k-mera (lijeve i desne) postoji pozitivan rezultat.

Takvo proširenje dovodi do problema rubnih k-mera sekvence koji nemaju susjeda s obje strane. Taj problem se rješava postojanjem skupa rubnih k-mera. Ako je traženi k-mer rubni dovoljno je da s jedne strane postoji susjed.

Primjer:

![Slika na kojoj se prikazuje tekst

Opis je generiran uz visoku pouzdanost](data:image/jpeg;base64,/9j/4AAQSkZJRgABAQEAYABgAAD/4RD0RXhpZgAATU0AKgAAAAgABAE7AAIAAAAOAAAISodpAAQAAAABAAAIWJydAAEAAAAcAAAQ0OocAAcAAAgMAAAAPgAAAAAc6gAAAAgAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAEpvc2lwYSBLZWxhdmEAAAWQAwACAAAAFAAAEKaQBAACAAAAFAAAELqSkQACAAAAAzg1AACSkgACAAAAAzg1AADqHAAHAAAIDAAACJoAAAAAHOoAAAAIAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA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Slika 2. Primjer dvostrukog filtera

Ukoliko Bloomov filter na Slici 2. vrati pozitivan rezultat vidimo da postoji jedan susjedni k-mer koji bi sigurno vratio pozitivan rezultat. No to bio uvjet koji je dovoljan da se radi o jednostranom Bloomovom filteru. S obzirom da se radi o dvostranom filteru te da traženi k-mer nije u skupu rubnih k-mera ukoliko ni jedan od njegovih desnih susjeda ne vrati lažno pozitivan rezultat za traženi k-mer se sa sigurnošću može reći da nije sadržan u sekvenci.

# Algoritmi za smanjenje veličine početnog skupa k-mera

Korištenjem niže opisanih algoritmima nastoji se smanjiti veličina početnog skupa   
k-mera koji se unose u Bloomov filter, odnosno memorija koju zauzima sam Bloomov filter, bez povećanja vjerojatnosti pojave lažno pozitivnih rezultata. Osnovna ideja ovih algoritama jest, također, činjenica da svaki k-mer *u* ima skupove prethodnika (*Lu*) i sljedbenika (*Ru*) koji se sastoje od k-mera koji prethode, odnosno slijede *u* nekoj od sekvenci. Ako možemo garantirati da je u Bloomov filter pohranjen barem jedan od prethodnika i barem jedan od sljedbenika k-mera *u*, tada možemo ustvrditi postojanje promatranog k-mera bez da ga pohranimo u Bloomov filter. Na taj način smanjuje se veličina početnog skupa k-mera koju je potrebno inicijalno pohraniti u Bloomov filter.

Neka je *Pvu* skup pozicija svih k-mera koji se u sekvenci pojavljuju prije k-mera *u*, a *Auw* skup pozicija svih k-mera koji se u sekvenci pojavljuju nakon k-mera *u*. Tada, *∀ v ∈ Lu*i *∀ w ∈ Ru* definiramo skup udaljenosti:

*Su(v, w) = {iw – iv | iv ∈ Pvu,  iw ∈ Auw}*

Ako za neku preskočnu duljinu *s*, vrijedi da je min *Su(v, w)* ≤ *s* za neki *v* i *w,* tada možemo ustvrditi prisutnost k-mera *u* bez da ga pohranimo u Bloomov filter.

Za određivanje početnog skupa k-mera koji će biti pohranjeni u Bloomov filter koristi se jedan od sljedeća dva pristupa:

1. Olabavljeno prorjeđivanje (*Relaxed k-mer sparsification*)

Neka je zadan skup k-mera *U*. Potrebno je pronaći najmanji podskup *K* skupa *U* takav da za svaki k-mer *u ∈ U* vrijedi ili je *u ∈ K* ili *∃ v ∈ Lu ∩ K* i   
*∃ w ∈ Ru ∩ K* takvi da min *Su(v, w)* ≤ *s*.

1. Strogo prorjeđivanje (*Strict k-mer sparsification*)

Neka je zadan skup k-mera *U*. Potrebno je pronaći najmanji podskup *K* skupa *U* takav da za svaki k-mer *u ∈ U* vrijedi ili je *u ∈ K* ili *∃ v ∈ Lu ∩ K* i   
*∃ w ∈ Ru ∩ K* takvi da *s* ∈ *Su(v, w)*.

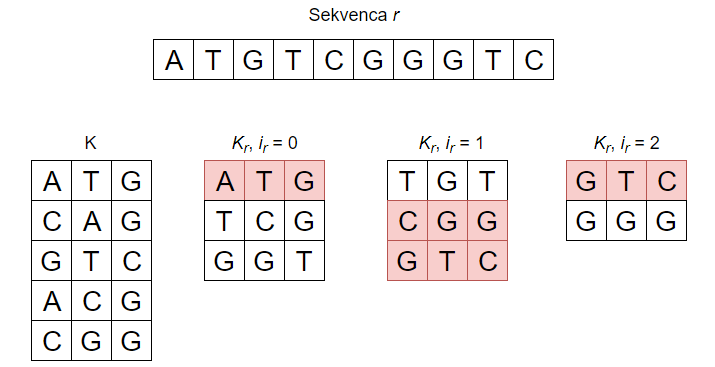
Određivanje početnog skupa k-mera korištenjem navedenih pristupa bilo bi jednostavno, kada bismo na raspolaganju imali sekvence koje se ne preklapaju – npr. poznati genom – rješenje bi tada bilo u početni skup dodati svaki *s*-ti k-mer (algoritam 3.3). Međutim, na raspolaganju će nam uglavnom biti dostupan skup preklapajućih sekvenci nekog genoma te je iz njih potrebno konstruirati što je bolji mogući skup *K*. Tim problemom bave se sljedeća tri algoritma.

## Prorjeđivanje odabirom najbolje pozicije u sekvenci

*(Best Index match per read sparsification)*

Ovaj algoritam za određivanje početnog skupa k-mera *K* iz niza preklapajućih sekvenci koristi pohlepni pristup: iz svake sekvence *r* u početni skup *K* dodaje se svaki *s*-ti k-mer počevši od pozicije *ir.* Početna pozicija *ir* odabire se na način da odgovarajući skup odabranih k-mera iz sekvence *r* ima najveći presjek s početnim skupom k-mera *K* konstruiranim u prethodnih r - 1 iteracija.

Primjer:



Slika 3. Prorjeđivanje odabirom najbolje pozicije u sekvenci, s=2

Na Slici 3. prikazana je jedna iteracija ovog algoritma. Skup *K* je početni skup k-mera konstruiran u prethodnih *r - 1* iteracija. Iz zadane sekvence *r* u skup *K* dodaje se svaki *s-*ti k-mer počevši od pozicije *ir*. *ir*  se odabire na način da odgovarajući skup k-mera *Kr* ima najveći presjek početnim skupom k-mera *K.* Presjek skupova *Kr* i skupa *K* na Slici 3. označen je rozom bojom. Najveći presjek dobije se ako je *ir* = 2 te će u skup *K* biti dodani sljedeći k-meri: *TGT, CGG, GTC.* Nakon toga algoritam kreće u sljedeću iteraciju.

## Prorjeđivanje aproksimacije minimalnog skupa preklapanja

*(Sparsification through approximate hitting set)*

U ovom algoritmu kreće se od pretpostavke da je s=1, odnosno da vrijedi sljedeće:

* k-mer je u Bloomovom filteru, znači da u Bloomovom filteru postoje susjedi koji su udaljeni za dvije baze od njega, a prvi susjedi (udaljeni za jednu bazu) su pokriveni s njim
* k-mer nije u Bloomovom filteru pa u njemu moraju postojati njegovi prvi susjedi (udaljeni za jednu bazu)

Algoritam za svaki k-mer radi listu lijevih i desnih susjeda odnosno listu svih prethodnika i listu svih sljedbenika. U nastavku algoritma koristi se pohlepni algoritam koji uzima onaj k-mer koji se nalazi u najviše skupova prethodnika i sljedbenika ostalih k-mera. Ovaj postupak može se vidjeti na *Slici 4.* Vidi se da se k-mer GT pojavljuje u najviše ostalih setova susjedstva.

![Slika na kojoj se prikazuje elektronički

Opis je generiran uz visoku pouzdanost](data:image/jpeg;base64,/9j/4AAQSkZJRgABAQEAYABgAAD/4RD0RXhpZgAATU0AKgAAAAgABAE7AAIAAAAOAAAISodpAAQAAAABAAAIWJydAAEAAAAcAAAQ0OocAAcAAAgMAAAAPgAAAAAc6gAAAAgAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAEpvc2lwYSBLZWxhdmEAAAWQAwACAAAAFAAAEKaQBAACAAAAFAAAELqSkQACAAAAAzI4AACSkgACAAAAAzI4AADqHAAHAAAIDAAACJoAAAAAHOoAAAAIAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAyMDE4OjAxOjE0IDE2OjE1OjE5ADIwMTg6MDE6MTQgMTY6MTU6MTkAAABKAG8AcwBpAHAAYQAgAEsAZQBsAGEAdgBhAAAA/+ELIGh0dHA6Ly9ucy5hZG9iZS5jb20veGFwLzEuMC8APD94cGFja2V0IGJlZ2luPSfvu78nIGlkPSdXNU0wTXBDZWhpSHpyZVN6TlRjemtjOWQnPz4NCjx4OnhtcG1ldGEgeG1sbnM6eD0iYWRvYmU6bnM6bWV0YS8iPjxyZGY6UkRGIHhtbG5zOnJkZj0iaHR0cDovL3d3dy53My5vcmcvMTk5OS8wMi8yMi1yZGYtc3ludGF4LW5zIyI+PHJkZjpEZXNjcmlwdGlvbiByZGY6YWJvdXQ9InV1aWQ6ZmFmNWJkZDUtYmEzZC0xMWRhLWFkMzEtZDMzZDc1MTgyZjFiIiB4bWxuczpkYz0iaHR0cDovL3B1cmwub3JnL2RjL2VsZW1lbnRzLzEuMS8iLz48cmRmOkRlc2NyaXB0aW9uIHJkZjphYm91dD0idXVpZDpmYWY1YmRkNS1iYTNkLTExZGEtYWQzMS1kMzNkNzUxODJmMWIiIHhtbG5zOnhtcD0iaHR0cDovL25zLmFkb2JlLmNvbS94YXAvMS4wLyI+PHhtcDpDcmVhdGVEYXRlPjIwMTgtMDEtMTRUMTY6MTU6MTkuMjgzPC94bXA6Q3JlYXRlRGF0ZT48L3JkZjpEZXNjcmlwdGlvbj48cmRmOkRlc2NyaXB0aW9uIHJkZjphYm91dD0idXVpZDpmYWY1YmRkNS1iYTNkLTExZGEtYWQzMS1kMzNkNzUxODJmMWIiIHhtbG5zOmRjPSJodHRwOi8vcHVybC5vcmcvZGMvZWxlbWVudHMvMS4xLyI+PGRjOmNyZWF0b3I+PHJkZjpTZXEgeG1sbnM6cmRmPSJodHRwOi8vd3d3LnczLm9yZy8xOTk5LzAyLzIyLXJkZi1zeW50YXgtbnMjIj48cmRmOmxpPkpvc2lwYSBLZWxhdmE8L3JkZjpsaT48L3JkZjpTZXE+DQoJCQk8L2RjOmNyZWF0b3I+PC9yZGY6RGVzY3JpcHRpb24+PC9yZGY6UkRGPjwveDp4bXBtZXRhPg0KICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAKICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgIAogICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgICAgCiAgICAgICAgICAgICAgICAgICAgICAgICAgICA8P3hwYWNrZXQgZW5kPSd3Jz8+/9sAQwAHBQUGBQQHBgUGCAcHCAoRCwoJCQoVDxAMERgVGhkYFRgXGx4nIRsdJR0XGCIuIiUoKSssKxogLzMvKjInKisq/9sAQwEHCAgKCQoUCwsUKhwYHCoqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioqKioq/8AAEQgBhwIyAwEiAAIRAQMRAf/EAB8AAAEFAQEBAQEBAAAAAAAAAAABAgMEBQYHCAkKC//EALUQAAIBAwMCBAMFBQQEAAABfQECAwAEEQUSITFBBhNRYQcicRQygZGhCCNCscEVUtHwJDNicoIJChYXGBkaJSYnKCkqNDU2Nzg5OkNERUZHSElKU1RVVldYWVpjZGVmZ2hpanN0dXZ3eHl6g4SFhoeIiYqSk5SVlpeYmZqio6Slpqeoqaqys7S1tre4ubrCw8TFxsfIycrS09TV1tfY2drh4uPk5ebn6Onq8fLz9PX29/j5+v/EAB8BAAMBAQEBAQEBAQEAAAAAAAABAgMEBQYHCAkKC//EALURAAIBAgQEAwQHBQQEAAECdwABAgMRBAUhMQYSQVEHYXETIjKBCBRCkaGxwQkjM1LwFWJy0QoWJDThJfEXGBkaJicoKSo1Njc4OTpDREVGR0hJSlNUVVZXWFlaY2RlZmdoaWpzdHV2d3h5eoKDhIWGh4iJipKTlJWWl5iZmqKjpKWmp6ipqrKztLW2t7i5usLDxMXGx8jJytLT1NXW19jZ2uLj5OXm5+jp6vLz9PX29/j5+v/aAAwDAQACEQMRAD8A+kaKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAx/FjasnhPUW8OqzamID9nCbd2f9nd8u7GcZ4zjNcV4Z1nw5deJrCy0XxHremawqrJeaN4hlu3luI2QnYI7lsBwcNuiz909VNegavbX93pcsWkX4sLw4MU7QiVQQQcMp6qQMHBBweCDzXPz6Rr3iO603/hI7DSbCHTryO9SSyvZLqSR0Bwo3Qx7Ac8nLcZGOcgj8X3f1/wOuwP4fv8A6/4PTcgn8e3KW97rEOjLL4bsLh4J777XiYiNtkkqQ7MNGrAgkuGwrEKeM51t4wnsfGPiXS7YS6tqc2oxJp+nG4Kqkf2aJnck58uIEklgDycAFiAap+Faw3N3aQ6D4Xuba6vJLgaxe2iS3sKyOXZPLaJlkIJZVYuAAVyp2/Ne1D4d3M2sa3rtgbKHWpL+G90q6OQyeXAsZilYLnY2JAQM8PnrSXS/9bf8H9Cn1t8v0Nebxulpp3iOa/svJudDlEf2dZt32neitEVJA++X2jjqD1ptz4t1WTUL620TQor8aUif2iz33lFZGQOYoR5ZEjhSD8xjHzKM9cU9d8EX2seO9J1hJ7aGw2xNq1qSzNO8DM9vt4wQruSSccAVT1r4ded4l1XUrTw/4Z1k6sVkabW4d0lnIEEeU/dP5iYVTsJTkH5vm+U1t5/5f56/gLS/9df8jV/4Ta71O/s7bwtpEeoC80xNTjnu7s20aozYCtiN2Degwe+cY5ba+O5tWsdITRNKSbVdThlmNpdXXlR26xMEl3yKjk4chRtQ7uvA5q9o/hmXSNdt7hJYXtbfSIrAbI1iJZHJLbEUIoIPRcAdAMViaV4N1zw/DpV9p0ljc6jYx3dvLazTvFBNFNP5oIkCMVZSF/gIOWHHWnpfy1/N2/QnX8vyV/1Nm+8R6xZaZbtJoMcN64laf7Te7LO2SPlpHuFRsKRgrlAx7hcHEngzxZD4w0ea8hSBWt7qS1lNrci4hZlx80coA3qQQQcA9iOKxdd8La5rl1pGp6rp3h/V5rPzw+lXcki2ybypR1kMblpECbdxjXIdiAvQ6ngnQtY0OPWTr1xZzzahqUl6jWgYKquiDYQR/CVIB7gA8EkUR3d+36r9L/1oOXS39b/8A6eiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACuSafxFqvinWLPTdYtrC2sGhVFksfOLb4wxOd69811tc3oP/I7eKv+utr/AOiBQAf2T4u/6Gmy/wDBR/8AbaP7J8Xf9DTZf+Cj/wC21e8S/wDCRf2K3/CHf2X/AGnvXb/avmeTt/iz5fzZ9K8vl8XfGCLx9D4RaLwQb+awN+sgW78oRh9mCc53Z9se9K93YdtLnoX9k+Lv+hpsv/BR/wDbaxdTl8YWPinQ9KXxJZsupfaNz/2UAE8tA3TzOc59R+NdH4V/4Sr+y5P+E3/sf7f5x8v+x/N8ry8DGfM53Z3e2MVmeIv+SleDf+37/wBEiqasItf2T4u/6Gmy/wDBR/8AbaP7J8Xf9DTZf+Cj/wC21uahf2ulabc3+oTLBa2sTSzSMeFVRkmvP7DxP8Q/F9mmq+E9H0TStJm+a1fXJJXmuU7P5cWAgPUZJP50gOk/snxd/wBDTZf+Cj/7bWN4rk8YeH/Dkuox+JbOVkmgj2jSgp/eTJHnJkPTfnp2q34U8Z6hf+Irzwv4r02HTNetIFuVFtMZILuEnHmRkgEANwVIyKm+Jv8AyINz/wBfdn/6VRUwLH9k+Lv+hpsv/BR/9to/snxd/wBDTZf+Cj/7bXSV5x4x1P4qaKNX1TR18HvotlFJcRrdC6NyY0XcQQpC7uD3AqW0txpNuyOj/snxd/0NNl/4KP8A7bR/ZPi7/oabL/wUf/ba5LwbrfxY8SWGja1cJ4Mj0e/EU8iRi6FwIWwWABJXfjOOSM16jVNWJTucF4Rk8YeIvCOnavJ4ls4mvIRIUOlBtvJ4yJBn8q2f7J8Xf9DTZf8Ago/+21X+Fv8AyS3QP+vQfzNQeJPG19beJ4vCvhHS49U114PtExuJvKt7KInAeVgCSSeiqMml5DL/APZPi7/oabL/AMFH/wBto/snxd/0NNl/4KP/ALbXNX3i7x14MWG/8baXo+oaPJMkU9zoRmEtpuOAzRyZ3rkgfKc89K9IVg6BlOVYZB9RT6XA4PSpPGGoeI9d05vEtmq6ZNDGrf2UCH3wrJnHmDH3sdT07Vs/2T4u/wChpsv/AAUf/bar+Gf+R+8a/wDX3a/+ksdXPGXi+z8GaGt9dQy3U88q29nZwf6y5mb7qL/j2FTsBH/ZPi7/AKGmy/8ABR/9to/snxd/0NNl/wCCj/7bWELz4tyxi9XS/CkMeN39myXM7T4/u+cBsDdvuke9dB4K8XQ+MtCa9S1ksbu3ne1vbKVgz206HDISOvqD3BqrAYeoSeMLTxdo2kDxLZldQhuZC40oDb5QjwMeZznf6jp3rZ/snxd/0NNl/wCCj/7bVfXP+SpeE/8Ar01D+UNdRced9ll+yeX5+w+V5udu7HGcc4z6VLdlcDn/AOyfF3/Q02X/AIKP/ttH9k+Lv+hpsv8AwUf/AG2vPfFfi74weD7XT59Ti8ESrf38VhELdbtiskmdpbJHy8c4yfau58K/8LF/tST/AITf/hF/sHkny/7I+0eb5mRjPmcbcbvfOKpag9DP8VyeMPD/AIcl1GPxLZysk0Ee0aUFP7yZI85Mh6b89O1bP9k+Lv8AoabL/wAFH/22q/xN/wCRBuf+vuz/APSqKuspAc3/AGT4u/6Gmy/8FH/22j+yfF3/AENNl/4KP/ttc54x1P4qaKNX1TR18HvotlFJcRrdC6NyY0XcQQpC7uD3Aqr4N1v4seJLDRtauE8GR6PfiKeRIxdC4ELYLAAkrvxnHJGaI+8D0Ot/snxd/wBDTZf+Cj/7bWN4Rk8YeIvCOnavJ4ls4mvIRIUOlBtvJ4yJBn8q72uT+Fv/ACS3QP8Ar0H8zQBY/snxd/0NNl/4KP8A7bR/ZPi7/oabL/wUf/ba0PE2u2/hjwtqOt3n+qsbd5iM/eIHC/UnA/GuZ+GfjXVfFFtqFj4ptLax13Tnjaa3tgyoYpYw8bAMSehIPPUULVtLoD0Sfc1v7J8Xf9DTZf8Ago/+20vhq91WTWNa07WLyK8awkhVJYrfyQQ8e8/Lk+uOvaujrm9B/wCR28Vf9dbX/wBECgDpKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAK5vQf+R28Vf9dbX/ANECukrm9B/5HbxV/wBdbX/0QKAOkrzG8/5Ok07/ALFeT/0ea9OrmJvBvnfFK28ZfbseRpbaf9j8n72ZN+/fu/DGPxpfaT9fyZV/dkvT80zp65HxF/yUrwb/ANv3/okV11cj4i/5KV4N/wC37/0SKZJnfHKG6n+C3iFbIMXEKO4Q8mMSKX/DaDn2zXSJNd3PgaCbwe1i1xJZRtYG73fZzlRt3bPm249K2JoY7iB4Z41kikUq6OMhgeCCO4rgI/htrOhI1t4D8a3eh6cWLLYXVnHfRQ55xHvIZR7biKXRrv8A1/ww97eV/wBP8jO0jXvHFl8V9I0bxtZeFWbUbO4eO60mKczIsYB2l5DwCSOMGun+Jv8AyINz/wBfdn/6VRVH4a+Hlto2uv4g1fVL3X9eePy/t96wAhQ9VijUBY1PoPfnmpPib/yINz/192f/AKVRVXRL+tyFe7f9bI6yuf8AHv8AyTjxJ/2Crn/0U1dBWfr+l/254b1LSvO8j7daS23m7d2zehXdjIzjOcZFZzTcWkbUpKNSLfcwfhR/ySLwv/2DIf8A0EV11ZHhPQv+EX8I6Xof2n7V/Z9slv5/l7PM2jGduTj6ZNa9ayd5NoximopM5P4W/wDJLdA/69B/M1zvghRb/HT4hxXfF1OLKaEsfvQeWRx7A8Gui+Fv/JLdA/69B/M0virwJB4h1S11r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X2K3lzJIQWwcZCnsaANuuEv/hxd22uXur+B/E1z4auNQfzLy3Fsl1bTSd5PKbG1z3IIzWv/AGn4x/6FnTP/AAct/wDGKP7T8Y/9Czpn/g5b/wCMUDv0KHh74fGw8RL4j8Ta3deItcjiMMNzPGsMVsh6iKJeFJ7nJPv1rs65v+0/GP8A0LOmf+Dlv/jFH9p+Mf8AoWdM/wDBy3/xincRF8Mf+SWeG/8AsHxf+g1J4q8EWPie5tL9bq60vV7HP2XUrFwssYPVTkEMh7qR+VZPhSLxn4e8JaZpD+HdNmaxt0gMh1dl37RjOBCcfma1/wC0/GP/AELOmf8Ag5b/AOMVIHP3Hw68Ua1C1l4q+Il9e6W/EltYWEVk8y/3XkXJII4IG3PNdxpOlWOh6TbaZpNuttZ2qCOGJOir+P8AOsf+0/GP/Qs6Z/4OW/8AjFH9p+Mf+hZ0z/wct/8AGKYdbh4d/wCRv8W/9ftv/wCksVdJXO+GLHVYNS1q/wBZtYLSTULiOVIoLgzBQsKR/eKrz8meneuioAKKKKAOc8R/8hvS/wDrlP8A+06qyxJPC8UyLJG6lXRhkMD1BFaOv6dfXd7ZXFhHDL5CyK6yylPvbcYOD/dNUf7P1z/nxtP/AAMP/wARWEk22elSnFU4q/8AVzh08A6royGDwX4tutHsSxZbK4tEvIos84j3YZR7ZIq/oHgaDStZbW9U1G71vWmTyxeXZAESnqscagKgPtXU/wBn65/z42n/AIGH/wCIo/s/XP8AnxtP/Aw//EUe8P8Ad9195na7/wAgo/8AXeH/ANGpWjVbUNF128szCLO0U+Yj5F2T91w39z2qz/Z+uf8APjaf+Bh/+IqbO+xrzw5Urr7ylrWnf2voN/pvm+T9stpIPM27tm9SucZGcZ6ZqDwzo3/CO+F9O0fz/tP2G3SHztmzftGM4ycfTJrU/s/XP+fG0/8AAw//ABFH9n65/wA+Np/4GH/4inZk80HbVff/AF2CtXwv/wAirpv/AF7r/Ksr+z9c/wCfG0/8DD/8RW5olpLYaHZ2lxt82GII205GR6VcE7nPiJRcLJ9S9RRRWpwnN+Ev+Qj4o/7DTf8ApPBXSVyFrB4n0bVdZbT9Gsb22vr43UckuomFgDHGmCoib+5nr3q3/afjH/oWdM/8HLf/ABigBmkeDf7K+IfiDxT9u83+2YreP7L5O3yfKXbnduO7P0GPeunrm/7T8Y/9Czpn/g5b/wCMUf2n4x/6FnTP/By3/wAYo2Vht3d2Vpf+Sz2v/Yvzf+lEVT+OPB58a6bY6dNf/ZbKK+iubuIQ7/tSIc+VnI2gnBzz06VkPB4zbxxFrv8Awj2mhY9Oez8r+12wS0ivu3eT/s4xjv1rZ/tPxj/0LOmf+Dlv/jFHW4jpBx0rlfCHgo+ENX1+a11DzdP1e8+2xWRh2/ZZGHz4fcdwY4OMDGKm/tPxj/0LOmf+Dlv/AIxR/afjH/oWdM/8HLf/ABijZ3DpYPG//IO0v/sNWH/pQldJXG6lD4q1xrC3u9E0+zgh1C2uZJU1NpWCxyq5AXyVycD1rsqACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACub8ff8AIpn/AK/7H/0rhrpK5vx9/wAimf8Ar/sf/SuGgDpK87ufj18NrO7mtrnxJsmhdo5F+w3J2sDgjIjx1FeiV538ev8Akh/iH/ch/wDR0dTJuKuXCPNJR7l3w/8AGLwJ4p1y30fQdd+1X9xu8qH7HOm7apY8sgA4BPJrt6q6Z/yCLP8A64J/6CKtVpJJOxlF3Vzl/hl/ySzw1/2DYP8A0AVV8TfFnwT4O1k6V4j1r7HeqiyGL7JNJ8rdDlEI/WrXwy/5JZ4a/wCwbB/6AK6dvun6VnJtK5Z5wP2gvhiSAPE3J/6cLn/43XpAIZQR0PSvMv2fP+SP2f8A193X/o5q9Nq3ayCStJrs2vuZzfhz/kbPF3/X/B/6SQ10lc34c/5Gzxd/1/wf+kkNdJSEFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFFFFABRRRQAUUUUAFc34+/5FM/8AX/Y/+lcNdJXN+Pv+RTP/AF/2P/pXDQB0lcJ8a9OvdV+Duu2Wl2dxe3UqRCOC2iaSR8TIThVBJ4BP4V2/nw/89U/76FHnw/8APVP++hUyXMrFQlyyUuxFpysml2qOpVlhQEEYIO0VZqPz4f8Anqn/AH0KPPh/56p/30KuTu7kRVlY5v4Zf8ks8Nf9g2D/ANAFdOfun6Vyvw0ljX4W+Gg0ig/2bBwWH9wV0/nw/wDPVP8AvoVLV1YZwHwM0y/0j4V2lpqtlcWNyt1cMYbmJo3AMrEHawB5HNeh1H58P/PVP++hR58P/PVP++hVDbu2+7b+85/w5/yNni7/AK/4P/SSGukrmvDTBvFfi4qQR9vg5B/6dIa6WkIKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKr31hZ6nZSWepWkF5ay48yC4jEiPg5GVPB5AP4UUUAY3/AAgHg3/oUtC/8FsP/wATR/wgHg3/AKFLQv8AwWw//E0UUAH/AAgHg3/oUtC/8FsP/wATR/wgHg3/AKFLQv8AwWw//E0UUAH/AAr/AMG/9CloX/gth/8AiaP+EA8G/wDQpaF/4LYf/iaKKAD/AIQDwb/0KWhf+C2H/wCJo/4QDwb/ANCloX/gth/+JoooA1NL0XS9EgeDRdNs9Pidt7x2kCxKzYxkhQMnAHNXaKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigAooooAKKKKACiiigD//2Q==)

Slika 4. Prvi korak algoritma hitting set

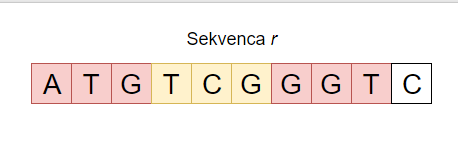
U sljedećem koraku uklanjanja se odabran k-mer iz setova susjedstva ostalih k-mera te se ponovno pohlepnim postupkom odabire sljedeći k-mer.

## Prorjeđivanje nepreklapajućih sekvenci

*(Single sequence sparsification)*

U posebnom slučaju u kojem na raspolaganju imamo nepreklapajuće sekvence (npr. poznati genom) problem strogog prorjeđivanja može se riješiti na jednostavan način: iz svake sekvence *r* u početni skup *K* dodaje se svaki *s*-ti k-mer počevši od početka sekvence.

Primjer:



Slika 5. Prorjeđivanje nepreklapajućih sekvenci, s=2

Na Slici 6. prikazana je jedna iteracija ovog algoritma. Iz sekvence *r* u početni skup *K* dodaje se svaki *s*-ti k-mer počevši od početka sekvence. U ovom slučaju u skup *K* bit će dodani sljedeći k-meri: *ATG, TCG, GGT.*

# Rezultati testiranja

Rezultati testiranja provedeni su nad dijelom podataka koji se koriste za testiranja varijanti Bloomova filtera u proučavanom radu. Dio testova nije bilo moguće ponoviti ni za jednu implementaciju zbog prevelike veličine određenih podataka i memorijskog ograničenja računala (podaci iz rada pod nazivom SRR514250\_1 i SRR553460).

Podaci na kojima se provodi testiranje prikazani su u tablici 1. Podaci pod nazivom ERR233214\_1 i SRR1031159\_1 odgovaraju podacima korištenim u originalnom radu.

Prema rezultatima testova za memorijsko opterećenje iz zadanog rada za ta dva algoritam Hitting set zahtjeva memoriju koja je puno veća od memorije naših računala (42 GB i 28 GB). S obzirom da se podaci nisu mogli ni inicijalizirat nije bilo mogućnosti za izmjerit ostale rezultate za usporedbu s radom te u tablicama ne postoje podaci za ta dva skupa podataka i algoritam hitting set.

Mali set podataka SRR1031159\_1e4 iz tablice potreban je kako bi mogli istestirati hitting set.

Tablica . Podaci za testiranje

|  |  |  |  |
| --- | --- | --- | --- |
| NAZIV | TIP | BROJ SEKVENCI | DULJINA SEKVENCE |
| ERR233214\_1 | Cijeli genom bakterije Pseudomonas aeruginosa | 7 571 879 | 92 |
| SRR1031159\_1 | Cijeli genom | 674 989 | 101 |
| SRR1031159\_1e4 | Dio genoma SRR1031159\_1 | 10 000 | 101 |

Prilikom svih mjerenja duljina k-mera je bila 20 kako bi rezultati bili usporedivi s rezultatima u originalnom radu. Set testnih upita sadrži 106 nasumičnih k-mera dobivenih uniformno s mutacijom na jednoj bazi. Bloomov filter inicijaliziran je s veličinom koja je 10 puta veća od ukupnog broja k-mera.

Podaci u svim tablicama i grafovima su srednja vrijednost od 10 mjerenja.

Tablica 2. Postotak lažno pozitivnih

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| NAZIV | Klasičan  Bloomov filter | 1-kBF | 2-kBF | Best match | Hitting set |
| ERR233214\_1 | 0.0322 | 0.0101 | 0.00089 | 0.0273 | - |
| SRR1031159\_1 | 0.0327 | 0.0101 | 0.00089 | 0.0272 | - |
| SRR1031159\_1e4 | 0.0327 | 0.0101 | 0.00086 | 0.0271 |  |

U tablici 2. prikazani su postoci za lažno pozitivne rezultate na različitim varijantama Bloomovog filtera. Dobiveni podaci odgovaraju mjerenjima dobivenim u zadanom radu.

U tablici se može primijetiti da filteri 1-kBF i 2-kBF, kojima je cilj smanjenje broja lažno pozitivnih, imaju manji udio lažno pozitivnih rezultata od klasičnog Bloomovog filtera. Također može se primijetiti i da algoritmi iz skupine algoritama kojima je cilj smanjiti veličinu početnog skupa k-mera koji se unose u Bloomov filter bez pogoršanja udjela lažno pozitivnih u tome uspijevaju, odnosno ne povećavaju udio lažno pozitivnih rezultata.

U gore prikazanom grafu vidi se da vrijeme potrebno za izvršavanje svih milijun upita drastično raste s upotrebom druge skupine filtera. 2-kBF se pokazao kao dobar pristup za smanjenje lažno pozitivnih rezultata uz mali dodatni utrošak vremena i memorije. Algoritmi koji prorjeđuju skup k-mera nisu pokazali isplativost s obzirom na utrošak memorije prilikom inicijalizacije, postotka lažno pozitivnih i dodatnog utroška vremena.

Tablica 3. Podaci za 2-kBF

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| NAZIV | Broj k-mera | Broj rubnih k-mera | Broj potencijalnih k-mera | Inicijalizacija  (fold change) |
| ERR233214\_1 | 41 766 273 | 1 328 281 | 6 310 923 | 3.425 |
| SRR1031159\_1 | 29 937 099 | 733 730 | 1 088 645 | 1.506 |
| SRR1031159\_1e4 | 586 095 | 15 941 | 16 420 | 0.0298 |

U tablici 3. vidljiv je broj dodatnih k-mera koji je potrebno zapamtiti zbog uvjeta na rubne k-mere. Vidljivo je da ipak nije potrebno spremiti baš sve rubne k-mere s obzirom da možemo izbaciti one rubne koji se pojavljuju i na drugim mjestima u sekvencama. Skup rubnih k-mera je takav da u presjeku sa skupom k-mera koji se ne nalaze na rubovima daje prazan skup. Broj dodatnih k-mera koje pohranjujemo je zanemariv kada su podaci takvi da sadrže manji broj sekvenci. Na primjer ako je ulazni podatak jedna sekvenca (sastavljeni genom) broj potencijalno rubnih k-mera je 2.

Tablica 4. Podaci za prorijeđeni k-mer Bloomov filter

|  |  |  |  |
| --- | --- | --- | --- |
| NAZIV | Broj k-mera | | |
| Klasičan | Best match | Hitting set |
| ERR233214\_1 | 41 766 273 | 21 775 771 | - |
| SRR1031159\_1 | 29 937 099 | 15 121 029 | - |
| SRR1031159\_1e4 | 586 095 | 293 283 | 335 359 |

Tablica 4. prikazuje odnos broja k-mera spremljenih u klasičnom Bloomovom filteru i u prorijeđenim Bloomovim filterima. Prorijeđeni filteri pohranjuju gotovo dva puta manje k-mera. Broj spremljenih k-mera bi se mogao dodatno smanjiti prorjeđivanjem skupa rubnih k-mera.

U tablicama 5 i 6 prikazana su mjerenja za inicijalizaciju. Pod inicijalizacijom se podrazumijeva podjela sekvenci na k-mere, određivanje rubnih k-mera te popunjavanje Bloomovih filtera.

Tablica 5. Memorija potrebna za inicijalizaciju [MB]

|  |  |  |  |
| --- | --- | --- | --- |
| NAZIV | 2-kBF | Best match | Hitting set |
| ERR233214\_1 | 2039 | 1573 | - |
| SRR1031159\_1 | 1589 | 1030 | - |
| SRR1031159\_1e4 | 39 | 35 | 424 |

U tablici 6. prikazano je vrijeme potrebno za inicijalizaciju. Vrijeme koje je potrebno za inicijaliziraju se očekivano razlikuje od rezultata dobivenih u radu jer ovisi o računalu na kojem se testovi pokreću.

Tablica 6. Vrijeme potrebno za inicijalizaciju [s]

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| NAZIV | Klasičan  Bloomov filter | 1-kBF | 2-kBF | Best match | Hitting set |
| ERR233214\_1 | 25.7 | 25.813 | 276.0868 | 588.906 | - |
| SRR1031159\_1 | 18.37813 | 18.35837 | 52.20602 | 68.38462 | - |
| SRR1031159\_1e4 | 0.2838 | 0.2817 | 0.6591 | 0.8879 | 15.908 |

# Zaključak

Ovaj rad prikazuje razne nadogradnje Bloomovog filtera za ispitivanje postojanja k-mera od kojih su se metode koje smanjuju postotak lažno pozitivnih rezultata pokazale kao jednostavne i vrlo učinkovite.

Dvostrani k-mer Bloomov filter (2-kBF) daje čak oko 35 puta manji postotak lažno pozitivnih rezultata u odnosu na klasični Bloomov filter uz minimalan dodatni vremenski utrošak prilikom ispitivanja pripadnosti.

Metode koje stvaraju prorijeđene Bloomove filtere zahtijevaju veći memorijski i vremenski utrošak tijekom inicijalizacije, a daju otprilike jednak postotak lažno pozitivnih rezultata kao i klasični Bloomov filter. Memorija koju zauzimaju tako izgrađeni filteri je otprilike dvostruko manja od klasičnog, ali je upitna isplativost takvog filtera s obzirom na inicijalizaciju i postotak lažno pozitivnih rezultata. Za prorijeđene filtere treba poraditi na algoritmima za inicijalizaciju da bi se pokazali kao učinkoviti.

# Literatura

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