SVEUČILIŠTE U ZAGREBU

**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-merova. K-merovi su kraći podnizovi zadane sekvence jednake duljine. Sekvencu želimo podijeliti na više dijelova, odnosno k-merova, jer algoritmi zasnovani na upotrebi k-merova uvelike poboljšavaju usporedbu sekvenci [1].

Sekvenca zbog svoje veličine može generirati stotine milijuna k-merova. Pohranjivanje tako velikog broja k-merova 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].

# Algoritmi za smanjenje broja lažno pozitivih rezultata

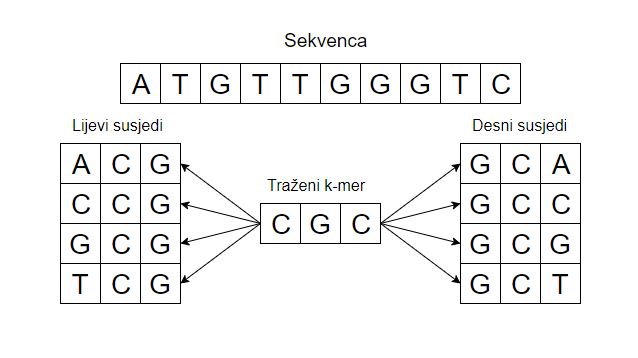
Prilikom testiranja Bloomovog filtera na postojanje nekog k-mera u skupu, on može vratiti pozitivan ili negativan rezultat. U slučaju negativnog rezultata, zbog same definicije Bloomovog filtera sigurni smo da se traženi k-mer ne nalazi u početnom skupu. Međutim, u slučaju pozitivnog rezultata, k-mer 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-merovi, 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-merova sekvence koji nemaju susjeda s obje strane. Taj problem se rješava postojanjem skupa rubnih k-merova. 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-merova 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-merova

Korištenjem niže opisanih algoritmima nastoji se smanjiti veličina početnog skupa   
k-merova 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-merova koji prethode, odnosno slijede *u* 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-merova koju je potrebno inicijalno pohraniti u Bloomov filter.

Neka je *Pvu* skup pozicija svih k-merova koji se u sekvenci pojavljuju prije k-mera *u*, a *Auw* skup pozicija svih k-merova 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-merova 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-merova *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-merova *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)*.

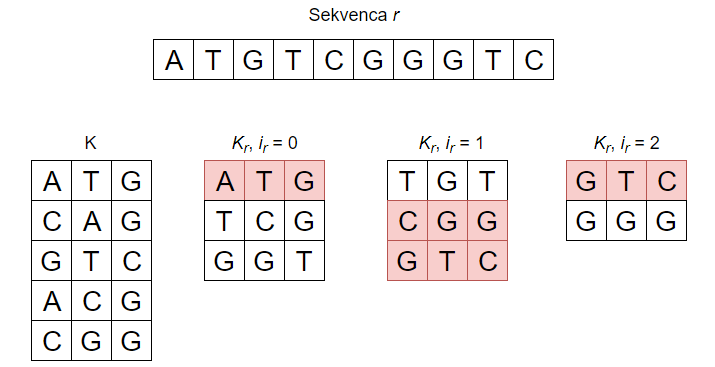
Određivanje početnog skupa k-merova korištenjem navedenih pristupa bilo bi jednostavno, kada bismo na raspolaganju imali sekvence koje se ne preklapaju – npr. poznati genom – rješenje bi tada bilo u početni skup dodati svaki *s*-ti k-mer (algoritam 3.3). Međutim, na raspolaganju će nam uglavnom biti dostupan skup preklapajućih sekvenci nekog genoma te je iz njih potrebno konstruiriati 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-merova *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-merova iz sekvence *r* ima najveći presjek s početnim skupom k-merova *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-merova 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-merova *Kr* ima najveći presjek početnim skupom k-merova *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-merovi: *TGT, CGG, GTC.* Nakon toga algoritam kreće u sljedeću iteraciju.

## Četiri

Bla bla

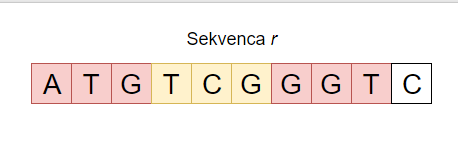
Slika 4.

## 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 5. 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-merovi: *ATG, TCG, GGT.*

# Rezultati testiranja

# Zaključak

Nešto pametno o radu :D

# Literatura

1. D. Pellow, D. Filippova, C. Kingsford, Improving Bloom Filter Performance on Sequence Data Using k-mer Bloom Filters, Journal of computational biology, Volume 24, Number 6, 2017
2. <https://en.wikipedia.org/wiki/Bloom_filter>, pristupano 13.01.2018.